

# Unveiling an Atypical Response to HIV-1 Infection by the Patient Carrier of the Beta-S Globin Gene and Duffy Antigen Gene Double Mutation

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## Abstract

It is generally accepted that red blood cell mutations have an effect on the carrier's response when exposed to certain pathogen agents. The present study was designed, in the Democratic Republic of Congo (DRC), to investigate the potential impact of the Duffy gene mutation associated with the carrier high susceptibility for HIV infection and, the low AIDS progress of the patient carrier of sickle-cell trait gene.

A descriptive cross-sectional study was conducted over a period of four years from 2013 to 2017 in Lubumbashi (Capital of the Haut-Katanga, the Southernmost province of the Democratic Republic of Congo). Three cohorts were identified including: 194 patients with homozygous ( $\beta\text{S}/\beta\text{S}$ ) mutation of sickle-cell anemia (*i.e.* Sickle Cell Disease, SCD) and their susceptibility to HIV-1 infection; 21 HIV-1 infected heterozygous ( $\beta\text{A}/\beta\text{S}$ ) patients; 59 none  $\beta\text{S}$  carrier ( $\beta\text{A}/\beta\text{A}$ ) of the general population. Furthermore, HIV-1 infected were also divided in two categories with or without antiretroviral therapy (ART). All patients were subjected to HIV-1 antibody and viral load detection, CD4+T-cell count, sickle-cell trait diagnosis (*i.e.*  $\beta\text{S}$  gene) and, Duffy antigen detection.

All participants were Duffy group negative (Duffy-46C/C). Among 194 homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients, only two were found HIV-1 positive with a rate of 1.03% as compared to 4% observed in DRC from the general population. One of the two homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients HIV-1 positive, without ARV, showed an HIV-1 viral load of 14,185 copies/ml, when the non-carriers ( $\beta\text{A}/\beta\text{A}$ ) HIV-1 positive patients, with ARV therapy, have a four-time higher viral load average of 56,088 copies/ml. Moreover, two heterozygous ( $\beta\text{A}/\beta\text{S}$ ) patients HIV-1 positive patients, without ARV therapy, presented a significant ( $p < 0.01$ ) lower viral load average of 7,401 copies/ml as compared to the non-carriers ( $\beta\text{A}/\beta\text{A}$ ) HIV-1 positive patients either with or without ARV therapy. The homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients presented a CD4+T- cell count of of 450 CD4/mm<sup>3</sup> respectively higher as compared to 244 and 431 CD4/mm<sup>3</sup> of the two HIV-1 positive heterozygous ( $\beta\text{A}/\beta\text{S}$ ) patients and the 8 non-carriers ( $\beta\text{A}/\beta\text{A}$ ) HIV-1 positive patients, all without ARV therapy. However, when one compared the CD4+T- cell count of heterozygous ( $\beta\text{A}/\beta\text{S}$ ) HIV-1 patients and  $\beta\text{A}/\beta\text{A}$  HIV-1 patients under ARV therapy, the former presented a significant ( $p < 0.01$ ) higher CD4 count than the latter.

In conclusion, the one homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD Duffy-46C/C HIV-1 positive patients without ARV therapy, showed, a higher CD4+T- cell count, without significant reduction of viral load, without any AIDS clinical signs. Furthermore, the heterozygous ( $\beta\text{A}/\beta\text{S}$ ) HIV-1 patients disclosed a significant higher CD4+T- cell count as well as a viral load reduction, therefore appeared to be better responder to ARV therapy than the ( $\beta\text{A}/\beta\text{A}$ ) HIV-1 positive patients. As it has been generally observed, African homozygous and heterozygous ( $\beta\text{A}/\beta\text{S}$ ) patients, systematically bared the double  $\beta\text{S}$  and Duffy -46C/C mutations and appear less susceptible to HIV-1 infection, whereas the two simultaneous mutations demonstrated a potential to work in synergy acting to slow progression of HIV disease.

**Keywords:** Human immunodeficiency virus; HIV-1; Duffy -46 C/C; Sickle cell diseases

## Introduction

It is known and well documented that genetic selective pressures occur from *Plasmodium falciparum*, *P. vivax* or *P. knowlesi* infection of red blood cells, such led to the selection of their cell membrane antigens, hemoglobin and enzymes thus associated to several mutation including, among others: Duffy antigen negative mutation which prevents the expression of the Duffy antigen on the surface of red blood cells; Sickle cell disease (SCD)  $\beta$  globin mutation; Thalassemia, with an absence or reduction of  $\alpha$  or  $\beta$  globin string; Deficit in glucose 6 phosphate dehydrogenase [1]. Moreover, various studies confirm that a large majority 88 to 100% of African black population is Duffy group negative (Duffy-46C/C) as well as 68% of African American population [2]. Although, the Duffy-46C/C mutation confers resistance to malaria due to *P. vivax* and *P. knowlesi* parasites, this protection is associated with a high susceptibility to HIV infection [2,3]. Indeed, the absence of the Duffy antigen appears to increase by 40% the risk by HIV infection and to be responsible of 11% of HIV prevalence in Africa [1,3-6]. Surprisingly, previous studies on homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients and HIV infection show that they were less susceptible to HIV and became slow progressors of the clinical expression of AIDS as compared to the general population ( $\beta\text{A}/\beta\text{A}$ ) without  $\beta$  globin mutation [7-10]. However, these studies did not take into account that this homozygous sickle patients have a high chance to be also Duffy negative, a mutation largely endemic in Africa.

In the context of these two apparently contradictory situations one can hypothesize that the two mutations have either an antagonistic effect or synergistic effect depending of the phase of infection. Meanwhile, the low seroprevalence of HIV found in the homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients appears to be associated to the  $\beta\text{S}$  gene mutation independently of the Duffy negative status [10,11].

Our study hypothesizes on the potential interactions of the Duffy-46C/C mutation (*i.e.* lack of Duffy Antigen Receptor for Chemokine, DARC) and  $\beta$  globin mutation of the homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients' carrier of both mutations, that would control their susceptibility (low prevalence) and slow progression of HIV infection. For this purpose and in order to compare the HIV infection among each participant and their status against Duffy and  $\beta$  globin mutation, we selected three cohorts including: One randomly selected homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD carriers (*i.e.* Sickle cell disease) patients; One heterozygous ( $\beta\text{A}/\beta\text{S}$ ) carriers HIV tested positive; And unmutated  $\beta\text{A}/\beta\text{A}$  individuals HIV tested positive.

## Methods

### Study site and population

The study was conducted in the Haut-Katanga Province

(Democratic Republic of the Congo, DRC) over a period of four years from December 2013 to November 2017. One cohort of the study population was randomly recruited among the patients attending three of the sickle cell care centers of the city of Lubumbashi (Haut-Katanga Province capital), both genders were represented ranging from 6 months to 40 years old (SM TS1; TS1). The two other cohorts were randomly selected HIV-1 positive patients including  $\beta\text{S}$  heterozygous carriers and  $\beta\text{A}/\beta\text{A}$  patients, from the HIV Center of the Swende Reference General Hospital (Lubumbashi).

### Sampling

From all 310 participants, 5ml of whole blood were collected on EDTA. After a selection made on the quality of the sample and, the inclusion and exclusion criteria, 274 samples remained qualified for the present study.

### Genetics

All participants were tested for the mutations  $\beta$  (SCD) and Duffy as well for HIV-1 antibody and viral load and, CD4+T- cell rate when confirmed HIV-1 seropositive.

### Sickle cell blood test

Hemoglobin electrophoresis was carried out on cellulose acetate at pH 8.5 alkaline to identify homozygous ( $\beta\text{S}/\beta\text{S}$ ) SCD patients, heterozygous ( $\beta\text{A}/\beta\text{S}$ ) carriers and,  $\beta\text{A}/\beta\text{A}$  non-SCD HIV-1 positive patients. Duffy antigen detection was done using indirect Coombs test.

### HIV diagnostic

HIV-1 antibody detection was done by the Alere Determine™ HIV-1/2 test [12], while the quantification of the viral load used the Abbot Realtime HIV-1 Viral Load Assay following manufactory guideline. CD4+T- cell rate was done by flow cytometry as previously described [13]. The potential mode of HIV transmission was estimated and recorded including vertical transmission from mother to newborn and, the number of blood transfusion.

### Data analysis

The Epi Info 7 software was used to interpret the results. Yates' correction for continuity (or Yates' chi-square test) was used at a significance threshold of  $p < 0.05$  for comparing two variable means (*i.e.* HIV versus genetic markers) for independence.

### Ethics

This study was approved by Kinshasa University Institutional Review Board and the National Ethics Committee (UNILU/EMC/071/2017). Written informed consent was obtained from the parents or legal

representatives of all subjects before study enrollment. Oral consent was obtained from each child assisted by their parents or legal representatives. A special authorization was obtained from the Director of the Sickle Cell Anemia Care Centers of Lubumbashi.

## Results

Among 310 homozygous ( $\beta S/\beta S$ ) patients selected, 194 blood samples were qualified (volume, conservation, etc.) to be tested. Also, 80 HIV-1 randomly selected positive patients, from the HIV outpatient clinic of the Swende Reference General Hospital, including 21 heterozygous ( $\beta A/\beta S$ ) patients, and 59 nonmutated ( $\beta A/\beta A$ ) patients were used as a comparative cohort (Table 1). Out of 194 homozygous ( $\beta S/\beta S$ ) SCD patients, 2 males tested positive for HIV antibodies (1.03%), from the age groups of 6 to 11 years, and 7 and 9 years. One of these two patient was absent and was not additionally tested (i.e. viral load, CD4).

HIV patients were either under treatment with WHO recommended antiretroviral therapy (ARV) or not treated at all (Table 2). The HIV-1 viral load of the  $\beta S/\beta S$  patient without ARV treatment, was of 14,185 copies/ml, as compared to mean values of 11,7084 copies/ml (of the 2  $\beta A/\beta S$  patients) and 81,229 copies/ml of 8  $\beta A/\beta A$  patients. Also, when compared the respective mean viral load among  $\beta A/\beta S$  and  $\beta A/\beta A$  HIV-1 positive patient under ARV treatment the difference is highly significant ( $p < 0.005$ ). Ultimately, besides an elevated HIV viral load, the clinical presentation of this young homozygous ( $\beta S/\beta S$ ) SCD patient did not show any signs associated with HIV infection, and his seropositivity was found only during the systematic HIV serology test performed on the cohort of homozygous ( $\beta S/\beta S$ ) SCD patients of this study.

A CD4 count of 450 CD4/mm<sup>3</sup> for the  $\beta S/\beta S$  HIV-1 patient (without ARV) was not significantly different ( $p > 0.05$ ) as compared to the CD4 count of the  $\beta A/\beta A$

Hemoglobin marker	Participant		HIV positive** (%)
	Origin*	Number tested	
$\beta S/\beta S$	SCD Center	194	2 (1.03)
$\beta A/\beta S$	HGR	21	21 (100)
$\beta A/\beta A$	HGR	59	59 (100)
Total		274	82

\*SCD: Sickle Cell Disease Center of Lubumbashi, DRC; HGR: Hôpital Général de Référence Sendwe (Swende General Referring Hospital) of Lubumbashi, DRC; \*\*HIV tested by Determine test

**Table 1:** Selected cohorts of Duffy blood group negative (Duffy-46C/C) negative patients tested for hemoglobin subunit beta ( $\beta$ ) and its variant (S) versus HIV infection status (Lubumbashi, Democratic Republic of Congo, 2013-2017).

Hemoglobin marker	Without ARV		With ARV	
	Number tested	Viral load *	Number tested	Viral load
$\beta S/\beta S$	1	14,185	0	0.00
$\beta A/\beta S$	2	11,7084 ± 5	19	7,401
$\beta A/\beta A$	8	81,220 ± 1	51	56,088 ± 3
Total	11		70	

\* mean value of Log of HIV copies by milliliter (copies/ml); \*\* One of the two  $\beta S/\beta S$ -HIV+ identified was missing during the study and not tested

**Table 2:** HIV viral load and antiretroviral (ARV) treatment among selected children study group from six to fifteen years old age class.

Hemoglobin marker	Without ARV		With ARV	
	Number tested	CD4*	Number tested	CD4
βS/βS	1	450	0	0.00
βA/βS	2	244 (± 53)	19	478 (± 57)**
βA/βA	8	431 (± 62)	50	364 (± 42)**
Total	11		69	

\*CD4 T cell count/mm<sup>3</sup>; \*\* Student Test: CD4 count of βA/βS versus βA/βA with with antiretroviral therapy (ARV): t-value = 3.98762. p-value = 0.000081; Children without ARV age class for the βS/βS, βA/βS and βA/βA cohorts was respectively of 6 to 11, 5 to 10 and 5 to 10; Children with ARV age class for βS/βS, βA/βS and βA/βA cohorts was respectively of 6 to 11, 11 to 15 and 11 to 15.

**Table 3:** T cell test value of HIV positive selected children by age class (6 to 15-year-old) and their antiretroviral treatment status.

βS and βA/βA HIV-1 positive patient with or with or without ARV. However, when we compare the response of the HIV patients under ARV of the βA/βA versus βA/βS, the latter presented a significantly higher CD4/mm<sup>3</sup> count (p<0.01) than for the βA/βA HIV-1 positive patient (Table 3).

From parent interviews as well as clinical data, the HIV mode of transmission was considered and revealed that 79 of 82 children (96.3%) were infected by the vertical pathway, while, surprisingly of a 1.5 female to male sex ratio, 3 male children (3, 7%) contracted HIV through blood transfusion (SM TS3; TS4; TS5).

## Discussion

Accordingly, with previously reported studies [14-16], the present results are in favor of an association between Duffy -46C/C and βS mutations on the HIV low susceptibility as well on the slow progression of infection observed in homozygous and heterozygous (βS) DARC negative patients.

In terms of HIV seroprevalence (*i.e.* infection) our results show an atypical association with the patients having the two obligatory mutations studied, Duffy -46C/C and βS. Indeed, if taken separately, these mutations have an antagonistic effect when the sickle cell mutation (βS) is associated with a reduced susceptibility to HIV infection and, the Duffy mutation (-46C/C) promotes susceptibility to HIV infection, however, these two mutations of a same carrier appear to have a synergistic effect and contribute to a low progression of HIV infection. Indeed, this low progression, after a certain stage of evolution of HIV infection, has been described among Duffy DARC negative patients [3] and secondarily concur with the βS mutation to slow down the progression of HIV infection.

In our observation of HIV-1 mother-to-child

transmission appears to be one of the main pathways for HIV transmission among children [17].

The scarcity of HIV-1-positive cases in homozygous (βS/βS) sickle-cell anemia did not allow us to make an accurate comparison of the average viral load and CD4 rates toward the (βA/βA) patients. Also, if our study showed that homozygous (βS/βS) SCD patient had the two compulsory mutations, other mutations such as α or β thalassemia, or G6PD were not studied and could potentially interfere on the HIV infectious pattern.

Ultimately, our observation is in favor of homozygous (βS/βS) SCD patients being less susceptible to HIV-1 infection. This suggests that the βS mutation appears to dominate that of Duffy -46C/C carriers and eventually contributing to slowing down HIV infection.

A comparative study including a cohort of HIV positive Indian homozygous (βS/βS) SCD Duffy positive patients could increase our understanding of the susceptibility to HIV infection and the progression of the clinical pattern of the disease.

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