

Nocturnal autonomic nervous system activity impairment in sickle cell trait carriers

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Summary

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Sickle cell trait (SCT) is a genetic disease affecting the synthesis of normal haemoglobin (Hb) and marked by the heterozygous presence of HbA and HbS. Some studies have suggested that SCT carriers might be prone to vascular alterations, cardiac ischaemia and arrhythmias leading, in some subjects, to sudden death. It is well known that a loss or a disequilibrium of autonomic activity are powerful predictors of sudden cardiac death. We hypothesized that SCT subjects might exhibit alterations in the activity of the autonomic nervous system that could constitute further risk factors for cardiac complications. Resting haemorheological parameters (η_b , blood viscosity; η_p , plasma viscosity; Hct, haematocrit; Tk, red blood cell rigidity), and sympathetic and parasympathetic indices of nocturnal autonomic activity (temporal and frequency analysis of heart rate variability) were thus compared between a group of nine SCT subjects and a group of nine control subjects. η_b was higher in the SCT group than in the control group while Hct, η_p and Tk were not different. Global variability (SDNN, SDNNIDX) and parasympathetic (PNN50, RMSSD, HF) indices were significantly lower in the SCT group compared with the control group, while the LF/HF ratio was highly increased, underlining a major sympathetic shift. The autonomic imbalance in SCT subjects was mainly related to lowered parasympathetic activity. Thus, our study suggests an additional global decrease and imbalance of autonomic nervous system activity to biological disorders of SCT carriers, that may constitute further risk factors for cardiac complications in this population.

Introduction

Sickle cell disease (SCD) is a genetic disease caused by the mutation of haemoglobin (Hb) A into HbS which mainly affects the people of African descent, because of the substitution of a single amino acid, valine for the glutamine. That affection determines a decrease in erythrocyte deformability and an increase in blood viscosity in response to a decrease in oxygen tension (Brandao *et al.*, 2003). Subjects homozygotes for the mutation (HbSS genotype) are prone to haemolytic anaemia and vaso-occlusive events that lead to the damage of most of the tissues causing painful-crisis and sometimes leading to death (Diop *et al.*, 2003). The most frequent complications are gallstones, femoral head necrosis, priapism, chronic leg ulceration, ophthalmic involvement, renal and myocardial infarctions. Cardiac function is also impaired in resting conditions in patients with SCD (James *et al.*, 1994). On the autonomic side,

Romero Mestre *et al.* (1997) have shown an alteration in 58.3% patients with SCD, and they suggested that this dysfunction could be involved in often reported sudden death.

Sickle cell trait (SCT) is the heterozygous form of the disease. SCT is clinically less severe than SCD and usually asymptomatic. However, SCT has sometimes been associated with splenic infarction, rhabdomyolysis, acute renal failure, cardiomyopathy and sudden death, particularly in condition of dehydration, heat stress, hypoxic states or physical exercise (Kerle & Runkle, 1996; Wirthwein *et al.*, 2001). Sickle cells are less deformable and adhere more to capillary endothelium than normal red blood cells (RBCs) which may cause occlusion and tissue ischaemia (Kerle & Runkle, 1996; Wirthwein *et al.*, 2001). Recently, RBCs from SCT subjects have also been described as poorly deformable in resting conditions, even when blood oxygen level was normal (Brandao *et al.*, 2003; Monchanin *et al.*, 2005). Alterations in blood rheology profile have been found in several pathologies, such

diabetes or peripheral vascular disease, and are known to participate to cardiovascular complications (Dintenfass, 1978; Brun et al., 1998; McHedlishvili & Maeda, 2001).

Estrade et al. (1989) found that subjects with SCT have a normal echocardiographic chamber size and normal left ventricular function in resting condition. Francis & Bleakley (1980) reported that cardiac responses to exercise did not differ between SCT and control subjects. In contrast, Alpert et al. (1982) found that 8.3% of children with SCT had electrocardiographic (ECG) changes during exercise which were suggestive of ischaemia, compared with 2.3% of the control subjects. Pearl et al. (1994), by comparing ECG between SCT carriers and control subjects in resting condition and during exercise both at sea level and at altitude, found significant differences in six ECG variables, the resting ECGs at sea level showing the greatest differences. However, it is difficult to conclude whether SCT is associated, or not, to dysfunction which might increase the risk of sudden death in extreme environmental conditions.

A loss or an imbalance of autonomic nervous system activity, usually assessed through heart rate variability (HRV) measurement, is a powerful and independent predictor of an adverse prognosis in patients with heart disease and in the general population (Task Force, 1996; Stauss, 2003). If altered in SCT subjects, this might contribute to an increased risk of death in that population. We hypothesized that SCT subjects might exhibit alterations in the activity of the autonomic nervous system that could constitute further risk factors for cardiac complications. We compared haemorheological parameters in resting conditions and HRV between a group of subjects with normal Hb and SCT subjects of the same age and physical fitness level.

Methods

Subjects

Nine black African subjects with (SCT group) and nine without (control group) SCT were included in the study after medical examination and after giving their informed consent. The study was approved by the Cameroon Ethics Committee at Yaounde.

To avoid the influence of physical fitness on HRV and haemorheological parameters, as already demonstrated in other studies (Brun et al., 1998; Pichot et al., 2002), all subjects were sportsmen (endurance sports) and had trained regularly (more than 10 h week⁻¹) for more than 10 years but none was cyclist. Subjects were recruited at the National Institute of Youth and Sport in Yaounde (INJS, Cameroon) and were submitted to a complete medical examination before admission. Exclusion criteria included apparent metabolic, muscle, heart, pulmonary diseases and malaria. Subjects with anaemia and/or alpha-thalassaemia were also excluded from the study.

Sickle cell trait detection

Testing for the presence of HbAS was performed by Hb electrophoresis (Dynatech) and identified as <50% HbS, more

than 50% HbA, and normal levels of HbA2 and HbF (Embury et al., 1982; Gozal et al., 1992). In the HbAS group, the range of HbS values was comprised between 33% and 47% (mean: $42 \pm 0.1\%$).

Protocol

All subjects came on the laboratory to determine haemorheological parameters in resting conditions. Then ECG Holter was set on each subject to provide a continuous ECG recording during the following night. Two days after, subjects performed exercise test on cycloergometer to assess physical performance.

Haemorheological measurements

Blood was drawn in EDTA tubes and to avoid RBC damage, haemorheological parameters were immediately determined. Blood viscosity (η_b) and plasma viscosity (η_p) were determined with a cone plane viscometer (Brookfield DVII+, CPE-40 spindle) at high shear rate (375 s⁻¹). Haematocrit (Hct) was assessed using a micro-method after blood microcentrifugation (Jouan Guetin-SA type 316). RBC rigidity index (Tk) was calculated at high shear rate (375 s⁻¹) according to the equation of Dintenfass (1985):

$$\eta_b = \eta_p \times [1 - (\text{Tk} \times \text{Hct})]^{-2.5}.$$

Where η_p , η_b and Hct are blood viscosity (mPa·s⁻¹), plasma viscosity (mPa·s⁻¹) and Hct (%), respectively.

HRV analysis

Holter ECG were analysed on a Stratascan (DelMar Reynolds, Hertford, GB) equipped with an HRV module. The sampling frequency was 128 Hz. Each R-R interval was manually validated before analysis. Only the night periods were analysed (midnight to 7 am) to avoid variations arising from differences in the subject's daily environment (Fortrat et al., 1999). The following indices of HRV were calculated according to the standards previously described in the literature (Task Force, 1996): the percentage of differences between adjacent normal RR intervals more than 50 ms (PNN50), the standard deviation of all normal RR intervals (SDNN), the square root of the mean of the sum of the squared differences between adjacent normal RR intervals (RMSSD), the standard deviation of the mean of all normal RR intervals for 5-min segments (SDANN), and the mean of the standard deviation of all normal RR intervals for all 5-min segments (SDNNIDX). Spectral analysis was performed using fast Fourier transform. The spectral indices were calculated as the mean of the values calculated on successive sets of 256 consecutive RR intervals during the night periods: the total power (Ptot, 0–0.50 Hz), the very low frequency (VLF, 0–0.04 Hz), the low frequency (LF, 0.04–0.15 Hz), the high frequency (HF, 0.15–0.40 Hz), the normalized low [LFnu = $100 \times \text{LF}/(\text{Ptot} - \text{VLF})$] and high frequency [HFnu = $100 \times \text{HF}/(\text{Ptot} - \text{VLF})$] and the LF/HF ratio.

All the calculated indices are recognized to provide a good estimation of the autonomic nervous system activity (Task Force, 1996). Some variables are mainly under the control of parasympathetic activity (PNN50, RMSSD, HF, HFnu) or reflect the global autonomic activity (SDNN, SDNNIDX, SDANN, Ptot). The very low frequency of the spectrum (VLF) contains partially parasympathetic activity, the low frequency indices (LF and LFnu) contain both sympathetic and parasympathetic activities, and the LF/HF ratio has been proposed as a marker for autonomic nervous system balance.

Exercise test

Each subject performed a progressive maximal exercise test on a mechanically braked ergometer (Ergomeca, Toulon, France) to assess physical performance. The test began with a 5-min warm-up at 30 W and then, load was increased by step of 30 W every minute until exhaustion (P_{peak} , maximal power). Pedalling speed remained constant (70 rpm) throughout the test.

Statistical analysis

Results are expressed as mean \pm SD. Anthropometric characteristics (age, weight and height), and P_{peak} were compared between the two groups using an unpaired Student's *t*-test. Haemorheological parameters and HRV data were compared between the two groups using a Mann-Whitney *U*-test (non-parametric test). Mathematical analyses were performed on a Power-Macintosh using MatLab[®] (MathWorks, Sèvres, France). Statistical analyses were performed with Statistica (v. 5.5; Statsoft, Tulsa, Oklahoma, USA). Significance level was defined as $P < 0.05$.

Results

Subjects characteristics and haemorheological parameters

Anthropometric and P_{peak} measures were not significantly different between the SCT group and the control group (Table 1). The SCT group had higher η_b than the control group, while Hct and η_p were similar in both groups (Table 2). The control group tended to have lower Tk values than the SCT group (Table 2, $P < 0.1$).

Table 1 Anthropometric data and maximal exercise power for the two groups during incremental exercise testing.

	Control group (<i>n</i> = 9)	SCT group (<i>n</i> = 9)
Age (years)	27.8 \pm 4.2	29.6 \pm 6.9
Weight (kg)	76.6 \pm 4.5	73.8 \pm 7.8
Height (cm)	177.6 \pm 7.5	176.4 \pm 6.6
P_{peak} (W)	259.0 \pm 42	248.9 \pm 21

Values are mean \pm SD.
SCT, sickle cell trait.

Table 2 Resting haemorheological parameters in control and sickle cell trait (SCT) groups.

	Control group	SCT group
η_b (mPa·s ⁻¹)	4.5 \pm 0.6	5.1 \pm 0.6*
η_p (mPa·s ⁻¹)	1.6 \pm 0.3	1.6 \pm 0.3
Hct (%)	44.2 \pm 6.6	45.4 \pm 4.8
Tk	0.78 \pm 0.06	0.82 \pm 0.06 [§]

Values are mean \pm SD.

η_b , Blood viscosity; η_p , plasma viscosity; Hct, haematocrit; Tk, index of RBC rigidity.

Significant difference between both groups * $P < 0.05$, [§] $P < 0.10$.

Nocturnal HRV analysis

Mean RR interval was significantly higher in the control group compared with the SCT group. Global autonomic activity, i.e. SDNN and SDNNIDX and parasympathetic, i.e. PNN50 and RMSSD, parameters were significantly lower in the SCT group compared with the control group (Table 3). No difference was observed for SDANN between the two groups. Using spectral analysis, Ptot was significantly lower in the SCT group compared to the control group (Table 4). The SCT group had lower values for HF than the control group (HF in the SCT group was around 30% of HF in the control group). LFnu was higher in the SCT group and HFnu was higher in the control group. The ratio LF/

Table 3 Raw values of time domain analysis on nocturnal period for both groups.

	Control group	SCT group
RR interval (ms)	1,226 \pm 103	1,040 \pm 142*
PNN50 (%)	26.3 \pm 9.6	15.8 \pm 7.8*
SDNN (ms)	164.3 \pm 51.9	118.7 \pm 42.0*
RMSSD (ms)	108.2 \pm 36.6	60.7 \pm 14.1**
SDANN (ms)	88.2 \pm 42.0	67.1 \pm 24.3
SDNNIDX (ms)	124.4 \pm 37.5	87.5 \pm 32.7*

Values are mean \pm SD.

Significant difference between both groups * $P < 0.05$, ** $P < 0.01$.

Table 4 Raw values of HRV Fourier analysis on nocturnal period for both groups.

	Control group	SCT group
Ptot (ms ² Hz ⁻¹)	7,396 \pm 3,531	4,292 \pm 3,080*
VLF (ms ² Hz ⁻¹)	4,323 \pm 2,108	2,777 \pm 2,372
LF (ms ² Hz ⁻¹)	1,453 \pm 900	872 \pm 521
HF (ms ² Hz ⁻¹)	1,581 \pm 824	576 \pm 269**
LFnu	44.3 \pm 13.2	55.1 \pm 9.6*
HFnu	55.8 \pm 13.2	44.9 \pm 9.6*
LF/HF	1.14 \pm 0.3	2.0 \pm 0.9*

Values are mean \pm SD.

HF, high frequency; HFnu, normalized high frequency; HRV, heart rate variability; LF, low frequency; LFnu, normalized low frequency; Ptot, total power; VLF, very low frequency.

Significant difference between both groups * $P < 0.05$; ** $P < 0.01$.

HF was two times higher in the SCT group compared with the control group. VLF and LF were not statistically different between the two populations.

Discussion

The present study mainly showed that (i) the SCT group had higher η_b and tended to have lower RBC deformability than the control subjects in resting condition and (ii) the SCT group had lower HRV, lower nocturnal HF power and higher LF/HF ratio than the control group.

SCT carriers and subjects with normal Hb trained regularly for several years in endurance sports (running and football). Both groups exhibited closed P_{peak} values, which were comprised in the range of P_{peak} measured by Gozal et al. (1992), suggesting that SCT and control athletes had the same physical fitness level. Thus, the differences in haemorheological parameters and HRV between the SCT group and the control group seemed to not be related to training or physical fitness levels. We chose to focus our analysis on men only because it has been demonstrated that HRV indexes differed with gender (Antelmi et al., 2004).

We observed lower mean RR interval (higher heart rate) and lower RMSSD and PNN50 in the SCT group compared with the control group. This suggests that autonomic vagal outflow was impaired in SCT carriers as confirmed by the lower HF, or fast-acting, component of HRV in the SCT group (Task Force, 1996; Stauss, 2003). The SCT group had also lower values for SDNN, SDNNIDX and Ptot than the control group which might suggest both lower vagal and sympathetic activity in this group (Task Force, 1996). However, although the SCT group had low values for LF power, no statistical difference was observed between the two groups ($P = 0.15$). VLF values that are supposed to be affected by temperature regulation and humoral systems (Stauss, 2003) were not different between the two groups. This is particularly important in the present study as we performed HRV recordings in Cameroon, i.e. in tropical condition, where external and nocturnal temperature was around 28°C. The LF/HF ratio was found to be close to 1 in the control group as well as it is in healthy subjects (Pichot et al., 2002), whilst the present experiments showed that its value rose up to 2 in the SCT group. The higher LF/HF ratio found in SCT carriers could traduce an autonomic imbalance (Montano et al., 1994) mainly due to a lower autonomic parasympathetic activity. Accordingly, comparison made after HF and LF normalization, which represents the relative value of each power component to the total power minus the VLF component (Task Force, 1996), confirmed that the relative contribution of cardiac parasympathetic activity was lower (lower HFnu) and the relative sympathetic activity was higher (higher LFnu) in the SCT group compared with the control group.

The possible mechanisms responsible for such impairment are unknown. In part it might be explained by the rheological alterations in η_b and RBC deformability found in the SCT group. These results are in accordance with previous studies which also reported RBC deformability impairment in subjects with SCT although the blood O_2 pressure was kept constant (Brandao

et al., 2003). Separate studies have reported haemorheological alterations and HRV impairment in cardiac disease and diabetes (Dintenfass, 1978; Task Force, 1996; Brun et al., 1998; McHedlishvili & Maeda, 2001; Kataoka et al., 2004) but it is not known whether the impairment of the blood rheological profile is directly the cause of HRV alterations. The role of haemorheological factors in coronary heart diseases has already been demonstrated in clinical studies (Baskurt et al., 1991). Dintenfass & Lake (1977) have reported significant correlation between ECG ST-segment depression and blood viscosity factors suggesting an involvement of blood rheology in cardiac activity impairment. In the narrowest capillaries, whose luminal diameter is less than the undeformed size of RBCs, the erythrocytes advance in a greatly deformed state and in a single file flow. A thin blood plasma layer is always preserved during flow between the outer membranes of RBCs and the luminal membranes of endothelial cells (McHedlishvili & Maeda, 2001). This plasma layer plays a lubricating role in preserving the blood flow in the lumina of microvessels. If RBCs are less deformable, as well as in the SCT group, the plasma layer between RBCs and endothelial membranes is reduced leading to an increased flow resistance (Driessen et al., 1984). Moreover, less deformable RBCs cannot pass through the narrowest capillaries or plug capillaries that favour plasma skimming (McHedlishvili & Maeda, 2001). All these microcirculatory alterations are usually responsible for the lack or the reduction of oxygen supply to tissues (Shiga et al., 1990; Sarelius, 1995). Heart usually adapts its beat frequency to provide adequate oxygen supply to tissues and to match the blood flow with the metabolic demand of tissues. Indeed, a low parasympathetic activity in the SCT group could be the adequate response of the organism in terms of physiological adaptation to increase heart beat frequency thus providing adequate O_2 supplies to tissues despite the haemorheological alterations. Moreover, Lahiri (1980) demonstrated that aortic chemoreceptors are able to sense arterial hypoxia and circulatory changes in oxygen flow. Further studies are needed to clarify these points and to really demonstrate that blood rheology impairment might cause HRV impairment.

Several studies have reported that a loss of HRV and the presence of a cardiac autonomic imbalance indicate severe cardiovascular diseases and predict a poor outcome (Task Force, 1996; Grippo et al., 2002) and, more particularly, a reduction in parasympathetic nerve function may induce fatal arrhythmia (Copie et al., 1996). It is well known that patients with SCD (HbSS genotype) are prone to cardiac complications (Diop et al., 2003) but our study is the first work which described a loss in HRV and impaired autonomic nervous system activity in SCT carriers (HbAS genotype) compared with subjects with normal haemoglobin (HbAA genotype). That could constitute a risk factor for cardiac complications and accidents in this population.

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