Original article

PCR-based screening of MNS glycophorin variants in patients suspected of having anti-Mi^a

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Abstract:

Background:MNS glycophorin variants such as glycoprotein (GP.) Mur, GP.Hop, GP.Bun, GP.HF, GP.Hut, and GP.Vw are known to carry multiple antigens, including Mi^a (MNS7). In East and Southeast Asians, an antibody to Mi^a is not only commonly found, but also clinically significant. ***Purpose and Methods:*** In this study, deoxyribonucleic acid (DNA) samples from 47 patients suspected of having anti-Mi^a by serological screening were genotyped for GP.Mur, GP.Hop, GP.Bun, GP.HF, GP.Hut, and GP.Vw variants using polymerase chain reaction (PCR) assay. ***Results:*** All patients were found to be negative for the presence of Mi^a-positive glycophorin variants. ***Conclusions:*** The presence of anti-Mi^a cannot be excluded in patients studied, as genotyping shows that Mi^a-positive MNS glycophorin variants are not present.

Keywords: MNS blood group system, MNS glycophorin variants, Anti-Mi^a, Polymerase chain reaction

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Introduction

The MNS blood group system (ISBT 002) was discovered after the ABO blood group system (ISBT 001)and before the Rhesus (Rh) blood group system (ISBT 004)[1]. The MNS system consists of 49 antigens that are the products of glycophorin A (GPA), glycophorin B (GPB) or hybrids thereof and are fully developed at birth[1-3].The genes encoding GPA (*GYPA*) and GPB (*GYPB*) are highly homologous with 95.5% sequence similarity[4]. In blood transfusion practices, the main antibodies of the MNS system considered are anti-M, anti-N, anti-S, anti-s, and anti-U. However, there have been reports of hemolytic transfusion reaction (HTR) and hemolytic disease of the fetus and new born (HDFN) as a result of antibodies to MNS glycophorin variants, including anti-Mi^a[1,5,6].

In the multi-ethnic Malaysian population, anti-Mi^a was the third most common alloantibody identified in general and antenatal patients with a prevalence of 0.2% (n=77/33,716)[7]. In Taiwan, Thailand, and China, anti-Mi^a was one of the most common alloantibodiesin patients with a prevalence of 1.5% (n=239/15,794)[8], 0.2% (n=43/18,627)[9], and 0.02% (n=7/37,548)[10], respectively. The antibody was alsodetected in 0.05% (n=10/20,786) of blood donors in Thailand[9]and anti-Mi^a/Mur was detected in 0.1% (n=19/20,283) of patients in China[11].

Many patients in our unit are suspected of having anti-Mi^a and cannot be confirmed by the current serological method. In this study, PCR assay was performed to detect Mi^a-positive MNS glycophorin variants, which were GP. Mur, GP. Hop, GP. Bun, GP.HF, GP.Hut, and GP. Vw in those patients.

Materials and Methods

Samples

Whole blood samples were collected from 47 patients in the Hospital Universiti Sains Malaysia (Hospital USM) based on purposive sampling. Patients who

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were positive in the antibody screening assay on cell III of ID-DiaCell, but negative for anti-MUT, anti-Mur, and other rare blood group antibodies in the antibody identification assays, and negative viral screening for human immunodeficiency virus (HIV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV) were included in the study demographic data were obtained from the record office.

Group, screen, and hold

Using standard blood bank methods, ABO and Rh D (RhD) blood groups of all samples were determined by using ABO/D+ combined with Reverse Grouping ID-Card (Bio-Rad, USA). Antibody screening and identification were done with indirect antiglobulin test (IAT) method using commercialized three and 11 cell panels, respectively by micro column gel agglutination method from Diamed-ID micro typing system (Diamed AG, 1785 Cressier, S/ Morat, Switzerland). Patients who were negative for antibody identification using Diamed-ID panel were subjected to antibody identification using the second antibody identification panel, Phenocell™ 0.8%. Details of the methods are described in the Supplementary Methods.

Polymerase chain reaction

Genomic DNA was extracted from whole blood samples using the NucleoSpin® Blood L (Macherey-Nagel, Duren, Germany)[12]. Human genomic DNA controls of known negative and positive GP.Mur genotype and phenotype were obtained from blood donors in the Transfusion Medicine Unit (TMU) of Hospital USM. Plasmid DNA that served as a GP.Mur-positive control was a generous gift from Dr. Shuangshi Wei (Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou, China). DNA samples and controls were amplified using primers (Table 1) under PCR conditions specified by Palacajornsuk et al.[13]. Two sets of PCRs were performed on a Veriti™ thermal cycler (Applied Biosystems, USA), consisting of CoralLoad PCR buffer (2.0 µl) (Qiagen, USA), Deoxyribonucleotide triphosphate (dNTP) blend (2.0 µl) (Fermentas, USA), Magnesium chloride ($MgCl_2$) (1.2 µl) (Qiagen, USA), primer mix F2/Rccgg or F1/RIN (1.0 µl of 10.0 µM), internal control primer mix FHGH5580/RHGH5967 (1.0 µl of 10.0 µM), Q-Solution (6.0 µl) (Qiagen, USA), HotStarTaq *Plus* DNA Polymerase (0.2 µl) (Qiagen, USA), and DNA template (2.0 µl of 100 ng). PCR products were electrophoresed on agarose gel (2%) and stained with Diamond Nucleic Acid Dye (Promega, USA) prior to visual analysis using the AlphaImager gel documentation system (Alpha Innotech, USA).

Ethical clearance

The study complied with the ethical guidelines and was approved by the Human Research Ethics Committee of USM [JEPeM-USM/259.3 (13)/14 September 2015-13 September 2017].

Results

Demographic profile

The majority of patients in this study (Table 2), with ages ranging from 21 to 40 years (51.0%) were Malays (83.0%). All patients were Rh positive, many of whom were A blood group (38.3%). Descriptive analysis revealed that the risk factors for antibody development in these patients were pregnancy (40.4%), blood transfusion (29.8%), and both blood transfusion and pregnancy (29.8%).

Serological analysis

All patients suspected of having anti-Mi^a (Fig.1) were also negative for other serologically identified blood group antibodies, including MUT and Mur (Fig. 2). Auto control tests were negative for all patients.

Genotyping analysis

GP.Mur/Hop/Bun (148 bp) and GP.Hut/HF (151 bp) (Fig. 3A) and GP.Vw (296 bp) (Fig. 3B) were not detected in all 47 patients.

Discussion

In this study, 47 patients who were positive for antibody screening cell III (Mi^a+), but negative for all antibody identification panel cells, including MUT and Mur were further investigated by genotyping. All patients included were found to be negative for GP.Mur, GP.Hop, GP.Bun, GP.HF, GP.Hut, and GP.Vw variants. Consequently, in the absence of Mi^a-positive glycophorin variants, anti-Mi^a cannot be excluded. This is consistent with Yousuf et al.[14], patients were suspected of having anti-Mi^a if positive for antibody screening cell III (Mi^a+), but negative in the antibody identification using Diamed-ID and Phenocell™ 0.8% panel cells.Additionally, antibody screening cell III was a GP.Mur phenotype natural screening cell, which can detect both immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies of MNS variants[15]. Thus, a positive reaction for antibody screening cell III could be either clinically insignificant IgM antibody or clinically significant IgG antibody of MNS variants.

Table 1. PCR primers

Primers	Sequences, 5'– 3'	Target(s)	Amplicon size (bp)	Reference sequence(s)
F2	ccc ttt ctc aac ttc tct tat atg cag ATA A	GP.Mur/Hop/Bun GP.Hut/HF	148 151	*GYP*Mur*: AF090739 *GYP*Hop*: KR815995 *GYP*Bun*: M60710.1 *GYP*Bun*: KR363627.1 *GYP*HF*: M81079.1
Rccgg	gag caa cta ttt aaa act aag aac ata cCG G			
F1	cag cat ttc tct aaa ggc taa ata aga aga tgt a	GP.Vw	296	*GYP*Vw*: M81826.1
RIN	CAT ATG TGT CCC GTT TGT GCA			
FHGH5580	TGC CTT CCC AAC CAT TCC CTT A	hGH	434	GH1: NG_011676.1
RHGH5967	cca ctc acG GAT TTC TGT TGT GTT TC			

Note: F2 is located in intron 2 and extends four nucleotides into exon 3, Rccgg is located in exon 3 and extends 28 nucleotides into intron 3. F1 is located in intron 2 and RIN is located in exon 3. FHGH5580 located in exon 2, RHGH5967 located in exon 3 and extends eight nucleotides into intron 3 of the human growth hormone (hGH) gene.

According to Gupta et al.[16], 23.2% (*n*=16/69) of patients with positive antibody screening, but inconclusive antibody identification was identified as having a definite antibody when retested at a later date. Nevertheless, 48.0% (*n*=33/69) of these patients had negative antibody screening. The situation in which a positive for gel test of antibody screening, but no defined pattern on antibody identification panel was classified as inconclusive antibody screening. On the other hand, positive antibody screening may also be associated with inconclusive antibody identification[16].A gel test in which dextran acrylamide gel placed in microtubes containing anti-IgG is reliable, accurate, and sensitive for the detection and identification of blood group antibodies[17,18]. Indeed,Weisbach et al.[19]found that the high-sensitivity of the microcolumn gel system produced higher false-positive ratesthan the conventional tube system.

The detection and confirmation of anti-Mi^a by serological methodremains unfeasible to date. There are no commercially available Mi^a panel cells for use in diagnostic laboratories. Chen et al.[20] revealed that the anti-Mi^a screening by manual polybrene method using known anti-Mi^a sera was insensitive (sensitivity rate of 67.2%). Furthermore, different methods have detected different frequencies of alloantibodies, including anti-Mi^a[9]. In the study, anti-Mi^a was identified with a frequency of 15.2% (*n*=53/349) by the conventional tube method, while 32.4% (*n*=181/558)by the column agglutination technology method. It is very clear that anti-Mi^{a}can cause HTR and HDFN[5,6].The present study has therefore shown the need for improved technologies to detect clinically relevant MNS blood group antibodies.

Conclusion

The presence of anti-Mi^a in patients studied cannot be excluded, as genotyping shows that thereare no Mi^a-positive MNS glycophorin variants.Patients with positive antibody screening, but no defined pattern of antibody identification entails retesting prior to blood transfusion. Anti-Mi^a should be considered as potentially clinically significant due to reported cases of HTR and HDFN. Henceforth, transfusion-dependent patients may be considered for Mi^a-negativeblood supply in clinically indicated condition.

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Conflict of interest:

All authors declare that they have no conflict of interest pertaining to the manuscript submitted to the Bangladesh Journal of Medical Science.

Individual contributions of the authors:

Conceptual work: Wan Suriana Wan Ab Rahman, Suharni Mohamad, Rosline Hassan

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Manuscript writing: Siti Nazihahasma Hassan

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Table 2. Demographic profile of patients

Characteristics	Sub-characteristics	Frequency (n) (%)
Gender	Male Female	15 (31.9) 32 (68.1)
Age	0-20 years old	3 (6.4)
	21-40 years old	24 (51.0)
	41-60 years old	10 (21.3)
	≥61 years old	10 (21.3)
Race	Malay Chinese Indian	39 (83.0) 5 (10.6) 3 (6.4)
Blood type	A B O AB	18 (38.3) 10 (21.3) 16 (34.0) 3 (6.4)

Note: Of these 47 patients, 14 (29.8%) have a history of blood transfusion, 19 (40.4%) have a history of pregnancy and 14 (29.8%) have a history of blood transfusion and pregnancy.

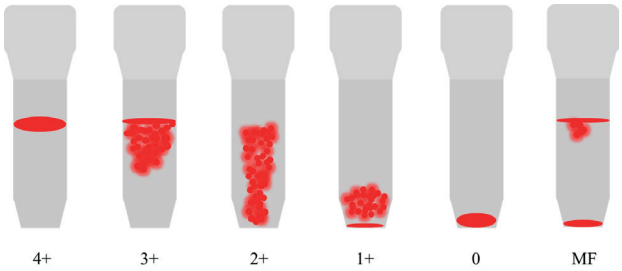

Fig. 1 Representative of antibody screening test, (3+) agglutination against cell III (Mi^a+).

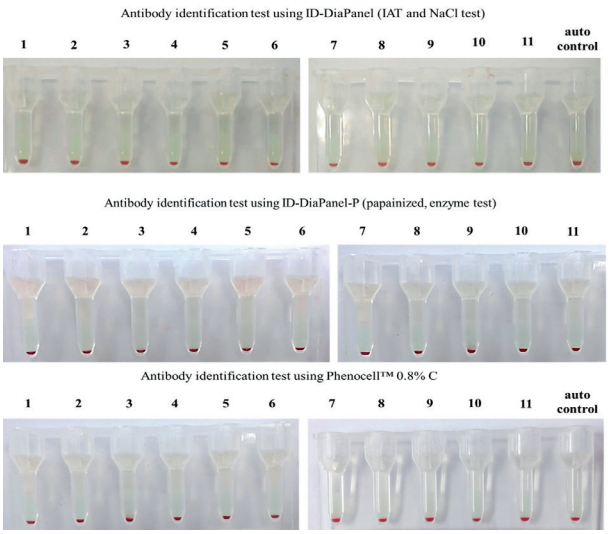

Fig. 2 Representative of antibody identification test, (0) negative against allpanel cells.

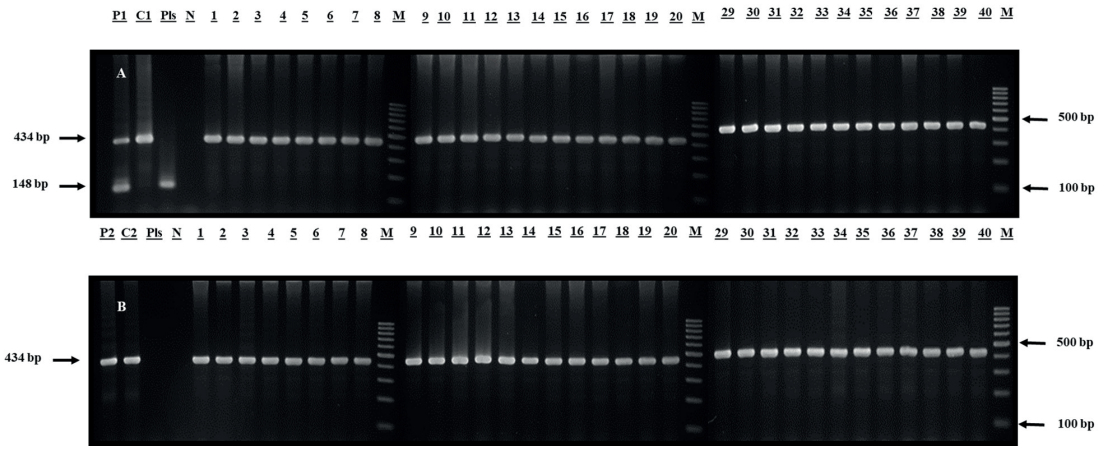

Fig. 3 [A] Representative electrophoretic analysis of PCR products genotyped by F2/Rccgg with hGH (434 bp) as an internal PCR control; Lane P1: Human GP.Mur positive control (148); Lane C1: Human GP.Mur negative control; Lane Pls: Plasmid GP.Mur positive control (148 bp); Lane N: Non-template control; Lane M: 100 bp DNA ladder, and Lane 1-47: Patient samples number 1 to 47. The GP.Mur/Hop/Bun (148 bp) and GP.Hut/HF (151 bp) were absent in all patients. The hGH (434 bp) is only present in human samples. [B] Representative electrophoretic analysis of PCR products genotyped by F1/RIN with hGH (434 bp) as an internal PCR control; Lane P2: Human GP.Mur positive control (148); Lane C2: Human GP.Mur negative control; Lane Pls: Plasmid GP.Mur positive control (148 bp); Lane N: Non-template control; Lane M: 100 bp DNA ladder, and Lane 1-47: Patient samples number 1 to 47. The GP.Vw (296 bp) was absent in all patients. F1/RIN amplified GP.Vw only, therefore, the band (148 bp) was absent in lane P2, lane C2 and lane Pls.

Supplementary Fig. 1 Grading of agglutination reactions using a gel card is shown as follows; (4+) reaction is represented by a solid band of agglutinated RBCs on the top of the gel matrix, (3+) reaction is represented by a predominant amount of agglutinated RBCs towards the top of the gel with a few agglutinated RBCs staggered below the agglutinated RBCs in the upper half of the gel matrix, (2+) reaction is represented by agglutinated RBCs dispersed throughout the gel with a few agglutinated RBCs at the bottom of the gel matrix, (1+) reaction is represented by agglutinated RBCs predominantly observed in the lower half of the gel matrix with some unagglutinated RBCs/pellet at the bottom of the gel matrix, (0/Negative) reaction is represented by a pellet of RBCs at the bottom, no agglutinated RBCs in the matrix of the gel and clear background, (MF) reaction is represented as a layer of agglutinated RBCs at the top of the gel matrix accompanied by RBCs pellet at the bottom of the gel matrix. A weak positive reaction might be observed, it is represented by a few agglutinated RBCs remaining in the gel matrix just above the RBCs pellet at the bottom of the gel matrix[18,21].

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