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Human Leukocyte Antigens and Host Factors are Associated with Good Health in Ageing Indian Population

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Abstract

Background: An efficient functional immune system is likely to be responsible for both good health and longevity. The human leukocyte antigen (HLA) system is responsible for many aspects of immunity.

Aims and objectives: This case-control study was carried out with to study the association of HLA with longevity and good health in Asian Indian population. The results of tissue-typing by serology and role of host factors in longevity was also evaluated.

Methods: A detailed questionnaire was administered to all 76 participants and 75 controls which included family history of longevity followed by a thorough clinical examination, routine hematological and biochemical testing. Tissue-typing in cases was performed by microlymphocytotoxicity (n=70), sequence specific primers (SSP) (n =06) or both (n=39) for class I antigens and by SSP for class II antigens. Statistical analysis was carried out by Chi-square test. P values and Odds ratio were calculated.

Results: A higher frequency of A*30, C*06, DRB1*13, DPB1*04:01 and *04:02 was present in the subjects whereas A*29, A*33, B*07, B*35, B*44, C*01, C*07, C*15 DRB1*04 was seen in the controls of which the antigens B*15, C*07 and C*15 were statistically significant. Longevity was found to be clustered in families. The study also brought out the polymorphism for DPB1 alleles min Asian Indians.

Keywords: Asian indian; Human leukocyte antigen; Sequence specific primers; Longevity and good health

Introduction

Ageing or senescence is associated with many physiological changes leading to enhanced susceptibility to diseases including malignancies culminating in death. Successful ageing implies presence of positive health with longevity. Longevity is influenced by both genetic and environmental factors.

In the last 25 years, many studies have searched for the impact of class I and class II HLA genes on human longevity. These studies have yielded conflicting results. In spite of the impressive amount of data generated, no clear consensus has been reached as to the role of HLA polymorphism on longevity [1]. It is likely that one of the genetic determinants of longevity reside in those polymorphisms for the immune system genes that regulate immune responses. Experiments performed on mice have suggested that the Major Histocompatibility Complex (MHC) is associated with the life span of the strains. Normal hematopoiesis and optimal functioning of T cells and thereby an intact immune system is is likely to be associated with longevity and good health [2,3]. Data from studies on many aspects of innate and acquired immunity suggests that a global well preserved immune function is associated with extended longevity and the immune system is continuously remodelled with ageing process [4].

We did not come across any studies on association of HLA antigens with good health in elderly or those based on Asian Indian population As per current estimates (2010) life expectancy for women is 67.57 years and that of men is 65.4 years [5,6] and the healthy life expectancy is 53.3 and 53.6 years respectively for Indian males and females [7].

Longevity is associated with positive or negative selection of alleles (or haplotypes) that respectively confer resistance or susceptibility to disease(s), via peptide presentation or via antigen non-specific control of the immune response. A study of DR frequency on 120 centenarians (79 women and 41 men) and 86 controls (53 women and 33 men) from Sardinia showed only DRB1*15 to be increased in the subjects [8]. A Chinese study from Shanghai which included 22 centenarians and 179 nonagenarians (mean age 93 ± 1.04 years) showed that A9 was highly associated (P=0.02), while A30, Cw07, Cw 03 and Cw 06 showed an inverse correlation (P<0.05) with longevity [9].

Materials and Methods

A retrospective case- control study was conducted at a tertiary care hospital over a two year period from February 2009 – January 2011. There were two groups which included 76 cases and 75 controls after matching for sex. Cases were carefully selected from healthy senior citizen population who had come to the hospital laboratory for routine lab tests, patients visiting Dental department or the eye OPD for cataract surgery. Controls comprised of 75 patients with end stage renal disease (ESRD) / hematological malignancies on waiting list for

renal or Bone Marrow Transplantation, whose tissue-typing was carried out in our laboratory for pre- transplant work up. As they were in poor health and most of them would not have ordinarily survived till 65 years age. The controls were matched for gender but not for age because this study compared longevity along with good health so the selection of controls for this study met the objective. Sample size was based on available budget and all samples were not typed for all antigens.

Inclusion criteria for cases

1. Age > 65 years

2. History of longevity in the family with at least one parent/sibling over 60 years age

3. Not more than two chronic diseases viz. Ischemic Heart Disease (IHD), Diabetes Mellitus (DM), Hypertension, Osteoarthritis

4. Regular physical activity equivalent to 30 minutes of walking four times a week.

5. Excellent mental health as concluded by interview

All cases were subjected to a medical examination including height and weight. Routine laboratory tests including CBC, blood sugar, urea, creatinine, lipid profile and urine examination was carried out. The criteria for physical and mental health were based on comprehensive history taking, exhaustive interview including a questionnaire, albeit not as exhaustive as SF -36 [10]. Informed consent from all subjects and approval of the Institution Ethical committee for the study was obtained.

Class I HLA typing was performed by serology using commercial kits from Innotrain (Germany) by standard microlymphocytotoxicity method. If the results were ambiguous or when only a single antigen was detected, low resolution DNA typing was carried out. Class II HLA typing was done by SSP using kits from Olerup (Austria). DNA was extracted from whole blood by column based method using Qiagen Kits (Germany). Thirty-nine samples of cases were typed for one or more Class I antigens by serology & SSP: while 12 were typed by SSP of which six were due to technical failure of serological typing. Low resolution typing for class I and II antigens by SSP except resolution for DPB1 locus. DPB1 typing is done only at high resolution. As it was the first time that we performed DPB1 typing we validated the results by Sequencing based typing for ten samples with kits from Genome Diagnostics (Utrecht, Netherlands). Protocol for SSP typing was as per the product insert.

The post PCR amplified products were examined by loading 10 µl in 2% molecular grade agarose gel and visualised with the help of UV Tec Gel documentation system (UK) and Stat score software was used for analysis. Further the analysis was confirmed with the help of tables manually when required. Relevant DRB3/4/5 and DQB1 associations were looked in to for all samples.

Results

The mean age for cases and controls was 71.3 and 34.6 years, respectively of which 60 (79%) were males. As per the history 56 cases (73.6%) had one parent who lived beyond 60 years age, and in 41 cases (56.5%) both parents survived beyond 60 years age. The number of cases who had one parent surviving beyond 70,80 and 90 years was 13, 27 and 16 respectively.

The height of 20 males among cases (33%) was >170 cms; all females were over 153 cms in height which is higher than general population and after accounting for decrease in height beyond 5th decade [11]. Only four cases were overweight. Thirty cases had no medical or surgical illness, 46 had some medical or surgical illness of which 15 had more than one illness (Table 1).

Illness	Cases
Essential Hypertension	22
None Insulin Dependent Diabetes Mellitus	13
Ischaemic Heart Disease	8
Late onset asthama	5
Low grade maligancy	3
Ttrivial surgical and or medical illness	10
Total	61

Table 1: Distribution of cases with respect to illness

In only 24/ 70 sample all six Class I antigens were detected on serology. Results were categorized as concordant when the antigen discrimination was at the private or public level and discrepant when one or more of the antigens were incorrectly or not identified. As expected the results were discrepant mainly for Cw antigen (Table 2), and where C locus had been tested along with others, discrepancy was on account of Cw antigen being typed incorrectly.

Antigen (s)	Number of samples	Concordant	Discrepant
typed Cw only	21	8	13
B only	5	3	2
A only	4	4	0
ABC	3	1	2
AB	3	3	0
AC	3	0	3
Total	39	19	20

Table 2: Results of HLA serology typing compared with SSP

The frequency of various HLA antigens and DPB1 allelels in cases and controls is shown in (Tables 3 and 4). Only three antigens C*15,C*07 and B*15 were significantly more frequent in the control population, while the antigens A*29 and allele DPB1*04:01 were more frequent in the control population but were not statistically significant (Table 5). Homozygosity for Class I HLA antigens was observed in four cases and 13 controls; homozygosity for class II antigens was present in nine cases and 19 controls. One sample from the cases showed homozygosity at both class I and II loci. A sample was considered homozygous if on SSP only one antigen was detected, as it was not possible to get samples from additional family members.

HLA - A	Freq Cases n=76	Freq Controls n=75	HLA-B	Freq N =72	Freq Controls 75	HLA C/Cw	Cases n =76	Controls n =73
01	21	20	07	15	9	01	6	13
02	25	21	08	4	1	02	7	3
03	05	9	13	07	6	03	16	14
23	03	5	15	07	23	04	21	18
24	26	25	18	01	2	05	2	1
26	03	1	21	1		06	15	10
28	06	3	27	06	2	07	19	40
11	15	20	35	18	25	*08	03	5
29	03	9	37	06	4	*12	09	8
30	06	1	38	02	2	*14	02	8
31	03	3	39	01	0	*15	10	42
32	03	2	40	17	20	*16	04	5
33	15	18	44	7	12			
34	02	1	47	1	3			
36	01	4	48	1	0			
68	05	6	51	12	7			
69	01	0	52	4	7			
80	0	3	53	1	1			
			55	3	9			
			56	2	6			
			57	8	6			
			58	7	9			

Table 3: Frequency of HLA -Class I antigens

Discussion

Sixty-five years was taken as cut off age for controls as at this age the country recognized its population as senior citizens and the same has been reduced to 60 years. Secondly this was the most chronologically aged population in good health that we could enrole for the study. All the subjects were physically and mentally fit and had been followed up for two years since enrolment. Only four subjects were overweight and a considerable proportion of cases were taller than average Indian height which is 164.7 years for males and 151.9 cms for females [12]. History of longevity was present in 73.6 % of cases. These host factors and regular physical activity may have contributed to good health and longevity of the cases. A study on Dutch population showed B40 to be negatively associated with ageing while DR5 was positively associated [13]. Another French study which included 533 centenarians and 229 nonagenarians for DR antigen association showed positive association of DR7 in men and DR 11 in women [14]. There was also an existence of homozygous advantage at DR locus at least at very advanced age which was not seen in present study [15]. Homozygosity at class I and II loci was more common in the controls, which is in agreement with the observation of some other workers in whose view heterozygosity is associated with better antigen presentation and hence better immune response and therefore may be more relevant to longevity and good health in the context of Indian population where infections are very common cause of morbidity and mortality. Our study showed a higher frequency of A*30, C*06, DRB1*13, DPB1*04:01 and *04:02 was present in the subjects whereas A*29, A*33, B*07, B*35, B*44, C*01, C*07, C*15 DRB1*04 was seen in the controls of which B*15, C*07 and C*15 were statistically significant. Discrepant results on Cw typing by serology have been described previously which could be attributed to a combination of low expression, lack of serological reagents and lack of information about distribution of C blanks [16].

The results of this study have been compared with those of some other workers in (Table 6). The difference in frequency of common antigens could be because of role of different HLA antigens in longevity in different ethnicities [7]. Our categorization of

homozygosity was not fully correct as this could have been so labeled only after testing samples from parents or by sequencing.

Limitations of this study include small sample size, and the fact that Cw typing was done by CDC for 34 subjects, while strengths include follow up for two years in OPD after enrolment, clinical correlation and the fact that this is one of the first few data of DPB1 typing in Asian Indians. As discussed by Izaks et al. [17], a minimum size of 320 persons for each sample is required to detect the difference in frequency if an antigen occurs. Most other studies have taken young population as controls which may also have shown longevity while the controls in current study had serious life threatening illness and were very unlikely to survive as long as the cases.

DRB1	Samples n =76	Controls n =75	DPB1	Samples n=48	Controls n = 45
*01	6 (1)	5(1)	*01:01	4	2
*15	32	42	*02:01	16	16
*16	2	1	*03:01	1	2
*03	11	5	*04:01	32	24
*04	7	11	*04:02	8	5
*07	12	25	*05:02	2	0
*08	6	5	*09:01	3	6
*09	1	1	*13:01	9	7
*10	14	17	*14:01	3	3
*11	11	12	*15:01	0	3
*12	2	8	*17:01	2	0
*13	18	15	*26:01	5	3
*14	12	8			
*13/*14	4	nil			
*08/*13	2	nil			

Table 4: Frequency of various Class II antigens in the samples; DPB1 allles with a frequency of 1 : *5:01, *06:02, *08:01, *10:01, *11:02, *13:02, *16:01, *23:01, *27:01, *28:01, *32:01,*35:01, *39:01, *46:01, *50:01, *53:01, *66:01, *70:01, *94:01, *96:01, *99:01

HLA type	Cases (n/sample size) (%)	Controls (n=sample size) (%)	Chi square value	p value
A*29	3	9	3.01	0.08
	-72	-75		
	-4.16	-12		
B*15	7	23	9.92	0.001
	-72	-75		
	-9.7	-30.66		
C*07	19	40	13.82	< 0.0001
	-76	-73		
	-25	-54.79		
C*15	10	42	32.27	<0.0001
	-76	-73		
	-13.15	-57.53		
DRB1* 15	32	42	2.67	0.1
	-75	-75		
	-42.66	-56		
DPB 1*04:01	17	24	1.72	0.1
	-48	-45		
	-66.66	-53.33		

Table 5: P values and Odds Ratios for HLA antigens / alleles with P values < 0.1

Authors	Ethnicity	Subjects	Controls	HLA Association
Lio et al. [8]	Sardinia	120 C (79 F + 41 M)	86 (53 F+ 33M)	DRB1 *15+ NS
Ma et al. [9]	Shanghai	22 C + 79 N	211 adults	A09 +; A30, Cw07,03, 06 – P < 0.05
Lagaay et al. [13] Izaaks et al.	Dutch	660 F (>85)	1010 F (20-35 years)	B40 –ve, DR5 +
Ivanova et al. [14]	French	533 C; 229 N	2950 adults	DR 11 –F, DR7-M, DR 13 both (P <0.01)
Izaks et al. [17]	Japan	129 C (100-110)	129 (20-75)	DR1 + NS
Vega et al.	Mexican	71(38 M, 33F)	99 (21-54 years)	DRB1*11
Present study	Asian Indian	76 (60 M, 16 F) > 65 yrs	75	B*15, C*07,C*15 -

Table 6: Comparison of present study with available literature

Conclusion

Host factors including, family history of longevity, weight, healthy life style and HLA are likely to have a role in longevity and good health. The study was first of such kind in Asian Indian population which showed significantly higher frequency of B*15, C*07 and C*15 in controls which may be negatively associated with ageing. The results are different from that in literature because of role of different HLA antigens in longevity in different ethnicities. There is an International Histocompatiblity Workshop project on same subject and new data is likely to emerge which may give impetus to more studies in India.

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