ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2022 November 21; 28(43): 6078-6205

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 28 Number 43 November 21, 2022

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
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EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/1007-9327/editorialboard.htm

PUBLICATION DATE
November 21, 2022

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World J Gastroenterol 2022 November 21; 28(43): 6078-6089

DOI: 10.3748/wjg.v28.i43.6078

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Kyoto classification of gastritis: Advances and future perspectives in endoscopic diagnosis of gastritis

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Jia J, China; Zhang J, China

Received: September 14, 2022
Peer-review started: September 14, 2022
First decision: October 3, 2022
Revised: October 6, 2022
Accepted: November 4, 2022
Article in press: November 4, 2022
Published online: November 21, 2022

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Abstract

This editorial provides an update of the recent evidence on the endoscopy-based Kyoto classification of gastritis, clarifying the shortcomings of the Kyoto classification, and providing prospects for future research, with particular focus on the histological subtypes of gastric cancer (GC) and *Helicobacter pylori* (*H. pylori*) infection status. The total Kyoto score is designed to express GC risk on a score ranging from 0 to 8, based on the following five endoscopic findings: Atrophy, intestinal metaplasia (IM), enlarged folds (EF), nodularity, and diffuse redness (DR). The total Kyoto score reflects *H. pylori* status as follows: 0, ≥ 2, and ≥ 4 indicate a normal stomach, *H. pylori*-infected gastritis, and gastritis at risk for GC, respectively. Regular arrangement of collecting venules (RAC) predicts non-infection; EF, nodularity, and DR predict current infection; map-like redness (MLR) predicts past infection; and atrophy and IM predict current or past infection. Atrophy, IM, and EF all increase the incidence of *H. pylori*-infected GC. MLR is a specific risk factor for *H. pylori*-eradicated GC, while RAC results in less GC. Diffuse-type GC can be induced by active inflammation, which presents as EF, nodularity, and atrophy on endoscopy, as well as neutrophil and mononuclear cell infiltration on histology. In contrast, intestinal-type GC develops *via* atrophy and IM, and is consistent between endoscopy and histology. However, this GC risk-scoring design needs to be improved.

Key Words: Kyoto classification; Gastritis; Endoscopy; Gastric cancer; Histology; *Helicobacter pylori*

Core Tip: Endoscopy-based Kyoto classification of gastritis assesses gastric cancer (GC) risk and *Helicobacter pylori* (*H. pylori*) infection status. Total Kyoto scores of 0, ≥ 2, and ≥ 4 indicate a normal stomach, *H. pylori*-infected gastritis, and gastritis at risk for GC, respectively. Atrophy, intestinal metaplasia (IM), and enlarged folds (EF) increase *H. pylori*-infected GC incidence. Map-like redness is a specific risk factor for *H. pylori*-eradicated GC, while regular arrangement of collecting venules result in less GC risk. Diffuse-type GC is induced by active inflammation, depicting EF, nodularity, and atrophy. Intestinal-type GC develops through atrophy and IM; however, the GC risk-scoring design still needs to be improved.

Citation: Toyoshima O, Nishizawa T. Kyoto classification of gastritis: Advances and future perspectives in endoscopic diagnosis of gastritis. *World J Gastroenterol* 2022; 28(43): 6078-6089
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6078.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6078

INTRODUCTION

The Kyoto classification of gastritis aims to match the endoscopic and histopathological findings of gastritis. It further aims to evaluate gastric cancer (GC) risk and *Helicobacter pylori* (*H. pylori*) infection of gastritis. The Kyoto classification was first advocated by the Japan Gastroenterological Endoscopy Society in 2013 and is widely used in recent clinical practice worldwide[1]. Technological advances in endoscopy have significantly improved the accuracy of identifying premalignant mucosal changes[2]. This editorial provides an update of the recent evidence on the Kyoto classification, clarifying the shortcomings of the Kyoto classification, and providing prospects for future research. This article is divided into the following four chapters: (1) *H. pylori* infection according to the Kyoto classification; (2) The histological consistency of the Kyoto classification; (3) Risk of GC according to the Kyoto classification; and (4) Future prospects in the Kyoto classification.

In the Kyoto classification, the total Kyoto score has been developed as a GC risk score. The total Kyoto score is calculated as the sum of the following 5 endoscopic findings: Atrophy, intestinal metaplasia (IM), enlarged folds (EF), nodularity, and diffuse redness (DR); and ranges from 0 to 8 (Table 1 and Figure 1)[1]. The Kyoto DR score includes the disappearance of the regular arrangement of collecting venules (RAC). Map-like redness (MLR) frequently appears after *H. pylori* eradication, and is generally pathologically consistent with IM[3]. This article describes the total Kyoto score and its five individual findings along with RAC and MLR.

GCs consist of two distinct histological subtypes: Lauren's diffuse and intestinal GC[4]. Diffuse-type GC develops directly from highly active inflammation, whereas intestinal-type GC develops through destruction and replacement of tissues, such as atrophy and IM, and is termed Correa's cascade[5-7]. GCs can also be described according to the different rates of incidence[8,9], lesion characteristics[10-12], and prognoses[13-16] as per the corresponding *H. pylori* infection status. In this editorial, we specifically describe the histological subtypes of GC and *H. pylori* infection status.

H. PYLORI INFECTION IN THE KYOTO CLASSIFICATION

H. pylori non-infection

Evidence of RAC as an indicator of non-infection has been reported in both in Japan[17,18] and several other countries[19-21], including in the west[22-24], as shown in Table 2. Two recent meta-analyses reported that the sensitivity and specificity of RAC for predicting non-infection were 78%-80% and 94%-97%, respectively[25,26]. The high reliability of RAC for non-infectious cases has also been verified.

H. pylori current and past infection

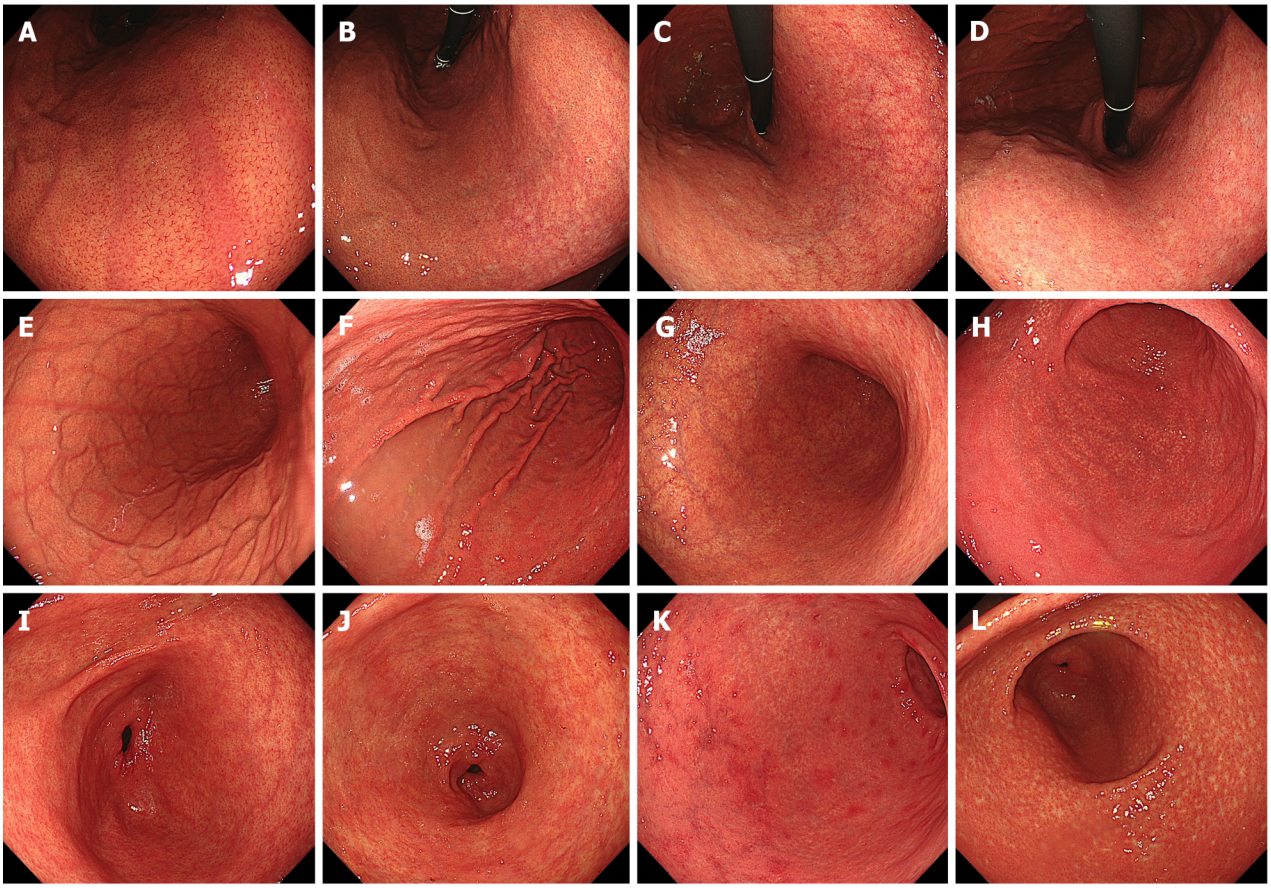
All five Kyoto scores, atrophy (61.1%-85.8% and 58.5%-85.3%)[19,23,27], IM (95.6% and 86.0%)[27], EF (96.6%-99.1% and 85.0%-85.3%)[17,27], nodularity (98.3%-100% and 76.5%-89.1%)[17,20,27,28], and DR (73.6%-97.6% and 65.0%-89.7%)[17,19,20,27], commonly offer high specificity and accuracy for categorizing current infections (Table 2).

Three studies have previously compared patients with non-infectious, current, and past infections, all of which reported that RAC was strongly correlated with non-infection [odds ratios (ORs) = 4.6-55.0]; MLR was a highly specific finding indicative of past infection (ORs = 7.8-12.9), and DR, EF, and nodularity provided high ORs of 10.5-26.4, 6.0-8.6, and 4.0-22.5, respectively, for current infection. Atrophy and IM were associated with both current (ORs = 1.9-21.6 and 4.3) and past infections (ORs = 1.9-22.8 and 4.4), respectively[17,19,25]. A previous study reported an algorithm with an accuracy of 80.0% for defining the presence of RAC as non-infection, DR and mucosal edema as current infection,

Table 1 Kyoto classification score

Endoscopic findings	Kyoto score		
	0	1	2
Atrophy[1]	None, C1	C2, C3	O1-O3
Intestinal metaplasia	None	Antrum	Corpus and antrum
Enlarged folds	Absence	Presence	-
Nodularity	Absence	Presence	-
Diffuse redness	None	Mild with RAC	Severe without RAC

[1]According to the Kimura-Takemoto classification[80].
RAC: Regular arrangement of collecting venules.



DOI: 10.3748/wjg.v28.i43.6078

Figure 1 Representative images of the Kyoto classification. A: Normal with regular arrangement of collecting venules; B: Atrophy score 1; C: Atrophy score 2; D: Intestinal metaplasia score 2; E: Normal; F: Enlarged folds score 1; G: Diffuse redness score 1; H: Diffuse redness score 2; I: Normal; J: Intestinal metaplasia score 1; K: Map-like redness; L: Nodularity score 1. A-D: Corpus lesser curvature; E-H: Corpus greater curvature; I-L: Antrum.

and MLR as post-eradication[24]. *H. pylori* eradication decreases the Kyoto EF, nodularity, and DR scores, but does not improve the Kyoto atrophy and IM scores[29]. These results indicate that the presence of RAC predicts non-infection; EF, nodularity, and DR predict current infection; MLR predicts past infection; and atrophy and IM predict current or past infection.

Total Kyoto score

Several studies have previously focused on the association between the total Kyoto score and *H. pylori* infection. The sensitivity and specificity of the total Kyoto score for current infection were good at 78.3%-98.7% and 92.0%-98.4%, respectively (Table 2)[27,30]. The area under the curve (AUC) of the total Kyoto score for predicting current infection was 0.85, with a cutoff value of 2[27]. Current infection rates increased stepwise, with total Kyoto scores of 0-1, 2-3, and ≥ 4 (8.6%, 61.4%, and 85.7%, respectively)

Table 2 Diagnostic performance of Kyoto classification for *Helicobacter pylori* infection

	Ref.	Country	No. of patients	Sensitivity	Specificity	Accuracy
Non-infection						
RAC	Garcés-Durán *et al*[22], 2019	Spain	140	100	49.0	65.0
	Yoshii *et al*[17], 2020	Japan	485	89.1	79.8	85.6
	Zhao *et al*[19], 2020	China	583	62.4	73.7	69.3
	Ebigbo *et al*[23], 2021	Germany	200	80.8	57.4	-
	Fiuza *et al*[20], 2021	Brazil	187	70.7	87.2	74.9
	Glover *et al*[24], 2021	UK	153	78.4	64.3	75.8
	Yuan *et al*[21], 2021	China	165	51.4	96.7	76.4
	Hirai *et al*[18], 2021	Japan	1761	93.2	83.2	90.6
Current infection						
Atrophy	Toyoshima *et al*[27], 2018	Japan	136	82.6	85.8	85.3
	Zhao *et al*[19], 2020	China	583	54.9	61.1	58.5
	Ebigbo *et al*[23], 2021	Germany	200	80.4	69.7	-
Intestinal metaplasia	Toyoshima *et al*[27], 2018	Japan	136	39.1	95.6	86.0
Enlarged folds	Toyoshima *et al*[27], 2018	Japan	136	17.4	99.1	85.3
	Yoshii *et al*[17], 2020	Japan	494	23.1	96.6	85.0
Nodularity	Toyoshima *et al*[27], 2018	Japan	136	8.7	100	84.6
	Yoshii *et al*[17], 2020	Japan	494	6.4	98.3	83.8
	Toyoshima *et al*[28], 2020	Japan	265	33.3	99.6	89.1
	Fiuza *et al*[20], 2021	Brazil	187	10.6	98.6	76.5
Diffuse redness	Toyoshima *et al*[27], 2018	Japan	136	52.2	93.8	86.8
	Yoshii *et al*[17], 2020	Japan	485	60.0	94.7	89.7
	Zhao *et al*[19], 2020	China	583	20.3	97.6	65.0
	Fiuza *et al*[20], 2021	Brazil	187	80.9	73.6	75.4
Total Kyoto score	Toyoshima *et al*[27], 2018[1]	Japan	136	78.3	92.0	89.7
	Sumi *et al*[30], 2022[2]	Japan	561	98.7	98.4	98.6

[1]The total Kyoto score ≥ 2 is defined as current infection.
[2]The total Kyoto score ≥ 1 is defined as current infection.
RAC: Regular arrangement of collecting venules.

[31]. The mean total Kyoto scores differed among patients with current, past, and non-infection (3.4, 1.1, and 0.0, respectively)[32]. A combination of the total Kyoto score and serum *H. pylori* antibody titer allows for the accurate diagnosis of current infection[33]. The total Kyoto score decreases from 3.9 to 2.8 following *H. pylori* eradication[29]. In summary, total Kyoto scores of 0 and ≥ 2 express non-infection and current infection, respectively.

HISTOLOGICAL CONSISTENCY OF KYOTO CLASSIFICATION

The purpose of the Kyoto classification is to match endoscopic and histological findings of gastritis. Regarding atrophy and IM, considerable evidence exists to indicate the consistency between endoscopy and histology. In recent studies, a high Kyoto atrophy score and severe endoscopic IM are associated with histologically advanced stages of operative link for gastritis assessment and operative link for gastric IM assessment, respectively[34,35].

Consistency between the endoscopic findings of the Kyoto scores and histological grading of the updated Sydney system (USS) scores has been examined individually. All five Kyoto scores were associated with histological inflammation, namely the USS score for neutrophil and mononuclear cell

infiltration, which is an indicator of *H. pylori* infection. The Kyoto atrophy and IM scores correlated with both histological atrophy and IM in the corpus[34,36]. Among *H. pylori*-infected patients, the Kyoto EF, nodularity, and DR scores indicated histologically high inflammation in the corpus[36-38]. In summary, the Kyoto atrophy and IM scores were concordant with histological corpus atrophy and IM scores. The Kyoto EF and nodularity scores were associated with the histological corpus inflammation.

GC RISK OF KYOTO CLASSIFICATION

Significant evidence to indicate endoscopic atrophy as a risk factor for GC has been accumulated. The incidence of GC based on atrophy is summarized in Table 3. GC incidences for mild, moderate, and severe atrophy are 0.06%-0.15%, 0.12%-0.34%, 0.31%-1.60%, respectively, indicating the severity of atrophy as a risk factor for GC development, even after *H. pylori* eradication[39-41]. A recent study from Western countries also showed that a Kyoto atrophy score of 2 was associated with GC development with a hazard ratio of 6.4 in patients with baseline IM[42].

The ORs for the histological subtypes of GC based on the Kyoto classification are summarized in Table 4. The Kyoto atrophy score is a predictor of GC with ORs of 2.5-7.4[43-45]. Two recent meta-analyses showed that a Kyoto atrophy score of 2 had high risk ratios (2.8-8.0 for developing GC)[46,47]. In an examination based on histological subtypes, a high Kyoto atrophy score was found to be associated with both diffuse-type and intestinal-type GCs with ORs of 2.3 and 6.2, respectively[44].

A high Kyoto IM score indicates a high risk for GC (OR = 1.6), especially intestinal-type GC (OR = 1.7), but a low risk for diffuse-type GC (OR = 0.2)[44,45,48,49]. In a direct comparison of diffuse-type and intestinal-type GCs, a high Kyoto IM score was associated with intestinal-type GC (ORs = 1.7-2.1)[44,49]. Furthermore, a high Kyoto IM score was associated with multiple GCs[50].

In a study on asymptomatic *H. pylori*-infected patients, the hazard ratio of patients with EF for GC development during the 5 years was high at 43.3[51]. In contrast, EF was associated with a low risk of intestinal-type GC (OR = 0.5)[44]. Furthermore, a direct comparison between diffuse-type and intestinal-type GCs indicated EF as a risk factor for diffuse-type GC (OR = 1.3)[44]. EF is reported to be an indicator of submucosal invasion in patients with GC (OR = 3.4; submucosal invasion *vs* intramucosal depth)[52].

The risk of nodularity is controversial. Previous studies found that nodularity was associated with a high risk for diffuse-type GC (OR = 10.0)[53], notably in young *H. pylori*-infected patients (OR = 64.2)[54]. In contrast, nodularity was described as a low risk factor for GC (OR = 0.5), especially intestinal-type GC (OR = 0.3)[44]. Nodularity decreases with age and the risk of intestinal-type GC increases with age[28]. Therefore, the risk of nodularity in GC should be stratified according to age.

Previously, RAC has been revealed as a predictor of non-GC[45]. Collectively, the Kyoto atrophy, EF, and nodularity scores were associated with diffuse-type GC, whereas the Kyoto atrophy and IM scores were related to intestinal-type GC, as shown in Figure 2.

GC risk after H. pylori eradication

Recently, the risk of GC after *H. pylori* eradication has been intensively investigated. Table 5 shows the risk of GC following *H. pylori* eradication. A Kyoto atrophy score of 2 and MLR are both indicators of GC after eradication, with ORs of 8.1 and 1.8-5.3, respectively[55-57]. Additionally, RAC was inversely associated with eradicated GC (ORs = 0.3-0.4)[56,58]. Studies have further revealed that the hazard ratios of Kyoto atrophy 2 and MLR for GC development were 4.9 and 3.6, respectively[55,59]. Take *et al*[41] previously reported the long-term incidence of GC after eradication based on endoscopic atrophy. The incidence of diffuse-type GC was higher in the second decade of follow-up than in the first decade. This increase was only observed in patients with mild-to-moderate gastric atrophy, indicating that even if atrophy is not severe, the risk of GC can persist long after eradication.

Total Kyoto score

The total Kyoto scores of patients with GC, *H. pylori*-infected GC, and *H. pylori*-eradicated GC were 4.0-4.6, 4.8-5.6, and 4.2, respectively[44,50,58,60]. A high total Kyoto score was associated not only with GC (ORs = 1.5-1.6), but also with both diffuse-type and intestinal-type GCs (ORs = 1.3 and 1.7, respectively, Table 4)[44,61]. Additionally, some investigators showed that the incidence of GC increased stepwise with the total Kyoto scores of 0-1, 2-3, ≥ 4, and that the AUC of the nomogram to predict GC using the total Kyoto score was 0.79[31,61]. Taken together, a total Kyoto score of 4 or more is useful for determining GC risks, including histological subtypes, even after *H. pylori* eradication.

FUTURE PERSPECTIVES IN KYOTO CASSIFICATION

The total Kyoto score was developed to evaluate GC risk, with a score ≥ 4 indicating risk. However, designing a method to simply add each component of the Kyoto score is problematic. First, the GC risks

Table 3 Gastric cancer incidence based on endoscopic atrophy

Ref.	Population	No. of subjects	No. of cancers	Duration, yr	Gastric cancer incidence, %/yr		
					Atrophy (mild)	Atrophy (moderate)	Atrophy (severe)
Shichijo *et al*[39], 2016[1,2]	Post eradication	573	21	6.2 ± 4.8	0.07	0.34	1.60
Kaji *et al*[40], 2019[3]	Screening	12941	63	3.7 ± 0.8	0.10	0.16	0.31
	Post eradication	2571	20	3.7 ± 0.8	0.06	0.12	0.42
Take *et al*[41], 2020[1]	Post eradication	2737	68	7.1 ± 5.4	0.15	0.29	0.67

[1]Mild, moderate, and severe atrophy represent Kimura-Takemoto's C1-2, C3-O1, and O2-3, respectively.
[2]Incidence was divided the incidence per 10 years by 10.
[3]Mild, moderate, and severe atrophy represent Kyoto atrophy scores 0, 1, and 2, respectively.

Table 4 Odds ratio for histological subtype of gastric cancer based on the Kyoto classification

	Ref.	*H. pylori* status	No. of subjects	No. of GC	No. of diffuse-type GC	No. of intestinal-type GC	OR for GC	OR for diffuse-type GC	OR for intestinal-type GC
Atrophy	Sekikawa *et al* [43], 2016	Current, past, and no infection	1823	29	3	26	7.4[1]		
	Toyoshima *et al* [44], 2021	Current infection	499	132	39	93	2.8	2.3	6.2
	Kawamura *et al* [45], 2022	Current, past, and no infection	380	115	19	96	2.5[1]		
Intestinal metaplasia	Shichijo *et al*[48], 2017	Current, past, and no infection	3392	107	22	85		0.2[2]	
	Toyoshima *et al* [44], 2021	Current infection	499	132	39	93	1.6		1.7
Enlarged folds	Nishibayashi *et al* [81], 2003	Current infection	276	135	69	66	5.0		
	Toyoshima *et al* [44], 2021	Current infection	499	132	39	93			0.5
Nodularity	Nishikawa *et al* [53], 2018	Current infection	674	25	9	16		10.0	
	Toyoshima *et al* [44], 2021	Current infection	499	132	39	93	0.5		0.3
RAC	Kawamura *et al* [45], 2022	Current, past, and no infection	380	115	19	96	0.2[3]		
Total Kyoto score	Toyoshima *et al* [44], 2021	Current infection	499	132	39	93	1.6	1.3	1.7
	Lin *et al*[61], 2022	Current, past, and no infection	1848	37	-	-	1.5		

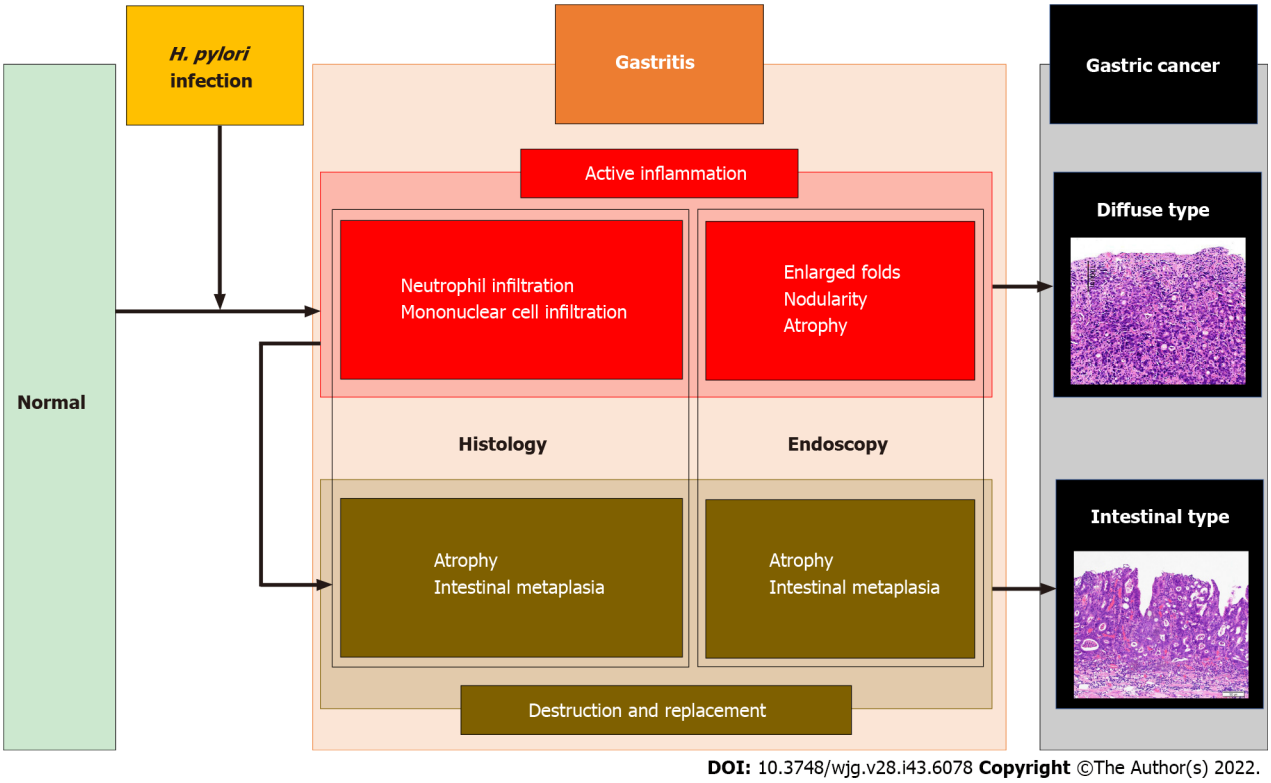
[1]Odds ratio for Kyoto score 2 *vs* Kyoto scores 0 + 1.
[2]Odds ratio for Kyoto scores 1 + 2 *vs* Kyoto score 0.
[3]Odds ratio for regular arrangement of collecting venules presence *vs* absence.
Odds ratios were calculated per 1 rank of Kyoto score. *H. pylori*: *Helicobacter pylori*; GC: Gastric cancer; OR: Odds ratio; RAC: Regular arrangement of collecting venules.

of the diffuse and intestinal types were distinctly different. For example, IM is associated with a high risk of intestinal-type GC but a low risk of diffuse-type GC. Conversely, EF and nodularity are high risk factors for diffuse-type GC, but indicate a low risk of intestinal-type GC (Table 4 and Figure 2). The majority of GC cases are classified as intestinal type, which indicates that the intestinal-type GC risk

Table 5 Odds ratio of gastric cancer after *Helicobacter pylori* eradication based on the Kyoto classification

	Ref.	No. of subjects	No. of GC	Odds ratio
Atrophy	Yan *et al*[57], 2021	1961	132	8.1[1]
Map-like redness	Moribata *et al*[55], 2016	122[2]	22[3]	5.3
	Majima *et al*[56], 2019	194	109	2.1
	Yan *et al*[57], 2021	1961	132	1.8
RAC	Majima *et al*[56], 2019	194	109	0.4
	Ohno *et al*[58], 2020	162	43	0.3

[1]Odds ratio for Kyoto atrophy score 2.
[2]Patients after endoscopic resection of gastric cancer.
[3]Metachronous gastric cancer.
GC: Gastric cancer; RAC: Regular arrangement of collecting venules.



DOI: 10.3748/wjg.v28.i43.6078

Figure 2 Pathogenesis of diffuse-type and intestinal-type gastric cancers. *H. pylori*: *Helicobacter pylori*.

may be overestimated, whereas the diffuse-type GC risk may be underestimated. Second, two points were assigned to Kyoto atrophy, IM, and DR scores in the total Kyoto score. The verification of the weighting of the total Kyoto score is a future task. Therefore, this scoring method should be revised in the future. A modified Kyoto score has been suggested as the sum of the following points: 2 points for invisible RAC, and 1 point each for Kyoto atrophy score 2, Kyoto IM score 2, and corpus MLR. Compared with the scores of 0-1, the ORs of the GC morbidity for the modified Kyoto scores of 2-3 and 4-5 were higher, at 8.6 and 28.0, respectively. Although statistical significance was not reached, the AUC of the modified Kyoto score had a higher predictive ability than that of the original total Kyoto score (0.75 *vs* 0.71, respectively)[45]. Furthermore, a scoring system specific to histological GC subtypes is needed. Third, MLR has been shown to predict GC after *H. pylori* eradication. Since IM manifests as MLR after *H. pylori* eradication[3], MLR may be more suitable than IM to assess the risk of eradicated GC.

In Western countries, RAC and endoscopic IM have been extensively studied; however, other endoscopic findings, such as atrophy, EF, nodularity, DR, and MLR, have been less extensively explored, and further studies in more varied populations are required. This article does not mention a

variety of important endoscopic findings, including spotty redness as a predictor of *H. pylori* infection [62]; xanthoma[57,63,64], foveolar hyperplastic polyp[65], refluxed bile[66], and a lack of fundic gland polyp[64] to predict GC; and depressive erosion and fundic gland polyp as indicators of functional dyspepsia[32,67]. Further research is required to confirm these findings. RAC provides high kappa values of intra-observer and inter-observer agreements of 0.88-0.91 and 0.74-0.79, respectively[21,68]; however, agreement between the other endoscopic findings needs to be clarified.

Autoimmune gastritis (AIG) is gaining attention as an important factor owing to the decrease in *H. pylori* infection[69]. Both severe endoscopic atrophy and a Kyoto IM score of 2 have been reported as AIG features[70,71]. Further steps should be taken to elucidate the differential diagnosis between AIG and *H. pylori*-associated gastritis using the Kyoto classification.

Recently, image-enhanced endoscopy (IEE) has been widely used in clinical practice. Two meta-analyses previously reported the utility of narrow-band imaging (NBI) for the diagnosis of IM[72,73]. Additionally, an improved diagnostic accuracy on using NBI, blue laser imaging, and linked color imaging have been reported[74-77]. In the future, research on endoscopic assessment using IEE, including texture and color enhancement imaging[78,79] will be required.

CONCLUSION

In conclusion, the total Kyoto score and individual Kyoto score, including atrophy, IM, EF, nodularity, and DR, can predict GC risk and *H. pylori* infection. Total Kyoto scores of 0, ≥ 2, and ≥ 4 indicate a normal stomach, *H. pylori*-infected gastritis, and gastritis at risk for GC, respectively; RAC predicts non-infection; EF, nodularity, and DR predict current infection; MLR predicts past infection; and atrophy and IM predict current or past infection. Atrophy, IM, and EF all increase in *H. pylori*-infected GC, MLR is a specific risk factor for *H. pylori*-eradicated GC, while RAC indicates a lesser GC risk. Diffuse-type GC can be induced by active inflammation, which presents as EF, nodularity, and atrophy on endoscopy, and neutrophil and mononuclear cell infiltration on histological examination. In contrast, intestinal-type GC develops *via* atrophy and IM, and is consistent on endoscopy and histology. However, the GC risk-scoring design still needs to be improved.

ACKNOWLEDGEMENTS

We would like to thank Dr. Hidenobu Watanabe to provide histological gastric cancer images.

FOOTNOTES

Author contributions: Toyoshima O and Nishizawa T contributed to this paper; Toyoshima O contributed to the design, writing of the manuscript, illustrations, and review of literature; Nishizawa T contributed to the design, discussion, and editing the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2022 November 21; 28(43): 6090-6098

DOI: 10.3748/wjg.v28.i43.6090

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

OPINION REVIEW

Current status of minimally invasive liver surgery for cancers

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Levi Sandri GB, Italy; Papadopoulos N, Greece

Received: August 25, 2022
Peer-review started: August 25, 2022
First decision: October 20, 2022
Revised: October 23, 2022
Accepted: November 6, 2022
Article in press: November 6, 2022
Published online: November 21, 2022

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Abstract

Hepatocellular carcinoma (HCC) patients have chronic liver disease with functional deterioration and multicentric oncogenicity. Liver surgeries for the patients should be planned on both oncological effects and sparing liver function. In colorectal patients with post-chemotherapy liver injury and multiple bilateral tumors, handling multiple tumors in a fragile/easy-to-bleed liver is an important issue. Liver surgery for biliary tract cancers is often performed as a resection of large-volume functioning liver with extensive lymphadenectomy and bile duct resection/reconstruction. Minimally invasive liver surgery (MILS) for HCC is applied with the advantages of laparoscopic for cases of cirrhosis or repeat resections. Small anatomical resections using the Glissonian, indocyanine green-guided, and hepatic vein-guided approaches are under discussion. In many cases of colorectal liver metastases, MILS is applied combined with chemotherapy owing to its advantage of better hemostasis. Two-stage hepatectomy and indocyanine green-guided tumor identification for multiple bilateral tumors are under discussion. In the case of biliary tract cancers, MILS with extensive lymphadenectomy and bile duct resection/reconstruction are developing. A robot-assisted procedure for dissection of major vessels and handling fragile livers may have advantages, and well-simulated robot-assisted procedure may decrease the difficulty for biliary tract cancers.

Key Words: Minimally invasive liver surgery; Laparoscopic liver resection; Robot-assisted liver resection; Hepatocellular carcinoma; Colorectal liver metastases; Biliary tract carcinoma

Core Tip: Minimally invasive liver surgery (MILS) for hepatocellular carcinoma is applied with the advantages of laparoscopic "caudal approach" for cases of cirrhosis or repeat resections. Small anatomical resections using newly developing approaches are under discussion. In many cases of colorectal liver metastases, MILS is applied combined with chemotherapy, owing to its advantage of better hemostasis. Two-stage hepatectomy and indocyanine green-guided tumor identification for multiple bilateral tumors are under discussion. In the case of biliary tract cancers, MILS with extensive lymphadenectomy and bile duct resection/reconstruction are developing. A robot-assisted procedure may have advantages.

Citation: Morise Z. Current status of minimally invasive liver surgery for cancers. *World J Gastroenterol* 2022; 28(43): 6090-6098
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6090.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6090

INTRODUCTION

Liver surgery for cancer is mainly performed in patients with hepatocellular carcinoma (HCC), liver metastasis of colorectal carcinoma (CRCLM), or biliary tract carcinomas (BTC)[1-4]. It is usually performed as curative-intent liver resection (LR) without other comparable alternatives, except for ablation therapy for small HCC and liver transplantation for patients with severe cirrhosis and non-advanced HCC. However, LR for each disease has its own specificity based on disease characteristics and background liver condition. This editorial describes the characteristics of LR for each disease. Thereafter, the advantages, disadvantages, and current status of the minimally invasive approach for each disease, and its potential are discussed.

LR FOR HCC

Patients with HCC mostly have a history of chronic liver disease (CLD), which causes functional deterioration and multicentric oncogenicity in the injured liver[1]. Therefore, depending on the tumor and pre-existing liver conditions, LR, ablation therapy, trans-arterial chemoembolization, liver transplantation, or recently emerged systemic chemo-immune therapy are chosen. The rates of LR application to primary HCC cases and the 5 year-survival rate thereafter are approximately 30% and 50%, respectively[1]. However, after a successful first treatment, many patients eventually enter a long-term treatment course with repeated treatments for recurrent/multicentric metachronous HCC raised from an oncogenic CLD background. One of the above-listed treatment options is selected based on the tumor and liver conditions of the patients at the time of each treatment. On LR indication, an evaluation of the liver function and, accordingly, estimation of resectable functional liver volume should be performed preoperatively[5], since HCC patients with CLD have a potential risk factor for postoperative morbidity and liver failure. Furthermore, potential repeated treatments over a long period of the treatment course should be considered in the treatment strategy of patients with HCC/CLD.

However, dissemination of cancer cells within the same portal territory as HCC tumors is well known, and anatomical resection of HCC is recommended[6]. Hemi-hepatectomy, sectionectomy, and sometimes segmentectomy, are widely accepted anatomical resections. However, since clear margins of segments one, five, six, and seven have sometimes been difficult to define, the Tokyo 2020 terminology of liver anatomy and resections was recently issued for segment or smaller anatomical resections[7]. These small anatomical resections and their combinations are under discussion for their oncological advantages. Liver surgeries for HCC patients should be planned to be appropriate in terms of both oncological effects on the currently existing HCC and sparing function of the liver with CLD, not only to minimize postoperative morbidity but also from the long-term perspective of potential repeated treatments.

LR FOR CRCLM

LR is the only curative-intent treatment for patients with CRCLM, with a 5-year overall survival rate of approximately 40%-50%[3]. However, due to the advancement of chemotherapy over the last few decades, combination strategies with chemotherapy, including adjuvant, neoadjuvant, and conversion strategies, for expanding indications and improving outcomes have become increasingly common[3]. Based on the current situation, patients with multiple tumors in the bilateral lobes often undergo LR. Several procedures are advocated to ensure that extended LR for multiple tumors is feasible and safer,

such as residual liver hypertrophy with percutaneous transhepatic portal embolization (PTPE)[8,9], two-stage hepatectomy[10], or associating liver partition or portal vein embolization for staged hepatectomy (ALPPS)[11]. In contrast, parenchymal-sparing LR is recommended for tumors occurring in small numbers, since tumor cell spreading *via* the portal vein system is rare, contrary to HCC[12]. Repeat LR for resectable recurrences improves long-term outcomes[13], and parenchymal-sparing LR reportedly improves salvageability and survival at recurrence within the liver[14]. The background liver condition is usually not fibrotic without CLD but is often associated with post-chemotherapy liver injury, such as steatosis and congestion of microcirculation with sinusoidal obstructive syndrome[15,16]. Liver steatosis can lead to liver fragility during surgery and postoperative elevation of transaminase levels. Congestion of microcirculation with sinusoidal obstruction can cause increased blood loss during surgery and, sometimes, postoperative morbidity, including ascites, similar to portal hypertension in patients with cirrhosis[17].

LIVER SURGERY FOR BTC

Liver surgery (LS) for BTC is performed in intrahepatic cholangiocarcinoma (ICC; peripheral and central types), perihilar cholangiocarcinoma, and gall bladder carcinoma[4]. Small peripheral ICC of the mass-forming type, often with CLD backgrounds and rarely with lymph node metastases, can be treated with an HCC-like approach, though it usually lacks an HCC-like tumor capsule with more invasive features than HCC[18]. Others have more aggressive features like spreading along the Glissonian pedicle with perineural, lymphatic, and venous invasions, as well as direct liver parenchymal invasion and lymph node metastases[4,18]. Since the invasion often involves hilar and intrahepatic Glissonian pedicles, LRs for those diseases sacrifice large volumes of functioning non-cancerous liver parenchyma to remove the cancer cells deeply infiltrated from the hilum into the peripheral Glissonian pedicle. PTPE is sometimes applied in such cases to enlarge the residual liver volume preoperatively[8]. Extensive lymphadenectomy and bile duct (sometimes including vasculature: Portal vein and hepatic artery) resection plus reconstruction are required. The patients mostly have a normal liver; however, sometimes damage from biliary obstruction may be present. In cases with jaundice, preoperative biliary drainage is needed[19].

MINIMALLY INVASIVE LIVER SURGERY

Laparoscopic liver resection (LLR) emerged in the early 1990s, and its indications have expanded thereafter[20,21]. The cancers previously mentioned are all within the indication of LLR. Less intraoperative blood loss, shorter hospital stays, and less morbidity in some conditions with comparable long-term outcomes have been generally reported with LLR for HCC and CRCLM[22,23]. LLRs for BTC, especially for those requiring extensive lymphadenectomy and bile duct (and vasculature) resection plus reconstruction, are now in the developmental stage with some early reports[24]. Although laparoscopic techniques for liver parenchymal transection, mobilization, and hemostasis have been established, those for extensive lymphadenectomy and bile duct (and vasculature) resection plus reconstruction are performed only in specialized centers. Robot-assisted LLR is also in its developmental stage and is performed mainly in specialized centers[25]. Moreover, devices that can be used in robotic procedures are still limited. However, some of the robotic procedures, such as bile duct/vasculature resection plus reconstruction, are expected to have advantages due to increased dexterity from stable endo-wrist instruments and stable high-definition three-dimensional visualization.

Minimally invasive LR for HCC

HCC with pre-existing CLD is thought to be the cancer for which the specific approach of minimally invasive LR (MILR) is most beneficial. In 2013, we presented the novel concept of the "caudal approach to LLR"[26]. Researchers followed[27,28] and defined the concept as a major conceptual changing of LLR in the statement of the 2nd international consensus conference[29]. LR is a procedure for handling the liver while it is protected inside the subphrenic "rib cage". In open LR, the cage is opened with a large subcostal incision and the liver is mobilized. In LLR, the laparoscope and instruments get directly into the space from the caudal direction with minimal damage on the cage, and minimal mobilization and compression on the liver[30]. This leads to minimal damage to the adherent structures as well as the liver. Since HCC patients mostly have underlying CLD, they have a higher risk of post-LR morbidity. LR patients are exposed to three types of surgical stress: (1) General and whole-body surgical stress; (2) Decreased liver function from the decrease of functioning liver parenchyma after LR; and (3) Surgery-induced damage on the environment structures and residual liver (such as disruption of collateral vessels in CLD patients by laparotomy/dissection of peritoneal attachments and parenchymal damage by compression). The third type of surgical stress can be reduced by laparoscopic-specific caudal approach[26-28] in LLR for HCC/CLD patients and that decreases short-term morbidity[30]. We

evaluated the short-term outcomes of liver surface small LLR for patients with severe CLD and our findings showed comparable short-term outcomes to those from patients of mild-to-moderate liver dysfunction[31]. Direct access to the surface tumor and minimal dissection of attachments made this surgery possible. This setting of LR can be achieved only with a laparoscopic approach. An international retrospective study using propensity score matching analysis of Child-Pugh B patients who underwent LR has shown that LLR is beneficial for these patients[32]. Moreover, LLR is thought to be advantageous also in less post-LR liver functional deterioration for those patients by smaller damage caused in surgical manipulation[30]. It can be beneficial for long-term and repeated treatment courses for the patients.

The treatment of intrahepatic recurrence is also important. Modifications of the anatomy and the formation of adhesions make repeating LR more difficult. Laparoscopic surgeries make following procedures easier from reduced formation of adhesions[33]. Furthermore, LLR allows for better visibility and manipulation even in a small surgical field between adhesions[30]. It can lead to unnecessity of total adhesiolysis in repeat procedures. Our international retrospective study for repeat LR compared laparoscopic and open approaches[34] and showed that laparoscopic repeat LR is feasible and has the short-term advantages of less bleeding and morbidity for selected patients. The overall survival curves were clearly separated, with a better tendency in LLR, though the disease-free survival curves were overlapped. The post-LR overall survival of HCC/CLD patients is determined not only by the treatment results of the resected lesion but by those of metachronous lesions and liver insufficiency during the long-term course[35,36]. During the long course of repeated treatments, patients with HCC/CLD should have adequate residual liver function after each intervention, which enables them to get future repeat treatments. We hypothesized that better overall survival after laparoscopic procedure may be from less liver functional deterioration[30]. Accompanied with less adhesion, it can make the repeat treatments easy to access and decrease deceased patients by liver insufficiency. LLR with the specific caudal approach, performed as a unique strong local treatment, is beneficial for patients with HCC/CLD.

However, LLR also has certain disadvantages. Disorientation can be easily happened due to lack of fine perceptible sensation and overview of the whole surgical field, and difficulty in performing precise intraoperative ultrasonography. Simulation and navigation from pre- and intra-operative imaging studies have been used to overcome these disadvantages. Well-simulated small anatomical resections in LLR can secure the tumor location in the resected area and provide adequate surgical margins, and lead to less postoperative bile leakage and less residual ischemic/congestive parenchyma, which possibly leads to recurrence[37]. For HCC for which anatomical resection is recommended, there are several reports of LLR that can lead to new developments, such as landmark (hepatic veins, *etc.*)-guided small anatomical resection[38], indocyanine green (ICG)-guided anatomical resection and tumor identification [39], and LLR with a Glissonian approach to more peripheral smaller branches from the hilum (cone unit resection)[40]. Robot-assisted LLR is an important emerging tool under discussion[25,41]. It could be advantageous, for example, in cases exposing a wide range of Glissonian pedicles and major hepatic veins.

MILR for CRCLM

As mentioned before, patients with post-chemotherapy liver injury and those with multiple tumors in bilateral lobes increasingly undergo MILR with recent advancements in neoadjuvant/conversion chemotherapy[15,16]. Handling multiple tumors in a fragile/easy-to-bleed liver is an important issue.

In MILR for CRCLM, disorientation can be easily happened due to lack of fine perceptible sensation and overview of the whole surgical field, and difficulty in performing precise intraoperative ultrasonography, as mentioned in MILR for HCC. Simulation and navigation from pre- and intraoperative imaging studies to assure tumors in the resected area with enough surgical margin are important. Landmark (such as hepatic veins)-oriented small anatomical resection[38], ICG-guided anatomical resection and tumor identification[39], and LLR with a Glissonian approach to more peripheral smaller branches from the hilum[40,42] are also applied to CRCLM. However, because the liver does not have CLD and the need for anatomical resection is low, LR for CRCLM with a large number of tumors is often planned as a combination of large anatomical resection plus partial resections or multiple partial resections. For the combination of large anatomical resection plus partial resections, the application of two-stage hepatectomy or ALPPS in MILR is now advocated[43,44]. For multiple partial resections, ICG-guided tumor identification is reported to be effective in addition to conventional intraoperative navigation[45,46].

In terms of handling fragile and easy-to-bleed injured liver after chemotherapy, bleeding can be controlled more easily in LLR than in open procedures by optimal visualization and pneumoperitoneum accompanied by Pringle's maneuver. However, handling a fragile liver can sometimes be difficult due to congenital motion restrictions in LLR, especially in complicated resections. Robot-assisted LLR may give us a chance to overcome this difficulty with its advantages, such as increased dexterity from stable endo-wrist instruments.

Theoretically, early recovery after MILR can be advantageous when it enables the earlier introduction of adjuvant chemotherapy. Although there are no solid data on the combination of MILR and chemotherapy, the combination of these treatments is a matter of further investigation.

Minimally invasive liver surgery for BTC

BTC is an emerging indication of minimally invasive liver surgery (MILS) under discussion[47]. Surgery for BTC consists of three different procedures: LR, lymphadenectomy, and resection plus reconstruction of the bile duct (and vasculature). The laparoscopic procedure for LR has already been established during its application in HCC and CRCLM. Lymphadenectomy around the hepatic hilum and hepatoduodenal ligaments is often performed in laparoscopic pancreatic surgery[48,49]. Laparoscopic resection plus reconstruction of the bile duct (and vasculature) for malignant diseases should be added to MILS for BTC, although there are reports of bile duct resection plus reconstruction for benign diseases[50].

Surgeries for small peripheral mass-forming ICC rarely with lymph node metastases and early-stage gall bladder carcinoma without invasion to the hepatic hilum or hepato-duodenal ligament (candidates for radical cholecystectomy) requires only peripheral LR and mild lymphadenectomy. They are the first good candidates for the MILS application. On the other hand, advanced gallbladder carcinoma, central (invasive) ICC, and perihilar carcinoma with pedicle invasions and a high potential for lymph node metastases should be handled with a complicated combination of LR, extended lymphadenectomy, and resection plus reconstruction of the bile duct (and vasculature). There are only a few reports of MILS applications in those conditions[24,51]. Among these, minimally invasive surgery for perihilar carcinoma is the most difficult. Bile duct dissection, division, and reconstruction should be performed at the more peripheral part of the small-sized bile duct to obtain a cancer-free margin. Under laparoscopic conditions, disorientation is easy to occur due to the lack of good overview/tactile sensation and motion restriction is also a congenital problem. Setting an adequate resection line without cancer invasion and performing reconstruction of the small orifice of the residual bile duct is more difficult than the open procedure. From this perspective, a well-simulated and planned robot-assisted procedure may be a potential tool to overcome this situation.

CONCLUSION

MILS for HCC with CLD background and recommendation for anatomical resection has been established and applied to most cases with advantages in cirrhotic patients and repeat LR by laparoscopic specific "caudal approach". Small anatomical resections using Glissonian approach, ICG-guided approach, landmark (*i.e.*, hepatic vein)-guided approach are under discussion and developments. Robot-assisted procedure for long range dissection of major vessels may have advantages.

MILS for CRCLM with combination treatments to chemotherapy has been established and applied to many cases with the advantage in better hemostasis by pneumoperitoneum and optimal visualization. LLRs with two-stage hepatectomy, ALPPS, ICG-guided tumor identification are under discussion and developments. Robot-assisted procedure for handling the fragile liver after chemotherapy may have advantages.

MILS for BTC with spreading invasion along Glissonian pedicle, lymph node metastases and direct invasion to liver parenchyma is developing, especially for extensive lymphadenectomy and bile duct (vasculatures) resection /reconstruction, and possible in experienced center. Well simulated and planned robot-assisted procedure may decrease the difficulty in setting tumor free resection line and reconstruction of small bile duct orifice (Table 1).

Table 1 Summary of current status of minimally invasive liver surgery for cancers

Disease	Backgrounds of disease and liver for surgery	Status of minimally invasive liver resection
HCC	Chronically injured background of liver[1,2]: (1) Deteriorated liver function–postoperative morbidity; and (2) Multicentric carcinogenesis–repeated treatments	Established/applied with some merit to most cases[21,29]: Advantages in LR of LC patients[30,31] and repeat LR[30,34] by laparoscopic specific "caudal approach"[26-28]
	Anatomical resection[6]	Under discussion and development: (1) Small anatomical resection using Glissonian approach[40,42], ICG-guided approach[39], hepatic vein-guided approach[38]; and (2) Robot for long-range dissection of major vessels?
CRCLM	Combination treatments with chemotherapy[3]: (1) Multiple bilateral tumors–TSH[10], ALPPS[11]; and (2) Injured liver–fragility from steatosis[15], easy bleeding form congestion[16,17]	Established/applied to many cases[20,23]: Advantages of better hemostasis[20,29] by pneumoperitoneum/optimal visualization
	Parenchymal sparing resection[12,14]	Under discussion and development: (1) LLR with TSH[43], ALPPS[44]–possible in experienced centers; (2) ICG-guided tumor identification [39,45]; and (3) Robot for handling the fragile liver?
BTC	Spreading invasion along Glissonian pedicle[4], LN metastases and direct invasion to liver parenchyma: (1) Extended LR of normal	Developing and possible in specialized center[24]: (1) Lymphadenectomy and bile duct (vasculature) resection/reconstruction in

	liver–PTPE[8]; (2) Lymphadenectomy; and (3) Bile duct (vasculature) resection/reconstruction-Needs of setting tumor free resection line and reconstruction of small bile duct orifice	specialized center[24,51]; and (2) Difficulties in setting tumor free resection line and reconstruction of small bile duct orifice: Well simulated robot-assisted procedure?

HCC: Hepatocellular carcinoma; CRCLM: Liver metastasis of colorectal carcinoma; BTC: Biliary tract carcinomas; LR: Liver resection; LLR: Laparoscopic liver resection; LC: Liver cirrhosis; ICG: Indocyanine green; TSH: Two-stage hepatectomy; ALPPS: Associating liver partition or portal vein embolization for staged hepatectomy; PTPE: Percutaneous transhepatic portal embolization; LN: Lymph node.

FOOTNOTES

Author contributions: Morise Z designed the overall concept and contributed to the writing of the manuscript and the review of literature.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2022 November 21; 28(43): 6099-6108

DOI: 10.3748/wjg.v28.i43.6099

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Management of non-alcoholic fatty liver disease patients with sleep apnea syndrome

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Pantelis AG, Greece; Villela-Nogueira CA, Brazil

Received: September 24, 2022
Peer-review started: September 24, 2022
First decision: October 18, 2022
Revised: October 20, 2022
Accepted: November 6, 2022
Article in press: November 6, 2022
Published online: November 21, 2022

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is strongly associated with sleep apnea syndrome (SAS). Many NAFLD patients have SAS, and obstructive sleep apnea hypopnea syndrome is also considered to be an independent risk factor for NAFLD, as it contributes to the progression of NAFLD *via* oxidative stress, lipid peroxidation, inflammation, and insulin resistance. This review aims to provide some recommendations for the management of NAFLD patients with SAS, including diet, exercise, weight loss, and continuous positive airway pressure. This review also highlights the importance of effective strategies in NAFLD prevention and treatment.

Key Words: Nonalcoholic fatty liver disease; Sleep apnea syndrome; Obesity; Obstructive sleep apnea hypopnea syndrome; Continuous positive airway pressure; Management

Core Tip: Nonalcoholic fatty liver disease (NAFLD) is strongly associated with sleep apnea syndrome (SAS). This minireview presents the relationship between NAFLD and SAS; addresses the role of obesity, insulin resistance, and oxidative stress, and emphasizes the management of NAFLD with SAS, which mainly includes lifestyle interventions and continuous positive airway pressure therapy. This review also highlights the importance of effective strategies in NAFLD prevention and treatment.

Citation: Sheng W, Ji G, Zhang L. Management of non-alcoholic fatty liver disease patients with sleep apnea syndrome. *World J Gastroenterol* 2022; 28(43): 6099-6108
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6099.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6099

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as hepatic steatosis greater than 5% and excludes causes such as alcohol, viral infection and hereditary factors[1]. Due to numerous studies that have characterized the association of NAFLD with metabolic syndromes, such as obesity and type 2 diabetes (T2DM), an international consensus in 2020 proposed renaming NAFLD as metabolic-associated fatty liver disease (MAFLD)[2]. NAFLD can progress into nonalcoholic steatohepatitis (NASH) with or without fibrosis. Currently, there are two main hypotheses about the pathogenesis of NASH. One is the "two-hit" hypothesis proposed by James and Day[3] in 1998: The first strike is induced by insulin resistance and excessive accumulation of hepatic lipids, while oxidative stress and inflammation are considered to compose the second strike. As research in this field continues to advance, the "parallel multihits" hypothesis is thought to more accurately explain the complex mechanisms of NAFLD progression, which involves genetic and epigenetic factors, insulin resistance, endoplasmic reticulum stress, oxidative stress, and gut dysbiosis[3-5].

NAFLD patients are prone to sleep apnea syndrome (SAS), a common respiratory disease. It is estimated that nearly 1 billion adults aged 30-69 years worldwide have SAS. A real-world study of uncomplicated NAFLD patients in South Korea showed that during a median follow-up of 5.3 years, 1351 patients (0.4%, total 334334) were diagnosed with SAS; among patients with a fatty liver index > 31.0, SAS occurred in 0.8% of patients[6]. Based on the differences in their pathogenesis, SAS is broadly categorized into three types: Obstructive sleep apnea hypopnea syndrome (OSAHS), central SAS and mixed SAS. OSAHS is the most common type of SAS, and obesity is a strong risk factor for OSAHS. Approximately 40% of individuals with obesity are reported to have OSAHS, and 70% of OSAHS patients have obesity[7]. The etiology of OSAHS is complex, with the main causes including anatomical narrowing of the upper airway and local soft tissue collapse. Obesity exacerbates upper respiratory obstruction due to compression of the pharyngeal cavity and airway by neck fat[8,9]. It has been reported that central obesity and neck circumference thickening are significantly and positively associated with the apnea hypoventilation index (AHI), and the AHI is also positively associated with insulin resistance[10]. In addition, the incidence of metabolic syndrome is elevated in patients with OSAHS compared to non-OSAHS; in particular, components of metabolic syndrome, such as T2DM, obesity, and dyslipidemia, are more strongly associated with OSAHS[11]. A German observational cohort study also found a higher incidence of hepatic steatosis in patients with moderate-to-severe OSAHS, and the assessment of a snoring index may help to identify the risks of associated liver disease in OSAHS patients[12]. More importantly, a previous meta-analysis found that OSAHS is associated with an increased risk of advanced fibrosis in NAFLD patients, independent of age, sex and body mass index (BMI)[13]. Another Italian observational cohort study suggested that evaluating the mean oxyhemoglobin percentage in obese patients with OSAHS is of great clinical value in identifying the risks of NAFLD progression[14]. However, because comorbidities coexist and are independently associated with systemic inflammation, it is very difficult to clarify the effects of OSAHS on the development and progression of NAFLD[15,16]. The exact mechanisms of NAFLD and SAS are still largely unknown, but some biological processes are considered to be integrated in NAFLD patients with SAS.

Hypoxia and oxidative stress

Lipid accumulation and subsequent oxidative stress in the liver are considered the initial causes of NAFLD progression and the development of NASH. The accumulation of free fatty acids in hepatocytes due to insulin resistance, increased *de novo* lipogenesis or excessive lipid uptake from dietary sources can disrupt the mitochondrial microstructure, leading to impaired fatty acid β-oxidation and the production of reactive oxygen species (ROS)[17,18]. Overwhelmed ROS can trigger oxidative stress, which is a stressful state involving an imbalance between oxidative and antioxidant actions, usually due to excess ROS interfering with endogenous antioxidant defense system[5]. Oxidative stress can exacerbate lipid accumulation in hepatocytes, disrupt the structure and function of mitochondria; activate Kupffer cells; and promote the release of inflammatory factors such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-8, thereby exacerbating the inflammatory response of hepatocytes[19].

OSAHS is characterized by chronic intermittent hypoxia (CIH) that occurs at night, and prolonged CIH can lead to tissue hypoxia and promote oxidative stress, lipid peroxidation, and systemic inflammation. The disturbed lipid metabolism and excessive oxidative stress in the liver during NAFLD progression may further affect OSAHS. Accordingly, the combination of OSAHS aggravates hepatic impairment, promotes lipid metabolism disorder and increases insulin resistance in patients with NAFLD[20-22]. This was also demonstrated by the positive correlation between the severity of CIH in OSAHS patients and the severity of liver fibrosis measured by liver elastography[23]. Evidence from clinical investigations suggests an increased incidence of NAFLD in patients with OSAHS even in the absence of obesity or metabolic syndrome, while the severity of NAFLD is parallelly associated with the severity of OSAHS in NAFLD patients with OSAHS[24]. More importantly, even in children with NAFLD, nocturnal hypoxia-induced oxidative stress promotes disease progression[25]. In addition, patients with OSAHS are at risk of hypoxemia and even hypercapnia due to the collapse of the pharynx

during sleep, resulting in upper airway obstruction and airflow restriction, as well as respiratory distress and even interruption of breathing, leading to a decrease in oxygen saturation.

CIH, insulin resistance and disorders of lipid metabolism caused by OSAHS may exacerbate NAFLD in patients with obesity and lead to an increased risk of NASH and more serious diseases[26,27]. It has been reported that polymorphisms of proinflammatory cytokine genes including the highly sensitive C-reactive protein, IL-6 and leptin receptor genes are associated with increased risk of OSA and NAFLD in Asian Indians[28]. Moreover, Asian Indian subjects carrying the Gly972Arg polymorphism of insulin receptor substrate 1 are predisposed to developing OSA and NAFLD[29]. However, as mentioned previously, we do not yet fully understand the association between OSAHS and NAFLD. Current evidence suggests that OSAHS-related CIH triggers excessive lipolysis, manifesting as an increase in plasma free fatty acids[30,31]. Excessive free fatty acids lead to ectopic fat accumulation, insulin resistance, vascular dysfunction and dyslipidemia[32,33], which may be one of the mechanisms by which OSAHS promotes the progression of NAFLD[34]. Furthermore, CIH has also been hypothesized to promote oxidative stress through increased ROS production and angiogenesis, increased sympathetic activation with elevated blood pressure, and systemic and vascular inflammation with endothelial dysfunction[35].

Diagnosis

The diagnosis of NAFLD is primarily an exclusionary diagnosis, which is different from the diagnostic criteria for MAFLD. Secondary causes of hepatic steatosis and causes of liver complications due to other diseases must be excluded to make the diagnosis of NAFLD, including alcohol and drug use, hepatic viral infections and autoimmune liver diseases, Wilson's disease and lipodystrophy[1,36,37]. Currently, the gold standard for NAFLD diagnosis (*e.g.*, assessment of steatosis, steatohepatitis and the stage of liver fibrosis) remains liver biopsy and histological staining; however, it is inappropriate to routinely perform liver biopsy; because it is invasive and may pose a risk of complications, sampling errors and expert interpretation inconsistencies. Therefore, many noninvasive assessment and clinical scoring systems are used to diagnose and assess the degree of steatosis and fibrosis in patients with NAFLD. For example, abdominal ultrasonography, as a noninvasive and convenient method, and controlled attenuation parameter measurement based on transient elastography for ultrasonography attenuation are commonly used to detect the extent of liver steatosis[38]. Magnetic resonance imaging (MRI) is also applied in the noninvasive diagnosis and grading of NAFLD, and is considered to be efficient in quantifying liver fat, stiffness, and visceral adipose tissue[39]. The further developed MRI-proton density fat fraction and proton magnetic resonance spectroscopy are reported to be more concise technologies for NAFLD diagnosis[40]. Furthermore, hepatologists from the American Gastroenterology Association and Chronic Liver Disease Foundation summarized several commonly used noninvasive scores[41]. Among them, the fibrosis-4 index is the most widely studied and preferred simple noninvasive algorithm and it is used to evaluate the degree of liver fibrosis based on age and levels of platelet, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[42].

Treatment

The recommended treatment for NAFLD focuses on lifestyle interventions, including dieting, exercise, weight loss, and promotion of energy expenditure. In 2020, the Asia Pacific Association for the Study of the Liver proposed clinical practice guidelines for NAFLD. This guideline points out that conservative management of lifestyle changes remains the best choice for the treatment of NAFLD[37]. Notably, when NAFLD patients are combined with SAS, supplemental treatment with continuous positive airway pressure (CPAP) is the most appropriate approach. CPAP therapy has been reported to help improve sleep apnea and hypoventilation, as well as stabilize or delay the progression of NAFLD[24]. Inflammation, a hypoxic environment and oxidative stress are key pathogenic mechanisms in patients with NAFLD and OSAHS. Therefore, antioxidants such as vitamin E may be effective, and anti-obesity medications have also shown considerable potential in the treatment of NAFLD and OSAHS[43,44]. The glucagon-like peptide-1 receptor agonist, liraglutide and semaglutide may improve AHI and protect against NASH and obesity, especially in relation to T2DM[45,46]. Notably, liraglutide at a dose of 3 mg is well tolerated and is effective at improving NAFLD and OSAHS[47,48].

Diet

A reasonable dietary structure and appropriate dietary intake are beneficial for obesity, especially for NAFLD patients with OSAHS. Severely obese patients with NAFLD should strictly limit calorice intake, and if necessary, scientific recipes should be prescribed by nutritional experts to help alter the metabolic pattern. A Mediterranean diet is recommended for patients with NAFLD and NASH. Based on high amounts of monounsaturated fatty acids derived from olive oil, fruits, grains, whole grains and low-fat dairy products, the Mediterranean dietary pattern can reduce the incidence of metabolic syndrome, obesity, T2DM and cardiovascular disease, as well as certain cancers[49-51]. Remarkably, the Mediterranean diet may potentially counteract the inflammation and oxidative stress that occur in OSAHS and improve upper-airway neuromuscular control and muscle force-generating capacity[52]. In an interdisciplinary weight loss and lifestyle intervention trial, participants who adhered to the Mediterranean diet

demonstrated greater weight loss as well as more significant improvement in OSAHS[53].

In addition, the diversity of foods should be increased for patients with NAFLD, and the foods need to be enriched for antioxidants and high-quality proteins, which can contribute to NAFLD and OSAHS improvement. However, NAFLD patients should reduce the consumption of specific foods, including red meat and overprocessed foods[54]. Moderate coffee consumption is allowed for NAFLD patients, but the intake of alcohol, fructose and sugar-containing drinks should be avoided because alcohol or sugar consumption can increase lipid synthesis, visceral fat accumulation and insulin resistance, and increase the risk of liver fibrosis in patients with NAFLD.

Exercise

Exercise is beneficial for NAFLD and SAS. Previous studies have shown that sedentary and reduced physical activity is associated with the progression of NAFLD and the development of moderate to severe OSAHS[55,56]. There is growing evidence showing that aerobic exercise training is beneficial for patients with SAS[57]. Active physical activity may reduce body weight, improve blood circulation, contribute to sleep quality and address excessive daytime sleepiness. Therefore, regular exercise facilitates weight loss and improves NAFLD and associated complications[58,59]. The recommended minimum level of exercise is 5 d per week (> 30 min each day) of moderate intensity aerobic exercise or 3 d per week (> 20 min each day) of vigorous exercise.

Metabolic bariatric surgery

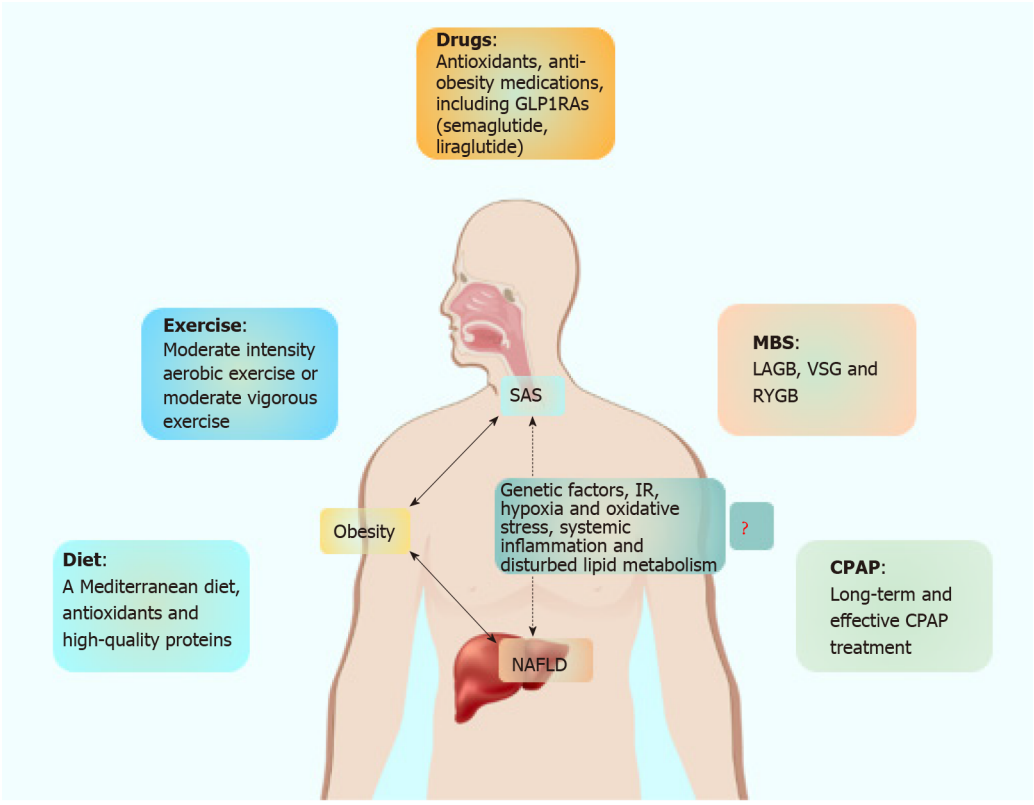
For NAFLD patients with severe obesity, weight loss is very difficult due to their large weight base and may require metabolic bariatric surgery (MBS) intervention. Over the past few decades, MBS has evolved from the simple gastric volume-limiting procedure laparoscopic adjustable gastric banding to surgical options with hormonal effects, including vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass (RYGB), both of which induce appetite changes by modulating intestinal and central hormones, thereby reducing food consumption and increasing satiety[60]. Usually, MBS is the most effective treatment for obesity and often results in dramatic improvements in glycemic control, insulin resistance and NAFLD[61,62].

SAS is also one of the comorbidities with the greatest response rate to MBS. The 6th IFSO Global Registry Report shows that MBS leads to a reduction of SAS ranging from 58%-65%, depending on the type of operation (LSG, RYGB, OAGB). Evidence from clinical studies has confirmed the efficacy of MBS for patients with obesity and OSAHS[63,64]. A retrospective study comparing the effect of VSG and RYGB on weight loss and their comorbidity remission also showed that both MBSs are effective in improving OSAHS symptoms[65]. Most surprisingly, in a study observing the effect of VSG in patients with a BMI ≥ 50 kg/m^2, complete remission was observed in all 13 patients with comorbid OSAHS[66]. MBS can effectively improve sleep apnea and nocturnal hypoxia, as well as hepatic steatosis and fibrosis [67,68]. More importantly, the severity of OSAHS determines the risk of NAFLD before MBS, and after surgery, it also determines the improvement of NAFLD[67].

CPAP

CPAP is the first-line recommendation for the treatment of OSAHS. For NAFLD patients with OSAHS, early intervention is even more important. Additionally, for obesity and OSAHS patients scheduled for MBS, guidelines recommend preoperative CPAP for at least 4 wk[69]. CPAP acts in multiple pathways to prevent airway collapse, reduce pharyngeal edema and upper airway resistance, increase the action of upper airway opening muscles through vagal reflexes, and restore chemoreceptor sensitivity and respiratory center drive. CPAP treatment has been reported to significantly reduce sleep apnea and hypoventilation in patients with OSAHS, improve quality of life; reduce daytime sleepiness, and decrease the occurrence of hypertension, diabetes, cardiovascular and cerebrovascular complications [70]. This may be because CPAP treatment reduces markers of oxidative stress and thus improves metabolic syndrome[71]. In addition, a randomized controlled trial showed that patients with OSAHS had increased markers of liver injury and atherogenic risk[72], whereas CPAP treatment helped stabilize or delay the progression of NAFLD and demonstrated improvements in metabolic and cardiovascular function[24]. A meta-analysis of 192 obesity patients combined with OSAHS showed a significant reduction in serum AST and ALT levels after CPAP treatment for 3 mo[73]. Another meta-analysis of six randomized controlled trials involving 699 subjects also showed that CPAP treatment reduces total cholesterol and triacylglycerol levels[74]. More importantly, CPAP treatment of patients with OSAHS significantly reduces serum inflammatory markers, including CRP and TNF-α[75]. More interestingly, in another randomized controlled trial, Sundaram *et al*[76] found that CPAP treatment reverses the parameters of liver injury, reduces oxidative stress in children with NAFLD can also be used to predict NAFLD progression in obese children with OSAHS.

Although these reports point to a beneficial effect of CPAP on NAFLD-associated parameters[73,74, 77,78], short-term (usually 4-12 wk) CPAP treatment may not be as effective[72,79,80]. The most significant result of CPAP treatment is a case report published in 2018 that reported the reversal of NAFLD and the normalization of liver enzymes and the associated lipidome in a patient with both NAFLD and severe OSAHS after 6 years of treatment[81]. In addition, long-term follow-up data based



DOI: 10.3748/wjg.v28.i43.6099

Figure 1 Management of nonalcoholic fatty liver disease with sleep apnea syndrome. Healthy lifestyle management remains the current first-line recommendation for nonalcoholic fatty liver disease (NAFLD) with sleep apnea syndrome (SAS), including diet, exercise, and weight loss. For patients with obesity and type 2 diabetes, drug intervention should also include anti-obesity medications and insulin-sensitizing drugs. Metabolic bariatric surgery can be considered when lifestyle changes and medical interventions are not effective. In addition, continuous positive airway pressure therapy may improve both intermittent hypoxia and liver injury in patients with NAFLD and SAS. MBS: Metabolic bariatric surgery; CPAP: Continuous positive airway pressure; IR: Insulin resistance; LAGB: Laparoscopic adjustable gastric banding; VSG: Vertical sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; SAS: Sleep apnea syndrome; NAFLD: Nonalcoholic fatty liver disease.

on a Taiwanese population also showed a lower cumulative incidence of NAFLD and cirrhosis in CPAP-treated patients than in nontreated patients[82].

Therefore, the beneficial effects of short-term CPAP therapy on OSAHS and NAFLD may be elusive, and although long-term and effective CPAP treatment can show significant improvements in both OSAHS and NAFLD, most patients are not amenable to long-term oral and nasal mask therapy. In addition, CPAP therapy is not yet commonly used due to the high cost of the device.

CONCLUSION

Obesity is a high-risk factor for NAFLD and SAS. Most patients living with obesity and metabolic syndrome are prone to SAS, and the severity of disease in patients with SAS is significantly associated with NAFLD progression. It is important to note that, even in nonobese patients, there is a relationship between NAFLD progression and SAS severity. SAS is usually one of the complications of NAFLD. However, at present, we can only confirm that lipid disorders, hypoxia, oxidative stress, and inflammation are involved, and a deeper understanding of the pathogenesis remains unclear. More in-depth studies are needed to elucidate the causal relationship between them. More importantly, an understanding of the pathogenesis will help us to develop more individualized treatment plans.

Collectively, healthy lifestyle management remains the most important strategy for the treatment of NAFLD, including diet, exercise and weight loss. Furthermore, the use of antioxidants such as vitamin E and the insulin-sensitizing drug pioglitazone is also necessary. It needs to be emphasized again that timely CPAP intervention is also important for NAFLD patients with SAS. We have summarized our recommendations for NAFLD combined with SAS management in Figure 1, and the improvements in SAS and NAFLD after intervention with lifestyle measures, medications and MBS are summarized in Table 1.

Table 1 Effects of current strategies on nonalcoholic fatty liver disease with sleep apnea syndrome

Category		Effects	Ref.
Lifestyle measures	Mediterranean diet	Inhibition of inflammation and oxidative stress that occur in OSAHS and improvement of upper-airway neuromuscular control and muscle force-generating capacity	[51]
	Dietary behavior change, moderate-intensity aerobic exercise, sleep hygiene, and tobacco and alcohol avoidance	Increases in adherence to the Mediterranean diet	[52]
		Reduced AHI and oxygen desaturation index	
		Increased sleep quality	
		Decease of body weight, fat mass, visceral adipose tissue, and neck, chest, and waist circumferences	
	Aerobic exercise training	Reduced body weight improved blood circulation, better sleep quality and less daytime sleepiness	[56-58]
Medications	Phentermine plus extended-release topiramate	Significant improvements in overnight oxygen saturation and reduction in blood pressure	[42]
	Liraglutide and semaglutide	Histological resolution of NASH and improved metabolic control	[43-48]
		Decreased AHI, body weight, SBP and HbA1c	
MBS	VSG or other MBS	Reduced AHI	[62-64]
	VSG	100% remission rate in patients with OSAHS who also underwent hiatal hernia repair	[65]
	MBS	Improved sleep apnea and nocturnal hypoxia, as well as liver steatosis and fibrosis	[66]

OSAHS: Obstructive sleep apnea hypopnea syndrome; AHI: Apnea hypoventilation index; NASH: Nonalcoholic steatohepatitis; SBP: Systolic blood pressure; HbA1c: Glycated hemoglobin; MBS: Metabolic bariatric surgery; VSG: Vertical sleeve gastrectomy.

FOOTNOTES

Author contributions: Sheng W performed the literature review and wrote the manuscript; Zhang L and Ji G conceptualized the idea, and critically reviewed and revised the manuscript; and all authors have read and approved the final manuscript.

Supported by the Project of Shanghai Hospital Development Center, No. SHDC2020CR4044.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

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World Journal of Gastroenterology

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World J Gastroenterol 2022 November 21; 28(43): 6109-6130

DOI: 10.3748/wjg.v28.i43.6109

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Differential analysis of intestinal microbiota and metabolites in mice with dextran sulfate sodium-induced colitis

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Kotlyarov S, Russia; Maslennikov R, Russia; Yang M, United States

Received: July 12, 2022
Peer-review started: July 12, 2022
First decision: September 26, 2022
Revised: October 4, 2022
Accepted: November 2, 2022
Article in press: November 2, 2022
Published online: November 21, 2022

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Abstract

BACKGROUND

Intestinal micro-ecological imbalances impair the intestinal barrier and induce intestinal inflammation, for example, ulcerative colitis (UC). According to the latest research, abnormalities in intestinal microbiota structure and their metabolites play a dominant role in UC progression; in addition, they could affect the mucus barrier based on different factors. Although numerous studies have confirmed the important role of intestinal microbiota in UC pathogenesis, the intricate connection between microbiota and metabolites and mucus barrier in UC occurrence remains unclear, and correlation analyses of differential microbiota and their metabolites under UC are relatively scarce.

AIM

To reveal the differential intestinal microbiota and metabolites in UC pathogenesis and explore more sensitive biomarker compositions.

METHODS

We used the antibiotic combination method to establish intestinal pseudo-aseptic mice; afterward, dextran sulfate sodium (DSS) was applied to establish an acute experimental colitis mice model. Colitis severity, assessed based on disease activity index, colorectal length, colorectal wet weight, and histological lesions, and mucus-related staining (mucopolysaccharide alcian blue and immunofluorescence of mucin), was compared between the pseudo-aseptic and bacterial colitis mice. Finally, differential intestinal microbiota, metabolites, and their association and correlations, were analyzed by 16s rDNA sequencing in combination with non-targeted metabolomics, through gas chromatography-mass spectrometry.

RESULTS

Compared with the pseudo-aseptic mice, intestinal bacteria positive mice were more severely ill and their intestinal mucus loss was more pronounced in DSS-induced colitis ($P < 0.05$), suggesting that different microbiota and metabolites could cause the different degrees of colitis. Subsequently, we observed that in addition to *Klebsiella*, and *Bacteroides*, which were widely associated with colitis, *Candidatus Stoquefichus*, *Anaerobiospirillum*, *Muribaculum*, and *Negativibacillus* may be involved in protection against colitis. Furthermore, differential metabolites of the microbiota were mainly enriched in the synthesis-related pathways of key structural sequences of mucin. In combination with the mucin-related staining and immunofluorescence results, the findings indicate that the differential microbiota and their metabolites potentially regulate the composition and function of mucus under colitis.

CONCLUSION

Microbiota and their metabolites are major factors regulating the composition and function of mucus, in turn influencing the function and structure of intestinal mucus barrier under colitis. The different microbiota and metabolites identified in the present study could be novel biomarkers for colitis.

Key Words: Ulcerative colitis; Gut microbiota; Metabolites; Dextran sulfate sodium; Mucin

Core Tip: We observed that the differences in microbiota and their metabolites can cause different degrees of colitis. The differential metabolites of the microbiota in colitis are mainly enriched in pathways related to the key structural sequence synthesis of mucin, and the different levels of the metabolites lead to differential expression of mucin and mucus. Therefore, the differential metabolites of colitis could regulate the composition and function of mucus. In addition, the differential intestinal microbiota and their metabolites in mice with colitis were largely associated with amino acid and energy metabolism.

Citation: Wang JL, Han X, Li JX, Shi R, Liu LL, Wang K, Liao YT, Jiang H, Zhang Y, Hu JC, Zhang LM, Shi L. Differential analysis of intestinal microbiota and metabolites in mice with dextran sulfate sodium-induced colitis. *World J Gastroenterol* 2022; 28(43): 6109-6130
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6109.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6109

INTRODUCTION

Ulcerative colitis (UC) is a chronic nonspecific inflammatory disease of the colorectum and has an unknown cause. UC is more likely to occur in young and middle-aged people. Its main clinical symptoms include diarrhea, mucopurulent bloody stools, and a variety of extraintestinal manifestations, such as peripheral arthritis, erythema nodosum, anterior uveitis, and others that seriously affect the quality of life of patients. The World Health Organization lists UC as one of the most difficult modern diseases and as one of the most significant research topics in the field of digestion because it is difficult to cure, easily recurs, has a high risk of cancer, and often requires lifelong medication.

UC pathogenesis is complex and considered to be mediated by genetic susceptibility, microbial dysregulation, and environmental factors[1]. The intestinal barrier is a complex and prominent defense system that not only prevents the transfer of various toxins through the intestines into tissues and organs but also prevents the invasion of endogenous microorganisms, which is particularly prominent in maintaining homeostasis in the intestine and even the body[2]. The intestinal barrier consists of a mechanical, chemical, biological, and immune barrier. The role of biological barriers in UC is receiving

increasing attention. The latest research shows that abnormalities of the intestinal microbiota and its metabolites play a more important role in the progression of the course of UC[3]. In addition to being involved in constituting a biological barrier, the intestinal microbiota and metabolites also have the functions of biological antagonism, defense against infection, participation in the maturation of the immune system, regulation of intestinal mucus, regulation of intestinal epithelial metabolism, and nutrition[3].

Studies have confirmed that the number of goblet cells in the intestine of aseptic reared mice decreases, their morphology becomes smaller, the mucus layer becomes thinner, and the permeability increases compared with conventionally reared mice[4]. Jakobsson *et al*[5] found that in C57BL/6J mice with two different intestinal microbiotas, although the thickness of the colonic mucus was similar, there was a significant difference in the permeability of the mucus barrier. Several other studies have found that intestinal microbiota and metabolites can affect mucus through the following effects: (1) Regulation of mucus synthesis: the microbiota's structures, such as lipopolysaccharides, flagella proteins, and inflammatory factors induced by induction, can directly regulate the mucus 2 protein, Mucin2 (MUC2) to produce mucus; probiotics can also provide the relevant nutrients required for MUC2 synthesis to upregulate its transcription; bacterial products and the inflammatory factor secretion caused by them also induce the formation of sulfotransferase, sulfate MUC2, and enhance the anti-inflammatory capacity of the intestine[6,7]; (2) Mucus secretion regulation: active substances, such as lipopolysaccharides and secondary bile salts, stimulate goblet cells to secrete MUC2 into the intestinal cavity to flush bacteria and resist invasion, but excessive MUC2 secretion causes goblet cell depletion and reduces the anti-infection ability of the intestinal mucosa[8,9]. It has also been suggested that inflammasomes can maintain the intestinal environment and microbiota homeostasis by promoting the production of interleukin-18 in sensing intestinal bacterial products or cell damage[10]; and (3) Mucus degradation regulation: primarily the slow degradation of probiotics represented by *Akkermansia muciniphila*, which is used to maintain the dynamic balance of the mucus layer and the rapid degradation of *Escherichia coli*, a representative of pathogenic bacteria that destroys the integrity of the mucus layer.

Therefore, the intestinal microbiota and its metabolites can directly constitute a biological barrier and affect the mucus barrier to play a multi-dimensional regulatory role. It can be seen that the intestinal microbiota and metabolites have a central position in the pathogenesis of UC. At present, although many studies have confirmed the important role of intestinal microbiota in the pathogenesis of UC, the intricate connection between microbiota and metabolites in UC occurrence remains unclear, and the correlation analysis of UC differential microbiota and its metabolites is relatively scarce, suggesting the need to carry out further studies.

Based on the intestinal microbiota and metabolism, this study uses a combination of antibiotics to establish pseudo-aseptic mice. We followed the internationally accepted dextran sulfate sodium method to establish a UC model. By comparing the severity of the disease, mucus-related protein expression, and 16s rDNA sequencing of pseudo-aseptic and bacterial UC mice, we evaluated the practical significance of intestinal microbiota in UC pathogenesis in multiple dimensions. Further, through microbiota sequencing combined with non-targeted metabolomics association analysis, we tried to reveal the differential intestinal microbiota and microbiota metabolites in UC pathogenesis, exploring more sensitive biomarker compositions and providing new ideas for diagnosing UC.

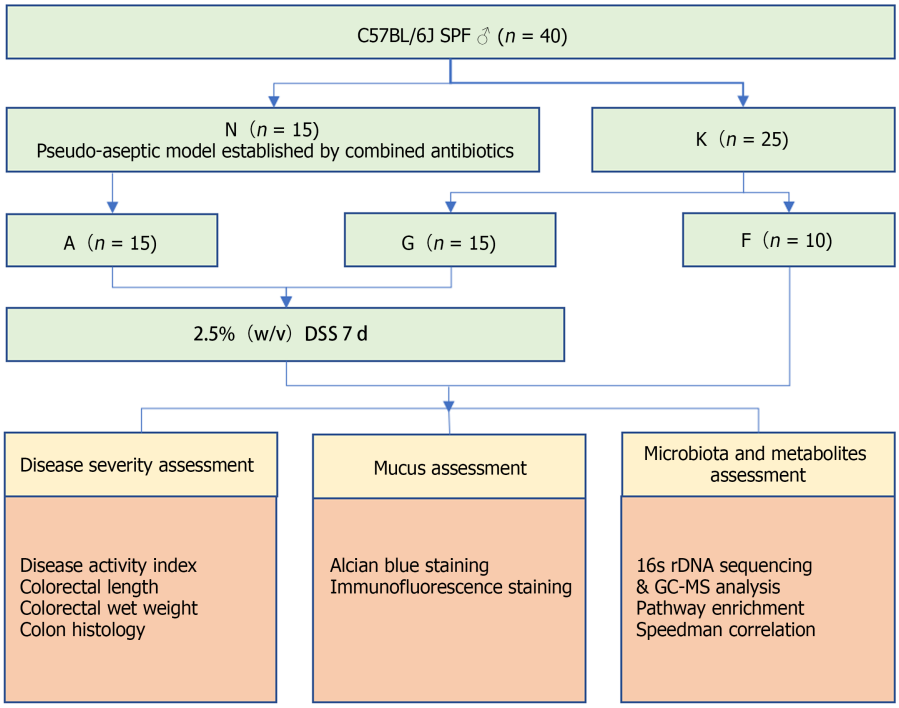
MATERIALS AND METHODS

Experimental animals

The animal protocol was designed to minimize pain or discomfort to the animals. Forty male C57BL/6J mice, specific pathogen free (SPF) grade, weighing 20 ± 2 g were purchased from SPF Biotechnology Co., Ltd. (Beijing, China, certificate No. SCXK-2019-0010). They were routinely raised in the SPF-level animal room of Beijing University of Chinese Medicine, with a 12-h light/night cycle, temperature of 22 ± 2 °C, and humidity of 50%-60% and were given access to unlimited food and drinking water. The experimental process complied with the ethical requirements formulated by the Laboratory Animal Ethics Subcommittee of the Academic Committee of Beijing University of Chinese Medicine (No. BUCM-2020-01162). The study design was shown in Figure 1.

Establishment of pseudo-aseptic models

Amphotericin B (catalog No. 1397-89-3), vancomycin hydrochloride (catalog No. 1404-93-9), neomycin sulfate (No. 1405-10-3), metronidazole (No. 443-48-1), and ampicillin sodium (No. 69-52-3) were all purchased from China Shanghai Macklin Biochemical Co., Ltd. After 1 wk of adaptive rearing of all mice, 40 mice were randomly divided into the pseudo-aseptic group (N group, n = 15) and the non-intervention group (K group, n = 25) using the random number table method. A schematic diagram of the model to replicate the pseudo-aseptic model of N group mice is shown in Figure 2A. Gastric lavage (0.1 mL/10 g) was first given with amphotericin B (1 mg/kg body weight) for 3 d, and from day 4, 1 mg/mL of ampicillin was added to drinking water. Meanwhile, mice were given a mixture of antibiotics



DOI: 10.3748/wjg.v28.i43.6109

Figure 1 Study design. N: Pseudo-aseptic group; K: Blank group; A: Microbiota$^-$ colitis group; F: Blank group; G: Microbiota$^+$ colitis group; DSS: Dextran sulfate sodium; GC-MS: Gas chromatography-mass spectrometry.

that included vancomycin 50 mg/kg body weight, neomycin 100 mg/kg body weight, metronidazole 100 mg/kg body weight, and amphotericin B 1 mg/kg body weight every 12 h for a total of 21 d (*i.e.*, 4-24 d)[11].

Establishment of a colitis model

Pseudo-aseptic mice were labeled as pseudo-aseptic colitis model groups (group A, n = 15), and non-intervention mice were randomly divided into the following two groups using the random number table method: Blank group (group F, n = 10) and bacterial colitis model group (group G, n = 15). In addition to the blank group, the remaining 30 mice were free to drink 2.5% (w/v) dextran sulfate sodium (DSS) solution (molecular weight: 36000-50000 Da; MP Biomedicals, Santa Ana, CA, United States) to establish an acute experimental colitis model.

Analysis of the disease activity index

The disease activity index (DAI) score includes the following three indicators: weight loss rate, stool consistency, and degree of fecal occult blood. The DAI score = (weight loss rate score + stool consistency score + fecal occult blood score)/3[12], the specific scoring criteria are shown in Table 1.

Measurement of colorectal length

The abdominal cavity was exposed, and the pelvis was cut from the anus with scissors. The fascia and other structures from the rectum were cut with forceps and ophthalmic scissors upwards. An ileocecal mass, a large bulge, was discovered, cutting from the lower end of the ileocecal to the rectum, and the colorectal length was measured.

Measurement of colorectal wet weight

The removed colorectal wet weight was measured before dipping in the phosphate buffer solution, and the intestinal tube was cut along the longitudinal axis before weighing. The feces in it were cleaned up, and the wet weight of the intestinal tube was recorded.

Colonic histological lesions score

After the specimen was fixed with 4% paraformaldehyde for 24 h, 4 μm sections were prepared after paraffin embedding. The histological lesions of the colon were observed under light microscopy after hematoxylin-eosin (HE) staining. The standard histological lesions scoring was used[13]. Table 2 provides the scores, with a total score of 0-16. One slice was made per mouse, and the average score of seven items per specimen was calculated.

Table 1 Disease activity index assessment standards

Score	Weight loss rate	Stool consistency	Fecal occult blood
0	< 1%	Normal	Negative
1	1%-5%		
2	5%-10%	Soft stool (mushy)	Positive
3	10%-15%		
4	> 15%	Watery stools (mostly near the anus)	Visible bloody stools or hematochezia

Weight loss rate = (weight the day before the material is taken - weight 2 d before the material is taken)/the weight of the day before the material is taken × 100%.

Table 2 Scoring criteria for histological lesions in the colon

Content	Score				
	0	1	2	3	4
Goblet cells	No loss	Mild loss	Moderate loss	Severe loss	
Mucosal thickness	No change	Mild thickening	Moderate thickening	Severe thickening	
Inflammatory cells	No infiltration	Mild increase	Moderate increase	Severe increase	
Submucosal inflammatory cell infiltration	No infiltration		Mild increase	Moderate increase	Severe increase
Degree of disruption of mucosal structures	No damage			Mild destruction	Moderate destruction
Percentage of epithelial cell ulcer area	No ulcers	0%-25%	25%-50%	50%-75%	75%-100%
Number of crypt abscesses	No	1–3	4–6	7–9	> 10

Colon tissue mucopolysaccharide alcian blue staining

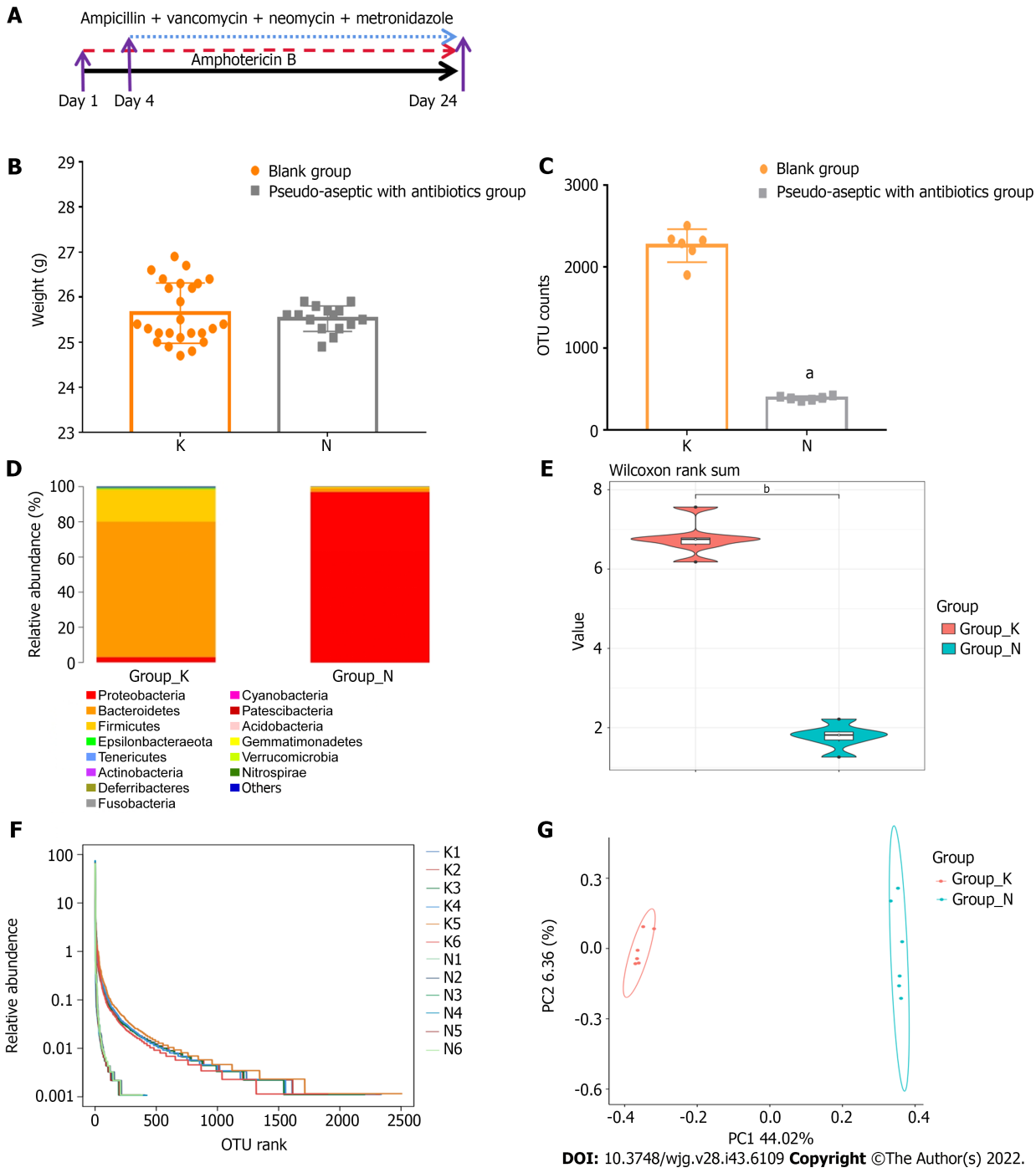
The paraffin sections were stained with alcian blue dye, and the HE staining step was continued. This was followed by washing, counterstaining, dehydrating, and clearing post-sealing. A panoramic scanner was used to scan the specimen, and the Caseviewer software (Hungarian 3D HISTECH, Pannoramic 250/MIDI, version 2) took pictures. Each sample was photographed after setting the white balance of the high magnification field of view (× 400), and Image J software (National Institutes of Health, United States) was used to analyze the images. The area of positive staining was selected for statistical analysis.

Colonic MUC2 immunofluorescence staining

Paraffin sections were de-waxed and then repaired by antigens. Primary antibodies (MUC2, 1:200, GB11344, Wuhan Servicebio Biotechnology Co., Ltd., Wuhan China), secondary incubated antibodies, and 4′,6-diamidino-2-phenylindole-stained nuclei were added after fluorescence quenching and serum blocking. Each sample was photographed and collected after setting a high magnification field of view (× 200) white balance; the image was analyzed using the Image J software mentioned above. The percentage of the positively stained area (area%) was selected for statistical analysis.

16s rDNA sequencing of the intestinal microbiota

The genomic DNA of the sample was extracted using a DNA extraction kit, followed by agarose gel electrophoresis. NanoDrop2000 was used to detect the concentration of the DNA. Takara's Tks Gflex DNA Polymerase was used for polymerase chain reaction (PCR), ensuring amplification efficiency and accuracy using genomic DNA as a template and specific primers with barcodes. Identification of the microbiota diversity corresponding V3-V4 regions was done using primers 343F and 798R. This study sample was assayed for mouse fecal microbiota, and the V3-V4 zone forward primer was set as 343F-5′-TACGGRAGGCAGCAG-3′, and the reverse primers used were: 798R-5′- AGGGTATCTAATCCT-3′. The PCR products were detected by electrophoresis, purified with magnetic beads after detection, purified as a two-round PCR template, amplified by two-round PCR, and then used for electrophoresis detection, purification with magnetic beads after detection, and Qubit quantification of the PCR products after purification. The PCR products were mixed in equal amounts according to PCR product concentrations and sequenced on a PCR machine (580BR10905; Bio-Rad Laboratories, Hercules, CA, United States). Using Vsearch (version 2.4.2) software, the high-quality sequence valid tags obtained by quality control were operational taxonomic unit (OTU) classified according to 97% similarity. The



DOI: 10.3748/wjg.v28.i43.6109

Figure 2 The combination of five antibiotics could effectively build an intestinal pseudo-aseptic mouse model. A: Antibiotics applied; B: Body weight; C: Operational taxonomic unit counts; D: Microbiota structure; E: Shannon index of alpha-diversity; F: Rank abundance of alpha-diversity; G: Beta-diversity. N: Pseudo-aseptic with antibiotics group; K: Blank group. Compared with the blank group, $^{a}P < 0.01$, $^{b}P < 0.05$.

sequence with the largest abundance in each OTU was selected as the representative sequence of the OTU. The Ribosomal Database Project classifier naive Bayesian classification algorithm was used to compare the representative sequence with the database to obtain the OTU annotation information.

Aseptic conditions were used to collect mouse feces. The feces were retained on clean filter paper, immediately picked up with disinfected forceps, placed in cryopreservation tubes, frozen in liquid nitrogen, and stored in a -80 °C ultra-low temperature freezer for subsequent use in the sequencing of fecal microbiota. The biological information for differential microbiota compared on a website (https://cloud.oebiotech.cn/task/) included the following: OTU abundance, community distribution statistics of microbiota, alpha diversity analysis, beta diversity analysis, and microbial multivariate statistical analysis [differential species heat map and 16S-based Kyoto Encyclopedia of Genes and Genomes (KEGG) function prediction].

Gas chromatography-mass spectrometry analysis and identification of intestinal microbiota metabolites

Gas chromatography-mass spectrometry (GC-MS) was used to detect and identify metabolites of the fecal microbiota. Processes included: sample pretreatment, metabolite extraction, metabolite derivatization, GC-MS detection, data pretreatment, and statistical analysis. The chromatographic conditions of this study were: DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J&W Scientific, Folsom, CA, United States), high purity helium carrier gas (purity not less than 99.999%), flow rate 1.0 mL/min, and a temperature of 260 °C at the inlet. The injection volume was 1 μL, the injection was not shunted, and the solvent was delayed by 5 min. Program heating was as follows: The initial temperature of the column oven was 60 °C, maintained for 0.5 min, heated to 125 °C at a rate of 8 °C/min, heated up to 210 °C at a rate of 5 °C/min, heated up to 270 °C at a rate of 10 °C/min, and heated up to 305 °C at a rate of 20 °C/min for 5 min. Mass spectrometry conditions: Electron bombardment ion source, ion source temperature of 230 °C, four-stage rod temperature of 150 °C, and electron energy of 70 eV. The scanning method was full scan mode, and the quality of scanning ranged from m/z 50-500. Metabolite data analysis included multivariate statistical analysis, univariate statistical analysis, differential metabolite screening, correlation analysis, and metabolic pathway enrichment analysis.

Statistical analysis

SPSS 22.0 software (SPSS Inc., Chicago, IBM, United States) was used to analyze all data. Measurements that conform to the normal distribution were expressed as mean ± SD, with *t*-test analysis for comparisons between two groups and one-way ANOVA for multi-group comparisons. Fischer's least significant difference was used for homogeneous variances and Tamhane's T2 for unequal variances. The measurement data of the abnormal distribution were expressed as median and quartile spacing (P25, P75), with the Mann–Whitney nonparametric test used for comparison between two groups and the Kruskal-Wallis nonparametric test used for multi-group comparison. The intergroup comparison of the counting data was expressed using median and interquartile spacing (P25, P75), and the Kruskal-Wallis nonparametric test was used for multi-group comparisons of the counting data. The statistical methods of the present study were reviewed by Prof. Zhao-Lan Liu from the Center for Evidence-based Chinese Medicine, Beijing University of Chinese Medicine.

RESULTS

Combination of five antibiotics could effectively establish an intestinal pseudo-aseptic mouse model

After 24 d of combined antibiotic application, there was no significant difference in body weight between pseudo-aseptic mice and mice in the non-intervention group (25.52 *vs* 25.64, $P > 0.05$, Figure 2B). The number of OTUs in the intestinal microbiota of non-intervention mice was significantly higher than that of pseudo-aseptic mice (2259.50 *vs* 389.83, $P < 0.01$, Figure 2C), indicating that the abundance of intestinal microbiota in pseudo-aseptic mice was significantly reduced. Subsequently, a columnar accumulation of species relative abundance at the phylum taxonomic level was plotted, showing the top 15 species in abundance, and it was found that the intestines of non-intervention mice were dominated by the *Bacteroidetes* (77.1%) and *Firmicutes* phyla (18.8%). In contrast, the intestinal communities of pseudo-aseptic mice were dominated by the *Proteobacteria* phylum (96.9%), with a distinctly single community structure (Figure 2D). A further selection of the Shannon index for the violin plot showed that the species richness and microbiota community diversity of the intestinal microbiota of the non-intervention mice were significantly greater than those of the pseudo-aseptic group (6.75 *vs* 1.78, $P < 0.01$, Figure 2E). The Rank Abundance analysis found that the span of the horizontal axis of the non-intervention mice curve was significantly larger than that of the pseudo-aseptic mice, indicating that the composition of the non-intervention mouse microbiota was relatively rich, and the span of the longitudinal axis of the non-intervention mice curve was smaller than that of the pseudo-aseptic mice, indicating that the species composition of the non-intervention mice microbiota was more uniform (Figure 2F). Further beta diversity analysis found that the two groups of microbiota differed substantially between groups and could be significantly separated (Figure 2G). Based on the above results, it was confirmed that the combined antibiotic method adopted by this study could effectively remove the intestinal microbiota of mice and establish an intestinal pseudo-aseptic mouse model, and its microbiota abundance, community structure, and alpha and beta diversity are significantly reduced.

Mice with bacteria were more severely ill in DSS-induced colitis

On day 2 of taking the DSS solution, stool changes and fecal occult blood test were positive, and the mice gradually lost weight from day 3 onwards. The symptoms gradually worsened with the prolongation of the DSS drinking time. During the entire molding period, four mice died in the pseudo-aseptic group and five mice died in the bacterial group. Performing DAI scoring on the last day of molding revealed an increase in both groups of colitis mice and greater severity of illness in the bacterial

mice (Figure 3A). After dissecting the intestinal tube, the length of the colorectum and the wet weight of the intestinal tube were measured, and it was found that the intestines of the two groups of colitis mice were significantly shortened and that the wet weight was significantly increased, especially in the bacterial mice. (Figures 3B-D). Subsequently, 10 mice in each group were selected for histological lesion evaluation of the intestinal mucosa, and the results showed that the four layers of colonic mucosa structure in the blank group were clear, the goblet cells were abundant, there was no obvious inflammatory cell infiltration, and the mucosal thickness was normal. In contrast, the mucosal structure of the colitis mice was destroyed; the four-layer structure disappeared, the inflammatory cells infiltrated, the submucosal layer was partially affected, and no obvious crypt abscess was seen (Figure 3E). The histological performance of pseudo-aseptic mice was between blank and bacterial mice, and the histological lesions were light. The score was significantly lower than that of bacterial mice (Figure 3F).

Intestinal mucus loss was more pronounced in DSS-induced colitis mice with bacteria

HE staining revealed that goblet cells in the intestinal mucosa of colitis mice were reduced. Then, through alcian blue staining, it was found that the colon mucosa of blank mice was rich in mucopolysaccharides and goblet cells. The mucopolysaccharides and goblet cells in the colons of mice with colitis were significantly reduced, the mucus layer was interrupted, and the goblet cells were significantly reduced and vacuolated (Figures 4A and B), and the characteristics of mucus of pseudo-aseptic mice came in between those of blank and bacterial mice. Immunofluorescence staining of MUC2, the most important structural and functional mucus protein, was significantly decreased ($P < 0.01$) in the intestines of the bacterial colitis model compared with those of the blank group. Expression of MUC2 in the pseudo-aseptic colitis mice came in between that of the blank and bacterial mice (Figures 4C and D).

The above two studies suggest that different intestinal microbiota have different effects on mucin and mucus, and the degree of colitis that eventually forms is different. The method of free drinking DSS solution can successfully establish a colitis model, but the symptoms of colitis, disease activity, intestinal mucosal histological structure, and mucus distribution in pseudo-aseptic mice are less than those in intestinal bacterial mice. The appearance of this difference may be related to the diversity of the intestinal microbiota or the difference in microbiota metabolites between pseudo-aseptic and bacterial colitis mice. The study then used 16s rDNA sequencing combined with non-targeted metabolomics technology to reveal this characteristic difference, further explore the potential differential intestinal microbiota and metabolites of colitis mice, and screen out the characteristic differential microbiota and metabolites in colitis.

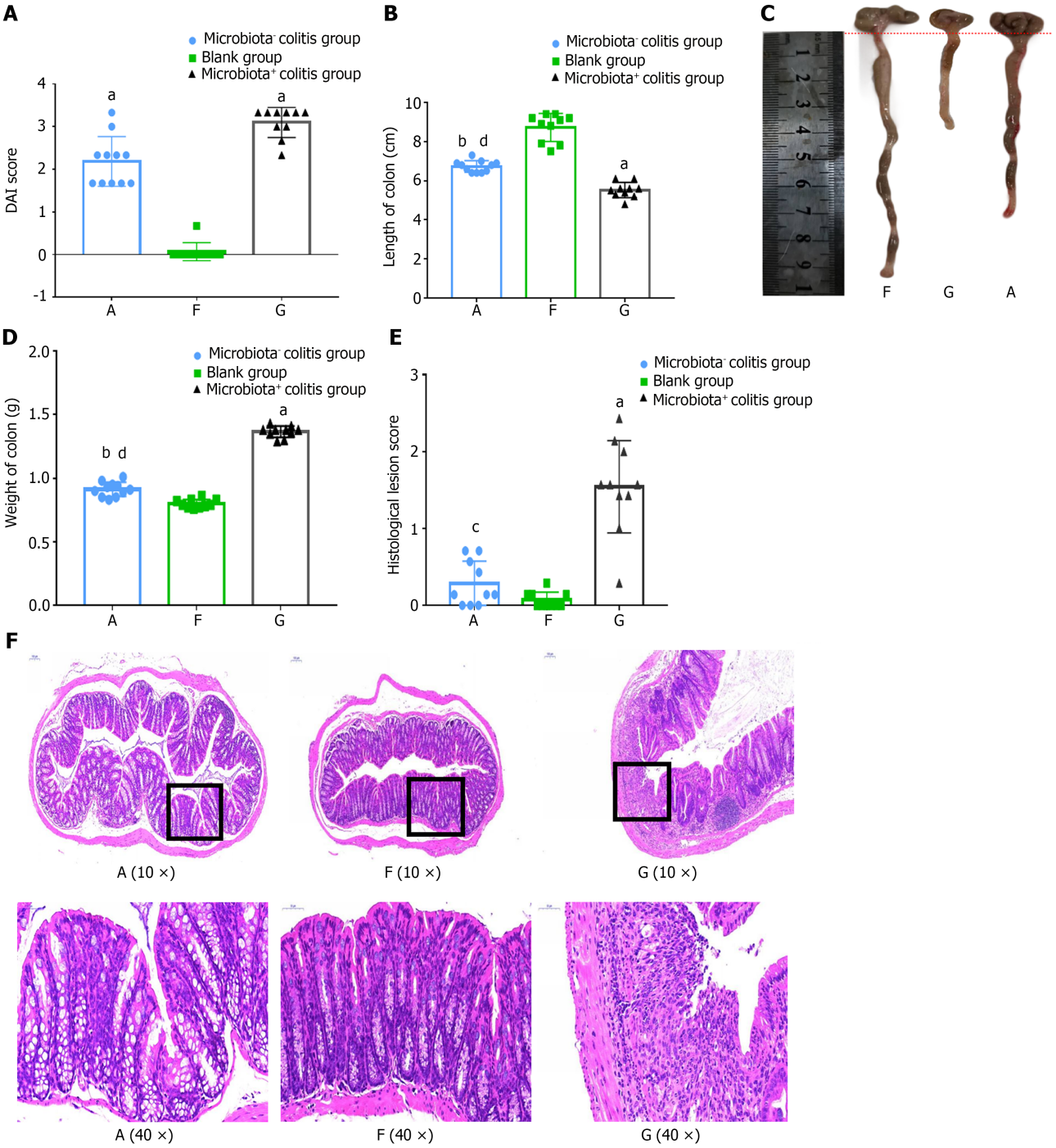
Analysis of different characteristic microbiota in DSS-induced colitis mice

Six mice were randomly selected from each group by the random number table method for 16s rDNA microbiota sequencing, and by comparing the number of OTUs, it was found that the OTU number of the intestinal microbiota of blank group mice was significantly higher than that of mice with bacteria ($P < 0.01$, Figure 5A). Subsequently, the diversity index dilution curve with goods coverage index and specaccum species accumulation curves were constructed, and the results showed that the two curves tended to be stable at the end, indicating that the sequencing data of each sample were sufficient and that sampling was sufficient (Figures 5B and C). The observed species index was used to plot the box plot and found that the alpha diversity of the intestinal microbiota of mice with bacterial colitis was significantly lower than that of the blank group (Figure 5D). Beta diversity analysis of the composition of the intestinal microbiota of the two groups of mice was further studied, and the results showed that the two groups were separated farther apart, which proved that there was a significant difference (Figure 5E).

Subsequently, Wilcoxon rank sum test was used for clustering at the genus taxon level of the microbiota, and Wilcoxon was performed on the different species of intestinal microbiota, and 50 different species with P (FDR) < 0.05 and names were selected for heat map cluster analysis (Figure 5F). Compared with that in the blank group, it was found that the following bacterial genera in the intestinal microbiota of mice with bacterial colitis were significantly increased ($P < 0.05$): *Anaerobiospirillums*, *Rikenellaceae RC9* gut group, *Prevotellaceae Ga6A1* group, *Escherichia-Shigella*, *Klebsiella*, *Anaerotruncus*, *Negativibacillus*, *Candidatus Stoquefichus*, *Blautia*, *Verrucomicrobium UBA1819*, *Helicobacter*, and *Lachnoclostridium*. The above bacterial genera were significantly elevated in mice with colitis and can be considered harmful bacteria that cause the occurrence or aggravation of colitis.

The bacterial genera that significantly decreased ($P < 0.05$) in the intestinal microbiota of mice with bacterial colitis were: *Bacteroides*, *Prevotellaceae UCG-001*, *Prevotellaceae NK3B31*, *Acetatifactor*, *Muribaculum*, *Lactobacillus*, *Eubacterium coprostanoligenes*, *Candidatus Saccharimonas*, and *Eubacterium brachy*. The above bacterial genera were significantly reduced in colitis mice, and we think that they may be able to prevent or mitigate the occurrence and development of colitis.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis was then used to predict the composition of differential microbial gene functions of known phylum level classifications, and the predicted KEGG results were displayed using cluster heat maps (Figure 5G). The study found that in the classification of the phylum level there were 28 KEGG signaling pathways with obvious enrichment of different microbiota in mice with colitis. Combined with the characteristics of the colitis disease itself and P (FDR) values, we deduced that the pathways involved were, from large to



DOI: 10.3748/wjg.v28.i43.6109

Figure 3 Mice with bacteria were more severely ill in dextran sulfate sodium-induced colitis. A: Disease activity index score; B: Colon length; C: Colon photos; D: Colon weight; E: Histological lesions score; F: Hematoxylin-eosin staining. A: Microbiota⁻ colitis group, F: Blank group, G: Microbiota⁺ colitis group. DAI: Disease activity index. Compared with the blank group, $^{a}P < 0.01$, $^{b}P < 0.05$. Compared with microbiota⁺ colitis group, $^{c}P < 0.01$, $^{d}P < 0.05$.

small according to the difference in significance: biodegradation and metabolism of symbionts, cell growth and death, biosynthesis and metabolism of glycans, energy metabolism, immune system diseases, digestive system, nucleotide metabolism, metabolic diseases, metabolism of terpenoids and polyketone compounds, amino acid metabolism, protein folding, classification and degradation, cancer, metabolism of cofactors and vitamins, immune system, metabolism of other amino acids, transport and catabolism, lipid metabolism, cellular processes and signaling, signaling molecules and interactions, and enzyme families. In summary, it was observed that the functional prediction of differential intestinal microbiota was mainly concentrated in the pathways related to the metabolism of various substances. Thus, we carried out further research on microbiota metabolism.

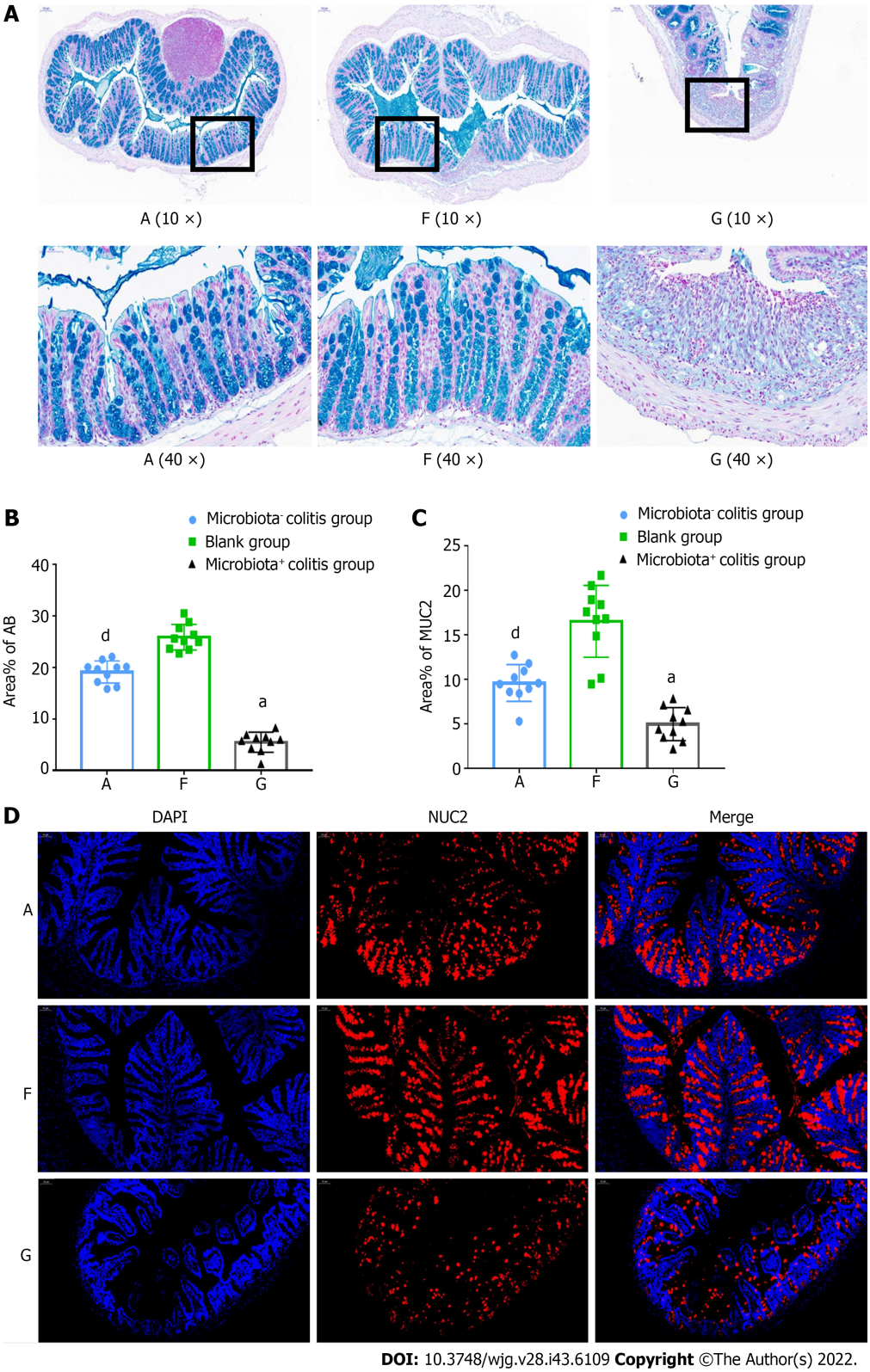


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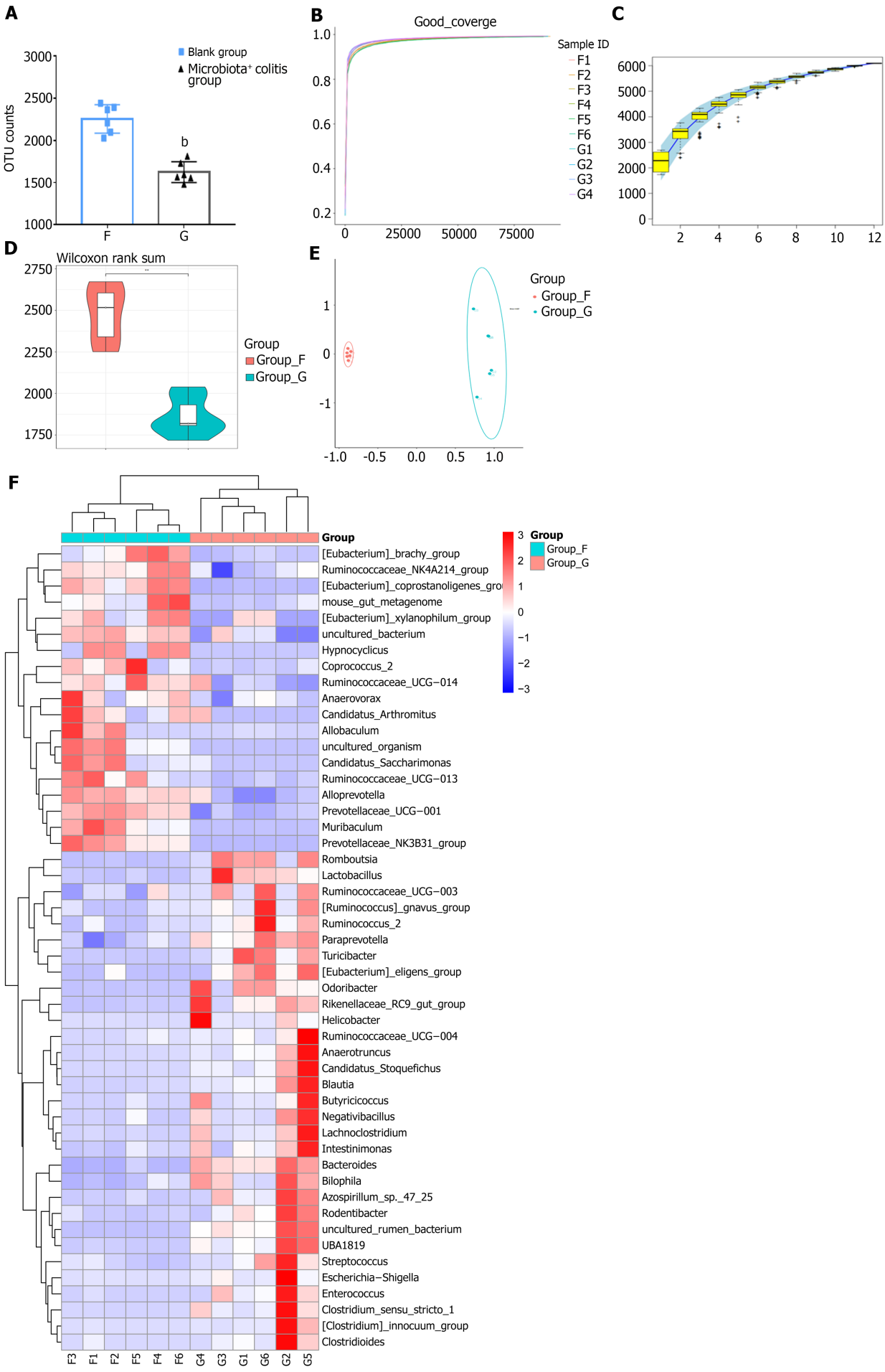
Figure 4 Intestinal mucus loss was more pronounced in dextran sulfate sodium-induced colitis mice with bacteria. A: Alcian blue staining; B: Percentage of positively stained areas; C: Percentage of MUC2-positive stained areas; D: Mucin2 (MUC2) immunofluorescence staining. A: Microbiota⁻ colitis group, F: Blank group, G: Microbiota⁺ colitis group. MUC2: Mucin2. Compared with the blank group, $^{a}P < 0.01$. Compared with the microbiota⁺ colitis group, $^{d}P < 0.05$.

Analysis of different microbiota metabolites in DSS-induced colitis mice

Orthogonal partial least-squares discrimination analysis was used to distinguish the overall differences in metabolic profiles between groups and look for differential metabolites between groups. It can be found that the microbiota metabolites of the blank group and the colitis mice have good polymerization, the intra-group difference is small, and the separation between the groups is obvious, indicating that there are obvious differences between the two groups of metabolites (Figure 6A). The *P* values and fold


A
Blank group
Microbiota+ colitis group
OTU counts
3000
2500
2000
1500
1000
b
F
G
B
Good_coverge
1.0
0.8
0.6
0.4
0.2
0
25000
50000
75000
Sample ID
F1
F2
F3
F4
F5
F6
G1
G2
G3
G4
C
6000
5000
4000
3000
2000
1000
0
2
4
6
8
10
12
D
Wilcoxon rank sum
2750
2500
2250
2000
1750
Group
Group_F
Group_G
E
Group
Group_F
Group_G
1
0
-1
-1.0
-0.5
0.0
0.5
1.0
F
Group
Group_F
Group_G
3
2
1
0
−1
−2
−3
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Ruminococcaceae_NK4A214_group
[Eubacterium]_coprostanoligenes_gro
mouse_gut_metagenome
[Eubacterium]_xylanophilum_group
uncultured_bacterium
Hypnocyclicus
Coprococcus_2
Ruminococcaceae_UCG−014
Anaerovorax
Candidatus_Arthromitus
Allobaculum
uncultured_organism
Candidatus_Saccharimonas
Ruminococcaceae_UCG−013
Alloprevotella
Prevotellaceae_UCG−001
Muribaculum
Prevotellaceae_NK3B31_group
Romboutsia
Lactobacillus
Ruminococcaceae_UCG−003
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Paraprevotella
Turicibacter
[Eubacterium]_eligens_group
Odoribacter
Rikenellaceae_RC9_gut_group
Helicobacter
Ruminococcaceae_UCG−004
Anaerotruncus
Candidatus_Stoquefichus
Blautia
Butyricicoccus
Negativibacillus
Lachnoclostridium
Intestinimonas
Bacteroides
Bilophila
Azospirillum_sp._47_25
Rodentibacter
uncultured_rumen_bacterium
UBA1819
Streptococcus
Escherichia−Shigella
Enterococcus
Clostridium_sensu_stricto_1
[Clostridium]_innocuum_group
Clostridioides
F3
F1
F2
F5
F4
F6
G4
G3
G1
G6
G2
G5

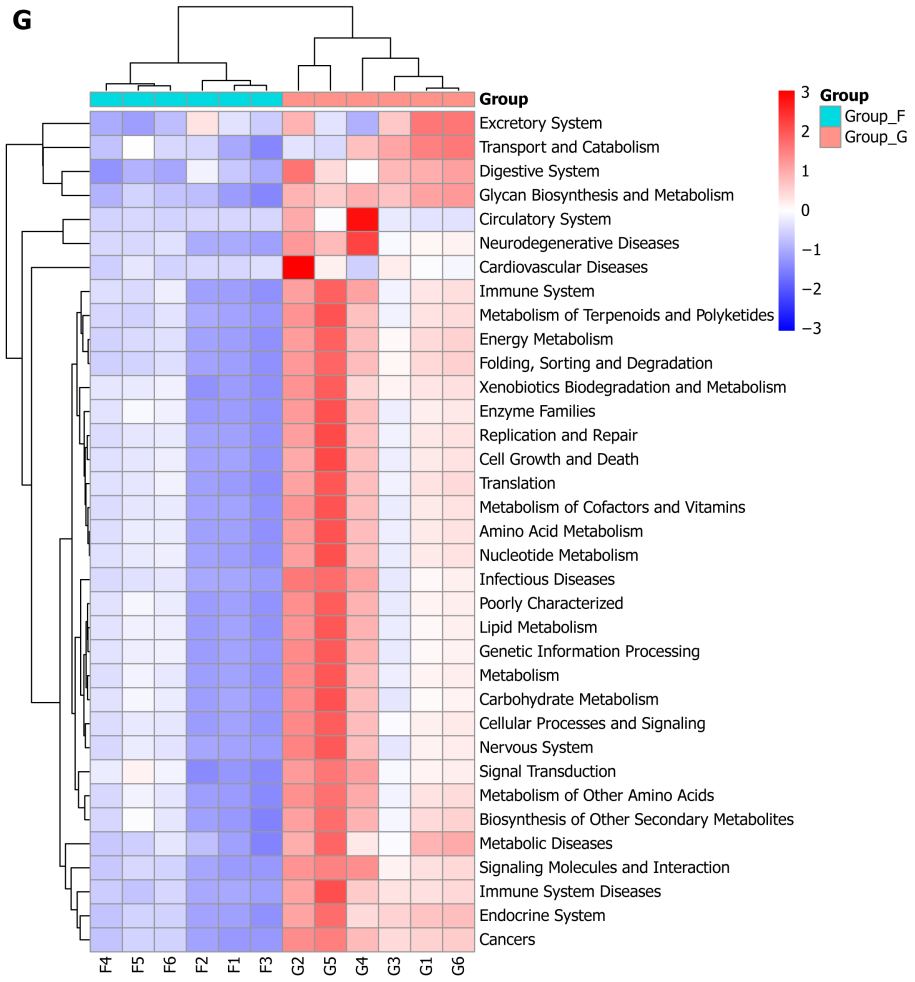

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Figure 5 Analysis of different characteristic microbiota in dextran sulfate sodium-induced colitis mice. A: Operational taxonomic unit counts; B: Goods coverage index dilution curve; C: Specaccum species accumulation curve; D: Observed species violin graph of alpha-diversity; E: Beta-diversity; F: Heat map cluster analysis of 50 different species at the genus level; G: Heat map cluster analysis of Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis with Kyoto Encyclopedia of Genes and Genomes pathways at the phylum level. F: Blank group; G: Microbiota^{+} colitis group. Compared with the blank group, $^{b}P < 0.01$.

change values of the two groups performed on the *t*-test were visualized using the volcano plot (Figure 6B), and the differential metabolites between the two groups were further screened (the standard was set to the variable weight value > 1, and the *P* value of the *t*-test < 0.05). Subsequently, all significantly different metabolites were hierarchically clustered, and a clustered heat map was plotted (Figure 6C). After analysis, it was found that there were 7 kinds of metabolites with significant downregulation in the intestinal microbiota of mice with bacterial colitis, and according to the significant differences, the order was: Galacturonic acid, maltose, lactitol, D-ribose, pyrophosphate, lactulose, and N-acetyl-5-hydroxytryptamine; there were 50 kinds of metabolites significantly upregulated in the intestinal microbiota of mice with bacterial colitis, the top 10 metabolites by the significant difference in size were: D-tagletose, o-phosphateserine, 5-methoxytryptamine, spermine, ribonic acid, creatinine, pinitol, sarcosine, 3-hydroxybutyric acid, and phenol (Table 3). Subsequently, through correlation data matrix analysis (Figure 6E), the degree of correlation between significantly different metabolites was quantified, and it was found that the significantly upregulated differential metabolites in colitis were most closely related to pyrophosphate, lactulose, galacturonic acid, maltose, and lactitol, and the significantly upregulated metabolites were closely related to o-phosphate, sonol, L-tryptophan, butylenediamine, 5-methoxytryptamine, spermine, p-hydroxyphenylpropionic acid, and l-lactic acid.

Based on the KEGG database, the study used the KEGG ID of differential metabolites to enrich the metabolic pathways of differential metabolites, and the *P* (FDR) < 0.05 in the metabolic pathway was used as the significance of the enrichment of the metabolic pathway, and the significant enrichment signaling pathway of the top 20 was selected for bubble mapping (Figure 6D). The analysis found that the differential metabolites of colitis mice were mainly enriched in: biosynthesis of aminoacyl tRNA; arginine and proline metabolism; ABC transporter; biosynthesis of valine, leucine, and isoleucine; cAMP

Table 3 Differential metabolites of gut microbiota in dextran sulfate sodium-colitis mice

VIP	*P* value	log2 (FC)	Metabolites	Expression
1.760	0.011	2.207	Digalacturonic acid	↓
2.128	0.017	3.470	D-maltose	↓
1.747	0.017	1.927	Lactitol	↓
1.898	0.000	-2.342	D-tagatose	↑
2.323	0.000	-3.519	O-phosphoserine	↑
1.111	0.000	-0.845	5-methoxytryptamine	↑
1.659	0.000	-1.867	Spermine	↑
2.493	0.000	-4.048	Ribonic acid	↑
1.726	0.001	-1.952	Creatinine	↑
2.983	0.001	-5.714	Pinitol	↑
1.716	0.001	-2.032	Sarcosine	↑
1.327	0.002	-1.259	3-hydroxybutyric acid	↑
1.593	0.002	-1.715	Phenol	↑
2.771	0.002	-5.062	Ascorbic acid	↑
2.181	0.003	-3.167	Montanic acid	↑
1.985	0.003	-2.701	L-tryptophan	↑
1.857	0.003	-2.522	2,3-dihydroxypyridine	↑
3.490	0.003	-5.993	Putrescine	↑
1.090	0.004	-0.853	Shikimic acid	↑
1.303	0.004	-1.191	Malonic acid	↑
1.539	0.004	-1.639	L-lactic acid	↑
1.010	0.004	-0.779	L-threonine	↑
1.549	0.005	-1.665	Xanthosine	↑
1.277	0.007	-1.301	hydroxy-3-(4'-hydroxy-3'4-methoxyphenyl)propionic acid	↑
1.297	0.007	-1.238	Leucine	↑
1.113	0.008	-0.947	7-hydroxynicotinic acid	↑
1.766	0.008	-2.298	3,6-anhydro-d-galactose	↑
2.103	0.009	-3.424	3-deoxyhexitol	↑
1.162	0.011	-1.079	Thymidine	↑
1.259	0.013	-1.171	4-aminobutyric acid	↑
1.118	0.015	-1.076	Aminomalonate	↑
2.490	0.016	-4.348	Maltitol	↑
2.607	0.016	-4.806	Butane-2,3-diol	↑
1.388	0.018	-1.622	(4-hydroxyphenyl)-4-propionic acid	↑
1.138	0.018	-0.998	Threose	↑
1.283	0.020	-1.266	2-hydroxybutanoic acid	↑
1.148	0.021	-1.130	3-hydroxypalmitic acid	↑
1.081	0.022	-0.983	Epicatechin	↑
1.219	0.022	-1.150	L-valine	↑
1.415	0.023	-1.560	Erythronic acid	↑
2.556	0.026	-2.451	Ornithine	↑

1.935	0.028	-3.003	Diacetone alcohol	↑
1.000	0.029	-0.917	Beta-glutamic acid	↑
1.442	0.029	-1.651	Sinapinic acid	↑
1.874	0.031	-2.768	Atropine	↑
1.211	0.031	-1.903	L-asparagine	↑
1.103	0.035	-0.988	4',5-dihydroxy-7-glucosyloxyflavanone	↑
1.334	0.037	-1.861	L-glutamine	↑
2.530	0.039	-4.230	Adenosine	↑
1.070	0.039	-0.948	Pyruvic acid	↑
1.051	0.041	-0.985	Cerotinic acid	↑
1.026	0.043	-0.942	L-tyrosine	↑
1.646	0.043	-2.361	Urocanic acid	↑

Up-arrows represent upregulated metabolites, and down-arrows represent downregulated metabolites in microbiota$^+$ colitis mice.

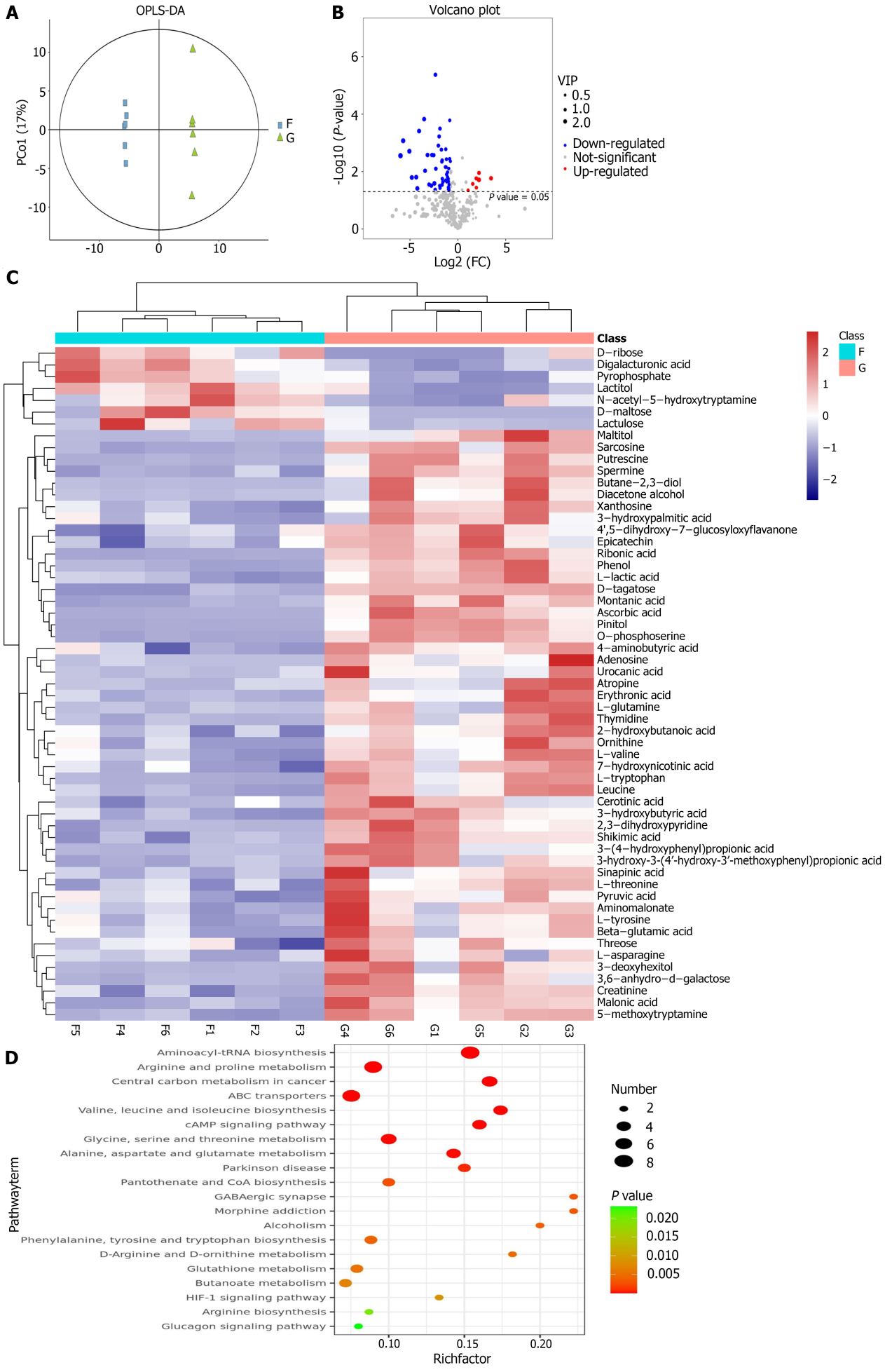
signaling pathway; metabolism of glycine, serine, and threonine; metabolism of alanine, aspartic acid, and glutamic acid; pantothenic acid and CoA biosynthesis; GABA synapses; biosynthesis of phenylalanine, tyrosine, and tryptophan; D-arginine and D-ornithine metabolism, glutathione metabolism, butyrate metabolism, and hypoxia-inducible factor-1 signaling pathways. In general, the differential metabolites of the intestinal microbiota of colitis mice are mainly concentrated in amino acid and energy metabolism.

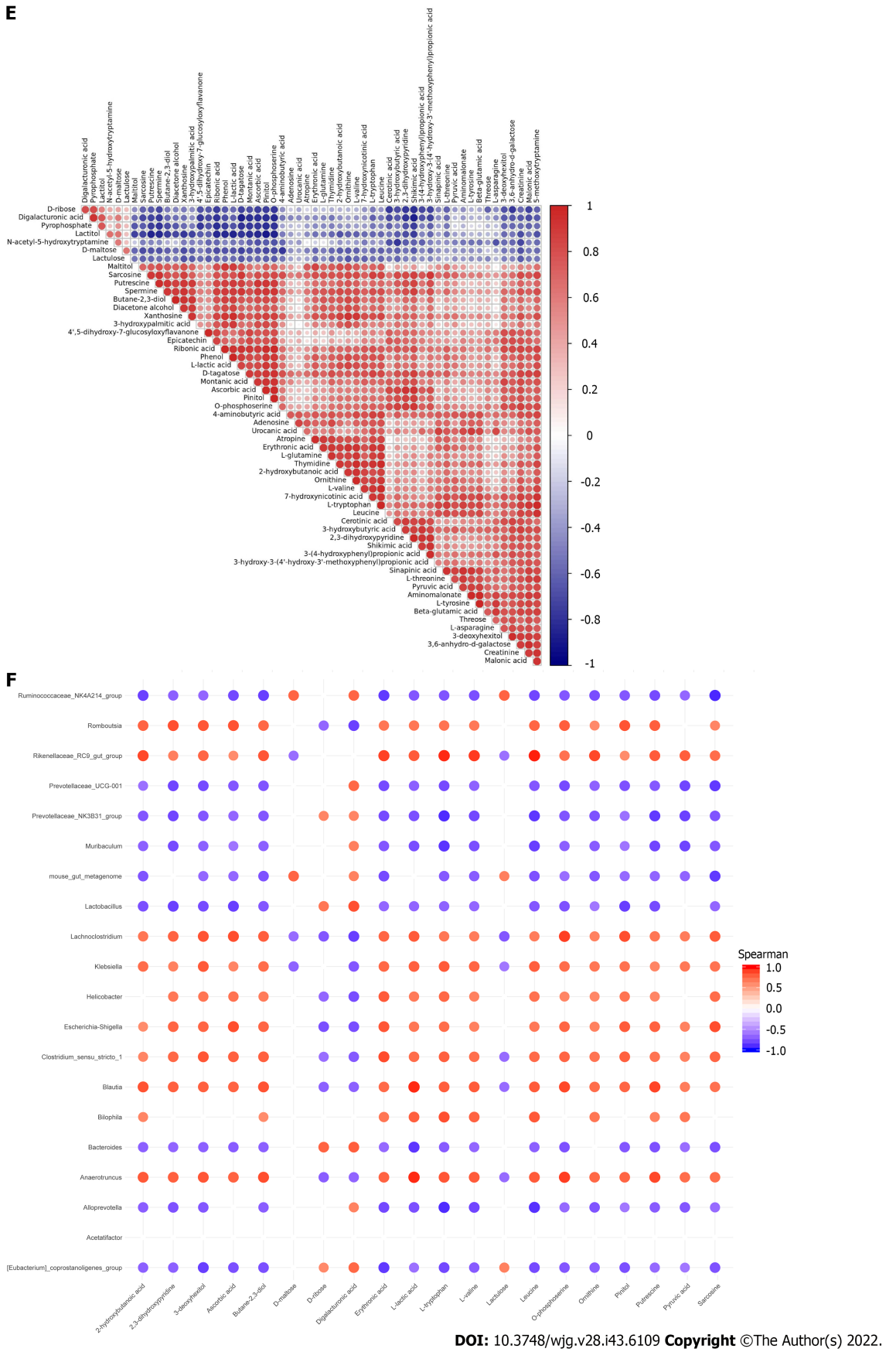
Finally, the correlation analysis of differential metabolites and differential microbiota species of the two groups of intestinal microbiota was carried out using Speedman correlation analysis, and the correlation matrix of the differential metabolites of top 20 and the microbiota at the genus taxonomic level was plotted (Figure 6F). The results showed that 3-deoxyhexanol, ascorbic acid, 2,3-butanediol, trihydroxybutyric acid, L-tryptophan, levo valine, leucine, o-phosphoserine, pineol, butylamine, and sarcosine were not only positively correlated with the genera significantly elevated in colitis ($P < 0.05$) but also significantly negatively correlated with the significantly reduced genera ($P < 0.05$), from which it can be speculated that the above substances are differential metabolites that can cause or aggravate colitis, and are positively correlated with their occurrence and development. Galacturonic acid was positively correlated with the significantly reduced genera in colitis ($P < 0.05$), while lactulose was negatively correlated with the significantly elevated genera ($P < 0.05$). Therefore, it can be considered that galacturonic acid and lactulose may play a beneficial role in the occurrence and development of colitis.

DISCUSSION

This study found that bacterial colitis caused more mucosal damage and inflammation than bacteria pseudo-aseptic colitis. The destruction of intestinal mucus was more pronounced, speculating that the intestinal microbiota contributed to colitis. The differences in the microbiota could determine the severity of colitis. However, in the past, the academic community has discussed the mechanism of the intestinal microbiota in UC, mostly based on research on the characteristics of the microbiota itself, such as flagellar protein, lipopolysaccharide, *etc*., often ignoring the co-metabolism of the host and the microbiota or the self-metabolism of the microbiota. Its metabolites can be used by the host intestinal epithelial cells again, thereby participating in and maintaining the intestinal microbiome balance of the host[14]. Modern research has gradually concluded that the intestinal microbiota in UC can affect the intestinal microenvironment through different characteristics of the species itself and metabolites produced by metabolism, including affecting the function of epithelial structures, changing the state of mucus synthesis, secretion, and degradation, and promoting or inhibiting immune responses and inflammatory responses[15,16].

We found that certain microbiota and metabolites were significantly elevated in colitis mice after comparing them to blank mice, suggesting that the microbiota and metabolites were involved in colitis or aggravated it. Significantly reduced microbiota and metabolites in colitis are deemed to have a protective effect on colitis. Further, the correlation analysis between the differential microbiota and metabolites and the analysis of KEGG pathway enrichment found that the intestinal differential microbiota and its metabolites in mice with colitis were mainly concentrated in amino acid and energy metabolism.


A
OPLS-DA
PCo1 (17%)
F
G
B
Volcano plot
-Log10 (P-value)
Log2 (FC)
P value = 0.05
VIP
0.5
1.0
2.0
Down-regulated
Not-significant
Up-regulated
C
Class
F
G
D-ribose
Digalacturonic acid
Pyrophosphate
Lactitol
N-acetyl-5-hydroxytryptamine
D-maltose
Lactulose
Maltitol
Sarcosine
Putrescine
Spermine
Butane-2,3-diol
Diacetone alcohol
Xanthosine
3-hydroxypalmitic acid
4',5-dihydroxy-7-glucosyloxyflavanone
Epicatechin
Ribonic acid
Phenol
L-lactic acid
D-tagatose
Montanic acid
Ascorbic acid
Pinitol
O-phosphoserine
4-aminobutyric acid
Adenosine
Urocanic acid
Atropine
Erythronic acid
L-glutamine
Thymidine
2-hydroxybutanoic acid
Ornithine
L-valine
7-hydroxynicotinic acid
L-tryptophan
Leucine
Cerotinic acid
3-hydroxybutyric acid
2,3-dihydroxypyridine
Shikimic acid
3-(4-hydroxyphenyl)propionic acid
3-hydroxy-3-(4'-hydroxy-3'-methoxyphenyl)propionic acid
Sinapinic acid
L-threonine
Pyruvic acid
Aminomalonate
L-tyrosine
Beta-glutamic acid
Threose
L-asparagine
3-deoxyhexitol
3,6-anhydro-d-galactose
Creatinine
Malonic acid
5-methoxytryptamine
F5
F4
F6
F1
F2
F3
G4
G6
G1
G5
G2
G3
D
Aminoacyl-tRNA biosynthesis
Arginine and proline metabolism
Central carbon metabolism in cancer
ABC transporters
Valine, leucine and isoleucine biosynthesis
cAMP signaling pathway
Glycine, serine and threonine metabolism
Alanine, aspartate and glutamate metabolism
Parkinson disease
Pantothenate and CoA biosynthesis
GABAergic synapse
Morphine addiction
Alcoholism
Phenylalanine, tyrosine and tryptophan biosynthesis
D-Arginine and D-ornithine metabolism
Glutathione metabolism
Butanoate metabolism
HIF-1 signaling pathway
Arginine biosynthesis
Glucagon signaling pathway
Pathwayterm
Richfactor
Number
P value



DOI: 10.3748/wjg.v28.i43.6109

Figure 6 Analysis of different microbiota metabolites in dextran sulfate sodium-induced colitis mice. A: Orthogonal partial least-squares discrimination analysis; B: Metabolite volcano plot; C: Clustered heat map of significantly expressed metabolites; D: Bubble mapping of the top 20 significant Kyoto Encyclopedia of Genes and Genomes pathways of metabolites; E: Correlation matrix analysis with significant metabolites; F: Correlation matrix of the top 20 differential metabolites and the microbiota at the genus levels. F: Blank group; G: Microbiota+ colitis group.

There are 10 to 100 trillion microorganisms in the human gastrointestinal tract, and in the past few decades, the impact of the gut microbiota on human health has received widespread interest from science and the general public. The gut microbiome comprises bacteria, viruses, fungi, and archaea that live in different states in the human gastrointestinal tract. More and more studies have confirmed that the intestinal microbiota is closely related to inflammatory bowel disease, metabolic disease, liver disease, hypertension, and other diseases[17,18]. As probiotics and other means to restore and rebuild the normal intestinal microbiota have shown good therapeutic effects for a variety of diseases, the intestinal microbiota is considered to be a new target for the occurrence or treatment of many diseases [19].

However, because the intestinal microbiota not only has a large number but also has a complex distribution and structural composition, the driving mechanism of the intestinal microbiota in many diseases is still unclear, which hinders the construction of intestinal microbiota models for specific diseases and the search for disease characteristic intestinal microbiota. Therefore, the development of aseptic mice is the most effective tool for conducting intestinal microbiota research, and aseptic mice can provide scientific methods for solving such problems[20].

Currently, two main methods have emerged in academia to study the effects of the microbiota on mouse physiology and disease: aseptic mice and antibiotic treatment regimens (pseudo-aseptic mice). Aseptic mice do not contain any bacteria, viruses, eukaryotic microorganisms, or other saprophytic or parasitic related life forms[21], but the high labor and cost of generating and maintaining them have resulted in many research teams not being able to use this model. In addition, this chronic lack of stimulation from foreign antigens can leave the development of its organs in an idealized aseptic state, leading to significant limitations in the structure and function of organs in the body, especially the immune system[22].

Scientists have created another method using multiple antibiotics for gut sterilization to solve this problem. Broad-spectrum antibiotic therapy usually eliminates most of the intestinal microbiota of mice. It can be easily applied to any genotype or condition in mice. Still, because it is impossible to eliminate the intestinal microbiota, it is generally referred to as pseudo-aseptic mice relative to aseptic mice[23]. Different antibiotics selectively deplete different members of the microbiota, with metronidazole and clindamycin targeting anaerobic bacteria; vancomycin being effective only against gram-positive bacteria; polymyxin B specifically targeting gram-negative bacteria[24]; amphotericin B having strong antimicrobial activity against candida spores, *etc*.; neomycin being effective against both gram-positive and gram-negative bacteria; and ampicillin being more effective against gram-positive bacteria, *Viridans streptococci* and *Enterococcus*[25]. Pseudo-aseptic mice can therefore be established by extensively depleting the intestinal microbiota by using different kinds of antibiotic mixtures. In this study, the establishment of intestinal pseudo-aseptic mice was built by combining species antibiotics, and the 16s rDNA sequencing of the microbiota was confirmed.

The pathogenesis of ulcerative colitis is complex, and most of the current views are that the interaction of multiple factors causes it. The academic community summarizes the pathogenesis of UC as follows: in genetically susceptible people, such as those suffering from depression and anxiety-related conditions, the intestinal microbiota involved in the intestinal barrier is destroyed, and the immune system function is disrupted, resulting in an excessive immune-inflammatory response[26]. The pathogenesis of UC involves multiple mechanisms, and the colitis model established by different methods is suitable for different research purposes, among which DSS is the most widely used chemical drug for the preparation of colitis models. In the DSS model, sulfated polysaccharides act as a direct chemical toxin of the colon epithelium by reducing the number of mucins and destroying the intestinal epithelial mucus layer[27], causing intestinal epithelial cells damage and structural destruction of the tightly connected complex, while breaking the distribution of the intrinsic intestinal microbiota and affecting the bacterial community structure and diversity[28]. The permeability of the intestinal barrier is increased, and harmful macromolecular antigens, bacteria, toxins, and other substances successfully pass through the intestinal barrier, causing abnormal responses to inherent and adaptive immunity and cascading amplification of inflammation and eventually forming colitis.

A growing number of epidemiological clues point to the onset of UC being closely related to dysbiosis of the gut microbiota. Colonic contents provide bacteria with a rich nutritional environment and become the main settlement of the intestinal microbiota, which provides energy and nutrients to the host through metabolites[29]. Under physiological circumstances, the intestinal microbiota mainly has the following functions: first, it forms a biological barrier with the intestinal mucosal epithelium, blocks exogenous antigenic substances from entering the intestinal mucosa, and produces different metabolic and secondary metabolites through its metabolism to provide energy for the intestinal epithelium; secondly, it participates in the differentiation of T lymphocytes, and jointly regulates mucosal immunity with lymphatic centers and immune cells to regulate immune function; thirdly, it participates in the metabolism and synthesis of a variety of amino acids, proteins, and other substances and participates in the composition of the mucosal barrier[30].

Studies have shown that bacteria that promote mucus degradation, such as *Ruminococcus* and *Entamoeba histolytica*, have been found in larger numbers in patients with UC compared to healthy individuals. *Listeria* that inhibits mucus synthesis has increased significantly, in contrast, *Lactobacillus* that promotes MUC2 secretion has decreased significantly in monocytogenes[31]. *Escherichia coli*

infection can disrupt the balance of intestinal flora and disrupt the intestinal microenvironment[32]. By colonizing the intestinal epithelial cells and secreting enterotoxins, *E. coli* damages the intestinal mucus layer, reduces the protein expression of tight junctions and adhesive junctions, impairs the intestinal mucosal barrier structure, increases intestinal permeability, and induces inflammatory responses and the production of cytokines, such as tumour necrosis factor-α, in addition to increasing MUC2 degradation, which is directly involved in UC pathogenesis[33]. In the stool specimens of patients with UC, the *Proteobacteria* phylum increased significantly, and the phyla *Firmicutes* and *Bacteroidetes* decreased significantly[34], accompanied by different degrees of expression changes in various amino acids, such as decreased valine and glutamate expression; increased tryptophan and isoleucine content; increased pyruvate metabolism; decreased citric acid expression; and decreased short-chain and medium-chain fatty acids in energy metabolism[35]. Other studies have shown that the protective microbiota of *Clostridium*, *Prevoella*, *Bifidobacterium*, and *Lactobacillus acidophilus* were significantly reduced in UC[36], while the invasive *Ekmanella* and *E. coli* were significantly increased. The metabolites of short-chain fatty acids, tryptophan, and bile acids[37] that provide nutrients and energy and protect the epithelium were also significantly reduced[38]. Besides *Klebsiella*, *Escherichia-Shigella*, *Bacteroides*, *Lactobacillus*, which are well-known microbiota in colitis, we also found *Candidatus Stoquefichus*, *Anaerobiospirillum*, *Muribaculum*, *Rikenellaceae RC9* gut group, *Candidatus Saccharimonas*, *Prevotellaceae Ga6A1* group, and *Negativibacillus*. We believe these are emerging microbiota that induces or protects against colitis.

Some studies have reported a certain relationship between other emerging flora and metabolites and colitis. For example, *Faecalibacterium* can influence goblet cell differentiation, mucin synthesis, and glycosylation in colonic epithelium, thereby regulating the intestinal mucus barrier[39]. *Lachnospiraceae* can degrade mucins and are major consumers of mucins. *Lachnospiraceae* over-deplete MUC2, reduce the thickness of the mucus layer and cause severe inflammation in the colon in DSS-induced UC mice[40]. Allobaculum mucolyticum secretes a high amount of mucin O-glycan targeting carbohydrate active enzymes, which enables it to efficiently degrade intestinal mucins, thereby degrading the host's protective mucus layer and disrupting the mucus barrier[41]. Blautia's excessive concentrations in the body may lead to the elevation of secondary bile acids, such as lithocholic acid and deoxycholic acid, which in turn induce UC[42]. A study observed significant differences in the contents of *Brautia* in the fecal and mucosal microbial communities of inflammatory bowel disease (IBD) patients and healthy people[43]. It has also been demonstrated intestinal inflammation in IBD patients is associated with increased *Faecalibaculum* relative abundance[44].

In addition, substantial changes have been evident in colitis, including the marked elevation in 3-deoxyhexanol and o-phosphoserine levels and the reduction in galacturonic acid and other microbiota-derived metabolites. Such findings have not been widely reported with regard to the occurrence and development of colitis. By reviewing the literature, we found that MUCs, as a large and complex class of glycosylated proteins, are characterized by important "mucin domains" composed of a protein core. This core contains proline, threonine, and serine, all three of which are known as rich-PTS sequences. Interestingly, through enrichment analysis, we found that these differential metabolites in this study are primarily enriched in the mucin domain of the rich-PTS sequence synthesis pathways. Levy *et al*[45] studies have shown that taurine and arginine can further promote the synthesis and expression of MUC2 by modulating the NLRP6 signaling pathway, thereby protecting the intestinal epithelium and mucus barrier. Therefore, we believe that these differential microbiota metabolites in colitis are likely to regulate the composition and function of mucus.

This study found differential intestinal microbiota and their metabolites in colitis. Preliminary studies, such as enrichment analysis, have initially found that these differential microbiota and metabolites are likely to be directly involved and affect the mucus barrier of the intestine. However, this study has shortcomings; namely, the lack of verification links for transplanting differentially expressed intestinal microbiota or metabolites into colitis mice; if an experimental design of microbiota or metabolite transplantation is added, we believe that the scientific nature of this study can be further improved, which is a research question we will evaluate further.

CONCLUSION

Candidatus Stoquefichus, Anaerobiospirillum, Rikenellaceae RC9 gut group, *Prevotellaceae Ga6A1* group and *Negativibacillus* are potentially emerging flora that induce or aggravate colitis, and *Candidatus Saccharimonas* and *Muribaculum* are potentially emerging flora that prevent or alleviate colitis. 3-Deoxyhexitol and o-phosphoserine may cause or aggravate colitis, whereas galacturonic acid may play beneficial roles in colitis alleviation and recovery. The differential metabolites of the flora are mainly enriched in the synthesis-related pathways of the rich-PTS sequence of the mucin MUC2 domain. They can influence the composition and function of mucus by regulating mucin expression, and finally act on the mucus barrier to induce aggravation or reduce colitis prevention.

ARTICLE HIGHLIGHTS

Research background

The role of gut microbiota in ulcerative colitis (UC) cannot be ignored; however, most of current research is only based on the microbiota itself, ignoring microbiota metabolism. Microorganisms can reduce many biologically active substances, such as short-chain fatty acids, which have strong immunomodulatory effects. Modern studies have reported that the destruction of the integrity of the mucus barrier is an early pathological change in UC. Different gut microbiota and their metabolites can influence the intestinal mucus barrier through different pathways, including altering epithelial structure, affecting mucin synthesis, secretion and degradation, and modulating immune responses.

Research motivation

At present, although many studies have confirmed the important role of gut microbiota in UC, the intricate relationship between microbiota and metabolites in UC has not been fully clarified. Association analysis of differential flora and their metabolites are required. It is worthwhile to conduct this study based on the intestinal mucus barrier to further reveal the potential differential biomarkers of UC.

Research objectives

The aim of the present study was to reveal the differential gut microbiota and metabolites that affect mucus in UC pathogenesis. The regulatory effect provides new evidence and new ideas for UC diagnosis.

Research methods

In the present study, based on the intestinal mucus barrier, taking the intestinal flora and metabolism as the breakthrough point, a combination of antibiotics was used to establish pseudo-aseptic mice, and the widely used dextran sodium sulfate was used to establish colitis mice. Disease severity, mucus-associated protein expression, bacterial 16s rDNA sequences, and non-targeted metabolomes of bacterial and bacterial colitis mice were examined. The tract microbiota and metabolites play potentially important roles in UC pathogenesis by affecting the mucus barrier.

Research results

This study found that: (1) The antibiotics combination can effectively remove the intestinal flora of mice, and reduce bacterial abundance, and α and β diversity, alter community structure, and successfully establish pseudo-aseptic mice; (2) Comparing the bacteria-bearing mice with the pseudo-aseptic mice, the bacteria-bearing mice had more severe colitis based on disease activity index, more severe intestinal mucosal damage, and more obvious intestinal mucus loss; (3) In the intestinal flora of colitis mice, *Candidatus Stoquefichus*, *Anaerobiospirillum*, *Rikenellaceae RC9* gut group, *Prevotellaceae Ga6A1* group, and *Negativibacillus* were significantly increased, while *Candidatus Saccharimonas* and *Muribaculum* were significantly decreased. The significantly up-regulated metabolites in the intestinal flora of colitis mice included 3-deoxyhexitol and ortho-phosphoserine, and the significantly down-regulated metabolites were galacturonic acid, *etc.*; and (4) Further enrichment analysis found that the above differential metabolites were mainly associated with amino acid and energy metabolism. Spearman correlation analysis found that 3-deoxyhexitol, o-phosphoserine, *etc.* were positively correlated with the occurrence and development of colitis, and galacturaldehyde was positively correlated with the occurrence and development of colitis. There was a significant positive correlation between galacturonic acid and lactulose and the reduction and colitis recovery.

Research conclusions

Candidatus Stoquefichus, Anaerobiospirillum, Rikenellaceae RC9 gut group, *Prevotellaceae Ga6A1* group, and *Negativibacillus* are potentially emerging flora that induce or aggravate colitis, and *Candidatus Saccharimonas* and *Muribaculum* are potentially emerging flora that prevent or alleviate colitis. 3-Deoxyhexitol and o-phosphoserine may cause or aggravate colitis, while galacturonic acid may play a beneficial role in colitis alleviation and recovery. The differential metabolites of the flora are mainly enriched in the synthesis-related pathways of the rich-PTS sequence of the mucin MUC2 domain. They can affect the composition and function of mucus by regulating the expression of mucin, and finally act on the mucus barrier to induce aggravation or reduce colitis prevention.

Research perspectives

This study identified some less-reported differential gut microbiota and their metabolites in colitis, which could affect UC progression by modulating mucin synthesis, altering mucus status and the mucus barrier. In future studies, we will carry out in-depth transplantation experiments of fecal bacteria and metabolites to further verify the experimental conclusions of this study.

ACKNOWLEDGEMENTS

We thank the Beijing University of Chinese Medicine and other institutions for funding this project, and all the authors for their efforts.

FOOTNOTES

Author contributions: Wang JL and Han X have contributed equally to this work, and they are co-first authors; Shi L and Li JX conceived and designed the study; Wang JL and Han X performed major experimental work; Wang JL and Shi R acquired and analyzed the results and edited the manuscript; Liu LL, Wang K, Liao YT, Jiang H, Zhang Y, Hu JC, and Zhang LM performed the experiments and statistical analyses; Shi L and Li JX revised the manuscript; All authors read and approved the final version of the manuscript.

Supported by the 13th Five-Year Plan for National Key R&D Program of China, No. 2018YFC1705405; Scientific Research Innovation Team Project of Beijing University of Chinese Medicine, No. 2019-JYB-TD004; New Faculty Startup Fund Program of BUCM, No. 2022-JYB-XJSJJ078; and National Natural Science Foundation of China, No. 82004113.

Institutional animal care and use committee statement: The animal study was reviewed and approved by the Animal Ethics Committee of Beijing University of Chinese Medicine. All animal experiments conformed to the internationally accepted principles for the care and use of laboratory animals (certificate No. SCXK-2019-0010, SPF Biotechnology Co., Ltd., Beijing, China; protocol No. BUCM-2020-01162, The Animal Ethics Committee of Beijing University of Chinese Medicine, Beijing, China).

Conflict-of-interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

Data sharing statement: All data are available upon reasonable request from LS, b01350@bucm.edu.cn.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

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S-Editor: Zhang H
L-Editor: A
P-Editor: Cai YX

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2022 November 21; 28(43): 6131-6156

DOI: 10.3748/wjg.v28.i43.6131

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Salvia miltiorrhiza extract may exert an anti-obesity effect in rats with high-fat diet-induced obesity by modulating gut microbiome and lipid metabolism

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Liao Z, Singapore; Nath L, India

Received: August 25, 2022
Peer-review started: August 25, 2022
First decision: September 2, 2022
Revised: September 21, 2022
Accepted: October 31, 2022
Article in press: October 31, 2022
Published online: November 21, 2022

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Abstract

BACKGROUND

Studies have shown that a high-fat diet (HFD) can alter gut microbiota (GM) homeostasis and participate in lipid metabolism disorders associated with obesity. Therefore, regulating the construction of GM with the balance of lipid metabolism has become essential for treating obesity. *Salvia miltiorrhiza* extract (Sal), a common traditional Chinese medicine, has been proven effective against atherosclerosis, hyperlipidemia, obesity, and other dyslipidemia-related diseases.

AIM

To investigate the anti-obesity effects of Sal in rats with HFD-induced obesity, and explore the underlying mechanism by focusing on GM and lipid metabolism.

METHODS

Obesity was induced in rats with an HFD for 7 wk, and Sal (0.675 g/1.35 g/2.70 g/kg/d) was administered to treat obese rats for 8 wk. The therapeutic effect was evaluated by body weight, body fat index, waistline, and serum lipid level. Lipid factors (cAMP, PKA, and HSL) in liver and fat homogenates were analyzed by ELISA. The effect of Sal on GM and lipid metabolism was assessed by 16S rRNA-based microbiota analysis and untargeted lipidomic analysis (LC-MS/MS),

respectively.

RESULTS

Sal treatment markedly reduced weight, body fat index, serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein, glucose, free fatty acid, hepatic lipid accumulation, and adipocyte vacuolation, and increased serum high-density lipoprotein (HDL-C) in rats with HFD-induced obesity. These effects were associated with increased concentrations of lipid factors such as cAMP, PKA, and HSL in the liver and adipose tissues, enhanced gut integrity, and improved lipid metabolism. GM analysis revealed that Sal could reverse HFD-induced dysbacteriosis by promoting the abundance of *Actinobacteriota* and *Proteobacteria*, and decreasing the growth of *Firmicutes* and *Desulfobacterita*. Furthermore, LC-MS/MS analysis indicated that Sal decreased TGs (TG18:2/18:2/20:4, TG16:0/18:2/22:6), DGs (DG14:0/22:6, DG22:6/22:6), CL (18:2/18:1/18:1/20:0), and increased ceramides (Cers; Cer d16:0/21:0, Cer d16:1/24:1), (O-acyl)-ω-hydroxy fatty acids (OAHFAs; OAHFA18:0/14:0) in the feces of rats. Spearman's correlation analysis further indicated that TGs, DGs, and CL were negatively related to the abundance of *Facklamia* and *Dubosiella,* and positively correlated with *Blautia* and *Quinella*, while OAHFAs and Cers were the opposite.

CONCLUSION

Sal has an anti-obesity effect by regulating the GM and lipid metabolism.

Key Words: *Salvia miltiorrhiza* extract; Obesity; Gut microbiota; Lipid metabolism; High fat diet

Core Tip: Obesity is a major public health issue today and an obesity-related change in gut microbiota composition and its metabolite profile has been demonstrated. As a commonly used traditional Chinese medicine, *Salvia miltiorrhiza* extract (Sal) has many pharmacological effects, including anticoagulant, anti-inflammation, antioxidation, anti-fibrosis, anti-tumor, and organ protection. Although it has not been documented, Sal has a regulatory effect on obesity, which may be related to the gut microbiota. In the present study, we found that Sal plays a role in weight loss, lowering serum lipid levels, regulating the gut microbiota, and improving intestinal fecal metabolites in obese rats.

Citation: Ai ZL, Zhang X, Ge W, Zhong YB, Wang HY, Zuo ZY, Liu DY. *Salvia miltiorrhiza* extract may exert an anti-obesity effect in rats with high-fat diet-induced obesity by modulating gut microbiome and lipid metabolism. *World J Gastroenterol* 2022; 28(43): 6131-6156
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6131.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6131

INTRODUCTION

Obesity, a condition whose incidence increases yearly, can lead to several chronic metabolic syndromes such as diabetes, hyperlipidemia, and atherosclerosis. According to 2016 epidemiological statistics, more than 1.9 billion adults worldwide suffer from obesity, and its prevalence reaches 70% in the United States[1]. According to the WHO, adults with a body mass index (BMI) > 25 are considered overweight, while adults with a BMI > 30 are considered obese[2].

Gut microbiota (GM) is among the key regulators of metabolism[3]. Dysregulation of the GM is closely connected with obesity and its complications[4]. Under physiological circumstances, there is a symbiotic relationship between the GM and the host, which keeps the intestinal dynamic equilibrium of the body and metabolism[5]. On the other hand, the GM-host imbalance[6] has been associated with the occurrence of many diseases, such as obesity, enteritis, and colitis. Recent data indicated that there are significant differences in the structural composition of the GM of obese patients and that of non-obese people. For example, fewer *Bacteroides* (beneficial bacteria which metabolize oligosaccharides and polysaccharides, providing nutrition to the host) and more *Pachyderma* were found in the intestinal tract of obese patients[7]. Consistent findings suggest that obesity is associated with decreased abundance in some taxa, such as *Bifidobacterium*, *Christensenella*, and *Ackermannia,* which are considered beneficial microbes. Moreover, as a key regulator of host metabolism, the GM can influence lipid metabolism and blood and tissue lipid levels in humans and rats[8]. The GM has a major role in the fermentation of carbohydrates, fermenting carbohydrates and producing short-chain fatty acids (SCFAs), such as acetic

acid, propionic acid, and butyric acid, to prevent and treat obesity and its complications. As a result, mounting evidence suggests treating obesity or obesity-related disease by improving the structure of the GM balance to regulate metabolism, particularly lipid metabolism.

Currently, bioactive substances of natural drugs are becoming increasingly popular as a new safe and effective approach to prevent and treat obesity. *Salvia miltiorrhiza* extract (Sal) is a traditional Chinese medicine that includes water-soluble components such as salyanolic acid, tanshinaldehyde, and comfrey acid that can inhibit early adipogenesis[9] and alleviate lipid metabolism disorders[10]. Moreover, its fat-soluble components, such as dihydrodanhinone I, tanshinone IIA and IIB, and cryptotanshinone can reduce glycerol release[11], promote adipocyte differentiation, and reduce the contents of triglycerides (TG) and cholesterol[12] to treat fatty liver or coronary atherosclerosis[13]. The main pharmacological effects of Sal include restraining the activation of IκB-α and NF-κB, inhibiting the oxidation of low-density lipoprotein (LDL-C) from regulating lipid metabolism processes and antioxidant effects, and improving the body's sensitivity to insulin by activating the AMPK pathway [14]. Sal is also used to treat atherosclerosis[15], hyperlipidemia, obesity, and other dyslipidemia-related diseases. Furthermore, Wang *et al*[16] found that the effect of Sal in regulating hepatic steatosis may be related to the intestinal flora. Hence, this study further investigated the anti-obesity effects of Sal in rats with high-fat diet (HFD)-induced obesity and explored the underlying mechanism by focusing on the GM and lipid metabolism.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (aged 7-8 wk, weighing 160 g ± 20 g) were provided by the Hunan Silaike Jingda Experimental Animal Co. Ltd. (Changsha, China; Animal Certificate Number: SCXK (Xiang) 2019-0004) and housed in specific pathogen-free conditions (23.0 °C ± 2.0 °C ambient temperature, 50%-60% relative humidity, and 12/12 h light/dark cycle) in Laboratory Animal Science and Technology Center of Jiangxi University of Traditional Chinese Medicine (Animal use license SYXK 2021-0007). The rats had free access to food and water throughout the experiment. The protocol (Permit Number: JZLLSC2021-236) was approved by the Jiangxi University of Chinese Medicine Animal Care and Use Committee and performed according to the guidelines prescribed by the committee. Experimental manipulation was performed after 7 d of acclimatization.

Drug

Salvia miltiorrhiza (batch number: 200701) was supplied by Baishixin Chinese Herbal Pieces Co., Ltd. (Millizhou, China). Preparation of *Salvia miltiorrhiza* extract was prepared as follows: The radix *Salvia miltiorrhiza* bunge was crushed into powder, dried at 58 °C, placed in 5000 mL round bottom flask, mixed with 75% ethanol (material-liquid ratio 1:10), soaked for 18 h, and placed in a water bath temperature 90 °C. A reflux extraction device was used to heat the reflux for 3 h, after which the filtrate was collected with four layers of gauze and left for 18 h after hot filtration.

The prepared Sal was freeze-dried with a vacuum freeze-dryer (Scientz-100F) and then smashed with zirconia beads using a mixer mill (MM 400, Retsch) at 30 Hz for 1.5 min. Then, 100 mg was dissolved with 1.2 mL of 70% methanol solution and vortexed for 30 s every 30 min 6 times, and the samples were placed in a refrigerator at 4 °C overnight. After centrifugation at 15984 *g* for 10 min, the extracts were filtered and then analyzed by ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS). Analytical conditions and mass spectrometry data were based on Wang *et al*[17] and Chen *et al*[18], respectively. Linear ion trap and triple quadrupole scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP) with the AB4500 QTRAP UPLC/MS/MS System operating in positive and negative ion mode (Figure 1A and B). For each period, a particular set of MRM transitions was observed by the metabolites eluted during this period (Figure 1C and D). Table 1 shows some of the metabolites identified in this study along with their metabolite numbers, integral values, and names.

Experimental design

According to previous studies[19-23], an HFD with purified ingredients and a total caloric value of 475 Kcal/100 g, with lard as the main source of fat (D12451, Research Diets Inc.) was used to induce obesity. The experiment scheme for the modeling of HFD-induced obesity in rats and drug administration is shown in Figure 2. At the initial phase, all the rats were divided into either a control group (given normal diet; n = 8) or an HFD group (given HFD; n = 40) for 7 wk and housed at an ambient temperature of 22.0 °C ± 1.0 °C. Then, the HFD groups were randomly subdivided into five groups: Control (HFD + normal saline), Sal_L (HFD + 0.675 g/kg/d Sal), Sal_M (HFD + 1.35 g/kg/d Sal), Sal_H (HFD + 2.70 g/kg/d Sal), and orlistat (HFD + 32.4 mg/kg/d). All the treatments lasted for 8 wk. The body weight was measured every 3 d and intake of food was measured per cage daily. After deducting the residual food from the initially supplied, the food intake (g/rat/wk) was determined. Rats were randomly selected from multiple cages, and cages were changed every 2 wk to control for potential cage

Table 1 Some metabolites detected in *Salvia miltiorrhiza* extract

Index	Compounds	Class I
pme2292	Putrescine	Alkaloids
pmf0096	Oxalic acid	Organic acids
Zmyn000268	2,3-Dihydroxypropanal	Others
pme2601	3-Hydroxypropanoic acid	Organic acids
MWS1787	2-Picoline; 2-Methylpyridine	Alkaloids
pma6298	3-Hydroxypyridine	Alkaloids
MWSmce460	2-Piperidone	Alkaloids
MWS1990	4-Pentenoic acid	Organic acids
MWSmce461	L-Azetidine-2-carboxylic acid	Alkaloids
MWStz073	5-Hydroxy-2-pyrrolidinone	Alkaloids

effects and sex/age differences.

Macroscopic observation

On the final day of the trial, all rats were euthanized with pentobarbital sodium (40 mg/kg intraperitoneally) and weighed, and the liver and fat were quickly removed and weighed after blood was drawn from the abdominal aorta. The body fat index (BFI) was calculated as follows: BFI = Total weight of fat/Body weight of rat × 100%.

Histological analysis

Fresh liver and adipose tissues were fixed with 4% polyformaldehyde (PFA) at 4 °C, embedded in paraffin, and then cut into 4-μm thick slices. Samples were then stained with hematoxylin-eosin (H&E) and observed under a light microscope to examine the histopathology according to the published criteria by Yerian *et al*[24] and Liew *et al*[25].

Determination of serum biochemical parameters

The serum was centrifuged for 15 min at 999 *g* at room temperature. Serum TG, total cholesterol (TC), high-density lipoprotein (HDL-C), LDL-C, glucose (GLU), and free fatty acids (FFAs) were measured with a Beckmann COULTERAU480 automatic biochemical analyzer.

Enzyme-linked immunosorbent assay (ELISA)

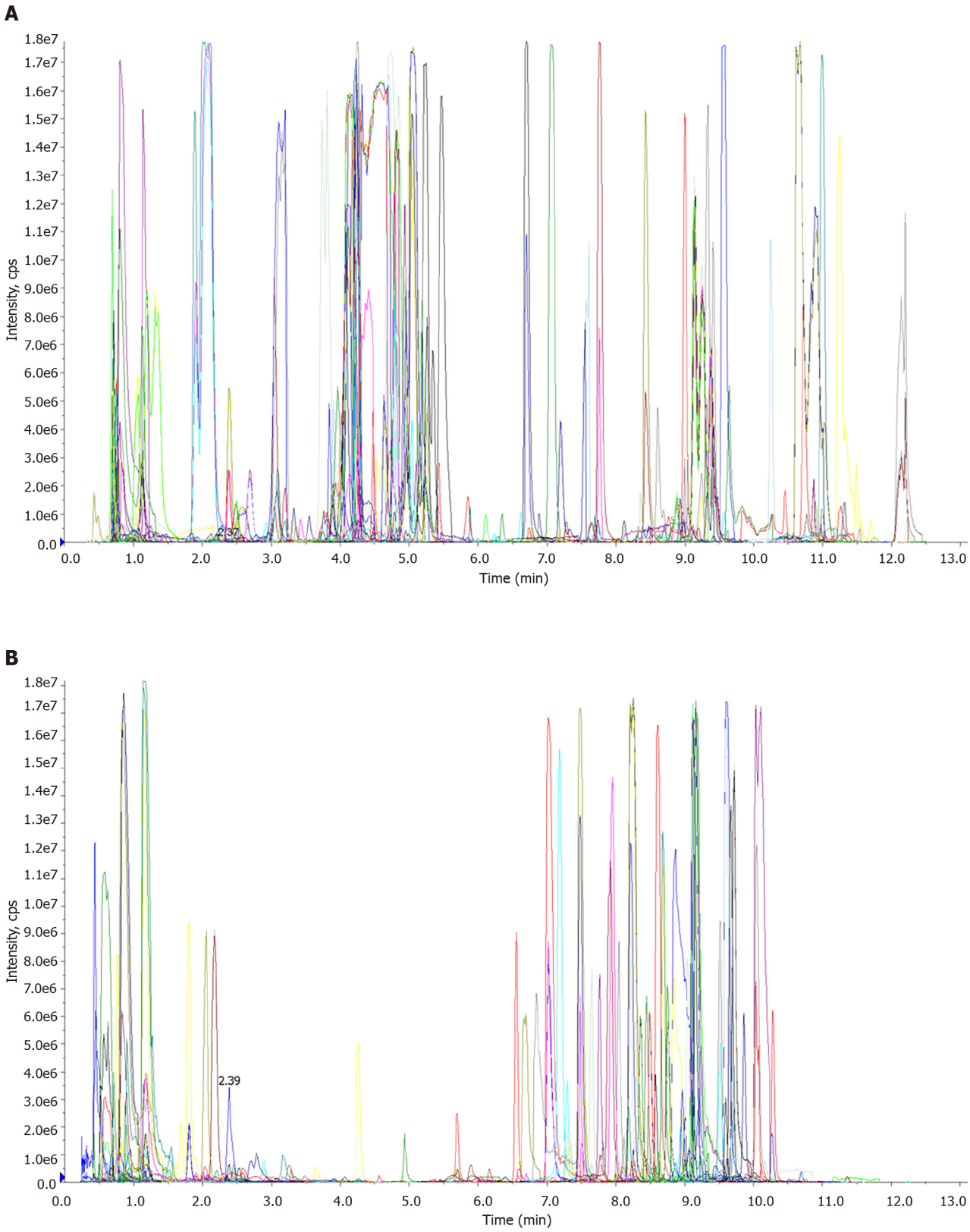
To detect the contents of cAMP in liver tissue, and hormone-sensitive lipase (HSL) and PKA in adipose tissue, parts of the liver and adipose tissue were homogenized under slow rotation (4 °C, 30 min) in 300 μL of RIPA buffer. The supernatant was obtained by centrifugation at 18759 *g* for 30 min. The concentrations of cAMP, HSL, and PKA were determined with commercial ELISA kits (Thermo Fisher Scientific, Waltham city, MA, United States), and the absorbance was measured at 450 nm with a microplate reader (Thermo, Varioskan, MA, United States).

Microbial diversity analysis

Intestinal contents of all rats were collected in cryopreservation tubes and preserved at -80 °C. The microbial diversity analysis was conducted using I-sanger (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China; www.i-sanger.com). Microbial community genomic DNA was extracted from intestinal contents using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, United States). The DNA extract was tested on a 1% agarose gel, and the concentration and purity of the DNA were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, United States). PCR reactions were run in triplicate.

PCR products were extracted from 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions, and quantified using a Quantus™ Fluorometer (Promega, United States). Purified amplicons were equimolar and end-to-end sequenced on the MiSeq PE300 platform/NovaSeq PE250 (United States) under the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were uploaded to the NCBI Sequence Read Archive (SRA).

Multiplexing of raw 16S rRNA gene sequencing reads was performed, followed by quality filtering achieved with Fast version 0.20.0 and merging with FLASH version 1.2.7. UPARSE version 7.1 was used for clustering operational taxonomic units (OTUs) with a 97 percent similarity cutoff. The RDP classifier version 2.2 was used for classification analysis of the 16S rRNA database with a confidence threshold of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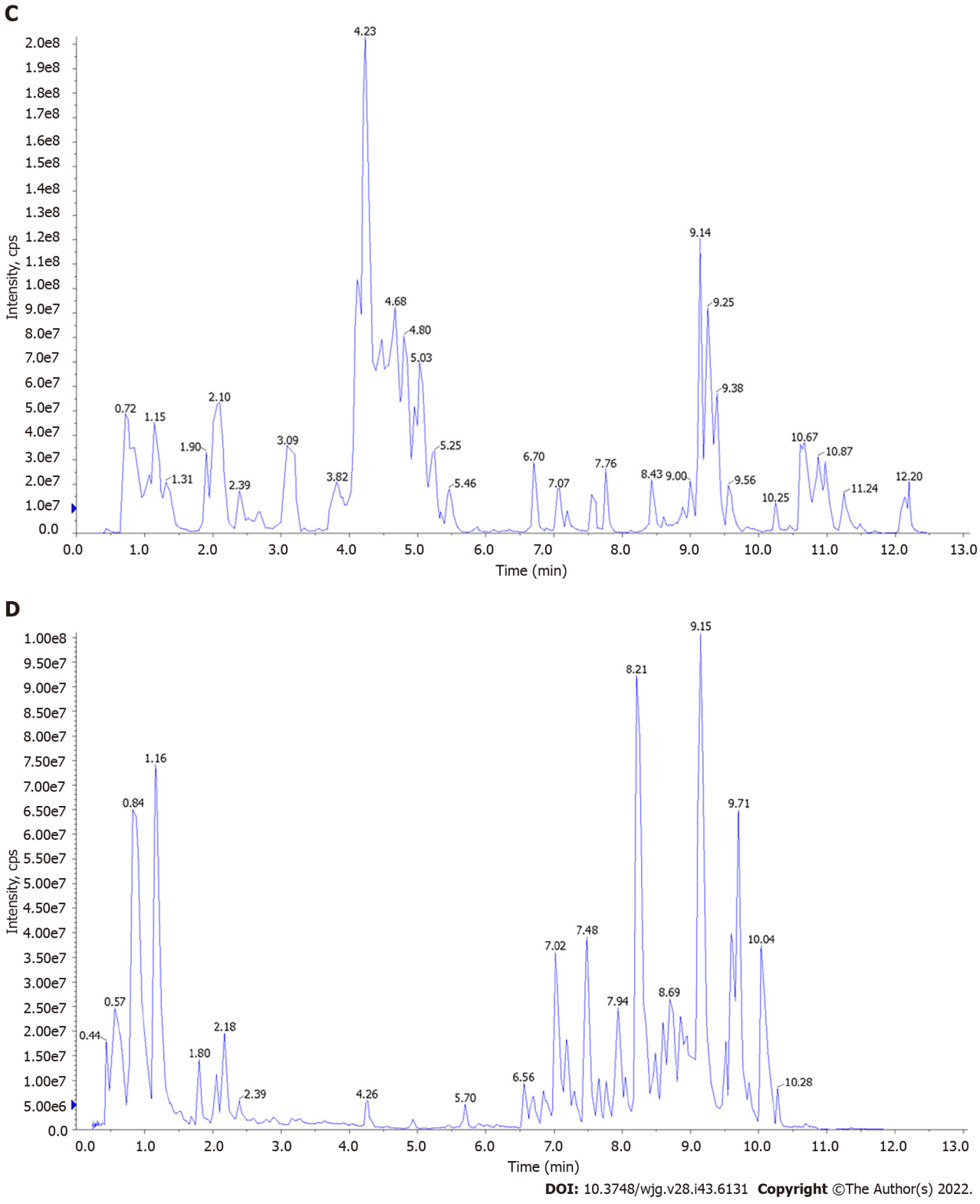

DOI: 10.3748/wjg.v28.i43.6131

Figure 1 UPLC-MS/MS analysis of *Salvia miltiorrhiza* extract. A: Total ions Current-N of QC_MS; B: Total ions Current-P of QC_MS; C: MRM detection of multimodal maps-N; D: MRM detection of multimodal maps-P.

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Figure 2 *In vivo* experiment scheme. Sal: *Salvia miltiorrhiza*.

70%. Calculation of the Shannon index and Sobs diversity was performed, and principal coordinates analysis (PCoA) was used to assess alpha diversity using Mothur (version v.1.30.1). The Kruskal-Wallis H test and the Wilcoxon rank-sum test were used to identify taxa that significantly differed (biomarkers) between groups ($P < 0.05$). I-sanger was used to perform community bar plot and heatmap analysis, correlation analysis, and co-occurrence network analysis.

LC-MS/MS analysis

A total of 50 mg of rat feces were mixed with 80 μL of methanol and 400 μL of methyl-tert-butyl ether for lipid extraction. The mixtures were vortexed for 30 s, followed by sonication for 30 min, and then precipitated at -20 °C for 30 min. The organic phase was separated by centrifugation at 18759 *g* for 15 min at 4 °C. Then, 350 μL of lipid extracts contained in the upper phase were transferred to EP vials and dried in a vacuum concentrator. Consequently, the lipid extract was re-dissolved in 100 μL of isopropanol:acetonitrile (1:1, v/v) solution, followed by 2 min vortexing and 5 min ultrasonication on an ice water bath. Finally, 80 μL of supernatant was transferred carefully to sample vials for LC-MS/MS analysis.

Mass spectral data were collected using a Thermo Q-Exactive Mass Spectrometer equipped with an electrospray ionization[26] source that can operate in either positive or negative ion mode. The raw data from the LC-MS analyses were imported into Lipid Search (Thermo, CA, United States) for peak detection, alignment, and identification. MS/MS fragments were used to identify the lipids. At least 80% of the lipidomic features detected in any set of samples were retained. After filtering, minimum lipid values were performed for specific samples with lipid levels below the lower limit of quantification, and each lipid profile was summed and pooled. After pooling procedures and imputation, log-transformed data were statistically analyzed to determine significant differences in metabolite levels between comparable groups.

Statistical analysis

Most of the data were statistically analyzed and figures produced using GraphPad Prism 7.0 software (San Diego, CA, United States). One-way ANOVA was performed on multiple groups, followed by Duncan's test to analyze statistical differences. For the data on the GM, we used the online platform of the Majorbio Cloud. The Wilcoxon rank-sum test was used to analyze alpha diversity. Weighted UniFrac distances were used to generate the PCoA plots. Kruskal-Wallis rank sum tests were used to analyze species differences between groups. All results are represented as the mean ± SE. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of Sal on body weight gain and fat accumulation in obese rats

After 8 wk of feeding, the body weight of HFD-induced obese rats was significantly higher ($P < 0.05$) compared to the control group ($n = 8$) (Figure 3A). However, the body weight decreased significantly in the Sal_H group ($n = 8$) from the 9th wk, and in Sal_L ($n = 8$) and Sal_M ($n = 8$) groups from the 10th wk compared with the HFD group ($P < 0.05$ for all). On the last day, the final body weight (Figure 3B), waistline (Figure 3C), and body fat index (Figure 3D) in the HFD group were observably increased compared with those of the control group, while Sal supplementation significantly reduced these indices without dose dependence ($P < 0.05$ for all). No significant difference was found among these groups in food intake (Figure 3E) ($P > 0.05$). The above results suggest that Sal can reduce the indices of HFD-induced obesity without affecting appetite intake.

Effects of Sal on serum lipid profiles, liver tissue, and adipose histopathology in HFD group rats

A long-time HFD intake often results in abnormal lipid metabolism[27]. Serum lipid content can reflect the lipid metabolism in the body, among which TG, TC, LDL-C, and HDL-C are the most critical

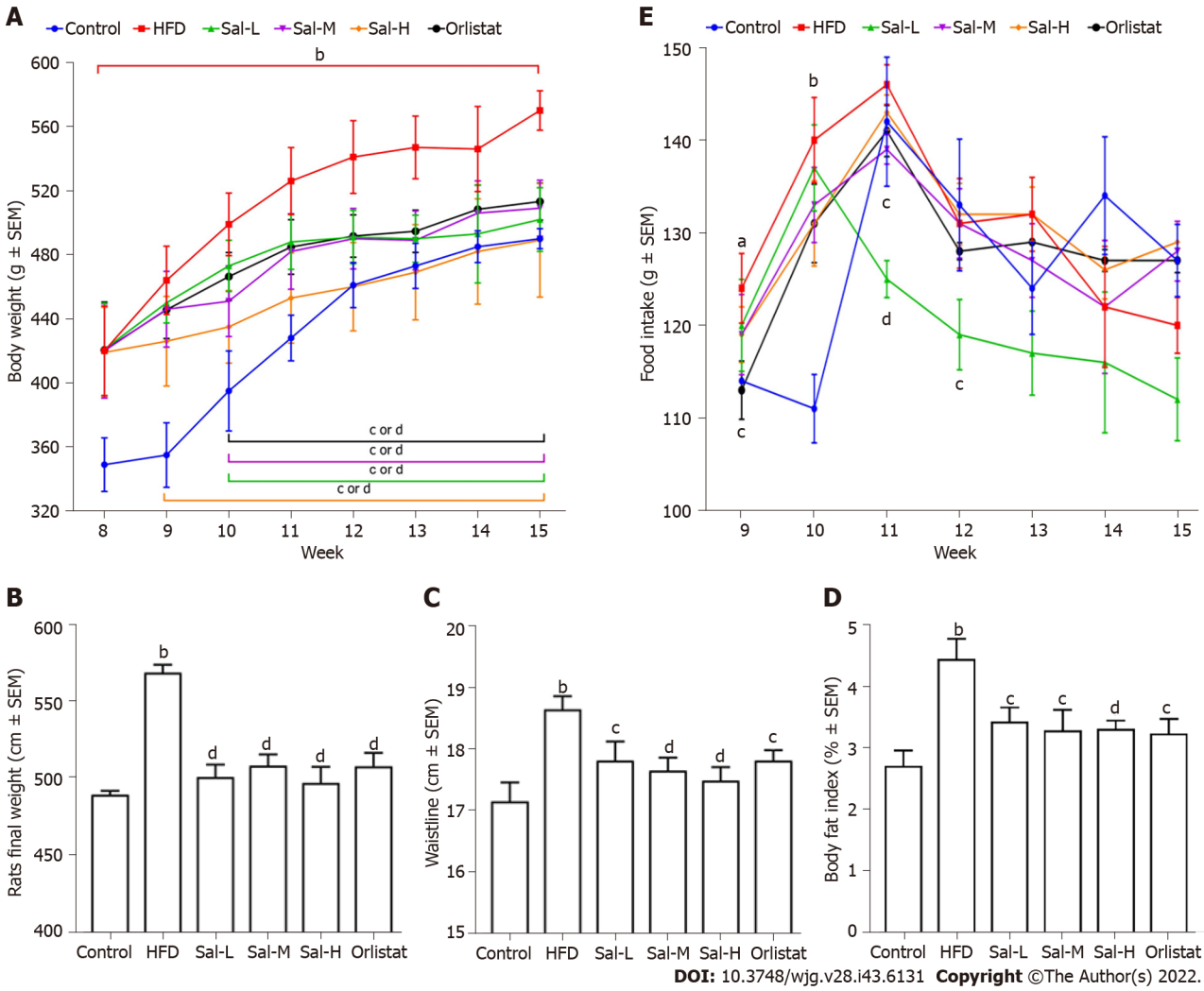

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Figure 3 Therapeutic evaluation of *Salvia miltiorrhiza* in ameliorating high-fat diet-induced obesity in rats. A: Body weight change; B: Final weight; C: Waistline; D: Body fat index; E: Food intake. Values are expressed as the mean ± SE and analyzed by one-way ANOVA followed by Duncan's multiple comparison test, $n = 8$. HFD: High-fat diet. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HFD group.

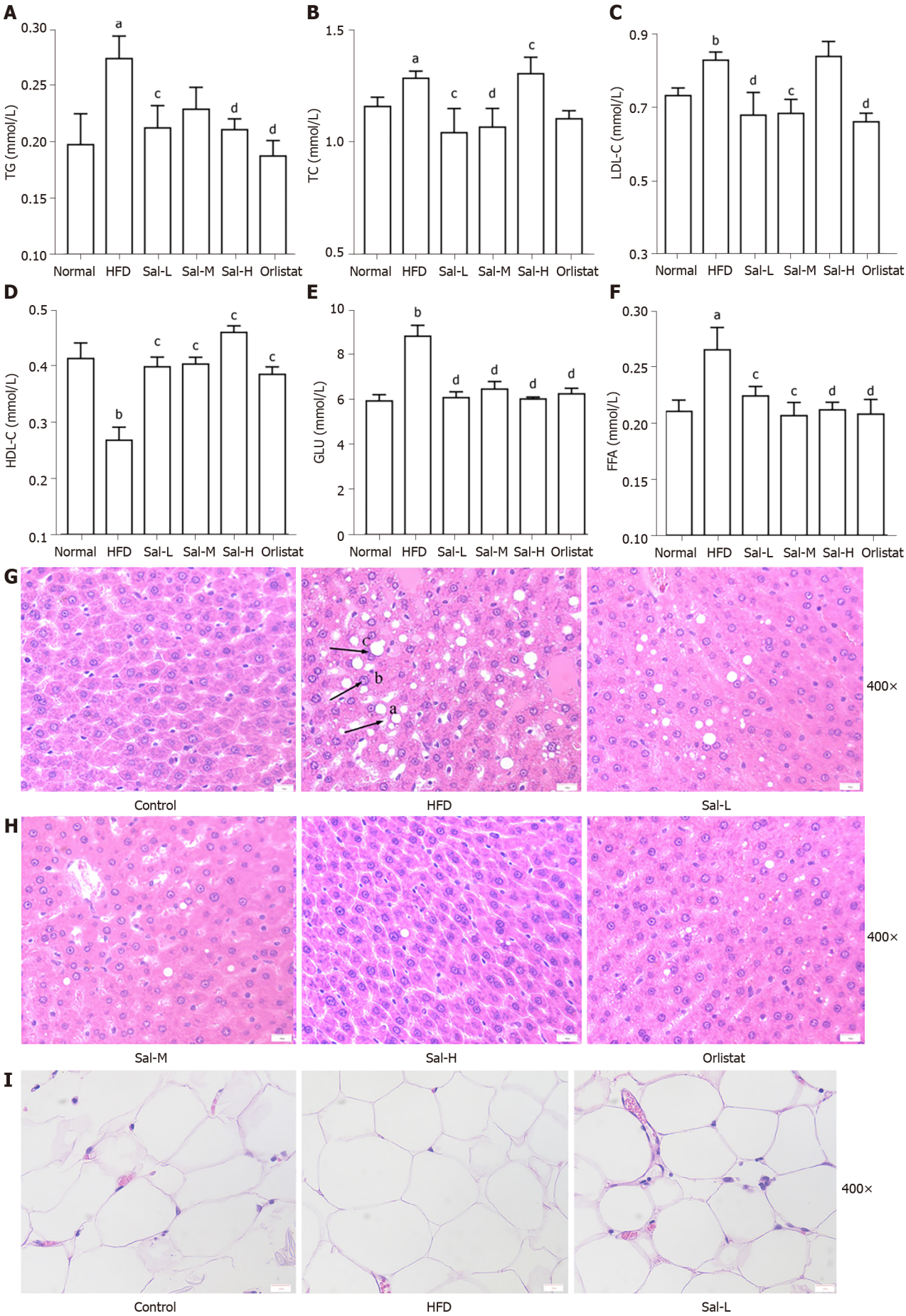
indicators to measure lipid metabolism. In our study, HFD feeding led to a significant increase in serum TG (Figure 4A), TC (Figure 4B), LDL-C (Figure 4C), GLU (Figure 4E), and FFAs (Figure 4F) levels and a significant decrease in the HDL-C (Figure 4D) level ($P < 0.05$ for all), while Sal_L, Sal_M, and orlistat significantly reduced TG and LDL-C levels without a dose-dependent relationship compared with the HFD group ($P < 0.05$ for all). However, Sal_H had no significant effect on TC or HDL-C ($P > 0.05$).

To assess the pathological injury in liver and fat tissues, pathological samples were prepared and stained using H&E. In the liver tissue in the control group, the hepatic lobule structure was ordered and tight (Figure 4G and H). By contrast, the sample in the HFD group exhibited pitting necrosis with balloon-like changes in hepatocytes, which revealed obvious accumulation of lipid droplets in the liver of HFD-induced obese rats. However, Sal and orlistat treatment alleviated these pathological changes. These data further suggest that the HFD intervention for 7 wk can lead to obesity, while Sal administration can effectively reduce dyslipidemia and hepatic lipid accumulation caused by HFD.

H&E-stained fat tissue was observed under a microscope, which showed that the outline of fat cells and volume became larger and the arrangement was loose in the HFD group (Figure 4I and J) compared with the control group. After treatment with Sal and orlistat, both the outline and volume of fat cells were reduced. The results showed that Sal alleviates hepatic steatosis and adipocyte hyperplasia in HFD-induced obese rats.

Sal increases the activities of cAMP, PKA, and HSL in HFD rats

The PKA and HSL signaling system is involved in promoting lipolysis and has a central role in regulating metabolism in all organ systems affected by obesity. In the present study, the levels of cAMP (Figure 5A) in liver tissue, and HSL (Figure 5B) and PKA (Figure 5C) in adipose tissue of the HFD group were significantly lower than those of the control group ($P < 0.05$ for all); contrarily, these levels were significantly increased in HFD-induced obese rats treated with Sal and orlistat ($P < 0.05$ *vs* HFD group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TG (mmol/L)
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0.25
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Normal HFD Sal-L Sal-M Sal-H Orlistat
B
TC (mmol/L)
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1.0
0.5
a
c
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c
Normal HFD Sal-L Sal-M Sal-H Orlistat
C
LDL-C (mmol/L)
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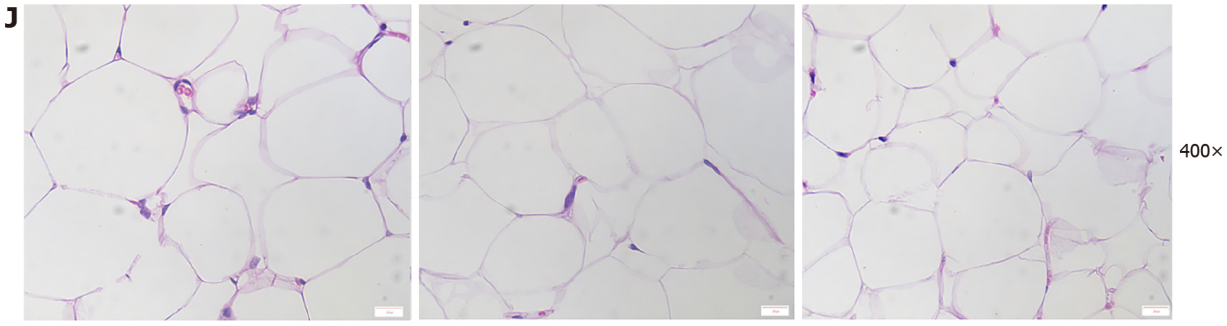
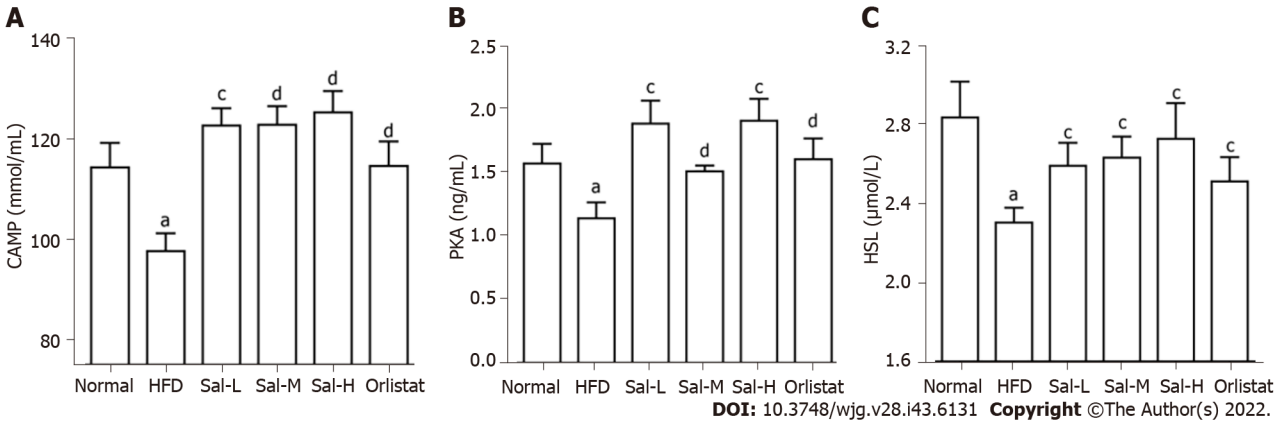


DOI: 10.3748/wjg.v28.i43.6131

Figure 4 Effects of *Salvia miltiorrhiza* on serum lipid profiles, glucose, free fatty acid levels, and histopathological changes of the liver and adipose tissue in HFD-fed rats. A-D: Serum triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein levels; E: Serum glucose level; F: Serum free fatty acid level; G and H: Representative images of hematoxylin-and eosin-stained (H&E) sections of liver tissue (400 ×) in the six groups (a: Lipid droplet accumulation; b: Punctate necrosis of hepatocytes with inflammatory cell infiltration; c: Balloon-like changes) (Scale bars: 20 μm); I and J: Representative images of H&E sections of adipose tissue (400 ×) in the six groups (Scale bars: 20 μm). Significance between groups was calculated using one-way ANOVA followed by Duncan's multiple comparison test. Data are shown as the mean ± SE. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HFD group. Error bars represent standard error.



DOI: 10.3748/wjg.v28.i43.6131

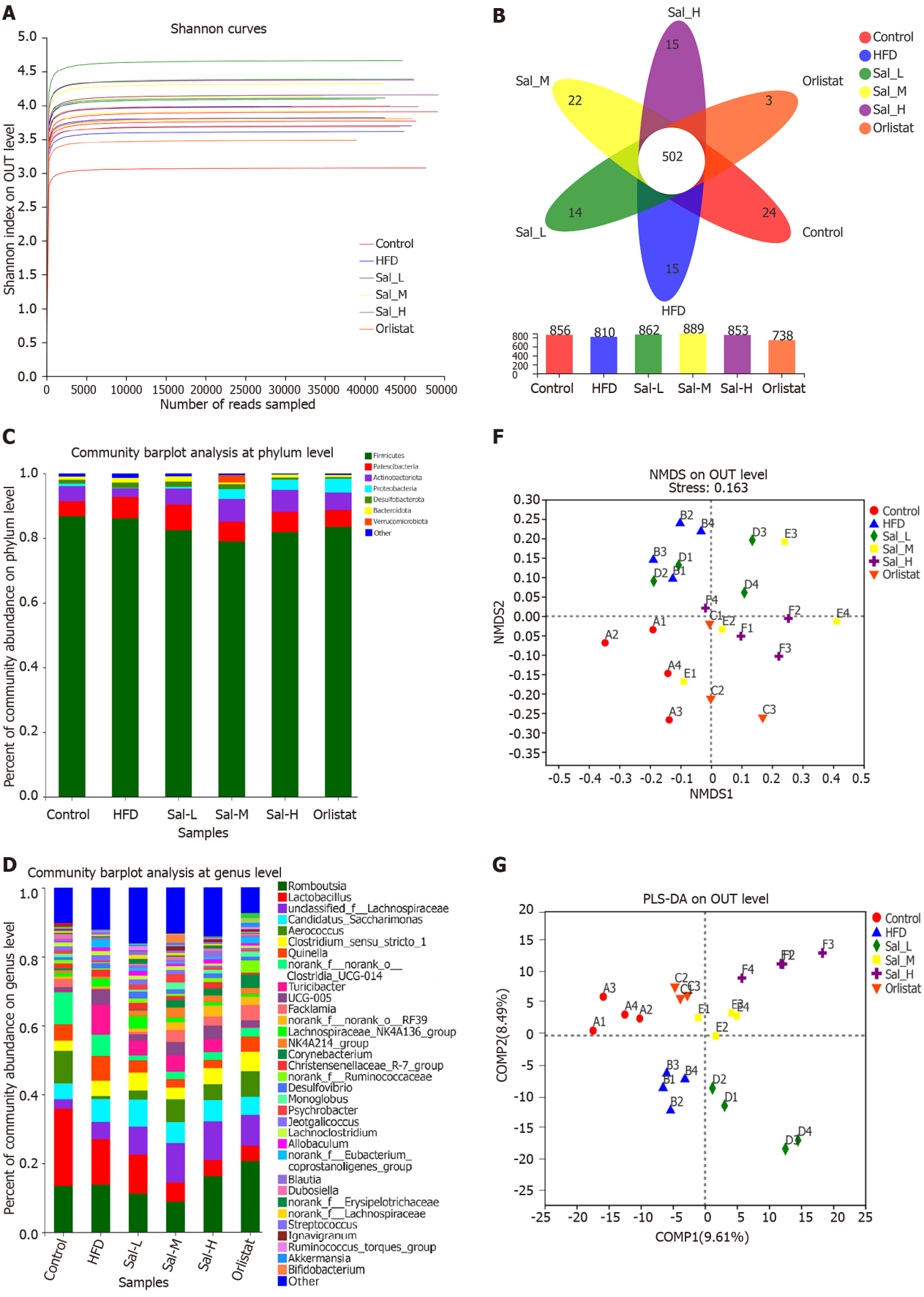
Figure 5 Levels of cAMP in liver tissue and HSL and PKA in adipose tissue of high-fat diet-fed rats. A: cAMP; B: PKA; C: HSL. Data are presented as the mean ± SE ($n = 8$). HFD: High-fat diet. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HFD group.

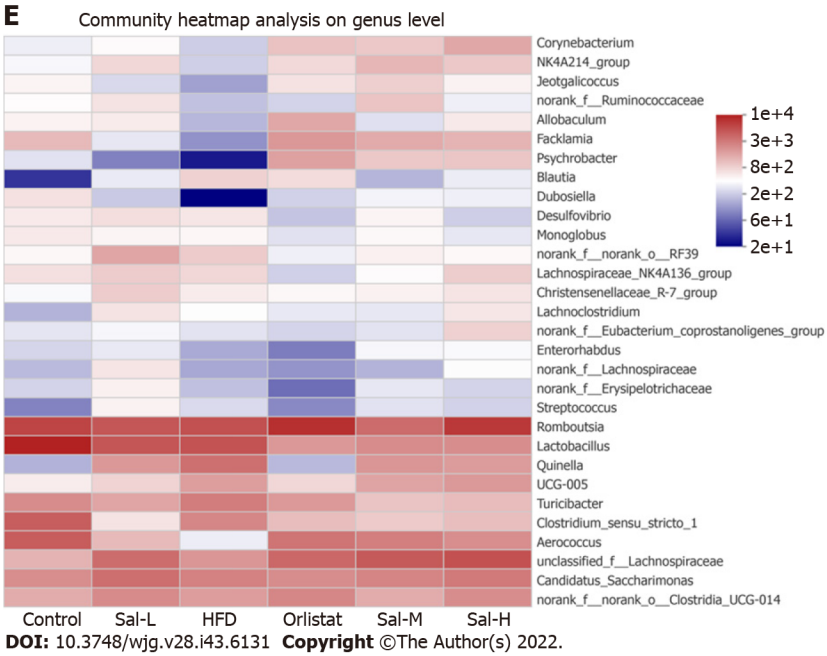
for all). The results indicate that Sal regulates lipid metabolism by enhancing lipolysis in obese rats induced with an HFD.

Sal improves the composition of gut microbiome in HFD rats

Many studies have shown that GM dysbiosis has an essential role in the pathogenetic process of human obesity and animal obesity induced by an HFD. Fecal samples from various groups were analyzed by 16S rRNA sequencing to investigate the regulatory effect of Sal on the GM composition in HFD-induced obese rats. Twenty-four samples yielded a total of 1224808 sequencing reads. The Shannon index curve (Figure 6A), reflecting the alpha diversity of the intestinal flora, shows an adequate amount of sample sequencing data; the Venn diagram (Figure 6B) shows the overlap among the six groups at the OTU level. A total of 856, 810, 862, 889, 853, and 738 OTUs were identified in the control, HFD, Sal_L, Sal_M, Sal_H and orlistat groups, respectively. Statistical analysis of bioinformatics for OTUs at 97% similar levels found that 502 OTUs overlapped among groups. Compared with the control group, the number of OTUs in the GM was reduced in the HFD group, while Sal reversed the change. We think the main reason for the invalid effect of orlistat on OTUs of GM is that there was an anomalous sample in the orlistat group.

To determine which bacteria were improved through Sal and thus intervened in the disease progression of obesity, we analyzed the composition of GM in different groups. The community barplot analysis at the phylum level (Figure 6C) showed that the relative abundances of *Proteobacteria* and *Actinobacteriota* decreased in the HFD group, and that of *Desulfobacterota* increased compared with those in the control, Sal, and orlistat groups. Compared with the control group at the genus level (Figure 6D), the relative abundances of *Aerococcus*, *Dubosiella*, *Psychrobacter*, and *norank_f_Lachnospiraceae* were significantly decreased, and those of *Quinella* and *Turicibacter* were increased in the HFD group. The


A
Shannon curves
Shannon index on OUT level
Number of reads sampled
Control
HFD
Sal_L
Sal_M
Sal_H
Orlistat
B
Sal_H
Sal_M
Orlistat
Sal_L
Control
HFD
15
22
3
502
14
24
15
856
810
862
889
853
738
Control
HFD
Sal-L
Sal-M
Sal-H
Orlistat
C
Community barplot analysis at phylum level
Percent of community abundance on phylum level
Samples
Firmicutes
Patescibacteria
Actinobacteriota
Proteobacteria
Desulfobacterota
Bacteroidota
Verrucomicrobiota
Other
F
NMDS on OUT level
Stress: 0.163
NMDS1
NMDS2
D
Community barplot analysis at genus level
Percent of community abundance on genus level
Samples
Romboutsia
Lactobacillus
unclassified_f__Lachnospiraceae
Candidatus_Saccharimonas
Aerococcus
Clostridium_sensu_stricto_1
Quinella
norank_f__norank_o__Clostridia_UCG-014
Turicibacter
UCG-005
Facklamia
norank_f__norank_o__RF39
Lachnospiraceae_NK4A136_group
NK4A214_group
Corynebacterium
Christensenellaceae_R-7_group
norank_f__Ruminococcaceae
Desulfovibrio
Monoglobus
Psychrobacter
Jeotgalicoccus
Lachnoclostridium
Allobaculum
norank_f__Eubacterium_coprostanoligenes_group
Blautia
Dubosiella
norank_f__Erysipelotrichaceae
norank_f__Lachnospiraceae
Streptococcus
Ignavigranum
Ruminococcus_torques_group
Akkermansia
Bifidobacterium
Other
G
PLS-DA on OUT level
COMP2(8.49%)
COMP1(9.61%)



DOI: 10.3748/wjg.v28.i43.6131

Figure 6 Beneficial effects of *Salvia miltiorrhiza* are associated with improved gut microbiome composition in high-fat diet-fed rats. A: α-diversity analysis: Shannon curves at the operational taxonomic unit (OTU) level; B: Venn diagram; C: Community bar plot analysis at the phylum level; D: Community bar plot analysis at the genus level; E: Community heatmap analysis of 30 species at the genus level; F: Non-metric multidimensional scaling (NMDS) at the OTU level; G: Partial least squares discriminant analysis (PLS-DA) score at the OTU level.

community heatmap analysis at the genus level (Figure 6E) showed that the relative abundance of *Blautia* was increased in the HFD group than in the control, Sal, and orlistat groups, while those of *Facklamia*, *Jeotgalicoccus*, *NK4A214_group*, and *Corynebacterium* were decreased.

Furthermore, β-diversity analysis, including non-metric multidimensional scaling (NMDS) and partial least squares discrimination analysis (PLS-DA), was used to assess the diversity variance among these six groups. NMDS (stress = 0.15) (Figure 6F) revealed that the GM composition of the HFD group was completely separated from that of the control group. Although the aggregation of the Sal_L group was similar to that of the HFD group, the two groups were significantly separated, and the aggregation of the control group was significantly enhanced as the dose of Sal increased.

PLS-DA (Figure 6G) was further used to analyze the similarity and differences among the grouped samples. It demonstrated that the species distribution of the HFD group was separated from that of each dose group of Sal and the control and orlistat groups, while the distance between Sal_M and the control group was shorter than that between the HFD and control groups. These findings suggest that Sal effectively improves GM composition in obese rats.

To further discover the regulatory effect of Sal on specific GM in obese rats, we analyzed species differences among the control, HFD, Sal_L, Sal_M, Sal_H, and orlistat groups. The genus-level Kruskal-Wallis H test bar plot (Figure 7A) showed that Sal and orlistat treatment markedly decreased the relative abundances of *Quinella* and *Blautia* in obese rats and significantly increased the abundances of *Facklamia*, *Corynebacterium*, *Psychrobacte*, and *norank_f_Ruminococcaceae*. In addition, the Wilcoxon rank-sum test at the genus level showed that compared with the control, Sal_L, Sal_M, Sal_H, and orlistat groups, the relative abundances of these species in the HFD group were significantly decreased, including *Facklamia*, *Jeotgalicoccus*, *Aerococcus*, and *Dubosiella*, while those of *Lactobacillus*, *Turicibacter*, and *Quinella* were increased significantly (Figure 7B-F). These findings suggest that Sal is important in treating obesity by regulating the GM composition in obese rats.

Correlation analysis of gut microbiota

To further clarify the distribution between groups and species, we analyzed the correlation of species abundance information among different samples through network analysis to obtain the coexistence relationship of species in environmental factors. The association and model prediction by network analysis at the genus level (Figure 7G) showed that the top species were most closely related to these six groups according to the degree of weighting (Table 2): *Romboutsia, Aerococcus, Turicibacter, Quinella, Facklamia*, *Corynebacterium* and *Desulfobacterota*. The evolution analysis by the phylogenetic tree at the genus level (Figure 7H) indicated that the top species of the closest consanguinity relation were *Romboutsia*, *Lactobacillus*, *unclassified_Lachnospiraceae*, *Aerococcus*, *Turicibacter*, and *Quinella*, which is consistent with the conclusion of community bar plot analysis, indicating that Sal exerts an anti-obesity role by regulating the above bacterial flora structure.

Table 2 Species nodes

Node name	Degree	Weighted degree
g_Romboutsia	6	36385.41667
g_Aerococcus	6	13881.41667
g_Turicibacter	6	10477.08333
g_Quinella	6	269.16667
g_Facklamia	6	6077.25000
g_Corynebacterium	6	4418.00000
g_Desulfovibrio	6	2716.58333

Node name represent species nodes. Degree is the degree of the node. Weighted degree, which means the degree weight of the node, is the number of species sequences corresponding to the node.

Finally, the functional prediction analysis (Figure 7I) revealed that these bacteria were primarily concentrated in energy production and conversion, amino acid transport and metabolism, carbohydrate transport and metabolism, biosynthesis, transport, and catabolism of secondary metabolites, lipid transport, and lipid oxidation, according to COG and KEGG orthology information and abundance. These findings suggest that the mechanism of Sal in obesity treatment involves energy and lipid metabolism.

Sal regulates lipid metabolism in obese rats

Lipid metabolism disorders are closely related to obesity and metabolic syndrome[28]. Here, non-targeted lipidomic studies of intestinal contents in rats were conducted to explore the effects of Sal on lipid metabolism. The number of differential metabolites between the control and HFD groups and between the HFD and the Sal groups is visualized in Figure 8A. In a statistically significant analysis, different metabolites were identified where $P < 0.05$ and VIP > 1 were used to identify the different metabolites. In order to understand the metabolic differences between the control, Sal, and HFD groups, the significant lipid data were analyzed using a heatmap of metabolite cluster analysis, which showed the variation of each lipid in each group directly and illustrated the relative increase (red) or decrease (blue) tendency in the HFD group compared with the control and Sal groups (Figure 8B). Interesting, the expression of the control group and the HFD group showed opposite trends; the Sal_M group was the closest to the control group trend in each Sal group, while the low and high dose groups of Sal showed the opposite trend when compared to the control group, which could be explained by the dose-response curve. Therefore, the Sal_M group was selected to make further comparisons of differential metabolites. Relative contents of identified lipids and fold changes in metabolites in the control and Sal_M groups compared with those in the HFD group were calculated to further investigate the magnitude of change in the significant lipids (Table 3). Increased TGs (TG18:2/18:2/20:4, TG16:0/18:2/22:6, TG16:0/14:0/22:6), DGs (DG14:0/22:6, DG22:6/22:6), and CL (18:2/18:1/18:1/20:0) were observed in the HFD rats. In contrast, the lipids including ceramides (Cers; Cer d18:0/20:4, Cer d16:0/21:0, Cer d16:1/24:1), (O-acyl)-ω-hydroxy fatty acids (OAHFAs; OAHFA18:0/14:0), and Hex1Cers (Hex1Cer d18:0/16:0 + O, Hex1Cer d18:1/18:2 + 2O, Hex1Cer t18:0/16:0 + O, Hex1Cer t18:1/18:1 + 2O) showed a decreasing tendency in HFD-induced obese rats. These results identified the up-regulated and down-regulated lipids in the obesity rat induced by an HFD.

In order to further obtain the variation of the expression trend of the different metabolites among the control, HFD, and Sal_M groups, we performed VIP value analysis (Figure 8C and D). TG (16:0/14:0/22:6), TG (16:0/18:2/22:6), TG (18:2/18:2/20:4), Cer (d16:0/21:0), and DG (22:6/22:6) were the most significant metabolites among these groups. These metabolites are closely related to the development of obesity and might have an important role in the metabolism dysfunction in HFD-induced obesity.

In pathway analysis, matched metabolic pathways were displayed based on the *P* value and KEGG pathway enrichment analysis (Figure 8E). A total of 7 pathways were found with $P < 0.05$, five of which were closely associated with obesity, including regulation of lipolysis in adipocytes, insulin resistance, glycerolipid metabolism, fat digestion and absorption, and cholesterol metabolism. In general, these results demonstrate that Sal may exert an anti-obese effect by regulating lipid metabolism in obese rats.

The above results show that Sal can effectively regulate the blood lipid level and GM composition of obese rats. The distance-based redundancy analysis (db-RDA analysis) and Spearman correlation heatmap were used further to investigate the correlation between lipid metabolism and the GM. Three sets of environmental factors were selected to establish the correlation between lipid metabolism and GM, including the physiological and biochemical indexes (FFAs, weight, BFI, and GLU), the key factors of lipid metabolism (cAMP, PKA, and HSL), and the lipid molecules screened by lipidomics. According

Table 3 Differential metabolites in feces after Sal treatment

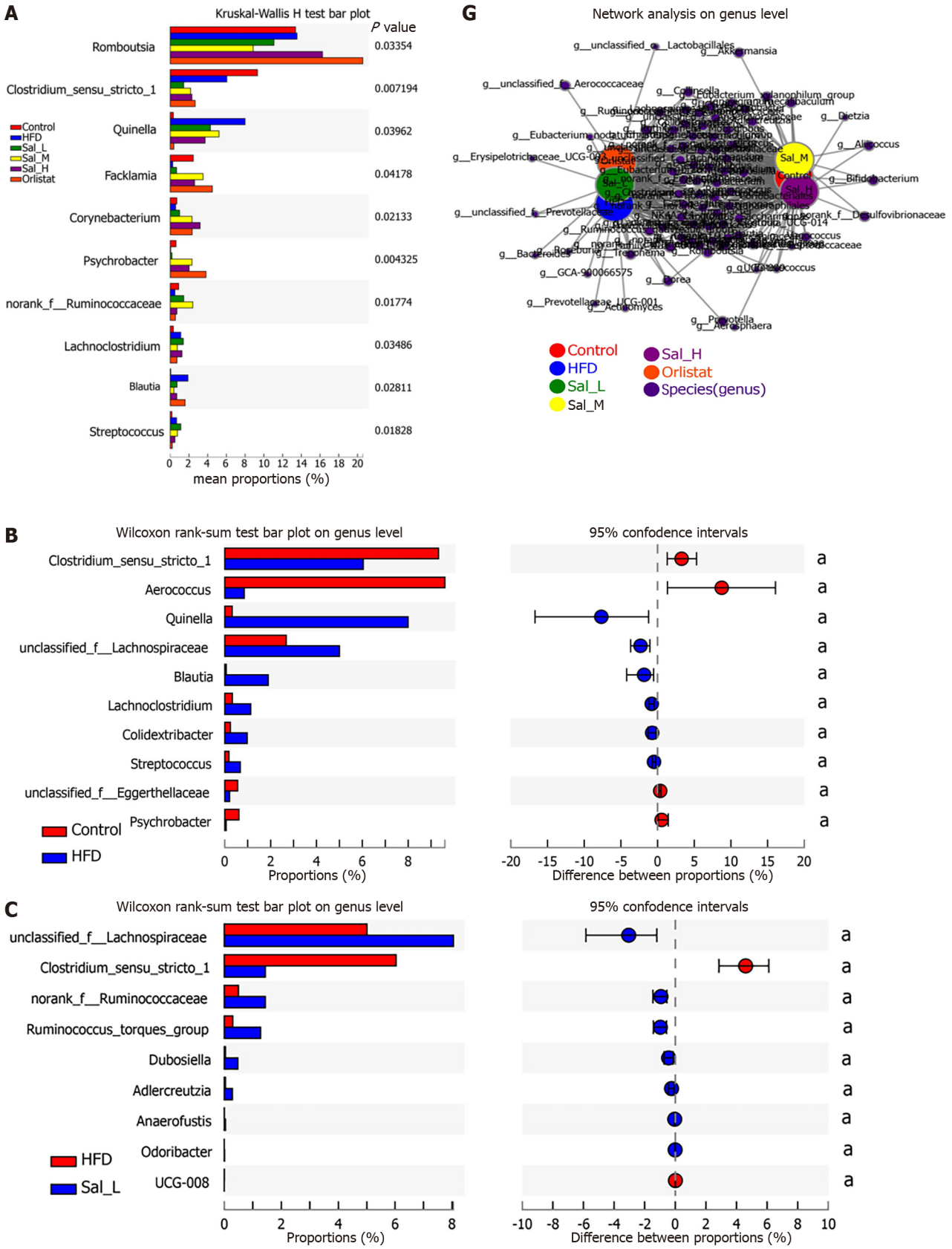
NO	Metabolite	Rt (min)	m/z	Formula	VIP		FC		Trend	
					H *vs* C	S *vs* H	H *vs* C	S *vs* H	H *vs* C	S *vs* H
1	Cer (d18:0/20:4)	6.72	588.54	$C_{38}H_{70}O_3N_1$	1.34	1.67	1.07	0.90	↓[a]	↑[c]
2	DG (14:0/22:6)	6.62	630.51	$C_{39}H_{68}O_5N_1$	1.51	1.91	0.91	1.14	↑[a]	↓[d]
3	DG (22:6/22:6)	6.56	730.54	$C_{47}H_{72}O_5N_1$	1.54	2.01	0.91	1.15	↑[a]	↓[c]
4	Hex1Cer (d18:0/16:0 + O)	6.01	718.58	$C_{40}H_{80}O_9N_1$	1.07	1.01	1.03	1.01	↓[a]	↑[c]
5	Hex1Cer (d18:1/18:2 + 2O)	5.76	756.56	$C_{42}H_{78}O_{10}N_1$	1.13	1.12	1.04	1.12	↓[a]	↑[c]
6	Hex1Cer (t18:0/16:0 + O)	5.79	734.58	$C_{40}H_{80}O_{10}N_1$	1.26	1.20	1.05	1.20	↓[a]	↑[c]
7	Hex1Cer (t18:1/18:1 + 2O)	5.80	756.56	$C_{47}H_{72}O_5N_1$	1.13	1.14	1.04	1.14	↓[a]	↑[c]
8	MGDG (16:1/17:2)	5.70	756.56	$C_{47}H_{72}O_5N_1$	1.13	1.14	1.04	0.96	↓[a]	↑[c]
9	TG (6:0/9:0/18:2)	4.39	615.46	$C_{36}H_{64}O_6Na_1$	1.23	1.36	0.95	1.06	↑[a]	↓[c]
10	TG (4:0/14:1/18:3)	6.29	631.49	$C_{39}H_{67}O_6$	1.34	1.52	0.93	1.08	↑[a]	↓[c]
11	TG (12:1e/6:0/18:4)	5.82	615.50	$C_{39}H_{67}O_5$	1.82	1.86	0.90	1.12	↑[b]	↓[c]
12	TG (16:0/14:0/22:6)	12.21	868.74	$C_{55}H_{98}O_6N_1$	1.87	2.27	0.87	1.18	↑[a]	↓[d]
13	TG (16:0/18:2/20:5)	12.82	877.73	$C_{57}H_{97}O_6$	1.46	1.68	0.94	1.08	↑[a]	↓[c]
14	TG (15:0/18:2/22:6)	11.72	895.74	$C_{58}H_{96}O_6Li_1$	1.45	1.92	0.92	1.13	↑[a]	↓[d]
15	TG (18:2/18:2/20:4)	11.79	920.77	$C_{59}H_{102}O_6N_1$	1.83	2.22	0.87	1.19	↑[a]	↓[c]
16	TG (16:0/18:2/22:6)	12.36	925.73	$C_{59}H_{98}O_6Na_1$	2.24	3.01	0.80	1.36	↑[a]	↓[e]
17	TG (18:2/17:1/22:6)	11.60	921.75	$C_{60}H_{98}O_6Li_1$	1.49	1.78	0.91	1.12	↑[a]	↓[c]
18	CL (18:2/18:1/18:1/20:0)	5.57	741.53	$C_{83}H_{152}O_{17}P_2$	1.50	1.31	0.95	1.05	↑[a]	↓[c]
19	Cer (d16:0/21:0)	8.47	626.57	$C_{38}H_{76}O_5N_1$	2.14	1.89	1.12	0.88	↓[a]	↑[c]
20	Cer (d16:1/24:1)	9.02	664.59	$C_{41}H_{78}O_5N_1$	1.87	1.66	1.11	0.89	↓[a]	↑[c]
21	OAHFA (18:0/14:0)	5.70	756.56	$C_{47}H_{72}O_5N_1$	1.32	1.18	0.95	0.94	↓[a]	↑[c]

[a]$P < 0.05$.
[b]$P < 0.01$ *vs* control group.
[c]$P < 0.05$.
[d]$P < 0.01$.
[e]$P < 0.01$ *vs* high-fat diet (HFD) group.
Control, HFD, and Sal_M ($n = 8$ per group) groups. ↑: Metabolite content increased; ↓: Metabolite content decreased; C: Control group; H: High-fat diet group; S: Sal_M group.

to the db-RDA analysis, the levels of FFAs, weight, BFI, and GLU of obese rats in the HFD group were opposite to the GM abundance in the control, Sal_L, Sal_M, and Sal_H groups at the genus level (Figure 8G). Analysis of the Spearman-related heatmap (Figure 8F) at the genus level found that TGs, DGs, and Cers were positively correlated with *Quinella*, *Desulfovibrio*, *Blautia*, and *Turicibacter*, and negatively correlated with *Aerococcus*, *Dubosiella*, *Facklamia*, and *Jeotgalicoccus*; cAMP, PKA, and HSL were positively correlated with *Corynebacterium*, *Aerococcus*, *Dubosiella*, and *Facklamia* and negatively correlated with *Desulfovibrio* and *Blautia* (Figure 8H), which agreed with our previous findings about the structure of the microbiome. The above analysis hints that Sal participates in the regulation of the microbial community structure and environmental characteristics, as well as the regulation of lipid metabolism, which helps to promote lipolysis and prevent adipogenesis.

DISCUSSION

In the present study, the weight losing effect of Sal on HFD-induced obese rats is evident, which was proven by the decrease in weight and BFI without changing food intake compared to the HFD groups with increased weight, obesity indexes, and lipid profiles. Moreover, we observed that Sal significantly down-regulated the levels of TG, TC, LDL-C, GLU, and FFAs in the serum of obese rats. Additionally, H&E analysis revealed that the liver tissue from HFD-induced obese rats treated with Sal displayed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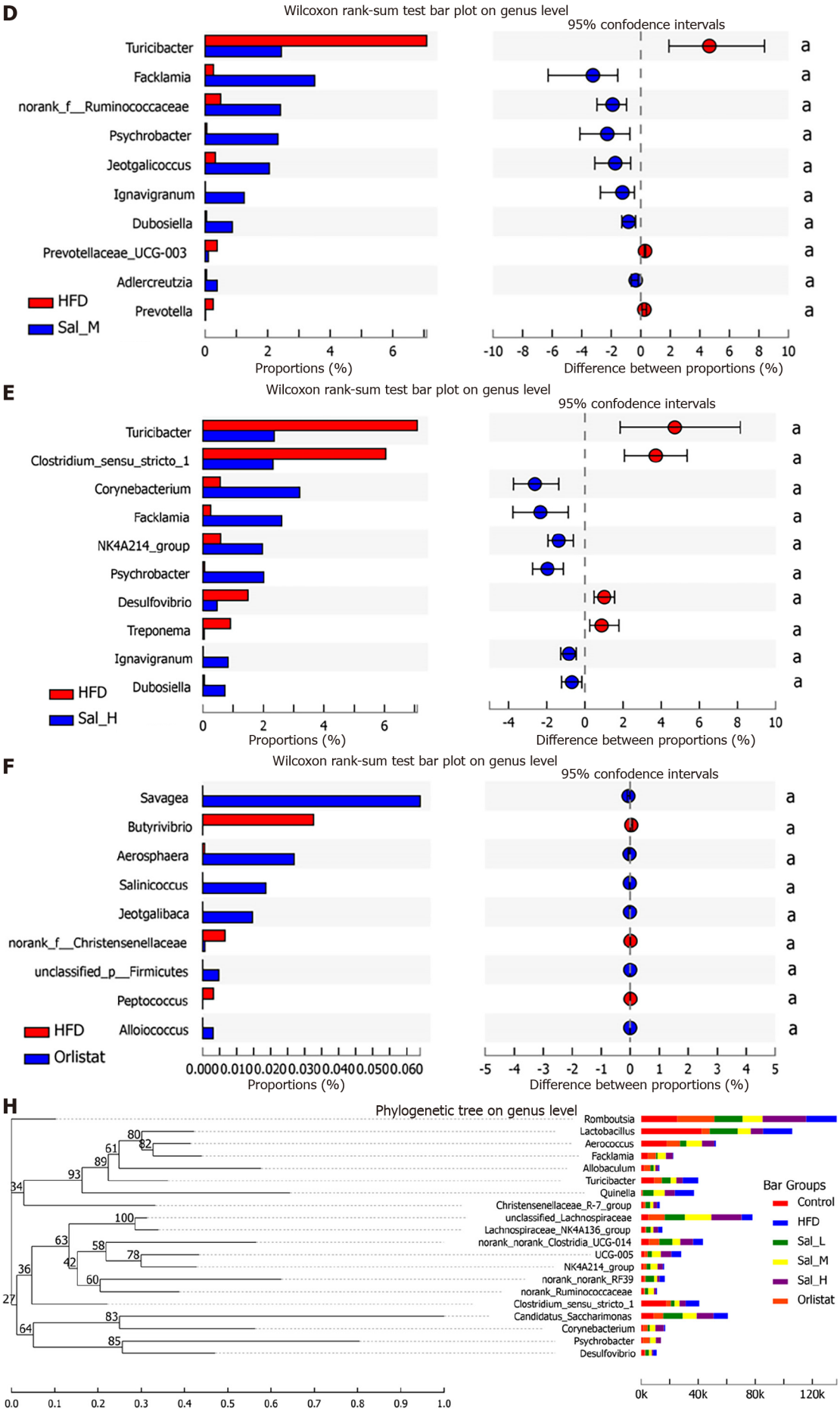
Kruskal-Wallis H test bar plot
P value
Romboutsia
Clostridium_sensu_stricto_1
Quinella
Facklamia
Corynebacterium
Psychrobacter
norank_f__Ruminococcaceae
Lachnoclostridium
Blautia
Streptococcus
0.03354
0.007194
0.03962
0.04178
0.02133
0.004325
0.01774
0.03486
0.02811
0.01828
Control
HFD
Sal_L
Sal_M
Sal_H
Orlistat
0 2 4 6 8 10 12 14 16 18 20
mean proportions (%)
G
Network analysis on genus level
Control
HFD
Sal_L
Sal_M
Sal_H
Orlistat
Species(genus)
B
Wilcoxon rank-sum test bar plot on genus level
Clostridium_sensu_stricto_1
Aerococcus
Quinella
unclassified_f__Lachnospiraceae
Blautia
Lachnoclostridium
Colidextribacter
Streptococcus
unclassified_f__Eggerthellaceae
Psychrobacter
Control
HFD
0 2 4 6 8
Proportions (%)
95% confodence intervals
a
-20 -15 -10 -5 0 5 10 15 20
Difference between proportions (%)
C
Wilcoxon rank-sum test bar plot on genus level
unclassified_f__Lachnospiraceae
Clostridium_sensu_stricto_1
norank_f__Ruminococcaceae
Ruminococcus_torques_group
Dubosiella
Adlercreutzia
Anaerofustis
Odoribacter
UCG-008
HFD
Sal_L
0 2 4 6 8
Proportions (%)
95% confodence intervals
a
-10 -8 -6 -4 -2 0 2 4 6 8 10
Difference between proportions (%)


D
Wilcoxon rank-sum test bar plot on genus level
95% confodence intervals
Turicibacter
Facklamia
norank_f__Ruminococcaceae
Psychrobacter
Jeotgalicoccus
Ignavigranum
Dubosiella
Prevotellaceae_UCG-003
Adlercreutzia
Prevotella
HFD
Sal_M
Proportions (%)
Difference between proportions (%)
E
Wilcoxon rank-sum test bar plot on genus level
95% confodence intervals
Turicibacter
Clostridium_sensu_stricto_1
Corynebacterium
Facklamia
NK4A214_group
Psychrobacter
Desulfovibrio
Treponema
Ignavigranum
Dubosiella
HFD
Sal_H
Proportions (%)
Difference between proportions (%)
F
Wilcoxon rank-sum test bar plot on genus level
95% confodence intervals
Savagea
Butyrivibrio
Aerosphaera
Salinicoccus
Jeotgalibaca
norank_f__Christensenellaceae
unclassified_p__Firmicutes
Peptococcus
Alloiococcus
HFD
Orlistat
Proportions (%)
Difference between proportions (%)
H
Phylogenetic tree on genus level
Romboutsia
Lactobacillus
Aerococcus
Facklamia
Allobaculum
Turicibacter
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UCG-005
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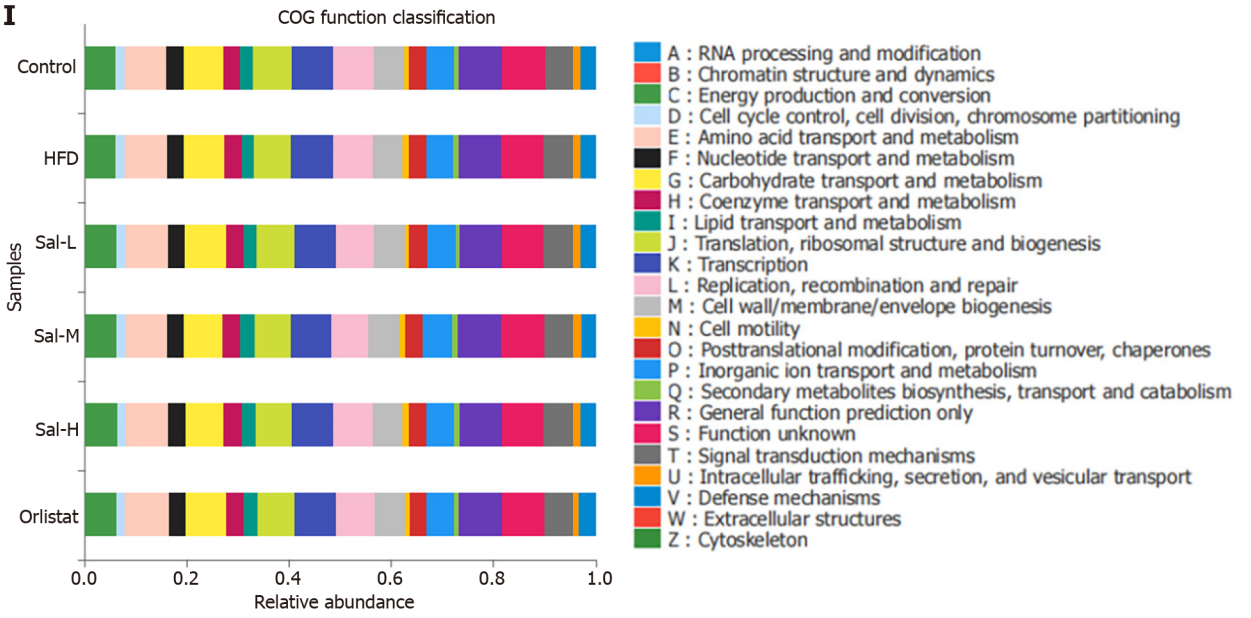
Psychrobacter
Desulfovibrio
Bar Groups
Control
HFD
Sal_L
Sal_M
Sal_H
Orlistat



DOI: 10.3748/wjg.v28.i43.6131

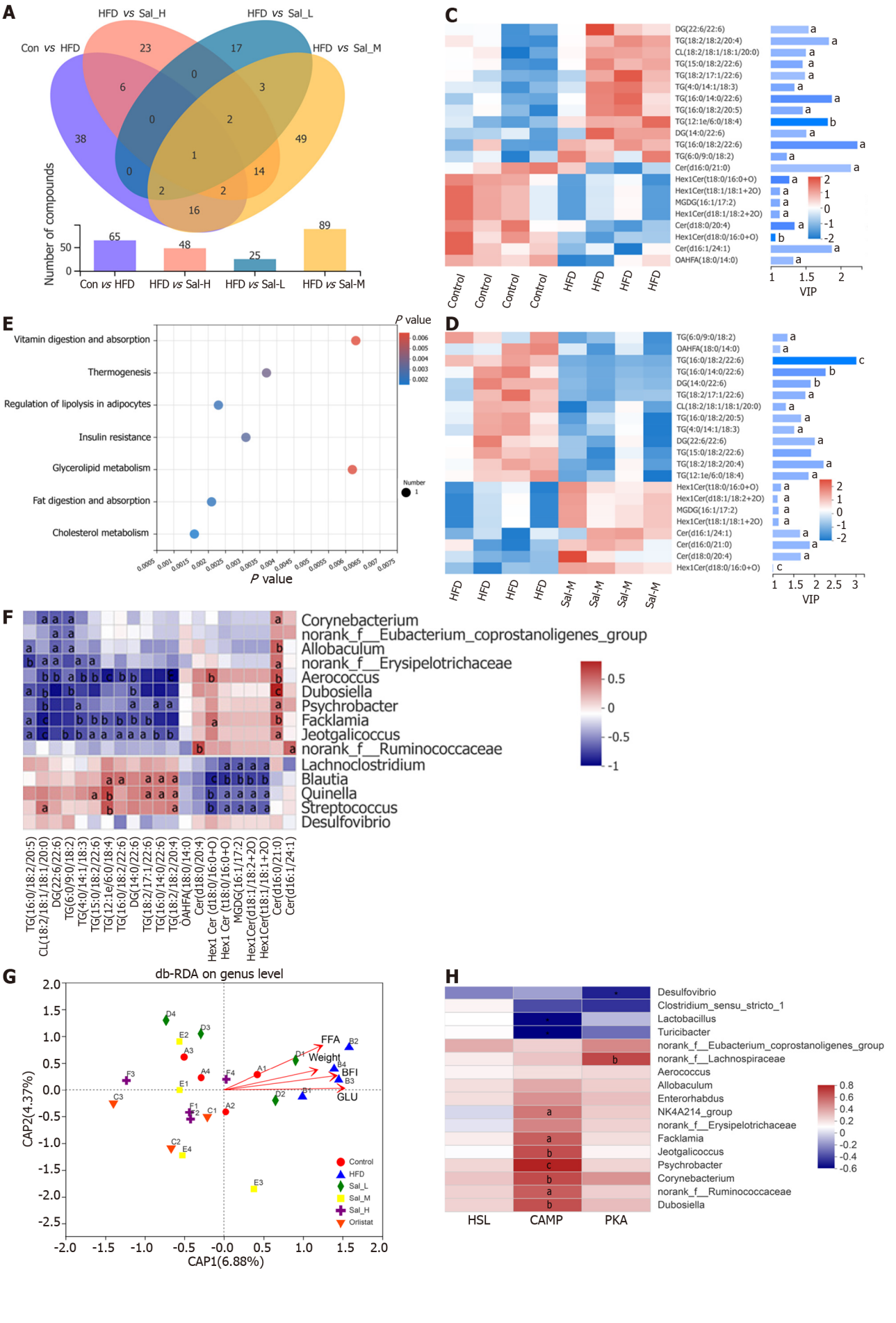
Figure 7 Effect of *Salvia miltiorrhiza* on intestinal microbiota composition in six groups of rats. A: Differential analysis among these six groups at the genus level; B-F: Differential analysis between control and high-fat diet (HFD) groups, HFD and Sal_L groups, HFD and Sal_M groups, HFD and Sal_H groups, and HFD and orlistat groups at the genus level ($^aP < 0.05$, $^bP < 0.01$); G: Network analysis at the genus level; H: Phylogenetic tree at the genus level; I: COG functional classification.

reduced mean adipocyte size, increased smaller adipocytes, and alleviated hepatic steatosis. Sal can improve dyslipidemia and reduce obesity by preventing the buildup of excess lipid compositions in the liver that can cause fatty liver and dyslipidemia in obese people. Similarly, after Sal intervention, smaller adipocytes were observed in adipose tissue. These results suggest that Sal has promising anti-obesity effects associated with reducing HFD-induced body weight independent of food intake.

Sal has been proven to have anti-inflammation[29] and antioxidation effects, which is widely used to treat cardiovascular disease[30], diabetes[31], fatty liver disease[32], and other dyslipidemia diseases [33]. Jung *et al*[9] reported that tanshinone, an ingredient in the Sal, can inhibit HFD-induced obesity by preventing early adipogenesis and improving lipid metabolism. Importantly, we found that Sal exerts anti-HFD-induced obesity effects by improving blood lipid levels, and regulating GM and metabolites, thus further suggesting the potential of Sal for clinical treatment of obesity.

As an environmental factor, the GM interaction with the host[34] has an essential role in the occurrence and development of obesity[35]. Previous studies reported lower GM diversity in obese mice than in the control group, and obese mice showed a decrease in *Bacteroides* and an increase in *Firmicutes* [36,37]. Armougom *et al*[38] found more *Methanobacter*, *Bacteroidetes*, and *Lactobacillus* in anorexic patients. GM imbalance is one of the pathogeneses of obesity. In this study, we found that obese rats had a different gut flora composition than lean rats, with higher abundances of *Desulfobacterota*, *Quinella*, and *Turicibacter* and lower abundances of *Jeotgalicoccus*, *NK4A214*, *Aerococcus*, *Dubosiella*, *Psychrobactor*, *norank_f_Lachnospiraceae*, and *norank_f_Erysipelotrichaceae* at the genus level and *Proteobacteria* and *Actinomycetes* at the phylum level. Studies have suggested that *Firmicutes*, including *Lachnoaceae*, *Erysipelotrichaceae*, *Oscillospiraceae*, and *Ruminococcaceae*, can promote energy absorption in obese and diabetic rats[39]. In this study, we found that Sal decreased the abundance of *Firmicutes*, including *norank_f_Lachnospiraceae* and *Dubosiella*, in HFD-induced obese rats. Similar results were observed in HFD-induced obese rats treated with polyphenols and procyanidins[40,41]. *Bacteroidetes*, a beneficial bacterium, have been reported to be negatively associated with obesity and hyperlipidemia. Previous studies have suggested that the proportion of *Bacteroidetes* and *Proteobacteria* is slightly higher than that of the control population[27]. Interestingly, when HFD was used to successfully induce obesity in rats, we found an increased abundance of *Bacteroidetes* at the phylum level. Our results are consistent with Schwiertz's evidence of a significant increase in *Bacteroidetes* in overweight and obese subjects[42]. We believe that this is because factors, such as heredity, diet, and environment, may have similar or even opposite effects on the flora. Lipid factors involved in metabolism may also interfere with the structure of GM through various mechanisms. Studies have shown that FXR agonists regulate lipid metabolism and GM by increasing the presence of *Ackermann* and decreasing the presence of *Desulfuricans* in obese mice[43], which is consistent with our findings.

One of the consequences of dyslipidemia is inefficient lipid metabolism, which in turn accelerates the development of obesity and dyslipidemia[44,45]. Lipid metabolism disorders provide the "first hit" in the progression of metabolic diseases such as obesity[46]. Hence, this study applied lipidomics to


A
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HFD vs Sal_L
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Number of compounds
Con vs HFD
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C
VIP
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Vitamin digestion and absorption
Thermogenesis
Regulation of lipolysis in adipocytes
Insulin resistance
Glycerolipid metabolism
Fat digestion and absorption
Cholesterol metabolism
Number
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Corynebacterium
norank_f__Eubacterium_coprostanoligenes_group
Allobaculum
norank_f__Erysipelotrichaceae
Aerococcus
Dubosiella
Psychrobacter
Facklamia
Jeotgalicoccus
norank_f__Ruminococcaceae
Lachnoclostridium
Blautia
Quinella
Streptococcus
Desulfovibrio
G
db-RDA on genus level
CAP1(6.88%)
CAP2(4.37%)
FFA
Weight
BFI
GLU
Control
HFD
Sal_L
Sal_M
Sal_H
Orlistat
H
Desulfovibrio
Clostridium_sensu_stricto_1
Lactobacillus
Turicibacter
norank_f__Eubacterium_coprostanoligenes_group
norank_f__Lachnospiraceae
Aerococcus
Allobaculum
Enterorhabdus
NK4A214_group
norank_f__Erysipelotrichaceae
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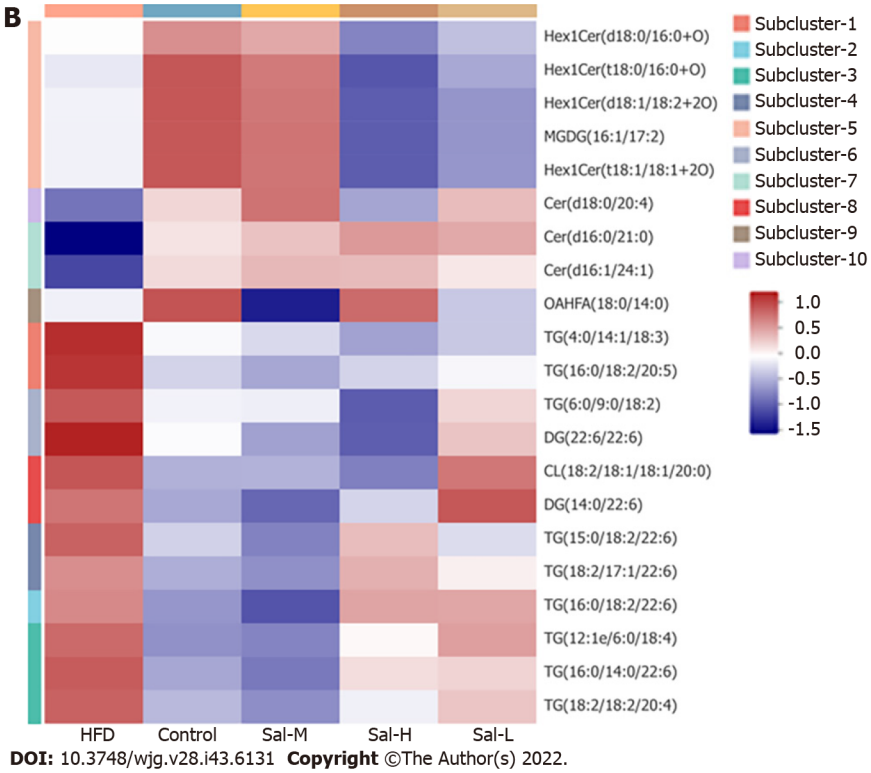

DOI: 10.3748/wjg.v28.i43.6131

Figure 8 Lipidomics analysis and associations of gut microbial species with environmental factors. A: Numbers of differential metabolites between the control and high-fat diet (HFD) groups, HFD and Sal_L groups, HFD and Sal_M groups, and HFD and Sal_H groups (Venn diagram); B: Hierarchical clustering of metabolites in Control, HFD, Sal_L, Sal_M, and Sal_H groups; C: Heatmap of the VIP expression profile of the metabolites between control and HFD groups; D: Heatmap of the VIP expression profile of the metabolites between HFD and Sal_M groups; E: KEGG pathway enrichment; F: Spearman's correlation between metabolites and gut microbiota; G: Correlation between free fatty acids, weight, body fat index, glucose, and microbial flora structure displayed by distance-based redundancy analysis (db-RDA analysis); H: Spearman's correlation between cAMP, PKA, HSL, and gut microbiota. $^{a}P < 0.05$, $^{b}P < 0.01$, or $^{c}P < 0.001$.

analyze the composition of lipid metabolites in obesity and observed the alteration of lipid metabolites by increasing the treatment of Sal to analyze the molecular mechanism of the anti-obesity and cholesterol-lowering effects of Sal, thus providing new insights into the treatment of obesity. A range of significant changes occurred in the lipidome with progressive obesity. Most notably, the contents of DGs, TGs, and CLs were increased in the HFD group compared to the control group, which was similar to previous studies[47]. Contrary, Sal decreased the levels of these lipid species in the lipidome profile, suggesting that Sal could improve the imbalance between HFD-induced lipid synthesis and catabolism. The concentration of FFAs in serum is related to lipid metabolism and glucose metabolism, and diseases such as diabetes and obesity can increase the concentration of FFAs. When energy metabolism is abnormal, FFAs will accelerate the accumulation of TG in hepatocytes or convert to lipid intermediates such as DGs, CLs, and Cer, depleting cellular functions and, in turn, leading to obesity and other metabolic disorder diseases[48]. Interestingly, we found that the supplementation of Sal effectively decreases the hepatic accumulation of TGs, DGs, and CLs. Evidence suggests that Cer is involved in obesity-induced metabolic disorders by various mechanisms, including inflammation, apoptosis, and autophagy. However, in our study, Cer was not entirely increased in the HFD-induced obese rats. We wondered whether Cer might interfere with obesity by participating in other pathways or whether the balance of sphingolipid (SP) metabolism, rather than Cer accumulation, is correlated with the development of obesity[49]. SP species are not only increased in obese patients but are also associated with hepatic oxidative stress, suggesting that these lipids may participate in the progression of obesity [50]. In general, TG accumulation is a sign of obesity and is positively correlated with hexosylceramide (HexCer); yet, in our experiment, Hex1Cer, which acts as a SP, was reduced in the HFD-induced obese rats and negatively correlated with TG. Part of that has to do with the small number of animals analyzed, and the role of Hex1Cer in obesity is not quite clear yet. Our results were consistent with Eisinger's study[51]. Some researchers also believe that pharmacological blockage of glucosylceramide prevents obesity and liver steatosis[3,52,53]. In addition, we found that a few fatty acids, such as OAHFA, a novel lipid in structure with functions such as stimulating insulin secretion and improving glycolipid transport *in vivo*[54,55], were increased in HFD-induced obese rats treated with Sal. These lipids are structurally novel lipids that are provided. It is worth mentioning that some beneficial lipids are important to the development of obesity, such as phosphatidylcholines[56], which can regulate lipid and cholesterol metabolism[57]. Although it did not appear in our study, it is also one of the focuses of our future research.

Pathway enrichment analysis can assess the biochemical pathways involved in significantly different metabolites and their functional classification. Significant pathways enriched by KEGG, including lipolysis in adipocytes, insulin resistance, glycerolipid metabolism, fat digestion and absorption, and cholesterol metabolism, have been shown to be closely associated with the development of obesity, among which metabolism of cholesterol had the greatest significance, with a *P*-value of 0.0016. Moreover, the dysfunction of glycerolipid metabolism may disturb the energy metabolism of hepatocytes and adipocytes. Furthermore, obese patients are often accompanied by hormonal dysregulation, which aggravates lipid metabolic disorders. For example, as one of the anti-lipolysis hormones, insulin has an important role in reducing lipolysis by reducing cAMP concentration in adipocytes. The increase of cAMP catalyzes the hydrolysis of triacylglycerol to fatty acids and glycerol and then completes the lipolysis process, and PKA with HSL is involved[2]. Similarly, increases in FFAs can also cause insulin-mediated decreases in glucose oxidation and utilization, increase insulin resistance, and cause hyperinsulinemia and obesity. In our study, the levels of cAMP, PKA, and HSL were decreased in the HFD group compared to the control group, while GLU and FFA were increased. Spearman-related heatmap analysis further showed that cAMP, PKA, and HSL had a negative correlation with the structure of the GM in the HFD group, while FFAs, weight, BFI, and GLU were positively correlated with GM.

It is widely believed that the regulatory effect of intestinal microbiota on lipid metabolism is closely related to bile acids and short-chain fatty acids (SCFAs). According to Zhang *et al*[58], increasing the SCFA-producing genera *Blautia* and *Allobaculum* could enhance intestinal integrity and reduce the body weight in obese mice[59], thus rivaling obesity. *Allobaculum* is a member of *Lachnospiraceae*, *Ruminococcaceae*, and *Erysipelotrichaceae*, a family that affects host metabolism[60] and protects body weight gain from HFD. *Allobaculum* can produce butyric acid and propionic acid[61,62]. In our research, analysis of the Spearman-related heatmap showed that lipids including TGs, DGs, and CL that increased in the HFD group were inversely associated with *Allobaculum* and positively related to *Blautia,* which suggests that Sal may improve *Allobaculum* but not *Blautia* to produce SCFAs and then regulate the GM and lipid metabolism. Acetate, propionate, and butyrate are examples of SCFAs produced by bacteria, and research showed that SCFA-induced activation of the PPAR pathway could modulate lipid metabolism by increasing energy consumption[63], reducing body weight, and decreasing liver TG accumulation [64]. It should be noted that the genus *Blautia,* as a member of the *Lachnospiraceae* family, could produce SCFAs, which can regulate inflammation and metabolism[65]. Consequently, the observation in our research that Sal promoted a decrease in *Lachnospiraceae* and *Allobaculum* indicated that Sal likely confers beneficial effects *via* modulating the GM composition and host lipid metabolism with SCFAs, which is consistent with previous reports[66,67]. However, whether *Lachnospiraceae* has a key role in the regulation of obesity and lipids still needs to be confirmed by accurate tests.

Desulfovibrio has been reported to be positively correlated with metabolism[68,69] and may increase after an HFD[70,71]. Similar to our findings, *Desulfovibrio* was reported to be decreased by the intervention of Sal. We also found that the improvement of gut microecology resulting in the increase of SCFAs may be responsible for the cholesterol-lowering effects of obesity. There is a reciprocal relationship between bile acids and cholesterol metabolism, which is closely related to intestinal flora. Bile acids are synthesized from cholesterol in the liver and have a role in maintaining cholesterol homeostasis and promoting lipid absorption[72]. Intestinal flora regulates lipid metabolism in the host by affecting bile acid composition. GM could influence bile acid metabolism by performing structural modifications, including oxidation, deconjugation, or hydroxylation[73]. It has been confirmed that bile acids and bile acid signaling pathways are involved in the control of plasma lipid and lipoprotein levels and that hepatic bile acid synthesis has an important role in the regulation of plasma TG levels in obese individuals[74], which is consistent with the present study that the cholesterol metabolism was most concentrated in KEGG pathway with the elevated cholesterol and TG in the serum and the significant elevation of TGs in lipidomics, while Sal may regulate the relationship between bile acids and cholesterol metabolism by reducing cholesterol accumulation and regulating lipid metabolism.

The above experimental results suggest that HFD-induced obesity causes dyslipidemia and dysregulation of GM and metabolites, accompanied by weight gain, hepatic steatosis, and abdominal fat accumulation. Importantly, we found that Sal effectively improved blood lipids and reshaped the balance of GM and lipid metabolism in obese rats (Figure 9), reversing weight gain and fat accumulation. Correlation analysis further demonstrated that Sal exerted anti-obesity effects through lipid metabolites of intestinal flora, which laid a good foundation for the subsequent study. In the following study, the key components of Sal's anti-obesity effects will be further investigated by pharmacokinetic and high-performance liquid chromatography techniques. The key flora and metabolites of Sal's anti-obesity effects will be further demonstrated by intestinal flora transplantation and lipid metabolite supplementation, and the key targets of Sal's anti-obesity effects will be further explored by molecular docking or protein interaction and gene enrichment and editing techniques, which will become a promising way to find new targets for obesity.

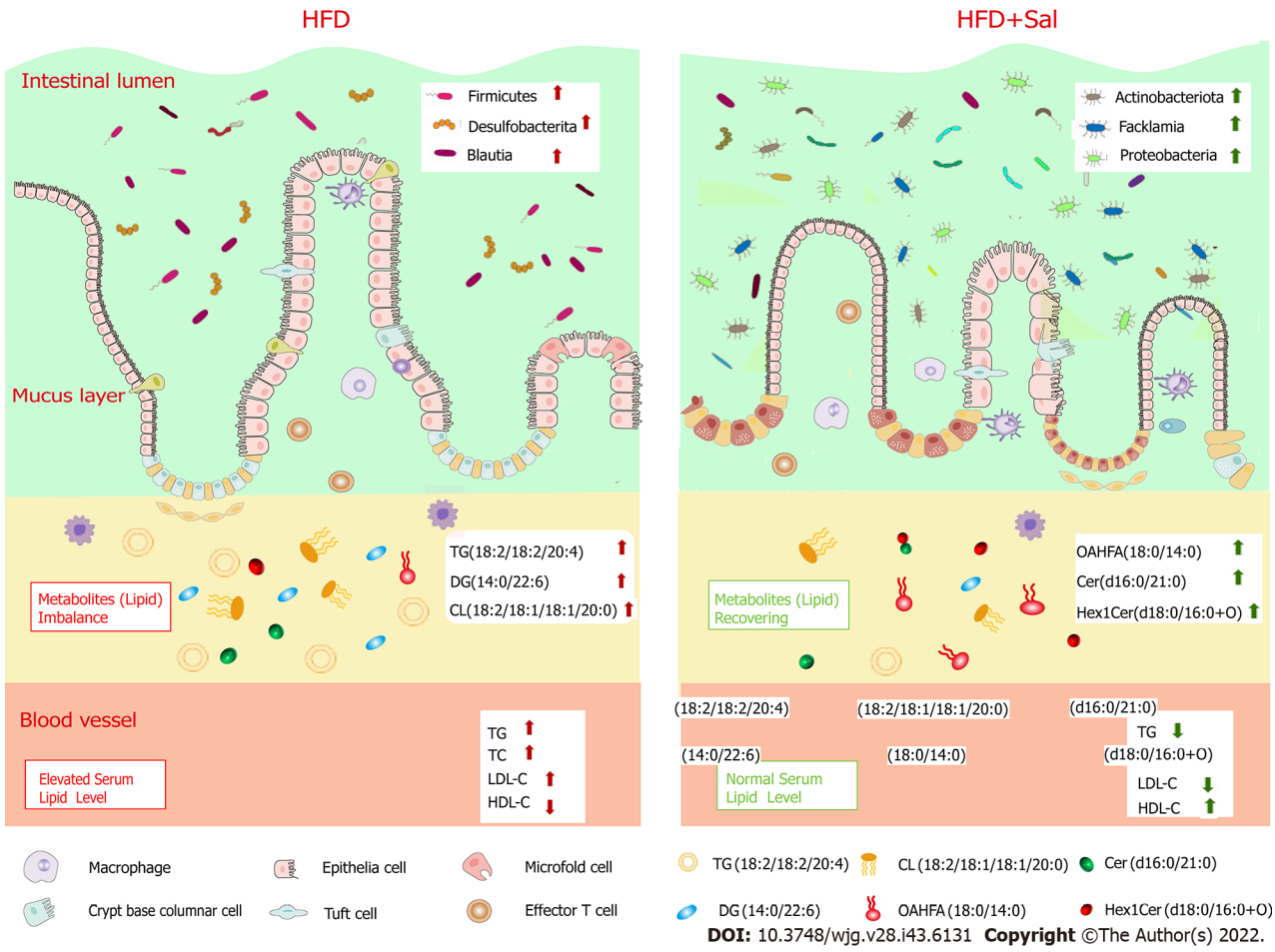

Figure 9 Graphical illustration of mechanism of action of *Salvia miltiorrhiza* to alleviate obesity. *Salvia miltiorrhiza* ameliorated obesity by reshaping the balance of gut microbiota, modulating the lipid metabolites, and improving blood lipids. Sal: *Salvia miltiorrhiza* extract; HFD: High-fat diet; TG: Triglycerides; TC: Total cholesterol; HDL-C: High-density lipoprotein; LDL-C: Low-density lipoprotein; GLU: Glucose; FFA: Free fatty acids.

CONCLUSION

Our results showed that Sal reduces body weight, body fat index, serum lipid level, hepatic lipid accumulation, and adipocyte vacuolation in HFD-induced obese rats, which may be associated with enhanced gut integrity and improved lipid metabolism. 16s RNA sequence analysis revealed that Sal could reverse HFD-induced dysbacteriosis while LC-MS/MS analysis indicated that Sal could improve the lipid composition of HFD rats, which provides research basis and evidence to study the mechanism of Sal in the treatment of obesity.

ARTICLE HIGHLIGHTS

Research background

Obesity is a world health problem. A growing number of studies have suggested that gut microbiota is an important regulator of host metabolism, and the dysregulation or imbalance of gut microbiota (GM) is closely related to obesity and its complications. Mounting evidence suggests that improving the structure of the GM balance to regulate metabolism, particularly lipid metabolism, is a viable strategy for treating obesity or obesity-related disease.

Research motivation

Salvia miltiorrhiza extract (Sal) has shown good efficacy in experimental obese rats induced by a high-fat diet (HFD). Also, disturbances in gut microbiota have been observed in various diseases, including metabolic disease. However, few studies have explored the role of Sal on gut microbiota and lipid metabolism when treating obesity.

Research objectives

To investigate whether Sal can alleviate obesity induced by an HFD by regulating gut microbiome and lipid metabolism.

Research methods

Rats were given an HFD (with purified ingredients and a total caloric value of 475 Kcal/100 g, with lard as the main source of fat) for 7 wk, while Sal (0.675 g/1.35 g/2.70 g/kg/d) was administered for 8 wk. Serum lipid test, liver and fat tissue histopathologic examination, ELISA, 16S RNA sequencing, and LC-MS/MS analysis were used to evaluate the efficacy of Sal on obesity.

Research results

Sal effectively improved blood lipids and reshaped the balance of gut microbiota and lipid metabolism in obese rats, reversing weight gain and fat accumulation caused by HFD. Correlation analysis further demonstrated that Sal exerted anti-obesity effects through lipid metabolites of intestinal flora, which laid a good foundation for the subsequent study.

Research conclusions

Sal may exert an anti-obesity effect in HFD-induced obese rats by modulating the gut microbiome and lipid metabolism.

Research perspectives

This study addressed an important topic of the development of obesity, *i.e.*, the role of gut microbiota and lipid metabolism in the development of obesity, using an extract from a Chinese herb that has been found to have anti-obesity effects in various diseases.

FOOTNOTES

Author contributions: Ai ZL and Zhang X contributed equally to this work; Zhong YB and Ge W performed the experiments; Liu DY, Wang HY, and Zuo ZY contributed reagents/materials/analytical tools; Liu DY and Ge W analyzed the data; Ai ZL and Liu DY wrote the paper; Zuo ZY and Wang HY conceived and designed the experiments.

Supported by the National Natural Science Foundation of China, No. 82060836; Jiangxi Province Graduate Student Innovation Special Fund Project, No. YC2021-B146; and Jiangxi University of Chinese Medicine Science and Technology Innovation Team Development Program, No. CXTD22008.

Institutional review board statement: The study was reviewed and approved by the Jiangxi University of Chinese Medicine (Formula-Pattern Research Center).

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Jiangxi University of Chinese Medicine Animal Care and Use Committee (Approval No. JZLLSC2021-236).

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional unpublished data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Country/Territory of origin: China

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S-Editor: Chen YL
L-Editor: Wang TQ
P-Editor: Yu HG

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2022 November 21; 28(43): 6157-6167

DOI: 10.3748/wjg.v28.i43.6157

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Upper gastrointestinal endoscopic findings in celiac disease at diagnosis: A multicenter international retrospective study

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Taavela J, Finland; Zhang X, United States; Zhang JW, China

Received: August 24, 2022
Peer-review started: August 24, 2022
First decision: September 8, 2022
Revised: September 22, 2022
Accepted: November 7, 2022
Article in press: November 7, 2022
Published online: November 21, 2022

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Abstract

BACKGROUND

Gastroduodenal endoscopy and biopsy following positive specific serology is considered the gold standard to diagnose celiac disease (CeD) in adults. Whether upper endoscopy helps detect comorbid conditions is unknown.

AIM

To investigate the prevalence of non-celiac endoscopic findings in patients in whom endoscopy was performed to confirm CeD diagnosis.

METHODS

This is an observational, descriptive, multicenter, retrospective study that reports endoscopic findings obtained in adult patients enrolled in local registries from four tertiary centers. We collected data reported on first endoscopy, indicated for investigation of CeD. Diagnosis of CeD was performed by histology (≥ Marsh 2

type mucosal damage) and specific serology. Two European and one North American center included biopsy-confirmed CeD following positive serology. A fourth center (South America) included symptomatic patients undergoing endoscopy, irrespective of CeD serology. The latter cohort included a non-CeD control group.

RESULTS

A total of 1328 patients (80% female; 35 years median age) were enrolled, of whom 95.6% had positive specific serology. In 135 patients, endoscopy revealed 163 abnormalities unrelated to CeD (prevalence: 10.1%). Erosive reflux esophagitis (6.4%), gastric erosions (2.0%), and suspicion of esophageal metaplasia (1.2%) were the most common findings. Biopsy-confirmed Barrett's esophagus was infrequent (0.2%). No endoscopic cancer was detected. Older patients (≥ 51 years of age) had a higher prevalence of endoscopic findings than those ≤ 50 ($P < 0.01$). Within the South American cohort, CeD was associated with a lower rate (8.2%) of comorbid endoscopic findings compared with controls (29.1%; $P < 0.001$). In the adjusted multivariate analysis of this cohort, having CeD was associated with a 72% reduction in the risk of any endoscopic abnormality ($P < 0.0001$), and having alarm symptoms was associated with a 37% reduction in the risk of finding at least one endoscopic lesion ($P < 0.02$).

CONCLUSION

In this large multicenter study, young adults with positive CeD serology had few comorbid endoscopic findings. Although patients over 51 years had a high prevalence of non-CeD gastroduodenal mucosal damage, no malignancy or premalignant lesions were found.

Key Words: Celiac disease; Upper gastrointestinal endoscopy; Concomitant endoscopic lesions; Malignancies; Multicenter study

Core Tip: We offer novel data on the prevalence of non-celiac endoscopic findings at the time of endoscopy performed to confirm celiac disease (CeD) diagnosis. Based on the very high performance of specific serology tests, the diagnosis of CeD without duodenal biopsy has been proposed in recent years. However, some guidelines do not recommend avoiding endoscopy because relevant comorbid diagnosis can be missed. Our results found that comorbid upper gastrointestinal endoscopic pathology is uncommon in patients with positive CeD serology at the time of diagnostic endoscopy suggesting that a non-biopsy strategy is unlikely to clinically miss significant concomitant endoscopic findings unrelated to CeD.

Citation: Stefanolo JP, Zingone F, Gizzi C, Marsilio I, Espinet ML, Smecuol EG, Khaouli M, Moreno ML, Pinto-Sánchez MI, Niveloni SI, Verdú EF, Ciacci C, Bai JC. Upper gastrointestinal endoscopic findings in celiac disease at diagnosis: A multicenter international retrospective study. *World J Gastroenterol* 2022; 28(43): 6157-6167
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6157.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6157

INTRODUCTION

Celiac disease (CeD) is one of the most common life-long chronic diseases affecting people with a genetic predisposition conferred by HLA-DQ2 or DQ8[1]. Current recommendations for diagnosing CeD in adult patients involve a combination of specific serology and a duodenal biopsy demonstrating some degree of intestinal atrophy[2,3]. When CeD is clinically suspected, upper gastroduodenal endoscopy with duodenal biopsy confirms diagnosis[4]. Based on the very high specificity and predictive values of specific serology tests[5], the diagnosis of CeD without duodenal biopsy has been proposed in recent years[6-8]. Indeed, European pediatric societies recommend a non-biopsy approach under specific and strict criteria[9,10]. However, other pediatric societies (*e.g.*, the North American Pediatric Gastroenterology Society) do not recommend this, in part because relevant comorbid diagnosis could be missed[11]. This is of particular concern in patients with alarm symptoms such as weight loss, anemia, or abdominal pain[2,12,13]. However, relatively few studies have explored this in-depth, particularly in adult patients undergoing endoscopy to confirm CeD diagnosis[14-16].

Thus, we conducted a multicenter study involving four cohorts of patients diagnosed in three countries to investigate the prevalence of coincidental upper gastrointestinal endoscopic findings in CeD patients at the time of diagnosis. We also compared upper gastrointestinal mucosal injury

diagnoses across centers and age groups. Finally, we studied the pathological findings in patients with a confirmed diagnosis of CeD *vs* those in whom the disease was ruled out.

MATERIALS AND METHODS

Design

We conducted a descriptive multicenter retrospective study on endoscopic findings from adult patients who met standard clinical, serological, and histological criteria for CeD. Patients from four different CeD-specialized centers were included. Two European cohorts (Universities of Naples/Salerno and Padua; Italy) and a North American cohort (McMaster University, Hamilton; Canada) recruited consecutive patients enrolled in local registers. CeD was diagnosed by positive serology and confirmed by biopsy. The Naples/Salerno cohort included consecutive patients seen between 1987 and 2021, the Padua cohort between 2017 and 2021, and the Hamilton cohort between 2018 and 2020. A fourth (Small Bowel Section, Dr. C. Bonorino Udaondo Gastroenterology Hospital, Buenos Aires; Argentina) included patients referred for endoscopy and duodenal biopsy due to the presence of symptoms and/or signs compatible with CeD but, irrespective of serology, all of them part of prior research and study[7,15]. Thus, the fourth cohort included CeD and non-CeD participants (controls). Figure 1 and Table 1 summarize the demographic characteristics of the cohorts. The Ethics and Research Board of the Dr. C. Bonorino Udaondo Gastroenterology Hospital approved the study because of the prospective design and intervention in the Buenos Aires cohort. Ethics approval was obtained from Hamilton Integrated Research Ethics Board (HiREB# 14460/5415). In Italy, Ethical Committee review was not required for retrospective studies while patient data remained anonymously coded.

Endoscopic procedures

In all CeD centers, experienced gastroenterologists performed upper gastrointestinal endoscopies and obtained duodenal biopsies *per* shared standard of care protocols. Endoscopic reports were generated using a standard format, and the data were entered into a common database. Duodenal biopsies were sent to each institution's experienced pathologist. A standard number of biopsies were taken when any endoscopic abnormality was detected (*e.g.*, endoscopic evidence of esophageal metaplasia). Endoscopic abnormalities were defined as follows[17]: (1) Erosive esophagitis: Esophageal mucosal damage characterized by one or more mucosal breaks that do not extend across the top of mucosal folds and confluent lesions or ulcers of any size; (2) Suspected esophageal metaplasia: Endoscopically suspected columnar mucosa without histological confirmation of specialized intestinal metaplasia; (3) Barrett's esophagus confirmed by biopsy: Metaplastic columnar epithelium replacing the stratified squamous epithelium in biopsies from suspected metaplasia or presence of intestinal metaplasia; (4) Gastric and duodenal erosions: Presence of erythema and erosions in stomach or duodenum; (5) Esophageal, gastric, or duodenal ulcers extending into the *muscularispropria*; and (6) Esophageal, gastric, or duodenal cancer: Suspected endoscopic lesions were confirmed by specialized pathology.

CeD diagnosis

CeD was diagnosed based on duodenal histology (Marsh's classification)[1,18]. Inclusion criteria were Marsh 2 enteropathy or higher and positive CeD-specific serology [presence of either anti-TTG immunoglobulin (Ig)A, Anti-EmA IgA, anti-DGP IgA/IgG]. When serology was negative, CeD was diagnosed based on histology and clinical response to the gluten free diet (GFD)[18]. If patients had known exposure to gluten before the endoscopy, intestinal biopsies were taken. As previously stated, the Buenos Aires cohort was part of a research study in which the diagnosis was made first on histological grounds and then confirmed by serology. The standard specific CeD test for all centers was IgA transglutaminase 2[5]. Patients with normal biopsy or minimal inflammation (Marsh 0 or 1) were excluded from the study, regardless of serology or GFD response. In the Hamilton cohort, diagnosis of seronegative CeD patients was based on histology and a clinical and histological response to the GFD.

Data analysis

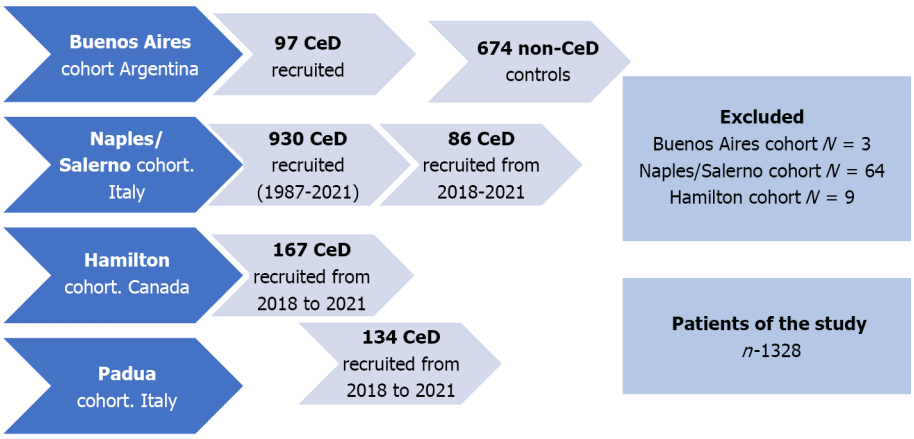
Statistical analysis was carried out using STATA (STATA version 14.0 Corp, College Station, TX, United States). Categorical variables were reported as frequencies and percentages, while continuous variables were reported as mean ± SD and/or median and 25%-75% interquartile ranges, according to their distribution. Comparisons of categorical variables between groups were made using the χ^2 test or Fisher's exact test. P values < 0.05 were considered statistically significant. For comparisons of continuous variables the analysis of variance test was used. Logistic regression was used to assess the risk of endoscopic lesions. The model included the report of significant lesions in endoscopy and/or histology reports as a dependent variable and factors such as age, sex, personal history, and signs/symptoms as independent variables.

Given the different recruitment times between centers, a subgroup analysis was performed to compare results in the Naples/Salerno cohort, focusing on cases diagnosed between 2018 and 2021 *vs*

Table 1 Demography, celiac disease serology, and endoscopic findings by cohorts and the overall population

Demographic data and upper GI endoscopic findings	Naples/Salerno cohort	Buenos Aires cohort	Hamilton cohort	Padua cohort	Overall CeD population
Total population	930 (70.0)	97 (7.3)	167 (12.6)	134 (10.1)	1328
Age in yr	34 (26-42)	35 (27-44)	39 (27-54)	35 (23-46)	35 (26-43)
Female sex	754 (81.1)	88 (90.7)	122 (73.0)	100 (74.6)	1064 (80.1)
Patients with positive serology	899 (96.7)	97 (100)	147 (88.0)	130 (97.0)	1269 (95.6)
Patients with at least one significant endoscopic abnormality	89 (9.6)	8 (8.2)	34 (20.4)	4 (3.0)	135 (10.2)
Reflux esophagitis with erosions	78 (8.4)	1 (1.0)	6 (3.6)	0	85 (6.4)
Esophageal peptic ulcers	0	0	1 (0.6)	0	1 (0.2)
Esophageal malignancy	0	0	0	0	0
Suspicion of esophageal metaplasia	3 (0.3)	0	13 (7.8)	0	16 (1.2)
Biopsy confirmed Barrett's esophagus	2 (0.2)	0	1(0.6)	0	3 (0.2)
Gastric erosions	0	7 (7.2)	16 (9.6)	4 (3.0)	27 (2.0)
Gastric ulcers	1 (0.1)	0	0	0	1 (0.1)
Gastric cancer	0	0	0	0	0
Duodenal erosions	0	0	15 (9.0)	0	15 (1.1)
Duodenal ulcers	8 (0.9)	1 (1.0)	6 (3.6)	0	15 (1.1)
Duodenal cancer	0	0	0	0	0

Data are presented as *n* (%) or median and 25% interquartile range. CeD: Celiac disease; GI: Gastrointestinal; IQR: Interquartile range.



DOI: 10.3748/wjg.v28.i43.6157

Figure 1 Flow chart of participants. CeD: Celiac disease.

previous endoscopies, estimating that such analysis could detect differences by using more actualized endoscopic protocols that were temporally concordant with those reported from patients collected in the Padua and Ontario cohorts.

RESULTS

Overall, 1404 patients were diagnosed with CeD and 1328 of them were included in the study (Figure 1). Patients with slightly positive serology but Marsh 0 or 1 (n = 76) were not diagnosed as CeD.

Demographic and clinical characteristics

The number of participants recruited varied between centers (Tables 1 and 2). The Naples/Salerno cohort contained most (70.0%) of the patients, while the Buenos Aires cohort had the fewest patients (7.3%). The European and North American centers differed in the length of time the celiac centers had been operational. The South American center included patients and controls over a specific time previously enrolled in a different study. There was a female predominance in all groups. There was no difference in the age at which diagnostic endoscopy was performed. There were no differences in baseline demographics across centers. The percentage of patients testing positive for celiac specific antibodies ranged from 88% (Hamilton) to 100% (Buenos Aires).

Endoscopic findings in CeD patients and age-related damage

Endoscopy revealed 163 distinct abnormalities in 135 patients with CeD (10.1%) (Table 1). The most common finding was erosive reflux esophagitis (6.4%), with the highest prevalence in the Naples/Salerno cohort (8.4%) and the lowest in the Buenos Aires (1%) and Padua (0%) cohorts. Peptic esophageal ulcers were only found in 1 patient within the total cohort. Although Barrett's esophagus was suspected in 1.2% of the patients, it was biopsy confirmed in 0.2% of cases (18.7% of those suspected and subsequently biopsied). The Hamilton cohort had a higher suspicion of metaplasia (n = 13), but Barrett's esophagus was confirmed in 1 patient (Table 1). Gastric ulcers were found in 1 patient (0.1%) within the Naples/Salerno cohort, while gastric erosions were found in 2.0% of the total population, with a higher prevalence in the Buenos Aires (7.2%) and the Hamilton (9.6%) cohorts. In the latter, 9.1% of patients with duodenal erosion were documented. Overall, 1.1% of duodenal ulcers were discovered, with a higher frequency encountered in the Hamilton cohort (3.6%). No cancers were reported at any level of the upper gastrointestinal (GI) tract of CeD patients.

Patients under the age of 50 had a lower risk of having at least one abnormality compared with patients over the age of 51 (P < 0.01). This indicated a 96.6% increase in lesions found in older patients (8.9% *vs* 17.5%), which was primarily driven by erosive esophagitis and gastric erosions (Table 2). We performed a subgroup analysis of the Naples/Salerno cohort, including patients diagnosed between 2018 and 2021. Compared with the overall Naples/Salerno cohort, patients diagnosed recently (n = 86) had a higher percentage of at least one significant endoscopic abnormality (29.2% *vs* 9.6%, respectively), owing to a higher proportion of cases with erosive reflux esophagitis (20.0% *vs* 8.4%) and duodenal ulcers (8.2% *vs* 0.9%, respectively). These endoscopic features were more common in the Naples/Salerno cohort (after 2018) than in the other cohorts (Padua and Hamilton) (P < 0.01). Compared with the Padua cohort, the Salerno cohort had a higher proportion of patients with at least one endoscopic abnormality (29.1% *vs* 3.0%; P < 0.01) (Supplementary Table 1).

Endoscopic findings in celiac patients and non-celiac controls from the Buenos Aires cohort

We compared CeD patients (n = 97) *vs* non-CeD controls (n = 674) (Table 3) using the Buenos Aires cohort. The median age at endoscopy in non-CeD controls was 11 years higher than in patients with CeD, and the percent of females was lower (P < 0.01 for both). Compared with patients with CeD, a higher proportion of controls were under the age of 50 (P < 0.001) (Table 3). CeD specific serology was positive in 1.3% of non-CeD controls. IgA transglutaminase positive levels in controls were less than three times the upper limit of normal. Endoscopic findings were more frequent in controls than in CeD patients (P < 0.001). In all age groups, gastric erosions were most common. Two control subjects, both older than 51, had a stomach adenocarcinoma and another a duodenal cancer at diagnostic endoscopy. In contrast, no cancers were discovered in CeD patients. Metaplasia was found in 1.0% of controls, with Barrett's esophagus being confirmed after biopsy in two of these cases. Controls over the age of 51 had 12.9% more frequent mucosal damage compared with younger subjects (overall prevalence 31.4% *vs* 27.8%, respectively).

The crude multivariate analysis based on CeD patients and non-CeD controls found that a CeD diagnosis and presence of alarm symptoms(weight loss, anemia, bleeding, dysphagia, epigastric pain, or history of malignancy) reduced the risk of having at least one lesion by 78.0% and 49.0% (P < 0.0001 for both), respectively. According to the adjusted multivariate analysis, having CeD was associated with a 72% reduction in the risk of any endoscopic lesion (P < 0.0001), and having alarm symptoms was associated with a 37% reduction in the risk of having at least one endoscopic lesion (P < 0.02; Table 4).

DISCUSSION

The study's main finding was that upper endoscopy performed concurrently with duodenal biopsies for CeD diagnosis revealed no concomitant damage in 92.0% of cases. Only 1.6% of CeD patients had relevant findings with the potential to progress to severe disease, comprised by esophageal and gastric ulcers and Barrett's esophagus. While 8.9% of patients demonstrated upper GI injury, only 1.3% potentially had dangerous lesions. The low yield of relevant concomitant findings in this study does not support the usefulness of upper endoscopy beyond the need of obtaining biopsies for the diagnosis of CeD.

Table 2 Demography, celiac disease serology, and endoscopic findings of the overall population and by the age of diagnosis

Demographic data and upper GI endoscopic findings	Overall CeD population	≤ 50 yr	51-60 yr	≥ 61 yr
Patients	1328	1140 (85.8)	114 (8.6)	74 (5.6)
Age in yr	35 (26-43)	33 (25-39)	55 (53-56)	67 (63-71)
Female sex	1064 (80.1)	931 (81.7)	82 (71.9)	51 (41.0)
Patients with positive serology	1269 (95.6)	1092 (95.8)	109 (95.6)	68 (91.9)
Patients with at least one significant endoscopic abnormalities	135 (10.1)	102 (8.9)[1]	20 (17.5)	13 (17.6)
Reflux esophagitis with erosions	85 (6.4)	69 (6.0)	12 (10.5)	4 (5.4)
Esophageal ulcers	1 (0.2)	1 (0.1)	0	0
Esophageal cancer	0	0	0	0
Suspicion of metaplasia	16 (1.2)	10 (0.9)	3 (2.6)	2 (2.7)
Biopsy confirmed Barrett's esophagus	3 (0.2)	2 (0.2)	0	1 (1.3)
Gastric erosions	27 (2.0)	20 (1.7)	3 (2.6)	4 (5.4)
Gastric ulcers	1 (0.1)	1 (0.1)	0	0
Gastric cancer	0	0	0	0
Duodenal erosions	15 (1.1)	8 (0.7)	3 (2.6)	4 (5.4)
Duodenal ulcers	15 (1.1)	10 (0.9)	4 (3.5)	1 (1.3)
Duodenal cancer	0	0	0	0

[1]≤ 50-years-old *vs* other age categorizations: *vs* patients > 51-years-old: $P < 0.01$. Data are presented as *n* (%) or median and 25% interquartile range.
CeD: Celiac disease; GI: Gastrointestinal.

The possibility of detecting important or relevant esophageal, gastric, or duodenal pathology during diagnostic endoscopy has been put forward as an added benefit to the confirmation of CeD. Previous findings in CeD patients include reflux esophagitis, esophageal eosinophilia or eosinophilic esophagitis (mostly in children), Barrett's esophagus, *Helicobacter pylori* (*H. pylori*) infection and autoimmune gastritis[14-16]. These were, however, reported in small populations and single center studies. Our study, which included cohorts from the European Union, North America, and South America, gathered the largest sample of patients reported to date. The sample size collectively obtained allowed for subgroup and age category comparisons. The majority of CeD patients were young and female, as expected. The Buenos Aires cohort was prospectively designed to diagnose symptomatic patients suspected of having CeD, which allowed for comparisons between CeD patients and controls biopsy (Marsh's 0 or 1 histology categorization).

Our findings in a large multicenter population confirm recent reports that adult patients with alarm symptoms have a very low prevalence of major endoscopic and histological findings in the upper GI tract other than CeD features at presentation and was comparable to that of patients without alarm symptoms[14,16]. The definition of what constitutes an alarming symptom for CeD at the time of diagnosis appears to be central to this analysis. Weight loss, iron deficiency anemia, pain, or malabsorption symptoms were prevalent among symptomatic patients, which constitutes the vast majority of currently diagnosed CeD patients since a case finding strategy is recommended[19]. However, our findings were limited to the upper GI tract, and the lower GI tract was not explored.

Erosive reflux esophagitis was the most common endoscopic finding at the time of diagnosis (6.4%). Notably, undiagnosed patients with classical or subclinical CeD frequently seek treatment for gastroesophageal reflux symptoms prior to diagnosis, which has been shown to be more common in subjects in whom CeD is ruled out or in those treated with the GFD[20]. We previously reported that up to 30% of newly diagnosed CeD patients perceive moderate to severe reflux symptoms, which does not respond to anti-reflux therapy prior to CeD diagnosis[21,22]. Most of these "non-responsive" patients to anti-reflux therapy will rapidly improve after starting the GFD. Surprisingly, between 2018 and 2021, the Naples/Salerno cohort revealed higher prevalence of overall endoscopic lesions, and specifically of erosive reflux esophagitis, compared with diagnoses made before that time. This could be attributed to the characteristics of the CeD population over time or to differences in the reporting of endoscopic and histology findings.

The possibility of missing severe lesions or potentially dangerous diseases in CeD patients if a diagnostic endoscopy is not performed has been a source of concern in CeD guidelines[2,4]. With respect to Barrett's esophagus or esophageal metaplasia, an Italian study published in 2005 showed

Table 3 Demography and endoscopic findings in celiac disease patients and non-celiac disease controls of the Buenos Aires cohort

Demographic data and upper GI endoscopic findings	CeD population	Non-CeD population	≤ 50 yr non-CeD	51-60 yr non-CeD	≥ 61 yr non-CeD
Patients	97 (12.6)	674 (87.4)	435 (64.5)	135 (20.0)	104 (15.4)
Age in yr	35 (27-44)	45 (33-55)[a]	37 (29-44)	55 (53-58)	68 (63-72)
Female sex	88 (90.7)	472 (70.0)[a]	312 (71.7)	92 (68.1)	68 (65.4)
Patients with positive serology	97 (100)	9 (1.3)[a]	6 (1.3)	3 (2.2)	0
Patients with at least one significant endoscopic abnormalities	8 (8.2)	196 (29.1)[b]	121 (27.8)	48 (35.6)	27 (26.0)
Reflux esophagitis with erosions	1 (1.0)	21 (3.1)	11 (2.5)	2 (1.5)	5 (4.8)
Esophageal ulcers	0	0	0	0	0
Esophageal cancer	0	0	0	0	0
Suspicion of metaplasia	0	7 (1.0)	5 (1.1)	2 (1.5)	0
Biopsy confirmed Barrett's esophagus	0	2 (0.3)	2 (0.5)	0	0
Gastric erosions	7 (7.22)	165 (24.5)[a]	103 (23.7)	43 (31.8)	19 (18.3)
Gastric ulcers	0 (0)	11 (1.6)	4(0.9)	2 (1.5)	5 (4.8)
Gastric cancer	0	2 (0.3)	0	1 (0.7)	1 (1.0)
Duodenal erosions	0	10 (1.5)	6 (1.4)	2 (1.5)	2 (1.9)
Duodenal ulcers	1 (1.0)	5 (0.7)	2 (0.5)	2 (1.5)	1 (1.0)
Duodenal cancer	0	1 (0.1)	1 (0.2)	0	0

[a]$P < 0.01$.
[b]$P < 0.001$.
Data are presented as *n* (%) or median and 25% interquartile range. Controls were grouped according to age at endoscopy. Comparisons between celiac disease patients *vs* non-celiac disease controls.
CeD: Celiac disease; GI: Gastrointestinal; IQR: Interquartile range.

Table 4 Crude and adjusted multivariate analysis for the Buenos Aires cohort

Independent variable	At least one endoscopic lesion[1] (Buenos Aires cohort)			
	OR (95%CI)	*P* value	Adjusted[2] OR (95%CI)	*P* value
Male sex	1.19 (0.83-1.69)	0.34	1.03 (0.72-1.48)	0.860
Age	1.01 (1.00-1.02)	0.14	1.00 (0.99-1.01)	0.550
Celiac disease	0.22 (0.10-0.46)	< 0.0001	0.28 (0.13-0.60)	0.001
Alarm symptoms[3]	0.51 (0.35-0.74)	< 0.0001	0.63 (0.43-0.93)	0.020

[1]At least one endoscopic lesion: Erosive esophagitis, esophageal ulcer, esophageal cancer, Barrett's esophagus, gastric erosion, gastric ulcer, gastric cancer, duodenal erosion, duodenal ulcer, duodenal cancer.
[2]Weight loss, anemia, bleeding, dysphagia, epigastric pain, neoplasia history.
[3]Weight loss, iron deficient anemia, malabsorption, chronic diarrhea.
CI: Confidence interval; M: Male; OR: Odds ratio.

metaplasia in 26.6% of CeD patients compared with 10.9% of the control population[23]. This was not confirmed in studies from the United States[24] and South America[15,21] nor by the present study. Reasons for this discrepancy could be related to differences in populations and in the definition of Barrett's esophagus, which required confirmation by biopsy in our study.

In the present study, we did not find mucosal eosinophilic infiltration. A pediatric prospective longitudinal study based on systematic esophageal biopsies found that diagnoses of eosinophilic esophagitis and/or eosinophilia were not clinically relevant, suggesting esophageal biopsy is not necessary in the absence of clinical suspicion[25]. A 2015 cross-sectional population study in the United States based on a national pathology database involving over 88000 CeD patients with both esophageal and duodenal biopsies, reported a slight increase in comorbid eosinophilic esophagitis and CeD[24]. However, no link

between reflux esophagitis or Barrett's esophagus and CeD has been reported. Finally, autoimmune atrophic gastritis was previously modestly associated with CeD[26]. Our study, as well as other population-based studies and systematic reviews, did not confirm the association[27,28].

An earlier prospective study[15] collected consecutive patients and non-CeD controls in a high-risk population for having CeD, and gastric and duodenal biopsies were performed systematically at the time of the diagnostic endoscopy CeD and biopsy. Gastric biopsies from untreated CeD patients also revealed a significantly higher intraepithelial lymphocyte count in the antrum and corpus when compared with controls[15,29,30]. According to an Irish study, 10% of CeD patients have lymphocytic gastritis, which is twice the rate of non-CeD controls[12,14]. These findings are attributed to *H. pylori* infection, autoimmune atrophic gastritis[15,26], or a pan-mucosal gluten-related inflammation[14,15,29,30].

Our study showed only 1 CeD patient had a gastric peptic ulcer. Previous studies found 18.1% of CeD children with gastric ulcers, with a higher prevalence in *H. pylori* negative patients and those with no history of nonsteroidal anti-inflammatory drug use[31,32]. The rate of *H. pylori* infection across centers was not consistently reported here, and this could explain the difference in results. Previous research, however, has shown that high rates of biopsy-confirmed *H. pylori* infection are not associated with an increased risk of malignancy in the long term[27,33]. However, several studies have also shown that when endoscopic appearance is normal, histological evaluation (both in the stomach and the esophagus) is not cost-effective, especially when performed in experienced academic centers[34-36].

There was no diagnosis of gastric adenocarcinoma in CeD. Despite the small number of cases studied, this is consistent with previous findings that the prevalence of other cancers (breast, colon, pulmonary, and gynecological cancers) in CeD appears to be lower than in the general population[27,28]. Small bowel carcinoma is extremely rare in the general population, and CeD patients are three times more likely to develop it[1,28]. However, malignancies in the duodenum are still uncommon at the time of CeD diagnosis, which implies diagnostic CeD endoscopy should not be recommended as surveillance for upper GI cancer[28]. Overall, the current findings, as well as those from previous studies, suggest that a biopsy-avoiding approach in adult patients who meet recommended and strict serological criteria for CeD is possible[12,37-40].

Study strengths included the multicenter design, the large number of patients diagnosed at specialized centers for CeD in whom confirmatory biopsy diagnosis was obtained, as well as the use of standard endoscopic protocols. Despite the small numbers in sub-analyses, the study also provided novel data related to the association of endoscopic findings according to age and time. Study limitations included the observational design, the retrospective collection of endoscopic reports (with potential missing data), the differences in time of enrollment across the four centers, the lack of systematic collection of biopsies from the esophagus and stomach, and the limited number of non-CeD controls. Although the current study suggests that missing potentially serious events is unlikely, this should be confirmed in a larger population.

CONCLUSION

In conclusion, this multicenter, retrospective study found that comorbid upper GI endoscopic pathology is uncommon in patients with positive CeD serology at the time of diagnostic endoscopy. The risk of severe or premalignant lesions is extremely low, and no malignancies were found in patients who displayed potential warning signs. Our findings suggest that a non-biopsy strategy for diagnosing CeD in adults is unlikely to miss clinically significant concomitant endoscopic findings unrelated to CeD. The results of this study should encourage future population-based or prospective studies in this area.

ARTICLE HIGHLIGHTS

Research background

Celiac disease (CeD) is currently diagnosed in adult patients using a combination of specific serology tests and a duodenal biopsy obtained through an upper endoscopy. Upper endoscopy is also considered necessary for CeD diagnosis because non-CeD comorbidities can be missed.

Research motivation

The prevalence of upper gastrointestinal comorbidities at the time of CeD diagnosis has received little attention.

Research objectives

To investigate the prevalence of coincidental upper gastrointestinal endoscopic findings at the time of diagnostic endoscopy in four cohorts of patients diagnosed in three different countries.

Research methods

We conducted a descriptive multicenter retrospective study reporting endoscopic findings from adult patients who met standard criteria for diagnosing CeD.

Research results

Of 1328 adult patients enrolled, 95.6% had positive specific serology. In 135 patients, endoscopy revealed 163 abnormalities unrelated to CeD (10.1%). Erosive reflux esophagitis (6.4%), gastric erosions (2.0%), and suspicion of esophageal metaplasia (1.2%) were the most common findings. Biopsy-confirmed Barrett's esophagus was infrequent (0.2%). No other neoplastic or malignancies lesions were detected. Patients with alarm symptoms or signs had a lower rate of concomitant findings.

Research conclusions

Adults with positive CeD serology had few comorbid endoscopic findings when CeD was diagnosed.

Research perspectives

These findings raise the possibility that adult patients who meet recommended and strict serological criteria for CeD could be diagnosed without undergoing endoscopy and biopsy.

FOOTNOTES

Author contributions: Stefanolo JP, Ciacci C, Zingone F, Gizzi C, Marsilio I, Espinet ML, Pinto-Sánchez MI, and Niveloni SI contributed with data acquisition; Stefanolo JP performed the statistical analysis; Stefanolo JP, Pinto-Sánchez MI, Ciacci C, Zingone F, and Bai JC contributed to study design; Verdú EF, Smecuol EG, Moreno ML contributed to critical analysis; Bai JC, Verdú EF, Ciacci C, Pinto-Sánchez MI and Zingone F contributed to writing and critical review of the manuscript; All authors read and approved the final manuscript.

Institutional review board statement: The Ethics and Research Board of the Dr. C. Bonorino Udaondo Gastroenterology Hospital approved the study because of the prospective design and intervention in the Buenos Aires cohort. Ethics approval was obtained from the Hamilton Integrated Research Ethics Board (HiREB# 14460/5415). In Italy, Ethical Committee review was not required for retrospective studies while patient data remained anonymously coded.

Informed consent statement: Informed consent was not required by the Ethics and Research Committee of the Hospital de Gastroenterología Dr. C. Bonorino Udaondo (Buenos Aires, Argentina) given the retrospective nature of the study and because this study was categorized as minimal risk by the Committee.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at jbai@intramed.net. Consent was not obtained, but the presented data are anonymized and risk of identification is low.

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S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Fan JR

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World Journal of Gastroenterology

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World J Gastroenterol 2022 November 21; 28(43): 6168-6202

DOI: 10.3748/wjg.v28.i43.6168

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

SCIENTOMETRICS

Integrity of the editing and publishing process is the basis for improving an academic journal's Impact Factor

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A, A
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ede MO, Nigeria; Li S, China; Link A, Germany; Watanabe T, Japan

Received: October 8, 2022
Peer-review started: October 8, 2022
First decision: October 20, 2022
Revised: October 27, 2022
Accepted: November 10, 2022
Article in press: November 10, 2022
Published online: November 21, 2022

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Abstract

BACKGROUND

Journal Impact Factor™ (JIF) is often used to evaluate the relative reputation and quality of academic journals in their respective fields, and can greatly influence the quality and scope of subsequent manuscript submissions. Therefore, many if not all academic journals are interested in increasing their JIF, to improve their academic impact.

AIM

To determine the importance of the integrity of the editorial and publication process in improving the academic influence of academic journals and the JIF of academic journals.

METHODS

In this paper, we describe our statistical analysis of bibliometric factors - including the 2021 JIFs released in the *Journal Citation Report*™ 2022, discipline rankings, received and published articles in 2019-2021, and webpage visits and downloads - for seven journals published by Baishideng Publishing Group (Baishideng) and indexed in Science Citation Index Expanded™; ultimately, we introduce and discuss the editing and publishing processes of Baishideng's journals in their entirety, as they form the basis for our objective of safeguarding and bolstering integrity in academic publication.

RESULTS

For the seven journals assessed, their 2021 JIFs were basically unchanged from 2020, with the current metric ranging from 5.374 for *World Journal of Gastroenterology* (*WJG*) to 1.534 for *World Journal of Clinical Cases* (*WJCC*). Further assessments of the journals' bibliometrics from 2019 to 2020, showed that *World Journal of Stem Cells* has the highest self-citation rate (1.43%) and *World Journal of*

Gastrointestinal Surgery has the lowest (0.21%). Additionally, the total 3012 articles published during this period were cited by more than 20000 articles in approximately 8000 academic journals. Of note, the 1102 articles published in *WJG* were cited by articles in 3059 journals, among which 171 journals have a JIF of > 10, including internationally renowned academic journals such as *CA-A Cancer Journal for Clinicians* (2021 JIF 286.130, record count: 1), *Lancet* (2021 JIF 202.731, record count: 4), *Nature Reviews Immunology* (2021 JIF 108.555, record count: 2), *Nature Reviews Gastroenterology & Hepatology* (2021 JIF 73.082, record count: 9), *Lancet Gastroenterology & Hepatology* (2021 JIF 45.042, record count: 8), *Gastroenterology* (2021 JIF 33.883, record count: 19), and *Gut* (2021 JIF 31.793, record count: 21). This suggests that Baishideng's journals have been widely recognized for their academic quality. In the *Reference Citation Analysis* (*RCA*) database, all seven Baishideng-published journals obtained a 2022 *Journal Article Influence Index* (*JAII*). For example, *WJG* has a 2022 *JAII* of 22.048, ranking 18th out of 102 journals in the field of gastroenterology & hepatology in the *RCA*, with 469909 total citations (6/102) and 21313 total articles (5/102). The numbers of manuscripts received and published in 2021 were both higher than those in 2019-2020. For example, *WJCC* received a total of 3650 manuscripts in 2021, which is 91.1% higher than those in 2019-2020 (average: 1910 papers/year). In 2021, *WJCC* published 1296 articles, representing an increase of 105.1% compared to those in 2019-2020 (average: 632 articles/year). The numbers of webpage visits and downloads received by the seven journals have increased year by year. For example, the number of total visits received by *WJG* in 2019-2021 was 1974052 in 2019, 2317835 in 2020 (increased by 17.4% compared with that in 2019), and 2652555 in 2021 (increased by 4.4% compared with that in 2020). The visitors were from more than 220 countries and regions worldwide, such as the United States, China, and the United Kingdom. Open access (OA) plays a vital role in improving the quality, efficiency, transparency, and integrity of academic journal publishing. From 2019 to 2021, a total of 5543 OA articles were published in the seven journals, of which 2083 (37.6%) were invited and published free-of-charge. During the same period, 1683 articles were published in *WJG*, and the authors were from more than 70 countries and regions. For the total 5543 articles published in the seven journals from 2019 to 2021, 3903 article quality tracking reports were received after the online publication of these articles. The quality of the articles was further evaluated through the Baishideng's article quality and author evaluation tracking system, with 4655 articles (84.0%) having received author evaluation and feedback, which contributes to tracking metrics for authors' satisfaction with the collective publication processes. From March 25, 2021 to June 28, 2022, the seven journals received a total of 424 reader evaluations and 229 letters from readers; this subsequent reader engagement demonstrates that the popularity of the published articles and the volume of their readership audience were improved through the reader evaluation system.

CONCLUSION

Ultimately, the findings from our bibliometric assessments indicate that establishing, promoting and actively practicing processes that safeguard and bolster the integrity of the editing and publication process also help to improve the academic influence of academic journals, which itself is the cornerstone for improving JIF.

Key Words: Journal Impact Factor; Academic journal; Editing process; Publishing process; Open access; Peer review; Language polishing; Academic influence

Core Tip: This study describes a statistical analysis of bibliometric factors including the 2021 Journal Impact Factor™ (JIF) released in the *Journal Citation Report*™ 2022, discipline rankings, received and published articles in 2019-2021, and webpage visits and downloads for seven journals published by Baishideng Publishing Group (Baishideng). This study also introduces and discusses the editing and publishing processes of Baishideng's journals in their entirety, as they form the basis for our objective of safeguarding and bolstering integrity in academic publication. The findings from these bibliometric assessments indicate that establishing, promoting, and actively practicing processes that safeguard and bolster the integrity of the editing and publication process also help to improve the academic influence of academic journals, which itself is the cornerstone for improving JIF.

Citation: Wang JL, Li X, Fan JR, Yan JP, Gong ZM, Zhao Y, Wang DM, Ma L, Ma N, Guo DM, Ma LS. Integrity of the editing and publishing process is the basis for improving an academic journal's Impact Factor. *World J Gastroenterol* 2022; 28(43): 6168-6202
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6168.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6168

INTRODUCTION

In June 2022, Clarivate Analytics officially released its *Journal Citation Report*™ (*JCR*) 2022, and more than 12000 academic journals received their new Journal Impact Factor™ (JIF). The concept of JIF as a metric of a journal's impact in a given field was first proposed by Eugene Garfield in 1955, and was calculated as follows for any given year: Number of that year's citations divided by the source items published in that journal during the previous 2 years[1]. Since then, JIF has been frequently used as a quantitative representation of the relative reputation and quality of academic journals in their respective fields[2]. Although JIF may be misused, it has an undeniable impact on the quality and scope of manuscript submissions[3-5]. Many authors use JIF when selecting an academic journal to which they will submit their manuscript, and the higher the JIF of the academic journal, the better its scientific reputation[6]. As such, managers of academic journals have focused their efforts on developing processes that will increase the respective JIFs and thereby improve the academic influence of each journal.

There are many factors affecting the JIF of an academic journal, such as whether the journal is open access (OA) and whether the journal is specialized[7]; however, few studies have focused on the impact of the integrity of the editing and publishing process on the improvement of the JIF of academic journals. In this study, we examined the importance of the integrity of the editorial and publication process in improving the JIF of academic journals by introducing and discussing the complete editing and publishing process of journals published by Baishideng Publishing Group (Baishideng), and statistically analyzing their 2021 JIFs released in the *JCR* 2022, published articles in recent years, and webpage visits and downloads for Baishideng's seven journals included in Science Citation Index Expanded (SCIE), a Web of Science™ Core Collection index.

MATERIALS AND METHODS

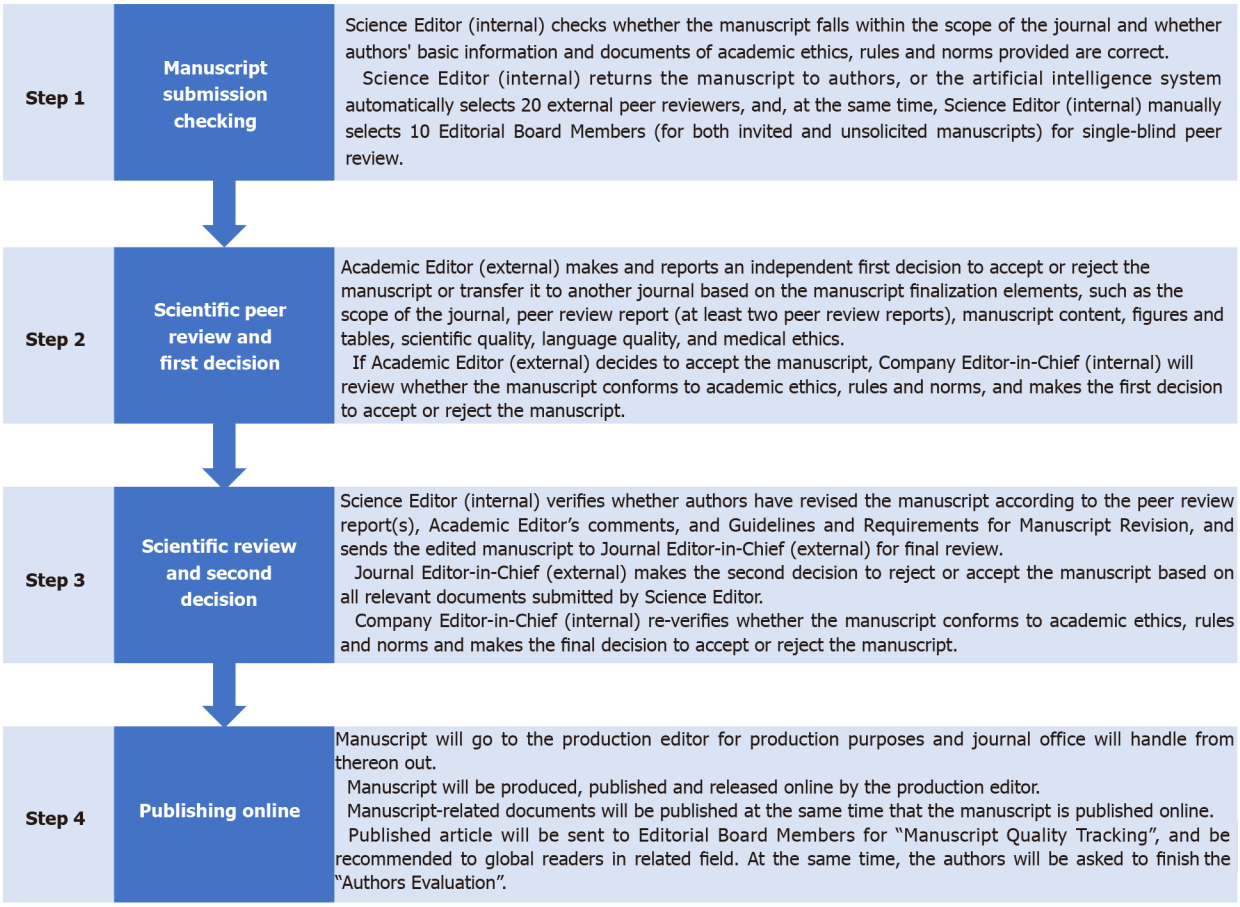
Baishideng's journals undergo a broad and thorough editing and publishing process

The seven SCIE-indexed journals published by Baishideng are all single-blind peer reviewed and OA. All manuscripts received by Baishideng's journals, both invited and freely submitted, are subjected to external peer review. The external peer review assesses the manuscripts for scientific quality, linguistic quality, and adherence to ethical standards and norms.

Baishideng established a broad and thorough academic journal editing and publishing process by focusing on the basic functions of academic journals, including: (1) Registration, to ensure the priority of authors' academic thoughts; (2) Peer review, to ensure the quality of the paper and obtain peer recognition; (3) Communication, to promote the dissemination of scientific findings; and (4) Document storage, to archive publications for future reference and textual research. Through the F6Publishing system, independently developed by Baishideng, each manuscript progresses stepwise through the complete editing and publishing process, including submission review, peer review, first decision, second decision, final acceptance, online publication[8], post-publication tracking of the quality of published articles, authors' evaluation of the publishing process, and readers' evaluation of the article (Figure 1).

Gathered data

We selected the seven SCIE-indexed journals from among the 47 total OA journals published by Baishideng, and performed systematic quantitative and statistical analyses based upon their 2021 JIFs, discipline rankings, and received and published articles in 2019-2021, including the main source countries/regions and the number of published articles. Furthermore, data were obtained from Web of Science, and the journals/articles citing the articles published in the respective seven Baishideng journals from 2019 to 2020 were statistically analyzed. In addition, we statistically analyzed the number of visits and downloads received by the seven journals' respective webpages, the number of non-conforming submissions identified in the submission preview, the number of peer reviewers and editorial board members who participated in the manuscript peer review, and the number of manuscripts that needed language polishing at the second decision, as well as the number of evaluations received *via* the Baishideng article quality tracking system, author evaluation system, and reader evaluation system from 2019 to 2021.



DOI: 10.3748/wjg.v28.i43.6168

Figure 1 The stepwise editing and publishing process of Baishideng journals. Each submitted article is subject to this process, with those of demonstrated quality in peer review progressing through the entire process to publication and post-publication qualitative and quantitative tracking.

RESULTS

Overview of 2021 JIFs of Baishideng's seven SCIE-indexed journals

In the *JCR* 2022, all seven of Baishideng's SCIE-indexed journals received their latest JIF. Compared with the JIFs of the seven journals received in 2020, the 2021 JIFs of three journals increased, and those of the remaining four slightly decreased, yielding an overall similarity between the 2 years (Table 1).

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Gastroenterology in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Gastroenterology* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Gastroenterology* (*WJG*) is 5.374, and the 5-year JIF is 5.715, ranking 31st among 93 journals in the field of gastroenterology and hepatology, located in quartile (Q) 2 (Figure 2). The 2021 JIF of *WJG* decreased by 0.368 from its 2020 JIF (5.742).

According to the *Reference Citation Analysis (RCA)* database, independently developed by Baishideng, *WJG* has a 2022 *Journal Article Influence Index* [2022 *JAII*, calculated as (Total Citations/Total Articles)] of 22.048, ranking 18th out of 102 journals in the field of gastroenterology & hepatology in the *RCA*, with 469909 total citations (6/102) and 21313 total articles (5/102).

From 2019 to 2020, *WJG* received a total of 3986 articles (average: 1993 articles/year), among which 1247 (31.3%) were invited and 2739 (68.7%) were freely submitted; the acceptance rate was 27.6%. During that same period, *WJG* published 1102 articles (average: 551 articles/year), among which 479 (43.5%) were invited and 623 (56.5%) were freely submitted. As of July 26, 2022, the articles published in *WJG* received a total of 12458 citations (without self-citations: 12341) by 11413 articles (without self-citations: 11314), yielding a self-citation rate of 0.94%; there were a total of 6387 citations in 2021. After excluding self-citations, the 11314 articles that cited the *WJG*-published articles were from 3059 journals (data from Web of Science, Table 2); among these journals, 171 (5.6%) had a JIF of > 10 (data from Web of Science), accounting for 25.9% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJG*-published articles include internationally renowned academic

Table 1 Comparison of the 2020 and 2021 Journal Impact Factors™ of the seven Baishideng journals indexed in Science Citation Index Expanded

Journal title	2021 JIF	2020 JIF	5-yr JIF	Category: Journal rank/total journals (quartile)
World Journal of Gastroenterology	5.374	5.742	5.715	Gastroenterology and Hepatology: 31/93 (Q2)
World Journal of Stem Cells	5.247	5.326	4.964	Cell and Tissue Engineering: 12/29 (Q2); Cell Biology: 86/194 (Q2)
World Journal of Diabetes	4.560	3.763	5.370	Endocrinology and Metabolism: 62/146 (Q2)
World Journal of Psychiatry	3.500	4.571	7.380	Psychiatry: 89/155 (Q3)
World Journal of Gastrointestinal Oncology	3.404	3.393	3.250	Oncology: 163/245 (Q3); Gastroenterology and Hepatology: 59/93 (Q3)
World Journal of Gastrointestinal Surgery	2.505	2.582	3.099	Surgery: 104/211 (Q2); Gastroenterology and Hepatology: 81/93 (Q4)
World Journal of Clinical Cases	1.534	1.337	1.599	Medicine, General and Internal: 135/172 (Q4)

JIF: Journal Impact Factor™; Q: Quartile.

DOI: 10.3748/wjg.v28.i43.6168

Figure 2 The 2021 Journal Impact Factor and category rank of *World Journal of Gastroenterology*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

journals such as *CA-A Cancer Journal for Clinicians* (2021 JIF 286.130, record count: 1), *Lancet* (2021 JIF 202.731, record count: 4), and *Nature Reviews Immunology* (2021 JIF 108.555, record count: 2), and renowned academic journals in the field of gastroenterology & hepatology such as *Nature Reviews Gastroenterology & Hepatology* (2021 JIF 73.082, record count: 9), *Lancet Gastroenterology & Hepatology* (2021 JIF 45.042, record count: 8), *Gastroenterology* (2021 JIF 33.883, record count: 19), and *Gut* (2021 JIF 31.793, record count: 21). From 2019 to 2020, the authors of the 1102 articles published in *WJG* were from 71 countries/regions, represented by 472 articles (42.8%) from China, 165 (15.0%) from the United States, 93 (8.4%) from Italy, 82 (7.4%) from Japan, 51 (4.6%) from South Korea, and 239 (21.7%) from other countries/regions (data from Web of Science, Figure 3). Of note, the article entitled "Gastrointestinal and hepatic manifestations of COVID-19: A comprehensive review", which was contributed by Cha *et al* [9] from Cleveland Clinic in the United States in 2020, is the article with the highest number of citations among the articles published in *WJG*. This article mainly reviews the gastrointestinal and hepatic manifestations of coronavirus disease 2019 (COVID-19). As of June 28, 2022, this article has been cited 77 times.

In 2021, *WJG* received a total of 2109 manuscripts, of which 1116 (52.9%) were invited and 993 (47.1%) were freely submitted; the acceptance rate was 27.5%, which was 5.8% higher than that in 2019-2020 (average: 1993/year). A total of 581 articles were published in *WJG* in 2021, including 360 (62.0%) invited and 221 (38.0%) freely submitted. As of June 28, 2022, the number of total citations was 1039. The number of articles published in *WJG* in 2021 was 5.4% higher than that in 2019-2020 (551 articles/year on average). The authors were from 56 countries/regions, represented by 160 articles (27.5%) from China, 59 (10.2%) from Italy, 53 (9.1%) from the United States, 38 (6.5%) from Japan, 20 (3.4%) from South Korea, 20 (3.4%) from Brazil, and 231 (39.7%) from other countries/regions (Figure 4).

Number of webpage visits and downloads received by *WJG* in 2019-2021: From 2019 to 2021, the *WJG* webpage received a total of 1974052 visits in 2019, 2317835 in 2020 (an increase of 17.4% compared with that in 2019), and 2652555 in 2021 (an increase of 14.4% compared with that in 2020), with visits from

Table 2 Rank and record count of journals that published articles that cited the 1102 articles published in *World Journal of Gastroenterology* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*Cancers*	241 (2.13)
2	*International Journal of Molecular Sciences*	241 (2.13)
3	*Frontiers in Oncology*	193 (1.706)
4	*Journal of Clinical Medicine*	138 (1.22)
5	*Nutrients*	119 (1.052)
6	*Scientific Reports*	111 (0.981)
7	*Frontiers in Pharmacology*	100 (0.884)
8	*Frontiers in Immunology*	92 (0.813)
9	*Frontiers in Medicine*	89 (0.787)
10	*World Journal of Clinical Cases*	77 (0.681)
11	*Cells*	76 (0.672)
12	*Digestive Diseases and Sciences*	73 (0.645)
13	*Medicine*	71 (0.628)
14	*Medicine Hagerstown*	70 (0.619)
15	*Cureus*	67 (0.592)
16	*Surgical Endoscopy*	67 (0.592)
17	*Surgical Endoscopy and Other Interventional Techniques*	67 (0.592)
18	*Diagnostics*	64 (0.566)
19	*Diagnostics Basel Switzerland*	64 (0.566)
20	*BMC Gastroenterology*	63 (0.557)
21	*Biomed Research International*	55 (0.486)
22	*Evidence Based Complementary and Alternative Medicine*	55 (0.486)
23	*Evidence Based Complementary and Alternative Medicine ECAM*	55 (0.486)
24	*PLOS One*	53 (0.468)
25	Other journals, *n* = 3025	9013 (79.662)

more than 220 countries and regions worldwide (Figure 5). The number of downloads was 1117432 in 2019, 2459893 in 2020 (an increase of 120.1% compared with that in 2019), and 3754483 in 2021 (an increase of 52.6% compared with that in 2020), with downloads from more than 200 countries and regions worldwide.

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Stem Cells in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Stem Cells* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Stem Cells* (*WJSC*) is 5.247, and the 5-year JIF is 4.964, ranking 12th among 29 journals in the field of cell & tissue engineering, located in Q2 (Figure 6), and ranking 86th among 194 journals in the field of cell biology. The 2021 JIF of *WJSC* decreased by 0.079 from its 2020 JIF (5.326).

According to the *RCA* database, *WJSC* has a 2022 *JAII* of 17.729, ranking 13th out of 32 journals in the field of cell & tissue engineering in the *RCA*, with 12056 total citations (18/32) and 680 total articles (20/32).

From 2019 to 2020, *WJSC* received a total of 396 articles (average: 198 articles/year), among which 282 (71.2%) were invited and 114 (28.8%) were freely submitted; the acceptance rate was 46.5%. During the same period, *WJSC* published 184 articles (average: 92 articles/year), among which 155 (84.2%) were invited and 29 (15.8%) were freely submitted. As of June 28, 2022, the articles published in *WJSC* received a total of 1956 citations (without self-citations: 1928) by 1813 articles (without self-citations: 1794), yielding a self-citation rate of 1.43%; there were a total of 1000 citations in 2021. After excluding

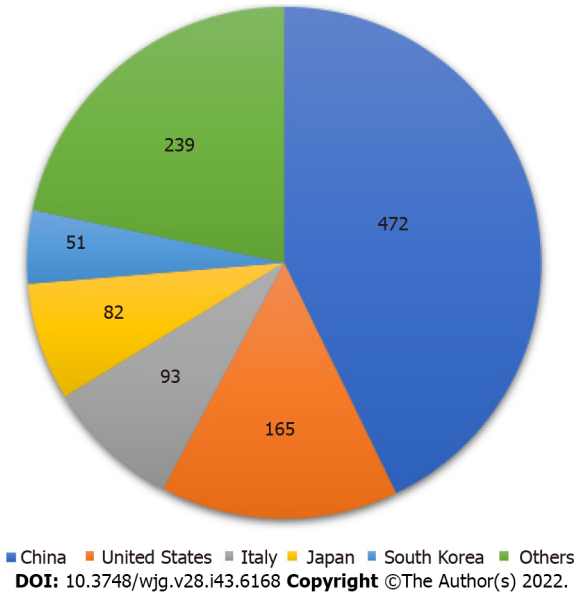


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Figure 3 Country sources and number of articles published in *World Journal of Gastroenterology* in 2019-2020.

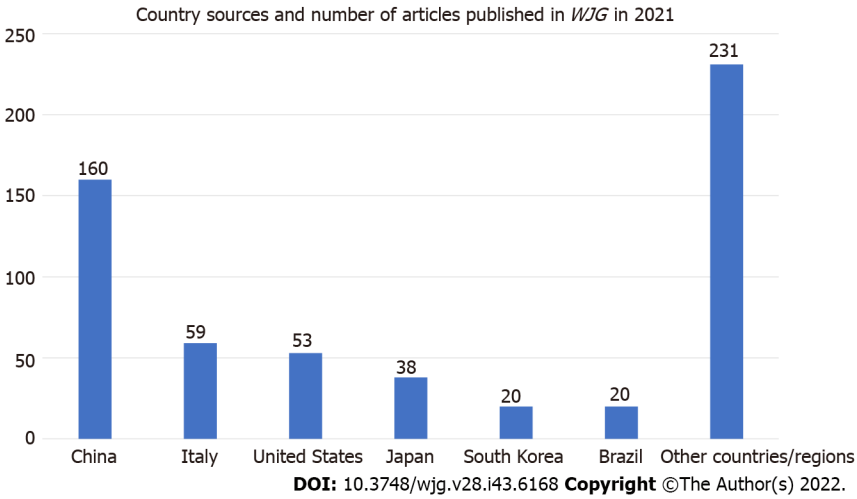

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Figure 4 Country sources and number of articles published in *World Journal of Gastroenterology* in 2021. *WJG*: *World Journal of Gastroenterology*.

self-citations, the 1794 articles that cited the *WJSC*-published articles were from 858 journals (data from Web of Science, Table 3); among these journals, 61 (7.1%) had a JIF of > 10 (data from Web of Science), accounting for 9.2% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJSC*-published articles include renowned academic journals in the field of cell biology such as *Nature Reviews Molecular Cell Biology* (2021 JIF 113.915, record count: 1) and *Trends in Cell Biology* (2021 JIF 21.167, record count: 1). From 2019 to 2020, the authors of the 184 articles published in *WJSC* were from 41 countries/regions, represented by 78 articles (42.4%) from China, 22 (12.0%) from the United States, 11 (6.0%) from Brazil, 51 (6.0%) from South Korea, 10 (5.4%) from Italy, and 52 (28.3%) from other countries/regions (data from Web of Science, Figure 7). Of note, the article entitled "Mesenchymal stem cells from different sources and their derived exosomes: A pre-clinical perspective", which was contributed by Associate Professor Álvarez-Viejo *et al*[10] from Hospital Universitario Central de Asturias, Spain in 2020, is the article with the highest number of citations among the articles published in *WJSC*. This article mainly reviews the research progress of different sources of mesenchymal stem cells and their derived apoplasts from a preclinical perspective. As of June 28, 2022, this article has been cited 29 times.

In 2021, *WJSC* received a total of 215 manuscripts, of which 167 (77.7%) were invited and 48 (22.3%) were freely submitted; the acceptance rate was 50.7%, which was 8.6% higher than that in 2019-2020 (average: 198 articles/year). A total of 109 articles were published in *WJSC* in 2021, including 96 (88.1%) invited and 13 (11.9%) freely submitted. As of June 28, 2022, the number of total citations was 164. The

Table 3 Rank and record count of journals that published articles that cited the 184 articles published in *World Journal of Stem Cells* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*International Journal of Molecular Sciences*	139 (7.748)
2	*Cells*	76 (4.236)
3	*Frontiers in Cell and Developmental Biology*	70 (3.902)
4	*Stem Cell Research Therapy*	69 (3.846)
5	*Stem Cells International*	44 (2.453)
6	*Cancers*	37 (2.062)
7	*Stem Cell Reviews and Reports*	24 (1.338)
8	*Frontiers in Immunology*	23 (1.282)
9	*Frontiers in Bioengineering and Biotechnology*	22 (1.226)
10	*Biomedicines*	18 (1.003)
11	*Scientific Reports*	18 (1.003)
12	*Pharmaceutics*	17 (0.948)
13	*Frontiers in Pharmacology*	16 (0.892)
14	*Current Stem Cell Research Therapy*	14 (0.780)
15	*Frontiers in Oncology*	12 (0.669)
16	*Biology*	10 (0.557)
17	*Biology Basel*	10 (0.557)
18	*Biomolecules*	10 (0.557)
19	*Molecules*	10 (0.557)
20	*Molecules Basel Switzerland*	10 (0.557)
21	*Applied Sciences Basel*	8 (0.446)
22	*Journal of Clinical Medicine*	8 (0.446)
23	*Molecular Biology Reports*	8 (0.446)
24	*Neural Regeneration Research*	8 (0.446)
25	Other journals, *n* = 834	1113 (62.040)

number of articles published in *WJSC* in 2021 was 18.5% higher than that in 2019-2020 (92 articles/year on average). The authors were from 28 countries/regions, represented by 26 articles (23.9%) from China, 12 (11.0%) from Italy, 8 (7.3%) from the United States, 6 (5.5%) from Brazil, 5 (4.6%) from India, and 52 (47.7%) from other countries/regions (Figure 8).

Number of webpage visits and downloads received by *WJSC* in 2019-2021: From 2019 to 2021, the *WJSC* webpage received a total of 99638 visits in 2019, 160901 in 2020 (an increase of 61.5% compared with that in 2019), and 192193 in 2021 (an increase of 19.4% compared with that in 2020), with visits from more than 180 countries and regions worldwide (Figure 9). The number of downloads was 51642 in 2019, 111236 in 2020 (an increase of 115.3% compared with that in 2019), and 180164 in 2021 (an increase of 62.0% compared with that in 2020), with downloads from more than 120 countries and regions worldwide.

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Diabetes in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Diabetes* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Diabetes* (*WJD*) is 4.560, and the 5-year JIF is 5.370, ranking 62nd among 146 journals in the field of endocrinology & metabolism, located in Q2 (Figure 10). The 2021 JIF of *WJD* increased by 0.797 from its 2020 JIF (3.763).

According to the *RCA* database, *WJD* has a 2022 *JAII* of 24.495, ranking 48th out of 149 journals in the field of endocrinology & metabolism in the *RCA*, with 20331 total citations (92/149) and 830 total articles (124/149).

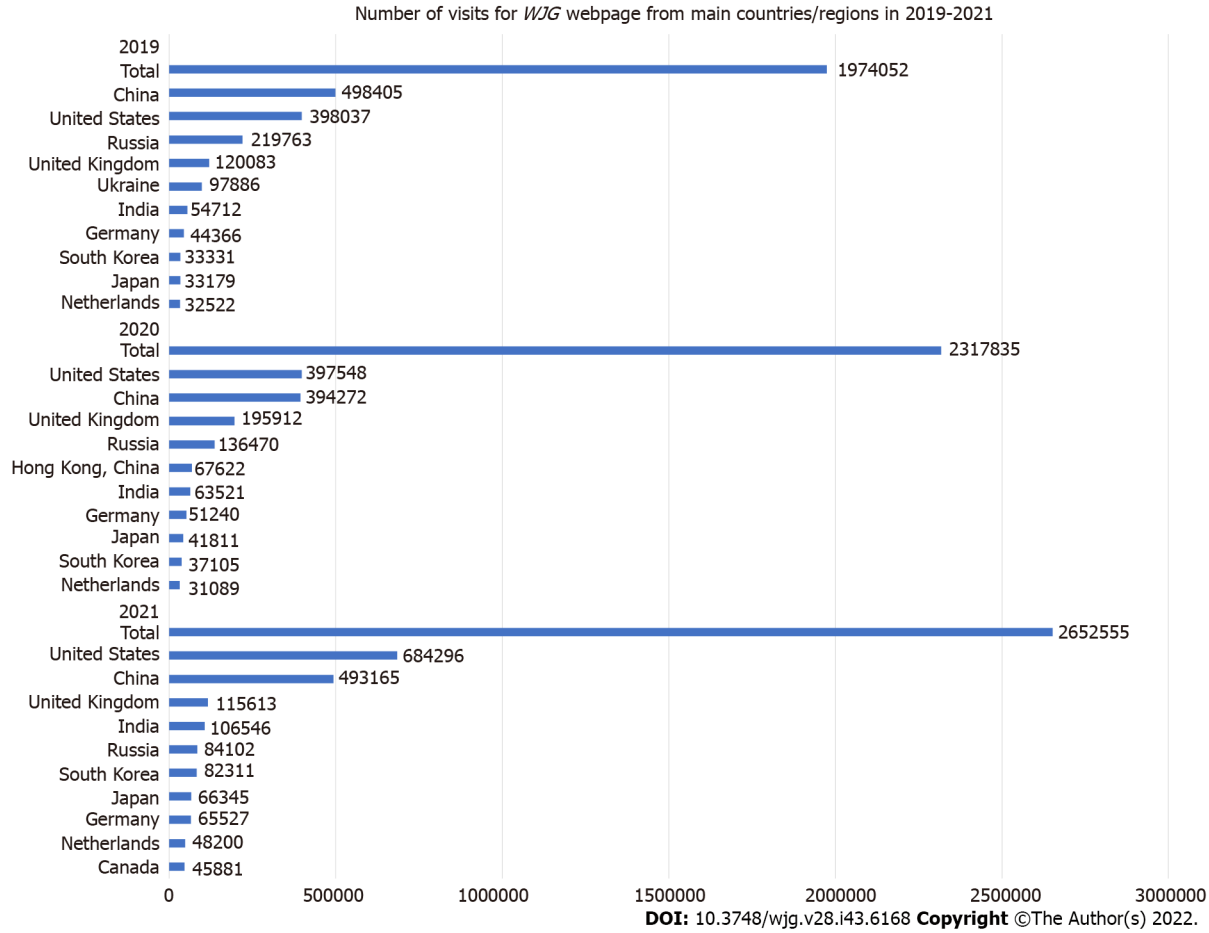

Figure 5 Number of visits to the *World Journal of Gastroenterology* webpage from main countries/regions in 2019-2021. *WJG*: *World Journal of Gastroenterology*.

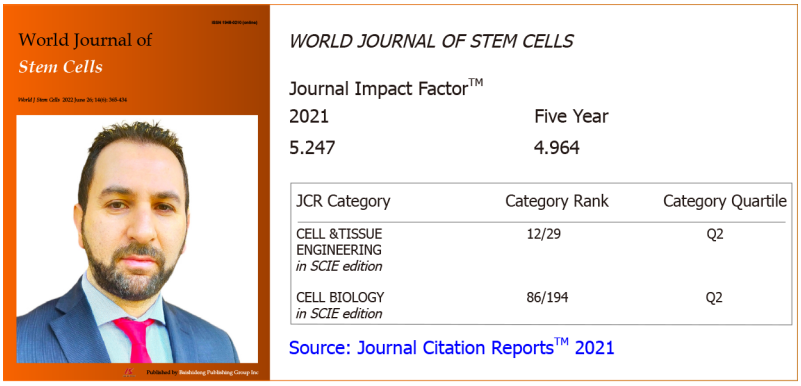

Figure 6 The 2021 Journal Impact Factor and category rank of *World Journal of Stem Cells*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

From 2019 to 2020, *WJD* received a total of 294 articles (average: 147 articles/year), among which 150 (51.0%) were invited and 144 (49.0%) were freely submitted; the acceptance rate was 37.4%. During the same period, *WJD* published 110 articles (average: 55 articles/year), among which 68 (61.8%) were invited and 42 (38.2%) were freely submitted. As of June 28, 2022, the articles published in *WJD* received a total of 964 citations (without self-citations: 959) by 931 articles (without self-citations: 927), yielding a self-citation rate of 0.52%; there were a total of 482 citations in 2021. After excluding self-citations, the 927 articles that cited the *WJD*-published articles were from 669 journals (data from Web of Science, Table 4); among these journals, 30 (4.5%) had a JIF of > 10 (data from Web of Science), accounting for 4.5% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJD*-published articles include renowned academic journals in the field of endocrinology &

Table 4 Rank and record count of journals that published articles that cited the 110 articles published in *World Journal of Diabetes* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*International Journal of Molecular Sciences*	35 (3.776)
2	*Frontiers in Endocrinology*	22 (2.373)
3	*Nutrients*	17 (1.834)
4	*International Journal of Environmental Research and Public Health*	15 (1.618)
5	*Journal of Clinical Medicine*	15 (1.618)
6	*Diabetes Metabolic Syndrome and Obesity Targets and Therapy*	14 (1.510)
7	*Diabetes Metabolic Syndrome*	13 (1.402)
8	*Diabetes Metabolic Syndrome Clinical Research Reviews*	13 (1.402)
9	*Cureus*	9 (0.971)
10	*Diabetes Therapy*	8 (0.863)
11	*Diabetes Therapy Research Treatment and Education of Diabetes and Related Disorders*	8 (0.863)
12	*Antioxidants*	7 (0.755)
13	*Antioxidants Basel Switzerland*	7 (0.755)
14	*BMJ Open Diabetes Research Care*	7 (0.755)
15	*Diabetes Research and Clinical Practice*	7 (0.755)
16	*Medicine*	7 (0.755)
17	*Medicine Hagerstown*	7 (0.755)
18	*Metabolites*	7 (0.755)
19	*PLOS One*	7 (0.755)
20	*Scientific Reports*	7 (0.755)
21	*Biomedicines*	6 (0.647)
22	*Cardiovascular Diabetology*	6 (0.647)
23	*Diabetes Obesity Metabolism*	6 (0.647)
24	*Frontiers in Pharmacology*	6 (0.647)
25	Other journals, *n* = 645	671 (72.384)

metabolism such as *Diabetes Care* (2021 JIF 17.152, record count: 3), *Obesity Reviews* (2021 JIF 10.867, record count: 2), and *Diabetologia* (2021 JIF 10.460, record count: 3).

From 2019 to 2020, the authors of the 110 articles published in *WJD* were from 42 countries/regions, represented by 23 articles (20.9%) from the United States, 18 (16.4%) from China, 8 (7.3%) from India, 6 (5.5%) from Greece, 5 (4.5%) from Turkey, and 50 (45.5%) from other countries/regions (data from Web of Science, Figure 11). Of note, the article entitled "Evaluation of oxidative stress levels in obesity and diabetes by the free oxygen radical test and free oxygen radical defense assays and correlations with anthropometric and laboratory parameters", which was contributed by Dr. Găman *et al*[11] from "Carol Davila" University of Medicine and Pharmacy, Romania in 2020, is the article with the highest number of citations among the articles published in *WJD*. This article focuses on the assessment of oxidative stress in obese and diabetic subjects and the possible correlation between oxidative stress and anthropometric and biochemical parameters. As of June 28, 2022, this article has been cited 39 times.

In 2021, *WJD* received a total of 300 manuscripts, of which 211 (70.3%) were invited and 89 (29.7%) were freely submitted; the acceptance rate was 49.0%, which was 104.1% higher than that in 2019-2020 (average: 147 articles/year). A total of 147 articles were published in *WJD* in 2021, including 111 (75.5%) invited and 36 (24.5%) freely submitted. As of June 28, 2022, the number of total citations was 286. The number of articles published in *WJD* in 2021 was 167.2% higher than that in 2019-2020 (55 articles/year on average). The authors were from 38 countries/regions, represented by 42 articles (28.6%) from China, 12 (8.2%) from the United States, 9 (6.1%) from India, 7 (4.8%) from Italy, 5 (3.4%) from Croatia, 5 (3.4%) from Turkey, and 67 (45.6%) from other countries/regions (Figure 12).

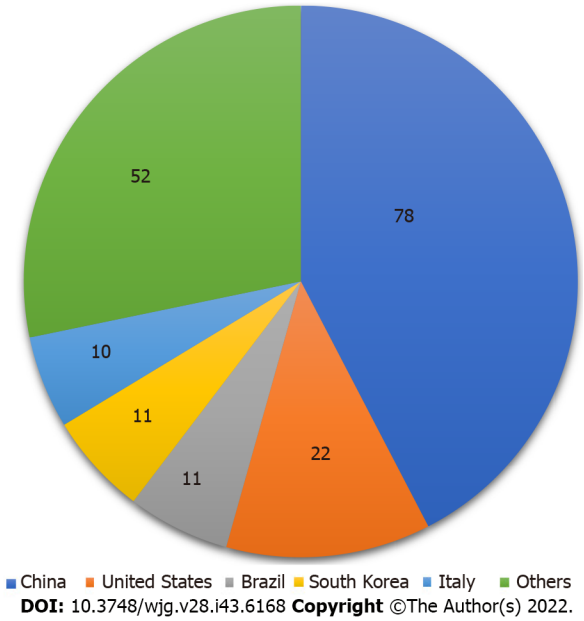

Figure 7 Country sources and number of articles published in *World Journal of Stem Cells* in 2019-2020.

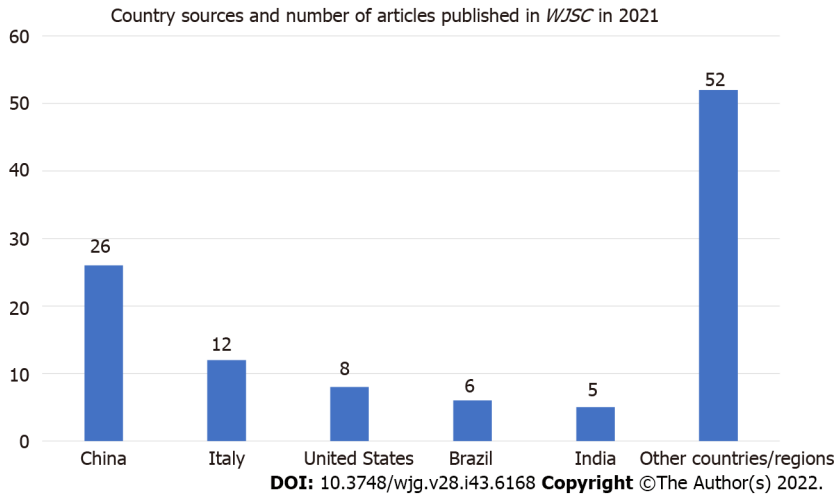

Figure 8 Country sources and number of articles published in *World Journal of Stem Cells* in 2021. *WJSC: World Journal of Stem Cells.*

Number of webpage visits and downloads received by *World Journal of Diabetes* in 2019-2021: From 2019 to 2021, *WJD* website received a total of 129630 visits in 2019, 189456 in 2020 (an increase of 46.2% compared with that in 2019), and 264148 in 2021 (an increase of 39.4% compared with that in 2020), with visits from more than 200 countries and regions worldwide (Figure 13). The number of downloads was 76163 in 2019, 137218 in 2020 (an increase of 80.2% compared with that in 2019), and 239021 in 2021 (an increase of 74.2% compared with that in 2020), with downloads from more than 170 countries and regions worldwide.

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Psychiatry in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Psychiatry* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Psychiatry* (*WJP*) is 3.500, and the 5-year JIF is 7.380, ranking 89th among 155 journals in the field of psychiatry, located in Q3 (Figure 14). The 2021 JIF of *WJP* decreased by 1.071 from its 2020 JIF (4.571).

According to the *RCA* database, *WJP* has a 2022 *JAII* of 15.402, ranking 93rd out of 222 journals in the field of psychiatry in the *RCA*, with 6238 total citations (165/222) and 405 total articles (193/222).

From 2019 to 2020, *WJP* received a total of 112 articles (average: 56 articles/year), among which 60 (53.6%) were invited and 52 (46.4%) were freely submitted; the acceptance rate was 31.3%. During the

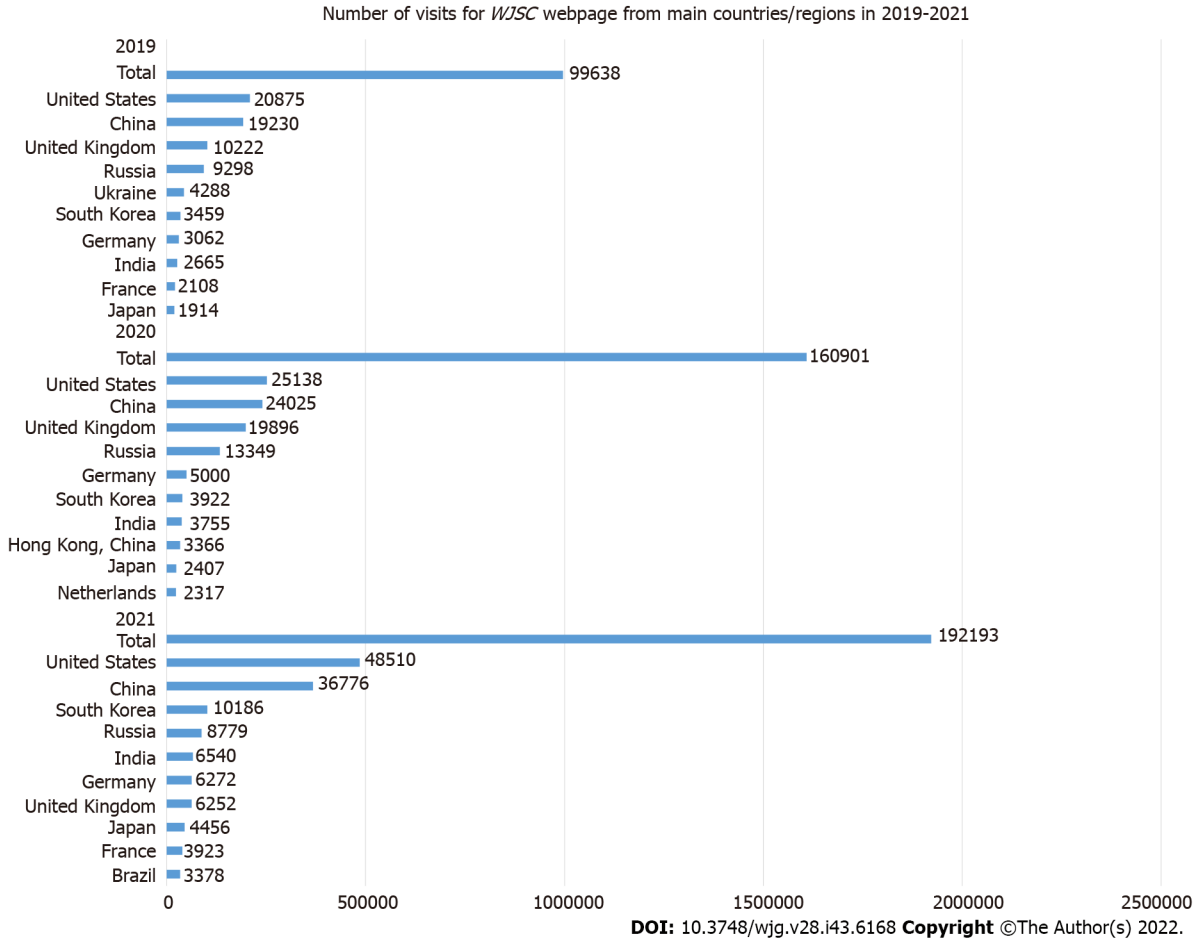

Figure 9 Number of visits to the *World Journal of Stem Cells* webpage from main countries/regions in 2019-2021. *WJSC*: *World Journal of Stem Cells*.

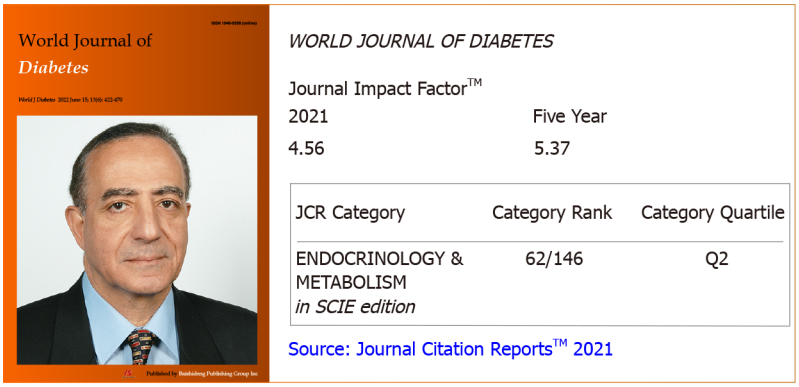

Figure 10 The 2021 Journal Impact Factor and category rank of *World Journal of Diabetes*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

same period, *WJP* published 35 articles (average: 18 articles/year), among which 25 (71.4%) were invited and 10 (28.6%) were freely submitted. As of June 28, 2022, the articles published in *WJP* received a total of 219 citations (without self-citations: 217) by 214 articles (without self-citations: 212), yielding a self-citation rate of 0.91%; there were a total of 121 citations in 2021. After excluding self-citations, the 212 articles that cited the *WJP*-published articles were from 76 journals (data from Web of Science, Table 5); among these journals, 9 (11.8%) had a JIF of > 10 (data from Web of Science), accounting for 1.4% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJP*-published articles include renowned academic journals in the field of psychiatry such as *World Psychiatry* (2021 JIF 79.683, record count: 1), *Asian Journal of Psychiatry* (2021 JIF 13.890, record count: 2), and *Molecular Psychiatry* (2021 JIF 13.437, record count: 1).

Table 5 Rank and record count of journals that published articles that cited the 35 articles published in *World Journal of Psychiatry* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*Frontiers in Psychiatry*	9 (4.245)
2	*Journal of Affective Disorders*	7 (3.302)
3	*Australian & New Zealand Journal of Psychiatry*	6 (1.415)
4	*BMC Psychiatry*	6 (2.830)
5	*International Journal of Environmental Research and Public Health*	6 (2.830)
6	*International Journal of Advanced Computer Science and Applications*	5 (2.358)
7	*European Psychiatry*	4 (1.887)
8	*Brain and Behavior*	3 (1.415)
9	*Journal of Clinical Medicine*	3 (1.415)
10	*Journal of Personalized Medicine*	3 (1.415)
11	*Psychiatria Danubina*	3 (1.415)
12	*Advances in Medical Education and Practice*	2 (0.943)
13	*Asian Journal of Psychiatry*	2 (0.943)
14	*Brain Sciences*	2 (0.943)
15	*Clinical Neuropsychiatry*	2 (0.943)
16	*European Archives of Psychiatry and Clinical Neuroscience*	2 (0.943)
17	*European Neuropsychopharmacology*	2 (0.943)
18	*Frontiers in Neuroscience*	2 (0.943)
19	*Frontiers in Psychology*	2 (0.943)
20	*Indian Journal of Psychiatry*	2 (0.943)
21	*Indian Journal of Psychological Medicine*	2 (0.943)
22	*Journal of Autism and Developmental Disorders*	2 (0.943)
23	Other journals, *n* = 54	135 (63.679)

From 2019 to 2020, the authors of the 35 articles published in *WJP* were from 22 countries/regions, represented by 10 articles (28.6%) from the United States, 8 (22.9%) from China, 3 (8.6%) from Australia, and 14 (40.0%) from other countries/regions (data from Web of Science, Figure 15). Of note, the article entitled "How to construct neuroscience-informed psychiatric classification? Towards nomothetic networks psychiatry", which was contributed by Professor Stoyanov and Maes *et al*[12] from Medical University of Plovdiv, Bulgaria in 2020, is the article with the highest number of citations among the articles published in *WJP*. This article mainly reviews how to construct neuroscience-informed psychiatric classification from the perspective of nomothetic networks psychiatry. As of June 28, 2022, this article has been cited 19 times.

In 2021, *WJP* received a total of 265 manuscripts, of which 222 (83.8%) were invited and 43 (16.2%) were freely submitted; the acceptance rate was 38.9%, which was 373.2% higher than that in 2019-2020 (average: 56/year). A total of 103 articles were published in *WJP* in 2021, including 93 (90.3%) invited and 10 (9.7%) freely submitted. As of June 28, 2022, the number of total citations was 146. The number of articles published in *WJP* in 2021 was 472.2% higher than that in 2019-2020 (18 articles/year on average). The authors were from 28 countries/regions, represented by 11 articles (10.7%) from China, 10 (9.7%) from Italy, 8 (7.8%) from the United States, 5 (4.9%) from Spain, 4 (3.9%) from Austria, 4 (3.9%) from Brazil, and 61 (59.2%) from other countries/regions (Figure 16).

Number of webpage visits and downloads received by *WJP* in 2019-2021: From 2019 to 2021, *WJP* received a total of 47123 visits in 2019, 82197 in 2020 (an increase of 74.4% compared with that in 2019), and 130151 in 2021 (an increase of 58.3% compared with that in 2020), with visits from more than 180 countries and regions worldwide (Figure 17). The number of downloads was 28838 in 2019, 59532 in 2020 (an increase of 106.4% compared with that in 2019), and 113939 in 2021 (an increase of 91.4% compared with that in 2020), with downloads from more than 130 countries and regions worldwide.

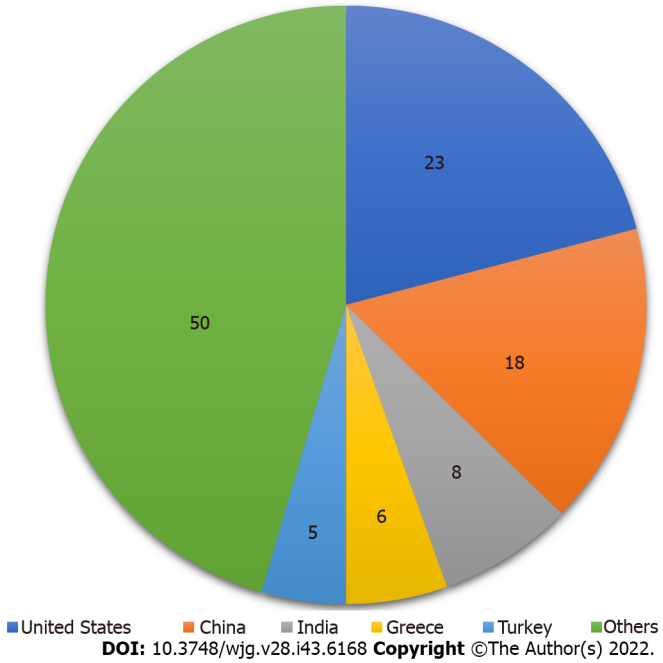

Figure 11 Country sources and number of articles published in *World Journal of Diabetes* in 2019-2020.

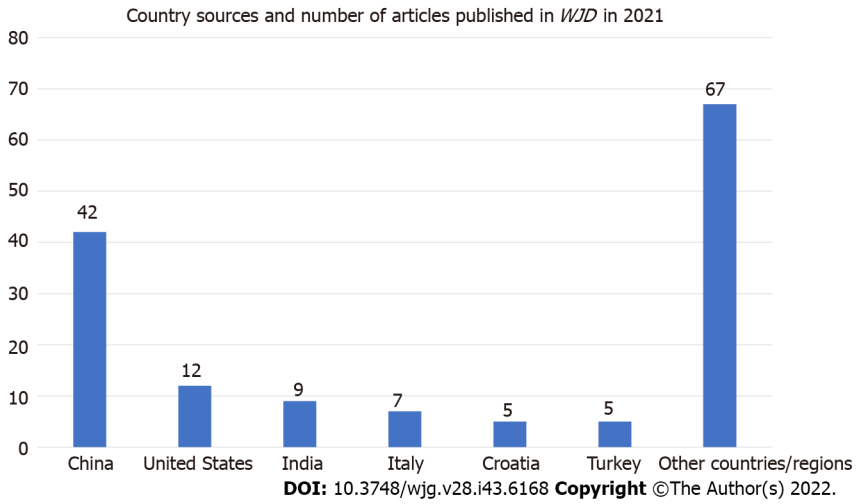

Figure 12 Country sources and number of articles published in *World Journal of Diabetes* in 2021. *WJD: World Journal of Diabetes.*

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Gastrointestinal Oncology in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Gastrointestinal Oncology* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Gastrointestinal Oncology* (*WJGO*) is 3.404, and the 5-year JIF is 3.250, ranking 162nd among 245 journals in the field of oncology, and ranking 59th among 93 journals in the field of cell biology, located in Q3 (Figure 18). The 2021 JIF of *WJGO* increased by 0.011 from its 2020 JIF (3.393).

According to the *RCA* database, *WJGO* has a 2022 *JAII* of 11.592, ranking 57th out of 102 journals in the field of gastroenterology & hepatology in the *RCA*, with 11627 total citations (63/102) and 1003 total articles (74/102).

From 2019 to 2020, *WJGO* received a total of 476 articles (average: 238 articles/year), among which 200 articles (42.0%) were invited and 276 (58.0%) were freely submitted; the acceptance rate was 45.8%. During the same period, *WJGO* published 218 articles (average: 109 articles/year), among which 81 (37.2%) were invited and 137 (62.8%) were freely submitted. As of June 28, 2022, the articles published in *WJGO* received a total of 1543 citations (without self-citations: 1533) by 1495 articles (without self-citations: 1486), yielding a self-citation rate of 0.65%; there were a total of 780 citations in 2021. After excluding self-citations, the 1486 articles that cited the *WJGO*-published articles were from 791 journals

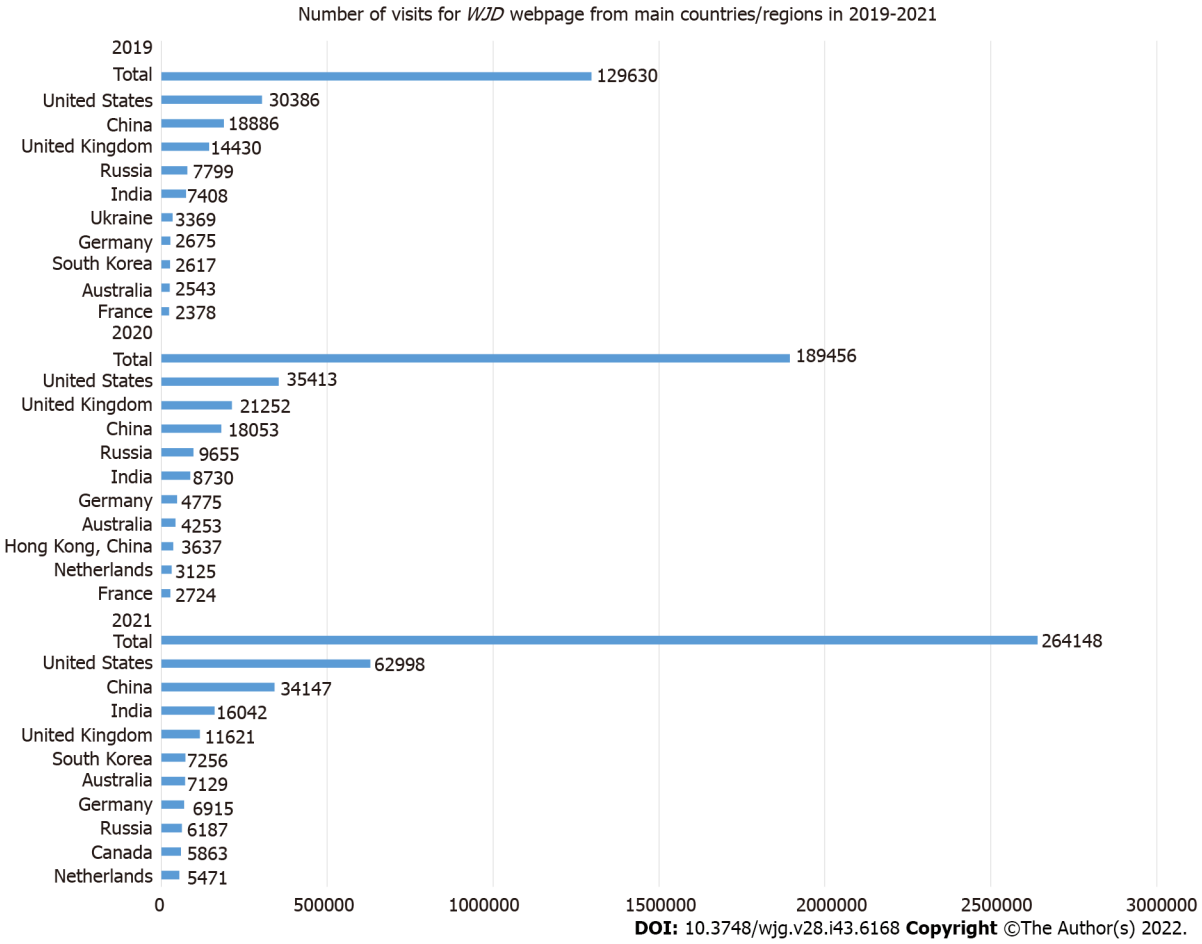

Figure 13 Number of visits to the *World Journal of Diabetes* webpage from main countries/regions in 2019-2021. *WJD*: *World Journal of Diabetes*.

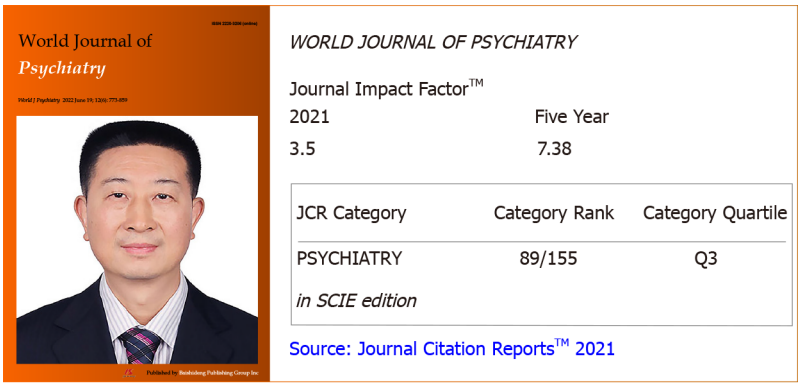

Figure 14 The 2021 Journal Impact Factor and category rank of *World Journal of Psychiatry*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

(data from Web of Science, Table 6); among these journals, 45 (5.7%) had a JIF of > 10 (data from Web of Science), accounting for 6.8% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJGO*-published articles include renowned academic journals in the field of oncology such as *CA-A Cancer Journal for Clinicians* (2021 JIF 286.130, record count: 1), *Nature Reviews Cancer* (2021 JIF 69.800, record count: 1), and *Nature Reviews Clinical Oncology* (2021 JIF 65.011, record count: 1). From 2019 to 2020, the authors of the 218 articles published in *WJGO* were from 31 countries/regions, represented by 112 articles (51.4%) from China, 29 (13.3%) from the United States, 16 (7.3%) from Japan, 10 (4.6%) from Italy, 8 (3.7%) from South Korea, and 43 (19.7%) from other countries/regions (data from Web of Science, Figure 19). Of note, the article entitled "Pancreatic ductal adenocarcinoma: Treatment hurdles, tumor microenvironment and immunotherapy", which was

Table 6 Rank and record count of journals that published articles that cited the 218 articles published in *World Journal of Gastrointestinal Oncology* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*Cancers*	70 (4.711)
2	*Frontiers in Oncology*	64 (4.307)
3	*International Journal of Molecular Sciences*	32 (2.153)
4	*Cells*	21 (1.413)
5	*BMC Cancer*	20 (1.346)
6	*Cancer Management and Research*	17 (1.144)
7	*Aging*	16 (1.077)
8	*Aging US*	16 (1.077)
9	*Biomedicines*	16 (1.077)
10	*Scientific Reports*	16 (1.077)
11	*Frontiers in Cell and Developmental Biology*	15 (1.009)
12	*Journal of Clinical Medicine*	15 (1.009)
13	*World Journal of Clinical Cases*	14 (0.942)
14	*Biomed Research International*	13 (0.875)
15	*European Review for Medical and Pharmacological Sciences*	13 (0.875)
16	*World Journal of Gastroenterology*	13 (0.875)
17	*Journal of Gastrointestinal Oncology*	12 (0.808)
18	*Frontiers in Genetics*	11 (0.740)
19	*Annals of Surgical Oncology*	10 (0.673)
20	*Frontiers in Pharmacology*	10 (0.673)
21	*Oncology Letters*	10 (0.673)
22	*PLOS One*	10 (0.673)
23	*Surgical Endoscopy*	10 (0.673)
24	Other journals, *n* = 768	1042 (70.121)

contributed by Sarantis *et al*[13] from National and Kapodistrian University of Athens, Greece in 2020, is the article with the highest number of citations among the articles published in *WJGO*. This article mainly reviews the latest research advances in the treatment barriers, tumor microenvironment, and immunotherapy for pancreatic ductal adenocarcinoma. As of June 28, 2022, this article has been cited 50 times.

In 2021, *WJGO* received a total of 424 manuscripts, of which 254 (59.9%) were invited and 170 (40.1%) were freely submitted; the acceptance rate was 37.0%, which was 78.2% higher than that in 2019-2020 (average: 238 articles/year). A total of 157 articles were published in *WJGO* in 2021, including 115 (73.2%) invited and 42 (26.8%) freely submitted. As of June 28, 2022, the number of total citations was 156. The number of articles published in *WJGO* in 2021 was 44.0% higher than that in 2019-2020 (109 articles/year on average). The authors were from 33 countries/regions, represented by 62 articles (39.5%) from China, 15 (9.6%) from Italy, 14 (8.9%) from the United States, 10 (6.4%) from Japan, 5 (3.2%) from South Korea, and 51 (32.5%) from other countries/regions (Figure 20).

Number of webpage visits and downloads received by *WJGO* in 2019-2021: From 2019 to 2021, the *WJGO* webpage received a total of 122230 visits in 2019, 179298 in 2020 (an increase of 46.7% compared with that in 2019), and 197781 in 2021 (an increase of 10.3% compared with that in 2020), with visits from more than 180 countries and regions worldwide (Figure 21). The number of downloads was 77505 in 2019, 165794 in 2020 (an increase of 113.9% compared with that in 2019), and 266672 in 2021 (an increase of 60.8% compared with that in 2020), with downloads from more than 130 countries and regions worldwide.

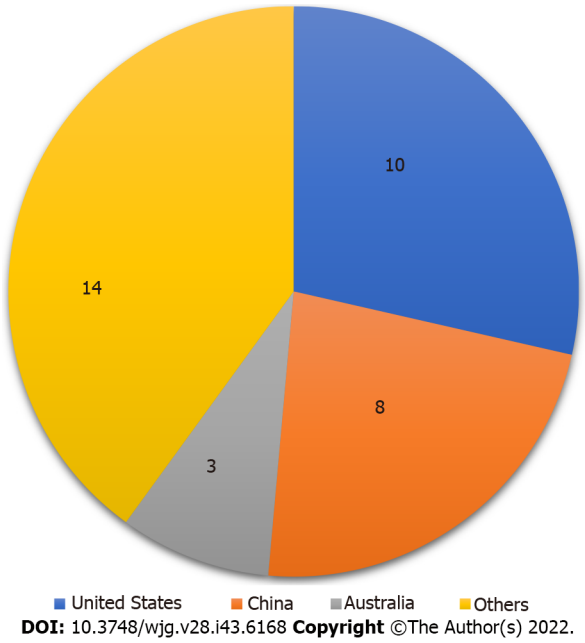

Figure 15 Country sources and number of articles published in *World Journal of Psychiatry* in 2019-2020.

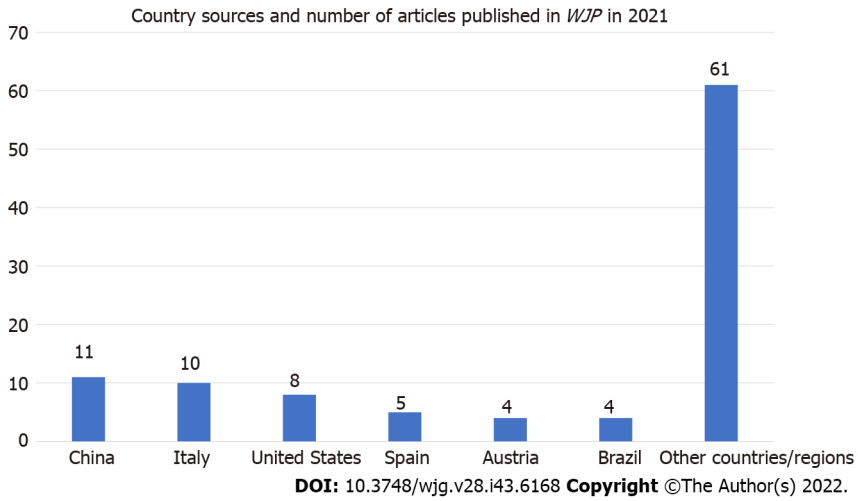

Figure 16 Country sources and number of articles published in *World Journal of Psychiatry* in 2021. *WJP*: *World Journal of Psychiatry*.

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Gastrointestinal Surgery in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Gastrointestinal Surgery* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Gastrointestinal Surgery* (*WJGS*) is 2.505, and the 5-year JIF is 3.099, ranking 104th among 211 journals in the field of surgery, located in Q2, and ranking 81st among 93 journals in the field of gastroenterology & hepatology, located in Q4 (Figure 22). The 2021 JIF of *WJGS* decreased by 0.077 from its 2020 JIF (2.582).

According to the *RCA* database, *WJGS* has a 2022 *JAII* of 10.615, ranking 62nd out of 102 journals in the field of gastroenterology & hepatology in the *RCA*, with 8694 total citations (71/102) and 819 total articles (80/102).

From 2019 to 2020, *WJGS* received a total of 297 articles (average: 149 articles/year), among which 113 (38.0%) were invited and 184 (62.0%) were freely submitted; the acceptance rate was 33.7%. During the same period, *WJGS* published 100 articles (average: 50 articles/year), among which 50 (50.0%) were invited and 50 (50.0%) were freely submitted. As of June 28, 2022, the articles published in *WJGS* received a total of 483 citations (without self-citations: 482) by 473 articles (without self-citations: 472), yielding a self-citation rate of 0.21%; there were a total of 242 citations in 2021. After excluding self-citations, the 472 articles that cited the *WJGS*-published articles were from 341 journals (data from Web of Science, Table 7); among these journals, 11 (3.2%) had a JIF of > 10 (data from Web of Science),

Table 7 Rank and record count of journals that published articles that cited the 100 articles published in *World Journal of Gastrointestinal Surgery* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*Frontiers in Oncology*	13 (2.754)
2	*Surgical Endoscopy*	13 (2.754)
3	*Surgical Endoscopy and Other Interventional Techniques*	13 (2.754)
4	*Cancers*	12 (2.542)
5	*Cureus*	11 (2.331)
6	*HPB*	10 (2.119)
7	*Journal of Gastrointestinal Surgery*	10 (2.119)
8	*Langenbeck's Archives of Surgery*	10 (2.119)
9	*Journal of Clinical Medicine*	9 (1.907)
10	*Diseases of the Esophagus*	8 (1.695)
11	*Updates in Surgery*	8 (1.695)
12	*World Journal of Clinical Cases*	7 (1.483)
13	*BMJ Case Reports*	6 (1.271)
14	*Frontiers in Surgery*	6 (1.271)
15	*Annals of Coloproctology*	5 (1.059)
16	*BMC Surgery*	5 (1.059)
17	*Egyptian Journal of Surgery*	5 (1.059)
18	*Indian Journal of Surgery*	5 (1.059)
19	*Scientific Reports*	5 (1.059)
20	*Annals of Coloproctology*	4 (0.847)
21	*Cureus Journal of Medical Science*	4 (0.847)
22	Other journals, *n* = 320	303 (64.195)

accounting for 1.7% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJGS*-published articles include renowned academic journals in the field of surgery such as *JAMA Surgery* (2021 JIF 16.681, record count: 1) and *British Journal of Surgery* (2021 JIF 11.122, record count: 1).

From 2019 to 2020, the authors of the 100 articles published in *WJGS* were from 31 countries/regions, represented by 18 articles (18.0%) from the United States, 12 (12.0%) from China, 8 (8.0%) from Japan, 7 (7.0%) from Greece, 7 (7.0%) from Italy, and 48 (48.0%) from other countries/regions (data from Web of Science, Figure 23). Of note, the article entitled "Carbohydrate antigen 19-9 - tumor marker: Past, present, and future", which was contributed by Dr. Lee *et al*[14] from Nanyang Technological University, Singapore in 2020, is the article with the highest number of citations among the articles published in *WJGS*. This article mainly reviews the related research on the tumor marker carbohydrate antigen 19-9. As of June 28, 2022, this article has been cited 31 times.

In 2021, *WJGS* received a total of 274 manuscripts, of which 141 (51.5%) were invited and 133 (48.5%) were freely submitted; the acceptance rate was 50.4%, which was 83.9% higher than that in 2019-2020 (average: 149 articles/year). A total of 138 articles were published in *WJGS* in 2021, including 86 (62.3%) invited and 52 (37.7%) freely submitted. As of June 28, 2022, the number of total citations was 119. The number of articles published in *WJGS* in 2021 was 176.0% higher than that in 2019-2020 (50 articles/year on average). The authors were from 30 countries/regions, represented by 47 articles (34.1%) from China, 16 (11.6%) from Italy, 12 (8.7%) from Japan, 8 (5.8%) from the United States, 5 (3.6%) from Singapore, 5 (3.6%) from Turkey, and 45 (32.6%) from other countries/regions (Figure 24).

Number of webpage visits and downloads received by *WJGS* in 2019-2021: From 2019 to 2021, the *WJGS* webpage received a total of 110712 visits in 2019, 137115 in 2020 (an increase of 23.8% compared with that in 2019), and 178025 in 2021 (an increase of 29.8% compared with that in 2020), with visits from more than 190 countries and regions worldwide (Figure 25). The number of downloads was 60397 in 2019, 111880 in 2020 (an increase of 85.2% compared with that in 2019), and 188632 in 2021 (an increase of 68.6% compared with that in 2020), with downloads from more than 150 countries and

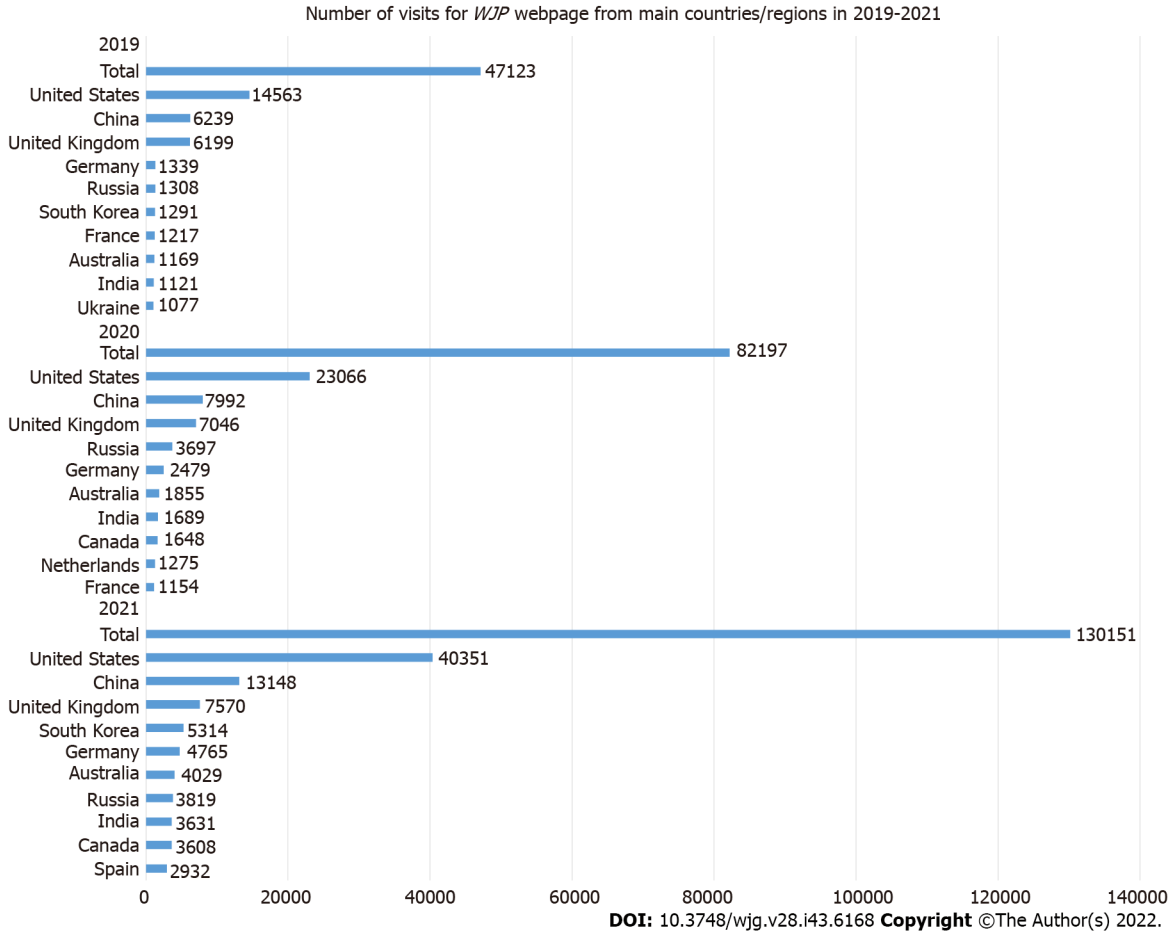

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Figure 17 Number of visits to the *World Journal of Psychiatry* webpage from main countries/regions in 2019-2021. *WJP*: *World Journal of Psychiatry*.

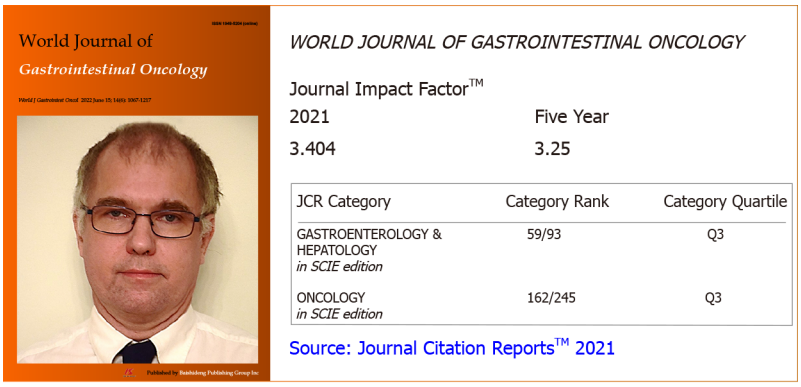

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Figure 18 The 2021 Journal Impact Factor and category rank of *World Journal of Gastrointestinal Oncology*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

regions worldwide.

The 2021 JIF, number of received and published articles, citations, and number of webpage visits and downloads for World Journal of Clinical Cases in 2019-2021

The 2021 JIF, number of received and published articles, and citations for *World Journal of Clinical Cases* in 2019-2021: According to the *JCR* 2022, the 2021 JIF of *World Journal of Clinical Cases* (*WJCC*) is 1.534, and the 5-year JIF is 1.599, ranking 135th among 172 journals in the field of general & internal medicine, located in Q4 (Figure 26). The 2021 JIF of *WJCC* increased by 0.197 from its 2020 JIF (1.337).

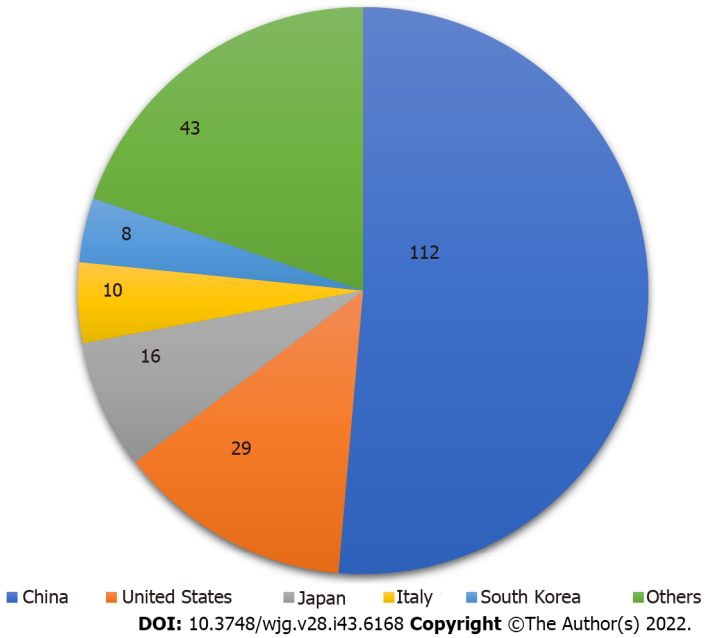

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Figure 19 Country sources and number of articles published in *World Journal of Gastrointestinal Oncology* in 2019-2020.

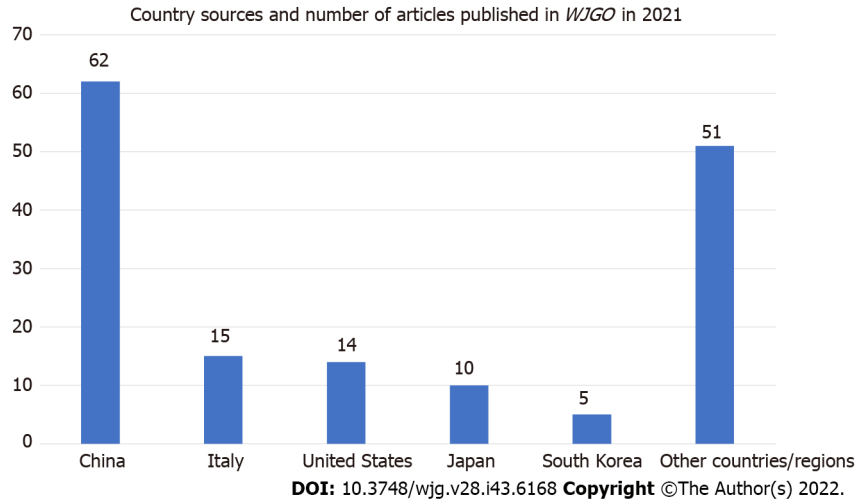

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Figure 20 Country sources and number of articles published in *World Journal of Gastrointestinal Oncology* in 2021. *WJGO*: *World Journal of Gastrointestinal Oncology*.

According to the *RCA* database, *WJCC* has a 2022 *JAII* of 3.046, ranking 174th out of 177 journals in the field of general & internal medicine in the *RCA*, with 12852 total citations (91/177) and 4219 total articles (52/177).

From 2019 to 2020, *WJCC* received a total of 3820 articles (average: 1910 articles/year), among which 485 (38.0%) were invited and 3335 (62.0%) were freely submitted; the acceptance rate was 33.1%. During that same period, *WJCC* published 1263 articles (average: 632 articles/year), among which 228 (18.1%) were invited and 1035 (81.9%) were freely submitted. As of June 28, 2022, the articles published in *WJCC* received a total of 3966 citations (without self-citations: 3933) by 3842 articles (without self-citations: 3815), yielding a self-citation rate of 0.83%; there were a total of 2112 citations in 2021. After excluding self-citations, the 3815 articles that cited the *WJCC*-published articles were from 2298 journals (data from Web of Science, Table 8); among these journals, 92 (4.0%) had a JIF of > 10 (data from Web of Science), accounting for 13.9% of the 660 total journals that had received a JIF of > 10 in the *JCR* 2022. Moreover, the journals citing the *WJCC*-published articles include renowned academic journals in the field of general & internal medicine such as *New England Journal of Medicine* (2021 JIF 176.079, record count: 1), *Annals of Internal Medicine* (2021 JIF 51.598, record count: 1), and *JAMA Internal Medicine* (2021 JIF 44.409, record count: 2).

Table 8 Rank and record count of journals that published articles that cited the 1263 articles published in *World Journal of Clinical Cases* in 2019-2020, *n* (%)

Rank	Publication title	Record count
1	*Cureus*	63 (1.651)
2	*International Journal of Molecular Sciences*	54 (1.415)
3	*Journal of Clinical Medicine*	44 (1.153)
4	*Medicine*	39 (1.022)
5	*Medicine Hagerstown*	39 (1.022)
6	*Frontiers in Oncology*	36 (0.944)
7	*Cancers*	31 (0.813)
8	*World Journal of Gastroenterology*	30 (0.786)
9	*Cureus Journal of Medical Science*	29 (0.760)
10	*International Journal of Environmental Research and Public Health*	29 (0.760)
11	*Scientific Reports*	29 (0.760)
12	*BMJ Case Reports*	24 (0.629)
13	*Diagnostics*	24 (0.629)
14	*Diagnostics Basel Switzerland*	24 (0.629)
15	*Nutrients*	23 (0.603)
16	*PLOS One*	22 (0.577)
17	*BMC Gastroenterology*	20 (0.524)
18	*Frontiers in Medicine*	20 (0.524)
19	*American Journal of Translational Research*	19 (0.498)
20	*Abdominal Radiology*	18 (0.472)
21	*Abdominal Radiology New York*	18 (0.472)
22	*Biomedicines*	16 (0.419)
23	*Cells*	16 (0.419)
24	*Frontiers in Immunology*	16 (0.419)
25	Other journals, *n* = 2274	3132 (82.097)

From 2019 to 2020, the authors of the 1263 articles published in *WJCC* were from 59 countries/regions, represented by 905 articles (71.7%) from China, 69 (5.5%) from South Korea, 55 (4.4%) from the United States, 49 (3.9%) from Japan, 43 (3.4%) from Italy, and 142 (11.2%) from other countries/regions (data from Web of Science, Figure 27). Of note, the article entitled "Fear can be more harmful than the severe acute respiratory syndrome coronavirus 2 in controlling the corona virus disease 2019 epidemic", which was contributed by Chief Physician Ren *et al*[15] from Beijing Aerospace General Hospital, China in 2020, is the article with the highest number of citations among the articles published in *WJCC*. This article mainly reviews the discrimination and prejudice caused by the fear of disease or misinformation after the outbreak of COVID-19. As of June 28, 2022, this article has been cited 146 times.

In 2021, *WJCC* received a total of 3650 manuscripts, of which 233 (6.4%) were invited and 3417 (93.6%) were freely submitted; the acceptance rate was 35.5%, which was 91.1% higher than that in 2019-2020 (average: 1910 articles/year). A total of 1296 articles were published in *WJCC* in 2021, including 136 (10.5%) invited and 1160 (89.5%) freely submitted. As of June 28, 2022, the number of total citations was 563. The number of articles published in *WJCC* in 2021 was 105.1% higher than that in 2019-2020 (632 articles/year on average). The authors were from 45 countries/regions, represented by 982 articles (75.8%) from China, 80 (6.2%) from South Korea, 38 (2.9%) from Japan, 32 (2.5%) from Taiwan, 20 (1.5%) from the United States, and 144 (11.1%) from other countries/regions (Figure 28).

Number of webpage visits and downloads received by *WJCC* in 2019-2021: From 2019 to 2021, the *WJCC* webpage received a total of 363959 visits in 2019, 712451 in 2020 (an increase of 95.8% compared with that in 2019), and 908002 in 2021 (an increase of 27.4% compared with that in 2020), with visits from more than 210 countries and regions worldwide (Figure 29). The number of downloads was 174808

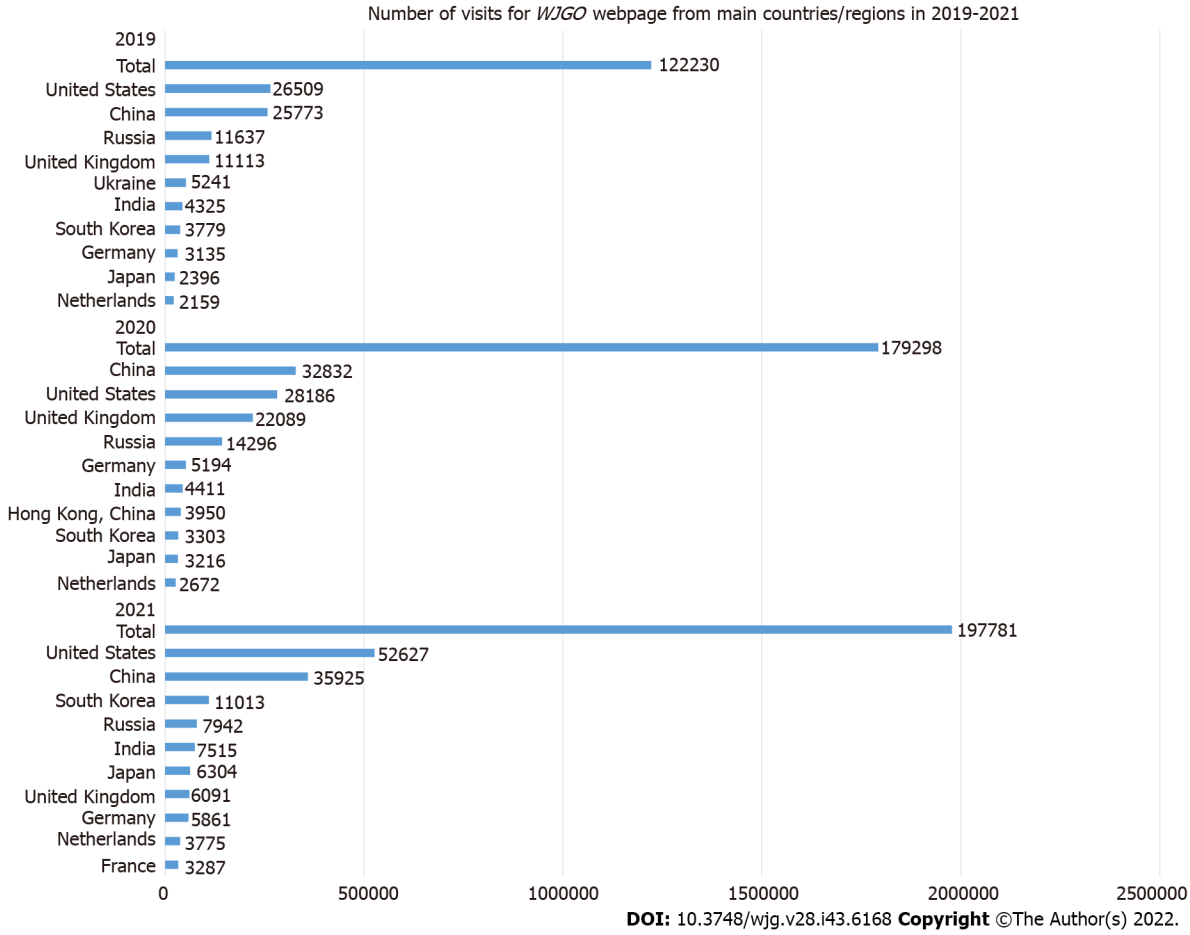

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Figure 21 Number of visits to the *World Journal of Gastrointestinal Oncology* webpage from main countries/regions in 2019-2021. *WJGO*: *World Journal of Gastrointestinal Oncology*.

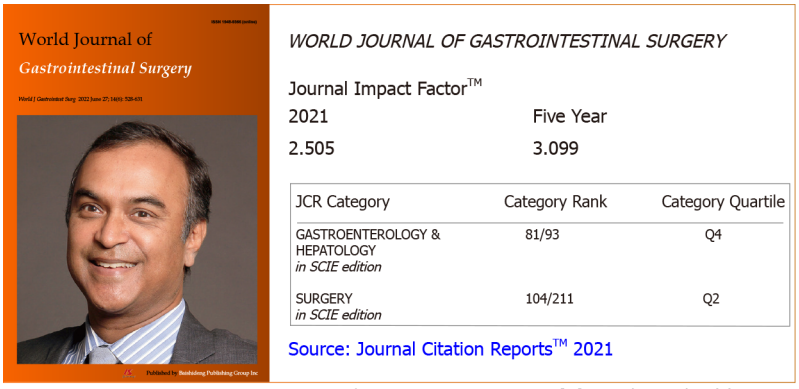

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Figure 22 The 2021 Journal Impact Factor and category rank of *World Journal of Gastrointestinal Surgery*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

in 2019, 442635 in 2020 (an increase of 153.2% compared with that in 2019), and 128.1% in 2021 (an increase of 52.6% compared with that in 2020), with downloads from more than 180 countries and regions worldwide.

Number of non-conforming submissions identified in the preview of submissions to the seven journals in 2019-2021

From 2019 to 2021, a total of 16618 articles were submitted by the authors to Baishideng's seven SCIE-indexed journals. After preview by the Baishideng editorial office, 856 manuscripts (5.2%) were found not to meet the requirements of Baishideng for receiving manuscripts, and the authors failed to modify

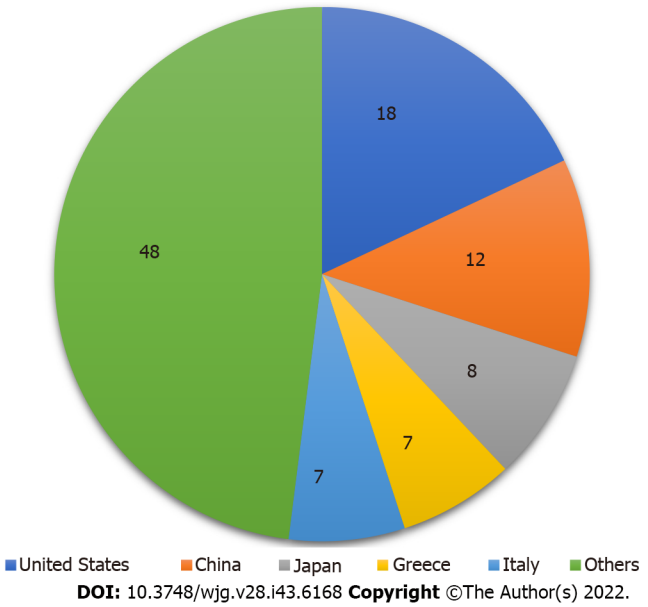

Figure 23 Country sources and number of articles published in *World Journal of Gastrointestinal Surgery* in 2019-2020.

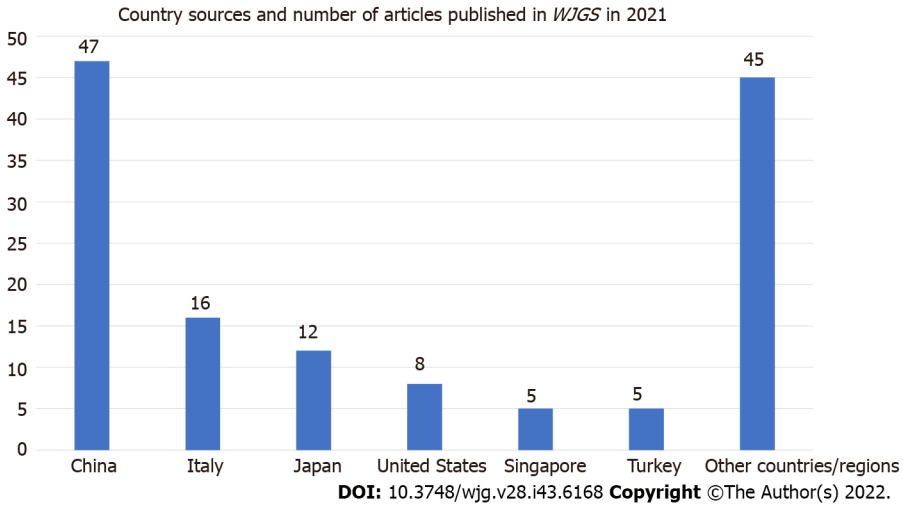

Figure 24 Country sources and number of articles published in *World Journal of Gastrointestinal Surgery* in 2021. *WJGS*: *World Journal of Gastrointestinal Surgery*.

and supplement the relevant documents within the prescribed time. As a result, these manuscripts failed to pass the submission preview and were not sent for peer review.

Number of peer reviewers and editorial board members involved in peer review of manuscripts submitted to the seven journals in 2019-2021

From 2019 to 2021, a total of 15762 manuscripts submitted to Baishideng's seven SCIE-indexed journals passed manuscript preview, and a total of 463297 peer reviews were performed by invited peer reviewers or editorial board members. Among these individuals, 30866 (6.7%) completed the peer review successfully, 66836 (14.4%) rejected the invitation to conduct peer review, 17976 (3.9%) agreed to conduct the peer review but failed to submit the peer review report on time, and 347619 (75.0%) failed to respond to the invitation. The success rate of manuscript peer review was low. We found that some of the peer reviewers' comments on the manuscript were only one-sentence in length, with no explanation for their suggestion of acceptance of the manuscript and no changes to the manuscript, and comments providing no guiding value in improving the quality of the manuscript (*e.g.*, "This is an interesting study. Recommend an acceptance.", "This manuscript is very well written.", "I recommend reject the study.", *etc*). Baishideng acknowledged all the peer reviewers and editorial board members who completed the manuscript peer review online, on the publisher's home page. For more details, please visit: https://www.f6publishing.com/highlyinfluentialpeerreviewers.

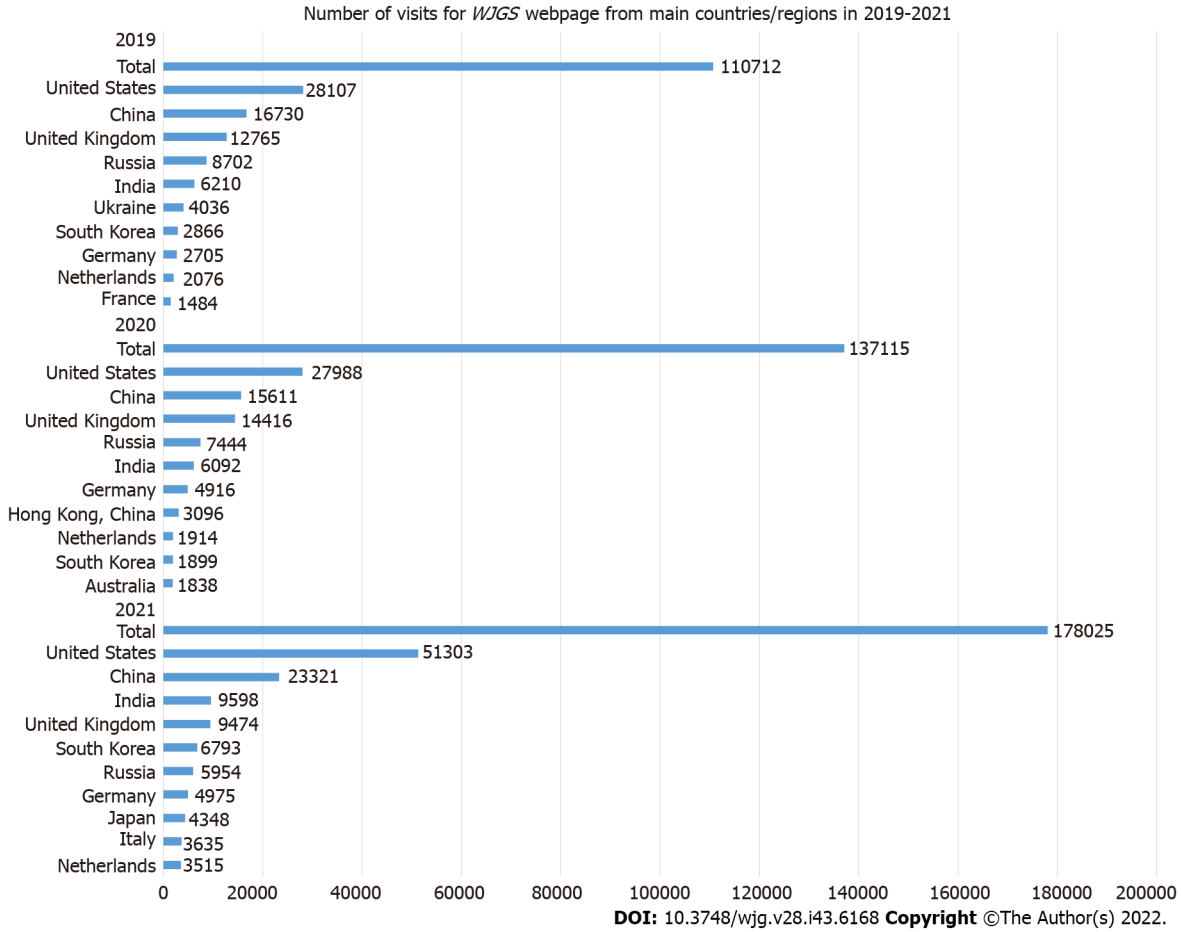

Figure 25 Number of visits to the *World Journal of Gastrointestinal Surgery* webpage from main countries/regions in 2019-2021. *WJGS*: *World Journal of Gastrointestinal Surgery*.

Figure 26 The 2021 Journal Impact Factor and category rank of *World Journal of Clinical Cases*. *JCR*: *Journal Citation Report*™; SCIE: Science Citation Index Expanded™.

Number of manuscripts linguistically polished in the seven journals in 2019-2021

From 2019 to 2021, a total of 5543 manuscripts were accepted and published online in Baishideng's seven SCIE-indexed journals. The grammatical content of all manuscripts submitted by non-native English speakers was verified based on Baishideng's manuscript language editing policy (https://www.wjgnet.com/bpg/gerinfo/240). Among them, 3406 (61.4%) were polished by Baishideng's language editors.

All seven journals adopt an OA publishing model

All seven of Baishideng's SCIE-indexed journals adopt the OA publishing model, that is, authors pay to

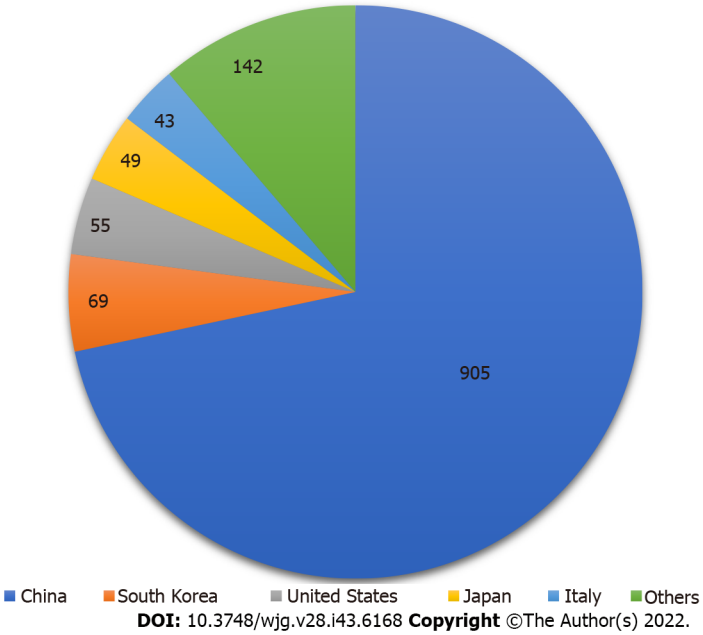

Figure 27 Country sources and number of articles published in *World Journal of Clinical Cases* in 2019-2020.

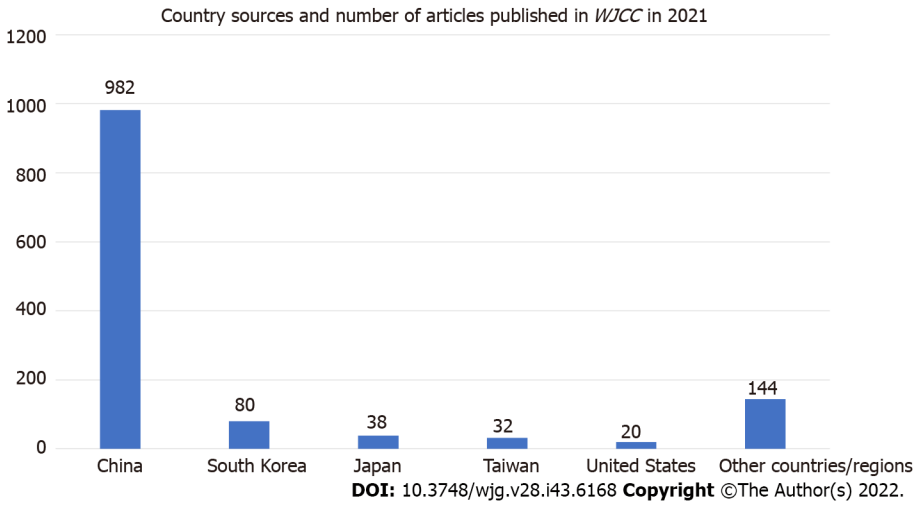

Figure 28 Country sources and number of articles published in *World Journal of Clinical Cases* in 2021. *WJCC*: *World Journal of Clinical Cases*.

publish and readers read for free. A total of 5543 articles were published in the seven journals from 2019 to 2021, all of which are OA articles with unlimited length, unlimited number of figures and tables, and unlimited number of references and citations, available for all readers to access and download online. Of these, 2083 (37.6%) articles were invited and published free of article processing charge (APC). At the same time, Baishideng has an APC discount policy, in which the APC will be reduced by 10% if the free contribution meets one of the following conditions: (1) Supported by a fund of national level or above; (2) Having a corresponding author who is a member of an association of national level or above; (3) Having a first author who is a young scholar under 45 years old; or (4) Having a corresponding author who is a member of the editorial board or who serves as a peer reviewer.

Quality tracking for articles published in the seven journals in 2019-2021

As of June 28, 2022, Baishideng has received 3903 quality-tracking evaluation reports from members of the Baishideng journal editorial boards for 5543 articles published in the seven journals from 2019 to 2021. The Baishideng editorial office verified and replied to every article tracking evaluation report received, and dealt with the important issues found and feedback by the editorial board members in a timely manner. Among them, 3765 reports (96.5%) gave positive comments or did not provide comments, 105 (2.7%) proposed revision opinions or pointed out article limitations, and 33 (0.8%) proposed editing and production quality problems. The Baishideng editorial office has asked the

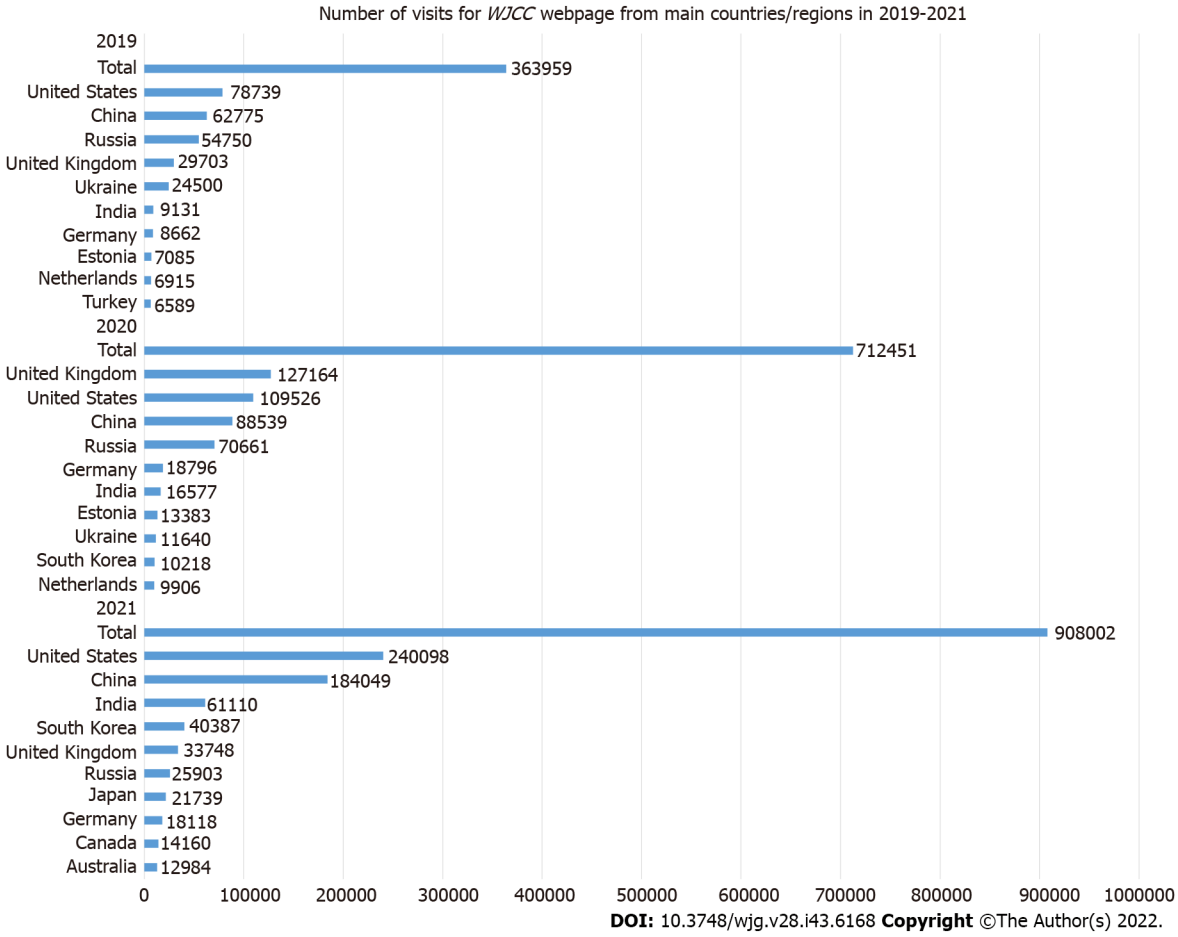

Figure 29 Number of visits to the *World Journal of Clinical Cases* webpage from main countries/regions in 2019-2021. *WJCC*: *World Journal of Clinical Cases*.

authors to clarify the problems raised by reviewers or write readers' letters for further discussion. The production department has verified and consequently revised the highlighted problems in the production process of articles.

Author evaluation for articles published in the seven journals in 2019-2021

As of June 28, 2022, Baishideng has received evaluation and feedback from the authors for 4655 (84.0%) of the 5543 articles published in the seven journals from 2019 to 2021. The Baishideng editorial office promptly verified and replied to the authors' comments received for each article. The vast majority of authors commented positively on Baishideng's peer review, as well as the editing and publishing process. As for the problems pointed out by some authors, the editorial office has verified each one-by-one and dealt with them accordingly. As for the suggestions given by the authors, we consequently organized discussions, reasonably optimized the publishing process of manuscripts, and improved the guidelines for authors.

Reader evaluation of articles published in the seven journals in 2019-2021

Since the launch of the reader evaluation system on March 25, 2021 to June 28, 2022, Baishideng's seven SCIE-indexed journals have received a total of 424 reader evaluations and 229 manuscripts of letters from readers. The Baishideng editorial office carefully read and responded to every reader comment received. For online reader comments with academic value, the Baishideng editorial office invited the commentors to write them as formal reader letters (Letters to the Editor) and submit them online through the F6Publishing system. In 2021, a total of 38 peer-reviewed Letters to the Editor were published online in Baishideng's journals.

DISCUSSION

JIF is still one of the most commonly used criterion for judging the quality of academic journals, although it has faced controversies since its inception. Regardless, increasing the JIF of academic

journals is an effective way of attracting high-quality contributions from scholars. How to improve the JIF of academic journals remains the primary issue of concern for academic journal publishers. Among the various factors influencing the JIF of academic journals, whether an academic journal is an OA journal and the integrity of its editing and publishing process exert substantive impacts on the number of articles it receives and subsequently publishes, the number of webpage visits and downloads it receives, and thus its JIF and academic influence in the overall discipline and topical field. The complete editing and publishing process of Baishideng's seven journals is introduced and discussed in detail in the following subsections.

Manuscript preview

For most academic journals worldwide, manuscripts are firstly previewed by the internal editors. It is the internal editors who usually decide to directly reject the manuscript or send it to the external reviewers. However, Baishideng journals are previewed in a different way. When the editorial office receives an author's contribution, the in-house assistant editor in the editorial office will verify whether the online submitted manuscript meets the requirements for Baishideng's formal receipt of a manuscript, including verification of whether the content of the manuscript falls within the scope of the journal, whether the basic information on the authors is correct (including the names, E-mail addresses, and ORCID information of all authors), and whether the manuscript document and the documents on ethics and academic rules and norms for the column that the manuscript belongs to are correctly provided [*i.e.*, (1) Institutional Review Board Approval Form or Document; (2) Institutional Animal Care and Use Committee Approval Form or Document; (3) Biostatistics Review Certificate; (4) Signed Informed Consent Form(s) or Document(s); and (5) Non-Native Speakers of English Editing Certificate]. If any of the above items do not meet the requirements, the manuscript will be returned to the authors for revision and resubmission; if the authors do not resubmit the manuscript within 7 d and do not reply with a reason, the manuscript will be rejected. If the manuscript meets the requirements, the F6Publishing artificial intelligence system will search the Baishideng database to identify relevant editorial board members and peer reviewers based on the keywords of the manuscript, and then invite five of the journal's editorial board members and 15 external peer reviewers to perform a single-blind peer review of the manuscript. At the same time, the in-house science editor will invite 3-5 members of the journal's editorial board to conduct a single-blind peer review of the manuscript.

Journals should have a policy for safeguarding research data submitted to them and to detect research misconduct and ensure the accuracy and reliability of the information published[16]. The main purpose of this step is to intercept submissions at risk of academic misconduct, prior to peer review. From 2019 to 2021, 5.2% of the submissions to Baishideng's seven SCIE-indexed journals were rejected at this stage, thus excluding the research that does not conform to the academic rules and norms, and effectively reducing the risk of publishing articles that may have academic misconduct. Compared with the figures in 2019-2020, the number of received articles by the seven journals in 2021 all increased (by 5.8% for *WJG*, 8.6% for *WJSC*, 104.1% for *WJD*, 373.2% for *WJP*, 78.2% for *WJGO*, 83.9% for *WJGS*, and 91.1% for *WJCC*). This increase in the number of articles received provides a solid foundation for the selection of high-quality articles published in the journals.

Manuscript peer review and first decision

Peer reviewers, in accordance with ethical norms for peer review, conduct a single-blind peer review of manuscripts submitted to Baishideng's journals to assess their scientific and linguistic quality. It is of critical importance to prevent citation manipulation by peer reviewers, and as such they are requested to cautiously recommend authors to cite their own published papers. If a peer reviewer recommends the author to cite his/her own papers, he/she must include a specific rationale as to why their inclusion is merited, so that the author can decide rationally on whether or not to cite the paper. If the peer reviewer cannot provide sufficient reasons for the request, their opinion will be deemed invalid, the editorial office will delete this comment and close the peer reviewer's account. In addition, the manuscript will be sent to other peer reviewers to review.

The peer reviewers' evaluation criteria for the scientific quality and language quality of the manuscript are as follows: (1) Scientific quality. Peer reviewers classify the scientific quality of the manuscript as Grade A (Excellent), Grade B (Very good), Grade C (Good), Grade D (Fair), or Grade E (Do not publish); (2) Language quality. Peer reviewers classify the language quality of the manuscript as Grade A (Priority publishing), Grade B (Minor language polishing), Grade C (A great deal of language polishing), or Grade D (Rejection); and (3) Conclusion. The conclusions and recommendations of peer reviewers on the manuscript are (A) Accept (High priority), (B) Accept (General priority), (C) Minor revision, (D) Major revision, and (E) Rejection. The reviewer will also state whether they would like to re-review the manuscript once it has been revised by the authors.

After a manuscript is peer reviewed by at least two peer-reviewer(s) and/or editorial board member(s), the first decision will be made by an Academic Editor (external), and an evaluation report of the first decision will be provided to the authors. The main task of this work is to verify and evaluate the academic and language quality of manuscripts, and suggest a first decision. The Academic Editors (external) are advised to consider the peer-review report; however, the comments by reviewers represent only a part of the information they will consider in making a first decision. The Academic

Editors (external) should also evaluate the manuscript based on the following: (1) Their own knowledge of the topic; (2) The manuscript's theme; (3) The main content of the manuscript; (4) The resolution of the figures and readability of the tables; (5) The appropriateness of the reference citations and the authors self-citations; (6) The language quality; (7) The medical ethics of the study; and (8) The study's adherence to academic norms. Then, the Academic Editors (external) will independently decide to accept or reject the manuscript, or suggest to the authors a transfer of the manuscript to another of Baishideng's journals that is more appropriate in scope and/or topic. They will also provide a summary of this decision, in order to help the authors and the journal to further improve the academic quality of the manuscript.

If the Academic Editor (external) decides to accept the manuscript, the Company Editor-in-Chief (internal) will review whether the manuscript conforms to academic ethics, rules and norms, and make the first decision to accept or reject the manuscript.

The peer review process is probably the most important process for a journal to ensure that it publishes original and high-quality research or commentary[17]. Peer review underpins the quality and integrity of scientific publishing[18]. Although author-suggested reviewers are not necessarily, as commonly assumed, less neutral than reviewers not suggested by the authors[19], in order to avoid academic misconduct arising from manipulation of manuscript peer review by authors or third parties, Baishideng does not accept author-suggested reviewers. In addition, Baishideng selects peer reviewers from a different country than the authors to review the manuscript in order to prevent potential conflicts of interest and other improper practices.

From 2019 to 2021, each manuscript submitted to Baishideng's seven SCIE-indexed journals was peer-reviewed by an average of 1.96 peer reviewers or editorial board members. The strict adherence to peer review processes kept the acceptance rates of these journals at a low level. From 2019 to 2020, the acceptance rates of *WJG*, *WJSC, WJD, WJP, WJGO, WJGS*, and *WJCC* were 27.6%, 46.5%, 37.4%, 31.3%, 45.8%, 33.7%, and 33.1%, respectively; the corresponding percentages in 2021 were 27.5%, 50.7%, 49.0%, 38.9%, 37.0%, 50.4%, and 35.5%. This practice has ensured not only the academic and language quality of the manuscripts but also the authenticity and standardization of the research involved in each manuscript, thus providing a guarantee for Baishideng journals to publish high-quality articles.

Second decision

After receiving the revised manuscript from the authors, the in-house science editor first checks whether the authors have made careful revisions to the manuscript according to the requirements and have answered the comments raised by the peer-reviewers, academic editor, and Company Editor-in-Chief, based on the peer review report, comments from the academic editor and Company Editor-in-Chief, Answering Reviewers document (submitted by the authors), and guidelines and requirements for revision of the manuscript. Then, the in-house science editor verifies whether all other manuscript-related documents submitted by the authors meet the journal's publication requirements. After the manuscript has been edited by the in-house science editor, it is sent to the Editor-in-Chief for a second decision, and then the Company Editor-in-Chief reviews the manuscript and makes the final decision on whether to accept or reject the manuscript.

Verification of documents related to the revised manuscript: The in-house science editor verifies the documents related to the revised manuscript based on the manuscript type. Different documents are required for different types of manuscripts, with some overlap. All manuscripts should include: (1) Revised manuscript file; (2) Answering Reviewers document - the authors should make a point-to-point response to the reviewers' comments and revise the manuscript accordingly; (3) Conflict-of-interest disclosure form; (4) Copyright license agreement; (5) Audio core tip; and (6) Bing Check Report. In the process of revising the manuscript, the authors' response to the reviewers' comments can help to greatly improve the quality of the manuscript[20]; therefore, it is particularly important for the science editor to verify the authors' revised manuscript.

After the document verification is completed and the revised manuscript passes duplication detection, the in-house science editor edits the manuscript and then sends it back to the authors for proofreading according to the guidelines and requirements for revision of the manuscript and the Science Editor Working Checklist; the latter (entitled, "Checklist of Responsibilities for Scientific Editors of the Baishideng Publishing Group Journals," and publicly available on the publisher's website) outlines the primary responsibilities of Baishideng's scientific editors. Then, the in-house science editor further edits and revises the authors' proofread manuscript. As Katz[21] said: "Anything published in professional literature is going to go through an editing process, and this is where dedicated experts get to make our work look better than it perhaps started off as". The editing and processing of manuscripts by science editors are important parts of the publishing process.

Language polishing at second decision: In order to strictly control and ensure the language quality of the manuscript, the in-house science editor verifies whether the manuscript needs to be sent to a Baishideng language editor for further language polishing according to Baishideng's English language publishing standard for manuscripts at the second decision. The purpose of language polishing is to improve the readability of the manuscript by modifying, polishing, and improving the language of the

full text from the aspects of grammar and spelling, syntax, articles/prepositions/conjunctions, punctuation, professional language, definitions and consistency of use of abbreviations, and so on without affecting the academic content or the original meaning of the manuscript.

Baishideng formulated the following rules for sending manuscripts to their language editors for language polishing according to whether the authors' native language is English or not: (1) For native English-speaking authors, the revised manuscripts submitted no longer need to be sent to a Baishideng language editor to polish the language; (2) For non-native English-speaking authors who have provided a language certificate (written affirmation by a professional agency attesting to the high quality of the manuscript's language) or those from countries/regions with relatively good repute for English proficiency, such as those from Japan, Korea, Greece, Turkey, India, Brazil, Taiwan, and Hong Kong, the manuscripts do not need to be sent to a Baishideng language editor to polish the language if the peer reviewers had rated the revised manuscript's language as Grade A or B and the authors have provided the language certificate; (3) For non-native English-speaking authors who are from countries/regions without a solid repute for English proficiency, such as China, if the revised manuscript's language was rated Grade A or B by the peer reviewers and the authors have provided a language certificate issued by any of the professional language editing companies that have been vetted and are recommended by Baishideng, they do not need to be sent to a Baishideng language editor to polish the language. The following language editing companies are vetted and deemed acceptable by Baishideng: Filipodia Publishing, MedE Editing Group, American Journal Experts, Nature Publishing Group Language Editing, Elsevier, Wiley, Medjaden, Editage, Enago, and Charlesworth; (4) For non-native English-speaking authors who provide a language certificate from an individual or other editing service provider not listed above, such manuscripts must be linguistically polished by a Baishideng language editor before they are accepted for publication; and (5) Finally, for authors from non-native English-speaking countries/regions who do not provide a language certificate, if their country/region has a solid repute for overall high level of English proficiency, the revised manuscript does not need to be sent to a Baishideng language editor to polish the language if the manuscript's language has been rated as Grade A by at least two peer reviewers.

At Baishideng, we believe that the feature of high-quality language in a manuscript can greatly improve the readability and hence understanding of its academic content. From 2019 to 2021, 61.4% of the articles published in Baishideng's seven SCIE-indexed journals were polished by professional language editors, which effectively improved the language quality of the articles.

XML and PDF document production: After the manuscript is proofread by the authors, further edited by the in-house science editor, and polished by the language editor, the in-house science editor regenerates the Microsoft Word document of the manuscript through the TINY Technologies editor built into the F6Publishing system. The Word document, including the complete textual content and corresponding tables of the manuscript, is then automatically converted into the XML simple text-based format through the F6Publishing system. After redrawing the figure(s) of the manuscript, the in-house science editor converts the image file(s) and the XML document into a PDF document through the F6Publishing system, and sends both the Word document and PDF document to the authors for verification of column type, titles (main and short), authors, affiliated institutions, abstract, text, and the figures and tables. If the authors find any errors in spelling or grammar, they will revise the manuscript (excluding images) in the Microsoft Word format with the Track Changes software feature enabled.

In order to prevent potential academic misconduct caused, in this proofreading process, by changes made by the authors, such as modifications that affect the content of the manuscript or drastic revision of the references, the following rules apply without exception: (1) Baishideng does not allow the manuscript title to be changed; (2) Baishideng does not allow the order of authors to be changed, the authors and corresponding author to be changed or deleted, or for any new authors to be added; (3) Baishideng does not allow any funding agency or grant number to be added or deleted; (4) Baishideng does not allow the manuscript text to be added to or deleted; (5) Baishideng does not allow any figures or tables to be added/added to or deleted; and (6) Baishideng does not allow manuscript references to be added or deleted.

Second decision: The in-house science editor proofreads and revises the manuscript and related documents, which culminates with submission of all the documents to the Director of Science Editor Development Department for review through the F6Publishing system. After verification and confirmation that the relevant documents of the manuscript are complete, the Director of Science Editor Development Department forwards the manuscript to the external Editor-in-Chief for the second decision. The external Editor-in-Chief makes a final assessment of the scientific and linguistic quality of the manuscript, based on all documents related to the manuscript submitted by the in-house science editor, and makes a second decision to reject or accept the manuscript. If the decision of the external Editor-in-Chief is to accept the manuscript, the in-house Editor-in-Chief double-checks and confirms whether the manuscript complies with the ethics and academic rules and norms or not, and makes the final decision to accept or reject the manuscript.

OA online publishing

After the manuscript is finally accepted, the production department publishes the electronic version (PDF) and the web version (HTML) of the article online. For this, the production director first determines the list of articles to be published in the current issue according to the journal publication plan, and sorts all manuscripts to be published in the current issue according to column type. After this, the in-house production editor adds the publication date, page numbers, website address, DOI, and other information to the articles on the basis of the XML and PDF documents of the manuscript submitted by the in-house science editor, to generate the electronic version (PDF) and the web version (HTML) of the article for online release. In addition, the production director reviews the current electronic version and the web version to check whether the page numbers are continuous, whether the figures and tables are complete, whether the picture quality meets the publishing requirements, and whether the numbers, units, and letter case in the articles conform to the publishing standards. Finally, the article is officially published online by the in-house production editor through the F6publishing system, and the authors are invited to submit the author evaluation by informing them of the publication information *via* email.

The Baishideng's seven SCIE-indexed journals involved in this study all adopt the OA publishing model. OA is a form of publishing model in which a publisher makes all articles and related content associated with a certain journal available immediately and for no-cost on the journal's website in perpetuity; these features are also the basis of the JCR award of a 'Gold OA' label. In this model, authors are often asked to bear the cost of publication, typically through an APC[22]. Among the 21430 journals listed in the *JCR* 2022, 5300 (24.7%) hold the Gold OA designation. Wassef *et al*[23] pointed out that OA articles are associated with a higher number of citations than subscription access articles. OA plays a vital role in improving the quality, efficiency, transparency, and integrity of academic journal publishing. Therefore, more and more academic journals are adopting the OA publishing model.

For Baishideng's seven SCIE-indexed journals, the number of published articles was higher in 2021 compared to the numbers in 2019-2020 (specifically, the increases were 5.4% for *WJG*, 18.5% for *WJSC*, 167.2% for *WJD*, 472.2% for *WJP*, 44.0% for *WJGO*, 176.0% *WJGS*, and 105.1% for *WJCC*). Throughout, the authors of these published articles are from many countries and regions around the world and an expansive trend is indicated; for example, in 2019 to 2020, the authors of articles published in *WJG* were from 71 countries/regions, and in 2021 alone the authors were from 56 countries/regions. According to a global cities research report by Elsevier in collaboration with the Administrative Center of Shanghai R&D Public Service Platform, Beijing (China), London (United Kingdom), Boston (United States), Tokyo (Japan), and Seoul (South Korea) had the highest number of active researchers between 2014 and 2018 [24]. Most of the Chinese cities in the study were in the top 10, reflecting China's increased efforts to cultivate, support, and introduce talent year after year. There may be a certain relationship between the author group and the regionality of the journal. The *WJG*, the first Baishideng English journal, was launched in China in 1995; thus, Baishideng journals may attract more authors from China. Moreover, the 3012 total articles published in the seven SCIE-indexed journals in 2019 to 2020 were cited by more than 20000 articles from approximately 8000 academic journals; among them, many articles were from journals with a JIF of > 10 (*WJG*, 171; *WJSC*, 61; *WJD*, 30; *WJP*, 9; *WJGO*, 45; *WJGS*, 11; *WJCC*, 92), including internationally renowned academic journals such as *CA-A Cancer Journal for Clinicians* (2021 JIF 286.130), *Lancet* (2021 JIF 202.731), *New England Journal of Medicine* (2021 JIF 176.079), *Nature Reviews Molecular Cell Biology* (2021 JIF 113.915), and *Nature Reviews Immunology* (2021 JIF 108.555). This suggests that the academic quality of articles published in Baishideng journals has been widely recognized.

Verification of ethics and academic rules and norms by Baishideng

Many scholars have found that most of the retracted articles in academic journals have academic misconduct[25-27]. In order to guarantee the academic ethics of articles and avoid academic misconduct, Baishideng formulated specific guidelines for each manuscript type that are customized for related ethics and documents relevant to the standards and norms for academic publication. First, for all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. This is mandatory and is one of the determining factors as to whether or not the manuscript will be sent for peer review or finally accepted. If the human and animal studies had received a waiver of the approval requirement from their ethics committee, the author(s) must provide an official statement to this effect made by their ethics committee. For the full compendium of guidelines for manuscript type and their related ethics and relevant documents/statements, please visit https://www.wjgnet.com/bpg/gerinfo/287. When authors return a revised manuscript during the peer review process, the in-house science editor rechecks whether the documents of academic rules and norms continue to correspond to the manuscript column and whether the non-native speakers of English language certificate is authentic; this is invariably followed by a new check of the full manuscript with iThenticate (CrossCheck) for duplication detection.

In order to make the process of manuscript peer review and publication more open and transparent, Baishideng publishes each article online along with the relevant documents such as those related to ethics and the international academic rules and regulations, including the peer-review report,

answering reviewers letter, conflict-of-interest statement, copyright license agreement, language certificate, institutional review board statement, informed consent statement, *etc*. Of particular importance, the authors are asked to provide the primary version (PDF) of the Institutional Review Board's official approval in the official language of the authors' country to the F6Publishing system; for example, authors from China should upload the Chinese version of the document. This plays an important role in eliminating academic misconduct in ethics approval documents.

For the 543 articles published from 2019 to 2021 in Baishideng's seven SCIE-indexed journals involved in this study, all were subject to and passed review of the above documents of ethics and academic standards and norms and other relevant documents; all of which are also published online, alongside the corresponding manuscript. Public disclosure of these ethics approval document(s) and the other relevant documents plays a supervisory role on its own, as it can effectively deter and dissuade academic misconduct. The ultimate outcome will be of benefit to the reputation of the academic journals, further promoting their ability to attract more high-quality manuscripts for peer review and publication and subsequently improving their JIF.

Baishideng's article quality tracking system

In order to continually improve the quality of its published articles, Baishideng not only conducts rigorous peer review of each manuscript but also provides an article quality tracking system for focused post-publication assessment. After an accepted article is published online, Baishideng, through the F6Publishing system, invites some of the journal editorial board members to re-evaluate the quality of the article, including its objectivity, credibility, and scientificity. These evaluation findings by the editorial board members are submitted online as tracking reports, which are immediately passed along to the authors and the Baishideng editorial office for verification and reference.

Post-publication peer review has been increasingly recognized as an important activity by which academic journals can control their academic quality[28-30]. As a newly released means of post-publication peer review (in 2018), the Baishideng article quality tracking system has been used to track 2.7% of the articles published between 2019 and 2021 in the seven SCIE-indexed journals. This activity has allowed editorial board members to put forth productive suggestions for refining the editing and production processes, bolstered by evidentiary explanations of limitations and quality problems that may have been overlooked. For example, an editorial board member (ID: 00182114) raised the following comments on an article reporting a retrospective study on survival and outcomes for co-infection of chronic hepatitis C with and without cirrhosis and COVID-19, which was contributed by Afify *et al*[31]: "Authors concluded that male gender, diabetes mellitus, and liver cirrhosis were the independent factors affecting mortality in COVID-19 patients. I agree to authors' opinion. … Please comment about the relationship between HCV and COVID-19". For the complete comments, please visit: https://www.wjgnet.com/1007-9327/full/v27/i42/7362.htm. In this way, the authors were able to address an additional important feature of their findings that may have been beyond the scope of their manuscript's focused content for the initial publication.

The launch of the article quality tracking system has proven to be greatly beneficial to Baishideng journals. It not only improves the academic quality of the published articles and the publishing standards but also has emerged as a useful platform for productive communication among the members of the editorial board, the authors, and the editorial office. In addition, the establishment of this system has benefited the editorial board members, improving their own academic influence, and the authors, facilitating rapid and wide dissemination of their articles.

Baishideng's author evaluation system

Authors are the foundation of academic journals. Without authors' writing and submitting articles for peer-reviewed publication, there would be no academic journals. Therefore, in order to better serve and understand the needs of its authors, Baishideng established and launched an author evaluation system in 2018. In this system, after the online publication of an article in any of Baishideng's journals, Baishideng invites the authors to personally evaluate the entire publication process of their articles *via* F6Publishing; their assessment involves a broad span of the process, including the manuscript submission system itself, peer review time, quality of peer review reports, manuscript editing quality, manuscript duplication detection system, quality of PDF and web version production, quality of figure and table production, the editorial office's ethical and academic regulatory requirements for the manuscript, and their overall satisfaction with the publication cycle of the article. For example, Patrone *et al*[32] provided a comment that then prompted an internal refinement of Baishideng's process: "The submission and editing process was fair. …The manuscript was sent in January 2021 and was finally accepted after several months. …during the year spent in the publishing process and therefore the value of the manuscript is somehow limited". For the complete author's comments, please visit: https://www.wjgnet.com/1948-5204/full/v13/i12/2203.htm.

Approximately 84.0% of the articles published from 2019 to 2021 in the seven SCIE-indexed journals underwent evaluation by and received feedback from the authors. Through the timely feedback of these authors, Baishideng has been able to gain a better understanding of author needs and has continued to provide better publishing services for them; ultimately, this has strengthened Baishideng's reputation among authors.

Baishideng's reader evaluation system

Journals have become deeply embedded in academic infrastructure[33]. As the vehicle of knowledge dissemination, academic journals are published for the purpose of presenting high-quality academic papers to readers. In order to better serve readers and share high-quality articles published in Baishideng journals in a timely manner, for readers to read and evaluate, Baishideng has established and launched a reader evaluation system. In this system, after the online publication of an article in any of Baishideng's journals, Baishideng will recommend and share the articles to relevant global scholars through the F6Publishing system. The accuracy of the target recipients of these recommendations is established by consideration of the article's subject area and research keywords. The invitation to scholars to read the full text of the articles (including title, abstract, research background, research methods, research results, discussion, figures, statistical methods, references, academic ethical statements, *etc*), is accompanied by the opportunity to provide an online evaluation of the scientific and linguistic quality of the articles from the perspective of readers. Scholars can make further comments on the research highlights of the article, the rationality of the research design, the integrity of the data, and the limitations and shortcomings of the study, and make suggestions for future research directions. For example, a reader (ID: 01714826) gave the following valuable advice on an article reporting a retrospective study on preoperative hemoglobin to albumin ratio for the short-term survival of gastric cancer patients, which was contributed by Hu *et al*[34]: "In this paper the authors used HAR as yet another measure to assess the prognosis of gastric cancer. … However, it would have been better if the authors included Hb and Albumin as independent parameters to predict prognosis along with the HAR data they have analyzed. Additionally, it would have been nice to mention whether other factors like UGI bleeding resulted in a low Hb…". For the complete reader's comments, please visit: https://www.wjgnet.com/1948-9366/coretip/v14/i6/580.htm.

After readers' online comments are posted, they are automatically sent to the authors for verification and reference. At the same time, readers can also write a formal letter about an article that they have read and submit it to us online for consideration of traditional publication. After the reader's letter has been peer-reviewed and finally accepted for publication, it will be published online. From 2019 to 2021, the articles published in the seven SCIE-indexed journals were read and downloaded by readers from all over the world; moreover, webpage visits and downloads are showing an increasing trend year by year for each. For example, the number of total visits received by the *WJG* webpage increased by 17.4% in 2020 compared with that in 2019, and by 14.4% in 2021 compared with that in 2020; the number of downloads increased by 120.1% in 2020 compared with that in 2019, and by 52.6% in 2021 compared with that in 2020. As a result, the academic influence of the seven SCIE-indexed journals was improved greatly. The reader evaluation system continues to be of great benefit, particularly as it has improved the readership volume of published articles, expanding the academic influence of each study's findings, improving citation counts, and enhancing the authors' influence among readers in their field and beyond.

CONCLUSION

This paper introduces the complete editing and publishing process of Baishideng's journals, providing a unique perspective on the publication of academic journals. In our experience, the integrity of the editing and publishing process of academic journals has a great impact on the number of articles received and published, and the number of webpage visits and downloads received by the journals, and thus is invaluable to expanding the influence of academic journals and improving their JIFs.

In our focused analyses of seven SCIE-indexed journals, we found that the JIFs increased for three from 2020 to 2021; for the other four, the JIFs decreased only slightly due to the influence of factors such as the increased numbers of published articles. Overall, the JIFs of these journals have steadily increased over time. Further findings were that *WJG* has the highest 2021 JIF (5.374) and *WJCC* has the lowest (1.534), while *WJSC* has the highest self-citation rate (1.43%) and *WJGS* has the lowest (0.21%). In Baishideng's *RCA* database, all seven of the SCIE-indexed journals received a 2022 *JAII*. For example, *WJG* has a 2022 *JAII* of 22.048, ranking 18th among 102 journals in the field of gastroenterology & hepatology included in the *RCA*; its total citations rank 6th and total articles rank 5th. Both the number of articles received and published by the seven SCIE-indexed journals increased. For example, compared with the figures in 2019-2020, the number of articles received by *WJCC* increased by 91.1% and the number of articles published increased by 105.1% in 2021. The articles published in the seven SCIE-indexed journals in 2019-2020 were cited by more than 20000 articles from approximately 8000 academic journals, including internationally renewed journals such as *CA-A Cancer Journal for Clinicians*, *Lancet*, and *New England Journal of Medicine*.

The webpage visits and downloads received by these journals have also increased year by year. For example, the total number of visits received by the *WJG* webpage was 1974052 in 2019, 2317835 in 2020 (an increase of 17.4% compared with that in 2019), and 2652555 in 2021 (an increase of 14.4% compared with that in 2020). The visitors were from more than 220 countries and regions worldwide, such as the United States, China, and the United Kingdom. A total of 5543 OA articles were published in the seven

SCIE-indexed journals from 2019 to 2021. OA plays a crucial role in improving the quality, efficiency, transparency, and integrity of academic journal publishing.

Following the online publication of all articles, through Baishideng's article quality tracking system, 96.5% of the article quality-tracking evaluation reports gave positive comments or did not put forward an opinion, providing further affirmation of the quality of the articles. Through the author evaluation system, 84.0% of the articles received author evaluations and feedback, and most of the authors gave positive evaluations. Through the reader evaluation system, the seven SCIE-indexed journals received a total of 424 reader evaluations and 229 letters from readers, both of which improved the readership volume and influence of the articles and their authors.

Baishideng's continual improvements to its journal editing and publishing process have greatly improved the academic influence of its journals and their publications, as demonstrated by its seven SCIE-indexed journals. Other publishers and academic journals will similarly benefit from actively tracking and refining the integrity of their editing and publishing process. The collective outcome could benefit all of science worldwide, as greater transparency and demonstrated adherence to ethical standards and norms will strengthen trust in scientific studies and their findings and in collaborative efforts worldwide. Moreover, open communication between editors, authors and readers will provide useful information on current and ongoing limitations to their editing and publication process, thereby providing insights through which they can improve their process and their JIFs.

In the face of all this potential benefit, however, many challenges still exist; these include: How to address the low success rate of peer review; how to improve the quality of manuscript peer review; how to avoid peer reviewers' misconduct more effectively, including attempts to force authors to cite the peer reviewer's own published articles, plagiarizing the author's articles, *etc*; how to avoid falsified ethical approval documents and language certificates more effectively; how to effectively avoid improper authorship or falsified signature on copyright license agreements; and, how to effectively train academic editors and science editors. These unresolved issues highlight the importance of collaborative support from authors, journal editors-in-chief, editorial board members, peer reviewers, academic editors, editors, readers, and publishers. Academic journals should always prioritize publishing high-quality innovative research, compliance with the international editing and publishing ethics standards and norms, establishment of an efficient article quality tracking system, author evaluation system, and reader evaluation system, and maintenance of a rigorous attitude towards ensuring a high-quality and ethical editing and publishing process and a high standard of service to create a solid academic exchange platform for the dissemination of researchers' academic achievements.

ARTICLE HIGHLIGHTS

Research background

Journal Impact Factor™ (JIF) is often used to evaluate the relative reputation and quality of academic journals in their respective fields. It can greatly influence the quality and scope of subsequent manuscript submissions. Few studies have focused on the impact of the integrity of the editing and publishing process on improvement of the JIF of academic journals.

Research motivation

This study introduces and discusses the editing and publishing processes of Baishideng's journals in their entirety, as they form the basis for our objective of safeguarding and bolstering integrity in academic publication.

Research objectives

This study aimed to explore the importance of the integrity of the editorial and publication process in improving the academic influence of academic journals and JIF of academic journals.

Research methods

In this paper, we describe our statistical analysis of bibliometric factors, such as the 2021 JIFs released in the *Journal Citation Report*™ 2022, discipline rankings, received and published articles in 2019-2021, and webpage visits and downloads for seven journals published by Baishideng Publishing Group (Baishideng) and indexed in Science Citation Index Expanded™. The editing and publishing processes of Baishideng's journals in their entirety were introduced and discussed, as they form the basis of safeguarding and bolstering integrity in academic publication.

Research results

For the seven journals assessed, their 2021 JIFs were basically unchanged from 2020. The 3012 articles published in 2019-2020 were cited by more than 20000 articles from approximately 8000 academic journals, including many journals with JIFs > 10. Baishideng's journals have been widely recognized for their academic quality. The numbers of manuscripts received and published in 2021 are both higher

than those in 2019-2020. The numbers of webpage visits and downloads received by the seven journals have increased year by year. The visitors were from all around the world. From 2019 to 2021, a total of 5543 open access articles were published in the seven journals, of which 2083 (37.6%) were invited and published free-of-charge. In addition, the quality of the articles was further evaluated through Baishideng's article quality, author evaluation tracking and reader evaluation systems.

Research conclusions

The findings from these bibliometric assessments indicate that establishing, promoting and actively practicing processes that safeguard and bolster the integrity of the editing and publication process also help to improve the academic influence of academic journals, which itself is the cornerstone for improving JIF.

Research perspectives

How to address the low success rate of peer review; how to improve the quality of manuscript peer review; how to avoid peer reviewers' misconduct more effectively, including attempts to force authors to cite the peer reviewer's own published articles, plagiarizing the author's articles, *etc*; how to avoid falsified ethical approval documents and language certificates more effectively; how to effectively avoid improper authorship or falsified signature on copyright license agreements; and, how to effectively train academic editors and science editors are still challenges and require further research in the future.

FOOTNOTES

Author contributions: Ma LS conceptualized and designed the study; Wang JL organized the study materials, performed the data collection and analysis, and wrote the first draft of the manuscript; and all authors reviewed and commented on each iterative version of the manuscript, and read and approved the final manuscript.

Conflict-of-interest statement: The authors are employees of the Baishideng Publishing Group Inc and declare that they have no other real or potential conflicts of interest to disclose.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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P-Editor: Wang JJ

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World Journal of Gastroenterology

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World J Gastroenterol 2022 November 21; 28(43): 6203-6205

DOI: 10.3748/wjg.v28.i43.6203

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CORRECTION

Correction to "MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation"

Zhen Zhang, Dong-Qiu Dai

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Dambrauskas Z, Lithuania; He D, China

Received: July 20, 2022
Peer-review started: July 20, 2022
First decision: September 26, 2022
Revised: September 29, 2022
Accepted: November 4, 2022
Article in press: November 4, 2022
Published online: November 21, 2022

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Abstract

Correction to "Zhang Z, Dai DQ. MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation. *World J Gastroenterol* 2019; 25: 1224-1237 [PMID: 30886505 DOI: 10.3748/ wjg.v25.i10.1224]". In this article, we found the following errors in Figure 4: Three images of the NC and miR-NC groups in the MGC-803 cell wound healing assay were misapplied during the preparation of submission; the mimcs and miR-NC icons were incorrectly edited in the image of the statistical chart. According to the reviewer's comments, we have re-analyzed the images of the wound-healing assay and revised the charts depicting the analyzed results. The corrected Figure is given in this correction.

Key Words: Correction; MicroRNA-596; Gastric cancer; Figure; Errors

Core Tip: This manuscript is to correct the images in Figure 4 of "Zhang Z, Dai DQ. MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation. *World J Gastroenterol* 2019; 25: 1224-1237 [PMID: 30886505 DOI: 10.3748/wjg.v25.i10.1224]".

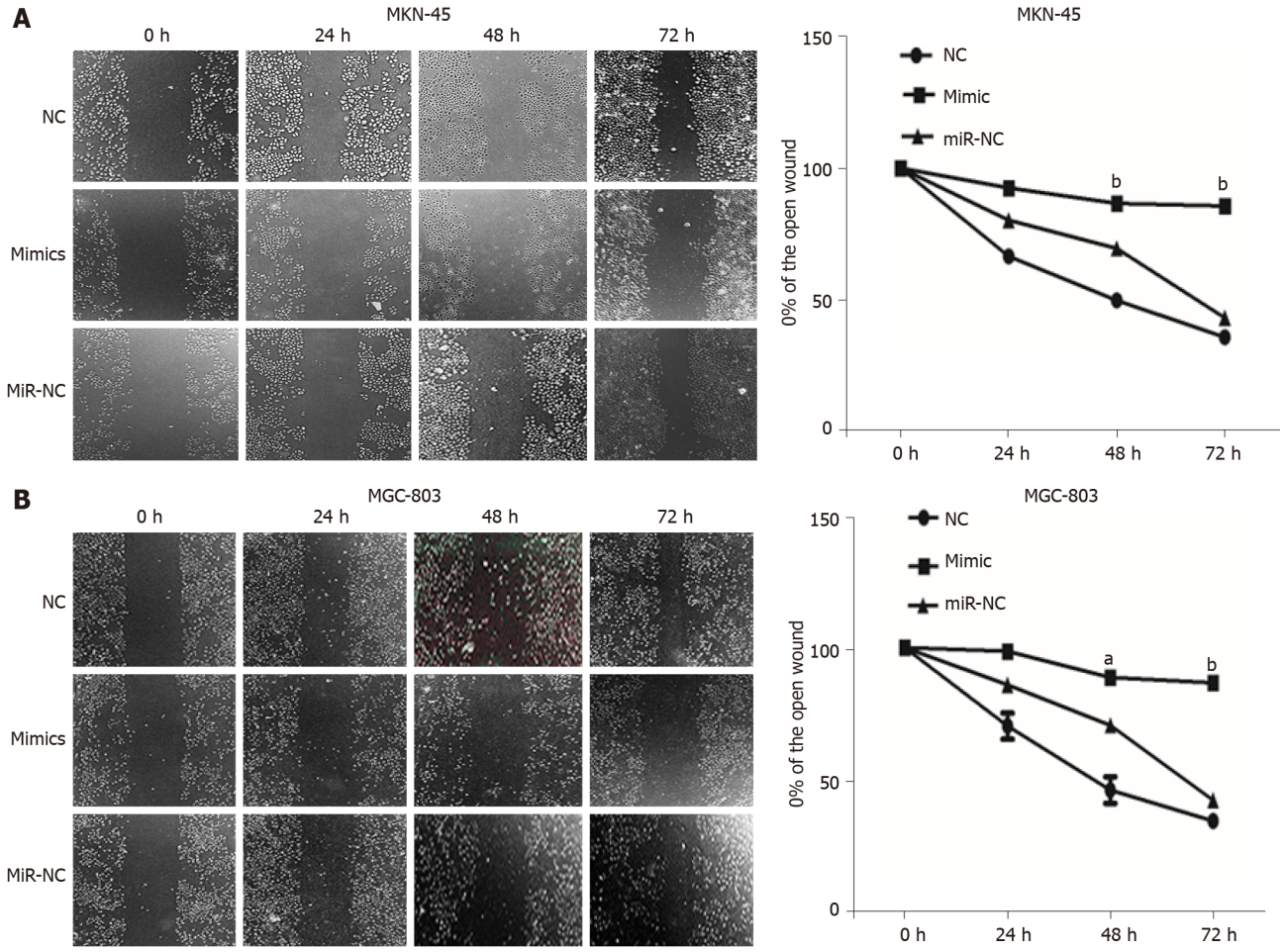
Citation: Zhang Z, Dai DQ. Correction to "MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation". *World J Gastroenterol* 2022; 28(43): 6203-6205
URL: https://www.wjgnet.com/1007-9327/full/v28/i43/6203.htm
DOI: https://dx.doi.org/10.3748/wjg.v28.i43.6203

TO THE EDITOR

Correction

Correction to: Zhang Z, Dai DQ. MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation. *World J Gastroenterol* 2019; 25: 1224-1237 [PMID: 30886505 DOI: 10.3748/wjg.v25.i10.1224].

In the original publication of the article[1], we found the following errors in Figures 4A and 4B (in this manuscript marked as Figure 1): Three images of the NC and miR-NC groups in the MGC-803 cell wound healing assay were misapplied during the preparation of submission; the mimcs and miR-NC icons were incorrectly edited in the image of the statistical chart. According to the reviewer's comments, we have re-analyzed the images of the wound-healing assay and revised the charts depicting the analyzed results. The corrected Figure is given in this correction. This correction will have no influence on the interpretation of the entire results and conclusion in this study. We apologize for any inconvenience this may cause.



DOI: 10.3748/wjg.v28.i43.6203

Figure 1 Wound healing assay for detecting cell migration in MKN-45 and MGC-803 cells transfected with miR-NC or microRNA-596 mimic.
A: MKN-45 cells; B: MGC-803 cells. [a]*P* < 0.05; [b]*P* < 0.01 *vs* miR-NC.

FOOTNOTES

Author contributions: Dai DQ and Zhang Z approved the final version of the article to be published.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: China

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

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