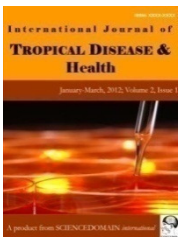

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Serum Iron Status and Haematological Profiles in Adult Nigerian Sickle Cell Anaemia Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAO source and wrote the proposal for the grant. Authors JAO, KSA, ADA and OGA designed the study, Authors JAO and OGA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KSA and ADA managed the analyses of the study. Author ADA coordinated sample collection while authors JAO and OGA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell anaemia (SCA) patients are prone to require long-term frequent blood transfusion because of chronic haemolysis and overlapping hyper-haemolysis. Hence, they become vulnerable to iron overload and its complications. On the other hand, iron deficiency has been documented among un-transfused SCA cases. Thus, there is a need to effectively and efficiently determine iron status of SCA patients.
Objective: We investigated whether adult SCA patients in steady state (SSt) or those with vaso-occlusive crisis (VOC) have significantly different iron status viz-a-viz Serum Iron (SI), Serum Ferritin (SF), Total Iron Binding Capacity (TIBC), Transferrin (TRF), percentage TFS and haematological parameters when compared with age and sex-matched controls.
Materials and Methods: Ninety subjects, comprising 30 SCA patients in SSt, 30 SCA patients with VOC and 30 HbAA, ethnicity, age and sex-matched controls (NC), were consecutively recruited. Serum samples were analysed for SF, TRF and TIBC using

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'WATER' HPLC 616 and 626; SI was determined using the Atomic Absorption Spectroscopic (AAS) method. Haematological parameters were determined using a Sysmex Kx21 auto-analyser.
Results: The SCA groups (VOC and SSt) had significantly lower SI, SF, TRF and TIBC compared to the control group. The VOC group had a significantly lower mean ferritin level but higher SI, TRF and percentage TFS levels compared to SSt group. The MCV, MCH (in SSt group) were significantly lower while MCHC was significantly higher in the SCA groups compared to the NC group. Using the normal ranges for all parameters, all parameters were within normal for the controls while TIBC was below normal and percentage TFS was higher in SCA groups. Percentage TFS was significantly higher in VOC compared to SSt group.
Conclusions: The study reported lower SI and TRF, lower MCV and MCH, below normal TIBC, within normal Ferritin but elevated WBC and platelet counts, elevated percentage TFS (more elevated in VOC than SSt) and higher MCHC in SCA patients. The use of percentage TFS as a marker of VOC is suggested.

Keywords: Adult Sickle Cell Anaemia; Vaso-occlusive; Steady state; Ferritin; Serum Iron; Haemolytic process.

1. INTRODUCTION

Sickle cell Anaemia (SCA) is a widespread genetic disorder characterized by red blood cells (RBCs) deformity to abnormal rigid and sickle shaped forms that result in chronic haemolytic anaemia, infarctive crisis, frequent infections and risks of serious complications. It occurs in high frequency in many tropical countries. The prevalence of sickle-cell disease is highest in sub-Saharan Africa with Nigeria having the highest burden. Approximately, 2% of the Nigerian population is affected by SCA [1,2]. The genetic abnormality is a mutation in position 6 of the beta (β) chain of haemoglobin (Hgb) where valine replaces glutamic acid [βs (6V)]. The homozygote state [βs (6V)/βs (6V)] known as SCA appears more severe than the heterozygote state [βs (6V) plus another abnormal Hgb feature, such as "C", "E", thalassemia etc.]. The clinical features of SCA begin in early childhood, deteriorating the quality of life with advancing age and also reducing life expectancy [3,4]. SCA patients suffer from various complications as a result of VOC such as hand-foot syndrome, stroke, acute chest pain, femoral head necrosis auto-splenectomy, hepatomegaly, priapism, renal failure, and heart failure and leg ulcers [5]. Severe bone pain crisis and hyperhaemolytic crisis, more often precipitated by malaria are the most common indications for periodic hospital admissions in SCA patients.

Physiologically, the human body effectively conserves iron, with loss being restricted to 1-2mg per day. In conditions such as SCA, where long term blood transfusions may be indicated, exogenous iron can accumulate, circulate as non-transferrin bound iron (NTBI), enter tissues, form reactive oxygen species (ROS) and finally result in organ damage [6]. Hence, iron overload is a presumed and unavoidable complication of multiple-transfused SCA patients.

Iron deficiency anaemia (IDA), on the other hand, is common amongst Nigerians due to low dietary intake, mal-absorption, excessive sweating and undue blood loss [7]. A study documented a prevalence of 12% of IDA among Nigerian blood donors [7]. However, patients with hereditary anaemias like SCA and thalassemia are not expected to develop IDA since recycling of iron from premature destruction of erythrocytes contributes to iron

stores [8,9]. However, contrary to this expectation, a report from USA suggested that iron deficiency was more common than expected in un-transfused SCA cases, and it is presumed that iron deficiency will be prevalent in SCA patients in tropical countries [9].

A SCA patient is prone to iron overload as an inevitable complication of episodic and/or chronic blood transfusion, but at the same time the patient is not immune to inherent and environmental factors that precipitate iron deficiency anaemia (IDA). Such factors, especially in the tropics, include poor nutrition, parasitic infestations like hookworm and schistosomiasis as well as varying bacterial infections that may disturb iron metabolism. In addition, poor absorption and metabolism of iron resultant from multiple mucosal/sub-mucosal infarcts and progressive multiple organ damage/failure, to which SCA patients are particularly highly susceptible to, are other factors that can precipitate iron deficiency anaemia.

Serum ferritin, serum iron and transferrin are known as acute phase reactants that are affected by both inflammation and infection. Hence, interpretations of these serum proteins may be difficult in SCA patients whose pathology is underlined by chronic inflammatory state and overlapping acute inflammatory events precipitated by infections. However, circulating ferritin has been identified as an indicator of iron status in the body, hence the need for its inclusion.

There are conflicting information in respect of published data on the iron profiles in SCA patients in tropical regions where poverty, under-nutrition, hookworm infestation and undue blood loss alter iron status. Whereas, the findings of Usanga et al. [10] of low iron stores among blood donors strongly corroborated this assertion. However, Aken'Ova et al. [11] concluded that SCA patients have adequate levels of iron and ferritin in their serum.

Therefore, the present study investigated whether adult SCA patients in steady state (SSt) or in vaso-occlusive crisis (VOC) have significantly different haematological parameters and iron status viz a viz serum iron, TIBC, Ferritin, Transferrin and percentage TFS) when compared with age and sex- matched control group (NC). This will serve to confirm the suspicion of iron overload or iron deficiency anaemia in this area of study.

2. MATERIALS AND METHODS

Ethical approval was obtained from the University of Ibadan/University College Hospital [UI/UCH] ethical board and written informed consent (attached to every questionnaire completed by each participant) was obtained from individual patients prior to recruitment into the study.

2.1 Study Design

This was a prospective, case controlled study, carried out in the Departments of Haematology and Chemical Pathology, University of Ibadan. A total of 90 subjects (18 – 40 years) comprising 60 subjects with sickle cell anaemia (SCA) and 30 apparently healthy individuals with HbAA genotype were recruited into this study. The SCA subjects were recruited from the Haematology Day Care Unit (HDCU), University College hospital, Ibadan and comprised 30 subjects in steady state and 30 subjects in vaso-occlusive crisis (VOC) [Table 1]. The SCA patients were those being followed up regularly at our Day-care and Outpatient clinics.

Inclusion criteria included only known HbS patients, aged between 18-40 years, attending clinic regularly. The subjects in steady state were those without crises and devoid of symptoms or signs attributable to acute illness in the preceding three months[10].Exclusion Criteria included refusal to sign consent, patients who were transfusion dependent and those who received transfusion in the last three months(for steady state only). The inclusion criteria for the controls were the same as for subjects except that the haemoglobin genotype was AA and they had no symptoms or signs attributable to acute illness in the preceding three months. Also, the exclusion criteria for the controls were the same as for subjects except that the haemoglobin genotype was AA and they had not received iron supplementation within three months prior to recruitment.

Record of previous blood transfusion obtained through completed questionnaire, revealed transfusion loads of 1–2 units in 90% and 3–6 units in 10% of the SCA patients.

Table 1. Mean age, sex and BMI of study groups

Group	Age (years)	Male	Female	BMI
Control (n=30)	26.1±4.8	14	16	22.5±2.6
HbS (SS) (n=30)	27.1±6.3	18	12	20.8±3.1
HbS (VOC) (n=30)	24.9±4.9	13	17	22.3±8.9

2.2 Blood Sample Collection

Approximately 6mls of venous blood was collected from each participant. 2mls of blood sample from each participant was put into an EDTA bottle, and this was used for the analysis of haematologic parameters using a Sysmex Kx21 auto-analyser [made in China]. The remaining 4mls of blood sample from each participant was put in iron-free plain bottles. The blood samples in the iron free plain bottles were allowed to clot. The serum sample separated from it was put into an aliquot and stored at -80ºC until analysed for iron studies.

2.3 Diagnostic Method

Serum iron was determined using the Atomic Absorption Spectroscopic method (AAS): Serum iron was measured by Ferrozine/ Magnesium carbonate method using the fully automated biochemistry I-Lab 650i auto-analyser [12]. Serum Ferritin, Transferrin and TIBC were analysed using High Performance Liquid Chromatography [HPLC] Water 616/626, which was controlled using working standards from the certified Reference Standards (1000 ppm) [13]. Ferritin, transferrin and TIBC were analysed using 249nm, 237nm and 228nm wavelengths respectively. The sensitivity and specificity of HPLC were 87.5% and 97.42% respectively while its positive and negative predictive values were 41.18 and 99.74 respectively.

2.4 Haematological Analysis

The procedure followed was based on the instruction manual of the Haematology Analyser (Sysmex Kx21). Total red blood cell (RBC) count (x 10^6/uL), haemoglobin content (Hb; g/dL), haematocrit (HCT; %), total number of white blood cells (WBC) or leukocytes (x 10^3/uL), lymphocyte count (LYM; x 10^3/uL), lymphocyte percentage (LYM; %), and platelet (PLT) count (x 10^3/uL) were assessed. Mean corpuscular volume (MCV; fL), mean corpuscular haemoglobin (MCH; pg), mean corpuscular haemoglobin concentration (MCHC; %), red

blood cell distribution width (RDW; Fl), platelet distribution width (PDW; Fl), mean platelet volume (MPV; Fl) and platelet larger cell ratio (P-LCR; %) were also calculated.

2.5 Statistical Analysis

The data were presented in Mean ± Standard Deviation [×±SD]. Student t test was used to determine the differences between the means by using Statistical Package for Social Sciences (SPSS) version 15. $P < 0.05$ was considered significant.

3. RESULTS

The demographic pattern of the subjects is as shown in Table 1. The 90 subjects i.e. 30 HbS in steady state (SSt), 30 HbS in Vaso-occlusive crisis (VOC) and 30 HbA as normal controls (NC) demonstrated close mean age, sex distribution and BMI.

According to Table 2 the SCA groups (VOC and SSt) had significantly ($p = .00$) lower serum Iron, Ferritin, TIBC and transferrin than the control group. The %TFS was significantly higher in VOC vs CC ($p = .00$) as well as in VOC vs SS group ($p = .03$). The SCA in the VOC group had comparable TIBC but significantly lower Ferritin ($p = .00$) and transferrin ($p = .02$). However, SI and % TFS were significantly higher in VOC group compared to SS group ($p = .00$ and $p = .02$, respectively).

According to Table 3 In comparing the SCA groups with the Control (NC), the mean values of Hct and Hgb were significantly lower ($p = .00$), the Platelet and the WBC were significantly elevated ($p = .00$) in SCA patients groups. The MCV was lower in VOC group and in SSt group ($p = 0.00$ and 0.05 respectively) while MCH was significantly reduced ($p = .03$) in the SSt group only. The MCHC mean values were comparable in the SCA groups but were significantly higher in the SCA groups compared to control (NC)($p = .00$)

The comparison of the VOC group with the SSt group showed that the mean values of PCV and Hgb in the two groups did not show any significant difference ($p = .09$ and $p = 0.17$ respectively). However, the mean WBC was significantly elevated in the VOC group (p=0-000) and the mean platelet count was significantly elevated in the SSt group ($p = .00$).Whereas, the MCH and the MCHC did not show any significant difference, the MCV was significantly higher in the VOC group.

According to Table 4 TIBC was below normal range (TIBC = 240-450mcg/dl) in all subjects in VOC and SSt groups. The mean serum iron was within normal range (Serum Iron = 50-175mcg/dl) in VOC and SSt groups. The ferritin and transferrin levels were within normal in most subjects in VOC (96.7% and 93.3% respectively) and in Steady State (SSt) (100%) and 76-7% in the NC group. The percentage transferrin saturation was above normal (%TFS = 20-50%) in 22(73.3%) in VOC group but completely within normal in the SSt group.

All subjects in control group had normal TIBC, ferritin, transferrin and iron within normal ranges except for 1(3.3%) and 11(36.3%) had iron and percentage saturation respectively above normal reference ranges.

Table 2. Shows the comparison of the mean values of SI, SF, TIBC, TRF and %TFS in the study groups

HbSS	VOC (×±SD) N=30	SSt (×±SD) N=30	NC (×±SD) N=30	VOCvs SSt P-value (P =.--)	VOCvs NC P-value (P =.--)	SSvsNC P-value (P =.--)
TIBC(mcg/dl)	206.44±13.50	201.10±15.78	318.44±25.47	.17	.00*	.00*
Ferritin (ng/ml)	73.67±7.00	108.40±50.40	216.12±14.55	.00*	.00*	.00*
Transferrin (mg/ml)	224.38±11.12	216.50±15.00	301.34±31.99	.02*	.00*	.00*
Serum Iron (umol/L)	110.44±19.56	76.52±4.88	155.19±9.7	.00*	.00*	.00*
%TFS	55.72±10.54	38.34±4.41	49.03±4.95	.00*	.00*	.03*

NB: P-value< .05 is considered significant

Table 3. Mean values of Haematological parameters in SCA patients (SS vs VOC) and Controls (CC)

HbSS	VOC ×±SD	SSt ×±SD	NC ×±SD	VOCvs SSt p-value (p = --)	VOCvsNC p-value (p = --)	SStvsNC p-value (p = --)
PCV (%)	24.2±5.8	21.7±4.5	40.1±3.5	.09	.00*	.00*
WBCx10^9/mm3	14.0±4.7	10.7±3.9	5.3±1.5	.00*	.00*	.00*
PLT x10^9/mm3	266.8±137.3	359.7±141.6	204.5±48.1	.00*	.00*	.00*
Hgb (g/dl)	8.3±1.5	7.7±1.7	12.9±1.2	.17	.00*	.00*
MCV(FL)	80.8±10.8	74±10.3	86.7±12.4	.00*	.00*	.05*
MCH (pg/dl)	29.8±7.9	26.8±4.2	28.9±3.3	.04	.03*	.88
MCHC (g/dl)	35.5±3.2	35.8±2.1	31.9±1.6	.82	.00*	.00*

NB: p-value< .05 is considered significant

Table 4. Showing proportion of subjects with determined levels of TIBC, serum ferritin, iron, transferrin and transferrin saturation (%TFS) that were within, below or above the stipulated normal ranges

	VOC			SSt			NC		
	Below	Within	Above	Below	Within	Above	Below	Within	Above
TIBC mcg/dl	30(100%)	_	_	30(100%)	_	_	_	30(100%)	_
Ferritin ng/dl	_	29(96.7%)	1(3.3%)	_	30(100%)	_	_	30(100%)	_
Iron mcg/dl	_	30(100%)	_	_	30(100%)	_	_	29(96.7%)	1(3.3%)
Transferrin g/dl	1(3.3%)	28(93.3%)	1(3.3%)	2(6.7%)	23(76.7%)	5(16.7%)	_	30(100%)	_
%TFS	-	8(26.7%)	22(73.3%)	_	30(100%)	_	_	19(63.3%)	11(36%)

Normal ranges: -%TFS=20-50%; TIBC = 240-450mcg/dl; Ferritin=12-300ng/dl; Serum Iron=50-175mcg/dl; Transferrin=200-400mg/dl

4. DISCUSSION

Iron deficiency, complicating SCD, is likely to worsen the clinical state of the disease since iron plays a central role in erythropoiesis and many other intracellular processes in all the tissues of the body [14]. It is also a universal cofactor for mitochondrial energy generation and supports the growth and differentiation of all cell types.

Diagnosis of iron deficiency in most patients, sickle anaemia inclusive, can be based on the measurement of a low serum iron and low serum ferritin with an elevated TIBC [15]. Serum ferritin and stainable iron in tissue stores decrease even in early stages of iron deficiency, as iron stores become depleted [15]. Other parameters such as Transferrin saturation (TSAT) and free erythrocyte protoporphyrin do not reach abnormal levels until tissue iron stores are completely depleted [15]. Only when iron stores are insufficient for haem synthesis (i.e. iron Deficiency Anaema (IDA)) do haemoglobin levels and red cell indices begin to decrease. In line with these and in accordance with findings (Table 4) of this study; although TIBC levels were below normal for SCA in steady state and in vaso-occlusive crisis and serum ferritin, as well as, transferrin levels were predominantly within normal; the percentage transferrin saturation, which indicates iron that is immediately available for erythropoiesis, was elevated in VOC group. This might be indicative of tissue iron depletion necessitating over saturation of transferrin receptor sites in order to provide iron for enhanced erythropoiesis in SCA in VOC group that had escalated haemolysis. On the contrary, the SCA in steady state displayed a grossly normal percentage TFS as a reflection of absence of exaggerated haemolytic process. Their serum ferritin remained within normal probably because ferritin is equally an acute phase reactant whose level increases in the face of infection and inflammation.

This index study showed that adult SCA subjects had a lower mean serum iron concentration than the controls. This finding is consistent with that of Jeyakumar et al. [16] and Aken'Ova et al. [11]. Since serum iron concentration represents a balance between intake, utilization and excretion, hence chronic haemolytic/ hyperhaemolytic process with constant release and mobilization of iron may explain the lower mean serum iron in SCA groups. It is however noteworthy that the mean serum iron was significantly increased in VOC group compared to SSt group because of escalated haemolysis in this group. Therefore, increased iron turnover may be a more plausible explanation than the one submitted by Koduri et al. [17] stating that one-third of the haemolysis in sickle cell anaemia subjects takes place in the intravascular space and is associated with excessive urinary loss of iron.

The mean serum ferritin was significantly lower among SCA compared to the control group Table 2). It was also significantly lower in the VOC group than the SSt group. Since serum ferritin is known to reflect mainly reticulo-endothelial iron stores [18]. It is considered to be a sensitive indicator of body iron stores and thus lower serum ferritin concentrations may reflect possible low body store. The lower body stores of ferritin among SCA groups especially, might be explained by increased mobilization and utilization of ferritin-iron from the stores to erythropoietic precursors in the bone marrow to meet increased demand for new red blood cells production in SCA patients. This explanation is buttressed by a significantly lower mean serum ferritin level in VOC group compared to the SSt group.

In the present study, low serum ferritin level was accompanied by reduced serum iron in sickle cell anaemia subjects. The reason for this pattern might be connected with the fact that SCD is not only underlined by chronic inflammatory state [19], it is also characterized by

chronic haemolytic/ hyperhaemolytic process. Therefore, chronic inflammation and repeated infections enhance hepcidin production [20,21]. It has been established that low serum ferritin (<12ng/dl) is virtually diagnostic of iron deficiency [22], using known normal range for ferritin, approximately 95% of SCA patients in this index study fell within normal range. Although serum ferritin can serve as surrogate marker for iron stores but it is also an acute-phase reactant and its levels can be elevated under conditions of chronic inflammation, liver diseases or malignancy, independent of iron status [23]. Therefore an elevated serum ferritin level does not necessarily imply that iron stores are adequate.

It seems that the iron profile in these SCA groups depicts Anaemia of chronic disease (ACD) since under these conditions termed ACD; serum iron is expected to be low, iron binding capacity is expected to be low to normal but serum ferritin may be low to high [23,24]. In these present study both serum iron and TIBC were below normal range and serum ferritin was within normal range in approximately 95% of the SCA patients.

Olubuyide et al. in 1983 [25] documented a mean Ferritin level of 296.3±61.9ng/ml in a Nigerian SCA group. The mean serum ferritin levels determined in the present study, of 73.67±7.00 ng/ml in SCA (in VOC) and108.40±50.4 ng/ml in SCA (in SSt), were considerably lower than the one determined over three decades ago. The low transfusion burden of 1–2 units in 90% and 3–6 in 10% may partly explain the difference in the mean levels. In addition, the analytical methods employed may account for the difference. In this case, HPLC was employed in carrying out the analysis.

The mean transferrin levels in the SCA group fell within normal range in 95% of the subjects but the percentage transferrin saturation was above normal range in over 73% of the SCA groups. Transferrin which is an iron transport protein carries iron to the bone marrow. Serum iron levels measure transferring bond iron while TIBC measures the amount of circulating transferring that is available to bind iron [26]. Transferrin saturation represents the ratio of serum iron to the TIBC, (as a measure of circulating transferring) x100. The plausible explanation for the finding of below normal range TIBC, within normal transferrin in 95% together with significantly lower TIBC and Transferrin in SCA groups compared to control might be increased production of transferrin being an acute phase reactant more so in SCA patients who are highly susceptible to recurrent infections.

According to Ontario Association of Medical Diagnostics on guidelines for use of serum tests of iron stores [27], neither iron overload nor iron deficiency anaemia can be categorically confirmed by this study (refer table 4). By this criteria IDA is defined with below normal range ferritin (Ferritin =12-300 ng/dl) (definite of IDA), or less than 40 ng/dl (for possible IDA) or <70 ng/dl in patients with inflammation (where patients with SCA belongs to); along with below normal serum iron (Normal serum Iron = 50-175 mcg/dl) but with elevated/normal TIBC. Therefore, in this index study, serum ferritin and iron were within normal with below normal TIBC (Table 4) and hence the SCA groups cannot be categorized as having IDA. Also, criteria for meeting iron overload (using same guideline) includes elevated serum iron and ferritin with low/normal TIBC along with %TFS >60% (males)/50% (females). Our study showed elevated %TFS in 22(73%) in VOC group only, high ferritin in 1(3.3%) in VOC group and 5(16.5%) in SSt group but with normal serum iron in the rest subjects. Therefore, iron overload cannot be considered.

As regards haematological parameters, the significantly reduced PCV and Hgb in the SCA group compared to the control group in this study confirmed the chronic haemolytic process on-going in SCA patients. In addition, significantly reduced mean MCV and MCH in SCA

compared to control groups correlate with significantly reduced serum iron, serum ferritin, demonstrated in SCA groups compared to the control group. This may indicate iron depletion but co-existence of alpha thalassaemia with SCA may also be accountable. However the thalassaemia status of these SCA patients was not pre-determined. Significantly elevated platelet count and white cell count in SCA patients compared to control group also confirmed chronic inflammation as a contributory pathogenic mechanism in SCA [28]. However, this study showed that platelet and white cells were significantly higher in the steady state SCA group compared to the SCA (VOC) group.

The finding of low iron status in SCA groups compared to the control might also be due to the low transfusion burden in the cohort of SCA patients studied; the tradition of avoiding iron-containing supplement by SCA patients as a means of preventing iron overload; the possibility of poor absorption and metabolism as a result of progressive multiple organ infarct/failure and, finally, poor nutrition as a result of low socio-economic status compounded by the high financial burden of managing the disease.

Limitations of this study may include inability to determine thalassaemia status of SCA patients, non-determination C-reactive proteins, hepcidin, free erythrocyte proptoporphyrin levels and bone assessment for iron store of the SCA patients.

5. CONCLUSION

Interpreting iron parameters in SCD patients remained complex and it is highly modified by chronic inflammatory state and chronic haemolytic/ hyperhaemolytic process. This study showed that iron status is lower in SCA groups. The VOC group had higher %TFS, serum iron, lower TIBC and lower ferritin compared to the steady state group. Therefore, higher %TFS may be a sensitive marker of VOC in sickle cell anaemia patients.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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