Original Article

Prevalence of Mia antigen and anti-Mia antibody among patients from a university hospital in Malaysia

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ABSTRACT

Background

Mia is a low-frequency red cell antigen associated with the MNS glycophorin variants, commonly found in individuals with the GP. Mur phenotype in Southeast Asia. The alloantibody, anti-Mia, is linked to hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. This study aimed to assess the prevalence of Mia antigen expression and the presence of anti-Mia antibodies.

Methods

This study was conducted at the Blood Bank unit of Universiti Kebangsaan Malaysia Medical Centre. The Mia antigen phenotyping was performed from June 2018 to September 2019 using random sampling of blood samples collected for pre-transfusion testing and analyzed using a monoclonal anti-Mia antibody. The prevalence of anti-Mia antibodies was evaluated from samples sent for antibody screening and identification over one year from October 2018 to September 2019.

Results

A total of 694 patients participated in the Mia antigen study, including 347 Malays, 233 Chinese, and 114 Indians. The overall prevalence of the Mia antigen was 18 out of 694 (2.6%). The highest frequency was observed in the Chinese group (4.7%), followed by Malays (1.7%) and Indians (0.9%), with statistical significance (p=0.038). For anti-Mia antibody study, 13,011 samples were screened for antibodies, resulting in 231 alloantibody positive cases (1.8%), with anti-Mia antibodies detected in 61 cases, reflecting a prevalence of 0.5%. ***Conclusion:*** Given Malaysia's diverse ethnic makeup, understanding Mia antigen frequency is essential. This study reveals a relatively low prevalence of the Mia antigen alongside a notable incidence of anti-Mia antibodies compared to neighboring countries.

Keywords

Mi^a antigen; Anti-Mi^a antibody; GP. Mur; Glycophorin; MNS variants; Malaysia

INTRODUCTION

Antigens of the MNS blood group system are present on human red blood cell membranes and are classified by the International Society of Blood Transfusion (ISBT 002) as the second blood group system. These antigens are glycoproteins, including glycophorin A (GPA), glycophorin B (GPB), and hybrid GPA/GPB molecules, encoded by the GYPA and GYPB genes, respectively[1]. The MNS system has 50 distinct antigens, making it second in complexity only to the Rh system (ISBT 004), which has 56

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antigens[2]. The main antigens in the MNS system are M, N, S, and s.

The Miltenberger subsystems include phenotypes with low-frequency antigens associated with the MNS blood group and were introduced by Cleghorn in 1966, named after a woman who developed the alloantibody anti-Mi^a after immunization from her antigen-positive fetus[3]. Initially, Miltenberger phenotypes were serologically defined based on reactivity patterns with various antisera[4]. They were first categorized into 5 classes, later expanded to 11 classes (Mi.I to Mi.XI)[1]. As research advanced, the outdated Miltenberger system was replaced with a glycophorin (GP)-based nomenclature, using 'GP' for glycophorin and 'GYP' for the gene[4,5]. The GP. Mur phenotype, previously known as Miltenberger subtype III (Mi.III), arises from the recombination of GYPA and GYPB into the GYP[B-A-B] hybrid gene, expressing antigens such as Mi^a, Mur, Hil, MINY, and MUT[1,6].

The incidence of the GP. Mur phenotype is found to be low in Caucasian (0.0098%)[7], Japanese (0.006%)[8], mainland Chinese from north of the Yangtze River (0%)[9], Northern India (0.1%) [10] and Australia (0.22%)[11]. However, relatively high frequencies of occurrence are noted in the general population of Southeast Asia and East Asia: Taiwan (7.3%)[12], Hong Kong (6.28%)[13], Southern China (6.5%)[14], mainland Chinese from south of the Yangtze River (4.3%)[9], Thailand (9.03%)[15], Vietnam (6%)[16] and Malaysia (3.8%)[17]. The frequency of the phenotype is notably higher among Taiwan's mountain indigenous groups, specifically the Ami, Yami, and Puyuma, with rates of 88.4%, 34.3%, and 21.2%, respectively[9].

Most earlier studies used serological methods with antibodies against Mi^a and/or Mur, but the Mi^a antigen is also present in GP. Vw, GP. Hut, GP. Hop, GP. Bun, and GP. HF phenotypes, while the Mur antigen appears in GP. Mur, GP. Hop, GP. Bun, and GP. Dane phenotype[1]. Due to overlapping antigens, recent studies employ genotyping for precise classification[18]. For instance, a Thai study with 1,041 subjects found 9.03% positive for anti-Mi^a antibodies, with genotyping revealing 8% as GP. Mur[15]. Another study in Taiwan has also conducted genotyping and proved that GP. Mur is the only prevalent Glycophorin subtype in Taiwan[19].

The Glycophorin subsystem antibodies are known to be clinically significant and can cause immediate and delayed transfusion reactions[12,20]. These antibodies are also reported to cause hemolytic disease of the fetus and newborn[21,22,23]. In Taiwan, anti-Mi^a was the most common alloantibody[12]. In Malaysia, anti-Mi^a was the most frequently detected alloantibody with an incidence of 30.4% (49/161) of all alloantibody's positive cases[24]. Understanding the prevalence of the Mi^a antigen is crucial in assessing immunization rates in Malaysia's diverse population.

This study aims to determine the prevalence of the Mi^a antigen using standardized anti-Mi^a monoclonal antibodies (Novaclone; Immucor) and corresponding anti-Mi^a antibodies with Mi^a positive RBCs (Biorad ID-DiaCell I-II-III Asia Mi^a+) among patients at a University Hospital in Peninsular Malaysia, focusing on its distribution across the three major ethnic groups in the country.

MATERIALS AND METHODS

Approval for this study was granted by the Research and Ethics Committee at Universiti Kebangsaan Malaysia (UKM) (Research number: FF-2018-192). This prospective, cross-sectional analytical study was conducted at the Blood Bank of UKM Medical Centre (UKMMC) and comprised two components. The first component focused on determining the prevalence of the Mi^a antigen, carried out from June 2018 to September 2019. The second component assessed the prevalence of the anti-Mi^a antibody, conducted from October 2018 to September 2019 over one year. The study population included blood samples from patients sent to the Blood Bank Unit at UKMMC for pre-transfusion testing. A random sampling method was employed for Mi^a antigen testing, while a universal sampling method was used for anti-Mi^a antibody testing, including all samples sent for routine antibody screening.

Mi^a antigen testing

A total of 694 blood samples were randomly selected for the study. Exclusions were made for patients with recent RBC transfusions (within the last 3 months), hemolyzed samples, insufficient volume, incomplete request forms, and incorrectly labeled samples. Testing was conducted following the manufacturer's

instructions for the Anti-Mi^a reagent (Novaclone; Immucor) using the tube method with standard hemagglutination techniques. A 2-4% suspension of washed RBCs was prepared in buffered isotonic saline. Fifty microliters (µL) of Anti-Mi^a murine monoclonal IgG antibody was added to a labeled test tube, followed by 50 µL of the RBC suspension. The mixture was incubated at room temperature for 10 minutes and then centrifuged for 15 seconds at 900-1000 rcf. After gently resuspending the red cell button, agglutination was assessed macroscopically. A reaction intensity of 1+ or higher was considered positive, with results graded from 1+ to 4+ based on agglutination levels[25]. Known Mi^a positive and negative RBCs served as controls, and results were recorded accordingly.

Anti-Mi^a antibody testing

A total of 13,011 blood samples were included for pre-transfusion testing. Antibody screening was conducted using three screening cells (Biorad ID-DiaCell I-II-III Asia Mi^a+). Plasma samples that tested positive were further analyzed using 11 red cells (Biorad ID-DiaPanel) for antibody identification. A typical anti-Mi^a case would show positive results with the Mi^a+ screening cell and negative results with the 11 Mi^a- red cells present in the antibody identification panel. Confirmation followed the "rule of three," requiring three reactive Mi^a+ and three non-reactive Mi^a- red cells, as outlined by the AABB Technical Manual based on Fisher's exact method[25]. Additionally, phenotyping of the patient's red cells was performed with a monoclonal anti-Mi^a antibody (Novaclone; Immucor), yielding a corresponding Mi^a antigen negative result. Positive reactions were graded from 1+ to 4+ based on agglutination level[25]. Results were recorded in the worksheet.

Data were collected and analyzed using IBM SPSS version 22. The results were expressed in percentages and significance were assessed using the Pearson Chi-Square test.

RESULTS

Mi^a antigen

A total of 694 randomly selected patient samples were tested, of which majority were Malays 50% (n=347), followed by Chinese 34% (n=233) and Indians 16% (n=114). The prevalence of Mi^a antigen among UKMMC patients was 2.6% (18/694). Chinese showed the highest frequency of 4.7% (11/233), followed by Malays 1.7% (6/347) and Indians 0.9% (1/114). Statistical analysis by Chi Square test shows a significant association between ethnicity and prevalence of Mi^a antigen with p=0.038. No significance difference seen between the male and female (Table 1).

Table 1: Prevalence of Mi^a antigen by overall population, ethnicity, and gender.

Variable		Frequency		Significance
		N	%	
Gender	Male	9/296	3.0	p value = 0.523 (>0.05)
	Female	9/398	2.3	
Ethnicity	Malay	6/347	1.7	p value = 0.038 (<0.05)
	Chinese	11/233	4.7	
	Indian	1/114	0.9	
Total		18/694	2.6	

Anti-Mi^a antibody

A total of 13,011 samples were sent for pre-transfusion testing to the blood bank over 1 year. From this, there were a total of 2.1% (267/13011) patients who had positive antibody screen. Alloantibodies were identified in 1.8% of patients (231/13011), while 0.3% of patients had autoantibodies (36/13011). Twenty types of red cell alloantibodies were detected, (Table 2) and anti-Mi^a was the most frequent with a prevalence of 0.5% (61/13011). Distribution of anti-Mi^a among the different ethnicities showed the highest record in Malays (n= 43), followed by Chinese (n=13), Indians (n=4) and one of Myanmar origin. Anti-Mi^a made up 26.4% (61/231) of the total alloantibody positive cases, followed by anti-E 23.4% (54/231), anti-c 11.7% (27/231) and anti-Lea 9.5% (22/231). Other antibodies encountered with decreasing frequency were anti-M, anti-Leb, anti-Jka, anti-S, anti-Jkb, anti-Fyb, anti-D, anti-C, anti-e and antibody against low incidence antigen (Table 2). There was only one case of the following antibodies, anti-Fya, anti-Jk3, likely anti-SDA, likely anti-Lua, anti-B, and antibody of undetermined significance (data tabulated as miscellaneous).

Table 2. Prevalence of anti-Mi^a and other specific red cell antibodies detected in patients.

Alloantibodies (n= 231)		Autoantibodies (n= 36)	
Antibody specificity	Antibody frequency	Antibody specificity	Antibody frequency
Mi^a	61 (26.4%)	Non-specific autoIgG	23 (63.9%)
E	54 (23.4%)	Non-specific cold autoantibody	9 (25%)
c	27 (11.7%)	Non-specific antibody against reagent	2 (5.6%)
Le^a	22 (9.5%)	Non-specific drug induced antibody	1 (2.8%)
M	16 (6.9%)	Auto anti-c	1 (2.8%)
Le^b	11 (4.8%)		
Jk^a	9 (3.9%)		
S	7 (3.0%)		
Jk^b	4 (1.7%)		
Fy^b	4 (1.7%)		
D	3 (1.3%)		
C	3 (1.3%)		
e	2 (0.9%)		
Antibody against low incidence antigen	2 (0.9%)		
Miscellaneous	6 (2.6%)		
Total antibodies	267 (100%)		

DISCUSSION

The Miltenberger series consists of phenotypes related to the MNS system, characterized by overlapping specificities of several low-frequency antigens. Until recently, 11 phenotypes (Mi I to Mi XI) were recognized. As the series evolved with advances in genetic testing, Tippett and colleagues proposed a glycophorin-based terminology in 1992[4]. Glycophorin classification has detected up to 22 phenotypes[1]. Each phenotype expresses between 1 to 6 antigens, with many antigens shared across multiple phenotypes[1]. The Glycophorin phenotypes include Mi^a, Mur, MUT, Hil, Vw, and others, totaling around 17 identified antigens[1]. Mi^a antigen can be found in 6 glycophorin phenotypes including GP. Mur, GP. Bun, GP. HF, GP. Hop, GP. Hut and GP. Vw[1]. Among these, GP. Mur is the most prevalent phenotype in Southeast Asia, resulting from a hybrid GYP(B-A-B) gene on the RBC membrane. The GP. Mur phenotype encompasses five antigens: Mi^a, MUT, Mur, Hil, and MINY[1]. Briefly, GP. Mur carries 5 antigens including Mi^a, and Mi^a is found in 6 hybrid glycophorins, in which GP. Mur is one of them. Anti-Mi^a antibody is the corresponding antibody against Mi^a antigen.

Our study found the prevalence of the Mi^a antigen to be 2.6% (18/694), which is lower compared to Southeast Asian counterparts like Taiwan, Hong Kong, Southern China, Thailand, and Vietnam. However, it is higher than the prevalence reported in India, Caucasian populations, Japanese individuals, and Australians. In this current study in Malaysia, the Chinese ethnic group exhibited the highest prevalence of Mia antigen at 4.7%, followed by Malays and Indians. This aligns with findings from other Southeast Asian and East Asian countries with Chinese populations. Our study showed the frequency of Mi^a antigens among Malays (1.7%) is similar to the GP. Mur frequency observed in Indonesians from a study on Filipino and Indonesian workers in Taiwan, where GP. Mur genotype frequencies were 7.6% and 2.1%, respectively[26]. This correlation reflects shared ancestry between Malays in Malaysia and Indonesians. Interestingly, the prevalence among Filipinos is comparable or slightly higher than that of Taiwanese at 7.3%[9,12]. Indians in our study had a low prevalence of Mi^a antigen (0.9%), slightly above the 0.1% observed in India[10]. The study by Makroo et al. (2016) involved blood donors from northern India, while Malaysia's Indian population mainly originates from southern India, suggesting both regions exhibit low prevalence of Mi^a antigen[10]. In Australia, the prevalence of Mi^a antigen was 0.22% in the blood donor population, likely due to the diverse demographics resulting from various immigration waves. Extended phenotyping of Mi^a-positive cases in Australia showed that most belonged to the GP. Mur phenotype, followed by GP. Vw, GP. Hut, and GP. Bun[11]. According to ISBT classification, a rare phenotype blood is defined as having a prevalence of less than 1 in 1000 individuals (0.1%)[27]. Therefore, our study findings of 2.6% as the prevalence of Mia antigen in Malaysia does not qualify as a rare blood phenotype and can be regarded as a significant antigen.

The GP. Mur phenotype is associated with enhanced RBC membrane stability due to increased levels of band 3 protein, making these RBCs more resistant to osmotic stress[28]. This property has led to the natural selection of GP. Mur in regions endemic to Plasmodium falciparum malaria, known to infect RBCs via glycophorins. A significant 88.4% of the indigenous Ami tribe in Eastern Taiwan are GP. Mur positive[6,9], an area where malaria has been endemic for centuries[29] . In southern China, 6.5% of the local population is GP. Mur positive[30] with historical records indicating malaria's presence in the region for 5000 years[6]. Thus, studying the prevalence of the GP. Mur phenotype in East Malaysia, specifically Sabah and Sarawak, which are also malaria-endemic, could be valuable[31].

Anti-Mi^a is the antibody corresponding to the Mi^a antigen. In our study, the prevalence of the anti-Mi^a antibody was 0.5% (61/13011), the highest frequency reported to date. Comparatively, the anti-Mi^a prevalence in other Southeast Asian and East Asian countries is lower, with 0.45% in Southern China[30], 0.28% in Taiwan[12], 0.057% in Hong Kong[32]and 0.19% in Thailand[33]. A similar study done in Malaysia by Prathiba et al. (2002)[17] showed prevalence of anti-Mi^a of 0.2%. From our study, anti-Mi^a antibody was also the most frequent alloantibody encountered, which made up 26.4% (61/231) of the total alloantibody positive cases, followed by anti-E, anti-c and anti-Lea. This result is comparable to a study by Yousuf et al. (2013)[24] which also demonstrated anti-Mi^a as the most frequently detected alloantibody with a prevalence of 30.4% (49/161) among total alloantibody positive cases. In contrast, Prathiba et al. (2002)[17] found anti-Mi^a to be the third most common antibody, with Lewis antibodies being the most prevalent, followed by those against Rhesus antigens. Blood banks in Taiwan have also shown that the frequency of anti-Mi^a is higher than the total of all Rh antibodies[34]. This is significant as it indicates a high rate of alloimmunization among our patients due to Mi^a antigen-positive RBCs from transfusions, transplants, or pregnancies, despite some cases of naturally occurring anti-Mi^a antibodies. The risk of developing anti-Mi^a antibodies in Mi^a-negative patients through blood transfusions is considerable in Malaysia, with 2.6% (our result) to 3.8% (Prathiba et al. 2002)[17] of the local population being Mi^a antigen positive.

Antibodies against glycophorin hybrids are immunogenic and have been associated with hemolytic disease of the fetus and newborn (HDFN) as well as hemolytic transfusion reactions (HTR). A total of 35 cases of HDFN and HTR linked to these antibodies have been reported. Among these, 27 cases (77%) were classified as HDFN, while 8 cases (23%) were HTR, with the majority (13/35; 37%) occurring in Asia[6]. Of the 27 HDFN cases, 12 (44%) were of moderate to high severity, necessitating multiple exchange transfusions, and one case was fatal. The antibodies identified were predominantly IgG, with titers ranging from 32 to 1024, indicating that strongly reactive IgG antibodies are a significant cause of HDFN[6]. A recent case of alloimmunization due to maternal anti-Mi^a antibody resulting in severe HDFN was reported in Korea in 2013, where massive fetomaternal hemorrhage led to severe anemia in the newborn, unresponsive to repeated transfusions, and resulting in hypoxic brain injury[35].

Additionally, a case of anti-Mur causing hydrops fetalis during a first pregnancy at 28 weeks of gestation was documented in Taiwan in 2002, where both the fetus and father had the GP. Mur phenotype, while the mother had anti-Mur antibodies[23]. Another case involving anti-Vw was reported in 2009 from Switzerland, where the prevalence of anti-Vw was notably high at 1.43%. This case illustrated severe HDFN in a neonate, requiring multiple transfusions and phototherapy due to hyporegenerative anemia induced by the antibody, which resulted in prolonged suppression of erythropoiesis. Indirect antiglobulin tests on maternal serum were strongly positive with the father's RBCs, confirming the presence of anti-Vw in the mother's serum, while the father tested positive for the Vw antigen[36].

While anti-Mi^a of the IgM type is typically naturally occurring and usually clinically insignificant[37], there has been a reported case of an IgM antibody reacting at 37°C that led to HTR[12]. For example, in Taiwan, a patient with anti-Mi^a developed intravascular hemolysis following a transfusion of GP. Mur positive blood[38]. To date, no published cases of HTR or HDFN have been reported in Malaysia or at our medical center. This may be attributed to the practice of providing Mi^a antigen-negative and crossmatch-compatible blood when a patient is found to have anti-Mi^a antibodies. In blood banks in Malaysia that do not have anti-Mi^a antibody reagents for phenotyping, the standard practice is to issue crossmatch-compatible blood to patients with anti-Mi^a antibody[39]. However, relying solely on crossmatching is not 100% effective in preventing HTR

or HDFN, as crossmatch-compatible blood may still contain the Mi^a antigen[40]. A study from Taiwan found that supplying GP. Mur antigen-negative blood reduced the rate of transfusion reactions in patients with anti-Mi^a antibody[40].

The serological method for Mi^a phenotyping that uses human polyclonal anti-Mi^a antibodies may yield false-negative results, leading to the transfusion of genotypically GP. Mur positive but serologically negative RBCs to patients with anti-Mi^a antibody[19] A comparative study conducted in Canada examined human polyclonal anti-Mi^a versus murine monoclonal anti-Mi^a (Novaclone; Immucor), finding that a sample with the GP. Vw phenotype tested negative with the human polyclonal anti-Mi^a but positive with the murine monoclonal anti-Mi^a[41]. Research by Fraser et. al, (2018)[42] demonstrated that the anti-Mi^a murine monoclonal antibody GAMA210 (Novaclone; Immucor) strongly reacted (3+-4+) with GP. Vw, GP. Hut, GP. Mur, GP. Hop, and GP. Bun RBCs, but was negative with GP. Hil RBCs. The monoclonal antibody GAMA210 accurately identified one Mi^a+ donor from 101 blood samples. In our study, the application of the anti-Mi^a monoclonal antibody revealed that 2.6% of patients were Mi^a+.

Currently, the standard practice in Malaysia includes Mi^a+ cells in antibody screening; however, Mi^a+ cells are often not available for antibody identification. It is recommended that at least three Mi^a+ cells and three Mi^a- cells be included to satisfy the rule of three in antibody identification, and the patient's RBCs should be typed as negative with the anti-Mi^a antibody. A limitation of this study is that ethnic groups from East Malaysia, specifically Sabah and Sarawak, were not included.

CONCLUSION

Our study indicates that the prevalence of the Mi^a antigen of 2.6%, is not considered as rare phenotype and can be regarded as a significant antigen. It is highest among the Chinese population in Malaysia, followed by Malays and Indians. Additionally, the prevalence of anti-Mi^a antibodies in Malaysia is higher than that reported in other countries. This finding highlights the need for measures to ensure the availability of Mi^a-negative crossmatched compatible blood for patients with anti-Mi^a antibodies, especially given the significant cases of HTR and HDFN associated with this antibody. It is also recommended that antibody screening be conducted for pregnant women during their antenatal bookings to identify anti-Mi^a antibodies early.

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Authors' Contribution

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REFERENCE

1. Lomas-Francis C. Miltenberger phenotypes are glycophorin variants: a review. *ISBT Science Series*. 2011; **6**:296–301.
2. International Society of Blood Transfusion. Red Cell Immunogenetics and Blood Group Terminology: Table of Blood Group Systems. 2024. https://www.isbtweb.org/resource/tableofbloodgroupsystems.html.
3. Cleghorn TE. A Memorandum on the Miltenberger Blood Groups. *Vox Sang*. 1966; **11**:219-222.
4. Tippett P, Reid ME, Poole J, Green CA, Daniels GL, Anstee DJ. The Miltenberger subsystem: is it obsolescent? *Transfus Med Rev*. 1992; **6**(3):170-182. doi: 10.1016/s0887-7963(92)70167-9.
5. Reid ME, Tippett P. Review of a terminology proposed to supersede Miltenberger. *Immunohematology*. 1993; **9**:91–95.
6. Heathcote DJ, Carroll TE, Flower RL. Sixty years of antibodies to MNS system hybrid glycophorins: what have we learned? *Transfus Med Rev*. 2011; **25**(2):111-124. doi: 10.1016/j.tmrv.2010.11.003.
7. Issitt PD. Applied Blood Group Serology. Montgomery Scientific Publications 3rd ed, 1985;352.
8. Uchikawa M, Tsuneyama H, Akaza T, Tadokoro K, Juji T. Miltenberger antigens in Japanese: Book of Abstracts 6th Regional Congress of International Society of Blood Transfusion, Western Pacific Region, 1995; 100.
9. Broadberry RE, Lin M. The distribution of the MiIII (GP.Mur) phenotype among the population of Taiwan. *Transfus Med*. 1996; **6**(2):145-148. https://doi.org/10.1046/j.1365-3148.1996.d01-64.x
10. Makroo RN, Bhatia A, Chowdhry M, Rosamma NL, Karna P. Frequency of Mi(a) antigen: A pilot study among blood donors. *Indian J Med Res*. 2016; **143**(5):633-5.
11. Lopez GH, Wilson B, Turner RM, Millard GM, Fraser NS, Roots NM, et al. Frequency of Mia (MNS7) and Classification of Mia-Positive Hybrid Glycophorins in an Australian Blood Donor Population. *Transfus Med Hemother*. 2020;**47**(4):279-286. doi: 10.1159/000504026.
12. Broadberry RE, Lin M. The incidence and significance of anti-'Mi^a' in Taiwan. *Transfusion*. 1994; **34**:349-352. doi: 10.1046/j.1537-2995.1994.34494233585.x.
13. Poole J, King MJ, Mak KH, Liew YW, Leong S, Chua KM. The MiIII phenotype among Chinese donors in Hong Kong: immunochemical and serological studies. *Transfus Med*. 1991; **1**(3):169-175.
14. Wei L, Lopez GH, Zhang Y, Wen J, Wang Z, Fu Y, et al. Genotyping analysis of MNS blood group GP(B-A-B) hybrid glycophorins in the Chinese Southern Han population using a high-resolution melting assay. *Transfusion.* 2018; **58**(7):1763-1771.
15. Palacajornsuk P, Nathalang O, Tantimavanich S, Bejrachandra S, Reid ME. Detection of MNS hybrid molecules in the Thai population using PCR-SSP technique. *Transfus Med*. 2007; **17**(3):169-174.
16. Huynh NT, Ford DS, Duyen TT, Huong MT. Jk and Mi.III phenotype frequencies in North Vietnam. *Immunohematology*. 2003; **19**(2):57–58.
17. Prathiba R, Lopez CG, Usin FM. The prevalence of GP Mur and anti-"Mi^a" in a tertiary hospital in Peninsula Malaysia. *Malays J Pathol*. 2002; **24**(2):95-98.
18. Hassan SN, Mohamad S, Hassan R, Wei S, Noor NHM, Ab Rahman WSW. PCR-based screening of MNS glycophorin variants in patients suspected of having anti-Mia. *Bangladesh Journal of Medical Science*. 2022; **21**(2), 432–437. doi: http://doi.org/10.3329/bjms.v21i2.58078.
19. Chen TD, Chen DP, Wang WT, Sun CF. MNSs blood group glycophorin variants in Taiwan: a genotype-serotype correlation study of 'Mi(a)' and St(a) with report of two new alleles for St(a). *PLoS One*. 2014; **9**(5): e98166. doi: 10.1371/journal.pone.0098166. eCollection 2014.
20. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. *Transfus Med Rev*. 2007; **21**(1):58-71. doi: 10.1016/j.tmrv.2006.08.003.
21. Lin CK, Mak KH, Szeto SC, Poon KH, Yuen CM, Chan NK, et al. First case of hemolytic disease of the newborn due to anti-Mur in Hong Kong. *Clin Lab Haematol*. 1996; **18**(1):19-22. doi: 10.1111/j.1365-2257.1996.tb00731.x.
22. Van den Bos AG, Steiner K. Hemolytic disease of the newborn caused by anti-MUT (MNS 35). *Vox Sang*. 2004; **87**(3):208-209.
23. Wu KH, Chang JG, Lin M, Shih MC, Lin HC, Lee CC, et al. Hydrops fetalis caused by anti-Mur in first pregnancy--a case report. *Transfus Med*. 2002; **12**(5):325-327. doi: 10.1046/j.1365-3148.2002.00394.x.
24. Yousuf R, Abdul Aziz S, Yusof N, Leong CF. Incidence of Red Cell Alloantibody among the Transfusion Recipients of Universiti Kebangsaan Malaysia Medical Centre. *Indian J Hematol Blood Transfus*. 2013; **29**(2):65-67. doi: 10.1007/s12288-012-0155-x.
25. Cohn C, Delaney M, Johnson S, Katz L, Schwartz J, eds. Technical Manual, 21st ed. Bethesda, MD: AABB. 2023.
26. Hsu K, Lin YC, Chao HP, Lee TY, Lin M, Chan YS. Assessing the frequencies of GP.Mur (Mi.III) in several Southeast Asian populations by PCR typing. *Transfus Apher Sci.* 2013; **49**(2):370-1.
27. Nance S, Scharberg EA, Thornton V, Yahalom V, Sareneva I, Lomas-Francis C. International rare donor panels: a review.

Vox Sang. 2016;**110**(3):209-218.

28. Hsu K, Chi N, Gucek M, Van Eyk JE, Cole RN, Lin M, et al. Miltenberger blood group antigen type III (Mi.III) enhances the expression of band 3. *Blood.* 2009;**114**(9):1919-28.
29. Taiwan Provincial Malaria Research Institute and WHO Malaria team in Taiwan. Malaria control and eradication in Taiwan, Progress report, May 1952 to June 1957. *Bull World Health* Organ. 1958; **19**:595-620.
30. Li Q, Duan S, Sun A, Liao Y, Wu Y, Wang H, et al. Study on the antigens and antibodies of Mur and Mi^a blood groups in southern China. *Transfus Apher Sci*. 2022;**61**(4):103378.
31. Hussin N, Lim YA, Goh PP, William T, Jelip J, Mudin RN. Updates on malaria incidence and profile in Malaysia from 2013 to 2017. *Malar J*. 2020;**19**(1):55. doi: 10.1186/s12936-020-3135-x.
32. Mak KH, Banks JA, Lubenko A, Chua KM, Torres de Jardine AL, Yan KF. A survey of the incidence of Miltenberger antibodies among Hong Kong Chinese blood donors. *Transfusion*. 1994; **34**(3): 238-241. doi: 10.1046/j.1537-2995.1994.34394196622.x..
33. Promwong C, Siammai S, Hassarin S, Buakaew J, Yeela T, Soisangwan P, Roxby D. Frequencies and specificities of red cell alloantibodies in the Southern Thai population. Asian *J Transfus Sci*. 2013;**7**(1):16-20. doi: 10.4103/0973-6247.
34. Lin M, Broadberry RE. Immunohematology in Taiwan. *Transfus Med Rev*. 1998; **12**(1):56-72. doi: 10.1016/s0887-7963(98)80090-4.
35. Park SW, Park JH, Kim Y, You JH, Cho HM, Kim EY, et al. A case of neonatal isoimmune haemolytic disease due to anti-Mi^a antibody with massive fetomaternal hemorrhage. *Korean J Perinatol*. 2013; **24**:310-314.
36. Waldvogel S, Natterer J, Canellini G, Truttmann A, Tissot JD. Severe neonatal hyporegenerative anemia due to anti-Vw (anti-MNS9) alloantibody. *J Perinat Med*. 2009; **37**:422-424. doi: 10.1515/JPM.2009.071.
37. Lin CK, Mak KH, Cheng G, Lao TT, Tang MH, Yuen CM, et al. Serologic characteristics, and clinical significance of Miltenberger antibodies among Chinese patients in Hong Kong. *Vox Sang* 1998; **74**:59–60. doi: 10.1046/j.1423-0410.1998.7410059.x.
38. Lin M, Broadberry RE. An intravascular hemolytic transfusion reaction due to anti-'Mi(a)' in Taiwan. *Vox Sang*. 1994; **67**(3):320. https://doi.org/10.1111/j.1423-0410.1994.tb01262.x.
39. Faraizah Abd Karim, Julia Abdullah, Nor Hafizah Ahmad, Thane Moze a/p Darumalinggam, Alvina Lau Voon Na, Shaik Farizan Shaik Daud et al (Editors). Handbook on Clinical Use of Blood. 3rd edition. National Blood Centre. Ministry of Health, Malaysia. 2020. P-1-22
40. Yang CA, Lin JA, Chang CW, Wu KH, Yeh SP, Ho CM, et al. Selection of GP.Mur antigen-negative RBC for blood recipients with anti-'Mi(a)' records decreases transfusion reaction rates in Taiwan. *Transfus Med.* 2016; **26**(5):349-354. doi: 10.1111/tme.12357.
41. Dolnik T, Lin X, Yang JC, Saldanha J, MacEachern J. Performance Characteristics of a Novel Anti-Mi^a Monoclonal Antibody-Based Reagent in a Canadian Population. Poster presented at 34th International Congress of the International Society of Blood Transfusion (ISBT), Dubai, UAE. 2016. https://www.postersessiononline.eu/173580348_eu/congresos/34isbt/aula/-P_375_34isbt.pdf.
42. Fraser NS, Roots NM, Lopez GH, Wilson B, Ekman S, Liew YW, et al. Comparison of two monoclonal antibodies for the detection of Mi^a (MNS7) in MNS hybrid glycophorins. *Pathology*. 2018; **50** (Supplement 1)**:** S104. https://doi.org/10.1016/j.pathol.2017.12.291.