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Original Article

Significance of intestinal alkaline phosphatase in predicting histological activity of pediatric inflammatory bowel disease

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ABSTRACT

Background. Intestinal alkaline phosphatase (iAP) is an intestinal brush border enzyme that is one of the factors involved in the pathogenesis of inflammatory bowel disease (IBD). The aim of the study was to investigate the relationship between iAP enzyme and histological inflammatory activity in patients with IBD.

Methods. A total of 44 children were enrolled in this study including IBD patients (n=24; 12 Crohn's disease [CD] and 12 ulcerative colitis [UC]) and controls (n=20). Anti-human iAP antibody stained ileocolonoscopic biopsy specimens were graded for the terminal ileum and each section of the colon. Hematoxylin-eosin stained sections were used to determine inflammatory activity. Histopathological findings were compared in pre- and post-treatment biopsies of each group and with the control group (CG).

Results. A low grade of iAP staining was detected in IBD patients compared to the CG (p=0.02). iAP was remarkably concentrated in the terminal ileum (TI) and especially in region 1, which involved the apical surface, brush border, and epithelial cells. A significant negative correlation was found between the grade of iAP staining and inflammatory activity both in pre- and post-treatment biopsies (p=0.02, p=0.008, respectively) in the terminal ileum of CD patients. Likewise, pre-treatment biopsies of UC and CD patients and biopsies of the CG were compared with each other according to the grade of iAP staining. There were significant negative correlations for CD patients compared to UC and the CG in region1 of TI, and regions 1 and 2 (lamina propria and goblet cells) of the colon (p= 0.015, p= 0.006, p<0.001, respectively).

Conclusions. As a histological marker, iAP can be of value in monitoring the histological activity of IBD, particularly in remarkable inflammation in the small intestine.

Key words: inflammatory bowel disease, intestinal alkaline phosphatase, disease activity.

Alkaline phosphatases (APs), a group of enzymes, are classified into two subtypes; tissue non-specific APs and tissue-specific APs. Tissue-specific APs cover placental, germ cell, and intestinal AP (iAP).[1] IAP is an intestinal brush border enzyme that is expressed on the apical surface of the microvillus of enterocytes and exists in both membrane-bound and soluble forms.[2,3] The soluble form is situated in the area between the microbiota, the food, and the host. iAP has a pivotal role in maintaining intestinal mucosal defense mainly through the detoxification of bacterial endotoxins (e.g. lipopolysaccharide).[4] Other roles of iAP in controlling inflammation are to regulate intestinal bicarbonate secretion, to decrease bacterial translocation and toll-like receptor 4 expression.[4,5]

Inflammatory bowel disease (IBD) is a chronic and relapsing inflammatory disease of the

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gastrointestinal system whose prevalence has increased worldwide particularly in industrialized societies. Even though various interrelated genetic and environmental factors have roles in the development of IBD, the pathogenesis of the disease is not accurately known. iAP enzyme is thought to be one of the factors involved in the pathogenesis. As is already known both iAP expression and activity decrease in inflammatory processes including necrotizing enterocolitis, celiac disease, and IBD.[6-9]

There are limited data on the relationship between iAP and inflammation of IBD within the childhood age group. The aim of the study was to investigate the relationship between iAP enzyme and histological inflammatory activity in patients with IBD.

Material and Methods

The study was conducted at the Pediatric Gastroenterology, Hepatology and Nutrition Division of Hacettepe University. The study protocol was approved by Hacettepe University Non-Interventional Clinical Researches Ethics Committee of the University (study approval number 13/400-20). This was a retrospective, single-center, and non-randomized case-control trial. Twenty-four children (12 males, 12 females) with IBD and twenty children comprising the control group, were enrolled in the study. The diagnosis of IBD was established according to the criteria of Porto.[10] Twelve of the patients (6 male; mean age, 11.5 years) had Crohn's disease (CD) and the other 12 (6 male; mean age, 12.9 years) had ulcerative colitis (UC). Demographic characteristics of patients are summarized in Table I. Twenty children (11 male; mean age, 12.7 years) who underwent an ileocolonoscopy for various symptoms (ie. chronic diarrhea, chronic abdominal pain, and bloody stool) and had normal biopsy results in the histopathological examination were enrolled as the control group.

All patients had received standard bowel preparation before the ileocolonoscopy. A regular colonoscope (Olympus GIF-Q260 video colonoscope) had been used for the examination. One to two biopsies had been taken with forceps from TI and each colonic segment (cecum, ascending, transverse, descending colon, and rectosigmoid colon). In total two ileocolonoscopies were performed on the IBD patients group; one before and one after the treatment. The time interval between pre-treatment endoscopy and post-treatment endoscopy was 6-48 months (median: 12 months). Each patient in the control group underwent only one ileocolonoscopy.

Pediatric Crohn's Disease Activity Index (PCDAI) and Pediatric Ulcerative Colitis Activity Index (PUCAI) were used for all IBD patients to measure the disease activity before and after treatment. The relevance between disease activity indexes (PCDAI and PUCAI) and histological findings were also evaluated. We compared PCDAI and PUCAI with inflammation score and iAP staining for TI and each colonic segment.

Paris Classification was also used for predicting the IBDs course by using the age and growth failure of the patients as well as the location and behavior of the disease.

Biopsies were fixed in formaldehyde and embedded in paraffin. Hematoxylin-eosin (H&E) sections from all biopsies from the terminal ileum and five colon segments were used to evaluate the presence and extent of neutrophilic infiltration, cryptitis, crypt abscess, epithelial erosion, and ulceration, by modified Riley score.[11] Modified Riley score classifies histological activity as; 0; neutrophils in epithelium, none crypts involved; 1, neutrophils in epithelium, <%25 crypts involved; 2, neutrophils in epithelium, ≥ %25-≤ %75 crypts involved; 3, neutrophils in epithelium, >%75 crypts involved; 4, neutrophils in lamina propria, mild but unequivocal increase; 5, neutrophils in lamina propria, moderate increase; 6, neutrophils in lamina propria, marked increase; 7, erosion or ulceration.

For immunohistochemical staining, an anti-human intestinal alkaline phosphatase antibody (1:100 dilution, ab95462; Abcam, Cambridge, MA, USA) was used and the immunohistochemical staining was graded as Grade 0: no staining; Grade 1: 1-25%; Grade 2: 26-50%; Grade 3: 51-74%; Grade 4: ≥ 75% of the tissue section. Since positive iAP staining was observed in different parts of the biopsies, such as the apical surface, epithelia, or lamina propria, this grading system was applied for two localizations: Region 1, composed of apical surface, brush border, and epithelial cells, and Region 2, composed of lamina propria and goblet cells. An experienced, blinded pathologist performed all histological evaluations by using an Olympus microscope.

Biopsies of the IBD patients and the control group were first evaluated individually for each patient and then compared with each other. The grade of H&E-stained sections was compared with the grade of iAP antibody-stained sections for both region 1 and region 2 of TI and each colonic segment. An average grade was attained by dividing the sum of individual colonic segmental scores by the number of colonic segments (cecum, ascending, transverse, descending colon, and rectosigmoid colon) both for H&E and iAP antibody. The colon was included in the statistical analysis as a single data. Finally, pre- and post-treatment biopsies of the patient group were compared with each other according to the grade of staining with H&E and iAP antibodies.

Statistical analysis

The data were analyzed using IBM SPSS software, version 21 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed when the p-value was <0.05. All results were expressed as median (minimum-maximum). Spearman's rank test was used for the association between quantitative variables. Kruskal-Wallis test was followed by the Mann-Whitney U-test for comparison between groups. Wilcoxon rank-sum test was used to compare pre and post treatment results.

Results

A total of 44 children were analyzed (24 IBD patients, 20 control). Demographic and clinical characteristics of patients are shown in Table I. The patients with CD were admitted mostly with complaints of diarrhea, while the UC patients with bloody stool, and the control group with complaints of abdominal pain.

The patients with CD had neither stricturing and penetrating disease nor perianal disease. All patients, except one with inflammation only in his terminal ileum and cecum, had involvements in the terminal ileum and all colon segments. There was no distal colitis or proctocolitis among the UC patients. Most of the patients had pancolitis (E4) (83.4 %) or extensive disease (E3) (Table I).

In the individual comparison of pre-treatment biopsies of UC and CD patients, and biopsies of the control group we found a statistically significant negative correlation between the grade of H&E-stained sections and iAP antibody-stained sections only in region 1 of TI in CD patients (p-value 0.02, r- value -0.686). In UC patients and the control group, there was no statistically significant correlation between the TI and colon (Table II).

Post- treatment biopsies of each of the IBD patients were evaluated individually and we found a negative correlation (p-value 0.008, r-value -0.747) between the grade of H&E-stained sections and iAP antibody-stained sections in region 1 of TI in CD patients. There was a negative correlation between the grade of H&E-stained sections and iAP antibody-stained sections in the colon of CD patients but it was not statically significant. In UC patients we found no significant correlation between the grade of H&E-stained sections and iAP antibody-stained sections for TI and any colonic segment (Table II).

Pre-treatment biopsies of IBD patients and biopsies of the control group, taken from the same region, were compared with each other. The first comparison was done according to the

Table I. Demographic and baseline characteristics of patients at diagnosis.

	CD (n=12)	UC (n=12)	UC (n=12)
Male/Female	6/6	6/6	11/9
Age at diagnosis (years)			
mean±SD	11.5±4,58	12.9±3,47	12.7±3,37
Paris classification age. n (%)			
A1a	3 (25%)	3 (25%)	
A1b	9 (75%)	9 (75%)	
Paris behaviour, n (%)			
B1	12 (100%)		
Paris location, n (%)			
L1	1 (8.3%)		
L4	11 (91.7%)		
Paris growth delay, (G1), n (%)	3 (25%)		
Paris extent			
E3		2 (16.6%)	
E4		10 (83.4%)	
Paris severity (S1), n (%)		3 (25%)	
Main symptom (%)	Diarrhea (41%)	Bloody stool (91%)	Abdominal pain (85%)
Laboratory findings, mean±SD			
Hb (g/dl)	10.7±1.78	10.5±3.27	13.2±0.96
WBC (x10^3/μl)	13±6.1	11.8±5.4	7.9±2.9
Plt (x10^3/μl)	539±132.1	497±231.7	298±92.7
Alb (g/dl)	4.0±0.63	3.9±0.98	4.7±0.3
ESR (mm/h)	52.2±31.7	26.8±18.5	14.3±17.5
CRP (mg/dl)	7.9±8.44	1.9±2.06	0.9±2.9
Fecal calprotectine (μg/g)	423±264.6	824±814.8	46±122.9 (n=9)

Hb: Hemoglobin, WBC: White blood cell, Plt: Platelet, Alb: Albumin, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive protein, CD: Crohn's disease, UC: Ulcerative colitis

Table II. Grades of pre-treatment and post-treatment ileocolonoscopic biopsy specimens stained for H&E and iAP according to the patient and control groups

		Hematoxylin-Eosin-stained sections		iAP antibody-stained sections			
		T. Ileum Mean (min-max)	Colon Mean (min-max)	T. Ileum Mean (min-max)		Colon Mean (min-max)	
				Region 1	Region 2	Region 1	Region 2
CD	Pre-treatment	4 (0-7)	2.94 (0-7)	1.4 (0-4)	2.02 (0-4)	2.13 (0-4)	2.25 (0-4)
	Post-treatment	2.5(0-7)	2.06(0-7)	1.81(0-4)	2.75(0-4)	2.19(0-4)	2.43(0-4)
	p-values	0.32	0.12	0.32	0.27	0.42	0.34
UC	Pre-treatment	1.3(0-7)	2.95 (0-7)	2.82 (0-4)	2.4 (0-4)	2.44 (0-4)	2.06(0-4)
	Post-treatment	1.27(0-7)	2.65(0-7)	2.87(0-4)	2.49(0-4)	2.48(0-4)	2.26(0-4)
	p-values	1.00	0.31	0.66	0.20	0.37	0.33
Control Group		0.68 (0-2)	1.02 (0-4)	2.84 (0-4)	2.05 (0-4)	2.45 (0-4)	2.8 (0-4)

Intestinal alkaline phosphatase. iAP; CD. Crohn's disease; UC. Ulcerative colitis; region 1. apical surface, brush border, and epithelial cells; region 2. lamina propria and goblet cells.

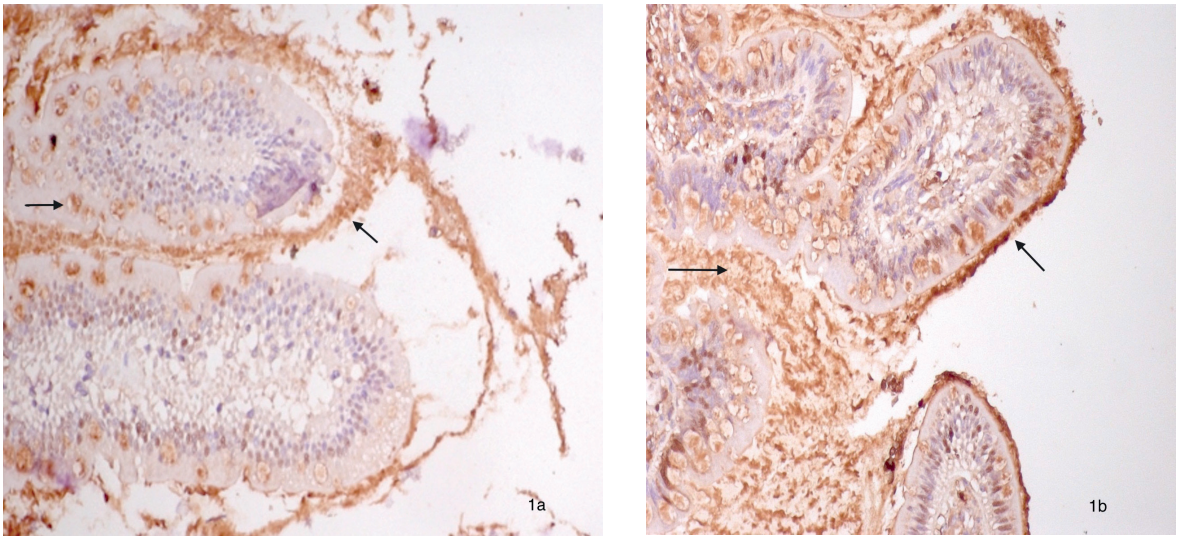

Fig. 1. Pre-treatment TI biopsy of a Crohn Disease patient stained for iAP (1a), low-intensity of iAP staining (arrow); TI biopsy of a control group patient stained for iAP (1b), high-intensity of iAP staining (arrow).

grade of H&E-stained sections. In the control group the grade of H&E-stained sections was lower both in TI and colon compared to IBD patients (p-value 0.005, < 0.001 respectively). Among the IBD patients, we determined that the grade of H&E staining was lower in TI of UC than in the CD patients but it was not statically significant. The second comparison was done according to the grade of iAP antibody-stained sections. In CD patients the grade of iAP staining of region 1 of TI was lower compared to patients with UC and the control group (p-value 0.015) (Fig. 1). We also found a significant difference between IBD patients and the control group, the grade of iAP staining of both region 1 and 2 of the colon were lower in IBD patients (p-value= 0.021, <0.001 respectively) (Fig. 2). This was the result of the low staining grade of CD patients with respect to the control group (p-value 0.006, <0.001 respectively).

Pre- and post-treatment biopsies of IBD patients were evaluated. We found a decrease in histologic grades of all CD (p-value for TI and colon 0.32, 0.12, respectively) and UC (p-value for TI and colon 1.00, 0.31, respectively) patients

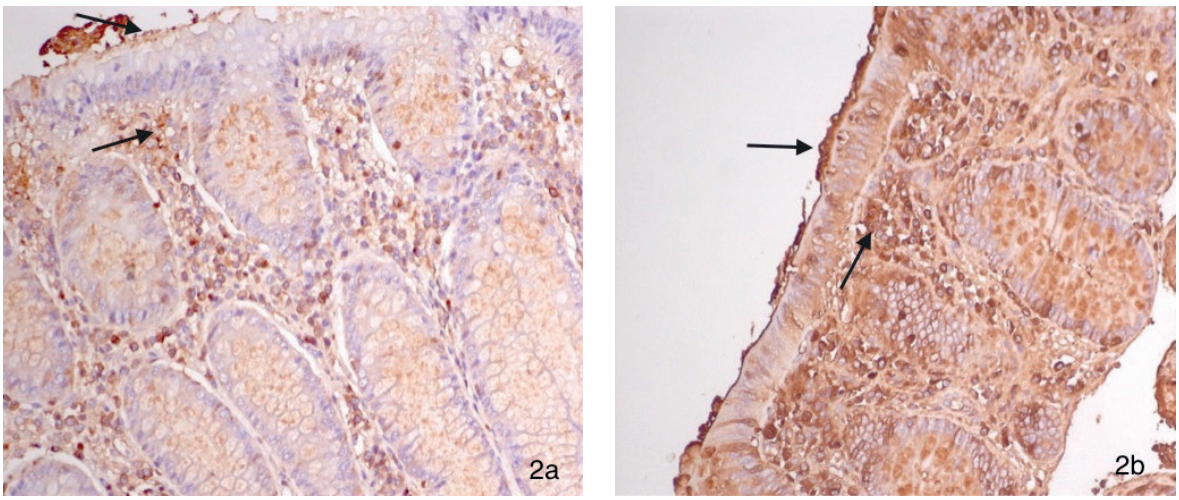

Fig. 2. Pre-treatment colon biopsy of a Crohn Disease patient stained for iAP (2a), low-intensity of iAP staining (arrow); colon biopsy of a control group patient stained for iAP (2b), high-intensity of iAP staining (arrow).

in post-treatment biopsies compared to pre-treatment biopsies (Table II). For TI and colon of CD and UC patients, we found no statistically significant difference neither for H&E nor for iAP staining grade.

We also evaluated the correlation between disease activity indices and histologic activity of IBD patients. IBD activity indices were calculated before and after treatment. Before treatment PCDAI scores were between 15-50 (median; 30), and PUCAI scores 15-75 (median; 42.5); after treatment 0-17.5 (median; 5), 0-55 (median 7.5), respectively. The time interval between pre-treatment endoscopy and post-treatment endoscopy was 6-48 months (median: 12 months). There was a positive correlation between PUCAI / PCDAI and the grade of staining with H&E and a negative correlation between PUCAI / PCDAI and the grade of staining with iAP in TI. However, this was not statistically significant.

Discussion

The interaction between gut microbiota and the innate immune system has a significant effect on intestinal homeostasis. It is thought that dysregulation of the balance between these two systems plays a considerable role in the pathogenesis of the inflammation in IBD and that luminal bacterial products may not trigger the disease but have a role in its progression by stimulating the local process.[8,12] The activation of Toll-like receptor 4 (TLR4) by bacterial lipopolysaccharide contributes to disease progression.

IAP is secreted into the intestinal lumen from the apical and basolateral domain of intestinal epithelial cells in 90-nm-diameter luminal vesicles, which also contain other functional proteins.[9] The physiological role of iAP in the intestine has remained a mystery for decades. As far as is known iAP is one of the major factors of mucosal defense. It attenuates the LPS-mediated inflammation by dephosphorylating and detoxifying lipopolysaccharide (LPS), the toxic cell component of the outer membrane of Gram-negative bacteria, which triggers the innate immune system by activating TLR4.[13,14] It has been shown that iAP reduces the activation of NF-κB by preventing the activation of TLR4, thereby inhibiting the MyD88-dependent inflammatory pathway. Although its pathomechanism is not fully understood, iAP deficiency and its decreased activity are thought to impair intestinal protective mechanisms in IBD patients.[8] In this context, we hypothesize that the more severe the grade of inflammation in IBD patients, the lower the iAP intensity and so the grade of staining with the iAP enzyme.

Previous studies have indicated that iAP is expressed substantially on the apical surface of the enterocytes and iAP enzyme expression is highest in the duodenum and least in the stomach and colon.[2,3,15] The existence and intensity of iAP were evaluated histologically in our study. We demonstrated the existence and the intensity of the enzyme itself with iAP specific antibody. We stained TI and colon specimens with anti-human iAP specific IgG type antibody and found that iAP was present, even in CD and UC patients and in the healthy control group, along the small intestine and colon with varied intensity. We also determined that it was concentrated predominantly in region 1 of TI, which involved the apical surface, brush border, and epithelial cells. The secretion of iAP from enterocytes could explain the more intense concentration of staining in region 1 than in region 2.

Histological assessment of biopsy specimens stained with H&E combined into clinical findings has already been used to predict diagnosis and to evaluate the response to treatment and disease activity. Modified Riley score was used to indicate the severity of inflammation for H&E stained specimens and in the study we used it in the grading of H&E stained specimens, as it is one of the most commonly preferred histological scoring systems of IBD to date.[16] We demonstrated that the grade of H&E-stained sections was positively correlated with the intensity of inflammation, as it was lowest in the healthy mucosa of TI and colon of CG.

Besides this existing histological assessment score, some studies have been reported about the utility of iAP as an additional negative inflammatory marker of the gastrointestinal tract. Some of these studies have reported that iAP is negatively correlated with the degree of inflammation.[3,8,17] Comparing the iAP activity and mRNA levels of iAP in inflamed and non-inflamed mucosa of IBD patients, Tuin et al.[8] found that both iAP activity and mRNA levels of iAP were reduced in inflamed mucosa and that iAP mRNA levels in the ileum were found to be 30 times higher than those in the human colon. Likewise, Molnár et al.[3] demonstrated that iAP protein level in the inflamed mucosa of children with CD and UC was significantly decreased compared to the control group. In the present study, as mentioned above, we evaluate the existence and intensity of iAP in TI and the colon. In the apical surface of TI of the children with CD, we found that as the degree of inflammation increases, the grade of staining with iAP decreases which were compatible with the findings of Molnár and Tuin.[3,8] We also found no significant correlation between the grade of H&E and iAP staining for TI and any colonic segment in UC patients and the control group. Tuin et al.[8] determined that when the epithelial layer within the colon is intact, iAP activity is absent in rats and they hypothesized that iAP only plays a role in the colon after the damage to the intestinal wall. Thus the negative correlation between histologic activity and iAP in TI of CD patients can be caused by the fact that there is intense inflammation in TI in CD and that there is mild or no inflammation in TI of UC patients. However, in the comparison of IBD patients and the control group we did not determine a decrease in the grade of staining with iAP despite obvious inflammation in the colon of UC patients. Following this finding iAP can only provide beneficial results when it is used in the case of both the presence of intense inflammation and the involvement of the small intestine.

In the comparison of pre-and post-treatment biopsies of CD and UC patients, we detected that histological score decreased and iAP staining score increased both in TI and colon in the post-treatment biopsies but this was not statistically significant. This finding can be explained by the fact that mucosal healing after therapies is associated with several variables including sex, presenting symptoms, disease extension, etc.[18] Additionally, mucosal healing lags behind symptomatic improvement depending on the chronicity of inflammation.[19,20]

PCDAI and PUCAI are frequently used in clinical practice because they are noninvasive and easy ways of predicting and monitoring disease activity in contrast with endoscopic procedures. But there are controversies about their accuracy in reflecting disease activity in children. Some reports claim that PCDAI is a utilizable measure of disease activity in children.[21] On the other hand Zubin et al.[22] determine that PCDAI is unreliable for endoscopic disease severity assessment.[23] Our results are consistent with the study of Zubin et al.[22] in that the correlations between both PUCAI / PCDAI and H&E and iAP staining were not statistically significant.

The first limitation of the study is the small sample size; larger-scale studies are required to evaluate the significance of iAP in IBD. There is no data about the alteration of iAP intensity by age in the gastrointestinal tract. Therefore age heterogeneity might reduce the comparability between the patients and the control group. The last limitation is that we were not able to use iAP activity and/or mRNA levels besides the histological intensity of iAP. This would have helped to strengthen the results and add some further information on this topic.

In conclusion, iAP as a histological marker can be of value in monitoring IBD activity, particularly in remarkable inflammation in the small intestine. The utility of iAP in predicting disease activity of the colon is a matter of debate.

Ethical approval

This study was conducted in adherence to the Declaration of Helsinki and approved

by Hacettepe University Non-interventional Clinical Researches Ethics Board with study approval number 13/400-20 and the participation involved informed concent.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBA, INST; data collection: BBA, HHG, BT; analysis and interpretation of results: BBA, EK; draft manuscript preparation: BBA, HD, HO, INST. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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