Case Report

Anti-Le^a IgG in a pregnant woman

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Abstract:

Antibodies of the Lewis blood group system are not generally considered to be clinically significant. Since the majority of them are IgM isotype, they do not react at the indirect antiglobulin (IAT) phase and do not cause hemolytic disease of the fetus and newborn (HDFN). Typically, the form of the IgG antibody is not found either in the general population or pregnant women. The Lewis antibodies are relatively common in the Thai population with an incidence of anti-Le^a (IgM) as 4.22%. Here we report a case of a pure IgG isotype of anti-Le^a antibody in a healthy 18-year-old pregnant woman. This was her first pregnancy, and she had no previous blood transfusion. The preoperative type and screen revealed her blood groups was A, RhD positive, Le(a-b-) phenotype. Her antibody screening was positive by column agglutination test (CAT). While the antibody identification assay by conventional tube test (CTT) disclosed the presence of anti-Le^a reacting only at the IAT phase. The IgG subtype of the detected antibody was confirmed by using a monospecific direct antiglobulin gel card. It appeared to be positive only in an anti-IgG column. No HDFN was observed. After the patient underwent a caesarean section, the baby was born healthy, with no clinical jaundice. She did not receive any blood transfusion. In conclusion, this is a case of pure IgG isotype anti-Le^a in pregnancy and considered to be the first case in Asian population and would raise the awareness of the clinical significance of this antibody.

Keywords : ● *Lewis antibody* ● *Anti-Le^a* ● *IgG antibody* ● *Pregnancy*

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รายงานผู้ป่วย

Anti-Le^{a} ชนิด IgG ในหญิงตั้งครรภ์

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บทคัดย่อ

แอนติบอดีต่อหมู่เลือดระบบ Lewis ส่วนใหญ่พบเป็นชนิด IgM ซึ่งไม่ทำปฏิกิริยาที่ระยะแอนติโกลบูลิน (indirect antiglobulin phase, IAT) และไม่มีความสำคัญทางคลินิก ในประชากรไทยพบการสร้างแอนติบอดีของระบบ Lewis ค่อนข้างสูง ถึงร้อยละ 4.22 ของผู้ที่มีแอนติบอดีทั้งหมดโดยเฉพาะ anti-Le^{a} ในลักษณะของ IgM แต่พบได้ยากในรูปของ IgG ในคนทั่วไป รายงานผู้ป่วย หญิงไทยตั้งครรภ์อายุ 18 ปี มีประวัติการตั้งครรภ์ครั้งนี้เป็นครรภ์แรกและไม่เคยมีประวัติได้รับเลือดมาก่อน แพทย์ได้ส่งตรวจ type and screen เพื่อเตรียมพร้อมสำหรับการผ่าตัดคลอด ผลการตรวจทางห้องปฏิบัติการพบว่าผู้ป่วยมีหมู่เลือด A, RhD positive การตรวจกรองแอนติบอดีในพลาสมาโดยวิธี column agglutination test (CAT) ให้ผลบวก จึงทำการตรวจหาชนิดของแอนติบอดีโดยวิธี conventional tube test (CTT) พบว่าเป็น anti-Le^{a} ที่ทำปฏิกิริยาเฉพาะที่ระยะ IAT เท่านั้น ทำการทดสอบยืนยันชนิดของ anti-Le^{a} ด้วยการนำเซลล์เม็ดเลือดแดงที่มีลักษณะการแสดงออกเป็น Le(a+b-) มาทำปฏิกิริยากับพลาสมาของผู้ป่วยที่ 37°C นาน 30 นาที แล้วนำเซลล์เม็ดเลือดแดงที่ได้จากการทำปฏิกิริยามาทดสอบกับ monospecific direct antiglobulin gel card พบให้ผลบวกเฉพาะที่ IgG column จึงสรุปว่า anti-Le^{a} ที่ผู้ป่วยสร้างเป็นชนิด IgG และผู้ป่วยมีลักษณะการแสดงออกเป็น Le(a-b-) จากการติดตามไม่พบภาวะเม็ดเลือดแดงแตกของทารกในครรภ์ ในการตั้งครรภ์นี้ ผู้ป่วยและบุตรแข็งแรงดี ไม่ต้องได้รับเลือด การรายงานนี้เป็นรายงานแรกในชาวเอเชียของแอนติบอดีต่อหมู่เลือด Lewis ที่พบแต่ชนิด IgG เพียงชนิดเดียว

คำสำคัญ : ● *แอนติบอดีระบบ Lewis* ● *Anti-Le^{a}* ● *แอนติบอดีชนิด IgG* ● *การตั้งครรภ์*
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Introduction

Antigens of the Lewis blood group system (ISBT 007) are glycosphingolipid molecules that differently expressed on various types of cell surface as well as secreted in body fluids. There are several Lewis antigens, but two main types are Le[a] (LE1) and Le[b] (LE2) which could manifest in four phenotypes - Le(a+b-), Le(a-b+), Le(a-b-), and Le(a+b+). The expression of these antigens on red blood cells occurs by adsorption from circulating antigens in secretions or plasma onto their membranes[1]. Le[a] and Le[b] are not antithetical antigens, but the variation depends on two fucosyltransferase enzymes encoded by *FUT3* (*Le*) and *FUT2* (*Se*) genes. The biosynthesis of the Lewis antigens is reviewed elsewhere[1-3]. The allele frequencies of *Le* and *le* genes in Thai blood donors were 0.58 and 0.42, respectively[4]. Regarding antibodies of the Lewis blood group, they are often naturally occurring, cold reacting and complying with the IgM isotype. The Lewis antibodies are created by Le(a-b-) individuals and generally considered to be clinically insignificant. Nonetheless, anti-Le[a] has the potential to fix complement and causes intravascular hemolysis[1]. The Lewis antibodies caused only a few reports of either acute or chronic hemolytic transfusion reaction[5-8].

In one recent report of acute hemolytic transfusion reaction (AHTR) caused by anti-Le[a] was in a sickle cell trait pregnant woman with gravida eight paras. She was at her 38 weeks of gestation and was admitted with severe urinary tract infection. Despite a pan-reactive cold-reacting antibody and anti-Le[a] were presented in the pre-transfusion sample, she was transfused with a prewarmed crossmatch-compatible red cell unit. She immediately developed the clinical and laboratory parameters of AHTR during transfusion. The post-transfusion blood sample did not have a newly identified antibody. The authors argued that the high thermal amplitude IgM or IgG anti-Le[a] would be the causation of AHTR in this patient[6]. Anti-Le[a] was also identified as causation of AHTR in two Black pregnant women. Both had negative antibody screening by the solid phase red cell adherence (SPRCA) assay but then experienced an AHTR. The pre- and post-transfusion samples revealed that anti-Le[a] was presented across all three phases of antibody identification and crossmatches[7]. Höglund and colleagues proposed another example of AHTR caused by anti-Le[a][8]. A Russian patient with chronic lymphocytic leukemia (CLL) with a cold agglutinin reactive at 32°C but not at 37°C. He was heavily transfused with red blood cells and developed ani-Le[a] antibody detected by the column agglutination test (CAT). His antigen typing showed Le(a-b-) phenotype. The patient was transfused with two units of crossmatch-compatible red blood cells using the indirect antiglobulin test (IAT) with the microcolumn at 37°C. The symptoms of AHTR rapidly developed after the second unit was administered, and the transfusion reaction investigation showed only a broad-spectrum reactive anti-Le[a] antibody in the post-transfusion plasma. The antibody showed an ability to fix complement in the presence of papainized cells; hence, the researchers postulated that the anti-Le[a] was of the IgM isotype[8].

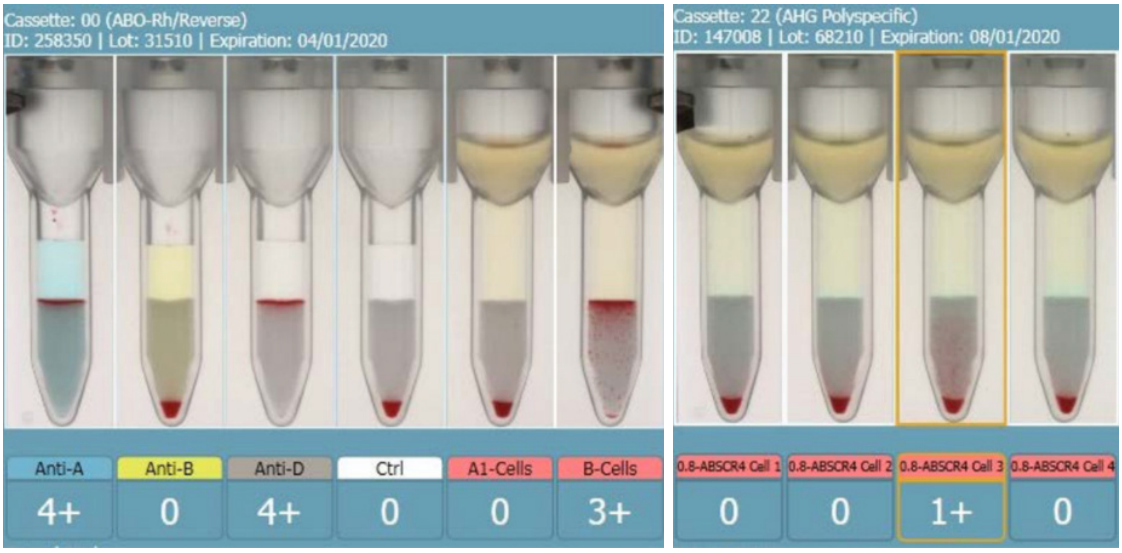
Since the Lewis antibodies are usually IgM in origin and the Lewis antigens are not well developed on fetal red blood cells; therefore, they do not cause HDFN. Lewis antibodies are relatively common. A recent study of red cell antibodies in 2,336 pregnancy in the Indian population reported the frequencies of anti-Le[a] and anti-Le[b] to be 2% and 12%, respectively, without such clinical significance[10]. While the frequency of Le[a] and Le[b] antibody among 9,124 Thai blood donors at Siriraj Hospital were 6.6% and 5.3%, respectively[9]. The incidences of Lewis antibodies in 10,579 patients collected between 2015 to 2019 collected from the Reference Laboratory, Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital were 4.22%, 2.33%, and 3.19% for anti-Le[a], -Le[b], and -Le[a]+Le[b], respectively. Of which, pregnant women had high prevalence across all types of the Lewis antibodies with the proportions of 21.52% (anti-Le[a]), 28.05% (anti-Le[b]), and 33.53% (anti-Le[a]+Le[b]) of the patients who produced Lewis antibodies.

The changes in Lewis phenotype during pregnancy is recognized as a result of increasing plasma lipoprotein in relation to the red cell mass. Subsequently, the Lewis substances are more attached to plasma lipoproteins, and less is available to bind to the red cell surface[11]. This transient Le(a-b-) phenotype could lead to antibodies production during gestation.

Case report

An 18-year-old Thai female presented to our hospital with labor pain at 38 weeks and four days of gestation in September 2019. The clinician requested for type and screen preoperative for a caesarean section. Previously, she had her antenatal care in a community hospital, and this is her first visit to Siriraj hospital. She is healthy and this gestation was her first pregnancy. She had no previous blood group typing, antibody screening, or blood transfusion. After entering the active stage of labor for six hours, she was transferred to the operative room for the caesarean delivery due to cephalopelvic disproportion. The operation was successful with no transfusion requirement. She delivered a healthy term male infant.

The patient's blood was sent to the blood bank, requested for type and screen for preoperative preparation. The type and screen were performed using CAT (Ortho Vision Max; Ortho-Clinical Diagnostics, Bridgend, UK). Figure 1a showed her blood as group A, RhD positive. The plasma was also applied to the four standard screening cells and showed a positive reaction (1+) in the O3 cells (Figure 1b). The antigram antigen profile of 0.8% Surgiscreen® (Ortho-Clinical Diagnostics) is depicted in Table 1. The antibody identification was then performed using saline conventional tube technique interpreting in three phases - five minutes saline at room temperature, incubation at 37°C for 30 minutes, and IAT phase with our in-house panel cells. The serum agglutinated with panel cell no. 2 and 9 in the IAT phase at 2+ reaction (Table 2). The autocontrol was negative across all phases. These results were in line with the Le^a antigen and classified as anti-Le^a. Due to an inadequacy of the sample, we were not able to test the patient's plasma with the enzyme-treated panel cells. The enzyme-treated red cells would react with an IgM component of the antibody, if any, and gave the hemolysis reaction. In addition, the identified antibody was confirmed to be anti-Le^a by using an adsorption technique. The procedure started with the incubation of patient's plasma with two known Le(a+b-) extra cells those were not from the first panel at 37°C



(a) (b)

Figure 1 The reactions of the patient's type and screen using CAT (a) ABO and D typing (b) Antibody screening test with four different screening cells

Table 1 Antibody screening test results (CAT method) using 4 screening cells

Screening cells	Rh					MNS					P1PK	Lewis		Kidd		Duffy		Kell		Diego		Test result by
No.	D	C	E	c	e	M	N	S	s	Mi^a	P1	Le^a	Le^b	Jk^a	Jk^b	Fy^a	Fy^b	K	k	Di^a	Di^b	CAT
1	+	+	0	0	+	+	+	0	+	+	0	0	+	+	0	+	0	0	+	/	/	0
2	+	0	+	+	0	+	0	+	0	0	+	0	0	0	+	+	+	0	+	/	/	0
3	0	0	0	+	+	+	+	0	+	0	0	+	0	+	+	0	+	+	+	/	/	**1+**
4	+	+	+	+	+	+	0	0	+	+	0	0	+	+	+	+	0	0	+	+	/	0

CAT = column agglutination technique; (/) = no available data

Table 2 Antibody identification results at room temperature, 37°C and IAT

Panel cells	Rh					MNS					P1PK	Lewis		Kidd		Duffy		Kell		Diego		Test result		
No.	D	C	E	c	e	M	N	S	s	Mi^a	P1	Le^a	Le^b	Jk^a	Jk^b	Fy^a	Fy^b	K	k	Di^a	Di^b	RT	37°C	IAT
1	+	+	+	+	+	0	+	0	+	+	+	0	+	+	+	+	0	0	+	0	+	0	0	0
2	+	0	+	+	+	+	0	0	+	0	0	+	0	+	+	+	+	+	+	0	+	0	0	**2+**
3	+	+	0	0	+	+	+	0	+	+	+	0	+	+	+	+	0	0	+	0	+	0	0	0
4	0	0	+	+	+	+	+	0	+	0	0	0	+	0	+	+	0	0	+	0	+	0	0	0
5	+	+	0	+	+	+	+	0	+	+	+	0	+	+	+	+	0	0	+	0	+	0	0	0
6	+	0	+	+	0	+	0	+	+	0	0	0	0	+	0	+	0	0	+	0	+	0	0	0
7	0	0	0	+	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	+	+	0	0	0
8	+	+	0	+	+	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	0	0	0
9	+	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	**2+**
10	+	0	+	+	0	+	+	0	+	0	0	0	0	0	+	+	0	0	+	0	+	0	0	0
11	+	+	0	0	+	+	0	+	+	0	0	0	0	+	+	+	0	0	+	+	+	0	0	0
																				Autocontrol		**0**	**0**	**0**

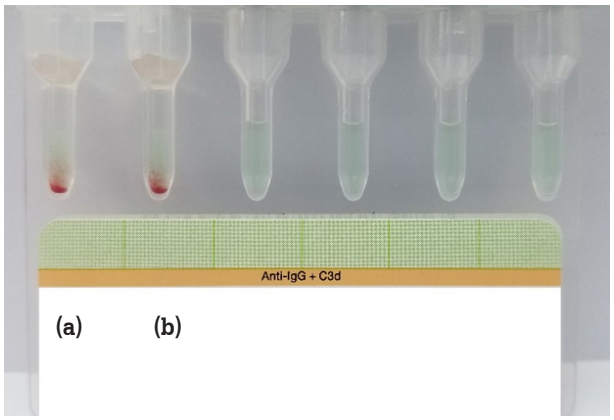


Figure 2 The positive reactions of polyspecific direct antiglobulin (DAT) gel card (anti-IgG+C3d, Bio-Rad Laboratories), when tested against patient's plasma, adsorbed with two extra Le(a+) cells from in-house panel cells I (a) cell no.9 and (b) cell no.6

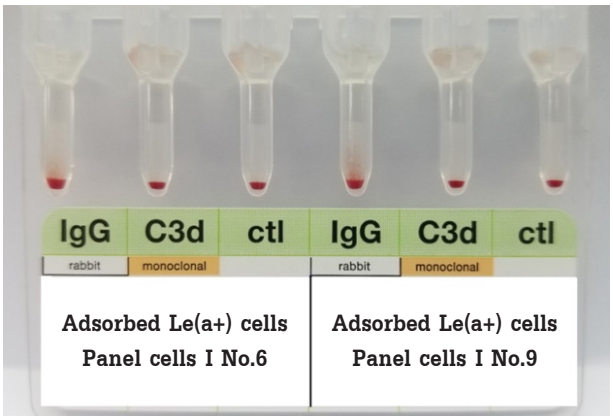


Figure 3 A monospecific DAT gel card showed the adsorbed Le(a+) cells with patient's plasma was reacted only at the anti-IgG column

for 30 minutes. The adsorbed Le(a+) cells were then tested and gave positive results with polyspecific direct antiglobulin (DAT) gel card (anti-IgG+C3d, Bio-Rad Laboratories, Cressier, Switzerland as demonstrated in Figure 2). Subsequently, two samples of the adsorbed Le(a+) cells were tested using the monospecific DAT gel card (Bio-Rad Laboratories) to determine the isotype of the antibody. The tested cells reacted only with the IgG antibody, as shown in Figure 3. This finding was corroborated with the antibody identification result. In conclusion, the phenotype of this patient was Le(a-b-), and the developed antibody was anti-Le^a, IgG isotype. Regarding the patient, she delivered a healthy and termed female baby without neonatal jaundice and did not require any transfusion.

Discussion

After reviewing the literature, as far as we are aware, there was only one case report of the pure IgG anti-Le^a [3] and one case that produced solely IgG anti-Le^b in a man with no transfusion history[3]. Similar to our findings, the antibody was potent and did not bind complement[3]. However, because the patient was pregnant at the time of testing, this antibody may be transiently developed from the diminishing of bound Lewis antigen to erythrocytes (Le(a-b-) phenotype)[11]. Post-partum follow-up of the antibody testing is needed for clarification.

In this patient, we performed antibody screening by using four O screening cells by CAT method (Table 1). This test is the routine antibody screening in our lab. Three of the four cells are commercial, and the other is our in-house O cells. This method would still enable us to detect antibodies that are commonly found in the Thais, e.g., anti-Mi^a and anti-Di^a. The immediate spin crossmatch that we use routinely would capture any significant IgM antibody that is not detected by the CAT technique.

When focusing on the transfusion support, *in vitro* antibody reactivity at 37°C or the IAT phase of testing usually is an indication of clinical significance, which could lead to a hemolytic transfusion reaction. Therefore, if this patient requires a transfusion, the compatible Le^a antigen-negative unit should be provided.

Regarding HDFN, some literature reported the association between neonatal jaundice and the delayed expression of the Le^a antigen[12], but none of HDFN caused by anti-Le^a was observed. Similarly, HDFN was not detected in this case. The absence of HDFN might be accounted for the incomplete transformation of the Le^a antigen on baby's red cells, and the phenotype will be expressed as a genuine phenotype after 6-7 years of life[13]. On the other hand, the Lewis substances could be detected in the baby after ten days[13].

This case report has limitations on the restriction of the blood sample due to the retrospective nature. To

confirm the subtype of immunoglobulin, a papain-treated red cell panel may be tested against the patient's serum. Generally, antibodies to the Lewis blood group system are enhanced by the enzyme-treated technique and can cause *in vitro* hemolysis according to the IgM component. The papain treatment could be helpful to differentiate and specify the subtype of the detected antibody. The genuine Lewis phenotype of this patient cannot be established since we cannot obtain the patient's saliva, or the post-partum follow-up blood sample.

Conclusions

This is the first report of the pure IgG Le^a antibody in a healthy Asian ethnic pregnant woman. She has never received a transfusion, and this is her first gestation. The antibody screening and identification showed the presence of anti-Le^a only at the IAT phase. The confirmation tests using both polyspecific and monospecific gel cards gave similar results of IgG type antibody. Although the clinical relevance of the IgG isotype of the anti-Le^a is still obscure, this report would raise the awareness of the importance of this antibody. Furthermore, unless there is evidence of clinical insignificance, the compatible antigen-negative blood unit should be transfused to the patient who has IAT reactive Lewis antibodies. Further follow up of the patient's phenotype in the postpartum phase, antibody production status, and possibly the genetic study of the *FUT3* mutation of this patient is warranted.

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