ORIGINAL ARTICLE

HLA DRB1 alleles in association with acute and chronic myeloid leukemia and evaluation of their role as markers for selection of the line of treatment

[1]Mona G. Morsi, [1]Maha M. El Gharbawy, [2]Nahla A. Hamed, [1]Heidi M. Gaber Ahmed, [1]Shams A. Arafa*

[1]Medical Microbiology & Immunology Department, Faculty of Medicine, Alexandria University
[2]Hematology Department, Faculty of Medicine, Alexandria University

Key words:
AML, CML, HLA-DRB1 typing

Corresponding Author:
Shams Abd El-Fattah Arafa, Medical Microbiology & Immunology Department, Faculty of Medicine, Alexandria University
Tel.: 01005759327
SHAMS.ARAFA@alexmed.edu.eg

ABSTRACT

Background: *The genes that encode the human leukocyte antigens (HLA) molecules are the most polymorphic in the human genome and have been considered as possible genetic risk factor in the development of acute and chronic leukemias.* ***Objective:*** *This study aimed at evaluating the role of HLA DRB1 alleles as markers for selection of the line of therapy in Acute myeloid leukemia (AML) and Chronic myeloid leukemia (CML) patients.* ***Methodology:*** *The study was conducted on: 20 AML, 20 CML cases and 20 healthy controls. Typing was done by the sequence-specific primer (PCR-SSP).* ***Results:*** *There was a highly statistical significance association between response to treatment and HLA DRB1 alleles as markers (MCP= .0001) in AML and CML patients. The HLA-DRB1 04*04 allele was found to be associated with good response to therapy in AML patients while, HLA-DRB1 07*15 allele was associated with bad response. In CML patients, HLA-DRB1 03*11 allele was found to be associated with good response to therapy while, HLA-DRB1 alleles 03*04 and 11*15 alleles were equally associated with bad response.* ***Conclusion:*** *Future researches are mandatory especially on larger scale to confirm whether these alleles are protective or even risk alleles regarding both AML and CML.*

INTRODUCTION

The human major histocompatibility complex (MHC) is known as the human leukocyte antigens (HLA) network since such antigens were first detected and characterized using leukocyte alloantibodies [1]. The HLA system has long been recognized as important antigens in transplantation, but HLA molecules have a key biological role in controlling immune response[2]. The human MHC charts about 3,600 kilobases of DNA to the short arm of chromosome 6 (6p21) [3].

The human MHC is grouped into three different regions: HLA-I, HLA-II and HLA-III based on their structure, tissue distribution and function. HLA class I proteins are coded by HLA-A, HLA-B, and HLA-C genes and are present on nearly every cell in the human body [4]. HLA class II proteins are coded by the genes HLA-DR, HLA-DQ, and HLA-DP [5,6]. Class II molecules are present in macrophages, B-cells, and other "antigen-presenting cells" (APCs). Class III region- does not encode HLA molecules, but includes complement genes for elements (C2, C4, factor B), 21-hydroxylase, tumor necrosis factors (TNFs) and some other components[3].

Leukemia is cancer of blood cells occurring in the bone marrow cell. The cell undergoes mutation and develop a form of leukemia. The cancer cells will grow and thrive better than normal cells once the marrow cell undergoes a leukemic shift. Over time, healthy cells are crowded out or killed by the cancer cells. The rate of development of leukemia and the way the cancerous cells replace normal marrow cells differ in each type of leukemia [7].

Acute myeloid leukemia (AML) is the commonest type of acute leukemia in adults especially above 50 years. Of all leukemias, AML constitutes more than eighty percent. Although its etiology is unknown it may evolve after being exposed to some risk factors as previous cytotoxic chemotherapy, benzene, ionizing radiation, genotoxic agents, chromosomal abnormalities, or after a previous hematologic disorder (bone marrow failure). The disorder develops in a malignantly transformed multipotential hematopoietic stem cell that undergoes multiple genomic alteration until an overt disease arises [8].

Chronic myeloid leukemia (CML) represents 15% of malignancy in adults. Although the age of onset is usually around 67 years; however, CML can develop at all ages. CML is distinguished by presence of

Philadelphia chromosome (Ph) in individuals with myeloproliferative neoplasms (MPN). Ph results from an unconditional translocation of the BCR-ABL fusion gene between chromosomes 9 and 22 [t (9;22)]; the outcome of such fusion is a protein with deregulated tyrosine kinase activity (p210) which plays a crucial role in CML pathogenesis. CML occurs in 3 different phases; chronic phase (CP), accelerated phase (AP), and blast crisis (BC), it is mostly diagnosed in the chronic phase [9, 10,11].

According to population studies conducted along past decades several diseases were associated with specific HLA alleles. Over a hundred disease were linked with classical HLA (class I and II) and non-HLA genes on MHC regions (e.g. complement C4) [12, 13]. The degree of correlation varies between diseases, and there is typically a lack of strong alignment between the HLA haplotypes and the associated disease, as the precise mechanisms overarching the most HLA-disease association are not clarified well and other environmental factors and genetic factors can also play a major role [14].

HLA studies have reported numerous links in multiple leukemias. Genetic interaction studies in leukemia are indeed valuable in pathogenesis interpretation, diagnosis and managing of leukemia with poor prognostic outcomes [14, 15]. Such connections may be connected to the marker itself, or may be a consequence of an interaction with another non-HLA locus within the MHC. Because of low recombination rates, the entire complex is expressed as a haplotype which produces exceptional levels of linkage disequilibrium (LD), that confuses many association studies [16].

The first human leukemia HLA research in 1967 showed an increase in the amount of HLA-A2 alleles in acute lymphoblastic leukemia (ALL) [17]. The strongest correlation between AML and HLA alleles was documented using a monoclonal antibody unique to HLA-DR53's HVR3 epitope [18]. Dorak et al identified multiple independent markers in the earliest genuinely molecular CML association review, as is the event with murine leukemia [15, 19].HLA trials in several leukemias have identified a variety of connections, but the utmost biologically important may be those observed in CML due to its powerful immunological background.

This study aimed at assessing the role of HLA DRB1 alleles as markers for selection of the line of therapy in AML and CML patients.

METHODOLOGY

Subjects:

The study was carried out on three groups, group I: 20 AML cases (10 responders and 10 non-responders to treatment), group II: 20 CML cases (10 responders and 10 non-responders), and group III: 20 healthy controls of matched age and sex.

Patients were selected from Hematology Department, Alexandria Main University Hospital. Ethical consent was taken from each subject. Approval of the ethical committee was obtained from Faculty of Medicine, Alexandria University.

Methods:

All enrollees were subjected to a full history taking. (Age, sex, drugs…. etc.), full clinical examination, routine investigations; CBC, Liver and Renal function tests, bone marrow examination for AML cases only, CML was diagnosed by detecting Breakpoint Cluster Region Abelson (BCR-ABL) gene and HLA typing.

HLA typing: Mononuclear separation of blood sample using lysis buffer. DNA extraction using ZYMO RESEARCH kit. Typing at the HLA-DRB1 loci by the sequence-specific primer (PCR-SSP) INNO-LIPA HLA-DRB1 Plus Fujirebio Europe, according to the manufacturer instructions.

RESULTS

Males constituted 55% in cases with AML & CML while percentage of males in control group was 50%. The mean age in the cases groups (With AML Vs with CML) was (37.8 ± 13.7), (38.9± 14.3) years respectively, while in control group it was (36.3± 14.2 years).

Although the study was carried out on 60 subjects, only 46 (12 cases of AML, 18 cases of CML and 16 controls) revealed results on HLA typing due to insufficient DNA yield.

By HLA typing: Twenty different alleles were detected and were distributed among the 3 groups. The commonest allele was HLA DRB1 04*04 which was detected in all the 3 groups.

There was a statistical significance difference in distribution of the HLA DRB1 alleles in the 3 different groups as illustrated in (table 1).

Table 1: Distribution of HLA DRB1 alleles in the 3 different groups

HLA DRB1 alleles	Cases				Control Group III (N=16)		Test of significance (p)
	Group I (N=12)		Group II (N=18)				
	No.	%	No.	%	No.	%	
04*04	6	50.0	1	5.6	5	31.25	(MCP= .0001*)
04*15	1	8.3	0	0	2	12.5	
07*07	2	16.7	0	0	0	0	
07*13	1	8.3	0	0	0	0	
07*15	2	16.7	0	0	0	0	
03*14	0	0	1	5.6	0	0	
01*01	0	0	1	5.6	0	0	
03*04	0	0	2	11.1	0	0	
03*07	0	0	1	5.6	0	0	
03*11	0	0	3	16.7	0	0	
03*13	0	0	1	5.6	2	12.5	
04*11	0	0	2	11.1	0	0	
07*11	0	0	1	5.6	1	6.25	
08*10	0	0	2	11.1	0	0	
11*13	0	0	1	5.6	0	0	
11*15	0	0	2	11.1	0	0	
03*03	0	0	0	0	1	6.25	
04*07	0	0	0	0	2	12.5	
11*11	0	0	0	0	1	6.25	
13*13	0	0	0	0	2	12.5	

MCP: Monte Carlo Exact p value *statistically significant

There was a highly statistical significance association between HLA DRB1 alleles as markers and response to treatment in AML and CML patients (MCP= .0001), as illustrated in (table 2).

Table 2: Association between HLA DRB1 alleles and responses to treatment

Treatment	HLADRB1 alleles	Cases				Test of significance (p)
		AML (N=12)		CML (N=18)		
		No.	%	No.	%	
Responders	**04*04**	5	41.7	0	0	**(MCP= .0001*)**
	04*15	1	8.3	0	0	
	07*07	2	16.7	0	0	
	07*13	1	8.3	0	0	
	03*14	0	0	1	5.6	
	03*07	0	0	1	5.6	
	03*11	0	0	3	16.7	
	04*11	0	0	1	5.6	
	07*11	0	0	1	5.6	
	08*10	0	0	2	11.1	
Non- Responders	**01*01**	0	0	1	5.6	
	03*04	0	0	2	11.1	
	03*13	0	0	1	5.6	
	04*04	1	8.3	1	5.6	
	04*11	0	0	1	5.6	
	11*13	0	0	1	5.6	
	11*15	0	0	2	11.1	
	07*15	2	16.7	0	0	

MCP: Monte Carlo Exact p value *statistically significant

The HLA-DRB1 04*04 allele was found to be linked with good response to therapy in AML cases while, HLA-DRB1 07*15 allele was connected to bad response. In CML patients, HLA-DRB1 03*11 allele was found to be linked with good therapy response while, HLA-DRB1 alleles 03*04 and 11*15 alleles were equally associated with bad response.

DISCUSSION

For decades it has been understood that the MHC plays a crucial role in the etiology and pathogenesis of certain diseases. HLA studies documented a number of links in leukemic patients. Leukemia genetic interaction studies are highly useful in the understanding the pathogenesis, in diagnosis and managing of leukemia especially those with bad prognosis [14,15].

AML is the commonest form of acute leukemia among adults. It is responsible for the highest annual deaths due to leukemias in the United States. Treatment options are mainly chemotherapy and hematopoietic stem cell transplantation (HSCT) depending on biologic elements like age, patient condition, and response to therapy that may have prognostic importance [10, 20, 21]. Although there have been many interactions between specific HLA antigens and enhanced vulnerability to numerous diseases, initial attempts to associate class I and II antigens with AML have not been scientifically sound, possibly due in part to the heterogeneity of AML [22]. Later, several HLA antigens were dramatically associated with higher percentages of remission, period of remission, and longevity. However, given the heterogeneity of both the immune response genes of the HLA system and the cytogenetic subtypes of AML, it is essential to analyze a large pool of cases in order to accept that genetically important relationships actually exist [22].

HLA trials in several leukemias have identified a variety of connections, but the extremely biologically important may be those observed in CML because of its powerful immunological background [19]. Chronic myeloid leukemia (CML) characterized by presence of Philadelphia chromosome (Ph) which results from an unconditional translocation of the BCR-ABL fusion gene between the chromosomes 9 and 22 [t (9;22)]. Several reports show a connection between t (9;22) (q34; q11) and numerous HLA alleles. This relationship indicates a causal role for T-cell cytotoxicity in pathophysiology of diseases associated with t (9;22) (q34; q11) BCR-ABL fusion proteins. Varying HLA alleles have specific preferences for the peptide sequence that they could present to T cells. A peptide must bind to the HLA molecule before it is presented to the T cell in order to obtain a T-cell response. In that a given HLA allele is able to bind and display only peptides with certain sequence limitations, an individual's ability to obtain an effective T-cell cytotoxic response to cells carrying unfamiliar or newly mutated proteins depends on the array of HLA alleles inherited. Therefore, individuals carrying those HLA alleles are able to attach to peptides derived from bcr-abl fusion transcripts can in theory be considered to have a biological advantage over individuals missing these specific HLA alleles in the fight against the disease [14, 15, 23, 24].

Triggering peripheral blood lymphocytes of healthy individuals with synthetic bcr-abl fusion peptide has resulted in induction of CD4+T lymphocytes which propagate specifically in response to induction with bcr-abl fusion peptide in an HLA class II-restricted manner. If protein-specific bcr-abl fusion and HLA class II-restricted CD4+T lymphocytes play an important role in resisting the advancement of CML, then in CML patients the levels of certain forms of HLA class II should be lower than the general population. Conversely, if such CD4+Tlymphocytes promote the growth of leukemia cells by generating growth factors for CML cells, then the levels of certain forms of HLA class II in CML patients should be higher than those in the rest of the population. The connection between HLA-DRB1 genotypes and CML forms were studied based on these hypotheses [24, 25].

A genetic modification caused by t (9;22) (q34; q11) in the form of P210 fusion protein is demonstrated in CML patients. In CML patients, it was suggested that P210 fusion protein as an endogenous protein is presented to the molecules of HLA class II. HLA class II alleles like HLA-DR1, -DR2, -DR3, -DR4 and -DR11 have recently been reported to be capable of presenting endogenous proteins such as synthetic Bcr-abl peptides that induce T-cell responses to be produced. HLA alleles that present this protein to the immune system result in a negative association with CML susceptibility. Many alleles do not express this protein adequately and predispose a positive correlation with CML [14, 24].

The present study aimed to evaluate the role of HLA DRB1 alleles as markers for selection of the line of therapy in AML and CML patients. The study was carried out on three different groups, group I: 20 AML cases (10 responders and 10 non-responders to treatment), group II: 20 CML cases (10 responders and 10 non-responders), and group III: 20 healthy controls of matched age and sex.

In our study, 20 different HLA-DRB1 alleles were typed. The commonest allele was HLA-DRB1 04*04 which was found in all the studied groups.

Concerning alleles HLA-DRB1 07*07, 07*13, 04*04, 04*15 and 07*15, they were found only in AML cases but not in CML. While the alleles HLA-DRB1 03*13, 03*04, 03*07, 03*11, 03*14, 01*01, 04*11, 07*11, 08*10, 11*13 and 11*15 were found only in CML patients and not in AML.

HLA-DRB1 03* allele was the commonest (8/18, 44%) among CML cases, and was not detected in AML cases, meanwhile; over 58% of AML belongs to HLA-DRB1 04*, and the rest belonged to HLA-DRB1 07*. This result was in accordance with Mundhada et al [23] who designed a case-control study to evaluate the correlation of HLA alleles with CML in 163 patients and 376 control subjects, a significant positive correlation between CML and certain alleles was observed including -DRB1*0301, -DRB1*0302, -DRB1*0901, -DRB1*1001, -DRB1*1201, -DRB1*1202, and-DRB1*1503.

In a research on Iranian patients; HLA Class II allele and haplotype frequencies of AML cases were compared with those of CML patients. The HLA-DRB1*16 allele frequency in AML cases was 7.5% while none of CML patient was positive for this allele (Pc=0.004). Also, a significant positive association with AML for HLA-DRB1 * 11 allele was obtained [14].

On comparing the cases to controls in our study, it was noticed that the alleles 03*03, 04*07, 11*11, and 13*13 were found only in controls and not in AML or CML patients. In a research done on Moroccan patients with leukemia showed that HLA-DRB1*01 has decreased in patients. Therefore, they considered this allele as it may be protective against leukemia, whereas DRB1*03, DRB1*04, DRB1*13 has been moderately increased in patients. It therefore might be regarded as susceptible alleles in leukemia disease [26] .This research results was contradictory to another study which find that in CML patients, the haplotype HLA-DRB1 * 0101/DQA1 * 0104/DQB1 * 05011 were significantly more common than controls [14].

Our study revealed a high statistical significance association in AML and CML patients together in response or not to treatment with HLA DRB1 alleles as markers as (MCP= .0001).

The HLA-DRB1 04*04 allele was noticed to be connected to good response to therapy in AML patients, so it might be regarded as a protective allele while, HLA-DRB1 07*15 allele was linked with bad response to therapy and thus could be regarded as a risk allele. In CML patients, HLA-DRB1 03*11 allele was noticed to be connected to good responsiveness to therapy and thus could be viewed as a protective allele while, HLA-DRB1 alleles 03*04 and 11*15 alleles were equally associated with bad response and might be considered as risk alleles.

According to studies conducted along past decades several diseases were linked with specific HLA alleles. The degree of correlation varies between diseases, and there is typically a lack of strong alignment between the HLA haplotype and the alleged disease, as the precise mechanisms overarching the most HLA-disease association are not clarified well and other environmental factors and genetic factors can also play a major role [14].

CONCLUSION

After reviewing our results and the results of various studies in different countries, it is obvious that a discrepancy between ours and theirs is present, furthermore, there is a discrepancy between the results of different researchers in different areas of the world, this can be clarified by the fact that the association between HLA antigens and diseases may be attributable to either direct effect of the marker antigen or to another antigen in linkage disequilibrium with it. Also, environmental factors and risk exposure are not similar between various populations. A drawback in our study is the restricted number of cases and poor DNA yield in some of the studied cases. Future researches are mandatory especially on larger scale to confirm whether these alleles are protective or even risk alleles regarding both AML and CML.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

REFERENCES

1. Terasaki PI. Clinical Transplants Los Angeles. Calif: UCLA Tissue Typing Laboratory; 1990.
2. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 1987;329(6139):512-8.
3. Beck S, Trowsdale J. The human major histocompatability complex: lessons from the DNA sequence. Annu Rev Genomics Hum Genet 2000; 1:117-37.
4. Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. Nomenclature for factors of the HLA system, 2004. Tissue Antigens 2005;65(4):301-69.
5. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology: 9th ed. Netherlands: Elsevier; 2017.
6. Dausset J, Soulillou JP. An interview with Jean Dausset. Am J Transplant 2004; 4(1):4-7.
7. Grimwade D, Hills RK. Independent prognostic factors for AML outcome. ASH Educ Program Book 2009; 2009(1):385-95.

8. Lowenberg B, Rowe JM. Introduction to the review series on advances in acute myeloid leukemia (AML). Blood. 2016;127(1):1.
9. Radich JP, Deininger M, Abboud CN, Altman JK, Berman E, Bhatia R, et al. Chronic Myeloid Leukemia, Version 1.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2018; 16(9):1108-35.
10. Provan D, Singer CR, Baglin T, Dokal I. Oxford handbook of clinical haematology. New York: Oxford University Press; 2009.
11. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
12. Shiina T, Inoko H, Kulski JK. An update of the HLA genomic region, locus information and disease associations: 2004. Tissue Antigens 2004;64(6):631-49.
13. Breban M, Gaston H. Immune mechanisms: adaptive immunity. In: Inman R, Sieper J (eds). Oxford Textbook of Axial Spondyloarthritis. UK: Oxford University Press; 2016. 63-8.
14. Khosravi F, Amirzargar A, Sarafnejad A, Nicknam MH, Alimoghadam K, Dianat S, et al. HLA class II allele and haplotype frequencies in Iranian patients with leukemia. Iran J Allergy Asthma Immunol 2007; 6(3):137-42.
15. Dorak MT, Chalmers EA, Gaffney D, Wilson DW, Galbraith I, Henderson N, et al. Human major histocompatibility complex contains several leukemia susceptibility genes. Leuk Lymphoma 1994; 12(3-4):211-22.
16. Lilly F, Boyse EA, Old LJ. Genetic basis of susceptibility to viral leukaemogenesis. Lancet 1964; 2(7371):1207-9.
17. Dausset J. The major histocompatibility complex in man. Science. 1981;213(4515):1469-74.
18. Gowans D, O'Sullivan A, Rollinson S, Roddam P, Groves M, Fegan C, et al. Allele and haplotype frequency at human leucocyte antigen class I/II and immunomodulatory cytokine loci in patients with myelodysplasia and acute myeloid leukaemia: in search of an autoimmune aetiology. Br J Haematol 2002;117(3):541-5.
19. Oguz FS, Kalayoglu S, Diler AS, Tozkir H, Sargin D, Carin M, et al. HLA system affects the age-at-onset in chronic myeloid leukemia. Am J Hematol 2003; 73(4):256-62.
20. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010; 115(3):453-74.
21. Tallman MS, Wang ES, Altman JK, Appelbaum FR, Bhatt VR, Bixby D, et al. Acute Myeloid Leukemia, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2019;17(6):721-49.
22. Joventino L, Stock W, Lane N, Daly K, Mick R, Le MB, et al. Certain HLA antigens are associated with specific morphologic and cytogenetic subsets of acute myeloid leukemia. Leukemia. 1995;9(3):433-9.
23. Mundhada S, Luthra R, Cano P. Association of HLA Class I and Class II genes with bcr-abl transcripts in leukemia patients with t(9;22) (q34;q11). BMC Cancer 2004;4(1):25.
24. 24. Clark RE, Dodi IA, Hill SC, Lill JR, Aubert G, Macintyre AR, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. Blood 2001;98(10):2887-93.
25. Yasukawa M, Ohminami H, Kojima K, Inokuchi K, Nishimura Y, Fujita S. Analysis of HLA-DRB1 alleles in Japanese patients with chronic myelogenous leukemia. Am J Hematol 2000;63(2):99-101.
26. 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.