The Profile of Human leukocyte antigen Class I in Patients with Schizophrenia

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Abstract

Background: Schizophrenia is a severe mental disorder that affects approximately one percent of the general population. It is a complex, chronic mental health disorder characterized by a spectrum of symptoms, including delusions, hallucinations, disorganized speech or behavior, and impaired cognitive ability. The pathogenesis of schizophrenia is influenced by many risk factors, most importantly environmental –genetic interplay. At the present time, in addition to environmental factors, genetic factors are assumed to play a role in the development of the schizophrenia. **Aim :** The present study was carried out to investigate the mapping of Human Leukocyte Antigen (HLA) class 1 (A&B) alleles in the selected sample of schizophrenic patients in Iraqi population. **Methods:** The extracted DNA was amplified for exon1, exon2, exon3, and exon4 of the HLA –A,B genes in 60 clinically diagnosed schizophrenic patients and 60 healthy control individuals (30 healthy first degree relative and 30 random unrelated healthy individuals with no family history of psychiatric illness .For statistical significance, measured OR is assessed by a special χ^2 formula . **Results:** One human leukocyte antigen -A gene (A*03) and two human leukocyte antigen - B genes (B*07 and B*40) significantly increased the risk of having schizophrenia compared to general population controls P value = 0.005, 0.005and 0.027 respectively. Human leukocyte antigen -B*40 gene increased the risk of having the disease by 3.3 times, while B42 decreased the risk by 4 times however fail to reach the statistical significance.**Conclusion**: HLA-A*03 allele and B*40 allele considered as significant risk factors for having schizophrenia. The presence of HLA-A*31, A*26 and A*68 alleles protects against having schizophrenia.

Key words: Schizophrenia , HLA, Allele, Genotype.

Introduction:

Schizophrenia (SZ) is a mental disorder characterized by abnormal social behavior and failure to understand reality **(1)** . It is a disabling disorder characterized by positive (delusions and hallucinations), negative (social withdrawal and apathy), and cognitive symptoms (poor executive function and memory). It affects around 1% of the population at some point in their lives, with onset characteristically during the period of brain development that follows puberty, and lasts until the end of the third decade **(2)**. Studies suggest that genetics, prenatal development, early environment, neurobiological , psychological and social processes are important contributory factors **(3)**. Indeed, SZ is a neural development disorder that may relate to several genetic and environmental factors **(4).**In the recent years, a considerable number of studies of gene-environment interactions in SZ have been performed, mainly employing a hypothesis-based genetic approach i.e., testing candidate genes hypothesized as involved in their pathogenesis **(5) .** Complex immune–brain interactions that affect neural development, survival, and function might have causal and therapeutic implications for many disorders of the CNS including psychiatric illness **(6).** An immune involvement is another longstanding hypothesis of schizophrenia, based on various lines of evidence including an association with human leukocyte antigen (HLA) status **(7)**. The role of autoimmunity has been addressed by epidemiological data linking autoimmune diseases and SZ which have recently been reviewed **(8).**The primary aim of this study was to evaluate whether the HLA –A,B genes were associated with the risk of schizophrenia in Iraqi population.

Materials and Methods

Subject **:** The current study was conducted during the period from first of April 2015 to the first of December 2015, 60 patient who attended the out patient department of psychiatry were diagnosed by a consultant psychiatrist in the psychiatry department in Al-Diwaniya teaching hospital on the basis of the structured clinical interview (DSM-IV) criteria for schizophrenia (American psychiatric association), and 60 healthy control individuals (30 healthy first degree relative and 30 random unrelated healthy individuals with no family history of psychiatric illness), were included in the study. The study population were assessed by verbal questionnaire regarding personal history age, sex, marital status, duration of illness (in the study group),and the coexistence of other chronic diseases, explanation and verbal consent was taken from the study population regarding the inclusion in the study and the retrieval of the required amount of blood sample.

Genotyping**:** The INNO- LiPA HLA- A, HLA- B Multiplex kit, for in vitro use, is intended for the nucleic acid amplification of the first to the fourth exon of the human leukocytes antigen (HLA) A&B locus. The reaction is performed by means of the polymerase chain reaction in a multiplex format. Genomic DNA was extracted from frozen blood of the study population, by using QIA amp DNA kit (QIAGEN, USA) and done according to company instructions.The extracted DNA was amplified for exon1, exon2, exon3, and exon4 of the HLA –A,B genes by using INNO-LiPA HLA-A,B Amplification Plus (FUJIREBIO, Belgium.

Statistical analysis: Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analyses were computer assisted using SPSS version 21 (Statistical Package for

Social Sciences). To measure the strength of association between 2 categorical variables, such as the presence of certain HLA-antigen and disease status the odds ratio (OR) was used.

Result

The results presented were based on the analysis of a random sample of 60 cases with an established diagnosis of schizophrenia. Their ages ranged between 14 and 75 years with a mean of 37.4 years. A paired sample of 30 healthy controls was selected from first degree relatives of 30 cases. Each of 30 randomly selected case had one matched healthy control. Their ages ranged between 21 and 65 years with a mean of 38.9 years. Another random sample of 30 general population healthy controls (group matched on age and gender) was included in the study. Their ages ranged between 15 and 60 years with a mean of 34.1 years. No important or statistically significant differences in mean age was observed between the 3 study groups. In addition males were more frequent than females in the 3 study groups. Again no statistically significant differences in gender distribution was observed between the 3 study groups, table (1). Half of the cases with schizophrenia (50%) were 20-29 years of age at diagnosis of the condition, while those younger than 20 years and older than 40 years constituted around one fifth of the cases. A positive family history of the disease was obtained in 70% of the cases. About one third (35%) of cases had their disease for less than 5 years and only a quarter (23.3%) had the condition for more than 14 years, table (2).

One HLA-A gene (A*03) allele and two HLA-B genes (B*07 and B*40) alleles significantly increased the risk of having schizophrenia compared to general population controls by 8.7, 8.7 and 3.8 times respectively. On the other hand, another four HLA-A genes, namely A*23, A*26, A*31 and A*68 alleles and one HLA-B gene (B*44) allele had a protective effect and significantly decreased the risk of having schizophrenia by 7.3, 14.5, 3.2, 8.8 and 20.5 times respectively. After adjusting the statistical significance for repeated comparisons involving the same group, only one HLA gene remained statistically significant, namely A*26. Allele, table(3).

The three HLA genes (A*03, A*31 and A*68) alleles that showed important associations with the risk of having schizophrenia compared to general population control had a similar important predictive association for the risk of the disease when compared to first degree relatives control group. The risk estimates, however were not significant statistically, because of a reduced sample size of cases group, since a paired (matched) analysis method was used. Only the presence of HLA-A*26 allele gene was associated with a statistically significant protective effect. It significantly reduced the risk of schizophrenia by 14.5 times compared to relatives control group. Two HLA-B genes (B*40 and B*42) alleles had an important predictive power for schizophrenia, although failed to reach the level of statistical significance. The presence of HLA-B*40 allele increased the risk of having the disease by 3.3 times, while B*42 allele decreased the risk by 4 times, table (4).

Discussion

Schizophrenia is a heritable brain illness with unknown pathogenic mechanisms. Schizophrenia's strongest genetic association at a population level involves variation in the major histocompatibility complex

(MHC) locus, but the genes and molecular mechanisms accounting for this have been challenging to identify **(9).** In this study the HLA genes were used as surrogate markers for predicting the risk of having schizophrenia compared to each of two control groups,(general population and first degree relatives).

HLA- A*03 allele that showed important associations with the risk of having schizophrenia compared to general population control had a similar important predictive association for the risk of the disease when compared to first degree relatives control group. The risk estimates, however were not significant statistically (OR=3.33, 95% CI OR= (0.92 - 12.1) (P=0.09, because of a reduced sample size of cases group, since a paired (matched) analysis method was used. Among HLA- A*31, A*26 and A*68 alleles that showed a protective effect and decreasing the risk of having schizophrenia, only the HLA-A*26 allele was associated with a statistically significant protective effect. It significantly reduced the risk of schizophrenia by 14.5 times (OR=0.11, 95% CI OR= (0.01 - 0.87), Inverse OR= 9.09) compared to relatives control group P=0.021. Two HLA-B genes (B*40 and B*42) alleles had an important predictive power for schizophrenia, although failed to reach the level of statistical significance. The presence of HLA-B*40 allele increased the risk of having the disease by 3.3 times (OR=3.33, 95% CI OR= (0.92 - 12.1), P= 0.09[NS], while (*B42) decreased the risk by 4 times(OR=0.25, 95% CI OR= (0.03 - 2.24, P=0.38[NS].

Debnath *et al.*, demonstrated results that indicate a marked elevation (60%) of the frequency of HLA-A*03allele ($P < 0.01$) in patients with schizophrenic disorder, compared with healthy control subjects **(10),** results which is highly comparable with present study . In addition, they also observed a non significant increased frequency of HLA-A*11 allele. Regarding HLA-B alleles they found that B*07 (22% vs. 10%), B*45 (14% vs. 5%).) also showed increased frequency but was not significant by comparison, our results revealed that B*07 allele significantly increase the risk of having schizophrenia. In contrary to our results they found that A*02 allele showed significant negative values in the paranoid schizophrenia. Rudduck *et al.*, also documented significant higher frequency of HLA-A*03 allele observed in patient with schizophrenia as compared with healthy controls in Swedish population **(11).**

Conclusions: HLA-A*03 allele and B*40 allele considered as significant risk factors for having schizophrenia. The presence of HLA-A*31, A*26 and A*68 alleles protects against having schizophrenia.

Table (1): Age and Gender Differences Between the 3 Study Groups.

		General population Controls		First Degree Relatives (Controls)		Cases (Schizophrenia)	
		NO.	%	NO.	%	NO.	%
1.	Gender						
	Female	9	30.0	13	43.3	14	23.3
	Male	21	70.0	17	56.7	46	76.7
	Total	30	100.0	30	100.0	60	100.0
	P= 0.15[NS]						
2.	Age group (years)						
	<30	12	40.0	7	23.3	19	31.7
	30-49	14	46.7	18	60.0	30	50.0
	50+	4	13.3	5	16.7	11	18.3
	Total	30	100.0	30	100.0	60	100.0
	Range	(15-60)		(21-65)		(14-75)	
	Mean	34.1		38.9		37.4	
	SD	11.9		11.3		12.7	
	SE	2.16		2.06		1.64	
	N	30		30		60	
	P=0.29[NS]						

Table (2): Frequency Distribution of Cases with Schizophrenia by Age of Onset, Duration of the Disease and Family History.

		NO.	%
1.	Age of onset groups (years)		
	<20	7	11.7
	20-29	30	50.0
	30-39	17	28.3
	40+	6	10.0
2.	Family history		
	Negative	18	30.0
	Positive	42	70.0
	Total	60	100.0
3.	Duration of the disease (years)-categories		
	<5	21	35.0
	5-9	11	18.3
	10-14	14	23.3
	15+	14	23.3
	Total	60	100.0

Table(3): Risk of Having Schizophrenia Compared to General Population Controls by HLA Alleles.

	OR	95% CI OR	Inverse OR	P Value	Adjusted P	EF	PF
HLA-A							
A*01	1.52	(0.56 - 4.16)	**	NS	NS	0.109	**
A*02	2.10	(0.81 - 5.47)	**	NS	NS	0.227	**
A*03	8.70	(1.89 - 40.07)	**	0.005	0.06[NS]	0.339	**
A*23	0.14	(0.03 - 0.73)	7.3	0.020	0.22[NS]	**	0.172
A*24	0.53	(0.16 - 1.74)	1.9	NS	NS	**	0.094
A*26	0.07	(0.01 - 0.34)	14.5	0.001	0.012	**	0.310
A*29	0.24	(0.02 - 2.73)	4.2	NS	NS	**	0.051
A*30	1.00	(0.09 - 11.5)	**	NS	NS	**	**
A*31	0.31	(0.1 - 0.93)	3.2	0.038	0.41[NS]	**	0.208
A*33	2.47	(0.5 - 12.22)	**	NS	NS	0.089	**
A*68	0.11	(0.02 - 0.59)	8.8	0.009	0.10[NS]	**	0.207
HLA-B							
B*07	8.70	(1.89 - 40.07)	**	0.005	0.06[NS]	0.339	**
B*08	0.16	(0.01 - 1.86)	6.2	NS	NS	**	**
B*14	0.49	(0.03 - 8.14)	2.0	NS	NS	**	0.017
B*15	0.53	(0.16 - 1.74)	1.9	NS	NS	**	0.094
B*18	2.80	(0.57 - 13.7)	**	NS	NS	0.107	**
B*35	1.10	(0.39 - 3.06)	**	NS	NS	0.022	**
B*37	0.16	(0.01 - 1.86)	6.2	NS	NS	**	**
B*38	0.31	(0.05 - 1.97)	3.2	NS	NS	**	0.069
B*40	3.76	(1.16 - 12.2)	**	0.027	0.3[NS]	0.269	**
B*41	1.00	(0.09 - 11.5)	**	NS	NS	**	**
B*42	0.22	(0.04 - 1.3)	4.5	NS	NS	**	0.103
B*44	0.05	(0.01 - 0.44)	20.5	0.007	0.076[NS]	**	**
B*49	0.22	(0.04 - 1.3)	4.5	NS	NS	**	0.103
B*50	0.46	(0.11 - 2)	2.2	NS	NS	**	0.071
B*51	1.38	(0.44 - 4.33)	**	NS	NS	0.060	**
B*52	0.55	(0.19 - 1.58)	1.8	NS	NS	**	0.120
B*55	3.22	(0.37 - 28.05)	**	NS	NS	0.069	**

Table(4): Risk of Having Schizophrenia Compared to 1st Degree Relative Control by HLA Alleles (Matched-Pared Design).

	OR	95% CI	inverse OR	P (McNemar)	Adjusted P	EF	PF
HLA-A							
HLAA*01	1.14	(0.41 - 3.14)	**	1[NS]	**	0.041	**
HLAA*02	1.29	(0.48 - 3.46)	**	0.8[NS]	**	0.090	**
HLAA*03	3.33	(0.92 - 12.1)	**	0.09[NS]	**	0.257	**
HLAA*23	**	**	**	**	**	**	**
HLAA*24	1.00	(0.29 - 3.45)	**	1[NS]	**	**	**
HLAA*26	0.11	(0.01 - 0.87)	9.09	0.021	0.21[NS]	**	0.211
HLAA*29	1.00	(0.06 - 15.99)	**	1[NS]	**	**	**
HLAA*30	1.00	(0.06 - 15.99)	**	1[NS]	**	**	**
HLAA*31	0.33	(0.07 - 1.64)	3.03	0.29[NS]	**	**	0.169
HLA-A*33	1.00	(0.25 - 4)	**	1[NS]	**	**	**
HLAA*68	0.25	(0.03 - 2.24)	4	0.38[NS]	**	**	0.090
HLA-B							
HLAB*07	1.50	(0.42 - 5.32)	**	0.75[NS]	**	0.111	**
HLAB*14	**	**	**	**	**	**	**
HLAB*15	1.00	(0.25 - 4)	**	1[NS]	**	**	**
HLAB*18	1.33	(0.3 - 5.94)	**	1[NS]	**	0.033	**
HLAB*35	0.83	(0.25 - 2.72)	1.2	1[NS]	**	**	0.046
HLAB*38	**	**	**	**	**	**	**
HLAB*40	3.33	(0.92 - 12.1)	**	0.09[NS]	**	0.257	**
HLAB*41	**	**	**	**	**	**	**
HLAB*42	0.25	(0.03 - 2.24)	4	0.38[NS]	**	**	0.090
HLAB*44	**	**	**	**	**	**	**
HLAB*49	0.50	(0.05 - 5.51)	2	1[NS]	**	**	0.032
HLAB*51	0.43	(0.11 - 1.66)	2.33	0.34[NS]	**	**	0.181
HLAB*52	1.00	(0.29 - 3.45)	**	1[NS]	**	**	**
HLAB*55	**	**	**	**	**	**	**

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