Escola Nacional de Saúde Pública
UNIVERSIDADE NOVA DE LISBOA

Sickle cell trait in São Tomé e Príncipe: a population-based prevalence study in women of reproductive age

Master of Public Health

Guilherme Bento Lago de Queiroz

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Dissertation submitted to fulfil the requirements for obtaining the degree of Master of Public Health, carried out under the scientific supervision of Prof. Andreia Leite and the co-supervision of Prof. Maria de Jesus Trovoada.

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Abstract

Background: Sickle Cell Disorder is Africa's most prevalent genetic disease. Yet, it remains a neglected condition, with high mortality under five, and a lack of population-based studies in the region. This is the first of its kind in São Tomé e Príncipe, aiming to estimate the prevalence of sickle cell trait and other haemoglobin variants in women of reproductive age and its associated factors.

Methods: We conducted a cluster survey in 35 neighbourhoods. Haemoglobin was assessed through point-of-care capillary electrophoresis or high-performance liquid chromatography, and sociodemographic data through questionnaires. The weighted prevalence of sickle cell trait and HbC was estimated with a 95% confidence interval (95% CI). For its association with age and individual and collective genetic heritage, we calculated weighted prevalence ratios (95% CI) through robust Poisson regression.

Findings: The prevalence of sickle cell trait in women of reproductive age in São Tomé e Príncipe (n = 376) was 13.45% (95% CI: 9.05-19.00). The prevalence of HbC carriers was 8.00% (95% CI: 4.71-12.00). Older age and speaking Forro or Angolar were positively associated with having sickle cell trait.

Interpretation: The prevalence of sickle cell trait in São Tomé e Príncipe ranks high in the West African region. The country should follow international guidelines, implementing neonatal screening and comprehensive healthcare management.

Keywords:

Sickle Cell Disorder; Haemoglobinopathy; Global Health; Genetics; Sub-Saharan Africa

Resumo

Introdução: A drepanococitose é a doença genética mais prevalente em áfrica. Contudo, tem sido negligenciada, implicando uma elevada mortalidade em crianças abaixo dos 5 anos de idade, com uma falta de estudos de base populacional na região. Este é o primeiro estudo do género em São Tomé e Príncipe, com o objetivo de estimar a prevalência de portadores de hemoglobina S e outras variantes da hemoglobina em mulheres em idade reprodutiva, assim como os seus fatores associados.

Métodos: Conduzimos um estudo de amostragem por conglomerados em 35 bairros são tomenses. A hemoglobina foi avaliada através de eletroforese capilar ou cromatografia líquida de alta performance, e os dados sociodemográficos através de questionários. As prevalências ponderadas de portadores de hemoglobina S e C foram estimadas com intervalos de confiança de 95% (95% IC). Para calcular a associação com idade e herança genética individual e coletiva, calculámos razões de prevalência (95% IC) através de regressão de Poisson robusta.

Resultados: A prevalência de portadores de hemoglobina S em mulheres de idade reprodutiva em São Tomé e Príncipe (n = 376) foi de 13.45% (95% IC: 9.05-19.00). A prevalência de portadoras de hemoglobina C foi de 8.00% (95% IC: 4.71-12.00). Idades mais avançadas e utilizar o dialeto Forro ou Angolar foram positivamente associadas com ser portador.

Interpretação: A prevalência de drepanocitose em São Tomé e Príncipe é das mais elevadas na região ocidental africana. O país deve seguir orientações internacionais e implementar rastreio neonatal e seguimento clínico adequado.

Palavras-chave:

Drepanocitose; Hemoglobinopatias; Saúde Global; Genética; África Subsariana.

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Introduction

Sickle Cell Disorder - Pathophysiology, Presentation and Prognosis

Sickle Cell Disorder (SCD) is a red blood cell condition of genetic origin. The disorder originates from a single nucleotide polymorphism (SNP) that converts a GAG codon to a GTG codon in exon 1 of the *HBB* gene, located on chromosome 11. Such mutation implies the replacement of one glutamate by one valine in the β-chain of haemoglobin, forming the so-called Haemoglobin S (HbS) (1).

As an autosomal recessive condition, both parents must have the mutation to have a 25% chance of a child being born with homozygosity for HbS and present SCD. Although in heterozygosity (HbAS genotype) there is no manifestation of the disease, the disease may still appear in heterozygous combinations of HbS with other haemoglobinopathies that interfere with the β-chain of haemoglobin, such as β-thalassaemia, Haemoglobin C (HbC), Haemoglobin D-Punjab (HbD) or Haemoglobin E (HbE).

HbS differs from other haemoglobins in that valine is a hydrophobic amino acid, which facilitates the polymerisation of haemoglobin during deoxygenation. The progression of this polymerisation depends on factors such as oxygenation, 3-diphosphoglycerate (2,3-DPG) concentration, pH, temperature, saline concentration and carbon monoxide. Under certain conditions, the number of haemoglobin aggregates increases significantly, forming robust polymers that lead the erythrocyte's shape to change. Through this process, the cell transforms leaves its usual discoid and flexible shape to assume a rigid sickle-shape. Incapable of flowing through the smaller vessels, the erythrocytes agglomerate inside the blood vessels, leading to vaso-occlusive phenomena at the capillary level (2).

SCD presentation consists of a series of symptoms that depend on the extent and severity of the vaso-occlusion. The most common symptom is the so-called sickle cell crisis, caused by peripheral micro-ischemic phenomena conditioning acute, excruciating pain affecting mainly the joints and extremities. Vaso-occlusion also leads to splenic dysfunction, which may cause splenomegaly and splenic sequestration and increase the vulnerability to infections. Pneumonia is one of the principal causes of death in children with SCD. Patients may also develop present haemolytic anaemia, implying frequent transfusions and progressive multi-organ dysfunction. Transitory cerebral or ischemic strokes, intrahepatic cholestasis, ulcerative lesions, priapism or pulmonary hypertension may also occur (3).

Without proper clinical follow-up, about 80% of patients do not survive beyond 5 years. However, with currently available therapeutic strategies, it is possible to achieve an average life expectancy above 50 years (4). The prognosis also depends not only on the genotype, with homozygous forms of HbS usually leading to more severe conditions, but also on the fetal haemoglobin (HbF) concentration, which plays a modulating role in the development of the disease (5).

Currently, the only cure for SCD is bone marrow transplantation, which implies high technical complexity, high costs and significant risks. Gene therapy, aimed at increasing HbF levels, has shown promising results but is still in clinical trials, and also involves high costs and technical complexity. In the meantime, the current therapeutic strategy relies on crises and complications prevention. Hydroxyurea is the most widely used drug to control the disease, leading to an increase in HbF and a significant improvement in patients' prognosis and quality of life. In addition, antibiotic prophylaxis and differentiated vaccination are used to prevent the most frequent infections (6). Education of patients, relatives and carers is also essential, with techniques to avoid risk factors, such as exposure to altitude, extreme temperatures or dehydration, and early identification of crises.

Sickle cell trait (SCT) is defined as having a HbAS genotype, with just one allele producing the defective form of haemoglobin. People with SCT, also known as carriers, do not have any manifestation of the disease, although some authors point out that it is a risk factor for renal medullary carcinoma, renal papillary necrosis, splenic infarction, exertional rhabdomyolysis and exercise-related sudden death, and possibly also to complicated hyphema, venous thromboembolism, fetal loss/demise, and low birth weight (7). On the other side, it is considered that SCT confers protection to severe cases of *Plasmodium falciparum* malaria, even if there's still no clear physiological justification for that finding (8). Carriers do not constitute, by themselves, a clinical or Public Health issue, but are essential to genetic counselling strategies and epidemiological studies like the present one.

The Public Health Problem

SCD is one of the most prevalent inherited disorders in the world, with particularly high prevalences in the sub-Saharan African region and India. The survival bias among carriers related to the protection against severe malaria is associated with increased prevalence in malaria-endemic regions [REF]. Countries such as the United States of America or Brazil, with a large population of African descent, also register high prevalences of the disorder (8).

In sub-Saharan Africa, the most affected countries are located in the central and western regions, with some regions registering prevalences of SCT above 20% (9). The lack of Public Health strategies and access to adequate healthcare and therapy leads to high mortality among these patients. However, given the widespread absence of diagnostic means and studies, in particular population-based, the true extent of the disorder is still unknown. Some of the few studies conducted in the region reveal that sickle-cell anaemia accounts for more than 4% of all infant mortality in Nigeria, for example (10). While the 2021 Global Burden of Disease (GBD) study estimates SCD cause-specific mortality to rank 40th across all causes of under-5 mortality, a 2023 revision revealed that SCD total mortality actually ranked as the 12th (11).

Although evidence is increasingly bringing this disorder to the fore, SCD has historically suffered from institutional neglect and community recognition (12), with some authors provocatively including it in the group of neglected tropical diseases (13). As stated in The Lancet Haematology in June 2023, it is “the most neglected global health problem” (14). This has occurred mainly due to structural and institutional racism over the last decades (15). Widely considered a disorder of the black population, without any lucrative pharmacological treatment, and non-infectious, it neither affects much nor poses great risks of spillover to the white population. SCD is thus related to the history of black power movements, such as the Black Panther Party in the United States of America (16) or the Movimento Negro Unificado in Brazil (17). All these societal and scientific efforts have increasingly raised the profile of SCD as an important public health problem, vulnerable to various proven strategies.

The World Health Organization (WHO) launched a strategic document on SCD in the WHO African Region for 2010-2020. It determines the following priority strategies: "a) improvements in health care provision: clinical and laboratory management at all levels of the health system, screening of newborns, training of health professionals, and development of protocols; (b) genetic counselling and testing; (c) geographical and financial accessibility to health-care services; (d) public awareness in schools, communities, health institution, media and associations; (e) establishment of patient support groups; advocacy; and policies on employment for SCD patients"(18). At the time of writing, many of the recommended actions remain unimplemented.

Nowadays, public health strategies are deemed one of the major weapons against the disorder, contradicting some installed ideas of the little potential of such strategies against the apparent fatality of genetic diseases. Newborn screening has proved to significantly reduce early mortality and increase life expectancy (19–21). However, to date, no African country has initiated national newborn screening for SCD, despite some successful regional initiatives. Furthermore, the identification of people with sickle cell trait can lead to genetic counselling strategies, as well as prenatal diagnosis with termination for medical reasons, and pre-implantation diagnosis if available.

Finally, one of the strategies to be encouraged is a focus on literacy for patients, their relatives, health professionals and society in general. The historical silencing imposed on SCD led to the stigmatization of patients. Mothers of SCD patients are often blamed for the transmission of the disorder, patients are socially excluded as lazy and vague and even healthcare workers are cited as undervaluing the pain of sickle cell crises or considering SCD patients as mere opioid addicts (22). Increasing knowledge about the disease is important not only to decrease the loneliness and discrimination of SCD patients and their relatives, but also to promote coping strategies between them, to strengthen inter-institutional cooperation for clinical and laboratory follow-up of patients, and for more and better research on SCD.

São Tomé e Príncipe

São Tomé e Príncipe is an equatorial country in the Gulf of Guinea, consisting of the island of São Tomé and the island of Príncipe. A former Portuguese colony, it still keeps Portuguese as the official language, being a member of the Community of Portuguese-Speaking Countries (CPLP). Although the country has a small and scattered territory, where one of the mottos is "we're all cousins" ("*somos todos primos*"), some neighbourhoods and localities may present genetic discrepancies.

São Tomé e Príncipe was uninhabited until its discovery by the Portuguese in the 1470s. Besides a small European contingent, mostly Portuguese, the island was initially occupied by both free and enslaved people from the coast of Guinea and by enslaved people from the coast of Angola. The first group is considered to be the ascent of an ethnic group currently known as Forro, more associated with the northern and western occupation of the island, and the second of the Angolares, more associated with the eastern region. From the beginning of the 19th century until independence in 1974, the country's economy was based on plantations (roças) of sugar, cocoa and coffee. Due to the refusal of the São Tomé people to participate in this situation analogous to slavery, the Portuguese were forced to hire workers from Angola and Cape Verde. Today, many of these plantations remain as communities of former workers and their descendants, who have given rise to a third ethnic group called Tonga (23). Previous studies point to a greater genetic similarity between Forros and Tongas, while the Angolares still show distinct genetic peculiarities (24–26). Since the prevalence of SCD varies considerably throughout the African territory, the different origins of the Sao Tomean population and the maintenance of their genetic lineages may influence the distribution of the disorder in the territory.

The country still lacks official figures on the sickle cell trait and SCD. The two previous hospital-based studies indicate a prevalence of sickle cell trait between 13.0% (27) and 14.1% (28). There is also a single study that points to a proportion of 1.9% of HbC (28), prevalent in other nearby countries and responsible for compound heterozygous forms of sickle cell disease (genotype HbSC). The GBD estimates that in 2021, SCD was the 5th cause of mortality under-5, responsible for 7.3 (4.5-10.7) of these deaths (29). Currently, the public health service has no diagnosis capacity and there are no national newborn screening strategies for SCD.

The WHO highlights the importance to carry out population-based prevalence studies, due to the support they provide for the justification and planning of public health actions. For this reason, we decided to carry out this study as the first nationwide study in São Tome and Principe, and one of the first in sub-Saharan Africa, aiming to estimate the prevalence of sickle cell trait and other haemoglobin variants, as well as their associations with age and individual and collective genetic inheritance. As a secondary objective, we intend to assess the knowledge of SCD and its association with sickle cell status or family members with SCD. We believe that this may be an important first step towards the design of future health plans that improve the lives of SCD patients in São Tomé and Príncipe and broaden knowledge of the disorder in sub-Saharan Africa.

Study Context

This study was promoted by Drepa Comunidade, a platform created by the Portuguese Association of Patients with Haemoglobinopathies (APPDH) to promote content and strategies about SCD in Portuguese (language) through the cooperation between patient associations and institutional structures of the CPLP. This study was born from a direct collaboration between the APPDH and the Associação Filhos da Meia Lua Vermelha (AFMLV) from São Tomé and Príncipe, involving health professionals from both organisations, as well as researchers from the Centro Nacional de Endemias of the Democratic Republic of São Tomé and Príncipe and the Centro de Investigação em Antropologia e Saúde da Universidade de Coimbra (CIAS-UC). This study eventually involved the Centro Hospitalar e Universitário de Coimbra (CHUC) and the Hospital Dr Ayres Menezes (HAM) in São Tomé. Finally, it evolved into this Master of Public Health at the National School of Public Health of the New University of Lisbon.

Adequacy to the Sustainable Development Goals (SDGs)

This work contributes directly to SDG 3 - "Good Health and Wellbeing" as it aims to ensure health and well-being for all, including SCD patients, historically neglected and stigmatized. It also intends to contribute to the reduction of the mortality of children under 5 years of age (SDG 3.2), by providing evidence support to implement strategies that decrease this important global cause of death.

Another of the main objectives of the study, which reflects the Drepacomunidade spirit, is to contribute to greater visibility of SCD, chronically neglected and a victim of structural and institutional racism. The study is therefore aligned with SDG 10 - "Reduce inequality within and among countries". By presenting evidence on the scale of the problem, especially in the African context, we hope to combat the erasure of this health problem by its racial and geographical connection (SDG 10.3). Likewise, we intend to do so far from the neocolonial paternalism into which some projects of this nature fall, ensuring the methodological and ethical rigour that both the theme and the target population deserve.

Finally, SDG 17.7 "Enhance North-South, South-South and triangular regional and international cooperation on and access to science, technology and innovation and enhance knowledge sharing on mutually agreed terms, including through improved coordination among existing mechanisms, in particular at the United Nations level, and through a global technology facilitation mechanism" is embodied in the whole institutional structure that supported this project. This project is based on the Drepacomunidade in an integrated perspective and frank cooperation. Then there is the involvement of entities of different natures: 1) academic, such as the National School of Public Health of the NOVA University of Lisbon and the Research Centre in Anthropology and Health of the University of Coimbra; 2) national scope, such as the Centro Nacional de Endemias; 3) hospitals, such as the Centro Hospitalar e Universitário de Coimbra and the Hospital Dr

Ayres Menezes; 4) civil society, with the direct involvement of the Portuguese and São Tomé and Príncipe patients associations.

Manuscript

In the next section, we present the manuscript of the study "Sickle cell trait in São Tomé and Príncipe: a population-based prevalence study", which serves as the main text of this Master of Public Health. The study was carried out between April 2022 and June 2023 and proves the high prevalence of sickle cell trait in the country. We expect it to serve as a basis for the implementation of public policies directed at the disorder. In the end, we attach the supplementary material submitted along with the manuscript, the questionnaires and the informed consent used in the study.

The study was submitted to The Lancet Haematology journal on July 06, 2023, and the manuscript was assigned the ID “thelancethaematology-D-23-00302”.

The findings of the study will also be disclosed during the Drepa Comunidade presentation at the symposium "From Newborn Screening to Gene Therapy for Sickle Cell Disease: Challenges and Prospects in Africa" to be held in Dar es Salaam, Tanzania, on 12-13 July 2023.

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Manuscript

Sickle cell trait in São Tomé e Príncipe: a population-based prevalence study in women of reproductive age

Guilherme Queiroz (1,2,3), **Celdidy Monteiro** (4), **Licínio Manco** (1,5), **Luís Relvas** (6), **Maria de Jesus Trovoada** (7), **Andreia Leite** (2,8), **Celeste Bento** (1,6)

1. Centro de Investigação em Antropologia e Saúde da Universidade de Coimbra (CIAS-UC), Coimbra, Portugal
2. NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, NOVA University Lisbon, Lisbon, Portugal
3. Unidade de Saúde Pública do Agrupamento de Centros de Saúde do Baixo Vouga, Administração Regional de Saúde do Centro, Aveiro, Portugal
4. Hospital Dr. Ayres de Menezes, São Tomé, República Democrática de São Tomé e Príncipe
5. Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra, Portugal
6. Unidade Funcional Hematologia Molecular, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
7. Centro Nacional de Endemias da República Democrática de São Tomé e Príncipe, São Tomé, República Democrática de São Tomé e Príncipe
8. Department of Epidemiology, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisbon, Portugal

Research in context

Evidence before this study

We conducted a comprehensive search using PubMed and Scielo databases, employing the keywords "Sao Tome" AND ("sickle cell" OR "falciforme"), to retrieve all relevant papers published until March 1, 2023. Furthermore, we directly contacted the Centro Nacional de Endemias da República Democrática de São Tomé e Príncipe and the Hospital Dr Ayres Menezes to inquire about any studies on sickle cell disorder. We also reviewed grey literature focusing on sickle cell disorder in Sub-Saharan Africa and performed data mining to gather information from relevant papers concerning the epidemiology of sickle cell disorder in the region.

Our search yielded only two hospital-based studies. The first was a master's thesis by our coauthor MJ Trovoada, conducted in 1994, which analysed cord blood samples from 136 newborns. The second study, conducted in 2022 by A Vasconcelos and colleagues, examined 511 pregnant women. A systematic analysis was published after March 2023 as part of the Global Burden of Disease Study 2021 but has not provided specific prevalence values or presented any new studies related to the country.

It is important to note that the country's public health service does not currently offer diagnostic services for sickle cell disorder or trait, and there is no national newborn screening program in place. As a result, there is a lack of available data on the prevalence and mortality of sickle cell disorder in the country. Additionally, the World Health Organization has acknowledged the absence of population-based studies on sickle cell disease in Sub-Saharan Africa.

Added value of this study

In this study, we used cluster sampling to produce the first population-based data on the prevalence of sickle cell trait in São Tomé e Príncipe, and one of the first in Sub-Saharian Africa. To ensure the utmost benefit for participants, we focused our research exclusively on women of reproductive age. By using point-of-care capillary electrophoresis, we could identify additional variants of hemoglobin and, by examining fetal hemoglobin levels, potentially detect cases of mild thalassemia. Furthermore, our study stood among the first in Sub-Saharan Africa to assess the participants' knowledge of sickle cell disorder, including its various manifestations.

Implications of all the available evidence

These results strongly advocate for the implementation of newborn screening programs for sickle cell disease and the enhancement of the national health service's capacity to diagnose haemoglobinopathies. Additionally, the association of local dialects with the disorder sheds new light on the country's history. Moreover, the participants' level of knowledge regarding sickle cell disease presents an opportunity to develop community-driven approaches and effective communication strategies. These initiatives can contribute to introducing genetic counselling services, promoting better recognition of the disorder and its complications besides reducing the associated stigma.

Introduction

Sickle cell disorder (SCD) is an autosomal recessive condition, characterised by a mutation in the haemoglobin β-chain gene (HBB:c.20A>T) that alters chain structures into Haemoglobin S (HbS). When subjected to hypoxia, HbS polymerises and forms fibrous precipitates, transforming the usual discoid, flexible red blood cell structure into a rigid, sickle-shaped structure.1 Heterozygotic, sickle cell trait (SCT) is a benign condition, assumed to confer protection against malaria1 and therefore an evolutionary advantage in endemic regions.2 Contrarily, in homozygosity (HbSS) or compound heterozygotic forms (e.g. HbSC, HbSD, HbSbetatal), SCD is associated with high morbidity, vaso-occlusive crises with episodes of severe pain, anaemia, susceptibility to infection due to functional asplenia and ischaemic events.3

Without proper clinical support, as observed in some of the African countries where it is most prevalent, the median survival of these patients is less than five years.4 On the other hand, if promptly diagnosed and followed up, under-five mortality can be reduced up to 10 times 5 and patients register average life expectancies above 50 years.6 Early case identification and implementation of comprehensive health care management (CHCM) is thus a fundamental strategy to tackle the disease and improve patients' lives.7,8 The identification of parental SCT also allows timely genetic counselling when available. In addition to demonstrated health gains, these strategies are highly cost-effective 9.

SCD is the most prevalent genetic disorder in Africa, where more than 1000 babies are born every day with the disease and 38,403 deaths from SCD were recorded in 2019, a 26% increase from 2000.10 In some sub-Saharan regions, SCT can be present in up to 40% of the population 2 and be responsible for more than 4% of all under-5 mortality.11 However, SCD has been constantly neglected by national and international agents, with insufficient funding and research on the disease and its impact, with a lack of population-based studies.7 São Tomé and Príncipe, the archipelagic country in the Gulf of Guinea, still lacks official numbers on carriers and patients. Two previous hospital-based studies point to an SCT prevalence between 13·00%12 and 14·10% (plus 1·92% of Hemoglobin C).13 A revision of the 2021 Global Burden of Disease estimated that SCD was the 5th cause of under-5 mortality in the country.14

São Tomé e Príncipe has no diagnosis capacity nor newborn screening strategies for SCD. This is the first nationwide study in the country, and one of the first in Sub-Saharan Africa, aiming to estimate the prevalence of SCT and other haemoglobin variants among women of reproductive age and its associations with age and individual and collective genetic heritage. As a secondary objective, we intend to assess literacy on SCD (sickle cell knowledge) and its association with sickle cell status or relatives with SCD.15 We believe this can be an important first step to designing future health plans to improve SCD patients in São Tomé e Príncipe and widen knowledge of the disease in Sub-Saharan Africa.

Methods

Study Design and Setting

This cross-sectional study was conducted in the two islands of the country, São Tomé and Príncipe and consisted of an on-site collection of capillary blood and structured questionnaires. Data were collected between 10 and 30 April 2023; the remaining laboratory and statistical analyses were conducted in May of the same year.

Participants

The target population of the study was women of reproductive age (15-49 years) living in São Tomé and Príncipe. We limited our analysis to this group as they would benefit the most from the results since the identified carriers could receive genetic counselling and test future offspring. As inclusion criteria, we considered women of this age, living in São Tomé and Príncipe. We excluded participants who had received a blood transfusion in the last three months, who were on a short or medium-term stay in the country (e.g. tourists, emigrants or expatriates), women who were sisters or mothers/daughters identified as such, and if they already had a clinical diagnosis of SCD and were referred to us by healthcare professionals to get the laboratory confirmation. In these last cases, the test was conducted but we excluded them from the study to avoid a positive selection bias.

Due to the inability to conduct a nationwide simple random sampling, we employed a two-stage cluster sampling, with neighbourhoods as primary sampling units. The sampling process is detailed in Supplementary File 1.

Study size

To calculate the sample size, we used the total number of women aged between 15 and 65 in São Tomé and Príncipe, identified in the 2012 Census: 48,983 women.16 Assuming an expected frequency of 10·00%, consistent with previous studies, and for a 95% confidence interval (95% CI), this implied a minimum sample size of 138 women.17 Based on analogous studies in the same region, we also assumed a design effect of

2·00,18 and considered a refusal to participate rate of 10·00%.19 Thus, we defined a minimum recruitment target of 304 women to achieve a minimum sample size of 276 participants. This value was employed in the clusters mentioned above.

Data sources/measurements

We used a drop of blood to conduct capillary electrophoresis, using the Point-of-care device Lab001 (ARKRAY Inc, Japan). Whenever it was not possible to perform this technique, we collected the samples on Guthrie filter paper, later analysed in Laboratório de Eritropatologia, at Centro Hospitalar e Universitário de Coimbra, Portugal, through high-performance liquid chromatography (HPLC) in the VARIANT II Hemoglobin Testing System (Bio-Rad Laboratories Inc., Hercules, CA, USA). If necessary, we performed Multiplex Ligation-dependent Probe Amplification (MLPA) and Sanger sequencing to confirm dubious diagnoses.

Participants were also asked to answer a questionnaire. Every question was read by one member of the team to ensure answers would not be limited due to poor reading abilities and was always done in the company of local volunteers who explained the questions if needed, avoiding interpretation issues and information bias.

Variables

The main outcome was the woman's HBB genotype, categorized as (1) "SCT" if she had a HbAS genotype, defined as a proportion of HbS between 20% and 45%; (2) "SCD" if she had a HbSS genotype, a pathological heterozygous combination of HbS with another haemoglobin variant (e.g. HbC, HbD), or an HBAS genotype if the proportion of HbA was below 30%; (3) "HbAC" if she had a heterozygous combination of HbC and HbA, defined as a proportion of HbC between 20% and 45%; (4) "normal" if she had a HbAA genotype, defined as a proportion of HbA above 70%.

As independent variables, we collected sociodemographic characteristics of the participants: age, civil status, nationality, education level (knowing how to read or write) and past childbirths. As a proxy of collective genetic heritage, we asked if they spoke any of the local dialects: Forro, Angolar, Lunguie or Cape Verdian Creole. For individual genetic heritage, if they identified any relative with the disease.

Quantitative variables were categorized into groups. Age was considered as seven intervals of five years (15-19 to 45-49); and pregnancies were considered as nulliparous (0), primiparous (1), multiparous (2-4) or grand multipara (>4). Local dialects spoken were also grouped as none, only forro, only angular and other combinations, including

Lunguie. The variable of identifying any relative with SCD was grouped as (1) none; (2) a direct descendent, i.e. son(s) or daughter(s); (3) “ascendant 1” - father, mother, or sibling or nephew (as both sibling and nephew with SCD imply a direct ascendant with at least SCT); (4) “ascendant 2” - a grandparent or a first-degree uncle/aunt or cousin; and (5) second-degree uncle/aunt or cousin.

Finally, for the assessment of sickle cell knowledge, we used as outcomes the term used for SCD (“bone disease” or sickle cell disease), the knowledge about the associated symptoms and how SCD was transmitted from one person to another.

Statistical methods

All the statistical analysis was conducted using R version 4.1.0. 20

First, we evaluated the suitability of the sampling design by calculating the intraclass correlation coefficient (ICC) using the R package “fishmethods”,21 and then the design effect, using the equation $Deff = 1 + ICC(n - 1)$, where n is the average number of subjects sampled per cluster.

For sample characterisation, we calculated the absolute and relative frequencies. Even if the sampling was proportional to the size of each neighbourhood, and to address difficulties in recruitment, we adjusted the analysis considering each sample weight, calculated as the ratio between each neighbourhood population and the number of participants selected in that neighbourhood. The adjustment was conducted using the R package “survey”.22 We then calculated the weighted prevalence of all the outcomes and the corresponding 95% CI. Haemoglobin proportions were represented using a boxplot to illustrate the dispersion of HbF in participants without Hb variants and with SCT, and of HbS in those with SCT.

The HW_TEST software (26) gave us the expected prevalences of each genotype based on the weighted tri-allelic frequencies, assuming a Hardy-Weinberg equilibrium (HWE) at birth. We also tested the HWE with Chi-square without correction, and alpha set at 5%.

To assess if the age group, the spoken local dialect or an identified relative with SCD was associated with a higher risk of having SCT, we calculated the weighted prevalence ratio (PR) and corresponding 95% CI, through the robust Poisson method,23 using the “sandwich” package.24 The association between a better knowledge of sickle cell and having SCT, or a relative with SCD, was also assessed using the same method.

Role of the funding source: CIAS-UC paid for the ARKRAY® Lab001 and reagents for all the laboratory work, the flight and travel expenses of CB, the flight to Príncipe of GQ and publishing fees. APPDH paid for the flights of GQ to São Tomé. Forum Hematologico de Coimbra paid for the impression of forms and informed consent.

Results

A total of 376 women were enrolled in this study, representing 35 neighbourhoods of the seven health districts of São Tomé and Príncipe. Supplementary File 1 presents detailed information on neighbourhood selection and the number of participants per cluster. The Intraclass Correlation Coefficient (ICC) for the presence of HbS was 0·02, thus implying a Design Effect of 1·25. The anonymised dataset is available according to the requirements in the data statement.

Table 1 - Characteristics of the participants: absolute and proportions, both of the sample and of the studied population, with a 95% confidence interval.

Variable	Group	N	Proportion (Sample)	Proportion (Weighted, 95% CI)
Age	15-19	29	7·71%	5·48% (2·48-10·00)
	20-24	54	14·36%	16·40%(12·80-21·00)
	25-29	60	15·96%	14·90% (9·60-22·00)
	30-34	77	20·48%	19·30% (14·10-25·00)
	35-40	65	17·29%	17·70% (13·60-22·00)
	40-44	42	11·17%	12·39% (8·58-17·00)
	40-49	49	13·03%	13·80% (10·30-18·00)
Civil Status	Single	311	82·71%	85·30% (79·20-90·00)
	Married	63	16·75%	14·53% (9·58-21·00)
	Other	2	0·53%	0·20% (0·03-1·00)
Nationality	São-Tomense	361	96·01%	96·40% (92·40-99·00)
	Cape Verdean (double)	13	3·46%	3·00% (1·12-7·00)
	Gabonese (double)	2	0·53%	0·44% (0·04-2·00)
Education	Knows to read and write	352	93·62%	94·10% (90·00-97·00)
	Only knows to read	13	3·46%	2·99% (1·11-6·00)
	Doesn't know how to read or write	11	2·92%	2·88% (1·19-6·00)
Local dialect spoken	None	101	26·86%	24·60% (19·20-31·00)
	Only Forro	158	42·02%	47·90% (36·80-59·00)
	Only Angolar	23	6·11%	4·08% (1·90-7·00)

	Only Cape Verdean	41	10·90%	8·26% (4·00-15·00)
	Other	53	14·10%	15·13% (9·96-22·00)
Past childbirths	Nulliparous (0)	55	14·63%	14·00% (11·0-17·00)
	Primiparous (1)	58	15·42%	18·10% (12·00-26·00)
	Multiparous (2-4)	205	54·52%	53·20% (45·90-60·00)
	Grand multipara (>4)	58	15·42%	14·70% (10·90-19·00)

Table 1 presents sample and weighted frequencies. The majority of the participants (67·98%) were between 20 and 39 years old. The low numbers registered below 19 years old were mainly due to the need for informed consent by the legal representative of underaged volunteers, usually not present at the collection site. Most women were single (82·71%), even though some advised they were partnered, as São Tomé e Príncipe polygamy practices and informal marriages are common practices. Most of them also knew how to read and write, and all of them were of São Tomense nationality, with a few having double nationality. The most spoken dialect was forro and around a quarter of the women only spoke Portuguese.

All the blood samples were processed successfully, 155 by point-of-care capillary electrophoresis and 221 by HPLC. The analysis of haemoglobin proportions according to sickle cell status for the point-of-care samples is presented in Supplementary File 2.

Table 2 - Genotype: absolute number, prevalence in the sample, and prevalence in the population (weighted), with a 95% confidence interval (95% CI).

Genotype	**N**	**Prevalence (Sample)**	**Prevalence (Weighted, 95% CI)**
HbAA	301	80·05%	77·20% (68·30-85·00)
HbAS	49	13·03%	13·45% (9·05-19·00)
HbAC	18	4·79%	6·79% (3·77-11·00)
HbSS	2	0·53%	1·38% (0·25-4·00)
HbSC	6	1·60%	1·20% (0·31-3·00)

Prevalence of SCT and other hemoglobinopathies

The weighted allele frequencies were 8·71% for the HbS allele and 3·99% for the HbC allele. Table 2 presents the prevalences of each genotype. The district with a higher proportion of participants with SCT was Região Autónoma do Príncipe, with 25·00% (5/20), followed by Lembá and Me-Zochi with 16·67% (5/30; 20/120), Água Grande with 14·17% (17/120), Caué with 13.33% (4/30), Cantagalo with 11·54% (3/26) and Lobata with 10·00% (3/30). The expected (weighted) prevalence of newborns with the HbSS genotype is 0·80% (0·40-1·20) and with HbSC is 0·70% (0·40-1·00). This means that between 8 and 22 of each 1000 newborns will develop SCD. The HWE could not be rejected (p = 0·30).

Additionally, we tested two participants with an HbAA genotype that presented levels of Faetal Haemoglobin (HbF) above 12%. One presented a heterozygous mutation known as Cape Verdean deltabeta deletion,25 and the other a frameshift (HBB:c.126-129delCTTT).

Individual and familiar history of SCD

More than half of the participants (63·03%) didn't know whether any relative had SCD. Among the 139 recognizing at least one relative with the condition, 32·37% had a grandparent or first-degree uncle or aunt, 39·57% had the mother, father, sibling or nephew, 13·67% had a son or daughter and 10·07% had a second-degree uncle or aunt. There were also 15 participants reporting that they suffered from SCD. Of these, one had HbS in homozygosity and three had SC hemoglobinopathy, six only had SCT and five had no single haemoglobin variant of interest.

Factors associated with SCT

Figure 1 presents prevalence ratios of SCT according to age, the spoken dialect and having a relative with SCD. Age was significantly associated with having SCT, especially in the groups above 30 years old. The dialect spoken by the participants registered different associations when compared to those who only spoke Portuguese. Speaking Forro or Angolar increased the prevalence by 9·29% (95% CI: 2·35-16·70) and 46·86% (95% CI: 23·54-73·88) respectively while speaking only Cape Verdean or other combinations lowered the prevalence by 55·12% (95% CI: 46·74-65·02) and 82·30% (95% CI: 71·35-94·93). When compared to those without any known relative with SCD, those who had a son or daughter posed the most significant probability to have SCT, with a prevalence 806·22% (95% CI: 699·20-925·60) above the reference one, decreasing when just had a mother, father, sibling or nephew and keeping a 113·95% (95% CI: 67·07-173·98) in first-degree relatives. Second-degree relatives were excluded from the analysis due to their small number.

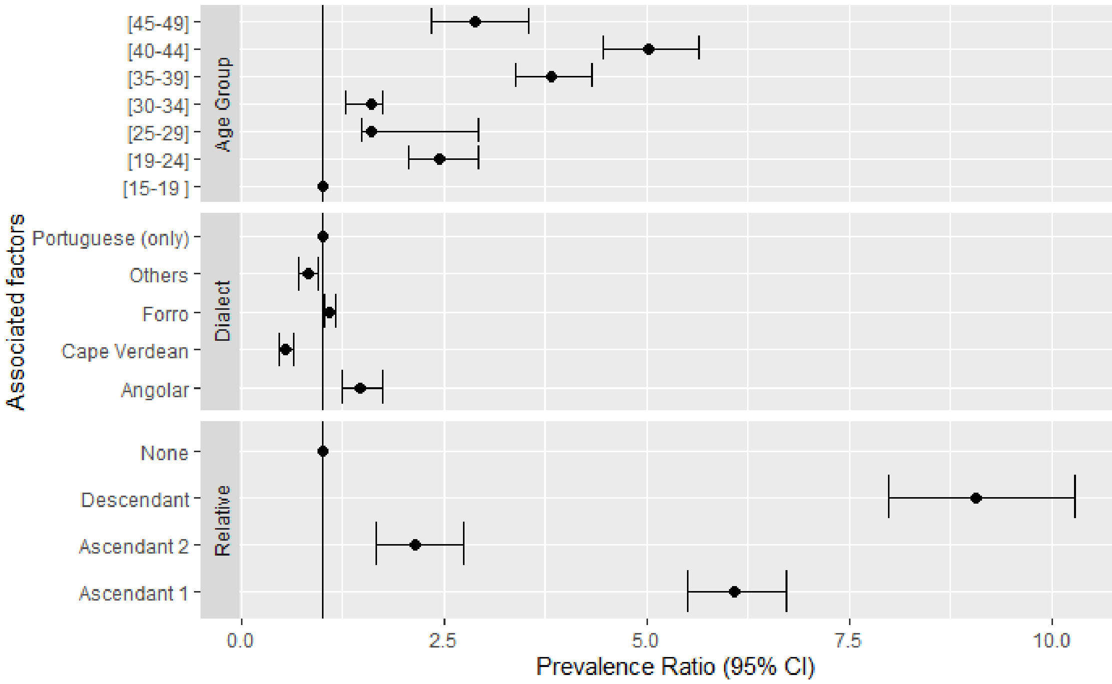

Figure 1 - Prevalence ratios (PR) of SCT, with 95% confidence intervals, for the association of age, dialect and relative with sickle cell disease with having sickle cell trait. Descendant - son or daughter; Ascendant 1 - father, mother, sibling or nephew

Sickle cell knowledge

When asked about their knowledge of SCD, only seven participants did not recognize the condition. The most used name was sickle cell disease, "cell disease" or just "cell" (69·65%), 45·26% named it "bone disease" and 14·90% would use both names. As for signs and symptoms, most women associated the disease with pain (79·25%), 14·36% with fatigue and 6·65% with anaemia. Knowledge of the transmission of SCD was scarcer, with 63·56% admitting they didn't know it and just 28·99% answering it was hereditary, from both mother and father.

When analysing the influence of each sickle cell status on sickle cell knowledge, we observe that people with SCT were 61·48% (95% CI: 59·54-63·48) less probable of being ignorant of SCD transmission, but 131·82% (95% CI: 98·70-170·47) more probable of being ignorant of its symptoms. On the other hand, those without relatives with SCD were 67·16% (95% CI: 59·34-75·36) and 148·69% (95% CI: 99.52-209.98) more probable of being ignorant of both transmission and symptoms, respectively.

Discussion

Our study estimated an SCT prevalence of 13·45% (95% CI: 9·05-19·00) among women of reproductive age in São Tomé and Príncipe. Due to its autosomal condition, we don't expect different prevalences of SCT according to sex.26 Thus, we expect that this value corresponds to the estimated prevalence of SCT in the whole population between 15 and 49 years in São Tomé e Príncipe. This prevalence is higher than those previously found in the country and ranks high in the interval of prevalences found in West Africa,2 highlighting the need to participate in the regional effort to tackle the disease.7

The prevalence of the HbC trait, 6·79% (95% CI: 3·77-11·00), though higher than the previously recorded, is the first recorded in a representative sample. The value is in line with the values observed in neighbouring countries.27 When looking at the HbSS genotype alone, we also obtained a prevalence [1·38 (95% CI: 0·25-4·00)] comparable to that of the region.28 In any case, these are only indicative values, as the study design only considered SCT prevalence. However, they show that future newborn screening strategies should not overlook HbC and beta-thalassemia.

The positive association of SCT with age has been previously reported,26 and may be related to its protection against malaria, increasing their long-term survival. As malaria elimination in São Tomé gets closer, these differences may disappear with time. However, the possibility of an HWE does not reflect the expected survival bias favouring the HbA allele frequency. This may imply a slight positive bias in the selection of SCD patients or an eventual balance between the negative survival bias of SCD and the positive one in SCT (malaria-related). Further research on comparative mortality between genotypes would be useful to clarify this.

Forro and mainly Angolar-speaking people had higher SCT prevalence, while the ones that spoke Cape Verdean creole or combinations of dialects had lower prevalences. This supports the theory of different genetic lineages according to the geographical origin of each ethnic group, as these findings are in line with the lower prevalences found in Cape Verde and higher in the Gulf of Guinea and Angola. On the other side, it also suggests a genetic difference between Tonga (Cape-Verdean descendants) and Forros which was previously discarded.29 Studies on the genetic diversity of São Tomé e Príncipe would be required to better comprehend the history of its colonisation.

The overall recognition of SCD was impressive, with most correctly naming the disease and identifying pain as a symptom. It would be useful for future campaigns to clarify the "bone disease" nomenclature and avoid eventual misdiagnoses of osteoarticular syndromes or dengue. Most of the participants correctly identified the transmission of the disease as hereditary, showing that there is a literacy base for health strategies addressing genetic counselling. The positive association of sickle cell status with sickle cell knowledge reveals a vulnerability of the target population to those strategies. The opposite was noted for people without close relatives with SCD and may imply that people without close contact with the disease need to be better informed about SCD. Data on signs and symptoms are difficult to interpret and may be due to the small number of people with both conditions. Since previous studies did not include the recognition of signs and symptoms,15 it would be useful to include it in future sickle cell knowledge scales.

The lack of recent demographic data on São Tomé e Príncipe is a limitation of this paper, as current estimates point to a population that almost doubled the one in the last census.30 This means that the distribution used in the sampling procedure may not correspond to the current one. However, in the first case, the major demographic flux was towards the capital (Água Grande district), already overrepresented in our sample as this tendency was already present in 2012.

Besides, when analysing age distribution in our sample, we realise that younger groups are underrepresented since they represent the majority of the population but not our sample. This may be related to the lack of random selection in the second stage of sampling, with collection sites in places where women this age do not go that frequently such as schools or health services. Although that may have influenced each location's results, we believe that the collection points choice tended to minimize this bias. This limitation may hinder our population-based estimates, yet we attempted to minimise it using weighted analyses.

Haemoglobin analysis was conducted with highly sensitive and specific tests to avoid misclassification. Data on literacy may also have been subjected to social-desirability bias, but we tended to minimize replications of answers among participants by controlling the interview settings.

The data hereby reported supports that SCD is an important health problem in São Tomé e Príncipe that shall be addressed by national strategies such as neonatal screening, updating of therapeutic guidelines and capacitation of healthcare workers. Neighbouring countries with lower prevalences of SCT have already addressed the issue, which was also set as a health priority by the Ministers of Health of the Region.10 Future research on SCD in the country should also focus on younger generations and on the quality of life and life expectancy of SCD patients, to better design support strategies. Data shows that there’s a solid base of knowledge encouraging future programmes that involve the community. São Tomé e Príncipe, with its high prevalence of SCT, but also its dimension, organization and culture, has the opportunity to be an example in Sub-Saharan Africa and significantly improve the lives of patients.

Ethical considerations

This study was approved by the Ethics Committee of São Tomé and Príncipe, identified as CESIC Case PC022_2022.

As the study is a population-based screening study, the research team decided to study only women of childbearing age, since they represent the group that can most benefit from a possible carrier diagnosis. Participants diagnosed as HbS carriers were guaranteed genetic counselling by Associação Filhos da Meia Lua Vermelha and, if possible, testing of their newborns. If the offspring is diagnosed with SCD at birth, clinical follow-up from the very first moment can improve the prognosis and quality of life of the patient. This ensures that the health information received by the participant corresponds to the proper response.

All participants signed an informed consent form before participating in the study. In the case of underage women, the consent was signed by a legal representative.

All data were anonymised by the first author and shared with the team in an aggregate form. Individual files and biological material are archived and protected in CIAS servers for 5 years, being destroyed thereafter.

Contributors

GQ and CB conceptualized the study, CM and MJT helped to adapt it to the context of São Tomé e Príncipe, and along with AL and MJT designed the methodology. GQ collected data in Região Autónoma do Príncipe and, with CM and CB, in São Tomé. CB, LM and LR were responsible for all laboratory analyses in Portugal. Statistical analysis was done by GQ and AL. GQ wrote the paper, with editing from AL, and all authors participated in the revision process.

Data Sharing

De-identified data and study protocol can be requested by e-mail to the corresponding author (GQ) and depends on the authorization of Centro de Investigação em Antropologia e Saúde and Centro Nacional de Endemias da República Democrática de São Tomé e Príncipe. The request must include a methodologically sound proposal with the full study protocol. All requests will be evaluated individually by both institutions, with data being available until 5 years following the publication. All shared data will ensure that the rights and privacy of the participants will be safeguarded during the process. The model code is available at https://gitlab.com/drepa-comunidade/sickle-cell-trait-in-stp.

Declaration of interests

The authors GQ and CB are associates of Associação Portuguesa de Pais e Doentes com Hemoglobinopatias, and CM is head of Associação Filhos da Meia Lua Vermelha. CM and MJT work in Centro Nacional de Endemias da República Democrática de São Tomé e Príncipe. LM and LR has no conflicts of interest. Their personal and professional involvement in the fight for sickle cell disease patients did not collide but enforced the methodological rigour and transparency of this study. The authors alone are responsible for the views expressed in this publication, and they do not necessarily represent the decisions, policies, or views of the government of República Democrática de São Tomé e Príncipe. CB has received honoraria from Arkray for a talk in a webinar. AL has received honoraria from Pfizer for a presentation at a scientific event.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly, DeepL and ChatGPT in order to ensure a rigorous translation from Portuguese to English, as well as to embellish the writing. After using these tools, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Appendix 1 – Supplementary Material

Supplementary File 1 - Cluster Sampling

Methods:

As primary sampling units (PSU), we used the census units “neighbourhoods”, as reported by the National Institute of Statistics (INE) of São Tomé and Príncipe. In the first stage, we randomly selected 35 neighbourhoods according to a probability proportional to their population size. For this purpose, we considered the female population between 15 and 65 years old of each neighbourhood published in the 2012 Census [REF]. Neighbourhoods with less than 50 people were excluded to ensure a minimum number of participants. Using Microsoft Excel, we listed the eligible neighbourhoods in alphabetical order. We calculated the cumulative population along this list, assigning each neighbourhood a number corresponding to the cumulative population achieved in its position. A number was then randomly drawn between 1 and the number assigned to the last neighbourhood, i.e. the total population considered, and the neighbourhood where it was achieved was selected and removed from the list until reaching a total of 35 selected neighbourhoods. This approach ensured that more populated neighbourhoods would have a higher probability of selection (probability proportion to size).

In the second stage, 10 participants were targeted in each neighbourhood, yielding a target number of 350 women. For technical reasons, such as electricity access, and to guarantee the legitimation of the study among the community, interviews and samples were collected at fixed points in the neighbourhoods, like health centres, schools or community centres. In dense urban areas with an intense circulation of people near adjacent neighbourhoods, we selected a common reference point that assisted people from the selected neighbourhoods (*e.g.* school, health post…). All collection points are listed in Table 2. To avoid selection bias, the survey was not announced by any means; whenever required, it was previewed with the responsible person for the collection point. In most cases, improvised volunteers would ask women to participate in the study as they walked by, and it was impossible to register refusals as initially considered. However, our overall perception was that adherence was near 100%.

Table 1: Characteristics of the selected neighbourhoods, collection point used and number of samples collected.

Neighbourhood	District	Population (2012)	Collection Point	N
Agostinho Neto	Lobata	992	Restaurant (Jardela)	10
Bela Vista	Lobata	517	Bela Vista Community Center	10
Maianço Roça	Lobata	209	Roça Mainço Community Center	10
Benga	Lembá	3589	Lembá Health Center	10
Diogo Vaz	Lembá	632	Diogo Vaz School	10
Rosema	Lembá	2587	Lembá Health Center	10
Água Lama	Mezochi	336	Bom Bom Health Center	10
Aldeamento Monte Café	Mezochi	194	Monte Café Hospital	10
Batepá	Mezochi	775	Trindade Hospital	10
Cabalo Molê	Mezochi	425	Trindade Hospital	10
C. Trindade Centro	Mezochi	1586	Trindade Hospital	10
Melhorada	Mezochi	656	Trindade Hospital / Caixão Grande Health Center	20
Praia Melão	Mezochi	2668	Bom Bom Health Center	10
Riba Mato	Mezochi	363	Bom Bom Health Center	10
Santa Margarida	Mezochi	384	Santa Margarida School	10
Uba Flor	Mezochi	588	Trindade Hospital	10
Bem Posta	Mezochi	120	Monte Café Hospital	10
Água Arroz	Água Grande	2238	Água Arroz Health Center	10
Almeirim	Água Grande	1591	Água Arroz Health Center	10
Boa Morte	Água Grande	3432	Boa Morte School	10
Hospital	Água Grande	1881	Hospital Community Center	10
Liberdade	Água Grande	512	Liberdade School	10
Madre de Deus	Água Grande	2469	Madre de Deus Health Center	10
Oquê-del-Rei	Água Grande	3465	Liberdade School	10
Praia Cruz	Água Grande	1652	Praia Gamboa Health Center	10
São João da Vargen	Água Grande	1793	Vila Fernanda Health Center	10

São Marçal	Água Grande	2866	Bom Bom Health Center / São Marçal Health Center	20
Vila Fernanda	Água Grande	802	Vila Fernanda Health Center	10
Água Izé	Cantagalo	1255	Água Izé Health Center	16
Cidade Santana Centro	Cantagalo	769	Santana Health Post	10
Beira Mar	Caué	510	São João dos Angolares School	10
Meven Ngay	Caué	406	São João dos Angolares School	10
Porto Alegre	Caué	795	Centre for Educational and Training Resources (CREF)	10
Lenta-Piá	RA Príncipe	1020	Santo António Hospital	11
Sundy	RA Príncipe	416	Sundy Health Center	9

Supplementary File 2 - Hemoglobin proportion analysis

Figure 1 depicts the distribution of the Hb variants in the sample studied with electrophoresis. This analysis excludes SCD cases (HbSS and HbSC) as the equipment was not able to give Hb proportion in the absence of HbA. Participants with SCT presented HbS proportions between 21.8% and 33.8%, with a mean of 29.0% (sd 3.0). HbC carriers presented similar values for HbC, with proportions between 27.0% and 34.8%, with a mean value of 32.0% (sd 2.84). In both SCT and HbC carriers, there was an overlapping of HbA proportion, with mean values of 58..4% (sd 2.6) and 58.0% (sd 2.3) respectively. On its side, HbF registered similar distributions between the three groups, with SCT cases registering slightly higher values - a mean value of 1.14% (sd 0.81) against 0.79% (sd 0.50) for HbC carriers and 0.99% (sd 1.34) for participants with no variant. Levels of HbF above 2.0% were observed in 15 participants, three with SCT and 12 without HbS or HbC, one of them (no SCT) with 12.8%.

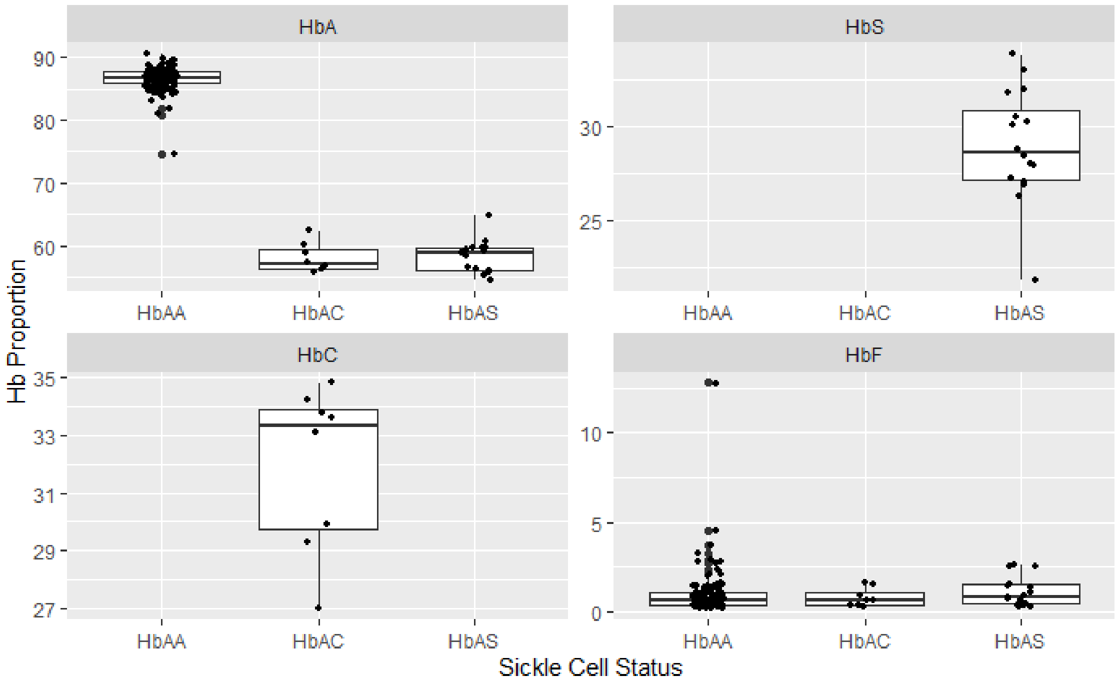

Figure 1: Hb variants proportion according to sickle cell status

Interpretation

The proportion of HbS in SCT is affected by the association with alpha thalassemia. HbS levels can be lower in the case of concomitant heterozygous alpha thalassemia, and much lower in the homozygous state. As the -alpha 3.7 deletion is a benign condition frequent in African countries, the lower values of HbS registered may be caused by double heterozygosity with the -alpha 3.7 deletion.

The high levels of HbF (> 2.0%) registered in some of the samples are not relevant, except for the one sample with 12.8% possibly due to a deltabeta thalassemia or a Hereditary Persistence of HbF. The molecular characterisation of these samples could elucidate the differences, but it is outside the scope of this project.

Appendix 2 – Questionnaire

Código:_ _ _ _ _ _ _

Portadores de Hemoglobina S em São Tomé e Príncipe: um estudo de prevalência em mulheres em idade fértil

Questionário sobre dados sociodemográficos, obstétricos e de conhecimento sobre anemia falciforme. Os dados serão tratados de acordo com o termo de consentimento informado.

Bairro: ______ **Data:** _ _ /05/ 2023 **Aceita participar no estudo?** ○ Sim ○ Não

Se não aceita, porquê? (pode selecionar mais que uma)
○ Medo de picada, sangue e/ou dor ○ Falta de tempo / inconveniência
○ Confidencialidade, cultura, religião ○ Não concorda / não interessa

Se aceitou participar, responda por favor às seguintes questões:

Idade: ______ **Estado civil:** ○ Solteira ○ Casada ○ Viúva ○ Divorciada

Nacionalidade : ○ São-Tomense ○ Portuguesa ○ Outra:_______________
(pode selecionar mais que uma nacionalidade)

Educação: ○ Sabe ler e escrever ○ Sabe ler mas não sabe escrever ○ Não sabe ler nem sabe escrever

Língua Falada: ○ Português ○ Forro ○ Angolar ○ Lunguie ○ Cabo-Verdiano ○ Outra
(pode selecionar mais que uma língua)

Quantas vezes já ficou grávida? ______ **Quantos partos já teve?** ______

Quais destes nomes de doença conhece?
○ Anemia Falciforme ○ Doença de Osso ○ Doença de Célula ○ Outro:____________________
(pode selecionar mais que um nome)

Quais dos sintomas associa a esta doença?
○ Tosse ○ Falta de ar ○ Dor ○ Cansaço ○ Febre ○ Inchaço
○ Olhos amarelos ○ Barriga inchada ○ Outro(s):____
(pode selecionar mais que um sintoma)

Tem algum familiar com suspeita ou diagnóstico desta doença?
○ Filho(a) ○ Pai ○ Mãe ○ Avô / Avó ○ Irmão / Irmã ○ Sobrinho(a)
○ Tio(a) / Primo(a) de 1º grau ○ Tio(a) / Primo(a) de 2º grau
(pode selecionar mais que um familiar)

Como se transmite a doença?
○ Mãe para Filho ○ Pai para Filho ○ Toque ○ Ar ○ Sexualmente
○ Contacto com fluidos infetados ○ Feitiço ○ Outro:____________________

Obrigado!

Appendix 3 – Informed Consent

Termo de Consentimento Informado

Portadores de Hemoglobina S em São Tomé e Príncipe:
um estudo de prevalência em mulheres em idade fértil

Investigadores Responsáveis: Guilherme Queiroz, Celdidy Monteiro, Teresa Ferreira, Maria de Jesus Trovoada, Luís Relvas, Licínio Manco, Andreia Leite, Celeste Bento

Instituição a que pertencem os Investigadores Responsáveis: Universidade de Coimbra, Portugal; Administração Regional de Saúde do Centro, Portugal; Centro Hospitalar e Universitário de Coimbra, Portugal; Hospital Fernando Fonseca, Lisboa, Portugal; Escola Nacional de Saúde Pública, Universidade NOVA de Lisboa, Portugal; Hospital Dr. Ayres de Menezes e Centro Nacional de Endemias S. Tomé e Principe

Nome da voluntária: ____ **Idade:** __

A Srª está a ser convidada a participar no projecto de pesquisa "Portadores de Hemoglobina S em São Tomé e Príncipe: um estudo de prevalência em mulheres em idade fértil", de responsabilidade da equipa de pesquisadores acima referida.

- Este estudo tem como objectivo a determinação da prevalência do alelo da Hemoglobina S presente em mulheres em idade fértil (15-49 anos), de modo a estudar os resultados obtidos e inferir, de acordo com esses mesmos resultados, o risco para a transmissão genética do alelo referenciado e possível origem de drepanocitose nos descendentes como resultado dessa mesma transmissão.
- O material biológico a colher para o estudo consistirá numa amostra de sangue capilar colhido por picada com lanceta para realização em exclusivo de testes associados ao estudo apresentado, nomeadamente uma eletroforese de hemoglobina, de modo a determinar a presença de Hemoglobina S.
- Uma segunda amostra de sangue capilar, utilizando a mesma picada, será armazenada numa tira de papel absorvente (papel filtro de Guthrie). Caso a doente revele outras variantes da Hemoglobina ou homozigotia para a Hemoglobina S, esta amostra servirá para caracterizar molecularmente o sangue da doente e obter um diagnóstico mais preciso.
- A colheita de sangue para o estudo será realizada em condições seguras e assépticas com contínua vigilância por parte dos investigadores presentes. A punção será sempre realizada por um profissional de saúde devidamente formado para o efeito.
- A punção para o exame laboratorial pode resultar em dor no local, durante ou depois da picada, ou manchas rochas transitórias denominadas de equimoses nas horas posteriores.
- A participante poderá, no final do estudo, ter acesso aos resultados apenas a si pertinentes, o que permitirá uma reflexão consciente e segura da sua parte acerca da sua descendência e dos riscos associados à transmissão deste mesmo gene entre os membros da sua família, de modo a permitir uma prevenção mais eficaz de possíveis doenças associadas à presença do gene referido.
- Para além da vigilância constante no momento e no pós-colheita por parte da equipa de investigadores, poderá entrar em contacto durante o período de estudo em qualquer ocasião através dos contactos que serão fornecidos. Nós estimulamos a si ou aos seus familiares a colocar questões ou curiosidades a qualquer momento do estudo através desses mesmos contactos.
- Todo e qualquer dano decorrente do desenvolvimento deste projecto de investigação, e caso necessite de atendimento, ficará a cargo da instituição envolvida e do grupo de pesquisadores e será da nossa inteira responsabilidade. O seu tratamento e acompanhamento contínuo fazem parte de sua participação neste estudo.
- Será garantida a confidencialidade das informações geradas e a sua total privacidade na pesquisa. Mais ninguém para além da equipa de pesquisadores que cuidará de si poderá aceder aos dados obtidos. O seu nome e dados pessoais não serão revelados, sendo que apenas os dados agregados serão utilizados propósitos educativos ou de publicação, e serão arquivados em servidores protegidos do CIAS durante 5 anos. Todo o material biológico e dados individuais serão destruídos até ao final deste período.
- Não haverá qualquer custo ou forma de pagamento para a paciente pela sua participação no estudo.
- A sua participação neste estudo é completamente voluntária e tem direito a recusar-se a participar ou interromper a sua participação a qualquer momento sem penalidades ou perda de benefícios aos quais tem direito. Em caso de decidir interromper a sua participação no estudo, a equipa de investigadores deve ser comunicada e a colheita de amostras e estudos recorrentes para os exames relativos à investigação será imediatamente interrompida.

Telefones para contacto: +239 999 0924 (Celdidy Monteiro)

Declaração de Consentimento

Eu, ____, nascida a __/__/____, declaro que li as informações no verso desta página, entendi o propósito deste estudo assim como os benefícios e riscos potenciais da participação no mesmo. Tive a oportunidade de fazer perguntas e todas foram gentilmente respondidas. Eu, por intermédio deste , dou livremente o meu consentimento para participar neste estudo.

Entendo que não receberei compensação monetária pela minha participação neste estudo e que essa participação é completamente voluntário e com voto de confidencialidade.

Eu recebi uma cópia assinada deste formulário de consentimento.

___________________________________ ____ / _____ / _____
(Assinatura da Participante) dia mês ano

____
(Nome da Participante – letra de forma)

Eu, abaixo assinado, expliquei completamente os detalhes relevantes deste estudo ao participante indicado acima.

____ ____ / ____ / ____
(Assinatura da pessoa que obteve o consentimento) dia mês ano

Termo de Consentimento Informado - menores

Portadores de Hemoglobina S em São Tomé e Príncipe:
um estudo de prevalência em mulheres em idade fértil

Investigadores Responsáveis: Guilherme Queiroz, Celdidy Monteiro, Teresa Ferreira, Maria de Jesus Trovoada, Luís Relvas, Licínio Manco, Andreia Leite, Celeste Bento

Instituição a que pertencem os Investigadores Responsáveis: Universidade de Coimbra, Portugal; Administração Regional de Saúde do Centro, Portugal; Centro Hospitalar e Universitário de Coimbra, Portugal; Hospital Fernando Fonseca, Lisboa, Portugal; Escola Nacional de Saúde Pública, Universidade NOVA de Lisboa, Portugal; Hospital Dr. Ayres de Menezes e Centro Nacional de Endemias S. Tomé e Principe

Nome da voluntária: ____ **Idade:** ______________

Nome da representante legal: ____ **Relação:**_____________

A Srª está a ser convidada a participar no projecto de pesquisa "Portadores de Hemoglobina S em São Tomé e Príncipe: um estudo de prevalência em mulheres em idade fértil", de responsabilidade da equipa de pesquisadores acima referida.

- Este estudo tem como objectivo a determinação da prevalência do alelo da Hemoglobina S presente em mulheres em idade fértil (15-49 anos), de modo a estudar os resultados obtidos e inferir, de acordo com esses mesmos resultados, o risco para a transmissão genética do alelo referenciado e possível origem de drepanocitose nos descendentes como resultado dessa mesma transmissão.
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- Para além da vigilância constante no momento e no pós-colheita por parte da equipa de investigadores, poderá entrar em contacto durante o período de estudo em qualquer ocasião através dos contactos que serão fornecidos. Nós estimulamos a si ou aos seus familiares a colocar questões ou curiosidades a qualquer momento do estudo através desses mesmos contactos.
- Todo e qualquer dano decorrente do desenvolvimento deste projecto de investigação, e caso necessite de atendimento, ficará a cargo da instituição envolvida e do grupo de pesquisadores e será da nossa inteira responsabilidade. O seu tratamento e acompanhamento contínuo fazem parte de sua participação neste estudo.
- Será garantida a confidencialidade das informações geradas e a sua total privacidade na pesquisa. Mais ninguém para além da equipa de pesquisadores que cuidará de si poderá aceder aos dados obtidos. O seu nome e dados pessoais não serão revelados, sendo que apenas os dados agregados serão utilizados propósitos educativos ou de publicação, e serão arquivados em servidores protegidos do CIAS durante 5 anos. Todo o material biológico e dados individuais serão destruídos até ao final deste período.
- Não haverá qualquer custo ou forma de pagamento para a paciente pela sua participação no estudo.
- A sua participação neste estudo é completamente voluntária e tem direito a recusar-se a participar ou interromper a sua participação a qualquer momento sem penalidades ou perda de benefícios aos quais tem direito. Em caso de decidir interromper a sua participação no estudo, a equipa de investigadores deve ser comunicada e a colheita de amostras e estudos recorrentes para os exames relativos à investigação será imediatamente interrompida.

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Declaração de Consentimento menores

Eu, ____, nascida a __/__/____, declaro que li as informações no verso desta página, entendi o propósito deste estudo assim como os benefícios e riscos potenciais da participação no mesmo. Tive a oportunidade de fazer perguntas e todas foram gentilmente respondidas. Eu, por intermédio deste , dou livremente o meu consentimento para participar neste estudo.

Entendo que nem eu ou a voluntária deste estudo receberemos compensação monetária pela minha participação neste estudo e que essa participação é completamente voluntário e com voto de confidencialidade.

Eu recebi uma cópia assinada deste formulário de consentimento.

__________________________________ ____ / _____ / _____
(Assinatura do Responsável Legal) dia mês ano

____
(Nome do Responsável Legal – letra de forma)

Eu, abaixo assinado, expliquei completamente os detalhes relevantes deste estudo ao participante indicado acima.

____ ____ / ____ / ____
(Assinatura da pessoa que obteve o consentimento) dia mês ano