

Relationship between ABO blood groups and the HBsAg positivity in blood donors

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Prior to the production of plasma-derived hepatitis B vaccine, HBsAg-positive blood units which had been collected from various blood banks of hospitals in Yangon area were tested again for confirmation and determination of the degree of HBsAg positivity by using the Counter Current Electrophoresis (CEP) method at the Hepatitis B Vaccine Plant, DMR (LM). Only those blood units with high titer of HBsAg positivity (i.e. titer $\geq 1/10$ dilution) were used for further production processes. In this study, ABO blood groups distribution in 6505 HBsAg-positive blood donors with low and high titers positivity of HBsAg were determined. It was found that ABO blood groups were equally distributed in HBsAg-positive blood donors. However, high titer of HBsAg positivity was apparently encountered in those donors with blood group AB compared to other blood groups. Donors with blood group AB were found to be more susceptible than others to have high titre positivity of HBsAg.

INTRODUCTION

Hepatitis B (HB) viral infection is a major public health problem worldwide. The HB vaccination is an effective protective measure against the HB viral infection and the plasma-derived HB vaccine entered the global market in 1981 [1]. In Myanmar, HB viral infection is regarded as one of the vaccine preventable diseases taking 8th position among the prioritized list of diseases and health conditions in National Health Plan (2006-2011) [2]. The plasma-derived HB vaccine was successfully developed by the Department of Medical Research, Lower Myanmar (DMR, LM) and has been distributed to the public since 1997 [3]. Prior to the production of plasma-derived hepatitis B vaccine, hepatitis B surface antigen (HBsAg) positive blood units collected from various blood banks of hospitals in Yangon area were tested again for confirmation and determination of the degree of HBsAg positivity by using the Counter Current Electrophoresis (CEP)

method. Only those blood units with high titer of HBsAg positivity i.e. titer $\geq 1/10$ dilution were used for further production processes. In this study, ABO blood groups distribution among the HBsAg-positive blood units collected from various blood banks and those blood units with high titre of HBsAg positivity were studied. This study was conducted with an aim to determine ABO blood groups distribution among HBsAg-positive blood donors, and to verify the relationship between ABO blood groups distribution and degree or titer of HBsAg positivity in the HBsAg-positive blood donors.

MATERIALS AND METHODS

This is a cross-sectional study, carried out at the Hepatitis B Vaccine Plant, DMR (LM). The HBsAg- positive blood units which had been already determined by ELISA test system (ORTHO antibody to HBsAg, USA) with known blood ABO groups from various hospitals in Yangon area, majority is from

the National Blood Centre, Yangon General Hospital, were collected for production of plasma-derived hepatitis B vaccine at the HB Vaccine Plant, DMR (LM). These blood units were tested again for degree of HBsAg positivity by using a semi quantitative method, i.e. Counter Current Electrophoresis (CEP) technique and also for anti-HCV, anti-HIV and anti-syphilis by using specific methods. Among them, those blood units with HBsAg positivity titer of $\geq 1/10$ dilution negativity for anti-HCV, anti-HIV and anti-syphilis were selected and pooled for further production processes [4].

Determination of the degree of HBsAg positivity

The CEP testing is based on electroendosmosis in which the HBsAg particles carrying a prevalent negative surface charge migrate toward the anode and anti-HBs immunoglobulins, raised in an experimental animal, with partial dissociation migrate toward the cathode at pH 8.6 in veronal buffer in CEP tank. A clear precipitation line was formed between them and results were read immediately after run [5].

In this study, the titres of serum samples of HBsAg-positive blood donors were determined and categorized by CEP method after serial dilution i.e. 1 in 5, 1 in 10, 1 in 20, 1 in 40, etc. with 1% agarose in 0.015 M veronal buffer containing 5-10% normal human serum.

Determination of the anti-HCV

The detection of anti-HCV in serum of these donors was performed by using Serodia-HCV Gelatin Particle Agglutination Test, Fujirebio Inc. of Japan. The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to HCV in serum/plasma specimens.

Determination of the anti-HIV

The anti-HIV was determined by the 3rd generation one step antibodies to HIV-1/HIV-2 test, Standard Diagnostics, Inc. of the Republic of Korea. It is an immunochromatographic test for the qualitative detection

of antibodies of all types specific to HIV-1 and HIV-2.

Determination of the anti-syphilis

Syphilis antibodies were identified by the Syphilis RPR test, Human Biochemicals and Diagnostics, Germany. It is a macroscopic, non-treponomal flocculation test that is used to detect and quantify reagin (syphilis) antibodies in blood samples.

In this study, a total of 6505 HBsAg-positive blood units with known ABO blood groups were studied for their degree of HBsAg positivity; i.e. titer of low ($< 1/10$) and high ($\geq 1/10$) dilutions in these donors. Baseline distribution of ABO blood groups in Myanmar population of other studies were mentioned for comparison [6, 7, 8]. Student unpaired 't' test and 'Chi square' test were used for statistical analysis.

RESULTS

Percentage distribution of the ABO blood groups in 6505 HBsAg-positive blood donors of this study and previous studies in Myanmar population are shown in Table 1.

Table 1. Percentage distribution of the ABO blood groups in Myanmar population of various studies

	Total cases studied	No. of patients with different blood groups			
		A	B	AB	O
Mya Tu & Khin Maung Lwin, 1967	10035	2539 (25.3%)	31613 (31.5%)	833 (8.2%)	3502 (34.9%)
Soe Min Thein, 1978	6223	1575 (25.3%)	1837 (30.1%)	504 (8.1%)	2271 (36.5%)
Thi Wa Tin, 1992	27672	6863 (24.8%)	8772 (31.9%)	2324 (8.4%)	9713 (35.1%)
Present study	6505	1544 (23.7%)	2130 (32.7%)	504 (7.8%)	2327 (35.8%)

Percentage of cases studied are shown in parentheses

Percentage distributions of A, B, AB and O blood groups in present study were 23.7%, 32.7%, 7.8% and 35.8%, respectively and were not apparently different from those of previous studies. Therefore, our study on

6505 blood donors with HBsAg positivity was assumed to be valid.

Table 2 depicts age and sex distribution in donors with low and high titers of HBsAg positivity. There was no significant difference in age and gender distribution in both low and high titers of HBsAg positivity.

Table 2. Age and sex distribution in donors with low and high titers of HBsAg positivity

	Degree of HBsAg positivity		Significance 'p' value
	Low titer (n=5342)	High titer (n=1164)	
Age (years)	27±4.86	30±1.85	>0.05 *
X± SE (range)	(18-45)	(18- 54)	(NS)
Sex	4578 : 763	970: 194	>0.05 **
Male : Female	(6:1)	(5:1)	(NS)

* Using Student's unpaired 't' test

** Using 'Chi square' test

NS = not significant at the 5% probability level

Table 3 summarizes the percentage distribution of ABO blood groups in donors with low and high titers of HBsAg positivity. It was found that high HBsAg titers were encountered in 17.9% of 6505 blood units. The percentage distributions of ABO blood groups in those donors with high titre of HBsAg positivity were found to be 17%, 17.5%, 32.5% and 17.9% in blood group A, B, AB and O, respectively indicating that high titer of HBsAg positivity was significantly encountered in blood group AB compared to others.

Table 3. ABO blood groups distribution in the HBsAg-positive blood donors with low and high titers of HBsAg positivity

Degree of HBsAg positivity (by CEP method)	No. of HBsAg-positive blood donors with different blood groups				
	A	B	AB	O	Total
Low HBsAg titer (<1/10 dilution)	1282	1757	340	19628	5341
	83%	82.5%	67.5%	4.30%	82.1%
High HBsAg titer (≥1/10 dilution)	262	373	164	365	1164
	17%	17.5%	32.5%	15.7%	17.9%
Total	1544	2130	504	2327	6505

$$X^2 = 82.92$$

Degree of freedom = 3

P value = <0.001

DISCUSSION

It is well documented that many diseases are associated with ABO blood groups; blood group A with higher incidence of cancer of stomach, pernicious anemia, and achlorhydria [9, 10], blood group O with duodenal ulcer and cholera [11, 12, 13.]. Regarding the hepatitis B viral infection, HB virus carrier i.e. HBsAg positivity were almost equally distributed in different ABO blood groups [14, 15] but Rhesus negative blood group donors were more susceptible than other to have HBsAg [16]. There have been some evidences of interaction between HB viral infection and blood cells [17, 18]. It is also a well known fact that higher degree of HBsAg positivity is usually associated with higher possibility of HBeAg, a marker of viral replication, and massive viral load leading to progressive and severe hepatic damage with poor prognosis.

In our study, regarding the prevalence of the HBsAg positivity among blood donors, it was found that the HBsAg positivity was almost equally distributed in different blood groups. Otherwise, there was no apparent association between frequency distribution of ABO blood groups and HBsAg carriers (Table 1). It is in accordance with the previous findings of Szmuness *et al.* [14] and Myo Aye *et al.* [15].

Regarding the degree of HBsAg positivity, age and gender factors were not related to the degree or titer of HBsAg positivity indicating that any age and sex have an equal chance of carrying HBsAg positivity and degree regardless of ABO blood groups (Table 2). Regarding the ABO blood groups with degree of HBsAg positivity, it is of interest to note that the HBsAg-positive donors with blood group AB were found to be more susceptible than other blood groups to carry HBsAg of high titer positivity. Otherwise, a person with blood group AB was found to be more likely to have high titer of HBsAg positivity than those with other blood groups (Table 3).

From the findings of our study, it could be concluded that HB viral infection followed by HBsAg positivity is not related to ABO blood groups and there was an equal chance of any person to carry HBsAg. Although a person with blood group AB also has an equal chance of being infected with HB viral infection, the possibility of high HBsAg titer was apparently found in donors with blood group AB compared to persons with other blood groups. Therefore, in clinical practice, persons with blood group AB should be more aware of HB infection prophylaxis than other persons and once being infected, they should take prompt and appropriate medical treatment to prevent further complications related to HB infection.

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