RESEARCH ARTICLE

Editorial Process: Submission:08/12/2023 Acceptance:01/22/2024

HLA-B*58 and HLA-B*27 Play a Role in the Development of Acute Leukemia: A Case Control Study

Narimane Habour Nouar[1], Nabil Yafour[2]*, Bouali Youcef Youcef[3], Rachid Bouhass[2], Mohammed Chekkal[4], Mohamed Brahimi[2], Mohamed Amine Bekadja[2], Tewfik Sahraoui[1]

Abstract

Background: Acute leukemia (AL) constitutes a group of malignant hematological diseases with multifactor origins. Some human leukocyte alleles (HLA) may be important genetic risk factors for development of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). It is still unknown whether there is a relationship between ALL and AML with some alleles of the major histocompatibility complex. Our study looks specifically at western and southwest Algerian populations. **Method:** Using the polymerase chain reaction with the sequence specific probe (PCR-SSP) method, we investigated the relationship of HLA-B alleles in 163 Algerian AL patients and 293 controls from the same ethnic origin. The study ran from 2013 – 2020. **Results:** Allele frequencies of HLA-B*27 and HLA-B*58 was higher in AL patients compared with control individuals; p=0.05 and p=0.03 respectively. Interestingly, all patients carrying HLA-B*27 allele and 88% of patients carrying HLA-B*58 allele had AML. However, there were no significant differences when we compared these results with the rest of AL group (HLA-B*X allele) (p=0.387). Response to induction chemotherapy treatment were comparable between the two patient groups 67% and 65% (p=0.978) respectively. **Conclusion:** These results suggest that the HLA-B*27 and HLA-B*58, may be factors predisposing individuals to acute leukemia, in west and southwest Algerian patients. A large-scale study is still needed to confirm these findings.

Keywords: HLA-B- Acute Leukemia- PCR SSP- HLA Typing- Algeria

Asian Pac J Cancer Prev, **25 (1)**, 169-173

Introduction

Characterized by the excessive proliferation of immature hematopoietic cells (blasts) in the bone marrow, acute leukemia (AL) constitutes a group of clonal, malignant, hematological diseases with multifactor origins [1, 2]. An accumulation of these cells in the peripheral blood leads to the development of fatal infections that can lead to death [3, 4]. Epidemiological studies indicate that acute lymphoblastic leukemia (ALL) affects mainly children, whereas acute myeloid leukemia (AML) affects young adults and its incidence increases with age [5, 3].

In Algeria, adult hematological malignancies account for 10% of all cancer pathologies and the evaluation of their frequency between 2011 and 2013 shows that AL ranks second after non Hodgkin lymphoma, a condition which accounts for 18% of all hematological malignancies in the country [6]. Three national investigations carried out over three different periods show that the incidence of AML is continuously increasing, with an estimated impact in 2017 of 1.9/100,000 inhabitants, with a median age at diagnosis of 44 years [7, 8].

A national epidemiological (January 2010 to December 2013) looking at ALL patients shows that the incidence over 4 years was 0.47 /100,000 inhabitants. Young people are the most affected 65.5% of these patients are under 30 years old with a median age at diagnosis of 31 years [9].

The Human leukocyte antigen (HLA) is the most polymorphic genetic system known in humans, containing the loci for genes encoding class I and II HLA antigens. These genes are on the short arm of human chromosome 6 [10, 11]. This arm is divided into class I (HLA-A, HLA-B, HLA-C) and class II (HLA-DR, HLA-DQ, HLA-DP) molecules. The function of these classes is activate the immune response by encoding membrane glycoproteins that act as mediators in the presentation of

*[1]Laboratory of Developmental Biology and Differentiation, BP 1510 El M'Naouer, Oran 1 Ahmed Ben Bella University, 31000, Oran, Algeria. [2]Hospital and University Establishment November 1, 1954, Department of Hematology and Cellular Therapy, BP 4166 Ibn Rochd, University of Oran 1, Ahmed Ben Bella, Faculty of Medicine, 31000 Oran, Algeria. [3]Hospital and University Establishment November 1, 1954, Immunology Department, 31000 Oran, Algeria. [4]Hospital and University Establishment November 1, 1954, Hemobiology Blood Transfusion Service, Blood Bank. Sincerely, 31000 Oran, Algeria. *For Correspondence: nabil.yafour@outlook.fr*

antigenic peptides to lymphocytes T CD8+ and T CD4+ respectively [12]. Thanks to multiple studies on the subject, we know that the HLA complex plays a role in AL pathogenesis [13].

Therefore, HLA alleles could be important genetic risk factors for AML and ALL patients. A study of a Moroccan population found that HLA-B*44 may be a presumptive predisposing factor in the development of leukemia [13]. By contrast, a Turkish study found that HLA-B*13 and HLA-B* 40 alleles were negatively associated with AML [14].

On the other hand, other associations between HLA genotypes and the onset of other cancers have been demonstrated, in fact , It has been shown that in Lynch syndrome carrier's, HLA genotype plays an important role in the presentation of frameshift peptides (FSP) antigens to the immune system, and may influence the likelihood of progression from precancerous lesions to cancer [15].

M. Zeddou et al. also found that class I HLA Allele predicted restricted antigenic coverages for Fap2 Protein of Fusobacterium Nucleatum, were Associated with colorectal cancer incidence [16].

Wang et al observed a protective effect of increased HLA diversity in cancers typical of autoimmune or infectious etiologies and high mutation burden, including non-Hodgkin lymphoma, Hodgkin lymphoma, and lung cancer [17].

The frequency of HLA alleles has not yet been determined in Algerian leukemic patients. In this study we analyzed HLA-B alleles using DNA-typing tests. It has been observed that the HLA-B locus has the highest number of allele variants because of its ability to present peptides derived from intracellular pathogens [18]. We estimated the incidence of HLA-B alleles in patients with AML and ALL compared to those from healthy control individuals to define for the first time, susceptible alleles for leukemia in Algerian AML an ALL patients.

Materials and Methods

Patients

The study population consisted of individual and unrelated patients who are hematopoietic stem cell transplantation candidates, referred from the hematology and cellular therapy unit at the main research hospital in Oran, EHU d'Oran 1er Novembre 1954 (Oran, Algeria).

All individuals were of Algerian origin and came from the west and southwest regions of the country. The sample set studied was composed of 163 patients with AL who were compared to 293 healthy donor controls of same ethnic origin, all recruited in the hematopoietic stem cell transplant department of 1st November Hospital in Oran .

HLA typing

DNA was extracted and purified from 200 µl of whole blood that has been collected in 5% EDTA, using QI Aamp ® DNA Blood mini kit, purchased from QIAGEN, following the manufacturer's instructions. DNA concentrations were obtained using MAESTRO GEN NANODROP. DNA amplification to genotype the HLA-B locus was carried out with polymerase chain reaction and using specific primers (PCR-SSP method) for HLA-B alleles (Micro SSP TM. HLA Class I B locus Specific DNA Typing Tray, ONE LAMBDA, INC). PCR amplifications were performed in BIORAD T100 thermal cycler ™. Amplified products were resolved using agarose gel electrophoresis.

Band interpretation analysis was performed using HLA Fusion ™ research 6.2 software purchased from ONE LAMBDA. All HLA typing was screened in the histocompatibility and cell exploration unit.

Statistical analysis

Statistical significance in HLA frequencies between patients and controls was determined using $\chi 2$ analysis and fisher's exact test, p-values of 0.05 or less were considered to be significant using Open Epi, version 3.

Results

Between 2013 and 2020, we studied 163 patients, 111 patients with AML (68.09%), 20 with ALL (18.01%), and 32 with unclassified AL (19.63%) (Table 1).

HLA-B allele incidence in west and southwest Algerian leukemic patients and control subjects

The distribution of HLA-B alleles in AML and ALL patients, and control subjects are summarized in Table 2. We identified 28 alleles of the HLA-B locus in the study group. Compared with the control group, we observed a positive association of the HLA-B*27 and HLA-B*58 alleles with the AL group (see Table 2).

*Patient characteristics of HLA-B*27 and HLA-B*58*

All patients (n=7) carrying HLA-B*27 allele and 88% (n=14) carrying HLA-B*58 allele had AML. However, there were no significant differences when we compared

Table 1. Patient Demographic Characteristics

Characteristics	Acute leukemia patients n (%)
Total patient	163
Median age, years, range	36 (15- 63)
Type of acute leukemia	
AML	111 (68)
ALL	29 (18)
Unclassified	23 (19)
Gender	
Female	67 (41)
Male	96 (59)
ABO groups	
A+	47 (29)
A-	4 (3)
B+	23 (14)
B-	0 (0)
AB+	8 (5)
AB-	2 (1)
O+	72 (44)
O-	7 (4)

Table 2. Acute Leukemia vs. Control Group: Comparison of HLA-B Allele Incidence

HLA -B Allele	HLA-B in Leukemic patients n=163	HLA B in normal subjects n=293	p value
2	0	1	0.9750
5	0	1	0.9750
7	27	42	0.5242
8	21	42	0.6669
13	11	23	0.6679
14	22	35	0.6311
15	16	43	0.1385
18	19	32	0.8114
27	7	3	0.05584
35	16	38	0.3179
37	3	6	>0.9999999
38	6	10	0.8815
39	5	9	0.9980
40	8	12	0.6847
41	4	8	>0.9999999
42	6	11	0.9684
44	27	64	0.1767
45	21	43	0.5974
47	1	0	0.9750
49	13	23	0.9620
50	20	37	0.9118
51	35	51	0.2889
52	11	13	0.2909
53	2	6	0.8177
55	3	10	0.5124
57	5	9	0.9980
58	16	14	0.03756
78	1	0	0.9750

these results with the rest of HLA-B*X allele AL group (Table 3).

*Response to treatment of HLA –B*58 and HLA-B*27 patients*

We then analyzed the response to induction chemotherapy treatment in patients with HLA-B*58 and HLA-B*27 genotype and compared them to the rest of the group HLA-B*X. There were no significant differences between the two groups (p= 0.978) (Table 3).

Table 3. Comparison between HLA-B*27/HLA-B*58 Allele and HLA B*X Allele in Acute Leukemia Patients

	AL HLA B*27/HLA B*58 allele n (%)	AL HLA B*X allele n (%)
Total evaluable patients	23	111
Age, median, range	38 (17-63)	36 (15-63)
Gender		
Male	12 (52)	69 (62)
Female	11 (48)	42 (38)
Type of acute leukemia		
AML	21 (91)	90 (81)
ALL	2 (9)	17 (15)
Unclassified	0	4 (4)
Response to induction chemotherapy		
Complete response	14 (67)	73 (65)
Failure	7 (33)	37 (34)
Not assessable	2 (9)	1 (1)

AL, acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; HLA-B*X allele, acute leukemia patient without others allele.

Discussion

The first study that investigated the relationship between the HLA system and hematological malignancies was published in 1967, showing HLA-A2 allele frequency to be higher in patients with AL [19]. Since then, several pathologies have shown a clear genetic link with the HLA system. Barion et al. found a positive association of HLA-B*07 allele with AML but not with ALL [20]. In contrast, Fernandez et al. found an opposite effect of HLA-B*40 in the genetic susceptibility to develop ALL or AML in Mexican population [21], and HLA-B*44 and HLA-B*51 were found to be presumptive predisposing factors in developing leukemia [22].

The variations between these results can be explained by the differences in immune responses to pathogens according to ethnicity and geographical zones within the populations studied. Brewerton et al. [23] and Schlosstein et al. [24] independently reported a very strong relationship between HLA-B27 and ankylosing spondylitis (AS). They found that 88–96% of the patients carried HLA-B*27 compared with 8%–4% of healthy controls, respectively. These data were later confirmed by many other groups, giving a relative risk (RR) of >100 to develop ankylosing spondylitis in individuals positive for HLA-B*27 [25-27]. The distribution of HLA alleles in different populations and their hypothetical haplotype associations have shown common features between certain populations [28, 29].

Mellemkjaer et al. reported that patients with AS and other rheumatological diseases may have an increased incidence of malignancies, notably leukemia in AS, and non-Hodgkin's lymphoma and leukemia in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [30].

Large-scale studies on the etiology of acute leukemia have been carried out in Algeria, such as on Myelodysplastic syndromes, a group of clonal disorders of hematopoietic stem cells with a high risk of transformation into acute myeloid leukemia [31]. The present study showed that HLA-B*27 and HLA B*58 are found to be increased in patients with AL compared to normal individuals. AU et al. [32] found that HLA-B*27 carriers may have an increased risk of AL and those with concomitant AS may be predisposed to lymphoid malignancies. It is still uncertain whether this is related to the treatment for the rheumatological disease, or caused by an intrinsically increased risk [32].

Luo et al. showed that HLA –B*58 allele can be associated with genetic susceptibility for patients with AL in Chinese populations [33]. These associations between certain HLA alleles and leukemia may be due to reduced immune surveillance, molecular mimicry by oncogenic microbes, or putative linkage disequilibrium with yet unidentified susceptibility genes in the studied populations [34]. In parallel, HLA plays an important role in immune surveillance, and HLA polymorphism may affect the ability of the immune system to recognize malignant cells and target them for elimination by T cells [35].

Bartakke et al [36], reported a case of a leukemia patient carrying HLA-B*27 allele, who developed reactive arthritis (ReA) during AML induction phase chemotherapy. This suggests that HLA-B*27 predisposes an individual to ReA during AML induction therapy and could investigated before treatment initiation.

In our study, there was no difference between the AL induction treatment response in patients with HLA-B*27 and HLA-B*58 alleles when compared with the rest of the study group. Finally, none of our patients carrying HLA –B*27 allele presented clinical manifestations of AS, suggesting that at least part of the increased risk of AL in HLA-B*27 carriers may be due to an HLA associated predisposition and not to autoimmune disease therapy.

In conclusion, the major finding of our study is that, in AL patients in west and southwest Algeria, a positive association may exist between HLA-B*27 and HLA-B*58 and AML. Extending these findings to a study with a larger cohort would be interesting in terms of confirm these results and contributing important new elements to the existing literature.

Author Contribution Statement

Narimane Habour ,conceiving the research idea and writing original draft for thesis work. Pr Nabil Yafour, Head of the Hematology and Cell Therapy Department where patients and controls were recruited, and Pr Tewfik Sahraoui are both thesis directors and did conceptualization, conceiving the research idea ,supervision and validation. Pr Youcef Bouali youcef and Pr Mohammed Chekal, head of department of the university hospital laboratories where the samples were taken and the HLA typing performed. Pr Mohammed Brahimi and Pr Mohamed Amine Bekkadja, for conceptualization and supervision. Rachid Bouhass, for the statistical analysis.

Acknowledgements

Funding statement

The present study benefited from in-house financial support from the 1st November hospital , and laboratory of development biology , Faculty of Nature and Life Sciences , Oran 1Ahmed Ben Bella university , Algeria.

Study and ethical Approval

The research committee of the Faculty of Nature and Life Sciences, Oran 1Ahmed Ben Bella university, approved this project.

Data availability

The data is accessible upon request to the author.

Conflicts of interests

The authors declare no conflict of interest.

References

1. Miller DR, Miller LP. Acute lymphoblastic leukemia in children: An update of clinical, biological, and therapeutic aspects. Crit Rev Oncol Hematol. 1990;10(2):131-64. https://doi.org/10.1016/1040-8428(90)90004-c.
2. Greaves MF. Aetiology of acute leukaemia. Lancet. 1997;349(9048):344-9. https://doi.org/10.1016/s0140-6736(96)09412-3.
3. Kumar CC. Genetic abnormalities and challenges in the treatment of acute myeloid leukemia. Genes Cancer. 2011;2(2):95-107. https://doi.org/10.1177/1947601911408076.
4. Greaves M. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer. 2006;6(3):193-203. https://doi.org/10.1038/nrc1816.
5. Belson M, Kingsley B, Holmes A. Risk factors for acute leukemia in children: A review. Environ Health Perspect. 2007;115(1):138-45. https://doi.org/10.1289/ehp.9023.
6. Hamladji R. Etat des lieux de la prise en charge des hémopathies malignes en algérie. Revue algérienne d'hématologie, n°8/9. 2013.
7. Bekadja M. Iiième enquête épidémiologique nationale des leucémies aigues myéloblastiques [15 eme congrès national d'hématologie tlemcen 2018]. 2018.
8. Benakli M. Approche épidémiologique des leucémies aigues myéloïdes en algérie. Revue algérienne d'hématologie. 2009.
9. Hamdi S, Bentahar I, Harbadji H, Grifi F, Bougherira S, Filali T, et al. Epidemiology of extranodal diffuse large b cell lymphomas in algeria: A study of the algerian group of extranodal lymphomas (galeg). Blood. 2016;128(22):5403
10. Begovich AB, McClure GR, Suraj VC, Helmuth RC, Fildes N, Bugawan TL, et al. Polymorphism, recombination, and linkage disequilibrium within the hla class ii region. J Immunol. 1992;148(1):249-58.
11. Bodmer JG, Albert ED, Bodmer W. Nomenclature for factors of thehla system. Immunogenetics. 1992;36(3):135-48.
12. Thorsby E. A short history of hla. Tissue Antigens. 2009;74(2):101-16. https://doi.org/10.1111/j.1399-0039.2009.01291.x.
13. Kabbaj M, Oudghiri M, Naya A, Naamane H, El Turk J, Bennani S, et al. Hla-a, -b, -drb1 alleles and haplotypes frequencies in moroccan patients with leukemia. Ann Biol Clin (Paris). 2010;68(3):291-6. https://doi.org/10.1684/abc.2010.0430.
14. Patiroglu T, Akar HH. Relationships of human leukocyte antigen-a, -b, -drb1 alleles, and haplotypes in 129 ethnic turkish patients with acute myeloblastic leukemia. Lab Med. 2015;46(3):195-9. https://doi.org/10.1309/lml8dsrktfuo27rm.
15. Ahadova A, Witt J, Haupt S, Gallon R, Hüneburg R, Nattermann J, et al. Is hla type a possible cancer risk modifier in lynch syndrome? Int J Cancer. 2023;152(10):2024-31. https://doi.org/10.1002/ijc.34312.
16. Zeddou M. Class i hla allele predicted restricted antigenic coverages for fap2 protein of fusobacterium nucleatum are associated with colorectal cancer incidence. Asian Pac J Cancer Prev. 2023;24(10):3629-36. https://doi.org/10.31557/apjcp.2023.24.10.3629.
17. Wang QL, Wang TM, Deng CM, Zhang WL, He YQ, Xue WQ, et al. Association of hla diversity with the risk of 25 cancers in the uk biobank. EBioMedicine. 2023;92:104588. https://doi.org/10.1016/j.ebiom.2023.104588.

18. McAdam SN, Boyson JE, Liu X, Garber TL, Hughes AL, Bontrop RE, et al. A uniquely high level of recombination at the hla-b locus. Proc Natl Acad Sci USA. 1994;91(13):5893-7. https://doi.org/10.1073/pnas.91.13.5893.
19. Cutoni ES, Mattiuz PL, RM T. Hsitocompatibility testing. Copenhagen:Munksgraad. 1967: Italy, Williams & Wilkins;14-24 June 1967. https://catalog.lib.msu.edu/Record/folio.in00000426038/Description.
20. Barion LA, Tsuneto L, Testa G V, al e. Associação entre hla e leucemia em uma população brasileira de etnia mista. Rev Assoc Med Bras. 2007;5(3):252-56.
21. Fernández-Torres J, Flores-Jiménez D, Arroyo-Pérez A, Granados J, López-Reyes A. Hla-b*40 allele plays a role in the development of acute leukemia in mexican population: A case-control study. Biomed Res Int. 2013;2013:705862. https://doi.org/10.1155/2013/705862.
22. Uçar F, Sönmez M, Erkut N, Balcı M, Yücel B, Yılmaz M, et al. Relation of hla-a, -b, -drb1 alleles and haplotypes in patients with acute leukemia: A case control study. Arch Med Res. 2011;42(4):305-10. https://doi.org/10.1016/j.arcmed.2011.06.003.
23. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and hl-a 27. Lancet. 1973;1(7809):904-7. https://doi.org/10.1016/s0140-6736(73)91360-3.
24. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM. High association of an hl-a antigen, w27, with ankylosing spondylitis. N Engl J Med. 1973;288(14):704-6. https://doi.org/10.1056/nejm197304052881403.
25. Flavell RA, Hafler DA. Autoimmunity. What is the turning point? Curr Opin Immunol. 1999;11(6):635-7. https://doi.org/10.1016/s0952-7915(99)00029-1.
26. Thorsby E. Invited anniversary review: Hla associated diseases. Hum Immunol. 1997;53(1):1-11. https://doi.org/10.1016/s0198-8859(97)00024-4.
27. Tiwari JL, Terasaki PI. Hla and disease associations. Springer science & business media. 2012.
28. Amirzargar A, Mytilineos J, Farjadian S, Doroudchi M, Scherer S, Opelz G, et al. Human leukocyte antigen class ii allele frequencies and haplotype association in iranian normal population. Hum Immunol. 2001;62(11):1234-8. https://doi.org/10.1016/s0198-8859(01)00320-2.
29. Saruhan-Direskeneli G, Uyar FA, Bakar S, Eraksoy M. Molecular analysis of hla-drb1, -dqa1 and -dqb1 polymorphism in turkey. Tissue Antigens. 2000;55(2):171-4. https://doi.org/10.1034/j.1399-0039.2000.550211.x.
30. Mellemkjaer L, Andersen V, Linet MS, Gridley G, Hoover R, Olsen JH. Non-hodgkin's lymphoma and other cancers among a cohort of patients with systemic lupus erythematosus. Arthritis Rheum. 1997;40(4):761-8. https://doi.org/10.1002/art.1780400424.
31. Bekadja MA, Fenaux P, Akrouf S, al e. Adults myelodysplastic syndromes in algeria: A study by the algerian mds group. Asian Pac J Cancer Bio. 2023;8(1).
32. Au WY, Hawkins BR, Cheng N, Lie AK, Liang R, Kwong YL. Risk of haematological malignancies in hla-b27 carriers. Br J Haematol. 2001;115(2):320-2. https://doi.org/10.1046/j.1365-2141.2001.03114.x.
33. Luo QZ, Li LX, Xie YB, Yan MY, Yu P. Association of hla-a, b and drb1 alleles with leukemia in han population in hunan province]. Nan Fang Yi Ke Da Xue Xue Bao. 2008;28(6):1016-8.
34. Dorak MT, Lawson T, Machulla HK, Darke C, Mills KI, Burnett AK. Unravelling an hla-dr association in childhood acute lymphoblastic leukemia. Blood. 1999;94(2):694-700.
35. Gragert L, Fingerson S, Albrecht M, Maiers M, Kalaycio M, Hill BT. Fine-mapping of hla associations with chronic lymphocytic leukemia in us populations. Blood. 2014;124(17):2657-65. https://doi.org/10.1182/blood-2014-02-558767.
36. Bartakke SP, Sampagar AA, Bafna VS, Patel P. Human leukocyte antigen-b27: The genetic predisposition leading to reactive arthritis during induction phase chemotherapy for acute myeloid leukemia. Indian J Med Paediatr Oncol. 2017;38(3):377-9. https://doi.org/10.4103/ijmpo.ijmpo_8_17.