Hematol Oncol Stem Cell Ther (2016) 9, 14–19

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/hemonc

ORIGINAL RESEARCH REPORT

Clinical events and their relation to the tumor necrosis factor-alpha and interleukin-10 genotypes in Sickle-Cell-Anemia patients

Jânio Emanuel Andrade Cavalcante [a], Rosângela Pinheiro Gonçalves Machado [b], Marilia Rocha Laurentino [a,*], Talyta Ellen de Jesus dos Santos [c], Izabel Cristina Justino Bandeira [c], Pedro Aurio Maia Filho [a], Monalisa Feliciano Figueiredo [d], Alice Maria Costa Martins [d], Romélia Pinheiro Gonçalves Lemes [d]

[a] *Post-Graduate Program in Pathology, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[b] *Post-Graduate Program in Development and Technological Innovation in Drugs, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[c] *Post-Graduate Program in Pharmaceutical Sciences, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[d] *Department of clinical and toxicological analysis, Federal University of Ceará, Fortaleza, Ceará, Brazil*

Received 5 May 2015; accepted 14 November 2015
Available online 2 December 2015

KEYWORDS
Anemia;
Cytokines;
Genetic;
Polymorphism;
Sickle cell

Abstract
Objective/background: Sickle-cell anemia (SCA) is a genetic blood disease characterized by chronic inflammation and a heterogeneous clinical picture. Serum tumor necrosis factor (TNF-alpha) and interleukin 10 (IL-10) levels are associated with the clinical course of SCA. This study aimed to evaluate the association between the frequency of the polymorphisms TNF-alpha-308 G → A, IL-10-1082 G → A, IL-10-819 C → T, and IL-10-592 A → C; serum TNF-alpha; and IL-10 levels, and the incidence of clinical events in SCA patients.
Methods: Polymerase chain reaction—restriction fragment length polymorphism and enzyme-linked immunosorbent assay were performed on 25 adults with SCA at the steady state; their data were compared with those for 26 healthy individuals.

* Corresponding author at: Department of Clinical and Toxicological Analysis, Federal University of Ceará, Rua Capitão Francisco Pedro, 1210, Rodolfo Teófilo, Fortaleza, Ceará 60430-370, Brazil.
E-mail address: marilialaurentino@gmail.com (M.R. Laurentino).

http://dx.doi.org/10.1016/j.hemonc.2015.11.002

Results: The most frequent genotype of the TNF-alpha polymorphism was GG (low producer), and the most frequent genotype of the IL-10 polymorphisms was ''low producer" (ACC ACC, ACC ATA, ATA ATA). The TNF-alpha levels were significantly higher in SCA in patients with acute chest syndrome (ACS). The IL-10 levels were reduced in polytransfusion and in patients with ACS. *Conclusion:* The patients presented prevalence of TNF-alpha and IL-10 low-profile producer. The cytokine serum levels presented an association with the presence of polytransfusion and ACS in SCA patients.

Introduction

Sickle-cell anemia (SCA) results from a point mutation, in which an adenine nucleobase in the sixth codon of the β-globin gene is replaced by a thymine (GAG → GTG). The molecular translation replaces glutamic acid with valine, thereby producing an abnormal form of hemoglobin called hemoglobin S [1]. Although the molecular lesion is limited to a single nucleotide, the SCA gene is pleiotropic and leads to multiple phenotypic expressions. SCA patients may present with various complications, such as recurrent episodes of vaso-occlusion, acute chest syndrome (ACS), stroke, infections, and priapism. These complications vary considerably among patients and over time [2—4].

The repeated polymerization of hemoglobin S can cause definitive damage to the structure of red blood cells, producing mainly intravascular hemolysis and vaso-occlusion, and triggering a cyclical cascade of reactions that culminates with the generation of reactive oxygen species and increased oxidative stress, reduced bioavailability of nitric oxide, endothelial injury, hypercoagulability, increased expression of adhesion molecules in blood and endothelial cells, ischemia/reperfusion injury, and chronic inflammation. Alone or in combination, these reactions are associated with inflammatory responses in various organs and can produce an array of other secondary pathological conditions [5].

For reasons not yet fully understood, the clinical outcome of SCA is highly variable. Several factors have been identified as modulators of clinical severity in SCA, including fetal hemoglobin (HbF) levels, association with α-thalassemia, β-globin haplotypes, and the presence of single-nucleotide polymorphisms (SNPs) [6—8]. SNPs are sites in which the genomic DNA sequence of a percentage of individuals in the population differs by a single base. It is the most common type of variation in the human genome [9].

Some authors have hypothesized that the phenotype of SCA patients is modulated by polymorphisms in genes involved in inflammation, cell interaction (vascular cell adhesion molecule 1, complement receptor 1, P-selectin, and alpha V integrin), modulation of oxidant injury, and nitric-oxide biology (nitric oxide synthase 2, nitric oxide synthase 3, and arginase, type II) [10—12].

The polymorphism tumor necrosis factor (TNF-alpha)-308 G → A has been shown to be associated with an increased risk of stroke in patients with SCA [13]. The polymorphism in the interleukin 10 (IL-10) gene is expressed in three genotypes: AA, AG, and GG, associated with low, intermediate, and high IL-10 production, respectively.

Synthesized mainly by activated mononuclear phagocytes, TNF-alpha is the main mediator of acute inflammatory response. It acts by recruiting neutrophils and monocytes to the site of inflammation through a variety of mechanisms [14]. IL-10 acts directly on cluster of differentiation 4^+ T cells inhibiting the proliferation and production of IL-2, interferon gamma, IL-4, IL-5, and TNF.

Importantly, cytokine-gene polymorphisms and serum concentrations of cytokines have been correlated with clinical heterogeneity in SCA. In addition, polymorphisms may act as modulating factors by inducing changes that can potentially interfere with important pathways involved in the pathophysiology of the disease. The phenotypes typically observed in SCA have been associated with polymorphisms in genes encoding cytokines. Thus, much attention has been given to the development of markers susceptible to specific SCA-related phenotypes and laboratory abnormalities with the purpose of preventing or minimizing clinical symptoms through early intervention. The objective of the present study was to evaluate the association between the frequency of the polymorphisms TNF-alpha-308 G → A, IL-10-1082 G → A, IL-10-819 C → T, and IL-10-592 A → C; serum TNF-alpha; and IL-10 levels, and the incidence of clinical events in a sample of Brazilian SCA patients.

Materials and methods

Participants

This analytical study was based on a cross-sectional sample of 25 adults diagnosed with SCA attending the hematology outpatient service of the Walter Cantídio University Hospital in Fortaleza, Brazil, in 2013 and 2014. All patients were at the steady state in accordance with the criteria proposed by Ballas (2012) [15]: absence of painful episodes and/or intercurrent illnesses, such as infections and inflammation in the 4 weeks preceding the study; no hospital admissions in the 3 days preceding the study; and no blood transfusions in the 4 months preceding the study. Patients with infectious diseases, hemoglobin profiles incompatible with SCA, history of blood transfusion within the preceding 4 months, and/or inflammatory episodes during the study were excluded. Clinical data, complete blood count, and HbF values were collected from the patients' medical records. Demographic data were obtained through interviews with the patients. The study protocol was approved by the Walter Cantídio University Hospital Research Ethics Committee. All patients and controls gave their informed written