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Biochemical and Haematological Profile in Patients with Alcohol Dependence Syndrome (ADS) Co-Morbid with Nicotine Dependence Syndrome (NDS)

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Authors' contributions

This work was carried out in collaboration between all authors. Author JBH designed the study, performed the statistical analysis, wrote the protocol, analyses of the study and wrote the first draft of the manuscript. Authors ARS and PJM managed the literature searches and proof reading of the study. Author MM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To compare and assess the biochemical and haematological parameters in patients with Alcohol dependent syndrome (ADS) co-morbid with Nicotine dependent syndrome (NDS) in comparison to those with only Alcohol dependent syndrome (ADS).
Study Design: Observational case control study.
Place and Duration of Study: Department of Biochemistry and Department of Psychiatry, Vailankanni Deaddiction centre, Father Muller Medical College, Mangalore.
Methodology: The subjects consisted of 30 male patients (age range 27-64 years) with Alcohol dependent syndrome co-morbid with Nicotine dependent syndrome (ADS+NDS), 30 male patients (age range 26-60 years) with Alcohol dependent syndrome (ADS) and 30 male normal healthy volunteers (age range 28-46 years). Activities of liver function marker enzymes and levels of

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haematological parameters were assayed by standard methods.
Results: Activities of alanine aminotransferases(ALT), aspartate aminotransferases(AST), alkaline phosphatase(ALP) and gamma glutamyl transferase(ɣGT) were significantly higher in patients with ADS+NDS and only ADS, in comparison to healthy controls (p< .001). The haemoglobin, RBC counts, polymorphonuclear cells were significantly decreased in only ADS group compared to controls (p< .01) and MCV and ESR were significantly increased in patients with ADS+NDS and only ADS (p<0.001). No significant difference was observed between patients with ADS+NDS and only ADS, with respect to biochemical and haematological parameters. There was significant correlation of ɣGT with ALT(r=0.72) and AST(r=0.88) in only ADS group and ALT with AST(r=0.79; r=0.83) in both ADS+NDS and ADS groups.
Conclusions: Significant changes were evident in biochemical and haematological parameters in patients with only Alcohol dependent syndrome. These parameters could serve as markers of alcoholism.

Keywords: Alcohol dependence syndrome; nicotine dependence syndrome; ɣGT; aminotransferases; MCV.

1. INTRODUCTION

Alcohol is a biochemical substance with multiple and varied effects in the body. Alcohol use disorder has been found to be associated with psychological, social and health problems world over. Alcohol consumption is related to more than 60 different medical conditions. Alcohol use disorders contribute significantly to the burden of disease in many developed countries where in mild forms discontinue without treatment, the more severe forms are underdiagnosed and undertreated [1]. Alcohol dependence and Nicotine dependence syndrome is defined as "A cluster of physiological, behavioural, and cognitive phenomena in which the use of a substance or a class of substances takes on a much higher priority for a given individual than other behaviours that once had greater value". Syndrome is clinically characterised by any three or more of the following have been present together. Craving, loss of control on substance use, physiological withdrawal state, evidence of tolerance, preoccupation with substance use, persistent substance use despite having medical or psychotic morbidity [2].

Alcohol and cigarette consumption have profound effects on genome wide DNA methylation and are common, often cryptic, co-morbid features of many psychiatric disorders [3]. The most common co-morbid substance addiction is nicotine. Smoking is prevalent in 84% of the people admitted for deaddiction treatment [4]. Smoking might encourage high level of alcohol consumption. The amount of alcohol and tobacco consumption in patients with heavy alcohol abuse increases the severity of liver damage [5].

Researchers have attempted to establish biomarkers of alcoholism and alcoholic liver disease [6]. There is paucity of studies which analyze and correlate biochemical and haematological parameters in individuals with alcohol dependence syndrome with co morbid nicotine dependence. The present study aimed to investigate the changes in biochemical and haematological parameters in patients with Alcohol dependent syndrome co morbid with Nicotine dependent syndrome-currently using substance (ADS+NDS-CUS) in comparison to only Alcohol dependent syndrome-currently using substance (ADS-CUS) patients.

2. MATERIALS AND METHODS

2.1 Study Design

The patients included in the study were diagnosed of alcohol dependence by the treating psychiatrist, based on The ICD-10 (the 10th revision of the International Statistical Classification of Diseases), classification of mental and behavioural disorders : diagnostic criteria for research of WHO [7,8], as 1) alcohol dependent syndrome - currently using substance (ADS-CUS) and 2) alcohol dependent syndrome co morbid with Nicotine dependent syndrome - currently using substance (ADS+NDS-CUS) admitted to Vailankanni Deaddiction centre, Father Muller Medical College, Mangalore. Patients with systemic illness such as non alcoholic liver disease, cancer, inflammatory diseases, infections, were excluded from the study.

Ninety males participated in this study. Thirty ADS-CUS patients (mean age ±SD 41.5±10.0).

Thirty ADS+NDS-CUS patients (mean age ±SD; 39.2±9.6). The control group consisted of 30 healthy volunteers (mean age ±SD; 35±5.1) without any history of alcohol abuse and smoking. Informed written consent was obtained from each subject after explanation of nature, purpose and potential risk of study. The study was approved by Institutional ethics committee. Blood was collected aseptically in EDTA and plain tubes, for analysis of haematological parameters and biochemical parameters respectively.

2.1.1 Assays done

Serum Total protein and Albumin were assayed by Biuret reagent [9] and Bromocresol green [10] respectively. Activities of alanine amino transferase [11] and aspartate amino transferase [12] were assayed by Modified IFCC method. γ – glutamyl transferase activity was assayed by carboxy substrate method [12]. The activity of alkaline phosphatase was assayed by PNPP Kinetic method [13]. Total bilirubin was assayed by diazo method [14]. Levels of Serum urea and creatinine were assayed by coupled enzymatic method [15] and Kinetic Jaffe's method [16] respectively. Haemoglobin concentration was measured by using cyanide free lytic reagent and other haematological parameters were determined by Beckman coulter, using the principle of electrical impedence.

2.2 Statistical Analysis

The results were analysed using SPSS version 23 software. Results were expressed as median (min-max.). Comparison in serum biochemical and haematological parameters between Alcohol dependent syndrome-currently using substance (ADS-CUS) and Alcohol dependent syndrome co morbid with Nicotine dependent syndrome – currently using substance (ADS+NDS-CUS) groups were made using Kruskal Wallis analysis and differences located using Mann-Whitney test. Pearson's correlation coefficient was used to measure the statistical dependence between two variables. Statistical significance was defined as $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Group Characteristics

The average time of dependence for alcohol was 17±7 years and 17±9 years in ADS-CUS and ADS+NDS-CUS respectively. The time of drinking of alcohol was categorised into 10 years and below, 11-20 years, and above 20 years. Among 11-20 years of alcohol use 53.3% (16/30) were in ADS-CUS (Alcohol dependent syndrome-currently using substance) group and 46.7% (14/30) were in ADS+NDS-CUS (Alcohol dependent syndrome co morbid with Nicotine dependent syndrome-currently using substance) group (Table 1).

3.1.1 Haematological parameters

There was no significant difference in eosinophils between control, ADS-CUS and ADS+NDS-CUS group (Table 2). There were no significant differences in any of the haematological parameters between ADS-CUS and ADS+NDS-CUS group. MCV and ESR were increased significantly ($p<.001$), Haemoglobin, RBC count and polymorphonuclear cells were decreased significantly ($p<.01$) in ADS-CUS group compared to controls.

Among the ADS+NDS-CUS and control group, lymphocytes was reduced significantly ($p<.001$), ESR and MCV was increased significantly ($p<.001$), Haemoglobin, RBC count and polymorphonuclear cells were not significant, in ADS+NDS-CUS group compared to controls. (Table 2).

Table 1. Group characteristics of the study subjects

Characteristics	C (n=30)	ADS-CUS (n=30)	ADS+NDS-CUS (n=30)
Age (mean±SD)	35±5.1	41.5±10.08	39.17±9.64
Alcohol dependence groups			
10 years and below	-	7 (23.3%)	8 (26.7%)
11- 20 years	-	16(53.3%)	14 (46.7%)
> 20 years	-	7 (23.3%)	8 (26.7%)

Table 2. Comparisons of the haematological parameters, in controls, alcohol dependent syndrome-currently using substance (ADS-CUS) and alcohol dependent syndrome with nicotine dependent syndrome – currently using substance groups (ADS+NDS-CUS).

Variables	C	ADS-CUS	ADS+NDS-CUS	P value[@]	p value[#]	p value[$]
Hb (gm%)	15.05 (14.5-15.5)	13.6 (13.2-15)	14.95 (13.8-16.3)	0.001*	0.894	0.073
MCV (fl)	84.9 (83.8-86.3)	94.5 (89.1-99.1)	94.8 (91.1-98.5)	0.000*	0.000*	0.909
RBC count (x 10^6) (cells/cu.mm.)	4.95 (4.8-5.3)	4.47 (4.1-5)	4.79 (4.3-5.1)	0.002*	0.091	0.202
Polymorpho-nuclear cells (%)	62 (60.7-64.2)	53 (48.2-66.7)	60 (53.7-68.2)	0.005*	0.161	0.096
Lymphocytes (%)	36 (34-37)	33.5 (20-39.2)	29 (20.7-35.2)	0.414	0.001*	0.176
Eosinophils	3 (3-3.2)	3 (2-5.2)	2 (1-5)	0.994	0.260	0.242
ESR (mm/h)	3 (2-3.2)	7.5 (4-15)	5 (4-11)	0.000*	0.000*	0.181

Data are median (min-max). Groups are compared using Kruskal Wallis test and differences located using Mannwhitney test. p value < .05 is taken statistical significant(*).
[@]p value: C/ADS-CUS
[#]p value: C/ADS+NDS-CUS
[$]p value: ADS-CUS/ADS+NDS-CUS.

Table 3. Comparisons of the biochemical parameters, in controls, alcohol dependent syndrome-currently using substance (ADS-CUS) and alcohol dependent syndrome co-morbid with nicotine dependent syndrome – currently using substance groups (ADS+NDS-CUS)

Variables	C	ADS-CUS	ADS+NDS-CUS	p value[@]	p value[#]	p value[$]
Total protein (g/dl)	7.1 (6.9-7.7)	7.2 (6.8-7.6)	7.14 (7-7.8)	0.894	0.982	0.830
Albumin (g/dl)	4.15 (3.9-4.49)	4.41 (4.1-4.69)	4.4 (4.2-4.6)	0.057	0.053	0.807
Total bilirubin (mg/dl)	0.69 (0.65-0.75)	0.74 (0.49-1.01)	0.79 (0.53-1.06)	0.657	0.225	0.631
Urea (mg/dl)	25.5 (24-27.25)	18.5 (12.7-21.2)	19 (15-24.5)	0.000*	0.000*	0.242
Creatinine (mg/dl)	0.88 (0.86-0.94)	0.79 (0.68-0.82)	0.81 (0.69-0.92)	0.000*	0.010*	0.225
ɣGT (IU/L)	19 (17-21.25)	151 (51.5-223.3)	106 (42.75-290.2)	0.000*	0.000*	0.482
AST (IU/L)	20 (18-22)	78 (42.75-117.5)	66 (45.5-143)	0.000*	0.000*	0.717
ALT (IU/L)	19.5 (18-21)	49 (26.25-81.5)	38.5 (29-60.75)	0.000*	0.000*	0.525
ALP (IU/L)	66 (61.75-72)	93 (74.5-106.75)	92.5 (72.25-107.5)	0.000*	0.000*	0.923

Data are median (min-max). Groups are compared using Kruskal Wallis test and differences located using Mannwhitney test. p value < .05 is taken statistical significant (*).
[@]p value: C/ADS-CUS; [#]p value: C/ADS+NDS-CUS; [$]p value: ADS-CUS/ADS+NDS-CUS

3.1.1.1 Biochemical parameters

There was no significant difference in serum proteins, albumin and total bilirubin between control, ADS-CUS and ADS+NDS-CUS group (Table 3 above). Serum enzymes amino transferases (ALT & AST), ɣ Glutamyl transferase (ɣGT), and ALP were increased

significantly (p<.001), in both the ADS-CUS and ADS+NDS-CUS group compared to controls. Urea and creatinine, were decreased significantly (p<.01) in ADS-CUS and ADS+NDS-CUS group respectively, compared to controls. There was no significant difference in the biochemical parameters between the ADS-CUS and ADS+NDS-CUS group (Table 3).

3.1.1.2 Correlation

A positive correlation was observed between ALT and lymphocytes (r=0.380, p=.03) and ALP and MCV (r=0.371, p=.04) in ADS-CUS and ADS+NDS-CUS groups respectively. A negative correlation was found between Total bilirubin and lymphocytes (r= -0.374, p=.04).

A highly significant positive correlation was found between ɣGT and AST (r = + 0.88, p< .001), GGT and ALT (r = + 0.72, p< .001), AST and ALT (r = + 0.79, p< .001), in ADS-CUS group. Highly Significant positive correlation was also found between ɣGT and AST (r = + 0.52, p< .001), and AST and ALT(r = + 0.83 p< .001), in ADS+NDS-CUS group. A significant negative correlation was found between creatinine and total bilirubin (r= - 0.48, p<.001) Table 4 and Figs. 1-3.

Alcohol has numerous adverse effects on the various types of blood cells, biochemical substances and their functions. The effects of alcohol on various tissues depend on its concentration in blood over time. The alcohol elimination rate varies among individuals and is influenced by factors such as chronic alcohol consumption, age, time of day, diet and smoking [17]. Alcohol has a variety of pathologic effects on haematopoiesis, it directly damages erythroid precursors, thereby contributing to macrocytosis, and it also interferes with heme synthesis and induces anaemia. Furthermore, chronic alcohol ingestion can lead to various types of haemolytic anaemia due to alterations in erythrocyte membrane lipids [18,19].

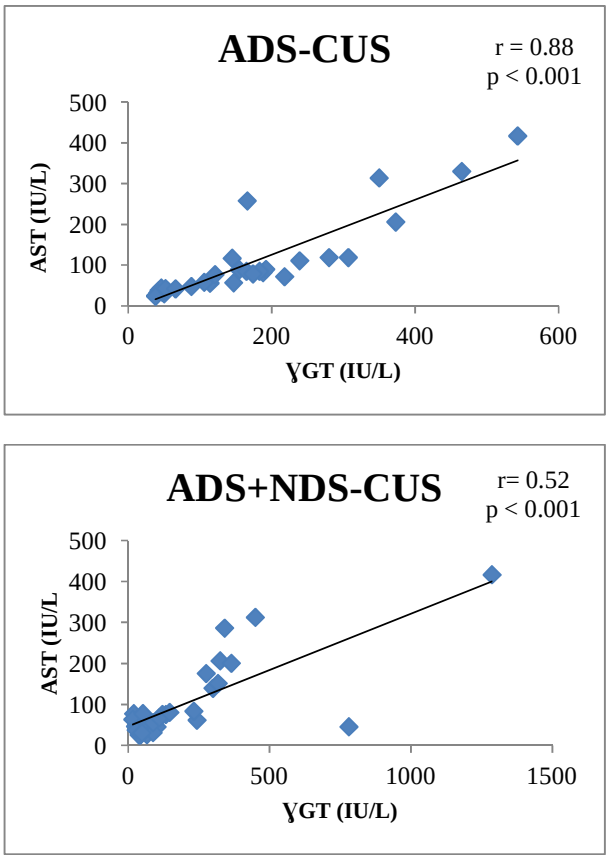

Fig. 1. Correlation between ɣGT and AST with ADS-CUS and ADS+NDS-CUS groups

Table 4. Correlation between biochemical parameters with ADS-CUS and ADS+NDS-CUS groups

Variable	ADS-CUS								ADS+NDS-CUS							
	Alb	**Ur**	**Cr**	**TB**	**ɣGT**	**AST**	**ALT**	**ALP**	**Alb**	**Ur**	**Cr**	**TB**	**ɣGT**	**AST**	**ALT**	**ALP**
TP	0.53*	0.06	0.07	-0.27	0.35	0.28	0.36*	0.23	0.63*	-0.37*	-0.08	-0.19	0.01	0.20	-0.01	0.32
Alb		0.15	0.18	-0.08	0.16	0.11	0.37*	-0.04		-0.37*	0.33	0.10	0.05	0.35	0.34	0.05
Ur			0.44*	-0.15	-0.31	-0.24	-0.05	-0.22			0.11	0.04	-0.33	-0.28	-0.16	-0.39*
Cr				-0.48**	-0.38*	-0.38*	-0.17	-0.12				0.18	-0.03	0.00	0.14	-0.27
TB					0.20	0.18	0.02	0.01					-0.14	0.12	0.31	-0.09
ɣGT						0.88**	0.72**	0.37*						0.52**	0.39*	0.39*
AST							0.79**	0.40*							0.83**	0.37*
ALT								0.16								0.11

(p<.005, **p <.001, TP: Total protein, Alb: Albumin, Ur: urea, Cr: Creatinine, TB: Total Bilirubin, ɣGT: gamma glutamyl transferase, AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase.)*

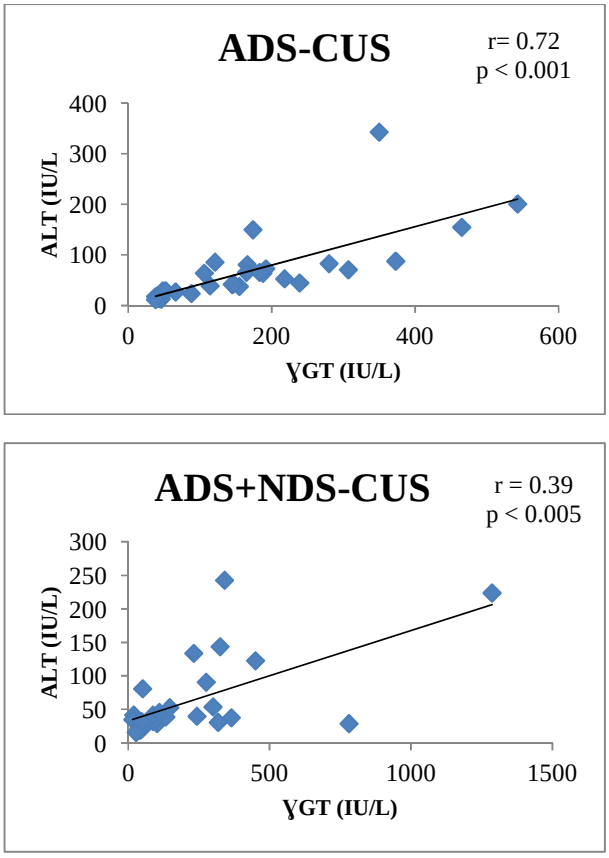

Fig. 2. Correlation between ɣGT and ALT with ADS-CUS and ADS+NDS-CUS groups

Haemoglobin percentage, RBC count and polymorphonuclear cells were significantly decreased in ADS-CUS group and not significant decrease in ADS+NDS-CUS group compared to controls. But the ADS+NDS-CUS group had higher levels of Haemoglobin percentage, RBC count and polymorphonuclear cells compared to ADS-CUS group, which was similar to other studies [20,21]. Elevated levels of Haemoglobin are correlated with increased number of erythrocytes, is consistent with other investigations [22]. High levels of RBC associated with blood viscosity which slows blood velocity and increases the risk of intravascular clotting, coronary vascular resistance, decreased coronary blood flow, and a predisposition to cause thrombosis [23]. Polycythemia leads to hypoxia, causing increased red blood cell production due to high levels of carboxy haemoglobin, with reduction in plasma volume. Thrombosis is a serious condition of polycythemia [24,19]. Whereas the MCV and ESR were significantly increased in both ADS-CUS and ADS+NDS-CUS group compared to controls because the RBC can survive for 120 days after it has been released into the circulation, an MCV level may remain elevated for up to 3 months after a person has stopped drinking. But increase in MCV has also been reported in other conditions such as thyroid disease, folate deficiency, recent blood loss, and a number of haematological conditions and liver diseases from other causes [25,6]. Lymphocytes are raised on consumption of moderate alcohol, but in chronic heavy drinking alcoholics lymphocytes are decreased [26]. The mechanism responsible for this effect is not known, however possibly alcohol triggers the release of hormones that alter lymphocyte adherence to endothelial cells lining blood vessels. In another study found Smoking decreases lymphocytes [27]. In the present study, the lymphocytes was reduced significantly in ADS+NDS-CUS group (smoking alcohol dependents) compared to controls, where as the levels of lymphocytes were higher in ADS-CUS (non smoking alcohol dependents) group compared to ADS+NDS-CUS group.

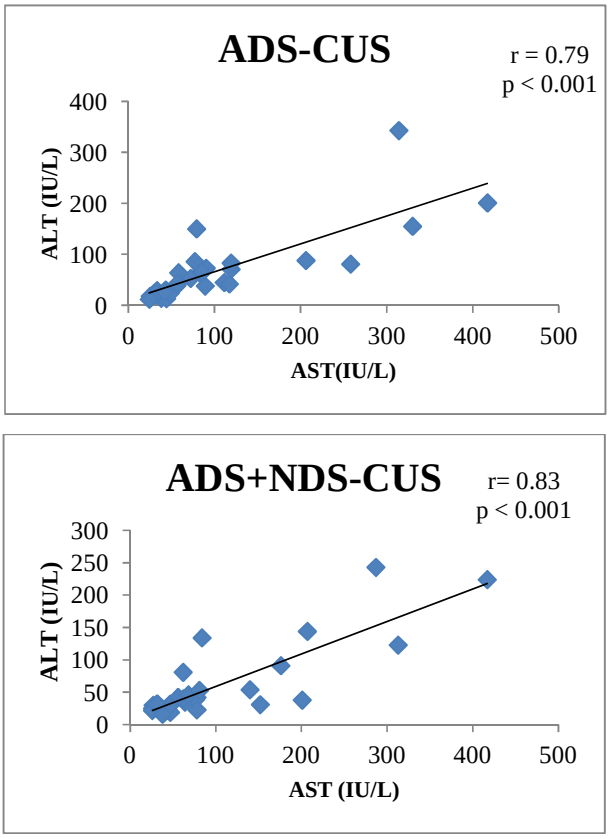

Fig. 3. Correlation between ALT and AST with ADS-CUS and ADS+NDS-CUS groups

The serum proteins, albumin, urea, creatinine, and total bilirubin in tested groups were within normal range. The rise in serum liver enzymes AST, ALT, ɣGT and ALP in ADS-CUS and ADS+NDS-CUS groups compared to control, findings agreed with several previous studies, that have reported on association between smoking, alcohol consumption and liver enzymes [28,29,6,30]. ɣGT characterises chronic misuse of alcohol. Our findings showed very high levels of ɣGT in both the tested groups. Previous studies have reported that serum AST and ɣGT are the most widely used markers for alcohol consumption [31,32]. In our study a highly significant positive correlation was observed between AST and ɣGT in both the tested groups (Fig. 1). Very high ɣGT demonstrate a more intense vulnerability to alcohol, a characteristic that appear to be stable over time [33]. Chronic alcohol abuse may increase the rate of nicotine metabolism, which then decreases over time after alcohol cessation [34]. Standard blood tests and mean corpuscular volume of red blood cells (MCV) have low sensitivity and specificity. Biomarkers for alcohol use that are more sensitive and specific than standard clinical assays exist, but they are not widely used because of their high cost and limited availability. These biomarkers include, carbohydrate-deficient transferrin, ethyl glucuronide, ethyl sulphate, phosphatidyl ethanol and fatty acid ethyl esters [35,36].

4. CONCLUSION

There were significant changes in biochemical and haematological parameters in patients with only Alcohol dependent syndrome. These parameters could serve as markers of alcoholism. This study had its limitations of small sample size, and we could not get exact information on amount of alcohol and nicotine use by the patients. There is a need for future studies with larger sample size, correlating the levels/activities of biochemical and haematological parameters with duration and dose of alcohol and nicotine use disorder.

CONSENT

All authors declare that informed written consent was obtained from each subject after explanation of nature, purpose and potential risk of study.

ETHICAL APPROVAL

All authors hereby declare that the study was approved by Father Muller institutional ethics committee. Number FMMC/FMIEC/2039/2014 dt 10.12.2014.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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