Case Report

Identification of a Rare Alloanti-GP.Hil (Anti-MNS20) in a Thai Patient

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Abstract:

A 35-year-old male Thai patient with severe anemia required two units of red blood cells (RBCs). The patient blood group was A, Rh(D) positive and had positive antibody screening. The antibody identification result was inconclusive. Thereafter, the blood samples were sent to the Reference Laboratory of the National Blood Centre, Thai Red Cross Society for further investigation. Antibody identification using in house panel cells demonstrated a suspicion of antibody to low-incidence of glycophorin (GP) phenotypes because two out of three panel cells with MNS7(+) were positive and all were negative with enzyme test. The autocontrol was negative. It was found that two panel cells with positive results were GP.Hil(+); whereas, one panel cell with negative result was GP.Hil(-). The patient RBCs tested with human anti-GP.Hil and found to be negative. Additionally, the patient plasma was also tested with two extra panel cells with GP.Hil(-) and showed negative results. In conclusion, this Thai patient had alloanti-GP.Hil and the GP.Hil(-) phenotype was confirmed. Therefore, two RBCs units of GP.Hil(-) with compatible crossmatch were provided. Interestingly, in a case of unusual antibody identification results suspected of antibody to GP phenotypes, additional testing with extra panel cells of those GP phenotypes and specific antisera is recommended to provide safe blood transfusions for a patient with rare antibody.

Keywords : ● *Anti-GP.Hil* ● *Rare antibody* ● *Low-incidence GP phenotypes*

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Identification of a Rare Alloanti-GP.Hil (Anti-MNS20) in a Thai Patient

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บทคัดย่อ

ผู้ป่วยชายไทยอายุ 35 ปี มีภาวะซีดมาก แพทย์จึงขอโลหิต 2 ยูนิตให้ผู้ป่วย ผู้ป่วยมีหมู่โลหิต A, Rh(D) positive ผลการตรวจทั้ง antibody screening และ antibody identification ให้ผลบวกแต่ไม่สามารถสรุปชนิดของแอนติบอดีได้ ธนาคารเลือดจึงส่งตัวอย่างโลหิตให้ฝ่ายห้องปฏิบัติการพิเศษ ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทยเพื่อตรวจหาชนิดของแอนติบอดี ซึ่งผลการทดสอบเพิ่มเติมกับ panel cells ที่เตรียมเอง คาดว่าผู้ป่วยน่าจะมีแอนติบอดีต่อ low-incidence glycophorin (GP) phenotypes เพราะพบปฏิกิริยาที่ให้ผลบวก กับ panel cells 2 ใน 3 รายที่เป็น MNS7(+) โดยที่ผล enzyme test ให้ผลลบกับทุก panel cells อีกทั้ง autocontrol ให้ผลลบ สำหรับ panel cells ทั้งสองรายที่ให้ผลบวกนั้นเป็น GP.Hil(+) ขณะที่ panel cells ที่ให้ผลลบเป็น GP.Hil(-) จึงคิดว่าผู้ป่วยรายนี้น่าจะมี anti-GP.Hil ซึ่งเมื่อทดสอบเม็ดโลหิตแดงผู้ป่วยกับ human anti-GP.Hil พบว่าให้ผลลบ นอกจากนี้ได้ทดสอบพลาสมาผู้ป่วยกับ extra panel cells อีก 2 รายที่เป็น GP.Hil(-) พบว่าให้ผลลบเช่นกัน สรุปว่าผู้ป่วยมีฟีโนไทป์เป็น GP.Hil(-) และสร้าง alloanti-GP.Hil ดังนั้นจึงเตรียมเม็ดโลหิตแดงจำนวน 2 ยูนิตที่เป็น GP.Hil(-) และผล crossmatch เข้ากันได้ให้กับผู้ป่วยรายนี้ ในกรณีที่ผลการตรวจชนิดของแอนติบอดีไม่สามารถสรุปผลได้ และคาดว่าน่าจะเป็นแอนติบอดีต่อ GP phenotypes ควรทดสอบเพิ่มเติมกับ extra panel cells ที่ทราบชนิดของ GP phenotypes และแอนติซีรัมที่จำเพาะเพื่อช่วยในการให้โลหิตแก่ผู้ป่วยที่มี rare antibody มีความปลอดภัยยิ่งขึ้น

คำสำคัญ : ● *Anti-GP.Hil* ● *Rare antibody* ● *Low-incidence GP phenotypes*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2559;26:373-8.

Introduction

Glycophorin (GP) phenotypes and associated antigens (previously the Miltenberger subsystem) is considered as low-incidence antigens in Caucasians, but MNS7 (Mi^a) is more common among Asians, especially GP.Mur (MNS10) phenotype.[1] Consequently, there are some case reports of delayed hemolytic transfusion reactions (DHTRs) or hemolytic disease of the fetus and newborn (HDFN) implicating anti-GP phenotypes. Therefore, a special requirement of positive MNS7 antigen should be included in both in house and commercial panel cells for Asian populations.[2-8]

In general, the GP phenotypes and the associated low-incidence antigens can be classified by serological testing with specific antisera, as shown in Table 1.[9] Regarding the findings of Cleghorn in 1966, red blood cells (RBCs) of the GP.Hil (MNS20) phenotype only reacted with anti-GP.Hil and did not react with some patients' sera with anti-Mi^a; whereas, the GP.Mur RBCs reacted with both anti-GP.Mur and anti-Mi^a sera.[10] Alloantibodies of GP.Hil are IgM and IgG and cause moderate HDFN.[1,11]

Case report

A 35-year-old male Thai patient with severe anemia, no previous history of transfusion from Rama II Hospital, required two units of RBCs, had positive antibody screening. The antibody identification result was inconclusive. Thereafter, the blood samples were sent to the Reference Laboratory of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand for further investigation.

Results

On 17 October 2014, the patient's samples were tested as follows:

ABO grouping and Rh(D) typing

ABO grouping and Rh(D) typing were performed by conventional tube test using anti-A, anti-B, anti-D and standard A, B and O cells (National Blood Centre, Thai Red Cross Society, Bangkok, Thailand)

ABO grouping = A

Rh(D) typing = Rh(D) positive

Antibody screening and identification

Antibody screening and identification were performed using screening cells (O1, O2, O4) and panel cells (Lot. 57100, Exp. date 13/11/2557, National Blood Centre, Thai Red Cross Society, Bangkok, Thailand). The methods performed were

1. Saline indirect antiglobulin test (Sal-IAT) using conventional tube test gave positive result with only O2 screening cells (Table 2) and panel cells No. 5 and No. 10 (Table 3).

Table 1 GP classification and associated antigens of the Miltenberger subsystem[9]

Terminology		Reaction of RBCs with antiserum to the following antigens										
Mi.	GP.	Mi^a	Vw	Hut	Mur	MUT	Hil	TSEN	MINY	Hop	Nob	DANE
Mi.I	GP.Vw	+	+	0	0	0	0	0	0	0	0	0
Mi.II	GP.Hut	+	0	+	0	+	0	0	0	0	0	0
Mi.III	GP.Mur	+	0	0	+	+	+	0	+	0	0	0
Mi.IV	GP.Hop	+	0	0	+	+	0	+	+	+	0	0
Mi.V	GP.Hil	0	0	0	0	0	+	0	+	0	0	0
Mi.VI	GP.Bun	+	0	0	+	+	+	0	+	+	0	0
Mi.VII	GP.Nob	0	0	0	0	0	0	0	0	0	+	0
Mi.VIII	GP.Joh	0	0	0	0	0	0	0	0	+	+	0
Mi.IX	GP.Dane	0	0	0	+	0	0	0	0	0	0	+
Mi.X	GP.HF	+	0	0	0	+	+	0	+	0	0	0
Mi.XI	GP.JL	0	0	0	0	0	0	+	+	0	0	0

Table 2 Antibody screening test results

No.	Rh					MNS					P	Lewis		Kidd		Duffy		Kell		Diego		CTT			Gel test	
	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	IAT	Enz
O1	+	+	0	0	+	+	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	0	0	0	0	0
O2	+	+	+	+	+	+	+	0	+	+	0	0	+	+	+	+	0	+	+	+	+	2+	1+	1+	2+	0
O4	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	0	0	+	0	+	0	0	0	0	0
Autocontrol																						0	0	0	W+	NT

CTT = conventional tube test; NT = not tested

Table 3 Antibody identification test results

No.	Rh					MNS					P	Lewis		Kidd		Duffy		Kell		Diego		CTT			Gel test	
	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	IAT	Enz
P1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	0	NT
P2	+	+	0	0	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	0	NT
P3	+	+	0	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	+	0	0	0	0	NT
P4	+	+	0	+	+	0	+	0	+	0	0	+	0	0	+	+	0	0	+	0	+	0	0	0	0	NT
P5	+	+	+	0	+	+	0	0	+	+	0	+	0	0	+	+	0	0	+	+	+	2+	2+	2+	3+	NT
P6	+	+	+	+	+	+	+	0	+	0	0	0	0	+	+	+	+	+	+	0	+	0	0	0	0	NT
P7	+	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	0	0	NT
P8	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	0	NT
P9	0	0	0	+	+	+	+	+	+	0	0	+	0	+	+	+	0	0	+	0	+	0	0	0	0	NT
P10	0	+	0	+	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	+	+	1+	2+	2+	2+	NT
P11	0	+	+	+	+	+	0	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	0	0	0	NT
Autocontrol																						0	0	0	W+	NT

CTT = conventional tube test; NT = not tested

2. Indirect antiglobulin test using gel test (LISS/ Coombs Card, Ortho ID-MTS, Raritan, NJ, USA) gave positive result with O2 screening cell but weakly positive autocontrol (Table 2). For antibody identification, panel cells No. 5 and No.10 gave positive reactions with weakly positive autocontrol (Table 3).

3. Enzyme test using gel test (Neutral gel Card, Ortho ID-MTS, Raritan, NJ, USA) and papain (Lot. 57010, Exp. date 28/10/2558, National Blood Centre, Thai Red Cross Society, Bangkok, Thailand) gave negative results with all screening cells (O1, O2, O4). As a result, enzyme test was not done in antibody identification.

The results of antibody screening and identification are shown in Tables 2 and 3. For antibody screening test using Sal-IAT and gel test, only O2 was positive and antibody identification demonstrated a suspicion of antibody to low-incidence of GP phenotypes because two out of three panel cells with Mi^a antigen (P5 and P10) were positive and all were negative with enzyme test. The autocontrol was negative. Additionally, the three panel cells with Mi^a antigen (P3, P5 and P10) were tested with anti-Vw, -Hut, -Hop, -Mur and anti-Hil. It was found that two panel cells (P5 and P10) were GP.Mur; whereas, one panel cell (P3) was GP.Hop, as shown in Table 4. Moreover, the patient RBCs tested with human anti-GP.Hil and found to be negative and to confirm antibody specificity, the patient plasma was also tested with two extra panel cells with GP.Hop(+), GP.Hil(-) and showed negative results (Table 5). Therefore, two RBCs units of GP.Hil(-) with compatible crossmatch were provided to this patient.

Concerning the high-prevalence of antibodies to MNS7 in Thai populations, the most common are anti-GP.Mur and anti-GP.Bun.[13,14] Moreover, a rare antibody to GP.Hop had been reported in a Thai patient.[15] For patients with antibodies specific to these GP. phenotypes, it would not be difficult to find antigen-negative blood because most of Thai donors are negative for these rare antigens.[13]

Conclusion

The GP.Hil(-) phenotype was confirmed in a Thai patient who had alloanti-GP.Hil. Interestingly, in a case of unusual antibody identification results suspected antibody to GP phenotypes and associated antigens, additional testing with extra panel cells of those GP phenotypes and specific antisera is recommended to provide safe blood transfusions.

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Table 4 Testing panel cells with specific antisera of different GPs

Panel cell	Anti-Vw	Anti-Hut	Anti-Hop	Anti-Mur	Anti-Hil	Interpretation
P3	0	0	2+	4+	0	GP.Hop
P5	0	0	0	4+	3+	GP.Mur
P10	0	0	0	4+	3+	GP.Mur

Table 5 Confirmation of anti-Hil by Sal-IAT

Phenotype	Sal-IAT		
	RT	37°C	IAT
GP.Hop(+), GP.Hil(-)	0	0	0
GP.Hop(+), GP.Hil(-)	0	0	0

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