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Research Article

Association of Positive Hepatitis B Serology Markers with Risk and Behavior Factors in Kazakhstan Blood Donors: A Cross Sectional Study

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Abstract

Background and Aim: A recent analysis of anti-HBc prevalence within Kazakhstan blood donors' population concluded a 17.2% rate. Considering these data are among the highest of infectivity results worldwide, this study sought to define an association of positive Hepatitis B serology markers with risk and behavior factors. **Methods:** The blood donors' samples were tested for anti-HBcore total (IgG/IgM**)** and anti-HBs, by CLIA on the Architect i2000SR platform (Abbott). Surrogate alanine transferase (ALT) markers for all blood donors were tested by kinetic method on the A25 analyzer (Biosystems). A questionnaire was developed to obtain the donors' socio-demographic characteristics, nutrition habits, cholesterol levels, history of smoking and alcohol consumption**.** Informed consent was obtained from all study participants**.** Statistics were calculated using the R software program (version 4.1.1, USA, 2021). **Results:** A group of 5709 blood donors participated in the study. Participants with positive for anti-HBc were on average older (41.8 vs 34.4 years, $p<0.001$), Kazakh (88.7% vs 83.0%, $p<0.001$), married (74.0% vs 55.6%, $p<0.001$), had a secondary education (70.1% vs. 59.4%, $p=0.03$), smoked (27.9% vs. 24.3%, $p=0.05$), had a longer smoking history (13.6±9.5 years vs. 9.8±8.5 years, $p<0.001$), and various hypercholestesterolaemia (6.2% vs 3.9%, $p=0.02$). Predominantly their main meal was dinner (17.0% vs 14.2%, $p=0.03$). Analysis for associations of socio-demographics characteristics, risk factors, and nutrition with anti-HBs, showed that those who have secondary education level (70.1%) were more likely to be anti-HBs-positive ($p=0.03$). No other significant correlations for anti-HBs were observed. **Conclusions:** The risk factors and behavior analysis highlighted the blood donors' overall health status. Positive Hepatitis B markers associated with statistically significant characteristics from socio-demography, nutrition habits or risk factors could support further pathogenesis studies looking for HBV treatment guides fulfilling the existing gaps.

Keywords: Hepatitis B; Anti HBcore; Risk factors; Blood donors

Abbreviations:

ALT: Alanine aminotransferase; Anti-HBs: Antibody to Hepatitis B surface; Anti-HBcore: Antibody to hepatitis B core antigen; CHB: Chronic hepatitis B infection; HBV: Hepatitis B infection; HBsAg: Hepatitis B surface antigen; HBV DNA: Hepatitis B infection DNA; HCV: Hepatitis C infection; HIV: Human immunodeficiency virus; WHO: The World Health Organization.

Introduction

According to the World Health Organization (WHO) approximately 1.5 million people become infected HBV each year [1], with around 240 million chronic carriers worldwide [2,3]. Kazakhstan is ranked as one of the countries with medium endemicity (2-7%) [4]. In terms of the prevalence of HBV in Kazakhstan, the peak incidence in 2020 was defined in West Kazakhstan, Kyzylorda region and in the city of Nur-Sultan rather than the national average prevalence [5].

Anti-HBc is considered as an informative marker of occult HBV infection and present in the chronic carrier state and at the end of an acute resolving infection [6].

The results of Croatian study [7] showed that the anti-HBc prevalence among blood donors is 1.32.%. The study by Meffre C. et al. [8] showed that the overall anti-HBc prevalence was 7.3% among metropolitan residents in France. In Germany population the anti-HBc prevalence of whole population was defined as 8.71% [9].

Considering these data are among the highest of infectivity results worldwide, another study of known risk and behavior factors and their association with positive HBV markers was performed in parallel. It was observed that tobacco smokers with hepatocellular carcinoma most often develop HBV infection [10,11]. Individuals with chronic HBV infection that consume high levels of alcohol are at a higher risk of alcoholic liver disease [12]. Also, patients with HBV-associated liver failure suffer from insufficient nutrition with high risk for the impairment of intestinal function [13]. Although HBV risk factors have been widely studied in Western countries, they are less analyzed and documented in Kazakhstan.

The primary objective of this study is to investigate the correlation between positive Hepatitis B serology markers and various risk and behavior factors. The aim is to address the current gap in knowledge and offer valuable insights into the epidemiology and determinants of HBV infection in Kazakhstan. Ultimately, the study intends to make a meaningful contribution through the development of targeted public health programs aimed at prevention and control of HBV infection within the country.

Materials and Methods

Study design

A cross-sectional study aimed at investigating anti-HBc was conducted in the period between June 1 to July 22, 2021 at the Scientific-Production Center of Transfusiology, Ministry of Healthcare, in Kazakhstan.

Approval for the study was obtained from the Ethics Commission (Decision of EC #5 from 20 August 2020). A specific donor questionnaire was created outlining the study purpose and to obtain information regarding the donors' socio-demographic characteristics, nutrition habits (eating vegetables and fruits), diet (main meal), at risk factors (smoking, alcohol consumption, dyslipidemia, HBV-infected family members, blood transfusion, intravenous or intramuscular procedures, tattooing or skin piercing, and surgical interventions). Also, cholesterol disorder was determined through self-reporting on the questionnaire.

Inclusion criteria

The study included blood donors who satisfied the donation criteria, which encompassed individuals aged 18 and above, with no history of blood donation within the past 3 months, weighing more than 50 kg, and testing negative for Hepatitis B, Hepatitis C, HIV, syphilis, gonorrhea, and other relevant laboratory parameters as per the stipulated requirements for blood donation. 68.9% of recruited donors were on regular basis of blood donation. HBsAg found to be positive for 3.4 % individuals from initial screening, while first time blood donors demonstrate more than twice higher HBV infectivity than regular blood donors [5].

Exclusion criteria

Potential blood donors were not engaged to participate in the study if did not meet inclusion criteria.

Laboratory analysis

The samples initially were tested for anti-HBc. If found to be positive with anti-HBc, further testing for anti-HBs was performed. All reagents for Hepatitis B screening were associated with the Architect i2000SR, Abbott system.

ALT markers were tested using Biosystems A25 analyzer. The confirmatory tests for infectious markers were evaluated by NAT in pools of six samples by Cobas TaqScreen MPX Test v.2.0

Statistical analysis

Statistical analysis was conducted by the R software (version 4.1.1, USA, 2021) [14]. Descriptive statistics were reported as proportions (%) for categorical variables and as means±standard deviations for continuous variables. The normality of the distribution was assessed using the Kolmogorov-Smirnov test.

Categorical variables were analyzed using Chi-square or Fisher's exact tests, while continuous independent variables were evaluated using Student's t-test or Mann-Whitney U test, depending on appropriateness. Adjusted odds ratios were utilized to present the associations between the risk factors and the prevalence of outcomes, specifically anti-HBc and anti-HBs results, while controlling for certain variables. In the final multivariable analysis model, the following variables were included and controlled for: age, ethnicity, and marital status. Statistical significance was considered at $p \leq 0.05$.

Results

Five thousand seven hundred and nine (5709) participants were enrolled in this study in the period between June 1 to July 22, 2021. Not all questions in the questionnaire were completed by the study participants. Thus, the demographics, risk factor and behavior total numbers do not add up to the participant total of 5709.

The average age of the participants engaged into the study was 35.69±10.57 year. The average age of participants with positive anti-HBc was 41.75±9.54, which may be related to the anti-HBV compulsory vaccination that started in 1998.

Most participants were of Kazakh nationality (84%), mainly having secondary education (68%), married (58.8%), non-smokers (75.7%) and denied alcohol consumption on the questionnaire (71.2%).

Regarding nutrition of donors, the main meal was lunch (54.0%), most often the participants prefer to eat 3 or more times a day (90.7%), having fruits and vegetables every day (68.4%) and for the majority (48.0%) meat and meat products were essential parts of the diet.

Among participants, a notable proportion (15.3%) had surgical interventions (including cosmetic surgery or organ removal). A total of 5.2% of the participants reported receiving intravenous or intramuscular treatments such as injections, acupuncture, tattoos, or piercings within the previous 4 months.

When compared to participants with anti-HBc negative results, those positive for anti-HBc were found to be smokers (27.9% vs. 24.3%, $p \leq 0.05$), had a longer smoking history (13.6±9.5 years vs. 9.8±8.5 years, $p < 0.001$), had a history of cholesterol problems (6.2% vs 3.9%, $p \leq 0.05$), and their main meal was dinner (17.0% vs 14.2%, $p \leq 0.05$) (Table 1).

Variables	Total N=5709	anti-HBc		p-value
		Negative	Positive	
		n=4726 (82.78)	n=983(17.22)	
Socio-demographic				
Age (mean (SD))	35.69 (10.57)	34.42 (10.33)	41.75 (9.54)	**<0.001**
Gender*, n (%)				
Females	1466	1220 (32.1)	246 (30.7)	0.46
Males	3139	2583 (67.9)	556 (69.3)	
Ethnicity*, n (%)				
Kazakh	3855	3146 (83.0)	709 (88.7)	**<0.001**
Russian	415	364 (9.6)	51 (6.4)	
Other ethnicities	320	281 (7.4)	39 (4.9)	
Education level*, n (%)				
Secondary level	2753	2269 (67.8)	484 (69.1)	0.48
Vocational level	549	464 (13.9)	85 (12.1)	
University level	745	614 (18.3)	131 (18.7)	
Married*, n (%)				

Yes	2354	1845 (55.6)	509 (74.0)	**<0.001**
No	1651	1472 (44.4)	179 (26.0)	
Risk and behavior factors				
Smoker*, n (%)				
Yes	1041	836 (16.9)	205 (19.6)	**0.05**
No	3130	2600 (83.2)	530 (80.4)	
Smoking experience (mean (SD)	10.56 (8.79)	9.83 (8.47)	13.62 (9.46)	**<0.001**
Alcohol consumption in the last 48 hours*, n (%)				0.16
Yes	1160	972 (19.3)	188 (16.6)	
No	2866	2346 (70.7)	520 (73.4)	
Main meal*, n (%)				**0.03**
All	515	417 (12.5)	98 (13.8)	
Breakfast	754	645 (19.3)	109 (15.4)	
Lunch	2187	1805 (54.0)	382 (53.8)	
Dinner	595	474 (14.2)	121 (17.0)	
Meals per day*, n (%)				
1-2 times	386	330 (9.6)	56 (7.6)	0.10
3 or more times	3770	3091 (90.4)	679 (92.4)	
Eating vegetables and fruits*, n (%)				
Several times a day	581	487 (14.7)	94 (13.6)	0.68
Every day	2737	2254 (68.1)	483 (69.7)	
Sometimes	683	567 (17.1)	116 (16.7)	
The percentage of food with meat and meat products*, n (%)				
≤ 25%	1113	935 (27.8)	178 (25.1)	0.37
26%-50%	1953	1606 (47.8)	347 (48.9)	
51%-75%	726	597 (17.8)	129 (18.2)	
> 76%	279	223 (6.6)	56 (7.9)	
Having dyslipidemia*, n (%)				
Yes	150	113 (3.9)	37 (6.2)	**0.02**
No	3359	2797 (96.1)	562 (93.8)	
Having family members had hepatitis in the last 6 months*, n (%)				
Yes	68	59 (1.7)	9 (1.2)	0.41
No	4096	3367 (98.3)	729 (98.8)	

Having transfusion of donated blood or its components in the last 12 months*, n (%)				
Yes	56	44 (1.1)	12 (1.4)	0.57
No	4653	3835 (98.9)	818 (98.6)	
Having intravenous or intramuscular injections, acupuncture, tattoos or piercings in the last 4 months*, n (%)				
Yes	247	201 (5.2)	46 (5.6)	0.71
No	4458	3678 (94.8)	780 (94.4)	
Having surgical interventions (including cosmetic surgery or organ removal) *, n (%)				
Yes	722	608 (15.7)	114 (13.7)	0.18
No	3989	3273 (84.3)	716 (86.3)	
*Variables in which partial information is missing				

Table 1: Risk factors and anti-HB core positive data.

*Number (percent) of participants missing answer for gender – 20 (9.5%); missing answer for ethnicity – 20 (9.5%); missing answer for education level – 56 (26.5%); missing answer for marital status – 63 (29.9%); missing answer for smoking–46 (21.8%); missing answer for alcohol consumption – 48 (22.7%); missing answer for main meal – 53 (25.1%); missing answer for meals per day – 46 (21.8%); missing answer for eating vegetables and fruits – 59 (28%); missing answer for the percentage of food with meat and meat products – 54 (25.6%); missing answer for having cholesterol disorder – 77 (36.5%); missing answer for family history of hepatitis – 46 (21.8%); missing answer for blood transfusions – 44 (20.9%); missing answer for Intravenous or intramuscular injections, acupuncture, tattoos or piercings – 45 (21.3%); missing answer for surgical interventions – 44 (20.9%);

Results demonstrated that those who have secondary education level (70.1%) were more likely to be anti-HBs-positive ($p \leq 0.05$) than other groups, which consist to majority of screened individuals. No difference in anti-HBs prevalence was observed for other risk factors (Table 2).

Variables	**anti-HBs**		**p-value**
	Negative	**Positive**	
	n=211(9.8%)	n=887 (90.2%)	
Socio-demographic			
Age (mean (SD))	40.02 (10.43)	41.27 (9.50)	0.117
Gender, n (%)			
Male	62 (22.5)	223 (21.1)	0.795
Female	129 (67.5)	493 (68.9)	
Ethnicity, n (%)			
Kazakh	161 (84.3)	629 (88.2)	0.094
Russian	12 (6.3)	47 (6.6)	
Other ethnicities	18 (9.4)	37 (5.2)	
Education level, n (%)			

Secondary level	92 (59.4)	439 (70.1)	0.031
Vocational level	27 (17.4)	73 (11.7)	
University level	36 (23.2)	114 (18.2)	
Married, n (%)			
Yes	105 (70.9)	454 (73.3)	0.627
No	43 (29.1)	165 (26.7)	
Risk and behavior factors			
Smoker, n (%)			
Yes	40 (24.2)	184 (28.1)	0.366
No	125 (75.8)	470 (71.9)	
Smoking experience (mean (SD))	15.18 (8.86)	13.15 (9.57)	0.306
Alcohol consumption in the last 48 hours, n (%)			
Yes	43 (26.4)	168 (26.7)	1.00
No	120 (73.6)	461 (73.3)	
Main meal, n (%)			
All	22 (13.9)	87 (13.7)	0.382
Breakfast	31 (19.6)	100 (15.8)	
Lunch	85 (53.8)	335 (52.8)	
Dinner	20 (12.7)	112 (17.7)	
Meals per day, n (%)			
1-2 times	6 (3.6)	54 (8.3)	0.062
3 or more times	159 (96.4)	600 (91.7)	
Eating vegetables and fruits, n (%)			
Several times a day	26 (17.1)	81 (13.0)	0.081
Every day	110 (72.4)	433 (69.7)	
Sometimes	16 (10.5)	107 (17.2)	
The percentage of food with meat and meat products, n (%)			
≤ 25%	39 (24.8)	160 (25.2)	0.507
26%-50%	80 (51.0)	311 (48.9)	
51%-75%	31 (19.7)	115 (18.1)	
> 76%	7 (4.5)	50 (7.9)	
Having cholesterol disorder, n (%)			
Yes	7 (5.2)	33 (6.2)	0.838
No	127 (94.8)	503 (93.8)	
Have family members had hepatitis in the last 6 month? n (%)			
Yes	2 (1.2)	8 (1.2)	1.00
No	163 (98.8)	648 (98.8)	

Having transfusion of donated blood or its components in the last 12 months, n (%)			
Yes	5 (3.0)	8 (1.1)	0.126
No	162 (97.0)	736 (98.9)	
Having intravenous or intramuscular injections, acupuncture, tattoos or piercings in the last 4 months			
Yes	8 (4.8)	41 (5.5)	0.856
No	158 (95.2)	699 (94.5)	
Having surgical interventions (including cosmetic surgery or organ removal), n (%)			
Yes	31 (18.6)	104 (14.0)	0.166
No	136 (81.4)	640 (86.0)	
ALT level, n (%)			
Normal	196 (94.2)	819 (95.7)	0.479
Elevated	12 (5.8)	37 (4.3)	

Table 2: Risk factors and anti-HBs positive data.

*Number (percent) of participants missing answer for gender – 20 (9.5%); missing answer for ethnicity – 20 (9.5%); missing answer for education level – 56 (26.5%); missing answer for marital status – 63 (29.9%); missing answer for smoking–46 (21.8%); missing answer for alcohol consumption – 48 (22.7%); missing answer for main meal – 53 (25.1%); missing answer for meals per day – 46 (21.8%); missing answer for eating vegetables and fruits – 59 (28%); missing answer for the percentage of food with meat and meat products – 54 (25.6%); missing answer for having cholesterol disorder – 77 (36.5%); missing answer for family history of hepatitis – 46 (21.8%); missing answer for blood transfusions – 44 (20.9%); missing answer for Intravenous or intramuscular injections, acupuncture, tattoos or piercings – 45 (21.3%); missing answer for surgical interventions – 44 (20.9%); missing answer for ALT level – 106 (50.2%).

It was also showed that age ($p<0.001$), ethnicity ($p<0.05$), marital status ($p<0.05$) were statistically associated with anti-HBc positivity from descriptive analysis (Table 3). Statistical analysis done to compare gender, nationality, education, marital status, family history of hepatitis, blood transfusion history, injections, or surgical interventions, with a one-year increase in age (adjOR=1.06, 95%CI:1.05-1.07), showed a 6% growth in odds for anti-HBc positivity. Blood donors with Russian ethnicity (adjOR=0.65, 95%CI:0.46-0.93) and representatives of other nationality (adjOR=0.56, 95%CI:0.37-0.85) were found to have lower odds with positive anti-HBc results when compared to Kazakhs. Among participants that indicated not married, the odds ratio of positive anti-HBc results were lower by 29% (adjOR = 0.71, 95%CI:0.57-0.89) compared to married participants when adjusting for other variables.

Analysis adjusting for gender, nationality, education, marital status, family history of hepatitis, blood transfusions, injections and surgery, showed that those who represented other nationality groups (adjOR=0.36, 95%CI:0.18-0.72) had lower odds of having anti-HBs comparing to the Kazakhs. Those reporting to have Vocational level of education had 50% lower odds of having anti-HBs than those who had a Secondary level (adjOR=0.50, 95%CI:0.28-0.89) (Table 3).

Variables	**anti-HBcore**		**anti-HBs**	
	Adjusted OR (95%CI)	**p-value**	**Adjusted OR (95%CI)**	**p-value**
Age	1.06 (1.05; 1.07)	<0.001	1.02 (1.00; 1.04)	0.09
Gender		0.32		

Females	Ref.		Ref.	
Males	1.11 (0.90; 1.38)		1.11 (0.71; 1.72)	0.64
Ethnicity				
Kazakh	Ref.		Ref.	
Russian	0.65 (0.46; 0.93)	0.03	0.80 (0.39; 1.63)	1.00
Other ethnicities	0.56 (0.37; 0.85)	0.01	0.36 (0.18; 0.72)	<0.01
Education level				
Secondary level	Ref.		Ref.	
Vocational level	0.77 (0.57; 1.04)	0.18	0.50 (0.28; 0.89)	0.04
University level	1.02 (0.79; 1.31)	1.00	0.78 (0.47; 1.30)	0.70
Married		<0.01		
Yes	Ref.		Ref.	
No	0.71 (0.57; 0.89)		0.92 (0.58; 1.46)	0.74
Have family members had hepatitis in the last 6 months?		0.61		
Yes	Ref.		Ref.	
No	1.23 (0.55; 2.71)		1.31 (0.26; 6.53)	0.74
Having transfusion of donated blood or its components in the last 12 months		0.92		
Yes	Ref.		Ref.	
No	0.96 (0.44; 2.10)		3.31 (0.89; 12.3)	0.07
Having intravenous or intramuscular injections, acupuncture, tattoos or piercings in the last 4 months		0.21		
Yes	Ref.		Ref.	
No	0.79 (0.55; 1.15)		0.89 (0.40; 1.99)	0.89
Having surgical interventions (including cosmetic surgery or organ removal)		0.07		
Yes	Ref.		Ref.	0.19

Table 3: Risk factors for anti-HBcore and anti-HBs positivity: Multivariable logistic regression analysis.

Discussion

Anti-HBc prevalence within Kazakhstan blood donors' population was recently shown to be 17.2% (983) rate [15]. Of the anti-HBc positive donors, 90.2% (887 from 983) were also positive for anti-HBs. The 30 to 39 years old age group exhibited the highest prevalence of anti-HBc (38.7%).

The results from additional studies that examined the association of HBsAg positivity with the sociodemographic of the Kazakh population [16,17] were consistent with the results of our study which correlates HBV positive markers (anti-HBs and anti-HBc) with participants' age, ethnicity, education level and marital status.

Antibodies against HBsAg develop in a person successfully vaccinated against hepatitis B virus [7]. One explanation for the higher prevalence of anti-HBc among the 30 - 39-year-old age group in this study, may be that these individuals were at a higher risk for HBV infection since the implementation of the vaccination program in Kazakhstan [4] occurred in 1998. The 30 – 39-year-old age group also were the most active donor group in our study.

The study conducted by Shaha et al. [18] found no significant association of positive anti-HBs markers with age, gender, and socioeconomic status. Meanwhile, a significant decrease in the development of anti-HBs, either through natural immunity or vaccination, was observed among individuals who reported smoking [18]. This finding contrasts with our earlier results regarding anti-HBc positivity and its association with this high-risk behavior. Prabina P et al. [19] demonstrated that cigarette smoking was seen in 52% of the HBV-infected patients. Further investigation is needed to understand whether the development of different antibodies against HBV may vary due to the mechanisms of the pathogenesis affected by viral nucleic acid and host intracellular or humoral response [20]. Since nicotine is metabolized by the liver enzymes, subsequent involvement of hepatocytes within various signaling pathways may play either a direct role to liver affinity of viral infection and / or an indirect role to lung cancer biochemistry and the further development of malignancies [21]. A known protective role of anti-HBs in the structure of hepatitis B immunoglobulins (HBIG) was confirmed to be highly effective for perinatal HBV [22] suggest of intrahepatic production of immunoglobulins are working in the immune response cascade [23].

According to the survey results, we found that participants positive for anti-HBc had hypercholestesterolaemia disorders, and their main meal was dinner. Additional published were not available to confirm our results. HBV induces multiple changes in hepatic lipid metabolism, at the same time increasing both lipid synthesis and lipolysis [23], which may be contributing to our findings. Another study indicated that HBV could induce the expression of the TLR2 gene, associated with a pathogen recognition receptor in the liver. Hepatic lipid accumulation involving genes related to cholesterol absorption and metabolism may also be associated with TLR2 gene overexpression [24]. Lipid homeostasis, manifesting as dyslipidemia, and initially observed obesity associated with lipoprotein receptor damage in the liver are considering the main factors for regulation of hepatic lipolysis [25]. Target gene analysis, using a whole genome expression profile among individuals with a high-fat diet, may offer not only a clearer picture of lipid metabolism in the liver [20] but may aid in understanding how HBV impacts the same pathway [26].

We found that anti-HBc positivity was observed in 23% of participants with elevated ALT, however, in a previous study this marker was determined in 17% with normal levels of ALT [15]. The discussions around the ALT value as surrogate marker for hepatitis diagnostics in early stage are still under debate. Studies have shown that elevated ALT levels definitely show dysfunction of hepatocytes or their response to any other aggressive interaction which could be either infection or toxically acting substance [27,28].

The main challenge today is global burden of Hepatitis B. Scientists are developing an effective control of HBV replication in early stage [29]. Insufficient treatment of HBV which results in patient death due to hepatocellular carcinoma, cirrhosis or liver failure suggest that further insights are needed to close the knowledge gap.

One of the key strengths in this study, is the inclusion of a large sample size, which provided increased statistical power for accurately estimating prevalence rates and assessing risk factors. However, independent risk factors were obtained using self-reported information, which could be biased and / or be missed. In addition, HBV vaccination status was not assessed in our study and the status of lipidaemia was obtained from self-assessment questionnaire and not confirmed through a laboratory test.

Conclusions

The comprehensive analysis of risk factors and behaviors in this study shed light on the overall health status of Kazakhstan blood donors. The identification of statistically significant associations between positive Hepatitis B markers and various socio-demographic factors, nutritional habits, and other risk factors provides valuable insights. These findings can serve as a foundation for further research into the pathogenesis of Hepatitis B, aiming to address existing gaps in HBV treatment guidelines. Additionally, the identification of high-risk groups for screening expansion and the development of fundamental testing approaches are crucial steps in filling the current knowledge gaps.

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Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article its supplementary materials.

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