Original Article

ABO Antibodies Among Group O Thai Blood Donors

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Abstract: *Previous reports have shown antibody titers in different parts of the world have either increased or decreased throughout time. The cause of changing titers was found to be environmental rather than hereditary. During the past 10 years, there was only few studies concerning ABO antibodies in group O Thai blood donors. In spite of existing clinical significance of ABO titers for platelet transfusion when ABO identical is not available as well as transfusion of group O blood to non-group O recipients in emergency lifesaving resuscitation where and situation when the red blood cells (packed red cells) and other forms of red cell components group O can not absolutely be obtained. The objective of this study was to further investigate for the level of anti-A and anti-B in group O Thai blood donors.* ***Materials and Methods:*** *The study included 100 group O Thai blood donors consisted of 53 males, 19-59 years and 47 females 21-55 years of age. The saline tube test at room temperature (22-24°C) was used for determining of anti-A and anti-B titers.* ***Results:*** *The study revealed that anti-B titers (4-1024) were higher than anti-A (4-512) titers. The finding also demonstrated the tendency of high anti-A and anti-B titers in female vs. male ($p = 0.057$ for anti-A: $p = 0.297$ for anti-B) and in young age group (19-40 years) tendency of higher anti-A and anti-B titers vs. old age group (> 40-60 years) ($p = 0.275$ for anti-A : $p = 0.013$ for anti-B). It was found that the range of anti-A titer was from 4-512 while anti-B titer was from 4-1024, and the overall titer ≤ 64 for anti-A was 84% while overall titer ≤ 64 for anti-B titer was 73%.* ***Discussion and Conclusion****: the change in anti-A and anti-B titers are most likely due to environmental factors rather than genetics. The obvious variable factors involved are age and gender of the individuals. Female blood donors possessed higher range of both anti-A and anti-B than male blood donors. Young age group (19-40 yrs.) had higher anti-A and anti-B titers than old age group (> 40-60 yrs.). Majority of blood donors had antibody titers ≤ 64 (84% for anti-A and 73% for anti-B). The regular population survey for anti-A and anti-B titers is beneficial for transfusion practice and may lead to guideline development for safe, efficient transfusion and in time.*

Key Words : ● *ABO blood groups* ● *Anti-A* ● *Anti-B* ● *Thai blood donors*

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Introduction

The possible factors that cause the changing of anti-A and anti-B titers are frequently debated and observed. In 1969, Springer and Horton had demonstrated that the level of ABO antibodies is most likely to be regulated by environmental factors, contrary to the older beliefs.[1]

From previous researches, there had been numerous evidences that the levels of ABO antibodies around the world have been changing, compared to the past. Such possibilities can be the effects of bacteria in guts, parasites, drugs and vaccines, and chemicals exposures[2]. The ever-changing lifestyles and newer biochemical innovations may have greatly affected the

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ABO antibody levels of donors. It was also known that anti-A and anti-B titers would vary among age, gender, diets[2,3] and the prevalence of ABO blood types in different populations.[3,4] Hereby, the trend on the levels of ABO antibody titers from different populations will differ, and it is essential to determine and study the ABO antibody titers by specific populations.

In 2011, studies[5] have confirmed the increased of ABO antibody titers in Northeastern Nigerian blood type O donors compared to the studies in different regions in 1974, 1985, 1990, and 2001.

On the other hand, Japan had a significant decrease in anti-A and anti-B titers among blood group O donors in the past two decades. In fact, blood donors with titers more than 100 are rare today in Japan.[4]

During the past 10 years, there was only few studies regarding ABO antibodies in group O Thai blood donors[4,6]. In spite of existing clinical significance of the low titers of anti-A and anti-B for platelet transfusion when ABO identical unit is not available as well as transfusion of group O blood to non-group O recipients in emergency lifesaving resuscitation where and situation when red blood cell components (packed red cell) and other forms of red cell components are absolutely not available.

The aim of this study was to further investigate for the titers of anti-A and anti-B among group O Thai blood donors.

Materials and Methods

All laboratory procedures were carried out at 22-24°C (room temperature), using saline method for antibody detection[7]. One hundred screened clotted-blood samples donated by different blood group O donors (47 females and 53 males) were all randomly obtained from the National Blood Centre, Thai Red Cross Society in June 2013. The calibrated auto-pipette was used to transfer 500 μL of the serum from the clotted blood samples and to perform two-folded dilution with 1% BSA solution. The sera were diluted in 12 x 75 mm test tubes. In each test, 100 μL of serum was dropped into 10 x 75 mm test tubes and was tested against 100 μL of 2% pooled group A red blood cells or 100 μL of 2% pooled group B red blood cells. The samples were incubated at room temperature for 15 minutes and were centrifuged using fixed speed centrifuge (3,400 rpm) for the calibrated spin time (15 seconds).

Results were determined by the end point of 1+ macroscopic agglutination. All samples were graded and tested by the same person. Anti- A and anti- B titers above 64 were considered as high-titers[15]. This study used applied Mann-Whitney U test for statistical analysis and using proportion (percentage) to undertake Chi Test in finding the significance values.

Results

The age distribution for male and female blood donors were shown in Table 1. The predominant age group for both genders was 31-50 years of age. The ratio males : females was 1.13 :1.

Table 2 showed the distribution of anti- A and anti-B titers in a total of 100 Thai blood group O donors. It was observed that anti-B titer (4-1024) was higher than anti-A titer (4-512). The range, mode, and percentage of donors with titer ≤ 64 for anti-A of total study were 4-512, 32, and 84.00%, respectively while for anti-B, they were 4-1024, 64, and 73.00%, respectively. In male donor anti-A, they were 4-128, 32, and 90.57%, respectively, while for anti-B, they were 4-256, 64, and 82.00%, respectively. In female donor anti-A, they were 4-512, 32, and 76.60%, respectively, while for anti-B, they were 4-1024, 32-64 and 68.10%, respectively. In young age group (19-40 yr.) anti-A, they were 4-256, 32, and 80.00%, respectively, while for anti-B they were 8-256, 64, and 62.00%, respectively. In old age group (> 40 yr.) anti-A, they were 8-512, 16, and 88.00%, respectively, while for anti-B, they were 4-1024, 64, and 84.00%, respectively.

Table 1. Gender and age distributions among 100 group O Thai blood donors

Age Group (yr.)	Male Blood Donors(n)	Female Blood Donors(n)	Total (n)
19-30	8	8	16
31-40	19	15	34
41-50	15	15	30
51-60	11	9	20
Total (n)	53	47	100
Males:Females = 1.13:1			

Table 2. Distributions of anti-A and anti-B titers in total 100 group O Thai blood donors

Titers		2	4	8	16	32	64	128	256	512	1,024
Anti-A	Total (n = 100)	-	2	7	28	34	13	13	2	1	-
	Male (n = 53)	-	1	2	18	20	7	5	-	-	-
	Female (n = 47)	-	1	5	10	14	6	8	2	1	-
	Young Age Group (19-40 yr.) (n = 50)	-	2	5	10	18	5	9	1	-	-
	Old Age Group (> 40-60 yr.) (n = 50)	-	-	2	18	16	8	4	1	1	-
Anti-B	Total (n = 100)	-	1	7	9	22	34	18	7	1	1
	Male (n = 53)	-	1	4	6	9	21	10	2	-	-
	Female (n = 47)	-	-	3	3	13	13	8	5	1	1
	Young Age Group (19-40 yr.) (n = 50)	-	-	5	4	7	15	13	6	-	-
	Old Age Group (> 40-60 yr.) (n = 50)	-	1	2	5	15	19	5	1	1	1

Table 3. Range, mode and percentage of total group O Thai blood donors with anti-A and anti-B titers of ≤ 64 for male and female, young and old age groups

		Range of Titer	Mode	Donor with Titer ≤ 64 (%)
Total Anti-A (n = 100)		4-512	32	84.00
Total Anti-B (n = 100)		4-1024	64	73.00
Male	**Anti-A**	4-128	32	90.57
(n = 53)	**Anti-B**	4-256	64	82.00
Female	**Anti-A**	4-512	32	76.60
(n = 47)	**Anti-B**	4-1024	32-64	68.10
19-40 yr.	**Anti-A**	4-256	32	80.00
(n = 50)	**Anti-B**	8-256	64	62.00
> 40-60 yr.	**Anti-A**	8-512	16	88.00
(n = 50)	**Anti-B**	4-1024	64	84.00

Summary of the Results

1. Tendency of high anti-A in females > males (23.40% vs. 9.40%, p = 0.057)

2. Tendency of high anti-A in young age group (19-40 yr.) > old age group (> 40-60 yr.) (20.00% vs. 12.00%, p = 0.275)

3. Tendency of high anti-B in females > males (31.90% vs. 22.60%, p = 0.297)

4. Significance of high anti-B in young age group (19-40 yr.) > old age group (> 40-60 yr.) (38.00% vs. 16.00%, p = 0.013)

Discussion

It was worth noting that the gender, age and anti-A, anti-B titers in total 100 group O Thai blood donors seemed to distribute normally (Table 1, 2), indicating that these random blood donor samples could nicely represent the Thai blood donor population.

The percentage of donors > 64 for anti-A and anti-B titers in this study was slightly decreased but the modes and titers of both anti-A and anti-B were higher as compared to the finding in Thai blood donors in 2005.[4] However, the obviously high percentage of donors with titers > 64 for anti-A (75.70%) and anti-B (80.00%) in 2012 study using the same technique for antibody determination and the same source of the blood donor population needs further investigation to confirm this finding[6]. The percentage of high titers for both anti-A and anti-B of Brazilian blood donors[15] was low as compared to the Thais at all periods of time. (Table 4). In overview of this study, contrarily to the observations in whites[3], anti-B titers are higher than the anti-A titers among Thai population (mongoloid). The ABO antibody titers clearly decreased within age of the donors, the anti-A and anti-B titers of the males are relatively lower than those of females. The exact reasons and factors for the variation of ABO antibody titers in Thai population from 2005 to 2013 are yet to be further investigated. However, previous researches had noted some magnificent discoveries that can be used to postulate and suggest the possible reasons behind this phenomenon.

Through their 1959 experiment on white leghorn chickens[8] and 1969 studies[1], Springer and Horton had postulated on that exposures to bacteria such as *E.coli* O (86) will increase antibody levels and how intestinal lesions help induce the production of ABO-antibodies in infants and adults.[1] Since bacterial interactions are highly associated with the changing titer levels, it is possible that new patterns of lifestyles and diets of Thais in 2013 may be the causes. For example, consumption of healthy foods like yoghurt has increased.[9] The bacteria presented in yoghurt, such as *Lactobacillus* species[10] can induce specific systemic immune responses of the consumer[11,12] which in turn can attribute to the stibility or increase of anti-A and anti-B titers.

Probiotics consumptions can also affect the titers; in 2009, a fatal transfusion occurred because the group A donor was detected to have anti-B titer of 16,384 after obtaining probiotic tablets.[13] Hence, this may also be the case that Thai populations in 2013 have increased ABO antibody titers because there are now more exposures toward vaccination, probiotics, and other unknown bacterial strains in the donors' surroundings.

Table 4. Comparison of percentage of donors with anti-A and anti-B titers > 64 among group O Thai blood donors throughout time

Year	2005[4]	2012[6]	2013	2010*
Titer > 64	%	%	%	%
Anti-A	24.00	75.70	16.00	10.19
Anti-B	29.00	80.00	27.00	4.31

**Brazilian blood donors[15]*

As stated, parasitic infestations were also known to induce the high antibody titers. Gastrointestinal parasites contain A-like and B-like antigens on their surfaces, which stimulate the host's productions of anti-A and anti-B antibodies.[14] Therefore, infected donors will certainly contain high titers of anti-A and anti-B.

Additionally, the finding in this study that young age group had significant higher anti-A and anti-B titers than old age group agreed with the previous studies proposed that ABO antibody titers usually decrease as people get older. The titer level reaches its peak during childhood and then starts to decrease afterwards.[3] Furthermore, there had been suggestions of using of ABO incompatible platelets from older male donors in order to reduce the risks of agglutination and hemolysis.[15]

To supply ABO group specific blood and platelets transfusion to all of the patients is difficult and challenging. Problems of using ABO-identical platelets arise due to the requirement for multiple platelet transfusions, the limited availability amount of ABO identical platelets in the region, and the short expiry date (5-days) of the platelets. Additionally, at present the blood supply in Thailand is satisfactory increasing under the responsibility of The National Blood Centre in remote areas or emergence resuscitations, it may not be able to obtain sufficient blood / blood components and not be able to separate blood components themselves; whole blood is occasionally transfused. For the practical point of view, since the percentage of group O Thai blood donors with anti-A and anti-B titers ≤ 64 is high (Table 2), one should be able to select the low titer unit without difficulty and delay by using only 1:64 or 1:50 (more practical for the laboratory) dilution for screening test. Moreover, the blood establishments or blood banks can perform the screening and pre-labeled group O apheresis/ pooled platelets units for non- group O transfusion[16]. Therefore in such cases, the risk of fatal agglutinations can be alleviated by the use of low titer group O blood. Late in World War II and all US combats since the Korean and Vietnam wars and onward in Iraq and Afghanistan only low anti-A , anti-B titer group O whole blood had been used in non-group O recipients. There were no reports of hemolytic reactions, in spite of a large number of units transfused.[17] Some European countries had started setting up strategies to establish barriers for the safety levels of titers by prohibiting the use of high-titer donor products.[18] By investigating through the prevalence of ABO antibody titers in certain population, this information can facilitate the usage of ABO-incompatible platelets toward patients in the safe and most efficient way.

Conclusion

Changing of anti-A and anti-B levels in group O blood donors is a well-known fact. Each country and ethnic group should perform regular survey of their blood donor population in order to provide guidelines in clinical uses. The titers as ≤ 64 of anti-A and anti-B antibodies in the plasma can be truly beneficial for blood transfusion services where preparation of blood into different components are not possible or unavailable. The statistic data from this study can be beneficial toward future clinical guidelines and awareness to screen and use only low titer ≤ 64 group O apheresis platelets and blood containing plasma to non-group O recipients in emergency lifesaving when the group specific blood components and any form of packed red cell components are absolutely unavailable. Hopefully, there will be further investigations and surveys among the Thai populations to gather more data and lead to safer blood transfusion.

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ABO Antibodies ในผู้บริจาคโลหิตไทย หมู่โอ

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บทคัดย่อ *รายงานจากส่วนต่างๆ ของโลกพบว่า ระดับ anti-A และ anti-B titerในประชากรเปลี่ยนแปลงตามกาลเวลาที่ผ่านไป ซึ่งมีทั้งที่เพิ่มขึ้นและลดลง สาเหตุของการเปลี่ยนแปลงนี้พบว่าเกิดจากสิ่งแวดล้อมมากกว่าการถ่ายทอดทางพันธุกรรม ในระยะ 10 ปีที่ผ่านมามีการศึกษาน้อยมากที่เกี่ยวกับ ABO antibodies ในผู้บริจาคโลหิตไทยหมู่ O ทั้งที่ความสำคัญทางคลินิกยังคงมีอยู่ ในการให้เกล็ดเลือดแก่ผู้ป่วย เมื่อไม่มีหมู่เลือด ABO ที่ตรงกัน รวมทั้งการให้เลือดหมู่ O แก่ผู้ป่วยที่ไม่ใช่หมู่ O ในการช่วยชีวิตฉุกเฉินในที่ห่างไกลหรือสถานการณ์ที่ไม่มีส่วนประกอบโลหิตชนิดเม็ดเลือดแดงเข้มข้น (packed red cells)หมู่ O ใช้เลย* ***วัตถุประสงค์*** *เพื่อศึกษาต่อถึงระดับของ anti-A และ anti-B titers ในผู้บริจาคโลหิตไทยหมู่ O* ***วัสดุและวิธีการ*** *ทำการศึกษาผู้บริจาคโลหิตไทยหมู่ O จำนวน 100 ราย เป็นผู้ชาย 52 ราย อายุ 19-59 ปี ผู้หญิง 48 ราย อายุ 21-55 ปี ด้วยวิธี saline tube test ทดสอบที่อุณหภูมิห้อง (22-24 °ซ)* ***ผลการศึกษา*** *พบว่า anti-B titer (4-1024) สูงกว่า anti-A (4-512) titerเล็กน้อย และมีความโน้มเอียงอย่างมีนัยสำคัญที่ anti-A และ anti-B titer ในกลุ่มผู้บริจาคโลหิตหญิงสูงกว่ากลุ่มผู้บริจาคโลหิตชาย ($p = 0.057$ สำหรับ anti-A: $p = 0.297$ สำหรับ anti-B) และกลุ่มอายุน้อย (19-40 ปี) anti-A และ anti-B ไตเตอร์สูงกว่ากลุ่มสูงอายุ (> 40-60 ปี) ($p = 0.275$ สำหรับ anti-A: $p = 0.013$ สำหรับ anti-B) พบว่า range ของ anti-A titer เท่ากับ 4-512 ส่วน anti-B เท่ากับ 4-1024 และร้อยละ84 ของผู้บริจาคโลหิตทั้งหมด มีanti-A titer ≤ 64 ในขณะที่ร้อยละ73 มี anti-B titer ≤ 64* ***วิจารณ์และสรุป*** *การเปลี่ยนแปลงของ anti-A และ anti-B ไตเตอร์เป็นไปได้มากที่เกิดจากปัจจัยสิ่งแวดล้อมมากกว่าพันธุกรรม ตัวแปรร่วมที่เห็นได้ชัดได้แก่อายุและเพศ ผู้บริจาคโลหิตหญิงมี range ของ antibodies ทั้ง Aและ Bสูงกว่าผู้บริจาคโลหิตผู้ชาย กลุ่มอายุน้อย (19-40 ปี) anti-A และ anti-B ไตเตอร์สูงกว่ากลุ่มสูงอายุ (> 40-60 ปี) ผู้บริจาคโลหิตส่วนใหญ่มีantibody titers ต่ำ ≤ 64 (84% สำหรับ anti-A และ 73% สำหรับ anti-B) การสำรวจแอนติบอดีไตเตอร์ในประชากรเป็นประจำ มีประโยชน์ในการให้เลือดแก่ผู้ป่วยและอาจนำไปสู่การพัฒนาเป็นแนวทางการปฏิบัติ ที่ทำให้การให้เลือดมีความปลอดภัย มีประสิทธิภาพ และทันเวลา*

Key Words : ● *ABO blood groups* ● *Anti-A* ● *Anti-B* ● *Thai blood donors*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2556;23:203-9.