Asian Journal of Medicine and Health

Volume 21, Issue 11, Page 27-35, 2023; Article no.AJMAH.101999
ISSN: 2456-8414

Evaluation of Thyroid Function of HIV Patients in Umunze, Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2023/v21i11916

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/101999

Short Research Article

Received: 04/05/2023
Accepted: 09/07/2023
Published: 16/09/2023

ABSTRACT

Background: Abnormal thyroid function tests have been detected at various phases in people living with Human immunodeficiency virus (HIV) and the effect of highly active antiretroviral therapy (HAART) not well understood. However, there is insufficient study among Nigerians living with HIV.

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Objective: This study is aimed to determine thyroid hormones in HIV positive individuals compared with HIV negative individuals in Umunze, Anambra State, Nigeria.
Materials and Methods: This cross-sectional study was carried out over a period of six months among 95 HIV positive and 30 HIV negative individuals attending USAID/FHI clinic of Immaculate Heart Hospital in Umunze, Nigeria. The subjects were divided into three groups on the basis of HAART (those on HAART [48] – GROUP 1, HAART naïve [47] – GROUP 2 and Control [30] – GROUP 3). The subjects were interviewed, examined and blood sample collected for determination of thyroid function parameters which include thyroid stimulating hormone (TSH), free triiodothyronine (fT_3), triiodothyronine (T_3), free thyroxine (fT_4), and thyroxine (T_4).
Results: Among the 125 subjects recruited for the study 67.2% were females and 32.8% males. The mean levels of TSH and fT_3 was found to be higher in group 1 subjects than in group 2 and the group 3 subjects. The mean level of T_4 was significantly higher in group 2 subjects than group 1 and the group 3 subjects. The level of T_3 was significantly lower in control subjects in comparison to both HAART and non-HAART subjects. Primary hypothyroidism is the commonest pattern of thyroid dysfunction among the HIV positive patients followed by isolated low fT_4.
Conclusion: Serum levels of thyroid hormones as shown in this study may be used as baseline periodic markers during antiretroviral therapy while people living with HIV may benefit from supplementation if appropriate. There is also need for a larger study to identify the risk factors for progression to overt thyroid disease in HIV infected subjects with thyroid autoimmunity.

Keywords: HIV infection; HAART; thyroid hormones; Hypothyroidism; Umunze Nigeria.

1. INTRODUCTION

In Nigeria, about 1.9million people are living with HIV with prevalence of 1.3% among adults aged 15 – 49years. 'HIV is one of the largest health problems today because of its pandemic status and severity characteristics' [1]. The disease is mainly characterized by a progressive loss of CD4+ T lymphocytes (CD4+), which cause immunosuppression and involvement by opportunistic diseases. The natural history of AIDS has been altered considerably by high activity antiretroviral therapy (HAART), which prevents the evolution of the loss of CD4+ to its final stage. Along with prevention campaigns, HAART contributes to the decline of the transmission and stabilization of the epidemic in many countries" [2].

"Nevertheless, several complications have been reported with the use of HAART, among them are hypertriglyceridemia, lipodystrophy, type 2 Diabetes mellitus, gonadal dysfunction and osteoporosis" [3]. "The mechanism by which HAART causes these changes has not been fully elucidated" [4]. "Another complication is immune reconstitution inflammatory syndrome (IRIS). This condition occurs in some patients receiving HAART who develop clinical deterioration by the reestablishment of immunity despite high CD4+ counts and a low plasma viral load. Immune reconstitution (IR) can be defined as an increased CD4+ count above 200cells/mm^3 in subjects who previously had CD4+ counts lower than 100 – 200 cells/mm^3" [4].

2. MATERIALS AND METHODS

2.1 Study Location

This is a hospital based study in Umunze, Anambra State, Nigeria.

2.2 Study Population

Participants in this study are known HIV positive patients and healthy individuals who are on routine medical checkup at USAID/FHI clinic of Immaculate Heart Hospital Umunze, Nigeria.

2.3 Method for Sample Collection

About 8ml of venous blood was collected by venipuncture from the cubital fossa into plain specimen tubes. It was allowed to clot, centrifuged and the resultant serum stored at -20°C until analyses was carried out for T_3, T_4, TSH, fT_3, and fT_4.

2.4 Sample Size

Formular proposed by Naing *et al* (2006) was adopted for the calculation of sample size of the study.

It states:

$$N = Z^2PQ / D^2$$

Where N = minimum sample size

- Z = standard normal deviate at 95% confidence interval which is 1.96
- P = least estimate of population prevalence from literature review
- D = test difference between two sub samples regarding a proportion, assuming an equal number of cases (D = 0.10).

$$Q = 1 - P$$

$$N = \frac{1.96^2 \times 0.5 (1 - 0.5)}{0.10^2}$$

N = 96.04

2.5 Research Design

This is a cross sectional investigation to study evidence of overt and subclinical thyroid disorders especially hypothyroidism among HIV positive subjects compared with HIV negative controls attending Immaculate Heart Hospital, Umunze, Nigeria.

2.6 Laboratory Analysis

Electrochemiluminescence assay procedure was used for the determination of serum thyroid stimulating hormone [TSH], thyroxine [T_4], triiodothyronine [T_3], free thyroxine [fT_4] and free triiodothyronine [fT_3].

2.7 Statistical Analysis

Data generated from this study was collated and analyzed using Statistical Package for Social Sciences Version 20. Frequency and percentages was used for distributions of variables. Chi-square and Fisher's exact was used to analyze dependent variable across the independent variables. Post hoc analysis involved pair wise comparisons using the z-test of two proportions was done after statistical significant chi-square of Fisher exact analysis. The choice of fisher's exact was based on the assumption minimum expected frequency is violated in choosing Pearson chi-square.T-test was used to analyze mean difference for two groups and One-way analysis of variance (anova) was used for groups more than two. Significant differences were considered at p-value less than 0.05. Post-hoc analysis for statistical significant comparison was done in anova, the result of post-hocs were express in superscript within the tables. Values with same superscript are not significantly different at p-value of 0.05. The categorical outcome was used as cross-tabulation against serostatus category to study the prevalence of hormonal dysfunction across the group of the serostatus.

3. RESULTS

The results obtained in this study are presented in Tables 1 to 5 and Figs. 1 to 2.

Table 1 shows the demographic and clinical characteristics of the study population such as gender, age group, HIV Status, HAART Status and HAART Duration. The number of subjects for the total study is 125, with 95 (76%) being seropositive which include 48 (38.4%) patients who were on HAART and 47 (37.6%) not on HAART; and 30 (24%) were seronegative individuals that served as control. 84(67.2%) were female and 41 (32.8%) were male. Based on HAART the population was grouped into three; those on haart - group 1, haart naïve – group 2 and controls – group 3. According to their age, the study population was into four; 30years below (32), 31-40years (64), 41-50years (18), 51-60years (11).

Table 2 shows the mean levels of various thyroid hormones between the HIV positive and HIV negative subjects was compared. The mean levels of TSH, T_4, T_3 and fT_3 were higher in HIV positive patients than the HIV negative subjects, while the level of fT_4 (25.1±7.5) in HIV positive patients was lower compared to HIV negative subjects (42.5±8.4). T-test showed that there was a significant ($p<0.05$) difference between the serostatus in the various thyroid hormone level except for fT_3 where the difference between the mean was statistically not significant ($p>0.05$).

Table 3 shows the average thyroid hormone level across three groups based on HAART administration (those on HAART, Those not on HAART and the control subjects). The results obtained shows that the level of TSH and fT_3 was significantly ($p<0.05$) higher in group 1 patients than group 2 and 3. The level of T_4 was significantly higher in group 2 than group 1 and 3 patients. The level of T_3 was significantly lower in group 3 subjects than group 1 and 2 patients.

Table 1. Demographic, clinical characteristics and the sero status category of the study population

Variables	Group	Frequency	Percentage	Mean±SD
Gender	Female	84	67.2	
	Male	41	32.8	
		125	100	
Age group	30 below	32	25.6	27.0±3.7
	31-40	64	51.2	36.1±2.7
	41-50	18	14.4	45.9±3.2
	51-60	11	8.8	57.0±1.9
HIV Status	Positive	95	76	
Control	Negative	30	24	
HAART Status	On HAART	48	38.4	
	Not On HAART	47	37.6	
	Control	30	24	
	Total	125	100	
Duration	1-2yrs	14	29.8	
	3-4yrs	23	48.9	
	5-6yrs	10	21.3	
	Total	47	100	

Table 2. level of the thyroid hormone in seropositive and seronegative persons

	Serostatus			
Variables	**Positive (n=95)**	**Negative (n=30)**	**t-value**	**p-value**
TSH	3.3±2.7	2±1.2	3.63	<0.001
T_4	10.2±2.1	8.6±1.5	3.68	<0.001
T_3	2.3±0.8	1.4±0.5	5.72	<0.001
fT_4	25.1±7.5	42.5±8.4	10.83	<0.001
fT_3	3±1	2.7±0.9	1.46	0.14

There is a significant difference ($p<0.05$) as shown in table 4 in the distribution of TSH, fT_4 and fT_3 dysfunction across the group. The TSH dysfunction was significantly lower in group 3 when compared to the patients in group 1 but not significant with patients in group 2. The prevalence of fT_4 dysfunction across the groups was significantly different from each other. fT_4 dysfunction was significantly higher in group 3 when compared to group 1 patients but not significant with patients in group 2. The prevalence of fT_3 dysfunction in patients on HAART was significantly higher than those not on HAART, but there was no significant difference in the prevalence of fT_3 dysfunction between group 1 and 3 patients.

Thyroid dysfunction across the duration on HAART for the patients on HAART as seen in table 5 shows that hormonal dysfunction was statistically not significant $p>0.05$.

In Fig. 1 the area under the curve (AUC) of the ROC quantifies the ability of the test to correctly classify the seropositive and the controls. The curve showed the AUC of all the biochemical markers against the Serostatus (patient and control). T_3 have the highest AUC which can be said to have the best validity test for serostatus among others with statistical significant AUC values of 0.83 followed by T_4 (0.74). Other variables have poor diagnostic validity value.

The AUC of the T_3 is 0.83 with 95% confidence interval of (0.75 - 0.91). This indicates that we would expect minimum 83% of the seropositive patient to be correctly identified by the by T_3. And the best cutoff point of T_3 that maximizes (sensitivity + specificity) is 1.5. The cutoff point has 92.6% true positive rate and 60% false positive rate.

The AUC of T_4 is 0.74 with 95% confidence interval of 0.64 to 0.84. This indicates that we would expect 74% of the seropositive to be correctly identified by the by T_4. And the best cutoff point of T_4 that maximizes (sensitivity + specificity) is 7.50. The cutoff point has 85.2% true positive rate and 30% false positive rate (1-specificity).

In Fig. 2 the area under the curve (AUC) of the ROC quantifies the ability of the test to correctly classify the HAART and Non-HAART patients. The curve showed the AUC of all the biochemical markers against the HIV HAART status (on HAART, and Non-HAART). The marker with highest AUC value was fT_4 (0.66) followed by TSH (0.63). The AUC values of the parameters are not good validity test to distinguish HIV subjects on HAART and those that are not.

Table 3. Thyroid Hormone level across the groups

Variables	On HAART	Not On HAART	Control	F-value	p-value
TSH	4±3.1[a]	2.5±2[b]	2±1.2[b]	8.61	<0.001
T_4	8.9±2.4[a]	11.4±0.5[b]	8.6±1.5[a]	35.31	<0.001
T_3	2.2±1.1[a]	2.4±0.5[a]	1.4±0.5[b]	17.49	<0.001
fT_4	27±8.5[a]	23.1±5.8[b]	42.5±8.4[c]	64.27	<0.001
fT_3	3.2±1.2[a]	2.8±0.7[b]	2.7±0.9[b]	3.851	0.024

a,b,c value with different superscript are significantly different from one another at p<0.05

Table 4. Distribution of Hormonal dysfunction across the groups

Variables	No	On HAART (%)	Not On HAART (%)	Control (%)	p-value
TSH	Normal	36 (75.0)	42 (89.4)	29 (96.7)	0.02
	Abnormal	12 (25.0)[a]	5 (10.6)[a,b]	1 (3.3)[b]	
T_4	Normal	46 (95.8)	47 (100)	30 (100)	0.34
	Abnormal	2 (4.2)	0 (0)	0 (0)	
fT_4	Normal	16 (33.3)	28 (59.6)	0 (0)	<0.001
	Abnormal	32 (66.7)[a]	19 (40.4)[b]	30 (100)[c]	
fT_3	Normal	41 (85.4)	47 (100.)	27 (90.0)	0.01
	Abnormal	7 (14.6)[a]	0 (0)[b]	3 (10.0)[a,b]	
	Total	48 (100)	47 (100)		

a,b,c value with different superscript across the row are significantly different from one another at p<0.05

Table 5. Distribution of thyroid hormone dysfunction across the duration of HAART for the patients on HAART

Variables	Group	1-2yrs	3-4yrs	5-6yrs	p-value
TSH	Normal	8 (57.1)	19 (82.6)	8(80.0)	0.21
	Abnormal	6 (42.9)	4 (17.4)	2(20.0)	
T_4	Normal	13 (92.9)	22 (95.7)	10(100)	1.00
	Abnormal	1 (7.1)	1 (4.3)	0 (0)	
fT_4	Normal	6 (42.9)	7 (30.4)	2 (20.0)	0.46
	Abnormal	8 (57.1)	16 (69.6)	8 (80.0)	
fT_3	Normal	11 (78.6)	19 (82.6)	10(100)	0.43
	Abnormal	3 (21.4)	4 (17.4)	0 (0)	
	Total	14 (100)	23 (100)	10(100)	

Table 6. Standard error of the variables for serostatus with p-value

Variables	AUC	Std. Error	p-value	95% CI (Lower-Upper)
TSH	0.61	0.05	0.06	0.52 - 0.71
T_4	0.74	0.05	<0.001	0.64 - 0.84
T_3	0.83	0.04	<0.001	0.75 - 0.91
fT_4	0.06	0.02	<0.001	0.02 - 0.10
fT_3	0.57	0.06	0.26	0.45 - 0.69

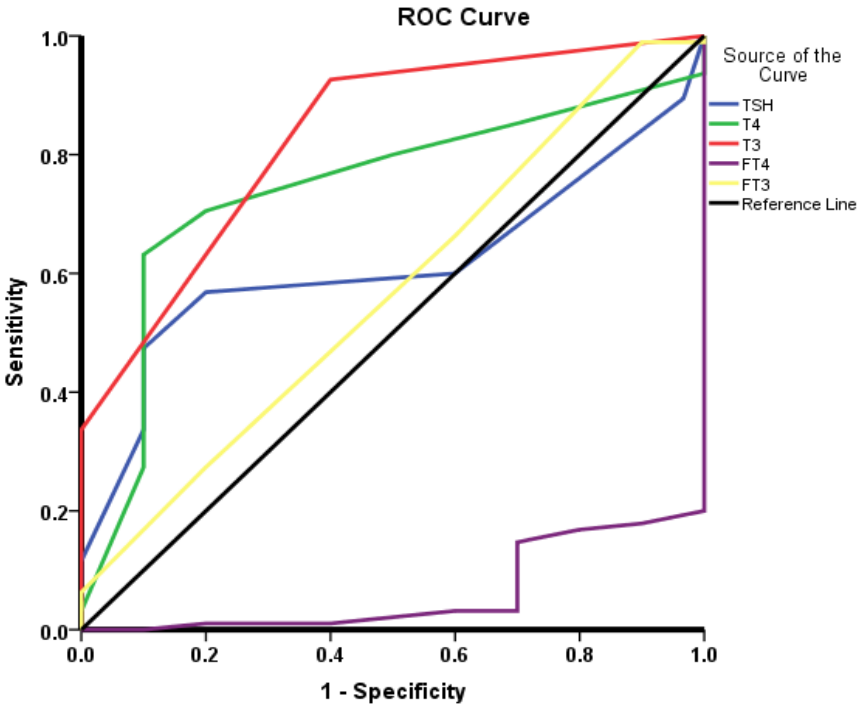

Fig. 1. Measure of diagnostic ability of the variables for serostatus using receiver operating characteristic (ROC) curve

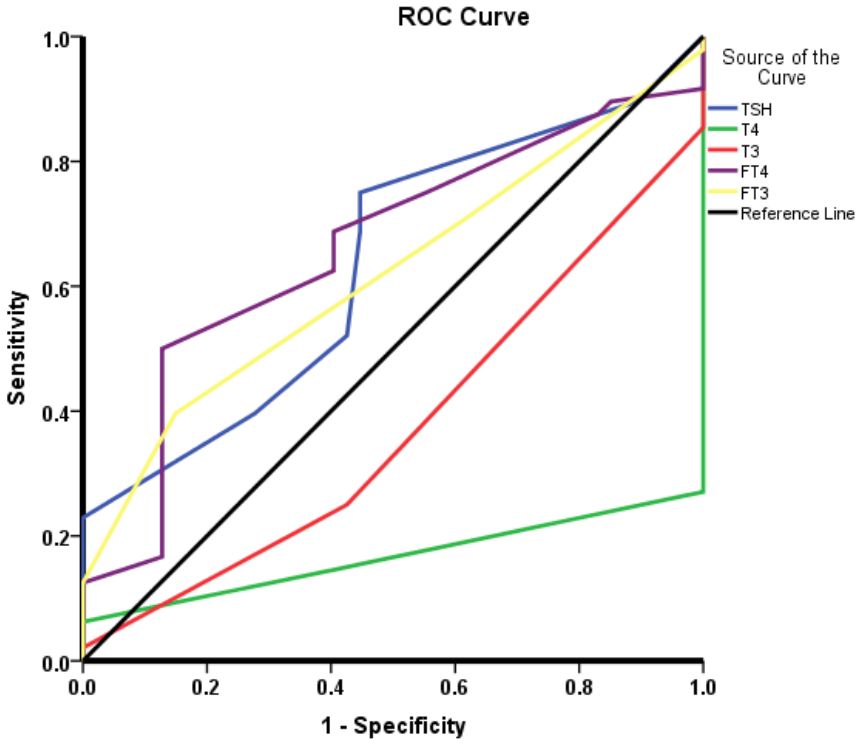

Fig. 2. Measure of diagnostic ability of the variables for haart administration using receiver operating characteristic (ROC) curve

Table 7. Standard error of the variables for haart administration with p-value

Variables	AUC	Std. Error	p-value	95% CI (Lower-Upper)
TSH	0.636	0.057	0.02	0.52 - 0.75
T_4	0.166	0.047	<0.001	0.07 - 0.26
T_3	0.375	0.057	0.04	0.26 - 0.49
fT_4	0.66	0.057	0.007	0.55 - 0.77
fT_3	0.62	0.058	0.04	0.51 - 0.73

4. DISCUSSION

This study aims at evaluation of thyroid function parameters and to determine the relationship between thyroid hormone levels in HIV and HAART duration. There are three groups on the basis of HAART – group 1 and 2 for those on HAART and naïve subjects respectively while group 3 are the negative control subjects.

The subjects in this study were also grouped into four based on age - 30years and below, between 31 and 40years, between 41 and 50years and between 51 and 60years. Their mean ages are 27.0±3.7, 36.1±2.7, 45.9±3 and 57.0±1.9 respectively. On the basis of duration of HIV, there are three groups - between 1-2yrs (29.8%), 3-4yrs (48.9%), and 5-6yrs (21.3%).

The study population according to the data generated in this study shows that more than 50% of the study population were young subjects aged 31 to 40years. This is similar to reports from two studies conducted in Osun and Enugu states were subjects aged 30-39years were found to have the highest percentage [58% and 40.9% respectively]. Mean age of the two study populations also supported the fact that many of the subjects were young individuals. This finding is also consistent with higher prevalence of HIV infection seen in the reproductive age group [15-49 years] compared to other age groups" [5,6]. Majority of the subjects were found to be females, suggesting that females were twice more likely to have HIV infection than males.

"Higher prevalence in females compared to males were also found in some studies carried out in the central and southern parts of Nigeria" [7,8]. "This is however contrary to what were found in some studies carried out in foreign countries where men dominated more than half of the study population" [9]. "The reason for the disparity may partly be due to increased homosexuality practice outside Nigeria" [CDC 2015]. Gender disparities in this study may also be due to the cultural practices in our various communities, which gives the men right to marry more than one wife. By so doing an infected husband can easily infect all his wives. Natural events which give females more opportunity to be screened than their male counter parts e.g. during antenatal care, child birth, child care, and immunization can also be a factor.

The mean levels of TSH, T_4, T_3 and fT_3 were found to be higher group 1 and 2 than the group 3 subjects. This is in contrast to what was reported in [10] but in agreement with [11] in India were fT_3 was lower with higher fT_4 and TSH among subjects with HIV compared with controls. In Ibadan Southwestern Nigeria, [12] also found higher TSH levels among HIV patients compared with controls. "It has been shown that abnormal thyroid function is not uncommon in HIV and there may be a number of contributory factors" [13,14,15]. However, the level of fT_4 in HIV positive was lower than HIV negative compared to fT_3 that is still within the normal reference range. Statistically using independent sample t-test there is a significant difference between the serostatus in the various thyroid hormone levels except for fT_3 that is not statistically significant.

This study shows increased level of TSH and fT_3 which is significantly high in group 1 subjects than the group 2 and 3 subjects. The increased level of TSH and fT_3 is also observed in Verma R.K et al., [16] and [17] studies in which thyroid dysfunction was significantly more frequent in the HAART group 1 than in group 2. Also T_4 is significantly higher on group 2 subjects than the group 1 and 3 subjects. T_3 is significantly lower in group 3 subjects than group 1 and 2 subjects. Therefore, TSH and fT_3 can be used as marker for the progression of thyroid abnormality in HIV infection.

There is a high significant TSH dysfunction in group 3 subjects compared to subjects in group 1 but not with group 2 subjects in the distribution of TSH, fT_4 and fT_3 dysfunction across the group. Prevalence of fT_4 dysfunction across the groups is significantly different from each other. There is no significant difference in the prevalence of fT_3 dysfunction between group 2 and 3 subjects. Across the duration for subjects on HAART which are grouped into 1-2yrs, 3-4yrs and 5-6yrs,

statistically there is no significant difference on the thyroid hormone dysfunction which could be as a result

The commonest pattern of abnormal thyroid function tests among subjects in this study was primary hypothyroidism, followed by isolated low fT4. Among the controls the most common thyroid dysfunction was subclinical hypothyroidism. Similar findings were reported by Ketsamathi et al. [18] in Bangkok. Studies carried out by Uloko *et al.,* [19] reported primary hypothyroidism as the most frequent thyroid abnormality among their study population. However, [13] in Toronto, Canada and [1] in Rio de Janeiro, Brazil, reported subclinical hypothyroidism as the commonest pattern of thyroid dysfunction among their subjects. The longer duration of HIV infection among subjects in those studies and the fact that many of the patients were not on HAART may explain the difference. Some studies have reported association between HAART use and overt hypothyroidism Uloko *et al* [19]. The isolated fT4 found in this study, were also reported by Rasoolinejad et al. [20] in Tehran, Iran and Abbiyesuku F.M et al [18] in Ibadan, Nigeria as the most common thyroid dysfunction among their subjects. This abnormality could be due to sick euthyroid syndrome in the setting of advanced HIV infection [21,22]. They could also be due to clinical and subclinical opportunistic infection.

5. CONCLUSION

Abnormal thyroid function test is common in people living with HIV. However, there is significant difference between serostatus in the various thyroid hormone levels except for fT_3 where the difference between the mean is statistically not significant. The commonest thyroid abnormality seen in this study is primary hypothyroidism among the HIV positive patients followed by isolated low fT_4. In the control subjects, the commonest thyroid dysfunction seen was subclinical hypothyroidism. The prevalence of fT_4 dysfunction across the groups is significantly different from each other with the prevalence of fT_3 dysfunction in patients on HAART. However, there is no significant difference in the prevalence of fT_3 dysfunction between control and HAART naïve subjects. Larger studies are required to determine the progression of thyroid dysfunction in HIV patients over time.

CONSENT

As per international or university standard, participants' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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