

August 7, 2018

Re: "Outcomes of furazolidone, amoxicillin-based quadruple therapy for *Helicobacter pylori* infection and predictors of failed eradication" (Manuscript No. 40462)

Dear Dr. Gong:

Thank you very much for your email of July 24, 2018 regarding this manuscript. We have revised the manuscript to address the comments of the reviewers. Our point-by-point responses are listed below. In the revised manuscript we have highlighted the changes that were made from the original manuscript by using the track changes mode.

We eagerly look forward to your response.

Sincerely,

Weiling Hu MD, PhD

Reviewer 1

1. Table 1. Please spell out percentage of females and more precisely.

We have corrected the number and percentage of females in Table 1.

Part of Table 1

Table 1. Baseline demographic and clinical characteristics (N=992)	
Variable	Information
Gender, N (%)	
Male	501 (50.5)
Female	491 (49.5)

2. Education level if possible e.g. 5th grade etc.

When we redefined the education variable into 6 categories by years of education (<7 years, 7-9 years, 10-12 years, 13-16 years, >16 years, or unknown), education level continued to demonstrate a lack of association with failed H. pylori eradication. We have now added the revised education variable in Table 1, Table 4, and in the Methods section.

“Educational levels were categorized by years of education (<7, 7-9, 10-12, 13-16, or >16 years).” (Page 9, line 13)

Part of Table 1 related to the education level.

Table 1. Baseline demographic and clinical characteristics (N=992)	
Variable	Information
Educational level, N (%)	
<7 years	164 (16.5)
7-9 years	249 (25.1)
10-12 years	197 (19.8)
13-16 years	229 (23.1)
>16 years	20 (2.0)
Unknown	133 (13.4)

Part of Table 4 related to the education level.

1 Table 4. Univariate and multivariate analyses for predictors of failed <i>H. pylori</i>					
2 eradication					
Factors		Eradication rate n/N (%)	P-value*	Multivariate†	
				OR 95%CI	P-value
Education†	<7 years	150/164 (91.5)	0.29	—	—
	7-9 years	237/249 (95.2)			
	10-12 years	187/197 (94.9)			
	13-16 years	219/229 (95.6)			
	>16 years	20/20 (100)			

3. Table 2. Side effects it is not clear and confusing With, % (95% CI) 130/144 90.3 (85.5-95.1)† Without, % (95% CI) 683/715 95.5 (94.0-97.0)

We calculated and compared the eradication rates among patients with (N=144) or without (N=715) adverse events effects during furazolidone-amoxicillin containing quadruple therapy. Eradication rate of *H. pylori* was higher in patients without (683/715, 95.5% vs. 130/144, 90.3%; mean difference=5.2%, 95%CI 0.7%-11.7%, $P=0.01$) compared to those with adverse events during therapy.

We have now clarified the inclusion of subgroup analysis in the Methods section and also provided the mean difference, 95%CI and P-value to clarify higher eradication rate among those without compared to those with adverse events during therapy in the footnotes of Table 2.

“Secondary endpoints were treatment-related adverse events and predictors of eradication failure. The primary endpoint was also analyzed by subgroups as patients receiving primary/rescue therapy and those with/without adverse events.” (Page 8, line 21)

Table 2. H. pylori eradication rates with furazolidone, amoxicillin-based quadruple therapy: overall and by subgroups

Variable	n/N	Eradication rate
Overall, % (95% CI)	937/992	94.5 (94.1-95.9)
Times		
Primary, % (95% CI)	800/842	95.0 (93.5-96.5)
Rescue, % (95% CI)	137/150	91.3 (86.8-95.8)
Adverse events*		
Without, % (95% CI)	683/715	95.5 (94.0-97.0) [†]
With, % (95% CI)	130/144	90.3 (85.5-95.1)

*: Patients who completed the study protocol were divided into two groups: without or with adverse events during therapy. Eradication rates of two groups were calculated and the difference between the two groups were analyzed.

[†]: Eradication rates were higher among patients without (mean difference=5.2%, 95%CI 0.7%-11.7%, $P=0.01$) compared to those with adverse events during therapy.

n: number of successful eradication;

N: number of total patients;

4. The authors should add reference: Mohammadi M, Attaran B, Malekzadeh R, Graham DY. Furazolidone, an Underutilized Drug for H. pylori Eradication: Lessons from Iran. Dig Dis Sci. 2017 Aug;62(8):1890-1896. doi: 10.1007/s10620-017-4628-5, which suggests the future studies as the one performed in this article.

We would like to thank the Reviewer for an excellent suggestion. Mohammadi et al. performed a systemic review of furazolidone-containing regimens in Iran.^[1] Based on their results, authors hypothesized that a 14-day quadruple therapy with a higher dose furazolidone dose (100mg t.i.d./b.i.d.) would have the highest eradication rates with the lowest side effect profile. Furthermore, the authors suggested future studies evaluating the impact of different PPIs and bismuth dosing in furazolidone-containing regimens on H. pylori eradication. Our study demonstrated high efficacy with nearly all patients (98%) receiving 14-day furazolidone, amoxicillin-containing quadruple therapy despite all patients receiving furazolidone 100mg b.i.d. dosing. Furthermore, the multivariate analysis also demonstrated that PPI types and bismuth are not associated with failed H. pylori eradication.

We have now referenced the study by Mohammadi et al. ^[48] and highlighted that 100mg b.i.d. dosing appears effective with a 14-day furazolidone, amoxicillin-containing therapy regardless of PPI types or bismuth doses in the Discussion section.

“Finally, our study showed that 14-day furazolidone, amoxicillin-based quadruple therapy led to a high eradication rate regardless of furazolidone dose (i.e. 200mg per day), bismuth dose, or PPI types previously raised as potential factors to successful *H. pylori* eradication ^[48].” (Page 18, line 11)

5. Minor linguistic correction “the carcinogenic effect in clinical settings remain speculatively. Last word should be changed to speculative.”

We have revised the word “speculatively” into “speculative” to the revised sentence to:

“The carcinogenetic effects of furazolidone suggested in early animal studies ^[12-15, 40] have remained speculative in clinical settings.” (Page 16, line18)

Reviewer 2

6. The retrospective type of the study and the absence of sensitivity tests in a large number of patients are the main drawbacks of this study.

We had acknowledged in the Discussion section that the retrospective study design allowing antibiotic sensitivity data in a small number of patients (clarithromycin in 17.3%, metronidazole in 73.1%, levofloxacin in 38.5%, amoxicillin in 0%, furazolidone in 3.8%) as the main limitation of the study. However in a recent study of 9,687 *H. pylori* strains evaluated from an adjacent city (Jiaxing) located 74 kilometers from Hangzhou, the resistance rates (clarithromycin in 17.8%, metronidazole in 95.5%, and levofloxacin in 19.7%) paralleled our results ^[2]. Another recent study of evaluating 545 *H. pylori* strains in pediatric population from Hangzhou also demonstrated similar *H. pylori* antibiotic resistance rates: clarithromycin-resistance in 20.6%, metronidazole-resistance in 68.8%, levofloxacin-resistance in 9.0%, and no resistance to amoxicillin and furazolidone ^[3].

We have referenced to two additional studies that demonstrated similar antibiotic resistance rates to those found in our subgroup.

“Furthermore, *H. pylori* culture and sensitivity were not performed in all enrolled patients. However, *H. pylori* antibiotic sensitivity data available in a subset of patients in our study paralleled findings from two recent large studies

from the same region ^[29,49].” (Page 18, line 19)

7. The authors have also to emphasize that more studies are needed and in other parts of the world using furazolidone-based quadruple regimens for Helicobacter pylori infection.

We have now suggested that future studies evaluating furazolidone, amoxicillin-based quadruple therapy regimens for H. pylori infection other than China and Iran are needed in the Discussion section.

“Future studies evaluating efficacy of furazolidone, amoxicillin-based quadruple therapy in areas other than Iran or China may be invaluable.” (Page 18, line 17)

Additional Corrections:

1. We would like to revise the title by adding “amoxicillin” to:

“Outcomes of furazolidone, amoxicillin-based quadruple therapy for Helicobacter pylori infection and predictors of failed eradication”

2. We have deleted the term “FABQT” and replaced them with furazolidone, amoxicillin-based quadruple therapy throughout the manuscript to improve clarity.
3. We have updated a reference that demonstrate H. pylori resistance pattern in Hangzhou in the Discussion section.^[3]

“A recent local study examining 545 H. pylori cultures obtained from children showed absence of furazolidone-resistant H. pylori, consistent with the low (4%) resistance rate shown at our center ^[29].” (Page 15, Paragraph 2)

4. We have revised Table 1 to improve clarity by: rounding the decimals to the tenth, changing “n” to “N” as total number of patients, and adding “n/N (%)” after “eradication rate”.
5. We have revised the title to Table 2 to improve clarity to:

“Table 2. H. pylori eradication rates with furazolidone, amoxicillin-based quadruple therapy: overall and by subgroups”
6. We have revised the title to Table 4 to improve clarity to:

“Table 4. Univariate and multivariate analyses for predictors of failed H. pylori eradication”

7. We would like to revise the order of the authors to: “Yawen Zhang^{1,2}, Weiling Hu^{1,2}, Yuan Cai¹, Wenfang Zheng^{1,2}, Qin Du³, John J. Kim⁴, John Y. Kao⁵, Ning Dai¹, Jianmin Si^{1,2}”.
8. We would like to revise the author contribution for one of the authors to: “John J. Kim analysed the data and drafted the paper.”
9. Additional minor grammatical corrections and format modification were performed throughout the manuscript and reference to improve the clarity.

References:

1. **Mohammadi M**, Attaran B, Malekzadeh R, Graham DY. Furazolidone, an Underutilized Drug for H.pylori Eradication: Lessons from Iran. Dig Dis Sci 2017;62:1890-1896. [PMID:28577244]
2. **Ji Z**, Han F, Meng F, Tu M, Yang N, Zhang J. The Association of Age and Antibiotic Resistance of Helicobacter Pylori: A Study in Jiaying City, Zhejiang Province, China. Medicine (Baltimore) 2016;95:e2831. [PMID:26937912]
3. **Shu X**, Yin G, Liu M, Peng K, Zhao H, Jiang M. Antibiotics resistance of Helicobacter pylori in children with upper gastrointestinal symptoms in Hangzhou, China. Helicobacter 2018;23:e12481. [PMID:29528162]