Description of F cells in Sickle Cell Anemia in Tanzania

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Background

•Sickle Cell Anemia (SCA) is a hereditary hemoglobin disorder that is characterized by the presence of hemoglobin (HbS). The deoxygenation of HbS results in structural deformability of red blood cells with pathological consequences in almost every organ system in the body. The phenotypic expression of SCD is highly variable, ranging asymtpomatic individuals to severe disease manifestations with high risk of morbidity and mortality. Tanzania ranks 5th in the world with the highest number of annual births of children with SCA after Nigeria, DRC, India and Angola.

•High levels of Hemoglobin F (HbF) have been associated with the milder forms of SCA disease. F cells are erythrocytes that contain measurable amount of HbF levels which are unevenly distributed within the cells. The distribution of HbF in F cells has been regarded as an important factor in the amelioration of SCA disease. The levels of HbF and F cells are highly genetically controlled but there is limited information on the epidemiological spectrum of F-cells and factors and mechanisms involved in influencing HbF and F cells.

Objectives

The overall objective is to determine the genetic determinants of F cells. The current report is the first step in this study and aims to provide a phenotypic description of the epidemiology of F cells in SCA in Tanzania. The specific objectives are 1) To determine the level of F-cells in Tanzania. 2) to evaluate the association between HbF and F cells in SCA patients 3) To determine the amount of HbF per F cell (HbF / Fcell)

Methodology: Population and Study design

The Muhimbili Sickle Cohort consists of prospective surveillance of SCA individuals in Dar-es-Salaam, Tanzania. It started in 2004, and has identified over 4,000 SCA individuals. A cohort of SCA individuals are part of a genome –wide association study (GWAS). The GWAS study, with genotyping done with Illumina Human Omnichip 2.5 platform with 1,827,523 SNPs typed for 1742 individuals.

This was a cross-sectional study conducted between November 2013 and April 2014. 107 SCA patients were randomly selected from the GWAS SCA cohort. 32 non-SCA individuals were selected as a control population.

Laboratory investigations included blood count (Sysmex) and HbF levels [(High Performance Liquid Chromatography (HPLC)] was done on venous blood. Anti- HbF antibody was used to stain the gamma chains to quantify F-cells by flow cytometry (FACSCalibur)

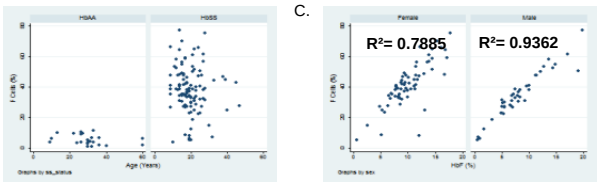
Results

Table 1: Description of the 139 individuals with HbF and F cell levels

	SCA	Non- SCA
Number (Male, Mean age)	107 (42 Male; 20yrs)	32 (18 Male; 31yrs)
Median HbF (%)	8.9 (IQR 6.9 - 11.6)	0.4 (IQR 0.2 - 1.1)
Median F cell (%)	38.3 (IQR 30.2 - 47.8)	4.6 (IQR 2.9 - 6.94)

• 3.8% of total SCA patients had RBC containing ≥ 10pg HbF per F cells

A. C.



B. D.

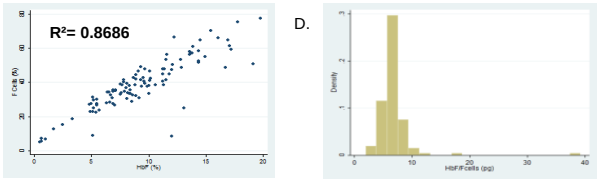


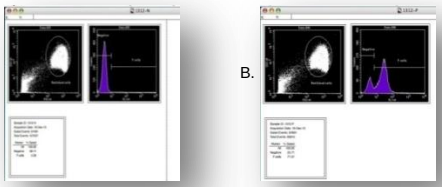
Figure 3: Fig 3a shows the association between % F cells with HbAA and HbSS . Fig 3b shows the correlation between HbF and F cells in all studied HbSS patients. Fig 3c shows a correlation between HbF and F cells with respect to sex and Fig 3d shows the distribution of HbF/ Fcell in SCA patients

Methodology: Phenotypic description of HbF and F cells

A. B. C.

Figure 1: Laboratory equipment used to obtain HbF and F cells. Figure 1a FBC (Sysmex), Fig 1b HbF (HPLC) and Fig 1c (FACSCalibur)

A. B.



HbF – 19.8%
F Cell – 77.37%

Figure 2: F cell quantification from Flow cytometry. Figure 2a and 2b represent unstained sample and stained sample respectively, of a SCA patient

Discussion and Conclusion

• As previously reported, this study confirms that HbF and F cell levels are high in SCA compared to non-SCA individuals.

•HbF and F-cell levels in SCA were higher in Tanzania compared to levels reported in Demogratic Republic of Congo where HbF [5.9% (1-27.5)]and F cells [2.19% (0 – 30.3)]. These suggest potential and significant differences in genetic modifiers in these two countries.

• There was a positive correlation between HbF and F cells (R^2 = 0.87). This suggests that the two variables might share common pathways.

• Males had stronger correlation between HbF and F cells compared to females (0.94 vs. 0.7752). One of the factors may be the presence of F cell production locus (FCP) on chromosome X which accounts for a wider variation in females

•3.8% of SCA patients studied had RBC containing ≥ 10pg HbF / F cell. Recent studies have hypothesized that a critical level of intracellular HbF of ≥ 10pg is thought to inhibit HbS polymer formation, hence a vital determinant of disease-severity than the total number of F cells or HbF levels. Further studies need to be done to determine spectrum of HbF per F cell within the Tanzanian SCA population

•The next step will be to explore the genetic factors determining F cells.

•The aim is to increase knowledge on the genetic and environmental factors that influence F-cells and HbF. This will contribute to increasing our understanding of factors and mechanisms involved in influencing HbF and F cells which in turn would lead to development of interventions for treatment of SCA.

Acknowledgement

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