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ORIGINAL ARTICLE

Characterization of biopsy proven non-alcoholic fatty liver disease in healthy non-obese and lean population of living liver donors: The impact of uric acid

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KEYWORDS
Non-alcoholic fatty liver disease;
Lean individuals;
Non-alcoholic steatohepatitis;
Liver biopsy

Summary
Background: Non-alcoholic fatty liver disease (NAFLD) is frequently seen among non-obese overweight individuals and lean subjects (those with normal body mass index). This study aimed to investigate prevalence and risk factors of biopsy proven NAFLD in a cluster of healthy non-obese and lean individuals.
Methods and Materials: In a retrospective study, adult (> 18 years) apparently healthy individuals who had donated liver to pediatric patients between July 2012 and October 2018 were included. Non-obese and lean individuals were defined as BMI < 30 kg/m^2 and BMI < 25 kg/m^2, respectively.
Results: Totally 310 patients were included. Seventy-six individuals (24.5%) had NAFL and 30 patients (9.67%) had non-alcoholic steatohepatitis (NASH) among non-obese population. In multivariate regression analysis, only higher BMI was marginally associated with NASH in non-obese compared to those without NASH (Odds ratio: 2.52, 95% CI: 0.097–6.54; $P = 0.05$). Totally, 246 individuals were lean. 55 individuals (22.3%) had NAFL and 20 individuals (8.2%) had NASH in their liver biopsies. In univariate analysis, serum triglyceride, cholesterol, LDL, ALT, alkaline phosphatase and uric acid were associated with NAFL among lean individuals ($P < 0.05$). In regression analysis, serum uric acid was associated with NAFL (Odds ratio: 1.70, 95% CI: 1.18–2.45; $P = 0.004$) and NASH in lean individuals (Odds ratio: 1.98, 95% CI: 1.27–3.10; $P = 0.003$).

Abbreviations: NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-Alcoholic Steatohepatitis; BMI, Body Mass Index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase.

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Conclusion: NAFLD/NASH is prevalent even in a healthy lean population when evaluated by liver biopsy. Higher BMI and serum uric acid were two major risks of NAFLD/NASH in non-obese and lean individuals.

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide and especially in the Middle Eastern countries [1,2]. Non-alcoholic steatohepatitis (NASH) is diagnosed when inflammation and hepatocyte injury are added to simple steatosis [3]. NASH might progress to advanced fibrosis and culminate in liver cirrhosis [4]. NAFLD is usually considered a hepatic component of the metabolic syndrome that is occurred in individuals with obesity and/or other metabolic abnormalities [5,6]. It has been suggested that multiple metabolic risk factors will increase risk of NAFLD [7]. Furthermore, obesity by itself has been reported to be an independent predictor of disease progression and long-term worse outcomes in patients with NAFLD [8]. Obesity might result in over expression of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in visceral adipose tissue leading to more severe disease and more rapid progression [9]. However, NAFLD is frequently seen among non-obese overweight individuals and more interestingly among lean subjects (those with normal body mass index) [10]. In an ultrasound based study of lean patients, prevalence of NAFLD was reported to be 17.5%. Elevated serum triglyceride and higher body mass index were associated with NAFLD in lean subjects [11]. However, ultrasonography may underestimate the true prevalence of NAFLD and cannot evaluate presence of NASH. Therefore, the epidemiology and risk factors of patients with non-obese and lean NAFLD have been less recognized especially among those with normal metabolic indices. This study aimed to investigate prevalence and risk factors of NAFLD/NASH in apparently healthy, metabolically normal non-obese and lean Iranian population.

Methods and Materials

Study subjects

This retrospective study was performed at Namazi hospital and Avicenna transplant hospital, Shiraz, Iran. Study population consisted of adult (>18 years) apparently healthy individuals who had donated liver to pediatric patients who were their first degree relatives between July 2012 and October 2018 in our transplant center. Liver biopsy had been performed, as a routine pre-transplant work-up, for all the study participants for evaluation of liver histology before living donor liver transplantation. Liver biopsies were performed by ultrasound guide using standard Tru-Cut needles and local anesthesia. Liver biopsy specimen were stained with Hematoxylin and eosin method and reviewed by 2 expert pathologists, blinded to clinical data of patients, checking for steatosis and steatohepatitis. Presence of fat droplets within cytoplasm displacing the nucleus to the cell periphery was defined as hepatic steatosis. Steatohepatitis was defined as presence of hepatic steatosis in addition to lobular inflammation or ballooning [12]. Individuals with history of significant alcohol consumption (> 10 g/day), chronic liver disease induced by hepatitis B and C infection, cholestatic liver diseases, autoimmune hepatitis, hepatobiliary malignancies and hemochromatosis were excluded from the study. NAFLD was defined as presence of steatosis in $\geq$ 5% of hepatocytes and absence of all above exclusion criteria [13]. Body mass index was calculated using this formula: Weight (kg)/[height (m)]^2. Non-obese individuals were those with BMI < 30 kg/m^2. Lean individuals were defined as participants with BMI < 25 kg/m^2. Data of participants including age, gender, weight, and height, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, liver function tests, lipid profile, fasting blood sugar, uric acid were collected using data gathering form. Impaired fasting glucose (IFG) was defined as fasting blood glucose form 100 mg/dL to 125 mg/dL based on the definition of American Diabetes Association (ADA).

Ethics and consent

The study was approved by the ethical committee of transplant ward, Shiraz University of Medical Sciences. Study participants had written informed consent to use their medical data for research purposes. The study was carried out in accordance with the Declaration of Helsinki as revised in Seoul 2008.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation for numeric variables and counts for categorical variables. Continuous data were compared using Student's t-test and categorical variables were compared by Chi-square test. Independent variables associated with NAFLD and NASH were analyzed by logistic regression. The first analysis was performed between those with and without NAFLD/NASH among non-obese participants (BMI < 30 kg/m^2). The next analysis was performed between those with and without NAFLD/NASH among lean participants (BMI < 25 kg/m^2). SPSS 18.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis and A P value of < 0.05 was considered statistically significant.

Results

Totally 310 patients were included. There were 180 female (58%) and 130 male (42%). Mean age of study population was 32.64 $\pm$ 7.04 years. Seventy six individuals (24.5%) had NAFL among non-obese population. The characteristics of patients with non- obese NAFL are outlined in Table 1. In univariate

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Table 1 Comparison of non-obese patients with and without NAFLD.

Univariate				Multivariate analysis		
	With NAFL	Without NAFL	*P*-value	OR	95% CI	*P*-value
Age (year)	33.85 ± 7.95	32.25 ± 6.69	0.095			
Sex (male/female)	34/42	96/138	0.511			
Height (cm)	167.48 ± 7.57	168.34 ± 9.61	0.623			
Weight (kg)	70.82 ± 11.40	65.52 ± 10.53	< 0.001			
BMI (kg/m^2)	25.43 ± 3.38	23.23 ± 3.17	< 0.001	1.46	1.09—1.95	0.011
FBS (mg/dL)	91.13 ± 10.85	88.43 ± 10.82	0.068			
TG (mg/dL)	118.73 ± 66.03	102.50 ± 59.38	0.053			
Cholesterol (mg/dL)	180.17 ± 34.88	166.67 ± 36.50	0.006	1.02	0.94—1.01	0.201
HDL (mg/dL)	45.87 ± 11.55	46.31 ± 10.69	0.780			
LDL (mg/dL)	108.5 ± 34.40	96.59 ± 29.22	0.008	1.04	1.001—1.088	0.042
AST (IU/L)	20.82 ± 8.33	19.84 ± 7.27	0.328			
ALT (IU/L)	23.82 ± 12.78	18.70 ± 10.83	0.001	1.029	0.98—1.07	0.177
ALK.Phos (IU/L)	209.97 ± 76.24	190.26 ± 64.18	0.030	1.005	0.996—1.014	0.275
TB (mg/dL)	0.78 ± 0.45	0.80 ± 0.43	0.607			
Albumin (mg/dL)	4.64 ± 0.41	4.60 ± 0.36	0.348			
Uric acid (mg/dL)	4.38 ± 1.59	3.38 ± 1.05	< 0.001	1.32	0.79—2.19	0.278
IFG (+)	22.2%	14.9%	0.151			

BMI: body mass index; FBS: fasting blood sugar; TG: Triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alk.phos: alkaline phosphatase; Chol: cholesterol; NAFL: non-alcoholic fatty liver; IFG: impaired fasting glucose; TB: total bilirubin.

Table 2 Characteristics of non-obese patients with and without NASH.

Univariate				Multivariate analysis		
	With NASH	Without NASH	*P*-value	OR	95% CI	*P*-value
Age (year)	32.92 ± 7.18	32.63 ± 7.06	0.792			
Sex (male/female)	14/16	117/163	0.533			
Height (cm)	170.22 ± 8.34	168.24 ± 9.36	0.623			
Weight (kg)	74.75 ± 11.02	66.11 ± 10.71	< 0.001			
BMI (kg/m^2)	27.22 ± 2.39	23.48 ± 3.27	0.001	2.52	0.097—6.54	0.056
FBS (mg/dL)	89.62 ± 10.34	98.83 ± 10.93	0.792			
TG (mg/dL)	142.96 ± 74.45	103.30 ± 59.01	0.001	1.002	0.98-1.02	0.834
Chol (mg/dL)	181 ± 34.81	169.30 ± 36.68	0.115			
HDL (mg/dL)	45.47 ± 9.07	46.24 ± 11.08	0.748			
LDL (mg/dL)	109.82 ± 38.63	98.73 ± 30.11	0.103			
AST (IU/L)	22.75 ± 11.02	19.86 ± 7.09	0.055	0.927	0.68—1.25	0.629
ALT (IU/L)	27.17 ± 12.70	19.32 ± 11.21	0.001	1.09	0.90—1.31	0.368
ALK.Phos (IU/L)	192.10 ± 68.96	195.57 ± 68.08	0.798			
TB (mg/dL)	0.67 ± 0.36	0.81 ± 0.44	0.124			
Albumin (mg/dL)	4.73 ± 0.36	4.60 ± 0.38	0.106			
Uric acid (mg/dL)	4.69 ± 1.47	3.50 ± 1.20	< 0.001	1.07	0.19—3.51	0.790
IFG	7.4%	17.8%	0.170			

BMI: body mass index; FBS: fasting blood sugar; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alk.phos: alkaline phosphatase; Chol: cholesterol; NASH: non-alcoholic steatohepatitis; IFG: impaired fasting glucose; TB: total bilirubin.

analysis, higher BMI, serum cholesterol, low-density lipoprotein (LDL), ALT, alkaline phosphatase and uric acid were associated with NAFL in non-obese individuals ($P < 0.05$). In regression analysis, higher BMI was independently associated with NAFL in non-obese individuals ($P < 0.05$) (Table 1). Thirty patients (9.67%) had NASH in liver biopsy. The comparison between non-obese patients with and without NASH are outlined in Table 2. In univariate analysis, BMI, serum triglyceride, AST, ALT and uric acid were associated with NASH ($P < 0.05$). In multivariate analysis, only higher BMI was marginally associated with NASH compared to those without NASH (Odds ratio: 2.52, 95% CI: 0.097—6.54; $P = 0.05$).

Totally, 246 patients were lean. A total of 55 individuals (22.3%) had NAFL in liver biopsy. Characteristics of lean

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Table 3 Characteristics of lean patients with and without NAFLD.

Univariate				Multivariate analysis		
	With NAFL	Without NAFL	*P*-value	OR	95% CI	*P*-value
Age (year)	33.86 ± 8.08	32.21 ± 6.82	0.148			
Sex (male/female)	25/30	79/113	0.664			
BMI (kg/m^2)	22.24 ± 1.27	21.70 ± 2.21	0.378			
FBS (mg/dL)	91.51 ± 10.33	88.40 ± 10.52	0.060			
TG (mg/dL)	120.77 ± 69.06	98.91 ± 57.45	0.022	1.002	0.99–1.01	0.647
Cholesterol (mg/dL)	180.05 ± 32.84	166.19 ± 37.90	0.017	0.998	0.97–1.02	0.869
HDL (mg/dL)	46.10 ± 12.56	46.59 ± 10.93	0.795			
LDL (mg/dL)	106.95 ± 31.62	95.77 ± 30.40	0.030	1.006	0.98–1.03	0.635
AST (IU/L)	21.50 ± 9.23	19.92 ± 7.29	0.185			
ALT (IU/L)	24.58 ± 13.84	18.61 ± 10.90	0.001	1.013	0.98–1.04	0.443
ALK.Phos (IU/L)	216.77 ± 71.22	189.05 ± 64.13	0.007	1.002	0.99–1.008	0.631
TB (mg/dL)	0.77 ± 0.40	0.81 ± 0.40	0.499			
Albumin (mg/dL)	4.64 ± 0.44	4.63 ± 0.36	0.828			
Uric acid (mg/dL)	4.43 ± 1.51	3.41 ± 1.05	< 0.001	1.70	1.18–2.45	0.004
IFG	34.3%	22.7%	0.144			

BMI: body mass index; FBS: fasting blood sugar; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alk.phos: alkaline phosphatase; Chol: cholesterol; NAFL: non-alcoholic fatty liver; IFG: impaired fasting glucose; TB: total bilirubin.

Table 4 Characteristics of lean patients with and without NASH.

Univariate				Multivariate analysis		
	With NASH	Without NASH	*P*-value	OR	95% CI	*P*-value
Age (year)	31.88 ± 6.64	32.64 ± 7.19	0.668			
Sex (male/female)	10/10	95/131	0.636			
BMI (kg/m^2)	22.47 ± 2.1	21.76 ± 2.15	0.747			
FBS (mg/dL)	90.84 ± 9.77	88.93 ± 10.56	0.450			
TG (mg/dL)	152.15 ± 79.00	99.81 ± 57.25	< 0.001	1.004	0.99–1.01	0.334
Cholesterol (mg/dL)	181.95 ± 35.82	168.62 ± 37.20	0.127			
HDL (mg/dL)	44.76 ± 9.42	46.67 ± 11.50	0.508			
LDL (mg/dL)	105.58 ± 36.10	97.89 ± 30.51	0.329			
AST (IU/L)	23.80 ± 12.50	20.01 ± 7.18	0.037	1.020	0.96–1.08	0.518
ALT (IU/L)	27.55 ± 13.43	19.37 ± 11.54	0.003	1.005	0.96–1.04	0.834
ALK.Phos (IU/L)	205.35 ± 76.25	194.43 ± 66.08	0.486			
TB (mg/dL)	0.64 ± 0.27	0.81 ± 0.41	0.069			
Albumin (mg/dL)	4.71 ± 0.32	4.62 ± 0.40	0.373			
Uric acid (mg/dL)	4.93 ± 1.40	3.50 ± 1.14	< 0.001	1.98	1.27–3.10	0.003
IFG	2.9%	9.8%	0.194			

BMI: body mass index; FBS: fasting blood sugar; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alk.phos: alkaline phosphatase; Chol: cholesterol; NASH: non-alcoholic steatohepatitis; IFG: impaired fasting glucose; TB: total bilirubin

patients with and without NAFL are outlined in Table 3. In univariate analysis, serum triglyceride, cholesterol, LDL, ALT, alkaline phosphatase and uric acid were associated with NAFL among lean individuals ($P < 0.05$). In regression analysis, serum uric acid was associated with NAFL in lean participants (Odds ratio: 1.70, 95% CI: 1.18–2.45; $P = 0.004$).

Among lean population, 20 individuals (8.2%) had NASH in their liver biopsies. In univaraite analysis, AST, ALT, uric acid, and triglyceride were associated with NASH among lean individuals. In regression analysis, higher serum uric acid was associated with NASH in lean individuals (Odds ratio: 1.98, 95% CI: 1.27–3.10; $P = 0.003$) (Table 4). Results of liver biopsies are outlined in Table 5. Serum uric acid was statistically different in patients with NASH, with only NAFL and those without NAFL (Fig. 1).

Discussion

The results of current study showed that prevalence of biopsy proven NAFL was 24.5% and 22.3% among non-obese (BMI < 30 kg/m^2) and lean (BMI < 25) study population,

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Table 5 Results of liver biopsy in individuals without NAFLD, with NAFLD and NASH.

	No NAFL	With NAFL	With NASH
Steatosis			
< 5%	36	0	0
5—33%	0	76	29
> 33% to 66%	0	0	1
> 66%	0	0	0
Lobular inflammation	1	5	30
Hepatocyte ballooning	0	2	30
Fibrosis	0	0	3

NASH: non-alcoholic steatohepatitis; NAFL: non-alcoholic fatty liver.

respectively. NASH was detected in 9.67% and 8.2% of non-obese (BMI < 30 kg/m^2) and lean (BMI < 25) study participants, respectively. Among non-obese study participants, BMI and serum LDL were independently associated with NAFL and BMI was associated with NASH independently. Among lean study participants, higher serum uric acid was an independent predictor of both NAFL and NASH in liver biopsies of study participants.

While majority of patients with NAFLD are obese, a considerable proportion of NAFLD patients have normal body weight. This phenotype is so called lean NAFLD and has attained attention among researchers [14]. Lean NAFLD is especially supposed to have a rising trend in Asian countries. It has been estimated that nearly 20% of patients with NAFLD are lean in Asian countries [15] and 8—19% of lean Asians have NAFLD [16]. The clinical implication and outcomes of this group of patients have not been well elucidated. Some authors have suggested that lean NAFLD might be an important cause of cryptogenic liver cirrhosis [17]. Others have reported that presence of NAFLD among lean individuals was associated with all cause and cardiovascular mortality [18]. Patient with lean NAFLD had severe lobular inflammation and ballooning compared to overweight patients with NAFLD [19]. Treatment of patients with lean NAFLD might be another dilemma. While weight reduction and dietary interventions are the major therapeutic options in NAFLD, the significance of these strategies in lean NAFLD have been less studied. Pharmacologic therapy might be more important in this group of patients with NAFLD [20].

The epidemiology, pathogenesis and prognosis of this subgroup of patients have not been well studied yet. In a cross-sectional study, 12% of study population with NAFLD was reported to have normal BMI [21]. In a study of Chinese patients with NAFLD, 41% of patients with NAFLD were lean. Lean NAFLD was associated with diabetes, hypertension and metabolic syndrome [22]. In a retrospective cohort of patients with biopsy proven NAFLD, lean patients with NAFLD had lower proportion of diabetes, hypertension and metabolic syndrome compared to obese patients. Lean patients were less likely to have NASH [23]. rs738409 C > G polymorphism in patatin-like phospholipase domain-containing protein 3 (PNPLA3) was associated with disease progression in lean patients with NAFLD [23]. In a cluster of biopsy proven NAFLD Japanese patients, 25% of men and 40% of women were lean and the prevalence of metabolic abnormalities were not different between obese and non-obese subjects [24]. The prevalence of lean NAFLD was reported to be 7.6% in a cross-sectional study of NAFLD patients diagnosed by liver biopsy. Higher hemoglobin level was a predictor of disease severity in this study [25].

All of these reviewed studies have reported proportion of lean NAFLD among patient with NAFLD and only few studies have addressed the prevalence of NAFLD among lean individuals. Younossi et al. reported that the prevalence of ultrasound diagnosed NAFLD among lean subjects was 18.7% of those 11.78% had NASH as defined by moderate to severe steatosis and elevated aminotransferase [26]. Patients with lean NAFLD were more likely to have younger age and female sex but less likely to have hypercholesterolemia and insulin resistance [26]. In a Chinese population of lean individuals, the prevalence of NAFLD was 8.16%; majority of them had mild NAFLD. They showed that serum uric acid was an independent predictor of NAFLD [27]. Both of these population-based studies have used ultrasound for diagnosis of NAFLD that might not be precise enough for estimation of hepatic steatosis and also cannot evaluate presence of NASH. It should be noted that using ultrasound might be inevitable due to invasiveness and possible harms of liver biopsy [28].

Uric acid might be involved in pathogenesis of NAFLD through several mechanisms. Elevated serum uric acid might induce insulin resistance [29] and activate renin-angiotensin system [30] that is involved in pathogenies of NAFLD [31]. Mitochondrial oxidative stress induced by hyperuricemia is another explanation for association of elevated uric acid and NAFLD [32]. Low serum magnesium has been reported to be associated with insulin resistance and NAFLD [33] and is inversely associated with serum uric acid [34]. NAFLD is strongly associated with the consumption of fructose

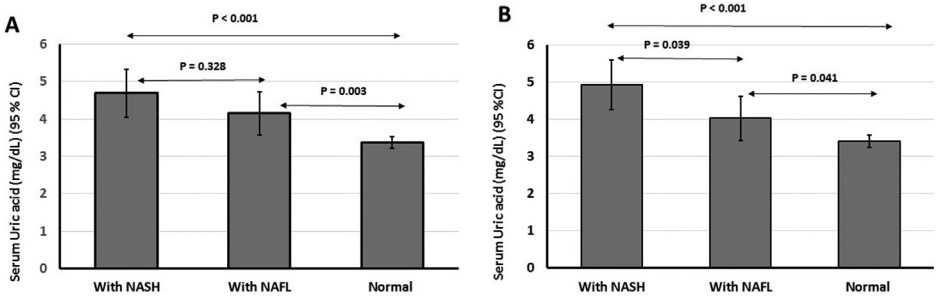

Figure 1 A. Comparison of serum uric acid in non-obese individuals with NASH, with NAFL and without NAFL. B. Comparison of serum uric acid in lean individuals with NASH, with NAFL and without NAFL.

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containing sugars. On the other hand, there is strong correlation between hyperuricemia and increased consumption of sugars containing fructose and glucose [35]. These evidences suggest the role of uric acid in fructose induced NAFLD. Fructose increases uric acid production from amino acids and uric acid is one of the end products of fructose metabolism [36]. Fructose induces mitochondrial oxidative stress mediated by uric acid activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [37]. The activtity of aconitase-2 is decreased by fructose and uric acid leading to activation of lipogenesis by stimulation of citrate lyase [37]. Fructose induced uric acid can also result in impairment of fatty acid oxidation via inhibition of enoyl A CoA hydratase and subsequent accumulation of lipids [38]. Activation of nuclear factor ƙ B (NFκB) and stimulation of monocyte chemoattractant protein-1 by uric acid are other proinflammatory mechanisms that might lead to NAFLD [39].

Our study is unique in reporting prevalence and risk factors of NAFLD/NASH diagnosed by liver biopsy in a cluster of apparently healthy, non-obese and lean population. Study participants were living liver donors and liver biopsies had been performed as a routine pre-transplant check-up. One limitation of the study is that none of the study participants had overt diabetes, and only 2 patients had dyslipidemia and hypertension. Therefore, the impact of these variables could not be investigated. However, this study is important showing NAFLD/NASH is prevalent even in a healthy lean population when evaluated by liver biopsy. It is noteworthy to know that most of the study participants had mild degrees of NAFLD/NASH in their liver biopsies and the clinical implication and outcomes of these patients is an interesting issue that should be clarified in future studies.

Guarantor of the article

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Authors' contribution

A.E.: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis.

S.N., A.S., K.K., B.G., S.A.M.: acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content.

Disclosure of interest

The authors declare that they have no competing interest.

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