Infectious complications in sickle cell disease and HLA polymorphism

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ABSTRACT

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

Besides, there is a striking variability of the clinical course of the disease between patients. This drew many scientists' attention.

Genetic factors have been investigated as potential factor risks for infection in sickle cell patients, for example: HLA system; genes encoding Fc Human Immunoglobulin G receptor IIA, mannose-binding protein, and myeloperoxidase.

Associations have been found, but all scientists agree that investigations have to be furthered.

We investigated an association between HLA DRB1 and DQB1 polymorphism and infectious complications in sickle cell patients.

Genotype frequencies were different between cases and controls for DRB1 locus. Indeed, HLA-DRB1*11 frequency was significantly higher in patients without infections than in patients with infections ($\chi^2 = 5.02$; $p = 0.025$).

KEY WORDS: sickle cell, infection, polymorphism, genetic association, HLA system

Abbreviations: SCD sickle cell disease, Hb haemoglobin, HLA human leukocyte antigen,

INTRODUCTION

Sickle cell disease (SCD) is a haemoglobinopathy characterized by the presence of the sickle haemoglobin (HbS), an atypical Hb which stands for normal Hb (HbA).

It is an autosomal recessive disorder. In 1956, Ingram determined that a point mutation in the gene coding the β chain of the haemoglobin molecule resulted in a single amino acid substitution (valine for glutamic acid) in the β globin chain, which, when present on both chromosomes in a patient, leads to sickle cell haemoglobin or Hb S (1).

SCD is the most important haemoglobinopahy since fifty millions persons are affected all over the world. In most of countries this pathology is a major public health problem.

SCD is very frequent in Africa, North America (USA), South America (Brazil) and in the West Indies. It is also found in population of Maghreb (Algeria, Morocco, and Tunisia),

Sicily, Greece, the Middle East, and Saudi Arabia. SCD spread in Western Europe: France, Britain, Portugal, Belgium, Germany…
In Martinique, an island of the French West Indies, 11% of the population is thought to have the sickle cell trait. (People with sickle cell trait have inherited the HbA gene form one parent and the HbS gene from the other).

During the third month of life of a sickle cell patient, vaso-occlusive crisis can occur. People with SCD also have to face anaemia, repeated infections and various debilitating complications such as stroke, acute chest syndrome, osteonecrosis of the femoral and the humeral heads, retinopathy, cardiomegaly, leg ulceration….
Acute painful vaso-occlusive crises are the most common and earliest, clinical manifestation of SCD. It is the hallmark manifestation of this disease. They are characterized by pain and fever.
Sickle cell disease is characterized by periods of stability, punctuated by episodes of severe pain involving the back, chest, abdomen, and joints. This syndrome is referred to as the acute painful crisis or vaso-occlusive crisis (2,3).

Before the systematic use of vaccines and prophylactic penicillin, bacterial infections were the leading cause of death in young children with SCD. Nevertheless, despite this prophylactic program, infections are still a significant cause of morbidity in older patients. (4,5). Patients with SCD have an increased susceptibility to infection particularly with encapsulated organisms: *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella spp* (6-10).
Bacterial infection may also be caused by intracellular organisms such as *Chlamydia pneumoniae, Mycoplasma pneumoniae, Salmonella spp, Escherichia coli* (10-12) 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* (9,12-16).

Besides, patients with SCD are at risk for 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* (9,12-16).

Furthermore, leg ulceration is a common complication of SCD. Because of its frequency, chronicity and resistance to therapy, it is an important cause of morbidity.
Leg ulcers can be very painful and can be worsened by a bacterial infection.
They appear to occur either spontaneously or as a result of local trauma and often persist for long durations of time.

All these clinical manifestations vary within patients which remains unexplained.
Besides, the reasons of the extreme susceptibility of sickle cell patients to infection are matter of debate.
Several mechanisms are believed to contribute to this susceptibility, a major factor being the early loss of splenic function.(17,18). However, this justification is incomplete 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.

Genetic bases of this susceptibility have been considered by scientists in the last twenty 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 HLA system (Human Leukocyte Antigen) plays a key role in immune system, scientists investigated the potential association between this system and infectious complications in sickle cell disease.
HLA system is a gene complex encoding glycoproteins involved in differentiating self from non-self. Human histocompatibility antigens are known as *Human Leukocyte Antigen* (H.L.A).
HLA molecules bind peptide fragments derived from pathogens and display them on cell surface for recognition by the appropriate T cells. Each T cell bears a cell surface receptor (T-cell receptor or TCR) that typically recognizes a peptide determinant bound to a molecule encoded by the HLA system.

Human histocompatibilty molecules, known as HLA molecules, are encoded by genes located on the short arm of chromosome 6.

There are three different types of HLA genes:

- class I with HLA-A, B and C genes, HLA-E, F and G genes and many other genes which functions are still unknown,
- class II with HLA-DR, DP and DQ notably,
- Class III. These genes encode other proteins related to the immune response. For example, genes for C4, C2, and B factor, components of the complement, and genes encoding critical cytokines such as TNF α and β (*Tumour Necrosis Factor*).

In this study we investigated HLA system class II genes association with infections 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, the cases, had already suffered from infections such as osteomyelitis, septicaemia. The others (35 patients), control patients, had never experienced 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 (23).

HLA typing

HLA class II typing was performed by polymerase chain reaction sequence specific oligonucleotide (PCR-SSO).

For HLA class II genes, the RSSO2B1 and RSSO2QB1 genotyping kits (One Lambda, Inc) were used respectively for HLA-DRB1 and HLA-DQB1 typing. Those kits were used according to the specifications of the manufacturer.

Statistical analysis

The association of the polymorphism of HLA system and infections in sickle cell patients was evaluated by the chi-square test and p value less than 0.05 was 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 shows the distribution of HLA-DRB1 alleles.

In this population, DRB1*13 and DRB1*15, were the more frequent alleles respectively with 20.8 and 19.4%.

In the control group, the predominant allele was DRB1*13 (13.6%) followed by DRB1*15 (17.1%) and DRB1*11 (17.1%). Likewise, for the cases, the predominant allele was DRB1*13 (23%), then, DRB1*15 (21.6%).

We could notice that the frequency of DRB1*11 was higher in patients without infections (17.1%) patients with infections (5.4%). Chi-square test revealed that this difference was significant ($\chi^2 = 5.02$, $p = 0.025$).

Results of frequency of HLA-DQB1 alleles are presented in table 3.

The predominant alleles were DQB1*06 (40.3%), then DQB1*05 (18.7%) and DQB1*03 (18.7%).

In the control group, the predominant allele was DQB1*06 (37.1%) followed by DQB1*05 (21.4%). For the cases, the predominant allele was DQB1*06 (43.2%) as well, but the second was DQB1*03 (18.9%).

There was no significant difference between the distribution of HLA-DQB1 alleles in patients with infections and in patients without infections.

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 this population, the offending pathogens were *Staphylococcus aureus* (34.9%), *Escherichia coli* (27.9%) and *Klebsiella* (11.6%).

There are a lot of candidate genes from the immune system for an association with infections in sickle cell patients.

Scientists investigated the polymorphism of the gene encoding Fc human immunoglobulin G Receptor II A (FcγRIIA) (19), and the polymorphism of the gene encoding the *mannose-binding protein* (MBP) (20) 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).

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.

Because HLA system plays a key role in the immune system, scientists investigated the potential association between this system and infectious complications in sickle cell disease.

Tamouza et al. worked on a population of 80 patients with sickle cell disease. Forty-three of them have presented major infectious complications (meningitis, septicaemia or osteomyelitis), the others (37 patients) have never experimented such complications.

The most frequent microrganisms involved in infections in the group of 43 patients were *Streptococcus pneumoniae*, *Salmonella spp*, *Staphylococcus aureus* and *Haemophilus influenzae*.

They found significantly more patients without infectious carrying HLA-DRB1*15 allele than did patients with infections ($\chi^2 = 10.47$, $p_c = 0.01$). They also found significantly more patients with severe infections carrying HLA-DQB1*03 allele than did the others ($\chi^2 = 9.41$, $p_C = 0.01$). These results suggest a protective effect of HLA-DRB1*15 allele against infections in patients with sickle cell disease. On the other hand, patients with SCD carrying HLA-DQB1*03 allele would be at increased risk for infectious complications (21).

On the contrary of Tamouza, we didn't found these associations. In our population, HLA-DRB1*15 frequency was not significantly different between cases and controls (respectively 21.6% and 17.1%). Likewise, HLA-DQB1*03 frequency was almost the same between the cases and the control group (respectively 18.9% and 18.6%). This could be explained by the fact that there is a linkage disequilibrium between DQB1*03 and alleles. Since those DRB1 alleles (DRB1*13 and DRB1*15) are well represented in this population (for the cases, respectively 23% and 21.6% and for the control group, respectively 18.6% and 17.6%), we could explain the reason why DQB1*03 frequency is almost the same between the control group and the cases.

However, our results showed that HLA-DRB1*11 was significantly more frequent in patients without infections than in patients who had already suffered from infections ($\chi^2 = 5.02$, $p= 0.025$, $p_C = 0.026$). Our results suggested that HLA-DRB1*11 would have a protective effect against infections.

In conclusion, our results suggest that HLA class II alleles could influence the clinical course of sickle cell disease. Indeed, we found that HLA-DRB1*11 allele could have a protective effect against infection.

Since other associations have been found between genetic factors and infections, we can consider that the propensity of sickle cell patients to develop infection could be the result of the action of various genetic modulators.

As our study has been conducted on a very small sample, and our results refute Tamouza's, the tests should be performed in a larger population.

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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. Allele frequencies at DRB1 locus

Séries alléliques	Control patients n = 70		Cases n = 74		χ^2	p	p_c
DRB1*	AF	%	AF	%			
01	5	7.1	3	4		NS	
03	7	10	11	14.9		NS	
04	1	1.4	1	1.3		NS	
07	6	8.6	3	4		NS	
08	5	7.1	8	10.8		NS	
09	1	1.4	2	2.7		NS	
10	1	1.4	1	1.3		NS	
11	12	17.1	4	5.4	χ^2=5.02	p=0.025	0.026
12	2	2.9	4	5.4		NS	
13	13	18.6	17	23		NS	
14	2	2.9	2	2.7		NS	
15	12	17.1	16	21.6		NS	
16	3	4.3	2	2.7		NS	

AF: allele frequency NS : not significant

Table 3. Allelic frequencies at DQB1 locus

Séries alléliques	Control patients n =70		Cases n =74		χ^2	p	p_c
DQB1*	AF	%	AF	%			
02	14	20	12	16.2		NS	
03	13	18.6	14	18.9		NS	
04	2	2.9	4	5.4		NS	
05	15	21.4	12	16.2		NS	
06	26	37.1	32	43.2		NS	

AF: allele frequency NS: not significant