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Molecular characterization of weaker variants of A and B in Indian population – The first report

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ABSTRACT

Background and objectives: The ABO blood group system is extremely important blood group system in transfusion medicine and weaker variants of A and B are subgroups of the system. From a Country like India with 1.2 billion population sporadic reports detecting weaker variants of A and B serologically are published. Therefore the main objective of the present study is to identify weaker variants of A and B serologically and characterize them at molecular level.

Materials and methods: Eight samples which were referred to us for resolving discrepancies in forward and reverse grouping were first phenotype in our laboratory by standard serologic techniques for ABO blood groups. Molecular genotyping for the ABO locus was done by PCR-SSCP. Altered SSCP patterns were analysed by DNA sequencing. Sequencing of intron 6 and exons 1–5 was done in one sample each.

Results and conclusion: Nine rare alleles affecting the normal expression of A and B antigens have been identified among Indians. They were two Aw06, one A209, one Ax20, two O05, one O49, one O56 and one O19 alleles. This is the first report demonstrating molecular studies on weaker variants of A and B from India.

1. Introduction

A and B antigens of the ABO blood group system are oligosaccharide antigens synthesized through series of enzymatic reactions, the final steps of which are catalysed by A and B glycosyltransferases which are the primary products of A and B genes respectively. A_1 and A_2 are the two major subgroups which are differentiated on the basis of reactivity of A_1 cells but not with A_2 cells with anti A_1 lectin (*Dolicos biflorus*). Apart from this, there are subgroups of A and B showing weaker expression of the respective antigens and are known as weaker variants of A and B respectively. The strength of agglutination reaction, the presence of mixed field agglutination, the presence of anti A_1 in the serum and the presence or absence of A, B and/or H substance in saliva help us to characterize weaker variants of A and B serologically [1].

There are sporadic reports from India, identifying weaker variants of A and B serologically [2–4]. In a large series of blood bank donors Bhatia and Sathe (1974) [3] reported

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[5] Performed DNA sequencing on the required samples.
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the incidence of weaker variants of A as 1:16700 individuals and weaker variants of B as 1:24000 individuals from Bombay. They observed that Ax–Bx variants were twice as common as Am–Bm variants among Bombay population. Recently the frequency of Ax has been reported as 1:14448 blood donors and that of Aend as 1:43344 donors from Chandigarh [4]. In this study the frequency of subgroups of B was much rarer than subgroups of A (1:86687). Sathe et al. [5] studied the levels of ABO glycosyltransferases in weaker variants of A and B and found to be considerably reduced.

The molecular structure of ABO gene was elucidated in 1990. Several studies have shown that there is a definite correlation between the nucleotide sequence variations in the coding region of the ABO gene and the quality and quantity of A or B antigens detectable on RBC surface [6–8].

Initially, Yamamoto et al. [9] identified alterations in the glycosyltransferases present in the individuals showing weaker variants serologically. Since then several alleles causing weaker expression of A and B have been characterized [10–13] and are deposited in db RBC antigen database (dbRBC, http://www.ncbi.nlm.nih.gov/gv/rbc/xslcgi.fcgi?cmd=bgmut/systems_info&system=abo). Till date there are no reports on molecular characterization of weaker variants of A and B from India. Here we report DNA alterations in 6 cases causing weaker variants of A and B for the first time from India.

2. Materials and methods

Several blood banks located in Mumbai referred either blood samples or individuals to the Dept. of Transfusion Medicine of the National Institute of Immunohaematology (NIIH) to resolve the ABO discrepancy in their cell & serum grouping. The blood samples from these individuals were collected intravenously in both EDTA & Plain vacationers. The saliva was also collected from these individuals. Their informed consent was taken. Forward and reverse grouping was performed by standard tube technique [14] using monoclonal antisera (Ortho diagnostics, USA). The phenotypes A_1 and A_2 were discriminated serologically using anti-A_1 prepared from Dolichos biflorus seeds indigenously. The weak presence of A or B antigen was determined by using different batches of appropriate monoclonal antisera. The antiserum which gave comparatively strong reaction was selected for adsorption–elution test. The test was carried out as described by Bhatia [14]. Hemagglutination inhibition test for secretor status in saliva was done to detect H, A or B substances in saliva.

The EDTA blood samples of weaker variants were taken for DNA analysis. The DNA was extracted from whole blood using standard phenol–chloroform technique [15]. The molecular method described by Yip [16] i.e. PCR followed by Single Strand Conformation Polymorphism (SSCP) was used to detect the polymorphism or mutation present in different fragments of exons 6 and 7. Three sets of specific primers were used here to amplify three fragments which were run using 9% T/1%C PAGE gel at 400 V for 2 and ½ h for SSCP as described previously.

After electrophoresis the bands were visualized by silver staining. The SSCP gel was stained by background free silver staining method [17]. For easy visualization of specific bands sodium thiosulphate was used in staining procedure to make the gel free from yellow or brown background by non-specific deposits of insoluble silver salts.

The samples which showed altered banding pattern in SSCP were characterized by sequencing. The DNA samples of case-1 and case-5 were sequenced for exon 1–5 and intron 6 of the ABO gene to look for the possible mutations as described earlier [18]. The DNA sequencing was carried out in the ABI Prism 310 Genetic Analyzer (Applied Bio-system, C.A. USA). The data was analysed using Macintosh sequence analysis 3.4 Software.

3. Results

Eight samples which were referred for resolving blood group discrepancy were first analysed by various serological techniques. Their results are shown in Table 1. In all the samples weaker expression of either A or B antigen was observed. Three samples showed A weak group, one sample showed B weak group, two samples showed A weak B while in two samples ABO group could not be determined. The categorization is shown in Table 1. They were further analysed by scanning exons 6 and 7 of the ABO gene to identify the molecular lesions. Whereever alterations were not detected remaining exons and introns were scanned.

Table 1
Serological results of weaker variants.

No. of cases	Forward grouping Antiseras						Blood group	Reverse grouping Pooled cells				Absorption/Elution studies		
	−A	−B	−A + B	$-A_1$	−D	−H		A Cells	B Cells	O Cells	O Cells + Pap	Anti-A	Anti-B	AntiA + B
Case 1	+w	0	+4	0	+4	+3	Ax type	0	0	0	0	+4	0	0
Case 2	0	+w	+4	0	+4	+2	Bx type	w	0	0	0	0	+4	0
Case 3	(+w)	0	(+w)	0	+4	+4	A_{end} type	0	+w	0	0	+4	0	+3
Case 4	(+w)	+4	+4	0	+4	+2	AxB type	(vw)	0	0	0	+4	NA	+4
Case 5	0	0	0	0	+4	+4	ND	0	(vw)	0	0	0	0	0
Case 6	+w	0	+3	0	+4	+2	Ax type	0	+4	0	0	+4	NA	+3
Case 7	(+w)	+4	+4	0	+4	+1	AxB type	(+w)	0	0	0	0	NA	0
Case 8	0	0	(+w)	0	+4	+4	ND	+4	+4	0	0	0	0	0

Footnotes: +w – weak reaction visible with naked eye, (+w) – weak reaction microscopically, (vw) – very weak reaction microscopically, ND – cannot detect, NA – not applicable.