

**A STUDY ON THE PREVALENCE OF MILTENBERGER BLOOD GROUP (GP.
MUR) OF ANTIGENS AND ANTIBODIES AMONG BLOOD DONORS AND
PATIENTS WITH DIFFERENT BLOOD GROUP IN HOSPITAL UNIVERSITI
SAINS MALAYSIA (HUSM)**

by

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Health Sciences (Biomedicine)

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CERTIFICATE

This is to certify that the dissertation entitled “A study on the prevalence of Miltenberger blood group (GP. Mur) of antigens and antibodies among blood donors and patients with different blood group in hospital Universiti Sains Malaysia (HUSM)” is the bonafide record of research work done by Mr. Muhammad Azrul Hafizi bin Ramli during the period from September 2012 to May 2013 under my supervision.

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LIST OF SYMBOLS AND ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AHG	Antihuman globulin
Arg	Arginine
DNA	Deoxyribonucleic acid
EDTA	Ethylenedianinetetraacetic acid
GP	Glycophorin (Protein)
GYP	Glycophorin (Gene)
GalNac	Galactose/N-acetylgalactosamine
Gal	Galactose
Glu	Glutamic acid
GSH	Group, Screen and Hold
HTR	Hemolytic Transfusion Reaction
HDNF	Hemolytic Disease Newborn and Fetus
His	Histidine
HUSM	Hospital Universiti Sains Malaysia
IAT	Indirect antiglobulin test
ISBT	International Society of Blood Transfusion
Ig	Immunoglobulin
LISS	Low Ionic Strength Solution
Mi	Miltenberger
Met	Methionine

mm	Milimeter
mL	Mililiter
NaCl	Sodium chloride
°C	Degree celsius
%	Percent
Rh	Rhesus
RBC	Red blood cell
r.p.m	Revolutions per minute
S.E	South East
SPSS	Statistical Package for the Social Sciences
Thr	Threonine
US	United states
μL	Microliter

ABSTRAK

Sistem MNS adalah sistem kumpulan darah kedua yang ditemui dan sekurang-kurangnya 16 daripada 46 antigen dalam hasil sistem MNS adalah daripada penggabungan semula genetik, menghasilkan glycophorin hibrid. Fenotip Mi (III) Miltenberger (GP Mur) subsistem adalah salah satu hibrid glycophorin yang dihasilkan oleh penggabungan semula genetik dalam sistem MNS. Insiden glycophorin hibrid ini adalah yang tertinggi dalam populasi Asia Timur dan antibodi yang dihasilkan adalah imunogenik terutamanya jenis IgG yang boleh menyebabkan tindak balas pemindahan hemolitik (HTR), penyakit bayi baru lahir hemolitik (HDNF) dan hydrops janin. Tujuan kajian ini adalah untuk mengesan kelaziman anti-Mur dan anti-MUT dalam kalangan pesakit dan antigen GP.Mur dalam penderma darah (individu yang sihat) dengan kumpulan darah yang berbeza di Hospital Universiti Sains Malaysia (HUSM). Sampel darah daripada 27,322 pesakit dengan kumpulan darah yang berbeza telah diskriminasi dengan kaedah serologi untuk antibodi anti-Mia dari September 2012 hingga Mac 2013 manakala 80 penderma darah dengan kumpulan darah yang berbeza telah dipilih untuk mengesan fenotip GP Mur dengan kaedah serologi. 57 % daripada penderma adalah Melayu manakala yang lain adalah berbangsa Cina. Anti-Mi (III) antibodi dikesan dalam 0.3 % daripada kedua-dua pesakit antenatal dan am. Daripada 83 pesakit, 15.7 % adalah positif bagi antibodi anti-Mur/MUT manakala yang lain telah disahkan sebagai kemungkinan lain-lain anti-Mi (III) antibodi. Berdasarkan kumpulan darah, 18.2 % daripada pesakit dari kumpulan darah A adalah positif bagi antibodi anti-Mur / Mut manakala 20.0 %, 8.7 % dan 15.4 % telah dikesan dari B, O dan kumpulan darah AB. Untuk fenotip GP.Mur, 5.0 % daripada penderma darah Melayu telah

dikesan mempunyai antigen GP.Mur manakala hanya 1.3 % telah dikenal pasti dalam penderma darah Cina. Hanya 5 % daripada penderma darah berbangsa melayu dari kumpulan darah A dan kumpulan darah O adalah GP Mur positif manakala 10 % Melayu dan 5 % daripada bangsa Cina mempunyai antigen GP.Mur dalam kumpulan darah AB. Berdasarkan keputusan, antigen GP Mur adalah lebih tinggi pada penderma darah Melayu dan banyak laporan masalah-masalah klinikal yang berkaitan dengan anti-Mi (III) antibodi, oleh itu adalah wajar bagi GP Mur sel-sel merah dimasukkan ke dalam panel pemeriksaan untuk kumpulan darah dan skrin prosedur di hospital USM.

ABSTRACT

The MNS system was the second blood group system discovered and at least 16 of the 46 antigens in the MNS system result from genetic recombination, producing a hybrid glycoprotein. The Mi (III) phenotype of the Miltenberger subsystem (GP. Mur) is one of hybrid glycoprotein produced by genetic recombination of MNS system. The incidence of this hybrid glycoprotein was highest in the East Asian populations and the antibodies developed were immunogenic especially IgG type which can cause hemolytic transfusion reaction (HTR), hemolytic disease of newborn and fetal (HDNF) and hydrops fetalis. The aim of the study was to detect the prevalence of anti-Mur and anti-MUT among patients and GP. Mur antigen in blood donors (healthy individuals) with different blood group in Hospital Universiti Sains Malaysia (HUSM). Blood samples from 27,322 patients with different blood group were screened by standard serological method for the anti Mi (III) antibody from September 2012 to March 2013 while 80 blood donors with different blood group were selected to detect GP. Mur phenotype by serological method. Fifty-seven percent of donors were Malay while others were Chinese. Anti-Mi (III) antibody were detected in 0.3 % of both antenatal and general patients. Out of 83 patients, 15.7 % were positive for anti-Mur/MUT antibodies while others were diagnosed as possible of other anti-Mi (III) antibodies. Based on blood group, 18.2 % of patients from A blood group were positive for anti-Mur/MUT antibodies while 20.0 %, 8.7 % and 15.4 % were detected from B, O and AB blood group. For GP. Mur phenotype, 5.0 % of Malay blood donors were detected to have GP. Mur antigen while only 1.3 % was identified in Chinese blood donors. Only 5 % of Malay blood donors from each A and O blood group were GP. Mur positive while 10 %

of Malay and 5 % of Chinese have GP. Mur antigen in AB blood group. Based on the findings that GP. Mur antigen were higher in Malay blood donors and with many reports of clinical problems associated with the anti-Mi (III) antibody, it is warranted that the GP Mur red cells be included in screening panels for group and screen procedures in our hospitals.

CHAPTER 1

INTRODUCTION

1.1 Background of Miltenberger (Mi) blood group systems

The “Miltenberger subsystem” was introduced by Cleghorn in 1966 based on reactivity with selected sera from Mrs Miltenberger (anti-Mia) of low-incidence MNS blood group antigens. The MNS blood group antigens are carried on glycophorin A (GPA), glycophorin B (GPB) or hybrid protein that arise from crossover or DNA conversion events within the Glycophorin (GYP) gene family (Palacajornsuk P. *et.al.*, 2007). Biochemical and molecular studies have shown that the Mi. cell classes are associated with altered forms of GPA molecules or hybrid molecules composed of sequences derived from GPA and GPB. These hybrid molecules originate from chromosomal misalignment and unequal crossing-over at meiosis or by the mechanism of gene conversion (Patricia *et.al.*, 1992). The Miltenberger subsystem grew to a complex of 11 classes and due to the molecular basis of the antigens in this subsystem they are grouped under the MNS blood group system (Palacajornsuk P. *et.al.*, 2007). The Miltenberger subsystem was established based on the naming of genetic glycoprotein variants rather than numbering (Damien *et.al.*, 2011) and their classification was summarized in the table 1.1.

Phenotype

Antigens

Class	Mi	Mi ^a	Vw	Hut	Mur	MUT	Hil	TSEN	MINY	Hop	Nob	DANE	Notes on incidence
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"Miltnerberger" series

GP.Vw	Mi. I	+	+	0	0	0	0	0	0	0	0	0	0.057 % in whites; 1.4 % in S.E. Switzerland; 0.04 % in Thais
GP.Hut	Mi. II	+	0	+	0	+	0	0	0	0	0	0	0.064 % in whites; 0.040 % in Thais
GP.Mur	Mi. III	+	0	0	+	+	+	0	+	0	0	0	Seen in most Asian populations; 5 % of Chinese and 9 % of Thais. Most other populations less than 0.01 %
GP.Hop	Mi. IV	+	0	0	+	+	0	+	+	+	0	0	Only 2 propositi known
GP.Hil	Mi. V	0	0	0	0	0	+	0	+	0	0	0	Most example seen in Europeans
GP.Bun	Mi. VI	+	0	0	+	+	+	0	+	+	0	0	Most populations less than 0.01 %; Thais 1.1 %
GP.Nob	Mi. VII	0	0	0	0	0	0	0	0	0	+	0	0.061 % of English blood donors
GP.Joh	Mi. VIII	0	0	0	0	0	0	0	0	+	+	0	Positive for Nob antigen
GP.Dane	Mi. IX	0	0	0	+	0	0	0	0	0	0	+	Most population less than 0.01 %; Danes 0.43 %
GP.Hf	Mi. X	+	0	0	0	+	+	0	+	0	0	0	Several propositi are known, all of Japanese ancestry
GP.JL	Mi. XI	0	0	0	0	0	0	+	+	0	0	0	Reported in S. China

Table 1.1 : Classification of Hybrid Glycophorins of the Obsolete Miltnerberger Subsystem and Related Variants

(Adapted from Damien *et.al.*, 2011).

Initially, there are variety terminologies that have been used to describe the “Miltenberger subsystem” such as “Miltenberger”, “Mia”, “Mi(a)” and “Mi (III)” and all these terminologies can be very confusing (Damien *et.al.*, 2011). Therefore, it was then suggested that the Miltenberger subsystem are defined by characteristic glycoprotein of variants (GP.) and named using an abbreviation of the name of the proband in which the variant was first described, e.g. Mi.I becomes GP.Vw, Mi.II becomes GP.Hut and Mi.III becomes GP.Mur (table 1.1) (Palacajornsuk P. *et.al.*, 2007).

Molecular studies have shown that antigens originally in the Miltenberger subsystem are carried on *GYP(A-B)*, *GYP(A-B-A)* and *GYP(B-A-B)* hybrids. The *GYP(A-B)* hybrids include GP.Hil and GP.JL; *GYP(A-B-A)* hybrids include GP.Vw, GP.Hut, GP.Nob, GP.Joh and GP.Danes; and *GYP(B-A-B)* hybrids include GP.Mur, GP.Hop, GP.Bun and GP.HF (figure 1.1) (Palacajornsuk P. *et.al.*, 2007).

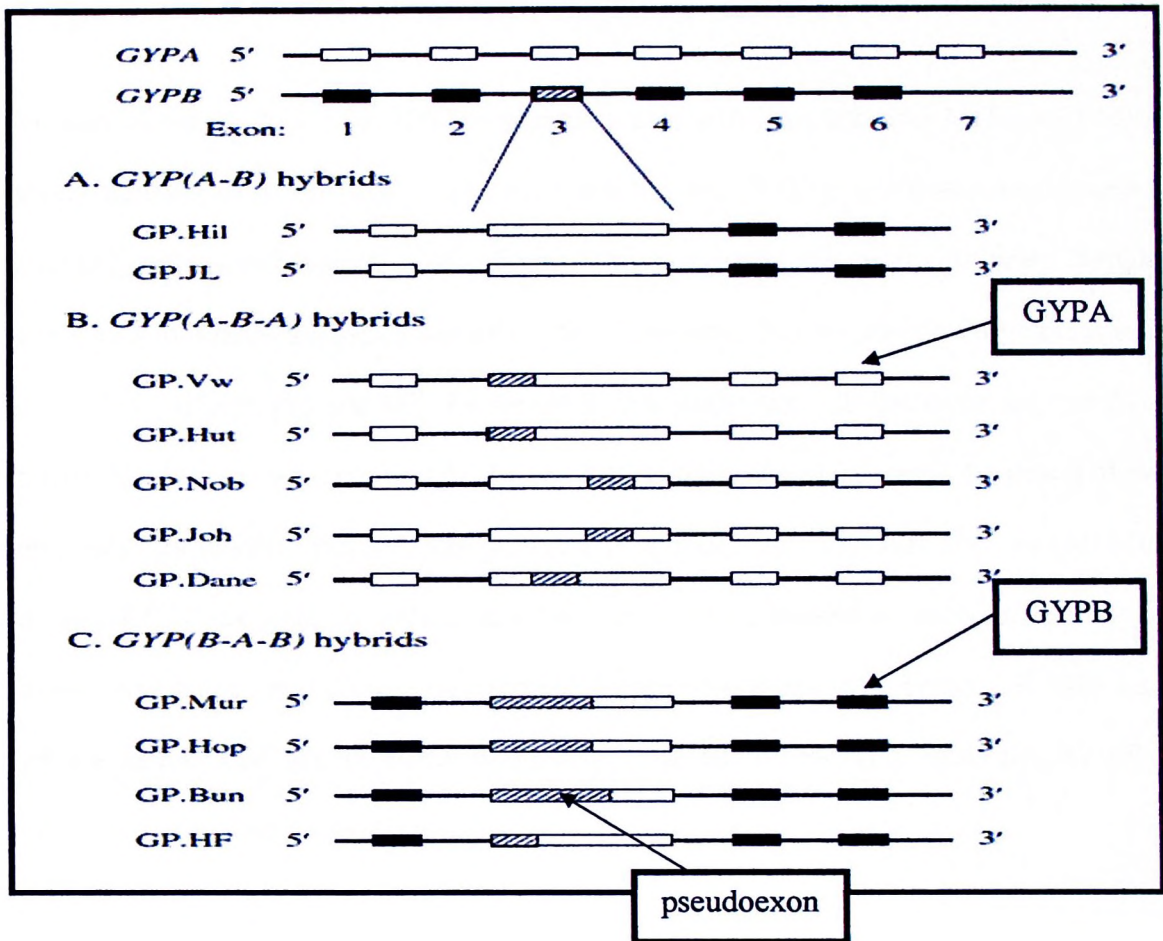


Figure 1.1 : Organization of MNS glycophorin hybrid genes (Adapted from (Palacajornsuk P. *et.al.*, 2007).

1.2 Aim of study

The aim of the study is to detect the prevalence of anti-Mur and anti-MUT antibodies among patients with positive screening panel for Mi (III), given that many positive screening cells were detected in blood bank laboratory even though it is initially thought to be a low incidence antigen. Basically, Mi (III) contain five important antigens such as Mur, MUT, MINY, Hil and Mi^a. However in this study, we will focussed only on Mur and MUT antigens and antibodies due to the availability of reagents to detect these antigens or antibodies. Besides, the presence of anti-Mi (III) antibody such as anti-Mur and anti-MUT are able to induce several complication condition such as Hemolytic Transfusion Reaction (HTR), Hemolytic Disease Newborn and Fetus (HDNF) and hydrops fetalis that are probably caused by reaction of the GP. Mur antigen after transfusion or during pregnancy.

In addition, because of many cases associated with the anti-Mi (III) antibody including HTR, HDNF and hydrops fetalis have been reported in Asian countries. Hereby, we want to justify that the GP. Mur red cells should be included in the screening panels for group and screen procedures in the countries with a significant Asian populations, especially in Malaysia in order to improve laboratory diagnosis and patient management.

1.3 Problem statement

The MNS blood group system, for example Miltenberger (Mi) is a highly polymorphic system that consists of many low incidence antigens. Even though Mi is a low incidence antigen but the frequency in Asian populations especially GP. Mur or Mi (III) can be regarded as high, so it is important to determine the prevalence of these antigens and antibodies in our population. Thus, I will take this opportunity to detect these antigens and antibodies among Malaysian, especially in Hospital Universiti Sains Malaysia (HUSM).