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Molecular identification of *Blastocystis hominis* isolates in patients with and without Gastrointestinal diseases

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Blastocystis hominis (*B. hominis*) is an anaerobic, single-cell protozoan, it is the most common protozoan in human fecal samples with potential pandemic distribution. The prevalence of *B. hominis* varies. The aim of this study was to detect prevalence of *B. hominis* in patients complain of gastrointestinal and non-gastrointestinal troubles. A case control study was conducted during the period from January 2022 to April 2023. Stool samples were collected from 100 patients (50 complain of gastrointestinal and 50 without gastrointestinal troubles) and examined by wet mount microscopic examination, stained by trichrome stain and conventional PCR molecular technique were used to confirm the diagnosis. PCR was the gold stander test for diagnosis. Also blood samples were collected for complete blood count. The prevalence of *B. hominis* in this study was 7/50 (14%) using PCR, in patients complain of gastrointestinal troubles, 5/50 (10%) using PCR, in patients complain of non-gastrointestinal troubles and 12/100 (12%) in total patients under study complain of gastrointestinal and without gastrointestinal troubles. There was increase in prevalence in rural area 5/14 (35.5%) in comparison to urban area 7/86 (8.1%) due to unsanitary living conditions, frequent animal contact, and consumption of tainted water and food. PCR is sensitive and specific test for diagnosis of *B. hominis* and its prevalence was high especially in patients with gastrointestinal troubles, so it is recommended to implement preventive measures and awareness programs regarding sanitation and personal hygiene.

Keywords: *Blastocystis hominis*, PCR, Prevalence

INTRODUCTION

A Russian scientist originally described the parasite *Blastocystis hominis* (*B. hominis*) in 1870, but it was overlooked due to its lack of taxonomic status. *Blastocystis hominis* was officially named after it was detected as a harmless yeast in a stool sample in 1912. It is now known to be the most common intestinal parasite. It is becoming more common, in contrast to other intestinal harmful parasites. In terms of the prevalence of this parasite, countries have been split into two groups: developed (prevalence up to 10%) and developing (prevalence between 50 and 60%). The high prevalence of this parasite is mainly due to lack of health improvements in terms of vaccinations and other protective measures (Badparva and Kheirandish, 2020).

B. hominis is one of the most prevalent parasites found in symptomatic and asymptomatic people's stool samples. It is spread by the feco-oral pathway and present in four main forms: vacuolar, granular, amoeboid and cysts. In fecal specimens, the vacuolated type (10-30 µm) most frequent. It was once thought to be a harmless yeast, but it is now recognized as a cause of human intestinal disease. The symptoms of disease caused by *B.hominis* rang from mild diarrhea to acute gastroenteritis. Water-resistant cysts, which are split into two kinds, cause infection Thin-walled *B.hominis* may produce autoinfection within the host, while the thick-walled *B.hominis* can cause direct infection transmission

to others via water and food (Eassa. 2016).

Despite the fact that it has been discovered for 100 years, only a few of its indicators, such as morphological traits, have been characterized, and other biological indices are not well-defined; thus, it is known as amysterious parasite. The pathogenic properties of this parasite are one of the ambiguities. Several biological investigations have lately described the parasite's heterogeneity and discovered its several forms, which have served as the foundation for a few epidemiologic and pathogenic studies (Badparva and Kheirandish, 2020).The source of human infectious *Blastocystis* is uncertain. *Blastocystis* occurs in many animals, including insects, reptiles, birds and mammals. Some evidence suggests that *Blastocystis* may not be host specific and that animal-to-human transmission is possible (Ning, et al. 2019).

Many patients who have *Blastocystis hominis* in their faeces show no signs or symptoms. The most common symptoms observed constantly by individuals who report symptoms such as diarrhoea, abdominal pain, and vomiting. Anal irritation, weight loss, constipation, and increased gas have also been described as symptoms. Even if *Blastocystis* is discovered in the stools, such symptoms could be caused by other illnesses, some patients infected with *B. hominis* develop skin allergy symptoms such as erythema, itching, and urticaria, where the cause is thought to be IgE released as a result of the immune system's response to the parasite's surface antigens (Badparva and Kheirandish, 2020).

Immunocompromised patients had a higher prevalence of Blastocystosis infection than the general population, according to their findings. Many countries currently lack adequate information on the prevalence rate. Nonetheless, new evidence suggests that *Blastocystis* should not be overlooked (Khorshidvand et al. 2020).

When the prevalence of *Blastocystis* was investigated, it was discovered that it did not exceed 5% among the populations of the major industrialized countries. Yet, it has been shown that up to 23% of the world's population is infected with it. It was noticed that in the least developed countries, the percentage might increase to effect the majority of the people in these countries (Mohamed et al. 2017).

For the detection of *B. hominis* in stool specimens, smears were less sensitive than short-term in vitro cultivation in Jones medium. In most laboratories, permanent trichrome staining is the gold standard for diagnosing *B. hominis* infection. However, the trichrome staining process may not be appropriate for field assessments. For the investigation of such infections, genotypic characterization by PCR has proven to be a valuable tool (Termmathurapoj et al. 2004).

B. hominis has a variety of morphological forms. The most well-known varieties include vacuolar, granular, ameboid, and cystic. Additional morphological types have been discovered using electron microscopy (a vacuolar and multi-vacuolar, of small dimensions are rarely present). Vacuolar and granular forms are the most typically observed in fresh stool samples and culture samples; they can be visualized by phase-contrast microscopy, light microscopy of native and stained sample preparations, and electron microscopy (Zierdt, 1991).

Several molecular methods have been utilized in recent years to classify *Blastocystis sp*. subtypes isolated from humans and animals in various parts of the world. The sequence variation in the small-subunit ribosomal RNA genes of isolates was studied utilizing random amplified polymorphic DNA (RAPD) employing four different arbitrary polymerase chain reaction (PCR) primers and PCR tests, followed by restriction fragment length polymorphism (RFLP) (Mohamed et al. 2017). So The aim of this study was to detect prevalence of B*. hominis* in patients complain of gastrointestinal and non-gastrointestinal troubles

MATERIALS AND METHODS

In this study a prospective, case-control method was conducted in King Faisal Medical Complex, Taif, Saudi Arabia. The study included 50 patients as cases with various gastrointestinal symptoms of various ages and sexes. In addition, a control group of 50 age-sex matched cohorts from the same population suffering from non-gastrointestinal conditions as urticaria and anemia for investigations also patients coming for preoperative investigation were recruited during the period from January 2022 to April 2023. Patients receive treatment for intestinal parasitic infection, those who have comorbid illness that have the ability to lower patient's immunity (cancers, renal dialysis, steroids intake, diabetes, and chronic debilitating illnesses) were excluded from the study. All of the participants were given fecal collection containers. Three stool samples were obtained from each participant after a detailed explanation of the protocol. Following the previous procedure (Garcia et al. 2017), feces were collected three times over the course of three days. The samples were carefully labelled and divided into two parts for wet, iodine mount, formol-ether concentration, Trichrome stain and other part for molecular diagnosis by PCR. Blood samples also withdrawn for complete blood count

Blastocystis hominis stool PCR:

Following the kit's protocol, the alcohol-kept fecal aliquots were subjected to DNA extraction using the genomic DNA purification Kit QIA amp® Fast DNA Stool Mini Kit (cat. No. 51604 Qiagen-Germany). DNA extracts were subjected to PCR amplification following a previously published assay (Videnska et al. 2019). The reaction setup and thermal cycles were conducted in a LightCycler (Roche Diagnostics Corporation, 100 Mannheim, Germany). With final concentrations closely

similar to the previously published assay, the Go Taq Hot Start Polymerase (Promega) and other PCR reagents adopted in the amplification reactions were used.

Data processing and statistical analysis:
The results were analyzed using computerized software program SPSS version 22. Ordinal data are compared using Chi-square test, exact Fisher test. Continuous numerical data are presented as mean and standard deviation and between-group differences are compared using the independent-samples t-test. P-values <0.05 are considered statistically significant.

Ethical considerations:
This study was approved by Taif University's and KFMC ethical council its IBR no. was (H-02-T-123).

This study was performed on 50 cases complain of gastrointestinal troubles (21 males and 29 females) with 39.54±17.79 mean age and 50 controls complain of non-gastrointestinal troubles (23 males and 27 females) with ***41.60±20.70*** mean age. Table-1 outlines the common demographic and clinical data of both cases and controls. Table 2 shows detection of *B. hominis* in stool specimens of cases and controls. The parasite was microscopically identified and confirmed in 12 participants by the specific PCR assay, with a documented overall prevalence rate of 12%, Table-2; Figure1. The parasite was identified with a prevalence rate of 12% and 10% by PCR and direct microscopic examination, respectively in cases and controls. There was a significant difference in the levels of positivity of *Blastocystis* infection between cases and controls, (P<0.05).

RESULTS

Table-1: Demographic and clinical data of both cases and controls

Variable		*Blastocystis* sp. Infection (n, %)		OR	95% CI for OR	*P* value
		Positive (n = 12)	Negative (n=88)			
Sex	Male (n = 44)	5 (41%)	39 (44%)	0.897	0.295-2.893	0.999
	Female (n = 56)	7 (58%)	49 (55%)			
Residence	Urban (n = 86)	7 (58%)	79 (90%)	0.159	0.046-0.588	0.012*
	Rural (n = 14)	5 (41%)	9 (10%)			
Gastric ulcer	Yes (n = 11)	1 (8%)	10 (11%)	0.709	0.060-4.336	0.999
	No (n= 89)	***11*** (***92***%)	78 (***89***%)			
Duodenal ulcer	Yes (n = 15)	0 (0%)	15 (17%)	0.000	0.000-1.548	0.204
	No (n= 85)	***12*** (***100***%)	73 (***8***3%)			
Atrophic gastritis	Yes (n = 5)	2 (16%)	3 (3%)	5.667	0.895-29.660	0.108
	No (n= 95)	***10*** (***8***3%)	85 (***97***%)			
IBD	Yes (n = 19)	4 (33%)	15 (17%)	2.433	0.731-8.642	0.234
	No (n= 81)	***8*** (***67***%)	73 (***8***3%)			
Urticaria	Yes (n = 7)	1 (8%)	6 (7%)	1.242	0.099-9.563	0.999
	No (n= 93)	***11*** (***92***%)	82 (***9***3%)			
AFI	Yes (n = 36)	4 (33%)	32 (36%)	0.797	0.251-2.589	0.999
	No (n= 59)	***8*** (***67***%)	51 (***58***%)			
POI	Yes (n = 7)	0 (0%)	7 (8%)	0.000	0.000-4.615	0.594
	No (n= 93)	***12*** (100%)	81 (***92***%)			

Abbreviations: OR: odd ratio; CI: confidence interval; IBD: Irritable Bowel Diseases, AFI: Anemia for investigation, POI: Preoperative investigation

Table 2: Hematological parameters in positive and negative *Blastocytis* infected patients with and without gastrointestinal diseases

Diseases	Gastrointestinal diseases		*P* value	Non- Gastrointestinal diseases		*P* value
	Positive (Mean±SD)	Negative (Mean±SD)		Positive (Mean±SD)	Negative (Mean±SD)	
WBC	8.84±1.90	7.62±2.79	0.05*	9.66±4.37	7.83±2.67	0.18
Neutrophils	5.54±2.37	4.80±1.94	0.21	5.38±3.54	4.56±1.96	0.42
Lymphocytes	2.37±1.67	2.37±0.89	0.20	3.80±1.12	2.67±1.17	0.08
Monocytes	1.29±0.56	0.88±0.48	0.02*	0.98±0.38	0.77±0.47	0.35
Eosinophiles	0.14±0.22	0.11±0.13	0.95	0.13±0.12	0.16±0.17	0.63

* Significant result

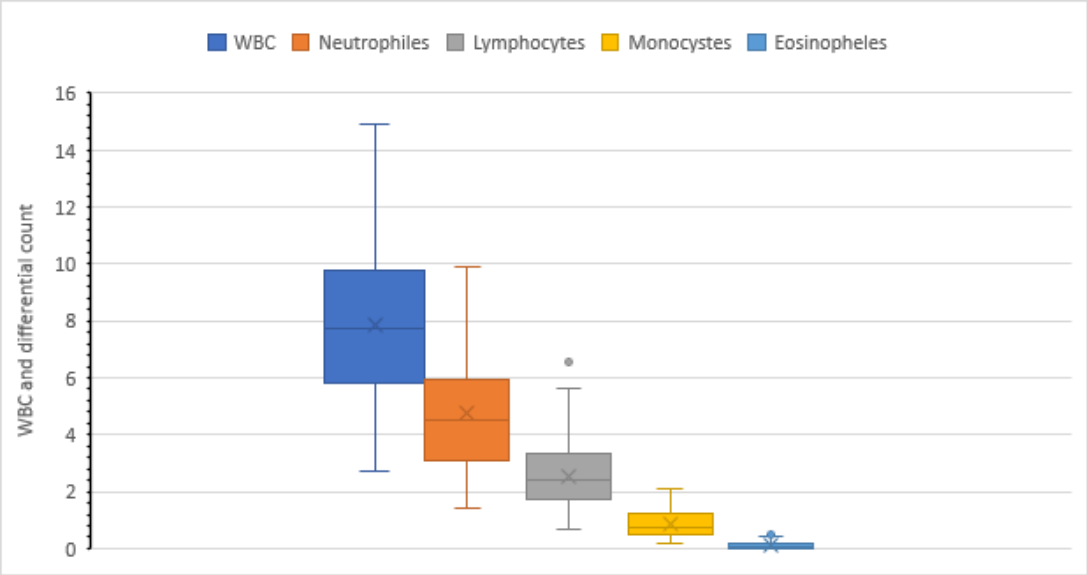


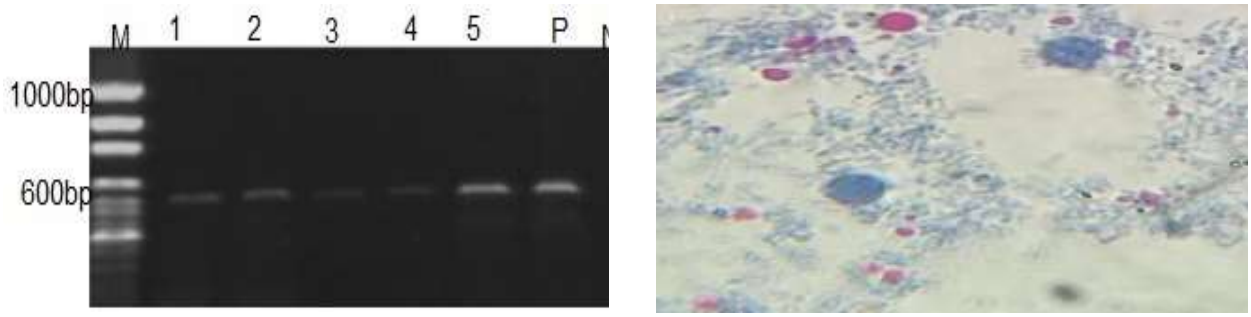
Figure 1: Box-and-whisker plot analysis for Hematological profile of patients

Results were submitted to the box-and-whisker plot analysis to detect the outliers, lines in the boxes signify the median values; the upper/lower border-lines of the boxes denote the 25th and 75th percentiles, respectively; and the upper/lower bars outside the boxes represent the 90th and 10th percentiles, respectively. While the separate dots are outliers excluded from the study.

Table 3: Diagnostic performance of microscopy versus the PCR tests in patients with and without gastrointestinal diseases

Test	Positive		Negative		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Agreements % (Kappa test)
	True	False	True	False					
Wet mount microscopy	10	0	90	2	85.7% (38.38- 88.18)	100% (96.97– 100.0)	100%	97.77% (92.9-98.56)	98.1% (0.353)
PCR assay	12	0	88	0	NA	NA	NA	NA	NA

Abbreviations: CI: confidence intervals; PPV, positive predictive value; NPV, negative predictive value; NA, not applicable.



A B

Figure 2 (A, B): A.Gel electrophoresis for *B. hominis* PCR. Lane M:1000-bp DNA marker; Lane 1-5: Five samples found to be positives for *B. hominis*. B, Trichrome stained *B. hominis* vacuolar type.

DISCUSSION

Blastocystis is a ubiquitous protozoan with global distribution, and it is the most common parasite eukaryote, invading the human intestinal tract and infecting approximately 1 billion people globally. The frequency of Blastocystosis infection varies greatly between countries, depending on sanitary conditions. Early findings from developed countries indicated a lower prevalence of *Blastocystis* (about 5%), whereas it appeared to be more frequent (approximately 30-60%) in developing countries (Alemu et al. 2011).

Many factors can influence the prevalence of Blastocystosis infection, including geographic location, host age, eating habits, immune status, and conditions such as HIV, hematological malignancies, immunosuppressive drug use, and solid organ transplants at risk of opportunistic infections; this parasite is frequently found in immunocompromised patients, indicating an opportunistic pathogenesis (Khorshidvand et al. 2020).

It is generally agreed that the prevalence of Blastocystosis infection, at various levels, is high in various developing countries. Owing to the high likelihood of intestinal parasites (Piubelli et al. 2019). When compared to developed countries, developing countries have a higher prevalence. Also, the incidence varies greatly across the country. Communities with inadequate hygiene, a lack of safe water supply, and a sewage system had a greater prevalence. Infection, however, was recorded in all areas and socioeconomic classes. The prevalence of *B. hominis* in this study were 7/50 (14%) in patients complain of gastrointestinal diseases, 5/50 (10%) in patients complain of non gastrointestinal diseases and 12/100 (12%) in total patients under study complain of gastrointestinal and without gastrointestinal diseases by PCR and 10(10%) by light microscopic examination of stool smears all the detected *B. hominis* is of vacuolar types this finding was going more or less with studies showed that the *Blastocystis hominis* prevalence was (13.5%) in Thailand (17.5%) in Saudi Arabia, also rates of prevalence were seen in European countries such as Italy (13.6%). In contrast to other studies which show high prevalence such as Malaysia (25.7%), China (32.6%),Turkey (56.3%) (Beyhan et al.2016). This discrepancy is highly dependent upon the diagnostic technique and the age groupings of the individuals under investigation and the geographic distribution.

Table 1 showed gender distribution among studied patients with and without gastrointestinal diseases where females represent 56% while males represent 44%. Tangi et al. 2016 agreed with our results where they found more female participants who are also more infected with intestinal parasites (10.5%) than men (8.9%), also study done by El-Shazly et al. 2005 agreed with our data. On the contrary to our results, The prevalence of *Blastocystis* in males and females was approximately the same (1:1 ratio) as mention by Dagci et al.2014 or had no correlation as shown by Yaicharoen et al.2006, while, Alver and Tore 2006 found that 32.1% of *Blastocystis* positive patients were females and 67.9% of them males. Other studies have also reported higher infection rates in men compared to women (Khoshnood et al. 2015). As most of our study cases are females can explain the discrepancy.

In table 1, also showed that urban resident positive *Blastocystis* patients were 7/86 with percentage of 8.86% while rural positive ones were 5/14 patients with percentage of 55.56% with statistical significant value, this result is in agreement with Viesy et al. 2022 where they found *Blastocystis*-positive was more common among rural people (59.3%) than urban subjects (40.7%), Shaker et al.2017 also agreed with our results as they concluded prevalence of *Blastocystis* in rural residents was higher than urban ones, and as the level of education increased, the prevalence of parasites decreased. This supposed to be related to people jobs. There are several reasons for the high level of infection among villagers, such as direct contact with animals, soil, lack of sanitary conditions, consumption and use of non-potable water and use of human fertilizers (Boonjaraspinyo et al. 2013).

The prevalence of *Blastocystis* positive cases is high in patients with atrophic gastritis 40%, follow by irritable bowel diseases (IBD) 21.1%, gastric ulcer 9%, duodenal

ulcer 0% as regard patients without gastrointestinal diseases the prevalence of *Blastocystis* positive cases is high in patients with urticaria 14% followed by anemia for investigation by 11% and 0% for preoperative patients.

The mean age of diseases patients with gastrointestinal diseases in our study was 39.54 ± 17.79. There were different findings regarding the relationship between age and gender with the *Blastocystis* infection. Qadri et al.1989 observed that the infection is mostly between the ages of 13-50 years with a 71.8% ratio, and 19.3% at ages over 50. In another study Dagci et al.2014 showed that *Blastocystis*-positive patients were predominantly between 20-29 years old. Unlike this, it was significantly higher between the ages 0-19 Ozçakir et al.2007 and the age distribution was determined homogenous with no significant correlation (Cirioni et al. 1999).

In the present study table 2 showed the CBC parameters of the gastrointestinal disease patients were expressed in mean ± SD, WBC, neutrophils, monocytes and eosinophils showed lower levels in negative *Blastocytes* infection than positive *Blastocytes* ones, while lymphocytes level very slightly increase in positive *Blastocytis* infected persons than negative *Blastocytes* ones. Salam et al.2015, the patients and there is also an increase in the count of monocytes compared to the control group. Increased neutrophils in patients' blood may be attributable to the fact that these cells are the body's first line of defense against pathogens. As a result, the introduction of a causative factor boosts and increases the amount of neutrophils to identify and ingestion of germs. Monocytes are white blood cells have the potential to travel from the bloodstream to the location allowing the infection to settle in the tissue and termed macrophages, which have a high capacity for engorgement pathogens, and some studies have shown that the infection of the *Blastocystis* stimulates a specialized locational response in the host, which include: T lymphocytes, monocytes, macrophages and natural killer cells (Nataša et al. 2017).

On the contrary to our results study done by Ahmad, 2019found a decrease in the lymphocytes compared to control group this result is relative to the result of a study in DhiQar, Iraq, which showed that there is a decrease in the lymphocytes in the case of intestinal parasitic infection (Daaj et al. 2017). Significance difference has been noticed between positive and negative *Blastocytis* infected patients with gastrointestinal diseases in monocytes ($P<0.05$), Non-significance difference has been found in all other CBC parameters between positive and negative *Blastocytis* infected patients with gastrointestinal diseases.

As shown in table 2 a significance increases has been found between *Blastocystis* infected patients with and without gastrointestinal diseases in WBC and monocytes ($P<0.05$). Non-significance difference was found as regard neutrophils, lymphocytes and eosinophiles. In contrast to the study conducted by Cheng et al.2003 which found that hemoglobin, neutrophil count, and hematocrit were decreased in subjects with *B. hominis* infections, as *Blastocystis hominis* is a possible factor in hematological abnormalities.

Table 3 showed that true positive cases are higher in PCR assay than in microscopy, this finding is in consensus with previous reports that molecular detection modalities are superior over microscopic examination and culture methods for the detection of *Blastocystis* from human stool samples (Cheng et al. 2011). Conversely, there are few studies suggesting the usefulness of culture methods over PCR (Santos and Rivera, 2013). The microscopy-based studies from India have shown a low prevalence of *Blastocystis* (Mohandas et al. 2002; Basak et al. 2013). But molecular detection modalities have revealed a dramatic increase in the frequency of detection of *Blastocystis* (Pandey et al. 2015).

CONCLUSIONS

Our study demonstrated a higher prevalence rate of *Blastocystis* infection among patients complain of gastrointestinal troubles 14 % (cases) than patients complain of non-gastrointestinal troubles 10% (control). *Blastocystis hominis* is a possible factor in hematological abnormalities. Another cohort study on a healthy population is recommended for determination of the cause and effect relationship. A potential research on subtyping the clinical isolates of *Blastocystis* was also suggested. We suggest screening for the presence of *Blastocystis* especially in those with atrophic gastritis and IBD.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/10.3390/antiox12081524/s1

Author contributions

Conceptualization,H.M.H.and K.A.I.; resources, A.A.A., M.A., H.J.B., M.M.A.,and M.R.A.; writing original draft preparation, K.A.I., S.A.Q. and E.A.A.; writing review and editing, M.M.A. K. M. A.and H.M.H.; statistical analysis: H.M.H., A.K.R. and O.M.K.; supervision, K.A.I. and A.A.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the King Faisal Medical Complex. IBR number (H-02-T-123).

Informed Consent Statement
Not applicable.

Data Availability Statement
All of the data is included in the article/Supplementary Material.

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Conflict of interest
The authors declare no conflict of interest.

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