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Human Leucocyte Antigen A Alleles in The Prediction of Post-Kidney Transplant Lymphoproliferative Disorders, A Single-Center Study

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ABSTRACT

Background: Post-transplant lymphoproliferative disorders (PTLPD) are frequently encountered after kidney transplantation. Human leucocyte antigen (HLA)A alleles were shown to be a predictor of development of these disorders.
Objective: Study of prediction of human leucocyte antigen HLA-A alleles in the development of PTLPD.
Methods: This study included 50 Egyptian kidney transplant (KT) recipients who underwent live-donor kidney transplantation, between 2010 to 2022. HLA-A alleles phenotyping was characterized by sequence specific oligonucleotide probes (SSOP). Nondestructive form of PTLPD was diagnosed by presence of lymphocytosis followed by assessment of, (CD45 and CD 19) mature B lymphocytes and blast cells (CD5 and CD10) with ratio of kappa/lambda by flow cytometry. The association between the development of this form and different HLA-A alleles was studied.
Results: Out of fifty recipients, five patients developed a non-destructive form of PTLPD in the median duration of 30 months (ranged, 15-50 months) post transplantation. A significant association was found between the expression of HLA-A01 (*P*=0.002, OR=1.020), CI (0.996-1.045) and HLA-A02 (*P*=0.042, OR=1.019), CI (1.001-1.037) and the development of PTLPD. Furthermore, HLA-A02 alleles were found to be a significant corelate with onset of development PTLPD.
Conclusion: Among Egyptian kidney transplant recipients, HLA-A01 and A02 alleles are good predictors of development of early non distractive form of PTLPD.
Keywords: Post-transplant lymphoproliferative disorders, Human leucocyte antigen A alleles, Kidney transplant.

INTRODUCTION

Human leucocyte antigens "HLA", which are programmed in the major histocompatibility complex "MHC" on chromosome 6, influence the human immune responses. HLA molecules are classified to class I and II. HLA class I is heterodimer of polymorphic-polypeptide chain and B2-microglobulin, which deliver peptides from an endogenous source to cytotoxic T-cells and expressed in somatic cells. HLA class II is heterodimer of alfa and beta polypeptide chains, that deliver extracellular peptides and presented them to helper T-cells [(1,2)].

T-cells recognize HLA molecules as pathogen and tumour peptides, which activate adaptive immune responses. With most polymorphic human genes, HLA haplotypes are greatly implicated in the causes and outcomes of immune conditions and hematopoietic malignancy [(1,2)]. Since a correlation between HLA and Hodgkin's lymphoma "HL" was first described in 1967, a lot of studies found links between HL risk with single nucleotide polymorphisms (SNPs) and HLA allele variants. Stratification of the population and the number and complexity of linkage disequilibrium in the MHC, on the other hand, have made it hard to pinpoint causal signals [(3)]. The associations between certain HLA phenotypes and the development of post-transplant lymphoproliferative disorders (PTLPD) have been reported in many studies. They showed that the role of HLA with tumour antigen presentation could be involved as either a genetic risk factors, prediction, or the disease protection [(1, 4-7)]. In this study, the role of HLA-A alleles as predictive tool for development of non-distractive form of PTLPD among Egyptian live-donor kidney transplant recipients was evaluated.

PATIENTS AND METHODS

A total of 50 kidney transplant recipients who received their renal allografts from live-related kidney donors between 2010-2022 were enrolled in this cross-sectional study. The inclusion criteria were first-time kidney transplantation, and age between 5 – 60-year-old. While exclusion criteria were patients with another organ transplantation, history of previous transplantation, or previous malignancy. All recipients were maintained on tacrolimus-based immunosuppression. Tacrolimus doses were adjusted to achieve a whole blood trough level between 4-8 ng/ml [(8)].

Ethical approval:

The required ethical approval for this study was obtained from the Ethical Committee of the University of Mansoura (IRB: MD. 20.5.328). The study was performed according to Helsinki standards and approval consent was obtained from each adult patient and from the caregiver of each child patient.

Clinical evaluation:

Recipients were thoroughly examined with special attention to any lymph node enlargement or splenomegaly.

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Laboratory evaluation

Pre-transplant evaluation:

HLA phenotyping was characterised according to European Federation for Immunogenetics [9]. QIAamp DNA Blood Mini Kits (#51104, QIAGEN, Germany) was used for extraction with an average of 6 µg of total DNA from 200 µl of whole human ETDA blood were carried out with Mini Spin Columns and proteinase K protocol and method according to manufactur .

Amplification by innolipa HLA-A (# 409306, Innogenetics, Belgium) was performed by conventional Polymerase Chain Reaction (PCR, thermal cycler ,USA) ,while detection by innolipa HLA-A (#409483, Innogenetics, Belgium) typing test was based on the sequence-specific oligonucleotide probe reverse hybridization assay, principles, and method according to manufacture. All probes were identified as positive on the INNO-LiPA HLA-A strips (Innogenetics, Belgium) and their HLA types were deduced by using a version of the LiPA. Interpretation software: LiRAS™ (genotyping and scoring).

Post-transplant screening for PTLPD

Hematological evaluation :

Complete blood count and differential count of white blood cells (WBC) for the detection of lymphocytosis (more than 3.8 $X10^9$/L) [10,12], was carried out using (SYSMEX, XN-1000, and SA-01).

Immunophenotyping was carried out to detect mature B cells by CD45 /CD19, immature blast cells (CD 5 and CD 10); and clonality (Kappa and Lambda) light chain.

According to WHO 2017, recipients were considered to be at high risk of development of PTLPD when following criteria were met**:** lymphocytosis followed by assessment of (CD45 and CD19) mature B lymphocytes and blast cells (CD5 and CD10) with ratio of kappa/lambda light chain by flow cytometry [10]. CD5 and CD10 <10% reveal normal value while kappa/lambda ratio from 1-5% is also normal [11,12].

Cells were isolated and characterized by lymph prep separation medium (#1672006, corning), which used to isolate mononuclear cells from an EDTA blood sample. Lymphocytes were stained with human conjugated antibody (Becton Dickinson, San Jose, CA, USA). CD38 Phycoerythrin Cy7 (PEcy7) (#335825 BD), CD45 Allophycocyanin H7 (APC H7) (#641417 BD), CD19 Phycoerythrin CF594 (PECF594) (#562294 BD), CD5 (PE) (#345782 BD) and CD10 (APC) (#332777 BD), Kappa light chain sIg*k* (FITC) and lambda light chain sIg*l* (PE) (#349516 BD).

Quantification of immune cells : labelled cells were identified by a wavelength of 488 and a wavelength of 633 using the FACS ARIA III cell sorter (Becton Dikinson). A total of 10,000 events were obtained and analyzed by Flow JO software (Becton Dikinson).

Statistical Methods:

The data were introduced to a PC using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 25.0. IBM Corporation, Armonk, New York). Data were expressed as median and range. The Chi Square test is utilized to analyse the association between two quantitative variables. Cox regression analysis of factors potentially related to survival and the Kaplan-Meier method was used to plot the progression free survival . All reported *p* values were two-tailed and $p < 0.05$ was considered significant.

RESULTS

Demographic data: the median age of the studied recipients was 20 years, (range, 7-60) years. The majority were adults (66%) and males (67%), the median duration post-transplantation was 30 (ranged,15-50) months.

HLA-A alleles interpretation: the deleted HLA-A phenotyping of the studied recipient is comprehensively described in **Table 1.**

Table 1: HLA A alleles results among all studied cases

		%
HLA A allele	**0**	**19**
	1	**17**
	2	**14**
	3	**8**
	9	**8**
	10	**6**
	11	**6**
	12	**5**
	19	**3**
	23	**3**
	24	**2**
	26	**2**
	28	**2**
	29	**2**
	30	**1**
	31	**1**
	32	**1**
	33	**19**
	34	**17**
	68	**14**
	69	**8**
	74	**8**

Post-transplant data

Clinical evaluation: no lymphadenopathy or splenomegaly were detected among our study population.

Laboratory evaluation

Immunophenotyping results among all studied :

A total of 43 recipients (86%) developed lymphocytosis and out of them five showed increases in percentage of mature B cells (CD45 and CD19). Those recipients were negative for blast cells (CD19 and CD5) and (CD19 and CD10), as well as kappa/lambada ratio were normal (**Figure 1).**

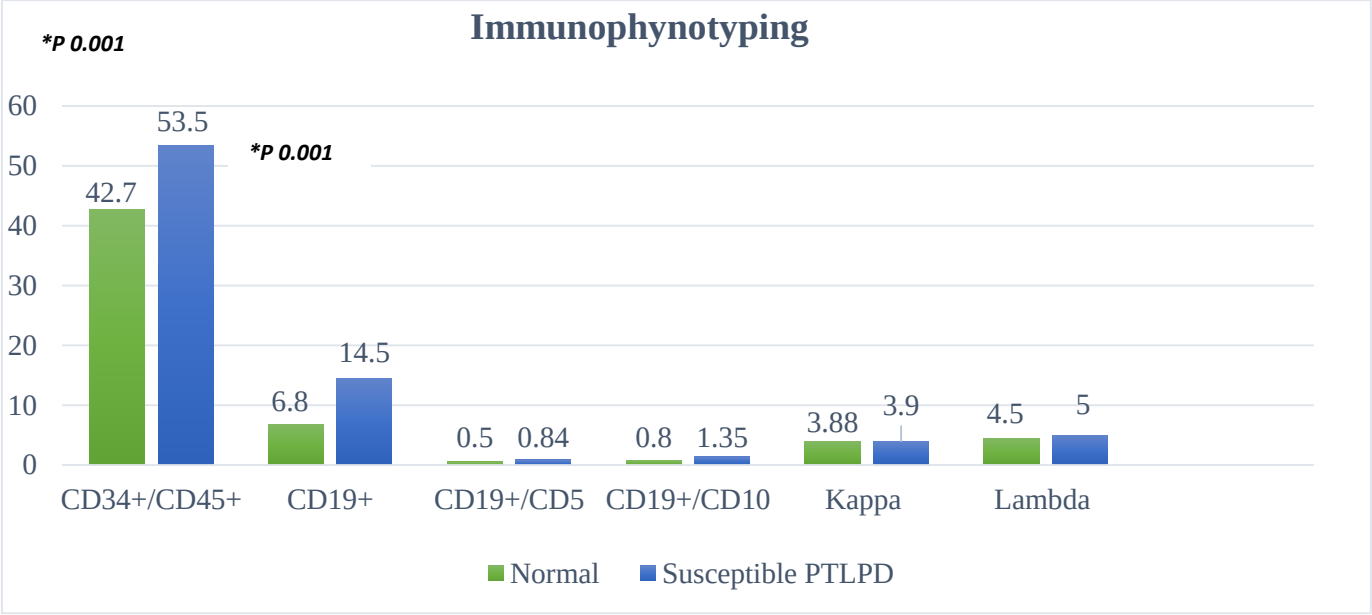


Figure 1. immunophenotyping among all study case

The results revealed that the proportions of immune cell subsets were comparable among the 2 groups (normal with increase lymphocyte level and susceptible PTLPD with lymphocytosis) with a significant difference. Accordingly, 10% of our recipients developed of non-destructive form of lymphoproliferative disorder based to **WHO, 2016 [(10)] (Table 2).**

Table 2: Association of LPD with age and increase of absolute lymphocytic count among all studied cases

			LPD		*P*
			Control	**Suspect**	
Age (years)		**Median (range)**	**15 (7-47)**	**28 (9-60)**	**<0.001**
Age group	**Child**	**N, %**	**15 (33.3%)**	**2 (40%)**	**0.003**
	Adult	**N, %**	**30 (66.7%)**	**3 (60%)**	
Lymphocytosis		**N, %**	**45 90%**	**5 10%**	**<0.001**

Prediction for development of PTLPD by study of HLA A alleles:

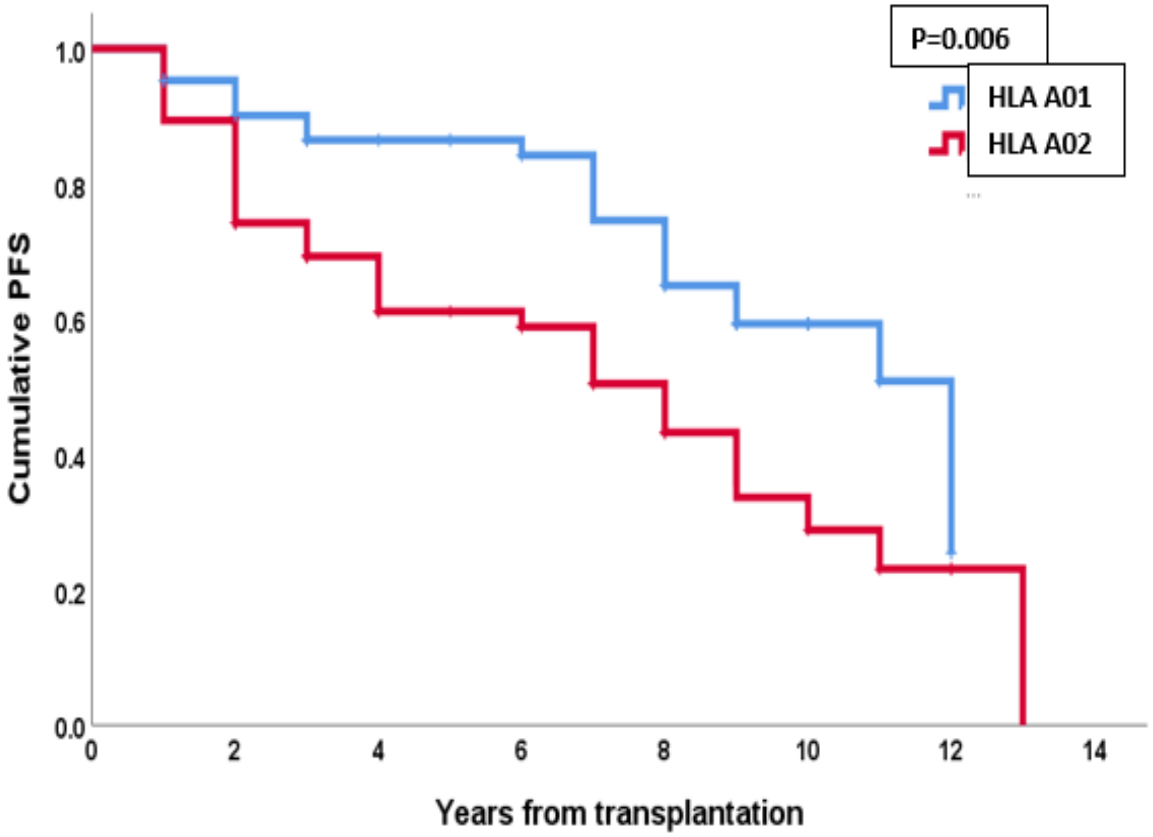
Cases suspected to have non-destructive PTLPD were associated with the presence of HLA-A01 and HLA-A02 (Table 3).

Table 3: Regression analysis for the prediction of LPD, using HLA-A genotypes as covariates

		LPD		**P**	**OR**	**95% CI**	
		Control	**Suspected**				
HLA A	**0**	23.3%	0%		Reference		
	1	40%	60%	**0.002**	1.020	0.996	1.045
	2	30%	70%	**0.042**	1.019	1.001	1.037

OR, Odds ratio; CI, confidence interval.

By multivariable analysis the onset of development of PTLPD performed by progressive free survival "PFS", was significantly earlier in recipients with expression of HLA-A01 and late in recipients with expression of HLA-A02 (**Figure 2)**. Cox regression analysis showed HLA-A02 predictor for PTLPD development by short PFS with (**Table 4)**.



PFS, progression free survival. HLA, Human leukocyte antigen

Figure 2: Progression-free survival (PFS) according to HLA-A.

Table 4. Cox regression for prediction of LPD

	Univariable				Multivariable			
	P	**HR**	**95% CI**		**P**	**HR**	**95% CI**	
HLA A 01	**0.002**	1.020	0.996	1.045	0.158	1.015	0.994	1.036
HLA A 02	**0.042**	1.019	1.001	1.037	**0.035**	1.015	1.001	1.029

HR, hazard ratio; CI, confidence interval.

DISCUSSION

Renal transplantation required a life-long immunosuppression maintenance therapy to prevent graft rejection [13]. These therapies are associated with increased risk of development de novo malignancies [13,14]. PTLPD is considered one of the serious malignancies, which are frequently encountered after solid organ transplantation [15]. Searching for a relative predictor for development of PTLPD is crucial for early detection and prevention [15,16]. In nontransplant population, several studies showed that HLA-A is one of these predictors as it was reported that HLA-A01 and HLA-A02 predicted the development of lymphoma subtypes [17,18].

So, the current study aimed to explore the role of HLA-A alleles as passible predictive markers for development of PTLPD among a group of Egyptian kidney transplant recipients. Out of fifty recipients, five patients were documented to have a non-destructive form of PTLPD evidenced by a significant lymphocytosis and abnormal increase in the percentage of mature B cells, which showed increase expression of CD45and CD19.

The expression of HLA-A01 and 02 was evidenced in all the five cases and was lacking in the remaining cases. On the other hand, there are conflicting reports, which showed that these alleles were protective against PTLPD. Most of this reports correlated this alleles with more advanced stages of PTLPD [19-21]. In addition, we were able to correlate between the expression of HLA-A01and A02 with the time of development of PTLPD. Moreover, recipients who express HLA-A02 developed the disease earlier than those express HLA-A01, and this was evidenced by the significantly shorter possessive free survival.

So, in our study, HLA-A01 was a good predictor for development of PTLPD, rather than protective against PTLPD as it was previously reported by **Brennan *et al.*** [22]

In spite of the novel finding reported in this study, yet there are some limitations which included the relatively small number of the studied recipient, and the need for HLA phenotyping by high resolution modalities.

CONCLUSION

Our study provided evidence that HLA-A01 and A02 alleles are good predictors of development of early non distractive form of PTLPD after kidney transplant.

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Author contribution: They contributed equally in the study.

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