Biochemical Profile of Nonalcoholic Fatty Liver Disease Patients in Eastern India with Histopathological Correlation

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Abstract Aminotransferase assay is often used as a screening test as well as an endpoint for resolution of disease in nonalcoholic fatty liver disease (NAFLD). Aim of the study was to evaluate the relationship of transaminase level with metabolic variables and histology in NAFLD. Single center observational study was conducted in a gastroenterology clinic at Cuttack in coastal Odisha. Subjects were consecutive patients presenting with functional bowel disease and undergoing abdominal sonography. All participants were evaluated for the presence of metabolic syndrome (MS), insulin resistance, liver function test and lipid profile. Various parameters were compared between NAFLD subjects and controls. 53.5 % of NAFLD had normal serum transaminases, whereas 20.8 % of healthy controls had transaminitis. NAFLD

patients had significantly higher BMI, fasting plasma glucose, serum transaminases, serum triglycerides, serum insulin and homeostatic model assessment (HOMA) IR than controls. NAFLD patients who had transaminitis had significantly higher incidence of MS and higher mean HOMA IR than those without. There was no significant difference in histopathological features between NAFLD with and without transaminitis. To conclude, over half of NAFLD subjects do not have transaminitis while transaminitis is present in a fifth of healthy people without fatty liver. Hence serum transaminase should not be used as screening test for NAFLD. NAFLD patients with transaminitis had a higher incidence of MS and insulin resistance than those without. However, there was no significant difference in histopathological features between these two groups.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) has now emerged as the commonest cause of chronic liver disease worldwide [1, 2]. Population based prevalence of NAFLD varies from 30 to 40 % in men and 15 to 20 % in women in the west [2]. A higher prevalence has been observed in people who have type 2 diabetes mellitus (T2DM) i.e., up to 70 % [3]. In coastal eastern India, one fourth of the general population have evidence of fatty liver on routine ultrasonographic screening [4]. NAFLD encompasses a wide histological spectrum, which ranges from simple benign macro-vesicular steatosis to non-alcoholic

steatohepatitis (NASH), NASH related liver cirrhosis, portal hypertension and ultimately hepatocellular carcinoma [5, 6]. Besides, patients of NAFLD also suffer from several extra-hepatic chronic complications including atherosclerosis and a high cardiovascular risk [7]. NASH, which is the more severe entity in the spectrum must be distinguished from the benign simple fatty liver. The former is more commonly associated with liver related (cirrhosis, end stage liver disease and hepatocellular carcinoma) and extra-hepatic (cardiovascular diseases) morbidity and mortality than the latter [8, 9]. Till date, liver biopsy, which is an invasive procedure and has several limitations [10], is the gold standard for diagnosis of NASH [11].

Currently, a lot of importance is being given to the levels of transaminases in assessment of liver diseases [12]. Besides, elevated level of aminotransferases in liver function test is also a very common reason for referral of patients to gastroenterology and/or hepatology clinics. Diagnosis of NAFLD is based on abnormal serum aspartate transaminase (AST) and alanine transaminase (ALT) in most of the studies [13, 14]. Previous studies have shown that a non-linear correlation exists between the level of ALT elevation and the histopathologic severity or grade of NAFLD [15, 16]. Similarly, in some studies, it has been demonstrated that higher ALT levels carried a higher risk of NASH [17]. On the contrary, there are several series which indicate patients with normal ALT can have histological features of NASH [18]. Further, in the last couple of decades some researchers have debated regarding the upper limit of normal level of serum transaminases. In 2002, Prati et al. [19] proposed a revision of the upper limit of normal of the serum transaminases to a lower level (30 in males and 19 in females). However, it has not been accepted universally among the hepatologists worldwide. As a result, there is ongoing controversy regarding the cut off for serum aminotransferases, at which a patient must be referred for liver biopsy to exclude NASH.

We conducted this study with an aim to explore the profile of liver enzymes in patients with NAFLD in eastern India, and also look at the possibility of histopathological correlation, if any, with the transaminase levels.

Subjects and Methods

This was a single center observational study conducted in a gastroenterology clinic at Cuttack in coastal Odisha. The subjects were the consecutive outpatients who attended the gastroenterology clinic during the period from January 2012 to August 2015.

Inclusion Criteria

Cases: Consecutive outpatients who presented with functional abdominal pain (ROME III criteria) [20], functional dyspepsia (ROME III criteria) [21] and/or irritable bowel syndrome (ROME III criteria) [22] who had fatty liver on ultrasonography were included in this study. These participants were in good health and with no abnormal findings on general and systemic physical examination, blood count, and trans-abdominal ultrasonography except for fatty liver.

Controls: Consecutive healthy individuals with functional abdominal pain (ROME III criteria) [20], functional dyspepsia (ROME III criteria) [21] and/or irritable bowel syndrome (ROME III criteria) [22] who had normal liver on trans-abdominal sonography were the controls for the study.

Exclusion Criteria

Patients with organic gastrointestinal disease diagnosed either clinically or by ultrasonography or gastro-intestinal endoscopy were excluded. Participants who had history of alcohol consumption exceeding 20 g/day, subjects with other liver diseases (hepatitis viruses A to E, autoimmune disease, Wilson's disease) and those on drugs which can induce fatty liver or insulin sensitization such as estrogens, amiodarone, methotrexate, tamoxifen, glitazones and metformin were also excluded from the study.

An informed consent was taken from each participant. Detailed anthropometric assessment including measurements of weight, height, and waist circumference (WC) and hip circumference (HC) were done. The WC was measured at a level midway between the lowest rib and the iliac crest and HC at the level of the great trochanter. Body mass index (BMI) was calculated as $\text{weight (kg)/height}^2 (\text{m}^2)$.

The measurements of fasting plasma glucose (FPG), post-glucose plasma glucose (PGPG), serum triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol and liver function tests were performed by standard laboratory methods. Serum insulin was estimated by electrochemiluminescence method by using standard kit (Roche-Diagnostics, USA) with autoanalyser Elecsys 2010 (Roche-Hitachi, Japan). Insulin Resistance (IR) was calculated using the homeostatic model assessment (HOMA) method using a mathematical model derived from FPG and plasma insulin. The value of HOMA was calculated by the following equation: $[\text{fasting insulin } (\mu\text{U/ml}) \times \text{FBG (mg/dl)}] / 405$, and depicted as HOMA-IR value [23].

Trans-abdominal ultrasonography was performed to detect fatty changes in the liver. Fatty liver was defined as per the standard criteria accepted by the American Gastroenterology Association [24] i.e., an increase in liver echogenicity with renal echogenicity as a reference, the

presence of enhancement and lack of differentiation in periportal intensity and vascular wall due to hyperchogenicity of the liver parenchyma.

The ATP III criteria [25] was used for diagnosis of metabolic syndrome (MS) in the study subjects (Table 1). Presence of more than two of the five criteria were needed to be present for diagnosis of MS. The cut off values for waist circumference used were 80 cm for males and 90 cm for females as per the Asian population criteria [26]. Other parameters were fasting plasma glucose ≥ 110 mg/dl, blood pressure $\geq 130/85$ mmHg or treatment with antihypertensives drugs, serum HDL cholesterol <40 mg/dl for males and <50 mg/dl for females and serum triglycerides ≥ 150 mg/dl or patients who are on treatment with hypolipidemic agents.

Liver biopsy was performed in 216 NAFLD patients who gave consent for the same. Liver biopsy was done using Bard Biopsy Gun 16 G, through the inter-coastal approach. The grading and staging of liver histopathology was done by pathologists according to the classification proposed by Kleiner et al. [27] using necroinflammatory activity for grading of NASH.

This study was approved by the institutional ethics' committee of Kalinga Gastroenterology Foundation, Cuttack.

Statistical Analysis

Normally distributed continuous variables were expressed as mean $\pm$ SD. Student's *t* test for unpaired data was used to compare the groups when the variables were normally distributed. Chi square test was used to compare differences between categorical variables. All calculations were performed in the statistical software, SPSS version 16. *p* value of <0.05 was taken as significant.

Results

1637 consecutive patients were screened for the study out of which 597 were excluded as per the exclusion criteria (Fig. 1). Finally after exclusion, a total of 1040 subjects

were enrolled for the study. 800 were males and 240 females. 939 subjects had ultrasonographic evidence of fatty liver. The remaining 101 had normal abdominal transabdominal sonographic scans and were taken as controls of the study. The sex distribution of the subjects with NAFLD and controls is depicted in Table 2. Table 3 shows the baseline characteristics of the NAFLD subjects and these have been compared with that of controls in Table 4. NAFLD subjects were older and had significantly higher BMI, FPG and triglycerides than the controls. Serum HDL was comparable between the two groups ($p = 0.239$), but serum AST and ALT were significantly elevated in NAFLD. Further, subjects with NAFLD were more insulin resistant than controls.

Serum transaminitis (either AST or ALT greater than 40 U/l) was observed in 46.5 % of patients in NAFLD whereas only 20.8 % of controls had the same (Table 5). We compared the baseline characteristics of NAFLD patients with or without transaminitis (Table 6). Patients with transaminitis were younger, had lower BMI, marginally lower FPG and higher GGT. All the remaining biochemical features were comparable in both groups (NAFLD with and without transaminitis).

On comparison of mean serum transaminases in various BMI groups no significant difference was observed (Table 7). The majority of the NAFLD (47.5 %) had a BMI between 25 and 29.9 i.e., grade 1 obesity.

Liver biopsy was done in 216 NAFLD patients. The histopathological features were compared between patients of NAFLD with and without transaminitis (Table 8). There was no significant difference in any of the histopathological features (steatosis, ballooning, lobular inflammation and fibrosis) between the two groups.

Presence of metabolic syndrome was detected by modified ATP III criteria [25] with Asian cut off for waist circumference [26]. Metabolic syndrome was present in 441 NAFLD patients (Fig. 2). Mean serum AST, ALT and mean HOMA IR were compared between NAFLD patients with and without metabolic syndrome (Table 9). NAFLD subjects with metabolic syndrome had significantly higher mean AST (44.8 ± 26.5 vs 29.1 ± 12.8 , $p < 0.001$), ALT (62.8 ± 39.0 vs 35.7 ± 22.3 , $p < 0.001$) and HOMA IR (3.07 ± 1.95 vs 2.02 ± 1.31 , $p < 0.001$) than those without.

Discussion

NAFLD has been found to have a strong association with raised AST and ALT [28–32] and it is one of the most common causes of unexplained mild elevation of serum transaminases [33, 34]. Along with the growing pandemic of obesity [34], the prevalence of NAFLD is steadily

Table 1 Clinical identification of the metabolic syndrome (ATP III criteria)

Risk factor	Defining level	
	For men	For women
Waist circumference	Men >102 cm	Women >88 cm
Triglycerides	≥ 150 mg/dl	
HDL cholesterol	Men <40 mg/dl	Women <50 mg/dl
Blood pressure	$\geq 130/85$ mmHg	
Fasting glucose	≥ 110 mg/dl	

Fig. 1 Study Consort diagram **Table 2** Sex distribution of NAFLD and Controls

Group	NAFLD (n = 939)	Controls (n = 101)	Total	p value*
Males	736 (78.4 %)	64 (63.4 %)	800	0.001
Females	203 (21.6 %)	37 (36.6 %)	240	

* Calculated by Chi squared method

increasing and has now become a major global health burden [35]. Consequently, NAFLD is considered as the first differential diagnosis of unexplained ALT elevation (after exclusion of viral hepatitis, drug induced liver injury and excessive alcohol intake). ALT and AST are markers of liver injury, and described as a useful surrogate measure of NAFLD. A definite but nonlinear relationship has been found to exist between serum transaminases and histological severity of NAFLD in several studies [15]. Hence patients with NAFLD and transaminitis are subjected to further work up to rule out NASH, the more severe entity of the NAFLD spectrum.

In our study, patients with NAFLD had higher BMI, higher FPG, higher serum triglycerides, lower serum HDL, higher serum AST and ALT, higher serum insulin and insulin resistance (HOMA IR) than controls (Tables 3, 4). Most of the previous studies too have shown that NAFLD is increasingly associated with obesity [36, 37],

hyperglycemia [37, 38], dyslipidemia [39], high serum AST and ALT [13, 14, 32, 33]. A positive correlation between insulin resistance and higher grades of NAFLD, rather than the degree of hyperglycemia in type 2 diabetes mellitus patients has been earlier demonstrated by Das et al. [40]. The mean AST and ALT in NAFLD subjects was 36.4 ± 21.9 and 48.4 ± 34.0 respectively. Most earlier studies reported that NAFLD is frequently associated with elevated serum transaminases [4, 41, 42, 43]. However, studies which are population based have shown that patients with NAFLD can have normal AST and ALT [18, 44, 45]. The subjects of our study were people attending a gastroenterology outpatient clinic and the mean values of serum transaminases were a little higher (Table 10).

The comparison of the distribution of subjects with metabolic syndrome in NAFLD patients with and without transaminitis revealed that elevated serum AST or ALT

Table 3 Baseline characteristics of NAFLD (n = 939)

Parameters	Range	Mean $\pm$ SD
Age (in years)	15–95	42.3 $\pm$ 10.9
BMI	17.2–37.2	26.1 $\pm$ 3.8
FPG (in mg/dl)	69–642	103.6 $\pm$ 29.72
PGPG (in mg/dl)	52–498	146.6 $\pm$ 63.22
Triglycerides (in mg/dl)	50–716	191.4 $\pm$ 98.5
Cholesterol (in mg/dl)	41–350	189.2 $\pm$ 42.6
HDL (in mg/dl)	15–178	43.1 $\pm$ 11.7
LDL (in mg/dl)	29–230	108.9 $\pm$ 33.9
VLDL (in mg/dl)	10–15	36.9 $\pm$ 17.7
Bilirubin (in mg/dl)	0.17–4.0	0.83 $\pm$ 0.42
AST (in U/l)	13–141	36.4 $\pm$ 21.9
ALT (in U/l)	12–208	48.4 $\pm$ 34.0
Alkaline Phosphatase (in U/l)	11–410	184.8 $\pm$ 65.7
Protein (in g/dl)	4.5–9.7	7.6 $\pm$ 0.6
Albumin (in g/dl)	2.4–6.0	4.4 $\pm$ 0.4
Globulin (in g/dl)	1.5–4.8	3.2 $\pm$ 0.5
GGT (in mg/dl)	9–411	36.0 $\pm$ 32.5
Insulin	2.1–330.3	10.8 $\pm$ 14.9
HOMA_IR	0.8–146	2.96 $\pm$ 6.4

Table 4 Baseline characteristics of NAFLD and controls

Parameters	NAFLD (n = 939)	Controls (n = 101)	p value
Age (in years)	42.3 $\pm$ 10.9	38.4 $\pm$ 14.1	0.001
BMI (in kg/m ²)	26.1 $\pm$ 3.8	21.1 $\pm$ 3.1	<0.001
FPG (in mg/dl)	101.1 $\pm$ 28.3	91.2 $\pm$ 10.7	0.001
TG (in mg/dl)	195.0 $\pm$ 106.3	129.6 $\pm$ 52.8	<0.001
HDL (in mg/dl)	43.2 $\pm$ 13.6	44.8 $\pm$ 7.9	0.239
AST (in U/l)	36.4 $\pm$ 21.9	29.9 $\pm$ 9.2	0.003
ALT (in U/l)	48.4 $\pm$ 34.0	35.7 $\pm$ 17.4	<0.001
Insulin	10.0 $\pm$ 5.1	5.3 $\pm$ 2.4	<0.001
HOMA IR	2.51 $\pm$ 1.72	1.23 $\pm$ 0.67	<0.001

predisposes an individual to greater risk for metabolic syndrome (Fig. 2). This appears consistent with the finding of serum ALT as an independent predictor of coronary events including all-cause mortality, incident cardiovascular disease and coronary heart disease events [46]. NASH patients have higher risk for liver and non-liver related mortality than patients with simple steatosis. However, it is not possible to perform liver biopsy in every patient of NAFLD due to its high incidence. Hence it is

important to stratify the NAFLD population and identify which subgroup needs more attention and regular follow up. NAFLD patients with transaminitis and higher values of HOMA IR are more prone for metabolic syndrome and hence cardiovascular disease.

In our study, we found that 20.8 % of our controls had elevated serum transaminase. This phenomenon has however been a common observation by many other researchers too. Tazawa et al. [47] reported abnormal serum ALT test in 24 % children; however of them only 64/77 (83 %) had fatty liver on ultrasound; remaining 17 % did not have any ultrasonographic evidence of fatty liver or other liver disease. Serdaroğlu et al. [48] too reported idiopathic hypertransaminasemia in 6.4 % subjects. In fact in a study by Çeltik et al. [49] the idiopathic rate was as high as 27.1 %. Iorio et al. [50] too reported idiopathic hypertransaminasemia in 31.7 % subjects. However, there is a possibility that this may have been due to conditions like celiac disease or occult hepatitis B/hepatitis C viral infection which were missed by routine investigations. Duodenal mucosal biopsy and serum tissue trans-glutaminase are needed to exclude celiac disease which has been known to cause asymptomatic elevation of serum transaminases in 3–4 % of cases of unexplained transaminitis in the West [51]. However, since celiac disease is extremely rare in our setting [52], these tests were not done. The prevalence of hepatitis B [53] and C [54] too is very low in Odisha. Hence, only exclusion of these infections by HBsAg and Anti-HCV assay by ELISA were performed for them. HBV DNA and HCV RNA were not tested for in our patients. By doing these tests we might have unearthed these as possible causes in only around 2–3 % of cases. Finally it would have been unethical to perform liver biopsies in these patients merely to diagnose asymptomatic NASH in a few of them.

We observed that in our study, patients with transaminitis were younger, had lower BMI, marginally lower FPG and higher GGT. However this is not unique to this study. In fact, similar findings have been reported in several previous studies. In a study by Adams et al. [6], baseline clinical and laboratory features were compared between NAFLD patients who underwent biopsy and those who did not. In this study, patients who were subjected to liver biopsy had lower BMI (32.6 $\pm$ 7.4) than others (33.5 $\pm$ 5.9) despite higher transaminase (AST: 69 $\pm$ 82 U/l vs 48 $\pm$ 43 U/l; p = 0.10). Singh et al. [4] too in their study found that patients with NASH had lower

Table 5 Transaminitis in NAFLD and controls

Group	Normal AST and ALT	AST or ALT >40	p value*
NAFLD (n = 939)	502 (53.5 %)	437 (46.5 %)	<0.001
Controls (n = 101)	80 (79.2 %)	21 (20.8 %)	

Table 6 Baseline characteristics in NAFLD with or without transaminitis

Parameters	Normal AST and ALT (n = 502)	AST or ALT > 40 (n = 437)	p value
Age (in years)	44.9 ± 11.1	39.3 ± 9.7	<0.001
BMI	27.3 ± 4.0	26.4 ± 3.4	<0.001
FBG (in mg/dl)	103.7 ± 31.9	98.2 ± 23.1	0.004
PGPG (in mg/dl)	146.9 ± 63.9	140.4 ± 53.6	0.117
Triglycerides (in mg/dl)	191.5 ± 97.2	199.0 ± 115.8	0.297
Cholesterol (in mg/dl)	182.6 ± 40.4	185.3 ± 52.9	0.392
HDL (in mg/dl)	42.3 ± 10.7	44.3 ± 16.3	0.028
LDL (in mg/dl)	104.2 ± 31.8	109.1 ± 38.3	0.039
VLDL (in mg/dl)	36.3 ± 16.8	36.7 ± 17.8	0.748
Bilirubin (in mg/dl)	0.8 ± 0.3	0.9 ± 0.5	<0.001
Alkaline Phosphatase (in U/l)	195.8 ± 65.9	189.2 ± 76.4	0.160
Protein (in g/dl)	7.5 ± 0.6	7.6 ± 0.6	0.032
Albumin (in g/dl)	4.3 ± 0.4	4.5 ± 0.5	0.001
GGT (in mg/dl)	28.3 ± 20.0	49.3 ± 43.6	<0.001
Insulin	9.3 ± 4.6	11.0 ± 5.7	<0.001
HOMA_IR	2.26 ± 1.66	2.79 ± 1.75	<0.001

Table 7 Mean BMI in different BMI ranges

BMI	<18 (n = 15)	18–22.9 (n = 117)	23–24.9 (n = 187)	25–29.9 (n = 446)	30–34.9 (n = 148)	≥35 (n = 26)
AST	35.6 ± 26.7	40.4 ± 30.8	37.6 ± 17.8	35.4 ± 19.0	35.8 ± 26.2	47.7 ± 27.7
ALT	38.2 ± 32.5	40.1 ± 37.4	49.9 ± 30.8	48.7 ± 34.3	45.4 ± 34.5	45.2 ± 25.6

Table 8 Comparison of histopathological features in NAFLD with or without transaminitis

Parameters	Normal AST and ALT (n = 53)	AST or ALT > 40 (n = 163)	p value
Steatosis			
<5 %	21	74	0.288
5–33 %	27	74	
33–66 %	4	15	
Ballooning			
None	16	62	0.064
Few	30	60	
Many	7	39	
Lobular inflammation			
Mild	23	70	0.352
Moderate	23	56	
Severe	0	4	
Fibrosis			
Stage 1	4	18	0.269
Stage 2	5	20	
Stage 3	1	0	
NAS			
0–2	13	46	0.564
3–4	20	59	
5–8	20	58	

Fig. 2 Bar diagram showing proportion of NAFLD patients with or without Metabolic Syndrome. **p* value calculated by Chi SQUARE method

**p* value calculated by Chi Square method.

Table 9 AST, ALT and HOMA IR in NAFLD patients with and without MS

Parameters	NAFLD without MS (n = 498)	NAFLD with MS (n = 441)	<i>p</i> value
AST (in U/l)	29.1 ± 12.8	44.8 ± 26.5	<0.001
ALT (in U/l)	35.7 ± 22.3	62.8 ± 39.0	<0.001
HOMA_IR	2.02 ± 1.31	3.07 ± 1.95	<0.001

Table 10 Mean AST and ALT in various studies

Study	Year	Number of Subjects	Diagnosis of NAFLD is based on	Mean AST	Mean ALT
Amarapurkar et al. [44]	2007	194	USG	24.3 ± 9.8	19.2 ± 4.5
Das et al. [40]	2010	42	USG	NA	35.3 ± 23.4
Singh et al. [4]	2013	632	USG	40.7 ± 22.5	52.9 ± 36.7
Treeprasertsuk et al. [43]	2012	309	USG and liver biopsy	51.6 ± 56.3	76.9 ± 48.5
Sanyal et al. [42]	2015	150	USG	33.3 ± 16	44.2 ± 22.0
Current study	2015	939	USG	36.4 ± 21.9	48.4 ± 34.0

incidence of obesity (BMI > 25) (50 vs 68.8 %), diabetes mellitus (6.3 vs 16.7 %) than NAFLD without NASH. In another study from our center, NAFLD patients without insulin resistance (HOMA IR < 2) were younger with lower BMI, higher serum AST and ALT levels as compared to those with insulin resistance (HOMA IR ≥ 2) [55]. Further studies are necessary to explain this paradoxical observation.

There are several limitations of this study. Being an observational study it is not devoid of selection and confounding bias. Also there may have been a selection bias for patients undergoing liver biopsy. Subjects with more severe higher serum transaminases and insulin resistance may have been more often referred for liver biopsy. However, over half of the patients who underwent liver

biopsy had normal transaminase values. Besides it is believed that trans abdominal ultrasound which was used as the screening test for fatty liver reliably diagnoses NAFLD only if steatosis is >33 % and patients with mild steatosis <33 % can be missed by sonography.

Conclusion

Despite the older belief that NAFLD is frequently associated with transaminitis, our study showed that more than half (53.5 %) of NAFLD subjects had normal serum transaminase levels, while paradoxically transaminitis was observed in one fifth (20.8 %) of normal healthy controls without fatty liver. Hence, serum transaminase level alone

should never be used as a screening test for NAFLD, otherwise a huge proportion of patients will be overlooked resulting in significant underestimation of the prevalence of fatty liver. However, although NAFLD subjects who had serum transaminitis were more insulin resistant with higher incidence of metabolic syndrome than those without, there was no significant difference in histopathological features between NAFLD with and without transaminitis. Therefore, although the presence of raised serum transaminase in patients of NAFLD indicates a higher metabolic risk, it is not a reflection of the degree of hepatic necro-inflammation. Besides, this also raises the possibility that hitherto unknown extra-metabolic factors, most likely the gut microbiota, might be influencing the degree of hepatic disease.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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