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Selecting the Best Gastric Anatomical Place for Biopsy to Detect *Helicobacter Pylori* in Iranian Morbid Obese Patients

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Abstract

Background: Obesity and *Helicobacter pylori* (*H. pylori*) infection are public health problems in the world and Iran. This study aimed to indicate the anatomical place with the most accurate results for *H. pylori*. According to gastric mapping, this study will be able to evaluate the prevalence of *H. pylori* based on the pathology of gastric mapping and the accuracy of the antral rapid urease test (RUT) based on endoscopic findings.

Methods: In this cross-sectional study, upper digestive endoscopy and gastric pathology were studied in 196 obese patients candidates for bariatric surgery. Statistical analyses were performed using a t-test and Chi-square/fisher's exact test to compare the groups. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and odds ratio (OR) were used to compare RUT and pathological *H. pylori* test of each of the six areas of the stomach. We set a positive test of the pathology of 6 regions of the stomach as our gold standard (in this study).

Results: The most common area of the stomach for pathological findings of *H. pylori* were incisura (116, 59.2%), greater curvature of the antrum (115, 58.3%), lesser curvature of the antrum (113, 57.7%), lesser curvature of the corpus (112, 57.1%), greater curvature of the corpus (111, 56.6%) and cardia (103, 52.6%). The prevalence of *H. pylori* was 58.2% (114 cases) and 61.2% (120 cases) with RUT and gastric pathology, respectively. Mild, moderate, and severe infection of *H. pylori* in cardia (58, 29.6%), greater and lesser curvature of the antrum (61, 31.1%), and greater curvature of the antrum (37, 18.9%) had the highest percentages of incidence comparing to other sites of the stomach, respectively. The most sensitive area for pathologic biopsy was incisura (96.6%, 95% confidence interval: 91.7, 98.7).

Conclusion: According to the highest sensitivity, PLR, NPV, and pathological findings of *H. pylori* in accordance with the lowest NLR in the incisura compared with other parts of the stomach, it is highly recommended to take the biopsy from the incisura instead of other anatomical places of stomach for detecting *H. pylori* specifically if our strategy is taking only one biopsy.

Keywords: Bariatric Surgery, Obesity, *Helicobacter pylori*, Endoscopy, Pathology

Conflicts of Interest: *None declared*
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Introduction

The prevalence of obesity has increased during the past several decades. Now, over 2 billion adults worldwide are

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↑What is "already known" in this topic:
Antrum is a usual anatomical place for biopsy to detect *H. pylori*. Some clinicians take more biopsies from other regions, specifically suspicious places of the stomach for this infection. No study has shown where is the best single gastric point for detecting *H. pylori* during endoscopy.

→What this article adds:
Considering highest accuracy (97.9%), Sensitivity (96.7%), Specificity (100%), positive (100%) and negative (95%) predictive value, positive likelihood ratio (735.7), and odds ratio (20), in accordance with lowest negative likelihood ratio (0.03), and the highest prevalence of *H. pylori* in pathological assessment between six anatomical points in the stomach, it seems that incisura is the optimal anatomical place for detecting *H. pylori* based on biopsy.

overweight or obese (1). Obesity is one of the main public health problems in Iran (2). The prevalence of obesity in populations above the age of 18 is 21.7% in Iran (2). Bariatric surgery is suggested in severely obese patients (BMI ≥ 40 kg/m^2) and those with BMI 35–39.9 kg/m^2 combined with comorbidities (3). In a systematic review study, the prevalence of infection caused by *Helicobacter pylori* (*H. pylori*) among the healthy population in Iran was reported to range from 30.6% to 82% (4), and in patients undergoing bariatric surgery varied from 10-67% (5). The number of biopsies from the stomach and anatomical places for detecting *H. pylori* seems a forgotten issue. Due to practical constraints for endoscopists and pathologists, increasing time of evaluation of biopsies, increasing time of anesthesiology, inducing higher anxiety for patients and families, and ethical limitations, everybody prefers to assess one best representative point instead of multiple samples. However, the question raised here is whether it can be possible to find such an anatomical place in the stomach. There are many studies about the comparison of RUT with different kits and pathologic diagnosis of *H. pylori* (6). However, debating for selecting the best place seems to be a point of agreement that may be due to not evaluation by studies like some other issues accepted as a default without deep evaluation by scientists. Reduction in BMI, triglyceride, and glucose levels in non-diabetic *H. pylori* treated patients after bariatric surgery was shown to be significantly greater than *H. pylori*-negative subjects (7). Pathologic findings such as chronic gastritis, chronic active gastritis, and secondary lymphoid follicle formation have a significant relationship with *H. pylori* infection in morbidly obese patients, but intestinal metaplasia and atrophy do not (8). The evidence about the benefit of *H. pylori* eradication before bariatric surgery is unclear, and obese patients have a significantly lower eradication rate than controls (9). The role of upper gastrointestinal endoscopy (UGIE) before bariatric surgery is controversial. American guidelines recommend that UGIE "may be used if suspicion of gastric pathology exists" (9). Conversely, European guidelines recommend all patients undergo UGIE before bariatric surgery, especially before Roux-en-Y Gastric Bypass (RYGB) (10). The present study aimed to evaluate the best place for detecting *H. pylori* infection, the prevalence of *H. pylori* based on the pathology of gastric mapping, and its association with endoscopic findings based on antral rapid urease test (RUT).

Methods

This is a cross-sectional study of 196 morbidly obese patients without any control group or randomization. Sampling was convenient. Considering type I error (α) equal to 5%, the prevalence of *H. pylori* equal to 71% (11), precision around this percentage equal to 5%, the sensitivity of detecting *H. pylori* equal to 90%, and using sensitivity estimation formula, our sample size was estimated as 196 cases. The study was conducted in the outpatient clinics of Rasoul-e-Akram hospital affiliated to Iran University of Medical Sciences (IUMS) between September 2015 and September 2016. Patient selection was based on the NIH Consensus Statement (BMI 40 kg/m^2 or BMI > 35 kg/m^2 with significant/major comorbidity) (12). After taking the medical history and demographic data such as the presence of comorbidities, all subjects had physical examinations, blood test screening, nutritional and psychological consultation, and upper gastrointestinal endoscopy. Lesions detected at endoscopy were sampled for pathological examination. *H. pylori* status was determined using an RUT from the antrum. Some data were extracted from the Iran National Obesity Surgery Database for patients undergoing bariatric surgery in our country, consisting of our hospital. UGIE was performed by four coordinated board-certified gastroenterologists using a conventional single-channel endoscope (CF, H170L). UGIE for the patients performed by the Sydney system (13) in which six specimens of three gastric zones (greater and lesser curvature of corpus (GCC, LCC), incisura, corpus, greater and lesser curvature of antrum (GCA, LCA)) were sampled. After fixation and processing of biopsies, they were stained with hematoxylin and eosin for general evaluation and with the modified Giemsa for *H. pylori*. Pathologic findings of the stomach were evaluated for the presence of *H. pylori*, chronic and active inflammation, epithelial atrophy, dysplasia, and intestinal metaplasia.

Hiatus hernia was classified according to the size of the herniated gastric chamber: small ≤3 cm; medium, 3-5 cm; and large ≥ 5 cm (14). All examiners were blinded to other parts' results consisting of blood test screening, physical examinations, endoscopic and pathologic findings.

Statistical methods

All statistical analyses were performed using SPSS, 21 Software. A P-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using a t-test and Chi-square or Fisher's exact test to compare the groups. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and odds ratio (OR) were used to compare RUT and pathological *H. pylori* test of each of the six areas of the stomach. We set a positive test of the pathology of 6 regions of the stomach as our gold standard (in this study). Mean, standard deviation (SD), frequency, and 95% confidence interval (CI) were also used in the descriptive analysis of variables.

Results

Most of the patients were female (169 patients, 86.2%). The mean ± SD of age and BMI were 39.9 ± 10.2 years and 45.9 ± 6.1 kg/m², respectively. *H. pylori* infection was detected with RUT in 114 (58.2%) and with pathology in 120 (61.2%) patients. Patients with positive RUT had a nine times higher chance of positive pathologic findings of *H. pylori* infection than those with negative RUT (OR=9.02) (Table 1). The most common area of the stomach for pathological findings of *H. pylori* was incisura (116, 59.2%) and GCA (115, 58.3%). Pathological findings, which were the anatomical place for incomplete metaplasia, was incisura (one case, 0.5%). Incisura, LCA, and LCC were the most prevalent areas for the pathological findings of extensive atrophy (each case, 0.5%) (Table

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Table 1. RUT (antral biopsy) and pathologic findings of *H. pylori*

		Pathologic *H. pylori* N (%)		*P*-value	OR (95% CI)
		Pos	Neg		
RUT, N (%)	Pos	93 (47.4)	21 (10.7)	<0.001	9.02
	Neg	27 (13.8)	55 (28.1)		(4.6, 17.4)

CI, confidence interval; *H. Pylori*, *Helicobacter pylori*; N, number; Neg, negative; OR, odds ratio; Pos, positive; RUT, rapid urease test.

Table 2. Pathologic findings of *H. pylori*, atrophy, and metaplasia in different anatomical areas of the stomach

	Pathologic *H. pylori* N (%)	Atrophy		Intestinal metaplasia	
		Focal	Extensive	Complete	Incomplete
Cardia	103 (52.6)	1 (0.5)	0	1 (0.5)	0
LCC	112 (57.1)	5 (2.6)	1 (0.5)	6 (3.1)	0
GCC	111 (56.6)	2 (1)	0	2 (1)	0
Incisura	116 (59.2)	10 (5.1)	1 (0.5)	3 (1.5)	1 (0.5)
GCA	115 (58.3)	22 (11.2)	0	17 (8.7)	0
LCA	113 (57.7)	14 (7.1)	1 (0.5)	11 (5.6)	0

GCA, greater curvature of antrum; GCC, greater curvature of corpus; *H. Pylori*, *Helicobacter pylori*; LCA, lesser curvature of antrum; LCC, lesser curvature of corpus; N, number.

Table 3. The severity of *H. pylori* infection in the six gastric areas

	The severity of *H. pylori* infection, N (%)				
	No	Mild	Moderate	Severe	*P*-value
Cardia	93 (47.4)	58 (29.6)	34 (17.3)	11 (5.6)	<0.001
LCC	84 (42.9)	43 (21.9)	47 (24)	22 (11.2)	
GCC	83 (42.3)	55 (28.1)	36 (18.4)	20 (10.2)	
Incisura	80 (40.8)	33 (16.8)	54 (27.6)	29 (14.8)	
GCA	81 (41.3)	17 (8.7)	61 (31.1)	37 (18.9)	
LCA	83 (42.3)	19 (9.7)	61 (31.1)	33 (16.8)	

GCA, greater curvature of antrum; GCC, Greater Curvature of Corpus; *H. Pylori*, *Helicobacter pylori*; LCA, lesser curvature of antrum; LCC, lesser curvature of corpus; N, number.

Table 4. Association of RUT (antral biopsy) and pathologic findings of *H. pylori* according to different areas of the stomach in morbidly obese patients

	Pathologic *H. pylori*	Endoscopic RUT, N (%)		*P*-value	OR (95% CI)
Region		Pos	Neg		
Cardia	Pos	80 (77.7)	23 (22.3)	<0.001	6.03 (3.2, 11.2)
	Neg	34 (36.6)	59 (63.4)		
GCA	Pos	89 (77.4)	26 (22.6)	<0.001	7.6 (4.0, 14.5)
	Neg	25 (30.9)	56 (69.1)		
LCA	Pos	86 (76.1)	27 (23.9)	<0.001	6.2 (3.3, 11.7)
	Neg	28 (33.7)	55 (66.3)		
Incisura	Pos	89 (76.7)	27 (23.3)	<0.001	7.2 (3.8, 13.7)
	Neg	25 (31.3)	55 (68.8)		
LCC	Pos	86 (76.8)	26 (23.2)	<0.001	6.6 (3.5, 12.4)
	Neg	28 (33.3)	56 (66.7)		
GCC	Pos	86 (76.1)	27 (23.9)	<0.001	6.2 (3.2, 11.7)
	Neg	28 (33.7)	55 (66.3)		

GCA, greater curvature of antrum; GCC, greater curvature of corpus; *H. Pylori*, *Helicobacter pylori*; LCA, lesser curvature of antrum; LCC: lesser curvature of corpus; N, number; RUT, rapid urease test; CI, confidence interval; OR, odds ratio.

2). The results showed that in 1176 gastric samples of 196 cases, there was a significant difference between the severity of *H. pylori* infection and six evaluated regions of the stomach ($P < 0.001$). Severe *H. pylori* infection was more common in the distal of the stomach (GCA, LCA, and incisura) than in the proximal (LCC, GCC, and cardia). The highest prevalences of mild, moderate, and severe *H. pylori* infection were in regions of the cardia, LCA, GCA, and GCA, respectively (Table 3). The results showed that there was a significant association between the existence of *H. pylori* infection in pathologic findings of six evaluated regions of the stomach and RUT (P=0.001), and this correlation was highest in GCA (OR=7.6, 95% CI: 4.0, 14.5), but the lowest in cardia (OR=6.03, 95% CI: 3.2, 11.2) (Table 4). Twenty-two patients (22.7%) with positive *H. pylori* in six pathologic areas of the stomach had negative endoscopic RUT. Twenty-one patients (27.6%) with negative *H. pylori* in six pathologic areas of the stomach had positive endoscopic RUT. The overall rate of false-positive (FP) and false-negative (FN) RUT were 27.6% (21 cases) and 32.9% (27 cases), respectively (Table 5).

Overall sensitivity, specificity, positive and negative predictive value (PPV and NPV), positive and negative likelihood ratio (PLR and NLR), accuracy, and OR (and their 95% CI) of RUT for diagnosis of *H. pylori* infection in comparison with pathology were 77.5% (95% CI: 69.2, 84.0), 72.4% (95% CI: 61.4, 81.2), 81.5% (95% CI: 73.5, 87.6), 67.1% (95% CI: 56.3, 76.3), 2.8 (95% CI: 1.9, 4.1), 0.3 (95% CI: 0.2, 0.4), 75.5% (95% CI: 69.0, 81.0), and 9.0 (95% CI: 4.6, 17.4), respectively. The most sensitive area, the highest OR, and the least NLR for pathologic

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Table 5. Association of RUT (antral biopsy) and pathologic findings according to the number of areas with positive *H. pylori* in morbidly obese patients

Pathologic *H. pylori* finding in six areas of the stomach	Endoscopic RUT, N (%)		*P*-value
	Pos.	Neg.	
0	21 (27.6)	55 (72.4)	<0.001
1	2 (100)	0	
2	2 (100)	0	
3	3 (100)	0	
4	4 (80)	1 (20)	
5	7 (63.6)	4 (36.4)	
6	75 (77.3)	22 (22.7)	

H. Pylori, *Helicobacter pylori*; N, number; Neg, negative; Pos, positive; RUT, rapid urease test.

biopsy was incisura (96.7% (95% CI: 91.7, 98.7), 20 (95% CI: 7.6, 51.9), and 0.03 (95% CI: 0.01, 0.08)) (Table 6).

Discussion

In our study, *H. pylori* infection was detected in the pathologic samples more than RUT. Incisura was the most common area of the stomach for pathological findings of *H. pylori* infection. Severe *H. pylori* infection was more common in the distal of the stomach than proximal. There was the highest correlation between pathological findings of *H. pylori* and RUT in GCA and incisura. FP and FN rates of RUT were 27.6*%* and 32.9*%*, respectively. Sensitivity, specificity, PPV, NPV, PLR, N LR, accuracy and diagnostic OR of RUT were 77.5%, 72.4%, 81.5%, 67.1%, 2.8, 0.3, 75.5%, and 9.0, respectively. A Meta-analysis performed in patients with partial gastrectomy for the diagnostic performance of RUT showed sensitivity, specificity, PLR, NLR, and diagnostic OR for *H. pylori* were 79%, 94%, 10.21, 0.28, and 49.02, respectively (6). The gold standard for detecting *H. pylori* infection in the mentioned meta-analysis was based on histology or RUT, while in this study, it was based on the positive test of at least one of 6 regions of the stomach pathology. Decreased specificity is related to increased false positives in this study. Few studies have also evaluated the optimal location of biopsy (15) which reported the best location to detect *H. pylori* infections in children was mid-antrum, at the lesser curvature (15), and antral lesser curvature at or near the incisura in adults (16). We did not find any study in this regard in obese cases. Based on this study, a study with 835 patients undergoing upper endoscopy revealed that severe atrophic, metaplastic, and chronic inflammatory changes were more frequently observed in the incisura angularis mucosa than in the antrum or corpus mucosae ($P < 0.05$) (17).

Regarding the agreement with other locations in the stomach (highest accuracy (97.9*%*), Sen. (96.7*%*), Spc. (100*%*), PPV (100*%*), NPV (95%), PLR (735.7), and OR (20), in accordance with lowest NLR (0.03)), the highest prevalence of *H. pylori* in pathological assessment, 2nd highest association between RUT and pathology for *H. pylori* detection, and 3rd place for the severity of *H. pylori* between six anatomical points in the stomach, it seems that incisura is the optimal anatomical place for detecting *H. pylori* based on biopsy. Hence, we also offer endoscopists to consider incisura instead of antrum if they want to do RUT or pathological assessment of *H. pylori* only in one place. The optimum place to detect *H. pylori* was mentioned to be the mid-antrum at lesser curvature (15) in one study in children, which seems to be much closer to the incisura in our study. This evidence is also in charge of considering incisura as the best single place to detect *H. pylori* in the stomach. It is plausible to take the biopsy from the incisura instead of other anatomical places (special antrum as a default place for biopsy) of the stomach if our plan is to take the biopsy only from one site. Previous studies have not evaluated obese patients as a separate group, have done biopsies from six sites (15), or 12 sites with a minimum of 8 specimens in each case(16), while we did biopsy from six locations in each case. The updated Sydney system recommends taking five biopsy specimens from different sites (18). Similar studies have a

Table 6. Sensitivity, specificity, positive and negative predictive value, accuracy and likelihood ratio, and odds ratio of RUT and six pathologic areas of the stomach in morbidly obese patients

	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	PLR (%) (95% CI)	NLR (%) (95% CI)	Accuracy (%) (95% CI)	OR (95% CI)
RUT	77.50 (69.24, 84.05)	72.37 (61.42, 81.16)	81.5 (73.47, 87.62)	67.07 (56.34, 76.28)	2.80 (1.925, 4.087)	0.31 (0.21, 0.44)	75.51 (69.04, 81.0)	9.0 (4.6-17.4)
Cardia	85.83 (78.48, 90.96)	100 (95.19, 100)	100 (96.4, 100)	81.72 (72.66, 88.26)	653.19 (1.33, 320067.65)	0.14 (0.091, 0.22)	91.33 (86.55, 94.51)	5.4 (3.5-8.4)
Incisura	96.67 (91.74, 98.70)	100 (95.19, 100)	100 (96.79, 100)	95.0 (87.84, 98.04)	735.63 (1.50, 360342.94)	0.03 (0.01, 0.08)	97.96 (94.87, 99.2)	20 (7.6-51.9)
GCA	95.83 (90.62, 98.21)	100 (95.19, 100)	100 (96.77, 100)	93.83 (86.35, 97.33)	729.29 (1.48, 357244.84)	0.04 (0.01, 0.09)	97.45 (94.17, 98.91)	16.2 (6.9-37.8)
LCA	94.17 (88.45, 97.15)	100 (95.19, 100)	100 (96.71, 100)	91.57 (83.6, 95.85)	716.60 (1.46, 351048.64)	0.05 (0.02, 0.12)	96.43 (92.81, 98.26)	11.8 (5.8-24)
LCC	93.33 (87.39, 96.58)	100 (95.19, 100)	100 (96.68, 100)	90.48 (82.32, 95.09)	710.26 (1.45, 347950.54)	0.06 (0.03, 0.13)	95.92 (92.15, 97.92)	10.5 (5.4-20.4)
GCC	94.07 (88.26, 97.1)	100 (95.19, 100)	100 (96.65,100)	91.57 (83.6, 95.85)	715.85 (1.46, 350682.04)	0.05 (0.02, 0.12)	96.39 (92.74, 98.24)	11.8 (5.8-24)

CI, confidence interval; GCA, greater curvature of antrum; GCC, greater curvature of corpus; LCA, lesser curvature of antrum; LCC, lesser curvature of the corpus, NLR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; PLR, positive likelihood ratio; OR, odds ratio; RUT, rapid urease test.

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comparable sample size to the present study: 206 children in the USA (15) have a similar pattern of *H. pylori* colonization severity (higher in distal parts compared to the upper zone of the stomach) (15). Similar to this study, gastritis grading was significantly higher in the presence of *H. pylori* infections, and *H. pylori* colonization was greater in the antrum than in the body (19). Another issue is the level of endemicity of *H. pylori* in different studies. The prevalence of *H. pylori* was 8% in children in the USA (15), and 50.7% in Iran (20). Although most of the indices used in this study are not related to the prevalence of *H. pylori* and our results can be relatively generalized to other regions of the world. Some of them, like PPV and NPV, should be updated in different regions based on the prevalence of *H. pylori*. The indication of endoscopy has not been similar in different studies. In one study, an endoscopy was done for screening (various medical reasons candidate for endoscopy) (15) in another one, subjects who were found to be infected with *H. Pylori* were evaluated with endoscopy (16), while, in our study, the patients are more homogenous and all of them was candidates of bariatric surgery, and we have done upper endoscopy in all of them irrespective of any symptoms or comorbidity. Hence, our results seem more generalizable. FP and FN of RUTs in this study were 27.6 and 32.9, respectively. FP results are related to other microorganisms, commercially available RUT kits, and delay in test restoration after 24 hours. The presence of intestinal metaplasia, antimicrobial drugs, and proton pump inhibitors (PPI) consumption may result in FN results (21). Regarding the FN result of RUT (32.9%) in our study, it is better to check the pathologic findings of *H. pylori* in symptomatic patients with negative RUT.

Conclusion

Incisura is the most sensitive area with the highest OR and the least NLR for pathologic biopsy to detect *H. pylori*. Negative RUT in symptomatic cases needs to be checked with pathologic findings for detecting *H. pylori*, specifically in highly prevalent areas like Iran.

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Ethical Approval

This study was conducted in accordance with the Helsinki Declaration. Also, the Ethics Committee of IUMS approved the study with the code number IR.IUMS.REC 1396.25669, and all patients signed an informed written consent form prior to entering the study. Also, all patients signed a written informed consent for publishing their data. In addition, the Institutional Board Review (IRB) approved this study on 21 July 2015.

List of Abbreviations

H. pylori: *Helicobacter pylori*
RUT: rapid urease test
PPV: positive predictive value
NPV: negative predictive value
PLR: positive likelihood ratio
NLR: negative likelihood ratio
OR: odds ratio
Kg: Kilogram
m2: square meter
BMI: body mass index
UGIE: upper gastrointestinal endoscopy
IUMS: Iran University of Medical Sciences
RYGB: Roux-en-Y Gastric Bypass
cm: centimeter
SPSS: Statistical Program for Social Sciences
SD: standard deviation
CI: confidence interval
GCA: greater curvature of the antrum
LCA: lesser curvature of the antrum
GCC: greater curvature of corpus
LCC: lesser curvature of corpus

Authors' contributions

AK, AFK: Conceptualization, proposal drafting, Project administration and validation;
AK, AZ, AP, MM, SAg, AFK: Data collection and curation;
AK, SA: Formal analysis, first drafting;
AZ, AP, MM, AFK: substantial commenting;
AK, SA, AZ, AP, MM, SAg, AFK: final drafting, responsibility of the whole manuscript.

Conflict of Interests

The authors declare that they have no competing interests.

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