



Original article

Serological characteristics of Lewis antibodies and their clinical significance – A case series

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ABSTRACT

Introduction: Lewis antibodies have been thought to play a small role in clinical transfusion practice, but recent reports suggest that they have gained more importance in the context of transfusion and transplantation. Data regarding the prevalence of Lewis antibodies and their clinical significance in the Indian context is very limited. Hence, this study was aimed at analyzing the serological characteristics and clinical significance of Lewis antibodies encountered in our patient and donor populations.

Methods: The retrospective data analyzed the records of red cell antibody screening results and the additional serological evaluation performed on the donor and patient samples included in the study.

Results: A total of 26 study subjects were noted to have Lewis antibodies (including 6 healthy donors and 20 patients). Of them, 13 individuals had anti-Le^b, 10 had anti-Le^a and the remaining three had an anti-Le^a/Le^b mixture. IgG Lewis antibodies were detected in 7 individuals. All cases of IgM Lewis antibodies detected were reacting at 37°C. Two patients were suspected of having hemolytic transfusion reactions due to Lewis antibodies. Antigen-negative cross-match compatible units were provided for transfusion in the recipients.

Conclusion: Lewis antibodies of the IgM class reacting at 37°C should be regarded as clinically important. The present study findings urge that the lab personnel look for the thermal amplitude of Lewis antibodies, irrespective of the fact that the antibody class and antigen-negative crossmatch compatible units should be provided to avoid hemolytic transfusion reactions.

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Introduction

Lewis antigens are widely distributed in the body and hence, they are called histoblood group antigens. Similar to ABH antigens, they are found in the pancreas, stomach, mucosa of small and large intestines, skeletal muscle, renal cortex and

adrenal glands.¹ Lewis antigens are believed to be produced by the exocrine glands of the small intestine and their synthesis is dependant on the presence of two enzymes encoded by the *Se* gene (*FUT2*) and *Le* gene (*FUT3*) located on chromosome 19p13.3 and 19q13.3, respectively. The *Le* gene produces a fucosyl transferase enzyme, which adds fucose directly to the type I precursor chain to form Le^a. The presence of *Se*

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(H transferase) and *Le* genes eventually creates the *Le^b* antigen by adding fucose to the type I H chain. Hence, *Le(a-b+)* individuals are always secretor and *Le(a+b-)* individuals are always non-secretors. Since red cells use type 2 chains, they cannot synthesize Lewis antigens, rather they are adsorbed from the plasma onto the red cell membrane.²

A total of 6 antigens (*Le^a*, *Le^b*, *Le^{ab}*, *Le^{bH}*, *ALe^b* and *BLe^b*) belong to the Lewis blood group system. Of them, *Le^a* and *Le^b* antigens are the most important and are frequently encountered in clinical practice.³ Accordingly, four Lewis antigen phenotypes have been described including *Le(a-b-)*, *Le(a-b+)*, *Le(a+b-)* and *Le(a+b+)*. The frequencies of Lewis antigen phenotypes, including *Le(a-b+)*, *Le(a+b-)*, *Le(a-b-)* and *Le(a+b+)*, among our studied donor population were 55%, 22.5%, 15.5% and 7%, respectively.

Lewis antibodies have been thought to play a small role in the clinical transfusion practice, but recent reports suggest that these antibodies have gained more importance in the context of transfusion and transplantation.⁴ Data regarding the prevalence of Lewis antibodies and their clinical significance in the Indian context is very limited. Hence, this study was aimed at analyzing the serological characteristics and clinical significance of Lewis antibodies encountered in our patient and donor populations.

Materials and methods

The retrospective observational study was conducted in the Department of Transfusion Medicine in a tertiary care hospital in South India from February 2016 to February 2021. The institutional ethics committee approval was obtained. Antibody screening results of all the patient and donor samples performed during the study period were traced and the data regarding the samples having Lewis antibodies were compiled.

Blood grouping for all samples was routinely performed by automated blood grouping equipment (IH-500, BioRad, Cressier, Switzerland) in column agglutination technology (CAT), using monoclonal antisera. In the case of a blood group discrepancy, blood grouping was repeated, using the conventional tube technique with monoclonal antisera (Tulip Diagnostics, Goa, India) and in-house pooled cells. At our center, preliminary antibody screening using pooled O cells was performed on all the donor samples. Donor samples testing positive during preliminary screening and those patient samples with blood group discrepancy/crossmatch incompatibility/samples of all RhD negative pregnant women were subjected to antibody screening, using commercial cell panels (Diacell, Biorad, Switzerland) in CAT.

Antibody identification was subsequently performed to detect the specificity of antibodies, using a commercial 11-cell panel (Diapanel Biorad, Switzerland) in CAT. Papain-treated red cells were used to augment the agglutination reaction in CAT. The direct antiglobulin test (DAT) was performed on all samples (before performing antigen phenotyping), using the monospecific Coombs gel card (DC screening II, Biorad, Cressier, Switzerland). The Lewis antigen (*Le^a* and *Le^b*) phenotyping was performed in CAT, using a single antigen testing card, as per manufacturer instructions (ID card,

Diacell anti-*Le^a* and anti-*Le^b*, Biorad, Cressier, Switzerland). The dithiothriitol (DTT) treatment of the serum was performed as described in the AABB Technical manual to detect the class (IgM or IgG) of the Lewis antibodies. The secretor status of the individuals was determined with the hemagglutination inhibition test. All the abovementioned procedures were performed as per the standard protocols of our department. The data were tabulated and the calculations were made using Excel software.

Results

During the study period, 48,236 donor samples and 7,864 patient samples were screened for unexpected antibodies. A total of 26 study subjects were noted to have Lewis antibodies, including 6 healthy donors (0.01%) and 20 patients (0.25%). Of these, 13 individuals had anti-*Le^b*, 10 had anti-*Le^a* and the remaining three had a mixture of anti-*Le^a*/*Le^b*. The serological characteristics of the Lewis antibodies among patients and donors have been studied separately.

Patients

The patient characteristics have been described in Table 1. Eleven patients had anti-*Le^b* antibodies, anti-*Le^a* was noted in seven patients and the remaining two had a mixture of anti-*Le^a*/*Le^b* antibodies. Of the study population, 35% (7 out of 20 patients) were pregnant women and two of them were multi-gravida. The individual clinical and laboratory characteristics have been summarized in Table 2. While a majority of them had IgM antibodies with a wide thermal amplitude (reacting at 4°C, 22°C and 37°C), the IgG class of Lewis antibodies were

Table 1 – An outline of the demographics and serological characteristics of patients.

Characteristics	N = 20
Mean Age in years (range)	43.7 (20 -78)
Gender	
Male	6
Female	14
Diagnosis	
Pregnant women	7
Chronic kidney disease	2
Hematological disorder/malignancy	2
Coronary artery disease	2
Diabetes mellitus	2
Neurological disorder	2
Chronic liver disease	1
Intertrochanteric fracture	1
Solid organ malignancy	1
Blood group distribution	
A	4
B	6
AB	7
O	3
No of individuals previously transfused	2
No. of samples with anti- <i>Le^a</i>	7 (5 IgM, 2 IgG)
No. of samples with anti- <i>Le^b</i>	11(10 IgM, 1 IgG)
No. of samples with anti- <i>Le^a</i> / <i>Le^b</i>	2 (1 IgM, 1 IgG)

Table 2 – Individual data of the patients.

Sex	Age	Associated clinical condition	Blood group	Transfusion history	DAT	Antibody characteristics			Secretor status	Lewis phenotype	Transfusion details(*)
						Specificity	Type	Thermal amplitude			
F	30	Antenatal	B negative	No	negative	Anti-Le ^a	IgG	37 ^o C	Secretor	Le (a-b-)	
F	25	Antenatal	A negative	No	negative	Anti-Le ^a	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	
F	20	Antenatal	B positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non- Secretor	Le (a-b-)	
F	23	Antenatal	AB negative	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	
F	26	Antenatal	O negative	No	negative	Anti-Le ^a	IgG	37 ^o C	Secretor	Le (a-b-)	
F	23	Antenatal	AB negative	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	
F	24	Antenatal	AB negative	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	
M	37	CKD	AB positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non- Secretor	Le (a-b-)	1 unit Le ^b – transfused
F	34	CKD	AB positive	No	negative	Anti-Le ^a /Le ^b	IgG	37 ^o C	Secretor	Le (a-b-)	
M	64	Low grade lymphom-a	O negative	Multiple, Last transfusion – a week before admission	C3d (1+)	Anti-Le ^a	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	ND*	2 units Le ^a -transfused
F	78	Chronic anemia	O positive	Multiple, Last transfusion- two days before admission	C3d(3+)	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non-Secretor	ND*	
F	65	CAD	B positive	No	negative	Anti-Le ^a	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	1 unit Le ^a -transfused
M	42	CAD	A positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non-Secretor	Le (a-b-)	
F	58	DKA	B positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non-Secretor	Le (a-b-)	
M	71	Diabetic foot ulcer	B positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non-Secretor	Le (a+b-)	
F	28	Acute SDH	A positive	No	negative	Anti-Le ^a	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	1 unit Le ^a - transfused
F	70	Myasthenia gravis	AB positive	No	negative	Anti-Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Non secretor	Le(a+b-)	2 units of Le ^b - transfused.
M	42	CLD	A positive	No	negative	Anti-Le ^a	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	1 unit Le ^a - transfused
M	67	Inter-trochanteric fracture	B positive	No	negative	Anti-Le ^b	IgG	37 ^o C	Non- Secretor	Le (a-b-)	1 unit Le ^b - transfused
F	48	Carcino-ma ovary	AB positive	No	negative	Anti-Le ^a /Le ^b	IgM	4 ^o C, 22 ^o C, 37 ^o C	Secretor	Le (a-b-)	

*ND- Not done in view of recent transfusion history; DAT- Direct antiglobulin test; CKD- Chronic kidney disease; CAD- Coronary artery disease; DKA- Diabetic Ketoacidosis; SDH- Subdural hemorrhage; CLD- Chronic liver disease; * - PRBCs transfused

detected in four patients. Twelve patients were identified to be secretors. Two patients were typed as Le(a+b-), while most of them were phenotyped as Le(a-b-).

Except for two patients, none of them had been previously transfused. One patient, recently diagnosed with low-grade lymphoma, was referred to our hospital for further management. The patient had been transfused with 2 units of packed red blood cells (PRBCs) at another center, a week prior to admission. The hemoglobin (Hb) was 5.8g/dL and DAT was positive for C3d only(1+). The patient was found to have IgM class anti-Le^a reacting at 37°C, while evaluating an incompatible crossmatch. Another elderly female received one unit of PRBCs, while being evaluated for anemia at a different center. Two days later, she was referred to our center because of the rapid decrease in Hb (from 6.2 to 4.8g/dL) post-transfusion. The DAT was positive for C3d (3+) only, with elevated serum lactate dehydrogenase levels and indirect hyperbilirubinemia. Anti-Le^b antibodies of the IgM class, reacting at 37°C were detected, while investigating the transfusion reaction. The pre-transfusion sample could not be traced and hence, the source of the immunization could not be determined. Lewis phenotyping could not be performed in both of the cases in view of the recent transfusion and the possibility of the Lewis-antibody-induced delayed hemolytic transfusion reaction was considered.

In total, 7 patients who required transfusion were transfused with 9 Lewis-antigen negative compatible units and the transfusion episodes were uneventful. Individual characteristics of the patients are shown in [Table 2](#).

Donors

Among the donors ($n = 6$), 3 had anti-Le^a, 2 had anti-Le^b, while one had a mixture of anti-Le^a/Le^b. All were males, aged from 23 to 54 years ([Table 3](#)). IgG class antibodies were detected in three donors and the remaining had IgM class antibodies reacting at 37°C. Two of them were non-secretors. All donors were typed Le(a-b-). None of them had been transfused. Individual characteristics of the donors have been tabulated in [Table 4](#).

Table 3 – An outline of the demographics and serological characteristics of donors.

Characteristics	N = 6
Mean Age in years (range)	29.8 (23-54)
Gender	
Male	6
Female	nil
Blood group distribution	
A	2
B	2
AB	1
O	1
No of individuals previously transfused	Nil
No. of samples with anti-Le ^a	3(1 IgM, 2 IgG)
No. of samples with anti-Le ^b	2(1 IgM, 1 IgG)
No. of samples with anti-Le ^a /Le ^b	1(IgM only)

Discussion

Those antibodies which can cause the hemolytic transfusion reaction (HTR), hemolytic disease of the fetus and newborn (HDFN) and reduced in-vivo survival of the transfused red cells are considered clinically significant. They are generally IgG antibodies reacting at 37°C.³ Lewis antibodies have been thought to lack significance and, considered benign due to the following reasons. Firstly, it is the soluble nature of the Lewis antigens which enables the donor red cells to shed their antigens. Secondly, these shed antigens often get neutralized by the recipient antibodies. Thirdly, these antibodies display a strong predilection for reacting at lower temperatures. Fourthly, Lewis antigens are weakly expressed by non-O group individuals when compared to O group individuals and hence, crossmatch incompatibility is not often encountered. Finally, Lewis antibodies are predominantly naturally occurring IgM antibodies and only occasional cases of the IgG class have been documented.^{3,5,6} In the present study, it was noted that, although the majority of the Lewis antibodies were of the IgM class, IgG class antibodies were not uncommon. In addition, all cases of IgM class Lewis antibodies displayed reactivity at 37°C, highlighting their clinical significance. Similar observations were made by Das *et al.*, in which 4 out of 7 individuals had IgG class Lewis antibodies⁷. Rarely has transfusion-induced Lewis alloimmunization been described in the literature.^{8,9}

At our center, antibody screening of the patient population is performed selectively, which could be the limitation of the present study. Active screening of all the patients would yield the actual prevalence of Lewis antibodies in this cohort. In one study, on screening 69,354 individuals, no case of anti-Le^b was found.⁷ In the present study, anti-Le^b was observed to be more frequent than anti-Le^a. The frequency of Lewis antibodies therefore tends to vary among different studies.¹⁰ Interestingly, it has been shown that the ABO blood group system can influence the expression of Lewis antigens. Especially the O group individuals have been shown to carry a significant amount of Lewis antigens on their red cells, compared to A/B/AB group individuals, which may influence the severity of hemolysis.^{5,8} Accordingly, O group individuals are less likely to produce Lewis antibodies, as seen in the present study.

Overall, there are two noteworthy features of Lewis antibodies which render them clinically relevant. 1, The hemolytic potential of Lewis antibodies has been ascribed to their complement-fixing ability, particularly the IgM class. The resultant instant intravascular hemolysis can even be fatal. Both acute and delayed HTRs have been documented with Lewis antibodies. Recently published literature highlights that the IgM class of Lewis antibodies (both anti-Le^a and -Le^b) reacting at 37°C can lead to acute hemolytic reactions in transfused recipients.⁴ In the current study, delayed hemolytic transfusion reaction, secondary to a Lewis antibody, was suspected in two patients, which was corroborated by the laboratory features of hemolysis (one had anti-Le^a and the other had anti-Le^b, IgM class antibodies, C3d positive DAT) and a temporal association with transfusion. 2, Individuals with a pre-existing Lewis antibody, are known to exhibit an

Table 4 – Individual data of the donors.

Sex	Age	Blood group	Transfusio- n history	DAT	Antibody characteristics			Secretor status	Lewis phenotype
					Specificity	Type	Thermal amplitude		
M	54	AB positive	No	negative	Anti-Le ^b	IgM	4°C, 22°C, 37°C	Non-Secretor	Le (a-b-)
M	25	B positive	No	negative	Anti-Le ^a	IgG	37°C	Secretor	Le (a-b-)
M	23	O positive	No	negative	Anti-Le ^a	IgM	4°C, 22°C, 37°C	Secretor	Le (a-b-)
M	24	A positive	No	negative	Anti-Le ^a	IgG	37°C	Secretor	Le (a-b-)
M	23	B positive	No	negative	Anti-Le ^b	IgG	37°C	Non-Secretor	Le (a-b-)
M	30	A Positive	No	negative	Anti-Le ^a /Le ^b	IgM	4°C, 22°C, 37°C	Secretor	Le (a-b-)

DAT- Direct antiglobulin test.

anamnestic reaction, subsequent to an antigen-incompatible transfusion.^{5,8}

Lewis antibodies are often found in the individuals having the Le(a-b-) phenotype and in the sera of pregnant women.¹¹ In a study on the rate of alloimmunization among 5,347 pregnant women, anti-D was the most frequent specificity observed (34.2%), followed by Lewis antibodies (17.7%).¹² In the present study, Lewis antibodies were most commonly found in pregnant women among the patient population, all with the Le(a-b-) phenotype. Hammar *et al.* observed that the frequency of the Le(a-b-) phenotype was significantly higher in pregnant women at the time of delivery (36%) when compared to non-pregnant women (11%). The null phenotype can appear as early as the 24th week of pregnancy due to the four-fold increase in the lipoprotein concentration, leading to the redistribution of the Lewis glycolipids on red cells. However, it is reversible and the original phenotype can be detected by 3 to 6 weeks after delivery.¹³ Despite occurring frequently amongst antenatal women, Lewis antibodies have rarely been implicated in HDFN as most of them are of the IgM class and Lewis antigens are poorly expressed at birth owing to their gut immaturity.^{2,3,4} In the present study, no case of Lewis antibody-related HDFN was observed, even though IgG antibodies were demonstrated in the sera of pregnant women.

Lewis antigens are usually present on tubular cells of the kidney tissue and may get adsorbed onto the glomerular and endothelial cells. Lewis antigens may generate cell-mediated or humoral response of a cytotoxic nature. Spitalnik *et al.* observed that those recipients with anti-Le^a or anti-Le^b antibodies who received Lewis incompatible kidney allografts later manifested with a 100% allograft rejection. Lewis negative recipients may develop antibodies on exposure to the Lewis antigens on the donor graft.¹⁴ Bortanyaska *et al.*, observed humoral rejection of the renal allografts due to complement-fixing Lewis antibodies formed post-transplant. Although Lewis antigen matching is not routinely recommended for renal transplants, for those with a past history of renal allograft rejection due to Lewis antibodies, the ensuing renal transplantation should be matched for Lewis antigens with the recipient.¹⁵

To conclude, most of the blood centers generally ignore evaluating naturally occurring IgM antibodies. But, the growing evidence on the clinical significance of Lewis antibodies demands more attention, thanks to the sensitive platforms

employed during pre-transfusion testing. The present study findings urge the lab personnel look for the thermal amplitude of Lewis antibodies, irrespective of the antibody class.⁹ IgM antibodies reacting at 37°C should be regarded as clinically important and antigen-negative crossmatch-compatible units should be provided to avoid hemolytic transfusion reactions.

Conflicts of interest

None.

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