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Complement C4 - A Genetic Marker in Arthritides

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ABSTRACT The present study aims at evaluating the role of complement C4 and its specific eletromorphic association with arthritides. C4 typing of the serum samples was carried out by PAGE on 41 juvenile arthritis, 101 rheumatoid arthritis, 25 juvenile control subject and 76 adult control subjects.Complement C4 phenotype distribution revealed an increased preponderance of FS phenotype (61.0%) in juvenile arthritis, whereas in rheumatoid arthritis both FF (12.9%) and FS (70.3%) phenotypes were predominant with a significant association of FF phenotype with the condition. This reflects an association of the F allele with the condition. C4 may be one of the etiological factor in the arthritides and its role in handling the immune complex may be altered in rheumatoid arthritis.

INTRODUCTION

Arthritides categorised as juvenile and rheumatoid arthritis, are a heterogeneous group of disorders, predominantly affecting the joints and causing inflammation of the synovial membrane leading to erosion and destruction of the joint cartilage. Heredity as well as environmental factors, in combination with humoral response may play a causative and pathogenic role in the aetiology of arthritides, with the common mode of inheritance reported to be multifactorial in nature (Virginia 1997).

Juvenile arthritis has been termed as a chronic arthritis beginning in childhood and for which no underlying cause is apparent, with the age at onset usually being 1- 14 years while rheumatoid arthritis, is an inflammatory disease in which the immune system attacks the tissues, particularly the joints and synovium, resulting in swelling, stiffness and loss of function in the joints and the age at onset was observed to be usually between 20-40 years (Millon et al. 1984).

Complement system, plays a major role by attacking the bacterial invaders and its activation leads to lysis of bacteria and foreign cells. Allotyping of complement proteins is clinically useful, wherein patients with deficiencies of complements are particularly susceptible to infections. Complement activation is an important effector mechanism in synovial inflammation of arthritis and serum levels of complement degradation products are found to be elevated in patients of juvenile arthritis (Mollnes and Paus 1986; Jarvis et al. 1993) suggesting the role of complements in the pathogenesis of juvenile arthritis (Anurag et al. 2002). Complement C4 is highly polymorphic of the complement components with many common alleles at each of its two closely linked loci C4A (acidic) and C4B (basic). Such diversity may be related to different intrinsic strengths among humans to defend against infections and susceptibilities to autoimmune diseases (Chung et al. 2002). Hence, a study on its polymorphic variation in arthritides may throw light on the role of complement C4 in immune disorders like rheumatoid arthritis and its polymorphic variation may help in genetic risk prediction and delineation of genetic heterogeneity of arthritides.

MATERIALS AND METHODS

The present study is based on arthritides patients referred to out patient unit of orthopaedic department at Andhra Vaidya Vidhan Parishad, King Koti, Hyderabad. Cases of juvenile arthritis (JA) and rheumatoid arthritis (RA) confirmed radiologically were considered for which the hospital was visited during the period 1998- 2002. As controls, juvenile and adult healthy individuals matched for age and sex were considered from voluntary blood donors from various blood camps organized by Lion's Club and dental checkup camps in schools in and around Hyderabad city. Blood samples from 41 juvenile arthritis, 101 rheumatoid arthritis, 25 juvenile controls and 76 healthy controls matched

for age and sex were collected.

5 ml of venous blood was collected from each patient/individual in sterilized vials, for obtaining serum samples. The samples were centrifuged at 2000 rpm for about 10 minutes and the clear supernatant serum from clotted blood was stored in eppendorff tubes at 4°C until further use.

An 8%, 1.0 mm PAGE gel was prepared taking 1ml tris (hydroxy methyl) methylamine solution, 2 ml acrylamide / bisacrylamide solution, 4 ml of ammonium persulfate solution and 1 ml distilled water following protocol of Davies (1964). After polymerization, the plates were fixed to the vertical slab gel electrophoretic unit, with 0.05 M tris glycine stock buffer diluted 10 folds and connected to the anodic and cathodic ends of the DC power supply.

The sample was prepared by adding a drop of bromophenol blue indicator to 40µl of serum mixed thoroughly and loaded on to the sample slot of the gel. The electrophoresis was carried out at 4°C for 2 hours at 20mA constant current. The run was carried out till the indicator migrated to the other end of the gel as a blue sharp band. The gel was then placed in a tray and the electromorphs of complement components were detected by immunofixation carried out using specific commercial antiserum obtained from Sigma chemicals (USA). Gels were stained using 0.025% coomassie brilliant blue R250 prepared in 40% methanol and 7% acetic acid. Later the gel was given several changes in destaining solution containing 7% acetic acid and 5% methanol until the clear bands appeared for phenotyping of the respective complement.

The data were computed for gene frequencies. Relative risk estimates, Woolf's test of association and test for Hardy- Weinberg (H-W) equilibrium (Emery et al. 1984) was carried out to identify any specific eletromorphic association with arthritides.

RESULTS

The frequency distribution of complement C4 phenotypes in control and disease groups is presented in Table 1. In the juvenile control group, 16.0% were of phenotype FF, 56.0% FS and 28.0% SS, while in the juvenile arthritis group 14.6% were FF, 61.0% FS and 24.4% SS types. In general, an increased frequency of FS phenotype was observed in both the control and disease groups.

In the adult control group, 6.6%individuals were of phenotype FF, 53.9% FS and 39.5% SS, while in rheumatoid arthritis, 12.9% were of phenotype FF, 70.3% of FS and 16.8% of SS. There was a significant increased risk of both FF (12.9%) and FS (70.3%) phenotypic individuals to rheumatoid arthritis condition (χ^2 = 11.893 df =1, p<0.01), thus highlighting an association of F allele with the disease condition.

Table 2 shows the relative risk estimates of C4 phenotypes in juvenile arthritis compared to the juvenile control group wherein the value was found to be 0.829 in SS vs other phenotype comparison (χ^2 = 0.11, df =1, p< 0.05), 0.952 in SS vs FF comparison (χ^2 = 0.003, df =1, p< 0.05). Similarly, for SS vs FS comparison, the relative risk was found to be 0.8 (χ^2 = 0.138, df =1, p< 0.05) and for FF vs FS comparison it was found to be 0.84 (χ^2 = 0.057, df =1, p< 0.05), with no significant association of C4 phenotypes with juvenile arthritis condition. This strengthens the view that juvenile arthritis may be a genetically controlled disorder rather than being autoimmune in nature.

The allele frequencies of C4 (Table 3) in juvenile arthritis were 0.55 for S allele and 0.45 for F allele, with no significant deviation of gene/ genotypic frequencies from the Hardy Weinberg equilibrium (χ^2= 2.195, df =1, p< 0.05).

Table 4 gives the relative risk estimates of C4 phenotypes in rheumatoid arthritis in comparison

Table 1: Distribution of complement C4 phenotypes in control and disease group

Subjects		*C4 Phenotypes*						*χ^2-value*
		FF		*FS*		*SS*		
	n	*n*	*%*	*n*	*%*	*n*	*%*	
Juvenile Control	25	4	16.0	14	56.0	7	28.0	
Juvenile Arthritis	41	6	14.6	25	61.0	10	24.4	0.061
Adult Control	76	5	6.6	41	53.9	30	39.5	
Rheumatoid Arthritis	101	13	12.9	71	70.3	17	16.8	11.893**

**p<0.01, df = 1

Table 2: Relative risk (RR) estimates of Complement C4 phenotypes in control and juvenile arthritis group

C4	*Control*	*Juvenile arthritis*		
		n	*RR*	χ^2
Phenotypes				
SS	7	10		
SS vs others	8	31	0.829	0.11
Phenotypes				
SS vs F	4	6	0.952	0.003
SS vs FS	14	25	0.8	0.138
FF vs FS	14	25	0.84	0.057

parisons, revealing a significant decrease of *S* allele in the disease group compared to the control group. Further, the C4 FF and FS phenotypes were found to be predominant in the disease group compared to the control group highlighting an increased predisposition of F allele to rheumatoid arthritis.

The frequencies of C4 alleles in both control and rheumatoid arthritis patients are presented in Table 5. The allele frequencies of S and F were found to be 0.66 and 0.34 respectively in the

Table 3: Distribution of C4 phenotype and allele frequencies in control and juvenile arthritis group

C4	*Phenotypic frequencies*				χ^2			*Allelic frequencies*	
Phenotype	*Control*		*Juvenile arthritis*		*Control*	*Juvenile arthritis*	*Allele*	*Control*	*Juvenile arthritis*
	Obs.	*Exp.*	*Obs.*	*Exp.*					
SS	7	7.84	10	12.36	0.465	2.195	*S*	0.56	0.55
FS	14	12.32	25	20.3					
FF	4	4.84	6	8.34			*F*	0.44	0.45

df = 2

Table 4: Relative Risk (RR) estimates of Complement C4 phenotypes in control and rheumatoid arthritis group

C4	*Control*	*Rheumatoid arthritis*		
		n	*RR*	χ^2
Phenotype				
SS	30	17		
SS vs others	46	84	0.31	10.87**
Phenotypes				
SS vs F	5	13	0.218	6.29*
SS vs FS	41	71	0.327	9.6**
FF vs FS	41	71	1.501	1.12

control group and 0.52 and 0.48 respectively in the disease group, with a significant deviation from Hardy-Weinberg equilibrium (χ^2= 16.69, df =1, p< 0.01) in the disease condition, further supporting the phenomenon of an increased activation of complement system associated with autoimmunity.

Alternatively, the high degree of polymorphism in C4 has led to speculation that susceptibility to some autoimmune diseases might be due to differences in the interaction of

Table 5: Distribution of C4 phenotype and allele frequencies in control and rheumatoid arthritis group

C4	*Phenotypic frequencies*				χ^2			*Allelic frequencies*	
Phenotype	*Control*		*Rheumatoid arthritis*		*Control*	*Rheumatoid arthritis*	*Allele*	*Control*	*Rheumatoid arthritis*
	Obs.	*Exp.*	*Obs.*	*Exp.*					
SS	30	33.52	17	27.27	3.36	16.69**	*S*	0.66	0.52
FS	41	33.89	71	50.5					
FF	5	8.59	13	23.23			*F*	0.34	0.48

**p<0.01, df = 2

to the control group, wherein SS vs other phenotypes comparison revealed the relative risk to be 0.310 (χ^2= 10.87, df =1, p< 0.01), while in SS vs FF comparison, the relative risk obtained was 0.218 (χ^2:= 6.29, df =1, p< 0.05), and that in SS vs FS comparison it was 0.327 (χ^2 = 9.6, df =1, p< 0.01) and in FF vs FS comparison, the relative risk was found to be 1.501 (χ^2 = 1.12, df =1, p< 0.05). However, the relative risk estimates were found to be significant in SS vs other com-

C4 variants with other polymorphic complement proteins, antigens and antibodies (Rittner and Bertrams 1981), which could be one of the possible mechanisms associated with rheumatoid arthritis.

DISCUSSION

The complement system is a group of serum proteins that is directly involved in the defense against microbial infections. Too little

complement C4A or C4B leads to recurrent bacterial and viral infections. This may eventually result in different types of diseases such as lupus, nephritis and possibly type 1 diabetes (Yu et al. 2002). Allotyping of complement proteins is clinically essential to identify its possible role in autoimmune disorder and infections. Further complement activation is an important effector mechanism in synovial inflammation of arthritis. Serum levels of complement degradation products are found to be elevated in patients of juvenile arthritis (Mollnes and Paus, 1986; Jarvis, et al., 1993) suggesting the role of complements as an etiological factor in arthritides. A study of complements for its polymorphic variation and possible electromorph association with arthritides may throw light on its role in autoimmune disorder like rheumatoid arthritis and specific phenotypic association, if any, with the condition may further throw light on the specific alleles which may encode for a protein with altered stability and/or altered immune complex handling, opsonisation and immune complex solubilization. An increased preponderance of C4 FS phenotypes in juvenile arthritis suggests that juvenile arthritis is a genetically controlled disorder, with reduced activation of complement system. Similarly significant association of FF and FS phenotypes to rheumatoid arthritis (χ^2=11.893, df =1, p< 0.01) reveals the disorder to an autoimmune condition, with the complement system playing a pivotal role. Hence, the association of C4 complements seems to be distinct in each type confirming the genetic heterogeneity of arthritides. In conclusion, the specific allelic/electromorph associations of complement C4 with rheumatoid arthritis may throw light on its variation in the immune complex handling. Further, differences in the interactions of C4 with specific allele combinations with other complement proteins may alter opsonisation, immune complex transport and/or immune complex solubilization functions of the C4 and that a genetically determined decrease in immune complex processing might be a causative factor in chronic inflammation associated with rheumatoid arthritis.

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