The relationship between diabetic nephropathy and human leucocyte antigens in patients with type I diabetes mellitus*

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In order to investigate the possible role of HLA-related genetic factors in the pathogenesis of diabetic nephropathy (DN), we determined urinary albumin excretion and HLA-typing in 55 type I diabetic patients who have a duration of diabetes longer than 10 years and have no hypertension or clinical proteinuria, and compared patients with incipient DN to ones without nephropathy with respect to frequency of HLA-antigens. We found that microalbuminuric patients had higher frequencies of HLA-A2 and 68 antigens than those of normoalbuminuric patients. No significant difference was detected in terms of other HLA-Class I and II antigens. The relative risk of microalbiminuria were found to be 5, 3.5 and 10 times greater in the patients with HLA-A2, B8 and A2+B8 antigens than in the patients without the expression of these antigens, respectively. For the presence of microalbimunira, while HLA-A2 had the highest sensitivity (80%), the combination of HLA-A2-B8 had the highest specificity (96%). In conclusion, these findings suggest that possesing of both HLA-A2 and B8 antigens confers a great genetic predisposition to diabetic nephropathy independent of glycémie state and blood pressure. So, HLA typing may be useful in defining high risk patients for nephropathy in early period of type I diabetes.
[Turk J Med Res 1996; 14(2):67-70]

Key **Words:** Nephropathy, HLA-antigens, Microalbuminuria, Diabetes Mellitus

Diabetic nephropathy (DN) is a serious complication developing in approximately 35% of the patients with insulin-dependent diabetes mellitus (IDDM) (1). It is known that, some patients develop DN despite relatively good metabolic regulation, while others escape the development of nephropathy despite very poor metabolic regulation. So, it can be concluded that individual susceptibility is dependent not only on metabolic regulation, but also on various factors (2).

Genetic factors have been suggested as risk markers for development of nephropathy in diabetes. For therapeutic reasons it is of the utmost importance to find out which patients are at risk long before the manifestation of renal insufficiency (3,4).

In this study, we aimed to investigate the possible role of HLA-related genetic factors in the pathogenesis of DN. Therefore, we determined urinary albumin excretion (UAE) and HLA-typing in 55 type I diabetic patients and compared patients with incipient DN (persistent microalbuminuria) to ones without nephropathy with respect to frequency of HLA-antigens.

Received: Nov. 18, 1995 **Accepted:** Feb. 24, 1996

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**Presented as a poster at the XII[th] International Congress of Nephrology, July 3-6, 1995, Madrid-Spain*

MATERIALS AND METHODS

This study included 55 patients (29 males, 26 females) with type I diabetes mellitus who have a duration of diabetes longer than 10 years and have no hypertension or clinical proteinuria. The patients with urinary infection, heart failure, hematuria or glycosuria, the factors that may lead to an increase in UAE, were excluded from the study.

Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one third of pulse pressure. HbA1c levels of the patients were measured by a chromatographic method (Glyc-AffinTM GHb-Isolab Inc, USA, Cat no: SG 6220). UAE was determined by immunopresipitation assay (SPQ Test System for Microalbumin-Incstar Corporation, USA, Cat no: 86072) in 24-hour urine samples. UEA in the range of 30-300 mg/24h. in at least 2 out of 3 consecutive urine collections as was considered as persistent microalbuminuria (incipient DN) (5).

HLA-A-B and -DR typing was carried out by the standard NIH two-stage microlymphocytotoxicity test on peripheral blood lymphocytes (6). Commercial trays (Lymphotype HLA-ABC 72 and DR 72-Biotest) were used for HLA-typing. T and B lymphocytes were seperated by immunomagnetic beads (Dynabeads®-HLA Class I and Class II Dynal). Dual fluorescent stains (Acridine or-

ange/ethidium bromide) were used for staining of cells and reading procedure of the wells was carried out with an inverted immunofluorescence microscope (7).

Student's-t (unpaired) and Chi-square tests were used for statistical analyses. Sensitivity and specificity values were calculated with classical formulas and the relative risk (RR) of a patient having microalbuminuria associated with expression of the certain HLA-antigen was calculated from the following formula (8).

$$RR = \frac{p' \times C''}{p'' \times C'}$$

P' and P": the numbers of diabetic patient with persistent microalbimunira possessing and lacking the particular HLA antigen, respectively.

C' and C": the numbers of diabetic controls with normoalbuminuria possessing and lacking the particular HLA antigen, respectively.

RESULTS

Of 55 patients, 30 (54.5%) had microalbuminuria and 25 (45.5%) normoalbuminuria. There was not significant difference between normoalbuminuric and microalbuminuric groups with respect to age, body weight, age at onset of diabetes, duration of diabetes, mean arterial blood pressure, HbA1c and serum creatinine levels (Table 1).

No significant difference was detected normoalbuminuric and microalbuminuric groups in terms of the frequency of HLA/Class II antigens (HLA-DR 1-9). When compared the frequency of HLA-Class I antigens, we found that microalbuminuric patients had higher frequency of HLA-A2 and HLA-B8 antigens than those of normoalbuminuric patients ($p<0.01$ and $p<0.05$ respectively). No significant difference was detected in terms of other Class I HLA-antigens.

HLA-antigens seen more frequently in microalbuminuric patients was shown in Table 2.

The relative risk of microalbuminuria were found to be 5, 3.5,10.2,2 and 2.5 times greater in the patients with HLA-A2, B8, B44 and DR3/DR4 antigens than in the patients without the expression of these antigens, respectively.

The sensitivity, specificity and diagnostic accuracy values of some HLA-antigens for microalbuminuria were shown in Table 3.

For the presence of microalbuminuri, while HLA-A2 had the highest sensitivity (80%), negative predictive val-

Table 1. The comparison of normoalbuminuric and microalbuminuric patients (mean±SD) (range)

Parameters	normoalbuminuric patients (n=25)	microalbuminuric patients (n=30)	t	P
Age (year)	28.8±9 (15-48)	28±9.3 (14-47)	0.32	>0.5
Weight (kg)	56.2±10 (38-85)	60±14.5 (32-90)	1.08	>0.05
Duration of diabetes (year)	13.7±4.0 (10-24)	14±3.4 (10-25)	0.31	>0.05
MAP (mmHg)	93.7±6.6 (83-107)	94±7.0 (80-110)	0.11	>0.05
Serum creatinine (mmol/L)	0.91±0.2 (0.5-1.4)	0.97±0.1 (0.6-1.3)	1.11	>0.05
HbA1c(%)	10.8+2.0 (7.9-14.5)	11 ±2.2 (7.8-15)	0.29	>0.05
Age at onset of diabetes (year)	15.0±8.3 (4-29)	14±7.5 (2-29)	0.48	>0.05
UAE (mg/day)	11.9±5.4 (4-22)	138±60 (36-225)	10.47	<0.001

MAP: mean arterial pressure, UAE: urinary albumin excretion, SD: standart deviation

Table 2. HLA antigens seen more frequently in microalbuminuric patients (RR>2)

	Normoalbuminuric patients n=25		Normoalbuminuric patients n=30				
HLA	n	%	n	%	X^2	P	Relative risk
A2	11	44	24	80	7.63	<0.01	5.09
B8	4	16	12	40	3.80	<0.05	3.52
A2+B8	1	4	9	30	6.19	<0.02	10.28
B44	3	12	7	23	1.17	>0.05	2.22
DR3/4	10	40	19	63	2.98	>0.05	2.52

RR: relative risk

Table 3. Diagnostic accuracy of some HLA-antigens for microalbuminuria

HLA	Sensitivity (%)	Specificity (%)	(+) predictive value (%)	(-) predictive value (%)	diagnostic accuracy (%)
A2	80	56	68	70	69
B8	40	84	75	53	60
A2+B8	30	96	90	53	60
DR3/4	63	60	65	57	61

ue (70%) and diagnostic accuracy (69%), the combination of HLA-A2+B8 had the highest specificity (96%) and positive predictive value (90%).

DISCUSSION

There is evidence that the HLA antigens may provide markers of susceptibility of diabetic microangiopathy (9,10), but the link with nephropathy has not been adequately examined. In contrast to previous studies we have focused on the association between HLA antigens and the earliest phases of diabetic renal disease, microalbuminuria.

It is known that genetic predisposition of IDDM is related to HLA-DR3 and DR4 antigens (11,12). Since HLA related factors play a role in the pathogenesis of IDDM, these factors may also have a role in the severity and course of the disease and the development of microangiographic complications. At the practical level, knowledge of the genetic determinants of DN is important because it may allow attention to be focused at an early stage on patients who are most at risk screening programmes for microalbumiruria may therefore be made more efficient.

There are controversial reports about the relationship between DN and HLA-antigens (13-20). Salardi et al, reported that the frequency of HLA-DR3/DR4 was found to be significantly higher in microalbuminuric patients than in normoalbuminuric ones (13). We also found a greater frequency of HLA-DR3/DR4 in microalbuminuric diabetics compared to normoalbuminuric diabetics, but the difference did not achieve statistical significance.

Walton et al did not show a difference in the frequencies of HLA class I and II antigens in diabetics with and without clinical proteinuria; their groups were not, however, matched for duration of diabetes or glycemic control (14).

Watts et al. reported a strong association between HLA-A2 antigen and microalbuminuria, in their study, done on 172 normotensive, insulin dependent diabetic patients without clinical proteinuria, and they calculated the RR of microalbuminuria associated with the expression of HLA-A2 as 2.52. But they did not find any relation between microalbuminuria and other HLA antigens (11). We also confirmed these findings in the present study, and additionally, we also found the frequency of HLA-B8 to be greater in microalbuminuric patients than in normoalbuminuric ones. The relative risk of microalbuminuria was detected as 5 in HLA-A2 (+) patients and as 10 in HLA-A2+B8 (+) patients. The ten-fold increased risk of microalbuminuria association with expression of HLA-A2 must be regarded as clinically significant.

In the present study, we found that HLA-A2 positivity was more sensitivite but less specific than HLA-B8 positivity for the presence of microalbuminuria in diabetics. Watts et al. also reported that HLA-A2 positivity was sensitive but not specific for the presence of microalbuminuria (11).

In conclusion, these findings suggest that possessing of both HLA-A2 and B8 antigens confers a great genetic predisposition to diabetic nephropathy independent of glycemic state and blood pressure. So, HLA typing may be useful in defining high risk patients for nephropathy in early period of type I diabetes.

Tip I diabetli hastalarda diabetik nefropati ve HLA ilişkisi

Diabetik nefropati patogenezinde HLA ile ilişkili genetik faktörlerin rolünü araştırmak amacıyla; diabet süresi 10 yıldan fazla olan, hipertansiyonu ve klinik proteinürisi bulunmayan Tip I diabetli 55 hastada üriner albumin ekskresyonu ölçümü ve HLA tayini yapılarak, başlangıç dönemde nefropatisi olan (mikroalbuminürik) ve olmayan (normoalbuminürik) vakalar HLA antijenlerinin sıklığı yönünden karşılaştırıldı. Mikroalbuminürik hastalarda HLA-A2 ve B8 antijenlerinin sıklığı normoalbuminürik gruba göre önemli derecede daha yüksek bulundu. Diğer antijenler bakımından ise iki grup arasında önemli bir farklılık tesbit edilemedi. HLA-A2, B8 ve A2+B8 antijenleri bulunan vakalarda bu antijenleri taşımayanlara göre mikroalbuminüri görülme riski sırasıyla 5, 3.5 ve 10 kat daha yüksek bulundu. Mikroalbuminüri mevcudiyeti için HLA-A2 antijeni en yüksek duyarlılığa (%80), HLA-A2+B8 birlikteliği ise en yüksek özgüllüğe (%96) sahipti. Sonuç olarak; Tip I diabetli hastalarda kan basıncı ve metabolik kontrolden bağımsız olarak HLA-A2+B8 birlikteliğinin nefropati gelişimi için büyük bir risk taşıdığı ve yüksek riskli hastaları erken dönemde belirlemede HLA doku tiplemesinden yararlanılabileceği sonucuna varıldı.
[Türk J Med Res 1996; 14(2):67-70]

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