Possible Association between Class-I HLA molecules & Idiopathic Nephrotic Syndrome

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Summary:

Background: *Nephrotic Syndrome (NS) is a clinical entity having multiple causes, characterized by increased glomerular permeability manifested by massive protein urea with variable tendency towards edema, hypoalbumineima and hyperlipidemia.*

Materials & Methods: *Microlymphocytotoxicity has been applied for Class-I HLA typing of 67 Iraqi Nephrotic syndrome children's blood samples in comparison with 107 apparent healthy controls.*

Results & Conclusions: *This study revealed that there is a significant difference in HLA-B21 molecule frequency among the nephrotic syndrome patients in comparison with control group ($P<0.0003$) with OR of 4.8. Negative association was observed between HLA-B5 & disease ($P < 0.0001$) with Inverse OR of 9.6. Neither HLA-A molecules nor HLA-Cw molecules showed significant frequencies. It was concluded that HLA-B molecules (B21) may associate with the disease development, while (B5) may be considered as a protective factor.*

Introduction:

Nephrotic syndrome [NS] is a clinical complex condition characterized by the presence of proteinuria, hypoalbumineima, edema, hyperlipidemia, hyperlipiduria, and hypercoagulability [1]. The prominent clinical features are proteinuria 3.0-3.5 gram / 24 hrs. [1] or exceeds 40 mg/hr [2]. It was denoted that the prevalence of NS is age-related [3]. About 80% of NS patients are children [3]. Congenital NS (CNS), is defined as an early onset NS, which can be classified into primary and secondary (or acquired forms) with many subtypes [4].

In children the most common variety is the idiopathic NS. The term "idiopathic" is often used to describe a heterogeneous group of proteinuric glomerulopathies that occur predominantly in children [2]. Over the past few years it has become recognized that idiopathic NS are caused by mutations in genes that encode structural components of the glomerular filter [5]

Familial, and sporadic cases clinically are characterized by therapy-resistance and eventual progression to end-stage renal failure [6].

Non-familial forms of NS are more common. Based on the renal biopsy findings, can be sub-divided into MCNS (minimal changes NS) and FSGS (focal segmental glomerulosclerosis) [7].

Minimal changes NS was observed in approximately 95% of NS [12]. In children, MCNS is the most common form of NS, occurring in 35-80% of the cases of NS, depending on ethnicity [8, 9].

The etiology and pathogenesis of idiopathic NS remain obscure. It was assumed that the two main forms (MCNS and FSGS) share the same immunopathogenetic mechanisms and several immunological alterations such as the presence of circulating factors which promote proteinuria and increased serum levels of autoreactive IgA antibodies and interleukins [10, 11, 12]. Immunogenetic studies of non-related patients conducted on distinct populations have shown associations with HLA antigens for idiopathic patients irrespective of the variant [13, 15]. This study is a trial to investigate if the Iraqi idiopathic NS children share the same immunogenetic markers.

Materials & Methods:

This study has been performed during the period between January / 2005 and July / 2005 in which a total of 76 blood samples of Iraqi children NS patients have been collected in addition to 107 samples for apparent healthy controls, who are matched the patient group in age and sex. For class-I HLA typing modified micro-lymphocytotoxicity test has been applied for all above samples [16-17].

All the patients have been diagnosed under supervision of consultant nephrologists in "Pediatric Protection Hospital" in Baghdad city.

Statistical Analysis:

Uni-variant and Multi-variant analysis have been applied for the data depending on logistic regression and the results were reported as odds ratio

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(ORs), which represented the increase or decrease in risk for NS [18].

Results:

I- Demographical Picture of NS patients:

According to gender, the patients included 38 males and 38 females (50% for each) while the control group included 53 females and 54 males (49.5%, 50.5% respectively). The distribution of those patients according to age groups was shown in figure 1 below which revealed that the majority of patients are less than 5 years (38.2%) while the school age patients were less (36.8%), whereas the teenage were the minor group (25%). Considering the age group the control group was included 18 (16.8%), 22 (20.6%), 34(31.8%), 33 (30.8%) [For under 5 years, school age (5-9), teenage (10-15) and over 16 years respectively] respectively. Table 1reveals that HLA-A11 has the highest frequency among NS patients in comparison with control group (OR= 2.5) though it is non-significantly.

No. negative association was observed between HLA-A antigen and the disease.

II- Class-I HLA Typing:

Microlymphocytotoxicity assay has been applied for HLA typing for Class-I antigens. The results of this test are shown in Table 2, 3 and 4, for HLA-A, HLA-B and HLA-Cw molecules

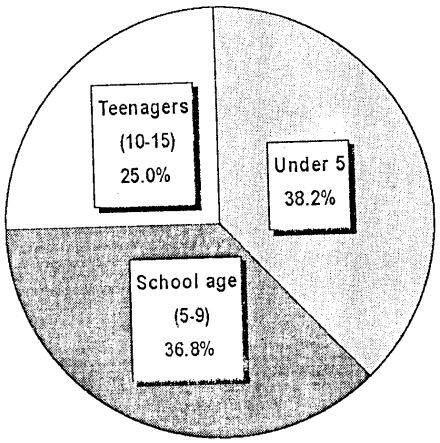

Figure 1: Age distribution of cases with nephritic syndrome.

Table 1: Frequencies of HLA class I (A) for NS patient, as compared with healthy controls.

	Cases (Nephrotic syndrome) (n=76)		Unrelated controls (n=107)		OR	Inverse OR	P value	Adjusted P	EF	PF
	N	%	N	%						
HLAtA	17	22.4	23	21.5	1.1	**	0.89	**	0.011	**
molegule	25	32.9	39	36.4	0.9	1.2	0.62	**	**	0.053
3	12	15.8	19	17.8	0.9	1.2	0.73	**	**	0.023
9	19	25.0	19	17.8	1.5	**	0.24	**	0.088	**
10	20	26.3	31	29.0	0.9	1.1	0.69	**	**	0.036
11	17	22.4	11	10.3	2.5	**	0.03	0.26[NS]	0.135	**
19	23	30.3	42	39.3	0.7	1.5	0.21	**	**	0.129
21	0	0.0	3	2.8	0.2	5.1	0.15	**	**	**
28	11	14.5	17	15.9	0.9	1.1	0.79	**	**	0.017
Blank	8	10.5	10	9.3	1.1	**	0.79	**	0.013	**

* = Not Done

Table 2 shows that the frequency of HLA-B molecules among the NS samples in comparison with healthy control. This table reveals that HLA-B21 participates in elevation the risk for NS development in highly significant manner in comparison with control group (OR= 4.8, P value < 0.0001, adjusted P = 0.002). On the other hand non-significant positive association of HLA-B molecule (B17) which assumes to be in high frequency

among patient cases in comparison with control (OR = 9.8, P < 0.003, adjusted P =0.8) .

An amazing high significantly, negative association is observed between B5 and the disease development (Inverse OR = 6.9, P < 0.0001, adjusted P =0.003).

There is no significant difference between patients' cases and control group in the frequency of Cw molecules as shown in Table 3 below.

Table 2: Frequencies of HLA class I (B) for NS patient, as compared with healthy controls.

	Cases (Nephrotic syndrome) (n=76)		Unrelated controls (n=107)		OR	Inverse OR	P value	Adjusted P	EF	PF
	N	%	N	%						
HLA-B molecule	5	6.6	35	32.7	0.1	9.6	0.000 HS	0.003	**	0.280
8	10	13.2	12	11.2	1.2	**	0.69	**	0.022	**
12	10	13.2	12	11.2	1.2	**	0.69	**	0.022	**
13	15	19.7	19	17.8	1.1	**	0.73	**	0.024	**
14	6	7.9	9	8.4	0.9	1.1	0.9	**	**	0.006
15	12	15.8	15	14.0	1.2	**	0.74	**	0.021	**
16	7	9.2	7	6.5	1.4	**	0.51	**	0.029	**
17	11	14.5	15	14.0	1.0	**	0.93	**	0.005	**
18	12	15.8	2	1.9	9.8	**	0.003 HS	0.08 NS	0.142	**
21	6	7.9	10	9.3	0.8	1.2	0.73	**	**	0.016
22	27	35.5	11	10.3	4.8	**	0.000 HS	**	0.281	**
27	7	9.2	10	9.3	1.0	1.0	0.98	**	**	0.001
35	3	3.9	5	4.7	0.8	1.2	0.81	**	**	0.008
37	1	1.3	0	0.0	4.3	**	0.24	**	0.010	**
40	5	6.6	3	2.8	2.4	**	0.23	**	0.039	**
41	1	1.3	1	0.9	1.4	**	0.81	**	0.004	**
42	2	2.6	2	1.9	1.4	**	0.73	**	0.008	**
47	0	0.0	2	1.9	0.3	3.6	0.27	**	**	**
53	1	1.3	1	0.9	1.4	**	0.81	**	0.004	**
57	2	2.6	1	0.9	2.9	**	0.39	**	0.017	**
58	0	0.0	4	3.7	0.2	6.7	0.09	**	**	**
73	0	0.0	5	4.7	0.1	8.2	0.05	**	**	**
Blank	0	0.0	2	1.9	0.3	3.6	0.27	**	**	**
	9	11.8	12	11.2						

** = Not done. HS= Highly significant, NS = Non-significant

Table 3: Frequencies of HLA class I (Cw) for NS patient, as compared with healthy controls.

	Cases (Nephrotic syndrome) (n=76)		Unrelated controls (n=107)		OR	Inverse OR	P value	Adjusted P	EF	PF
	N	%	N	%						
HLA-Cw molecule	7	9.2	13	12.1	0.7	1.4	0.53	**	**	0.032
3	9	11.8	18	16.8	0.7	1.5	0.35	**	**	0.056
4	21	27.6	29	27.1	1.0	**	0.94	**	0.007	**
5	17	22.4	29	27.1	0.8	1.3	0.47	**	**	0.061
6	14	18.4	29	27.1	0.6	1.6	0.17	**	**	0.106
7	21	27.6	19	17.8	1.8	**	0.11	**	0.120	**
8	25	32.9	28	26.2	1.4	**	0.32	**	0.091	**
Blank	5	6.6	11	10.3	0.6	1.6	0.39	**	**	0.040
	33	43.4	38	35.5						

** = Not Done.

Discussion:

The prevalence of NS is age-dependent. In the current study it was observed that majority of patients (38.2%) under five years of age. This result is comparable to other studies which showed that NS may occur during the first year of life, but it usually starts between 2-7 years of age [19, 20, 4]. On the contrary of other study, which denoted that NS cases where equally distributed between age group among Iraqi children [21]. This is true for the first two groups which characterized to some extent by weakness of natural defense mechanisms (in comparison with teenage group) particularly NK cells and thereby enhance the probability for infection and disease development [22]. Moreover, it is well known that the nature of diet habit of babies and children particularly high milk up take may increase the efforts on kidney for protein filtration and hence may encourage NS development.

No gender effect on the frequency of the disease was observed in this study, although other studies proposed high frequency of disease among males rather than females with male to female ratio of 2: 1 or even as high as 4:1 [23, 12]. This variation is related to the hormonal differences between male and female which results in high secretion of albumin in males rather than female [24].

Moreover, the variation in NS subtypes results in variation in Female: male ratio [24]

It is clear that the genetic parameters play an important role in disease development since 15.8% of patients are positive for family history of the disease, though this frequency is lower than that for the other [25]. This lower frequency may be related due to small sample size.

Genetic studies for HLA-Typing depend on variety of methods such as ELISA and PCR [26, 27], although the later are highly sensitive and specific but still not yet used in routine work and not easy available.

Microlymphocytotoxicity assay revealed that HLA-A molecules play non-significant role as an etiological factor for NS, though HLA-A11 molecule was noticed in high frequency among patients in comparison with control group (OR=2.5 , $P < 0.028$) . This result is in agreement with other regarding significant statistical association of A11 particularly among steroid-sensitive Nephrotic Syndrome patients [28]. Different result was reported considering this association, which differs by different populations, such as A31 association in Southern Turkey or no HLA-A molecules association with NS [29, 30].

Regarding the presence of HLA-B antigens and its proposed association with NS, Extensive studies have done with contraindicating results. The current study reveals statistically highly significant HLA-B17, B21 with OR of 9.8, and 4.8 respectively ($P< 0.0003$ and 0.0001 respectively). Those molecules may consider as risk factor which enhance the disease development. On the contrary of HLA-B5 which observed to show highly significant negative association with NS (Inverse OR = 6.9 and $P< 0.0001$). Other studies declared that there are positive associations with other HLA-B molecules such as B12 in European Children beside B8 while the other denoted that there is no effect for HLA-B molecules of the likelihood for disease development [2, 4, 29, 31].

There is no significant effect for HLA-Cw molecules on the probability of disease development in this study as well as the other studies denoted [32, 28].

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