Association of the +874 T/A IFN gamma polymorphism with infections in sickle cell disease

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Keywords: sickle cell, infection, polymorphism, genetic association, cytokine

Abbreviated title: IFN gamma polymorphism and sickle cell disease

ABSTRACT

Infectious complications are a leading cause of morbidity and mortality in patients with sickle cell disease. Several mechanisms are supposed to contribute to this susceptibility. The exact reasons of the propensity of sickle cell patients to infection are not clear and are still matter of debate.

Interferon gamma is a key cytokine involved namely in the defence against intracellular pathogens. We investigated a possible association of +874 T/A IFNγ polymorphism and infectious complications in sickle cell patients.

Seventy-two sickle cell patients were typed for +874 T/A IFNγ polymorphism.

Genotype frequencies were different between cases and controls. Indeed, T allele frequency was significantly higher in patients with infections than in patients without infections (χ^2 = 6.23 ; p = 0.013).

Our results suggest that +874 T/A IFNγ polymorphism is associated with infectious complications in sickle cell patients. The T allele could be involved in infections in sickle cell patients.

KEYWORDS: sickle cell, infection, polymorphism, genetic association, cytokine

Abbreviations: IFNγ interferon gamma, SCD sickle cell disease, Hb haemoglobin, SNP single nucleotide polymorphism, MBP mannose binding protein

INTRODUCTION

Sickle cell disease (SCD) is an inherited disorder caused by a point mutation in the β-globin gene resulting in the substitution of valine for glutamic acid at the position 6. This substitution leads to the presence of the sickle haemoglobin (HbS). Sickle haemoglobin is an atypical Hb which stands for normal Hb (HbA).

About fifty million persons are affected by this haemoglobinopathy all over the world. In most of countries, SCD is a major public health problem.

SCD is the first genetic disease in the Caribbean islands. This haemoglobinopathy is characterized by high mortality and morbidity, and various vaso occlusive symptoms very debilitating by their frequency and consequences. Besides, patients with SCD have to face very severe complications such as severe anemia, stroke, acute chest syndrome, osteonecrosis of femoral and humeral heads, infections…

In the last twenty years, before the systematic use of vaccines and prophylactic penicillin, bacterial infections were the leading cause of death in young children with SCD; nevertheless it is still a significant cause of morbidity in older patients (1,2). Patients with SCD have an increased susceptibility to infection particularly with encapsulated organisms: *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella spp* (3-7).
Bacterial infection may also be caused by intracellular organisms such as *Chlamydia pneumoniae, Mycoplasma pneumoniae, Salmonella spp, Escherichia coli* (7-9) and extracellular pathogens such as *Staphylococcus aureus*.
Meningitis and septicaemia are the more serious infections and most of the time are caused by *Streptococcus pneumoniae and Salmonella spp* (6,9-13).
These infections are all the more severe since they may cause other complications such as ostemomyelitis and leg ulcers.

Patients with SCD are at risk of osteomyelitis which is specific in SCD since it often occurs in ischemic bone. Moreover, this complication affects frequently femur, humerus, vertebra, ribs and sternum. Osteomyelitis occur far more frequently in patients with SCD than in general population and the offending pathogens are generally *Salmonella* and *Streptococcus pneumoniae* (6,9-13).

As for leg ulceration, this is a common complication in SCD. Because of its frequency, chronicity and resistance to therapy, this complication is an important cause of morbidity.
Leg ulcers vary considerably in size and can be very painful. Bacterial infection can worsen this complication. They appear to occur either spontaneously or as a result of local trauma and often persist for long durations of time.
The incidence of leg ulcers varies greatly:

- 70 percent of patients over 15 years old was reported in Jamaïca (14),
- 5 percent of patients over 15 years old in the United Sates (15), 10 to 15 percent of patients over 15 years old in West Africa(16) ,
- less than 1 percent of patients over 15 years old in India and Saudi Arabia (17,18).

All these clinical manifestations vary within patients which remains unexplained.
SCD is extremely heterogeneous phenotypically. Some patients are often sick whereas others never present complications. This matter of fact drew many scientists' attention. Reasons of

this variability are not clear. Genetic and environmental causes seem to be involved but are still the subject of discussions.
Haplotype differences cannot exclusively explain this variability.
Nowadays, the exact reasons of the propensity of sickle cell patients to develop infections are still unknown. Genetic basis of this susceptibility has been considered by scientists in the last ten years.
Some authors investigated the polymorphism of the gene encoding the Fc Receptor (FcγRIIA) (19), and the polymorphism of the gene encoding the *Mannose Binding protein* (M.B.P) (20) as they considered these genes as potential factor risks for infections in patients with sickle cell disease. Polymorphism of HLA system (21)and the gene encoding the myeloperoxidase (22) have also been studied.

Because interferon gamma (IFNγ) plays a key role in immune response against infection, we considered this interferon as a genetic modulator of infection in sickle cell patients.
Interferon gamma is the sole type II interferon. It is a pleiotropic cytokine produced by activated T cells and natural killer cells.
Antigen-presenting cells such as mononuclear phagocytes, dendritic cells, are target of IFNγ. In response to an infectious stimulus, this cytokine enhances the expression of Major Histocompatibility Complex (MHC) class II and class I molecules on the surface of those cells and so, increases antigen processing and presentation to T cells. IFNγ can also activate microbicidal effector functions of macrophages
In humans, IFN gene is located on chromosome 12 (12q15). It consists in four exons and three introns (23).
In this study, we focused on the mutation located in the first intron at nucleotide + 874 mutation + 874 G/A (24).
There are previous reports of an association of this single nucleotide polymorphism (SNP) and infectious diseases such as tuberculosis (25) and severe acute respiratory syndrome (26).

We hypothesized that this SNP is associated with infectious complications in sickle cell patients.

MATERIAL AND METHODS

This is a case-control study on sickle cell patients attending the Centre Intégré de la Drépanocytose (Centre Hospitalier du Lamentin, Martinique). The present study had local ethics committee approval, and written informed consent was obtained from all participants.

Seventy-two patients were included. Thirty-seven of them had already suffered from infections such as osteomyelitis, septicaemia. The others (35 patients) had never experimented any infection. There were 40 women and 32 men from 20 to 66 years old.

DNA isolation

Genomic DNA was isolated from peripheral blood collected on EDTA using a salting out method (27).

Genotyping

Polymorphism of IFNγ was determined using the CYTGEN kit (One Lambda, Inc) following manufacturer's instructions.

Statistical analysis

The association of IFNγ polymorphism and infections in sickle cell patients was evaluated by the chi-square test and p values smaller than of 0.05 were considered statistically significant.

RESULTS

In the case group, forty-three infections such as septicaemia, osteomyelitis, leg ulcerations, and infections of the urinary tract were counted.

Pathogens more frequently involved in those infections were *Staphylococcus aureus* (34.9%), *Escherichia* coli (27.9%) and *Klebsiella*(11.6%) (see table 1).

Table 2 presents the frequency of the different type of infections and the pathogens involved in these infections.

Results of IFNγ typing are presented on table 3.

Twenty-five patients in the control group (77.1 %) presented AA genotype whereas in the case group, there were seventeen (48.6%).

Only eight patients in the control group (22.9 %) presented TT or TA genotype whereas in the case group, there were nineteen (51.4%).

There are significantly more patients with infections carrying the T variant than patients without infections ($\chi^2 = 6.23$; $p = 0.013$).
T allele seems to be a risk factor for infection in sickle cell patients.

DISCUSSION

Patients with homozygous SCD (SS patients) are at increased risk of infection with *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Salmonella* spp, *Escherichia coli* and *Klebsiella* spp. In our population, the offending pathogens involved in the infections occurring belonged to this list.

Several mechanisms are believed to contribute to the susceptibility of sickle cell patients to infections, a major factor being the early loss of splenic function (28,29). However, this justification is insufficient since functional splenectomy often occurs after the first infectious complications.
The unanimous opinion is that opsonic defect is a factor contributing to infection, but the reasons of this defect are still discussed.

Nowadays, the exact reasons of the propensity of sickle cell patients to infection are still unknown. In the last ten years, genetic basis of the variability of the clinical course of SCD has been considered.

There are a lot of candidate genes from the immune system for an association with infections in sickle cell patients.
Scientists investigated polymorphism of the gene encoding Fc human immunoglobulin G Receptor II A (FcγRIIA) (19), and polymorphism of the gene encoding the *mannose-binding protein* as they considered these genes as potential risk factors for infections in patients with sickle cell disease.
Norris found that the H/H^{131} FcγRIIA genotype is overrepresented in sickle cell children with a history of *Haemophilus influenzae* type b infection whereas other studies suggested just the opposite: underrepresentation of H/H^{131} genotype in individuals with a history of encapsulated organism infection (19).
Neonato found an association between low-producing MBP genotypes and the absence of infection in children with SCD (20).

Tamouza found an association between HLA class II alleles and infections in sickle cell patients. According to this study, HLA-DQB1*03 is associated with severe infections whereas HLA-DRB1*15 confers a protective effect against infections (21).
More recently, Costa and co-workers investigated the gene encoding the myeloperoxidase as a genetic factor increasing susceptibility to infection in sickle cell patients (22). They studied the G to A polymorphism of the promoter of this gene (position -463) and found that the presence of A allele increased the susceptibility to infections.

Our results suggest that IFNγ gene could be involved in infectious complications in sickle cell patients. Indeed, the frequency of T allele is significantly higher in patients who had already suffered from infections than in patients who had never suffered from any infection.
Several authors found associations between some genes and the susceptibility to infections in sickle cell patients. They found that these genes might contribute to this susceptibility.
So, one can consider that the propensity of sickle cell patients to develop infection could be the result of the modulation of various genes since numerous genetic associations have been found.

Our results revealed, for the first time, that the + 874 T/A IFNγ SNP may be a genetic modulator of infectious complications in sickle cell patients ($\chi^2 = 6.23$; $p = 0.013$). We found an association between T allele and those complications.
These investigations have to be furthered in a larger sample in order to confirm this association. Such a result is of interest since it can provide a better management of sickle patients with the T variant.

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REFERENCES

(1) Leikin SL, Gallagher D, Kinney TR, Sloane D, Klug P, Rida W. Mortality in children and adolescents with sickle cell disease. Pediatrics 1989;84(3):500-508.

(2) Gill FM, Sleeper LA, Weiner SJ, Brown AK, Bellevue R, Grover R, et al. Clinical events in the first decade in a cohort of infants with sickle cell disease. Blood 1995;86(2):776-783.

(3) Granoff DM, Basden M. Haemophilus influenzae infections in Fresno County, California: A prospective study of the effects of age, race, and contact with a case on incidence of disease. Journal of Infectious Diseases 1980;141(1):40-46.

(4) Lobel JS, Bove KE. Clinicopathologic characteristics of septicemia in sickle cell disease. American Journal of Diseases of Children 1982;136(6):543-547.

(5) Powars D, Overturf G, Turner E. Is there an increased risk of Haemophilus influenzae septicemia in children with sickle cell anemia? Pediatrics 1983;71(6):927-931.

(6) Zarkowsky HS, Gallagher D, Gill FM. Bacteremia in sickle hemoglobinopathies. Journal of Pediatrics 1986;109(4):579-585.

(7) Magnus SA, Hambleton IR, Moosdeen F, Serjeant GR. Recurrent infections in homozygous sickle cell disease. Archives of Disease in Childhood 1999;80(6):537-541.

(8) Shulman ST, Bartlett J, Clyde Jr. WA, Ayoub EM. The unusual severity of Mycoplasmal pneumonia in children with sickle-cell disease. New England Journal of Medicine 1972;287(4):164-167.

(9) Wright J, Thomas P, Serjeant GR. Septicemia caused by salmonella infection: An overlooked complication of sickle cell disease. Journal of Pediatrics 1997;130(3):394-399.

(10) Robinson MG, Watson RJ. Pneumococcal meningitis in sickle-cell anemia. New England Journal of Medicine 1966;274(18):1006-1008.

(11) Overturf GD, Powars D, Baraff LJ. Bacterial meningitis and septicemia in sickle cell disease. American Journal of Diseases of Children 1977;131(7):784-787.

(12) Onwubalili JK. Sickle cell disease and infection. Journal of Infection 1983;7(1):2-20.

(13) Wong W-, Overturf GD, Powars DR. Infection caused by Streptococcus pneumoniae in children with sickle cell disease: Epidemiology, immunologic mechanisms, prophylaxis, and vaccination. Clinical Infectious Diseases 1992;14(5):1124-1136.

(14) Serjeant GR. Leg ulceration in sickle cell anemia. Archives of Internal Medicine 1974;133(4):690-694.

(15) Koshy M, Entsuah R, Koranda A, Kraus AP, Johnson R, Bellvue R, et al. Leg ulcers in patients with sickle cell disease. Blood 1989;74(4):1403-1408.

(16) Adedeji MO, Ukoli FA. Haematological factors associated with leg ulcer in sickle cell disease. Tropical and Geographical Medicine 1987;39(4):354-356.

(17) Kar BC, Kulozik AE, Sirr S. Sickle cell disease in Orissa State, India. Lancet 1986;2(8517):1198-1201.

(18) Padmos MA, Roberts GT, Sackey K, Kulozik A, Bail S, Morris JS, et al. Two different forms of homozygous sickle cell disease occur in Saudi Arabia. British Journal of Haematology 1991;79(1):93-98.

(19) Norris CF, Surrey S, Bunin GR, Schwartz E, Buchanan GR, McKenzie SE. Relationship between Fc receptor IIA polymorphism and infection in children with sickle cell disease. Journal of Pediatrics 1996;128(6):813-819.

(20) Neonato M-, Lu CY, Guilloud-Bataille M, Lapouméroulie C, Nabeel-Jassim H, Dabit D, et al. Genetic polymorphism of the mannose-binding protein gene in children with sickle cell disease: Identification of three new variant alleles and relationship to infections. European Journal of Human Genetics 1999;7(6):679-686.

(21) Tamouza R, Neonato M-, Busson M, Marzais F, Girot R, Labie D, et al. Infectious complications in sickle cell disease are influenced by HLA class II alleles. Human Immunology 2002;63(3):194-199.

(22) Costa RNP, Conran N, Albuquerque DM, Soares PH, Saad STO, Costa FF. Association of the G-463A myeloperoxidase polymorphism with infection in sickle cell anemia. Haematologica 2005;90(7):977-979.

(23) Gray PW, Goeddel DV. Structure of the human immune interferon gene. Nature 1982;298(5877):859-863.

(24) Pravica V, Perrey C, Stevens A, Lee J-, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-γ gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. Human Immunology 2000;61(9):863-866.

(25) Tso HW, Ip WK, Chong WP, Tam CM, Chiang AKS, Lau YL. Association of interferon gamma and interleukin 10 genes with tuberculosis in Hong Kong Chinese. Genes Immun. 2005;6(4):358-363.

(26) Chong WP, Ip WKE, Tso GHW, Ng MW, Wong WHS, Law HKW, et al. The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infectious Diseases 2006;6.

(27) Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 1988;16(3):1215.

(28) Winkelstein JA, Drachman RH. Deficiency of pneumococcal serum opsonizing activity in sickle-cell disease. New England Journal of Medicine 1968;279(9):459-466.

(29) Hand WL, King NL. Deficiency of serum bactericidal activity against Salmonella typhimurium in sickle cell anaemia. Clinical and Experimental Immunology 1977;30(2):262-270.

TABLES

Table 1. Frequency of the different offending pathogens identified in this population

Pathogens	Number of events	%
Bacteriodes ovatus	2	4.7
Enterobacter	3	7
Escherichia coli	12	27.9
Klebsiella	5	11.6
Proteus mirabilis	1	2.3
Pseudomonas aeruginosa	1	2.3
Salmonella sp.	4	9.3
Staphylococcus aureus	15	34.9

Table 2. Frequency of the different type of infections, and pathogens incriminated

Infections N = 43	Pathogens involved	%
Septicaemia	*Klebsiella*	25
N = 8 (19%)	*Escherichia coli*	25
	Bacteriodes ovatus	12.5
	Staphylococcus	12.5
	Enterobacter	12.5
	Salmonella sp	12.5
Osteomyelitis	*Salmonella sp*	50
N = 4 (9%)	*Bacteriodes ovatus*	25
	Staphylococcus aureus	25
Leg ulceration	*Staphylococcus aureus*	81.25
N = 16 (37%)	*Enterobacter*	6.25
	Proteus mirabilis	6.25
	Pseudomonas aeruginosa	6.25
Infections of the	*Escherichia coli*	66.7
urinary tract	*Klebsiella*	20
N = 15 (35%)	*Enterobacter*	6.7
	Salmonella	6.7

Table 3. Interferon gamma polymorphism at position +874 in sickle cell patients

Genotype	Control n = 35 F (%)	Case n = 37 F (%)	χ^2	p value	p_c value
AA	27 (77.1)	18 (48.6)			
TT+TA	8 (22.9)	19 (51.4)	6.23	0.013	0.024