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FREQUENCY OF TRANSFUSION TRANSMISSIBLE INFECTIONS AND POSSIBLE ASSOCIATIONS WITH THE ABO BLOOD TYPES IN BLOOD DONORS IN A REFERRAL HOSPITAL IN PAPUA NEW GUINEA.

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ABSTRACT:
Quick selection of blood free from blood borne infections is paramount during massive blood loss due to trauma and severe blood deficiencies. Therefore, the aim of this study was to determine the frequency of the ABO blood group antigens and their possible associations with Transfusion Transmissible Infections (TTIs), to help create a preliminary database for quick access to infection-free blood during such emergencies. This was an observational retrospective study which included all blood donor information recorded from January 2010 to June 2020 at the Nonga General Hospital Blood Transfusion Laboratory, East New Britain province, Papua New Guinea. All data were analyzed using Microsoft Excel Office Windows 10 version. Parametric statistics were used for analysis of the data, *p*-value less than 0.05 was considered significant. The chi-square test was used to determine if there were significant differences in infection rates between the ABO blood group antigens and variables of interest. Ethical clearance and consent were obtained from the relevant authorities. The order of frequency of the ABO blood group antigens in this population was O>A>B>AB (64%, 18%, 15%, 3%) respectively. The majority of the donors were males (74%). First time donors were 54% and voluntary donors were 85%. Most of the donors (43%) were in the 15-29 years age group. Males with blood type O were significantly associated (p=0.032) with TTIs. HBV/Syphilis co-infections and HBV/Syphilis/HIV triple infections were also significantly associated (p=<0.001) with blood type O. The prevalence rates of infections obtained in the present study were 14% among the young adults (15 to 29 years), 25% among males, 29% among voluntary donors, and 18% among first-time donors. This calls for increased public health educational awareness among the population in the study area.

Keywords: ABO blood group antigens, Frequency, Transfusion Transmissible Infections (TTIs), HIV, HBV, Syphilis, double infections, triple infections.

INTRODUCTION:

Blood and its products are widely used throughout the world for varying purposes. It is however, especially clinically important in blood transfusion practices as it is used as medicine to treat various blood component deficiencies. Apart from its therapeutic uses, it also serves as a medium to transport disease causing agents such as the human immunodeficiency virus (HIV), hepatitis C virus (HCV), Treponema *pallidum,* Plasmodium *species,* and the hepatitis B virus (HBV).

Apart from the Rhesus (Rh) blood group antigens, the ABO blood group antigens are of clinical significance because of their association with acute haemolytic transfusion reactions (AHTR) and Haemolytic Disease of the Foetus /Newborn (HDFN). They were the first blood group antigens to be discovered [1] and are exceedingly significant because individuals who do not have these antigens have antibodies against them.

Apart from associations with transfusion reactions, their association with diseases is well documented [2 – 3]. Epidemiological distribution of the ABO blood group antigens varies geographically, even within the same country [4], and consequently with frequency of disease infection associations [5 - 6]. For example; in various parts of India, the frequency of Blood type A is common in the Northern Eastern part of the country, blood group O in the Eastern, Southern and Central [4] and group B in the Northern Western part [7]. A study of healthy blood donors demonstrated that Blood group A was significantly associated with HIV and HBV infection [5], group AB with VDRL (syphilis) [8], while blood group B was associated with a lower risk of HBV infection [6]. Although Blood group O plays a protective role against Transfusion Transmissible Infections (TTIs) [5], those who are O Rh D positive are more at risk of developing TTIs [9]. They have a 12% increased risk of being infected with HBV [6]. In another study, the ABO blood types were found to have no association between HIV, HCV and syphilis, however found significant association with HBV infections [10]. In blood donors in Amman, Jordan, although the hepatitis B surface antigen was prevalent, no significant association between the ABO/Rh blood groups and TTIs were found [11], suggesting the need for continued improvement and health cultural education programs.

In Papua New Guinea (PNG), the frequency of the ABO blood group antigens and their possible associations with TTIs is sparse and therefore this study was done to determine the frequency of the blood group types and their possible disease associations to set a preliminary data base for their frequency distribution and associated TTIs in this setting.

The major objectives of this study were to determine the frequency of the ABO blood group antigens and their possible associations with Transfusion Transmissible Infections (TTIs), to

help create a preliminary database for quick access to infection-free blood during emergencies.

METHODOLOGY:

Data for this study was obtained retrospectively from Nonga General Hospital (NGH) laboratory data base in East New Britain province (ENBP), Papua New Guinea (PNG). The data included all donor information recorded from January 2010 to June 2020. The information gathered were from donors who have passed pre-screening tests; which included physical and medical checks and also serological screening.

Variables of interest included demographic information, frequency and type of donation, blood type and serological status.

Immuno-chromatographic tests (ICT) were used to screen for HIV, Syphilis and HBV. Screening for HCV was only started in 2018 and therefore was not included in this study. The ICT used for T. *pallidum* screening was the Abbott Determine Syphilis (TP) [12]. The HBV was detected using Determine TM HBsAg, Abbot Laboratories [13]. Detection of HIV follows a specific World Health Organization (WHO) approved national (PNG) algorithm; in that the initial testing is done using the Determine HIV-1/2 (Alere, MA, USA), and HIV-1/2 STAT-PAK (Chembio, Medford, NY) as confirmatory test [14]. The ABO antigens were identified using the forward and back grouping using commercial anti-sera to identify unknown antigens and known laboratory made washed red cells were used to identify the unknown antibodies in the back grouping [15]. Any inconclusive results were repeated by a second person to confirm blood phenotype.

The data was entered into Excel Spreadsheets (MS Office version 2010). Frequency and Chi-square test were used to compare differences in infections between categorical variables of interest; A *p*-value of less than 0.05 was considered statistically significant.

Ethical clearance was issued by the University of Papua New Guinea (UPNG) Research and Ethics Committee. Because of the retrospective nature of this study, consent to collect data from donors was not obtained. However, consent to use the data was obtained from the authorities in the NGH ENBP in PNG. Identification of all donors were kept confidential as approved by the ethics committee.

RESULTS:

A total of 7571 donors were recorded at the Nonga General Hospital Blood Transfusion Service (NGHBTS) from January 2010 to June 2020. Of these, 7477 (99%) were analyzed, the remaining 94 (1%) were excluded because of missing variables. Of the 7477 donors, the majority (74%) were males (Table 1). The mean age of the male donors was (33±12 years) and the age range was 16 to 87 years. Throughout the study period the mean age of the frequent male donors was 20±12 years. The mean age of female donors was 35± 12 years, and the age

range was 14 to 72 years. The mean age of the frequent female donors was 40±12 years.

Among the 7477 donors the frequency of the ABO blood group antigens in decreasing order was blood group O (64%), blood group A (18%), B (15%) and AB (3%). Most of these donors were males (74%), first-time donors (54%), and voluntary (85%), whose age group was 15-29 years (43%). The proportions of blood group antigens O and A in males were higher (64.0% and 18% respectively) than were B and AB (15.0% and 3.0% respectively). The same ABO antigens frequency sequence is seen in females, repeat and first-time donors, VDs and FRDs, and in the age groups (Table 1).

Of the 7477 donors, 33% (2462) were positive for TTIs. The prevalence rate of infection among the blood groups is presented in Table 2. The result shows that 21% (1562/7477) were blood group O, 6.0% (456/7477) were blood group A, 5.0% (372/7477) were blood group B, and 1.0% (72/7477) were blood group AB. Although the frequency of blood group O antigens was prevalent, using the Chi-square test and cross-tabulation, no statistically significant difference in infection rate was observed among the ABO blood group antigens (Table 2).

Among the genders, TTIs were high in males (25%; 1843/7477) than in females (8%; 619/7477). The difference was statistically significant in males with blood group O antigens than in females with blood group O antigens (p=0.032 vs p= 0.575) respectively. Although a high number of those infected were infected by a single pathogen (24%, 1805/7477), this was statistically insignificant (p=342). However, a significant difference in infection was observed among double and triple infections (p=<0.001); the majority of which were blood group O in males (Table 2).

Among repeat and first-time donors, a high proportion of TTIs were seen in first time donors (18%; 1324/7477) than in repeat donors (15%; 1138/7477). No significant difference in TTIs was observed between the different ABO blood types in both groups. This trend was also seen in VDs and FRDs, and among the sub-age groups (Table 2).

A high number of donors who had single infections were blood group O, and mostly infected with HBV (12%), followed by syphilis at 10%. The majority of those who had double infections were blood group O as well, and were mostly co-infected with HBV/Syphilis (3%), while HBV/HIV and HIV/Syphilis co-infections had equal rates of infection (2%) (Table 3). The results for triple infections are presented in Table 4. The Triple infections were also mostly seen in blood group O (1%). Infection in Blood type O was observed to be dominant in all three categories (single, double and triple) of infections.

Table 1: Distribution of the 7477 donors according to their ABO Blood group antigens, gender, type of donation, frequency and age-groups.

Blood Group	A n (%)	AB (%) n (%)	B n (%)	O n (%)	Totals n (%)
Antigens	1343 (18.0)	188 (3.0)	1120 (15.0)	4826 (64.0)	**7477**
Gender					
Males	1019 (18.0)	143 (3.0)	825 (15.0)	3536 (64.0)	5523 (74)
Females	324 (17.0)	45 (2.0)	295 (15.0)	1290 (66.0)	1954 (26)
Frequency of Donation					
Repeat	584 (17.0)	84 (2.4)	498 (14.4)	2288 (66.2)	3454 (46)
First time	759 (19.0)	104 (2.5)	622 (15.5)	2538 (63.0)	4023 (54)
Type of Donation					
VD	1122 (18.0)	154 (2.0)	964 (15.0)	4109 (65.0)	6349 (85)
FRD	221 (20.0)	34 (3.0)	156 (14.0)	717 (63.0)	1128 (15)
Age groups					
15-29	613 (19.0)	70 (2.0)	492 (16.0)	2006 (63.0)	3181(43)
30-44	425 (16.0)	84 (3.0)	380 (15.0)	1727 (66.0)	2616 (35)
45+	305 (18.0)	34 (2.0)	248 (15.0)	1093 (65.0)	1680 (22)

VD: Voluntary Donors; FRD: Family Replacement Donors

Table 2: Distribution of the 7477 donors according to frequency and prevalence of infection with TTIs among the ABO blood antigens, gender, type of infection, type of donation and age-groups. The Chi-square values are also shown.

Variables	**ABO Blood Group Antigens**				**Prevalence (95% CI)**	***p*=value (χ2)**
Blood Group Antigens	A n (%)	AB n (%)	B n (%)	O n (%)	(n=2462) 33.0 (32-34)	
	456 (6.0)	72 (1.0)	372 (5.0)	1562 (21.0)		0.281 (3.825)
Gender						
Male	343 (4.5)	61 (1.0)	258 (4.0)	1166 (16.0)	(n=1843) 25.0 (23.7-25.6)	0.032 (8.80)
Female	113 (1.5)	11 (0.2)	99 (1.0)	396 (5.0)	(n=619) 8.0 (7.7-8.9)	0.575 (0.58)
Type of Infection						
Single	314 (4.0)	37 (0.5)	284 (3.8)	1162 (16.0)	(n=1805) 24.0 (23-25)	0.342 (3.345)
Double	125 (1.8)	19 (0.3)	173 (1.0)	325 (4.0)	(n=534) 7.0 (7-8)	<0.001 (89.684)
Triple	17 (0.2)	16 (0.2)	15 (0.2)	75 (1.0)	(n=123) 2.0 (1-2)	<0.001 (56.857)
Frequency of Donation						
Repeat	192 (2.0)	36 (0.5)	169 (2.0)	741 (10.0)	(n=1138) 15.0 (14-16)	0.359 (3.219)
First time	264 (4.0)	36 (0.5)	203 (3.0)	821 (11.0)	(n=1324)	0.138 (5.508)

					18.0 (17-19)	
Type of Donation						
VD*	392 (5)	59 (0.8)	321 (4)	1373 (18)	(n=2145) 29.0 (28-30)	0.810 (0.966)
FRD*	64 (1)	13 (0.2)	51 (1)	189 (3)	(n=317) 4.0 (4-5)	0.125 (5.743)
Age-group						
15-29	198 (3)	28 (0.4)	164 (2)	681 (9)	(n=1071) 14.0 (14-15)	0.916 (0.051)
30-44	171 (2)	30 (0.4)	126 (2)	554 (7)	(n=881) 12.0 (11-13)	0.168 (5.051)
45+	87 (1)	14 (0.2)	82 (1)	327 (5)	(n=510) 7.0 (6-7)	0.844 (0.821)

*VD: Voluntary; FRD: Family Replacement Donor

Table 3: Distribution of the 7477 donors according to the prevalence of single, double and triple infections (HBV, HIV and syphilis) among the ABO blood group antigens.

Single Infections					
Blood group antigens	n	HBV n (%)	HIV n (%)	SYPHILIS n (%)	Prevalence (95%CI)
A	310	182 (2.4)	20 (0.2)	108 (1.4)	4 (3.7-4.6)
AB	49	32 (0.43)	2 (0.03)	15 (0.2)	1 (0.5-0.8)
B	284	167 (2.2)	8 (0.1)	109 (1.5)	4 (3.4-4.2)
O	1162	668 (8.9)	80 (1.01)	414 (5.54)	15.5 (14.7-16.4)
Totals	**1805**	**870 (11)**	**199 (3)**	**736 (10)**	**24 (23.2-25.1)**
Double Infections					
	n	HBV/HIV	HIV/SYPHILIS	HBV/SYPHILIS	
A	117	37 (0.5)	30 (0.4)	50 (0.7)	1.6 (1.3-1.9)
AB	19	5 (0.1)	5 (0.1)	9 (0.1)	0.3 (0.1-0.4)
B	73	17 (0.23)	18 (0.24)	38 (0.51)	1 (0.8-1.2)
O	325	86 (1.1)	84 (1.0)	155 (2.0)	4.1 (3.9-4.8)
Totals	**534**	**145 (2)**	**137 (2)**	**252 (3)**	**7 (6.6-7.7)**

Table 4: Distribution of the 7477 donors according to the prevalence of triple infections (HBV, HIV and syphilis) among the ABO blood group antigens.

Triple Infections HBV/HIV/SYPHILIS		
Blood group antigens	N (%)	Prevalence (95% CI)
A	29 (0.4)	0.4 (0.3 - 0.5)
AB	4 (0.05)	0.1 (0.00 - 0.11)
B	15 (0.2)	0.2 (0.10 – 0.30)
Q	75 (1.0)	1.0 (0.8 – 1.2)
Total	123 (1.65)	2.0 (1.4 – 1.9)

DISCUSSION:

Our results showed that the main donor population from January 2010 to June 2020 were young male voluntary donors who donated for the first time; the majority of which were blood type O, followed by blood group A, then blood group B and blood group AB (Table 1). According to some authors, a donor population comprising of young, voluntary and repeat donors represent the ideal population whose blood is supposedly low in bloodborne infections [16]. The low number of female donors in our present study is similar to the findings reported by others in some developing countries; this may be because of cultural and religious beliefs including normal physiological monthly shedding of blood in menstrual flow [17].

The high frequency of blood type O (64%) observed in the present study is similar to the results in several tribes in the Highlands region [18] and among the Gidra population [19] in Papua New Guinea. The latter authors noted that the variations seen in their study were partly due to the tribes' geographical isolation from each other and genetic drift [18], and migration [19]. Similarly, the same scenario was seen among males of the Pacific Islands Regimen in Port Moresby, which comprised of unrelated individuals serving in the disciplinary forces under the Australian administration, before independence [20].

In a country where blood is now mostly used for trauma and cancer cases [21], it is important that knowledge of the distribution of the ABO blood group antigens must be documented to assist in quick selection of the appropriate blood group for recipients, and in preventing and managing alloimmunization [7] during blood transfusion. The order of the frequency of the blood group antigens in the present study was O>A>B>AB. This is consistent with many other studies including, blood donors in the Central region of Saudi Arabia [22], Gabon [10], the National Blood Bank in Amman, Jordan [11], and the Indigenous Australians [23]; but in contrast to Prakash [24] in Eastern India and in Northern Indian [25] and also among blood donors in Gwalior, India [7]. The latter three studies in different parts of India showed frequency patterns of B>O>A>AB, while in Sikkim, India [4], the frequency pattern was A>O>B>AB. In all these studies, frequency of Blood group antigens AB was always low, similar to the value in the current study.

The distribution and frequency of the ABO blood group antigens is based on evolution over millions of years, such that a high frequency of blood group A is seen in Europe, while B and O in Asia and South America respectively [1]. Furthermore, current frequencies seen in different parts of the world are due to mix marriages between different races, migration and environmental factors including disease

epidemics. The spectrum of ABO antigens observed in this study is consistent with Ohashi et al. [18], who found that the predominant ABO blood group antigens in Oceania was O but which again varied geographically within the same region. The higher frequency of blood group O seen in this study is similar to the Gidra population of Western province but in contrast to the Balopa population of Manus Island [18]. A striking similarity between the Gidra setting and this study setting is the endemicity of malaria infections in these regions; which may have influenced the high frequency of the O blood group antigens as a result of selection pressure in malaria endemic settings favouring reduced susceptibility to P. *falciparum* infections in group O individuals [3, 26 - 27].

This study showed no statistically significant difference in TTIs between the ABO blood types and repeat and first-time donors, VDs and FRDs, age groups, females, and single infections, although TTIs were prevalent in Blood group O in all these groups (Table 2). This in contrast to Abegas [3] and Davidson [28] who reported in their reviews that red cell antigens interact and influence the risk of occurrence of both infectious and non-infectious diseases. In this study a significant difference in infection was observed between males (p=0.032) and the ABO blood types, and between double (p=<0.001) and triple infections (p=<0.001).

In contrast to the low number of female donors, who comprised an older population (mean age 40 ±12 years), the significant number of TTIs observed in males with blood group O could be due to the high number of young, male donors donating for the first time in this study. First time donors do not necessarily know their serological status and therefore the likelihood of them testing positive for TTIs is high [22], or it could be due to careless sexual behaviours as a high prevalence of donors who frequently donated throughout this period were mostly 20 years old. This is similar to Awili [29] who reported significantly high TTI prevalence in adolescent blood donors in Kenya in 2020. Moreover, the insignificantly low prevalent rate of TTI in females in this study could be due to the older female donor population who are probably more mature and educated, than a group of carefree and sexually active adolescence who were probably experimenting with perilous sex and wanted to have their tests done for free by donating blood.

The significant differences in TTIs between double and triple infections and the ABO blood types seen in this study, were mostly prevalent in blood group O donors. This is a cause for concern as this group of individuals were very young, supposed to have had vaccination against HBV infections, and therefore are supposedly healthy individuals. Apart from perilous sexual behaviour [29], factors such as insensitive tests [30], poor health education

awareness [31], scarcity of appropriate infrastructure, lack of trained personnel and financial constraints [32] are seen to be contributing to a reservoir of TTIs in blood donors in developing countries [16]. This calls for an increase in effective and efficient educational public health awareness in schools and in the media about sexually transmitted infections and their impact in this setting. In Papua New Guinea blood banks, Immunochromatographic tests (ICT) are commonly used for screening donor blood, and therefore, this could also be a contributing factor for the high number of TTIs seen among the universal donors. Lack of an effective documentation system [33] could also be a reason as positive donors could have been recorded more than once. An effective electronic data base system must be installed and sensitive confirmatory tests should be introduced to reduce the window period and also to eliminate the possibility of false positive tests resulting in the relatively high TTI counts as seen in this study.

Contrasting reports of associations of TTIs with blood group O have been reported. In some studies, blood group O was seen as playing a protective role against HBV and HIV [5, 10], while in another, it is more susceptible to all three infections, including syphilis) [11]. In this study, the most frequent disease seen was in donors with blood group O, although no significant difference in infection between the ABO blood group antigens was demonstrated (33%, 95%CI 32-34, p=0.281). This may suggest blood group O playing a protective role, similar to the findings by Batool et al. [5] but in contrast to Hrood et al. [11]. A case-control prospective study needs to be done to confirm the findings of this study to better guide selection of blood donors.

The order of frequency of TTIs in those that were infected with HBV and HIV was blood group O>A>B>AB and in those that were infected with syphilis was O>B>A>AB. In those that were infected with syphilis, infection in blood group B was higher than blood groups A and AB, after blood group O. Blood group AB was found to be significantly associated with syphilis in one study [8], which is in contrast to our present study. Although single infections were prevalent in this study (24%), no significant difference between the ABO blood antigens was seen. This could be a result of adherence to thorough pre-screening procedures or effective awareness campaigns, however, the high overall TTI prevalence observed and the significant association of blood type O with male donors and multiple infections is suggestive of perilous sexual activity as these are supposedly healthy individuals but are asymptomatic and therefore pose a high risk to recipients.

CONCLUSION:

The order of the frequency of the ABO Blood group antigens in this setting in decreasing order

is; O>A>B>AB. TTIs are prevalent in blood group antigens O and significantly associated with male donors, and double and triple infections. A review of existing pre-screening protocols is needed to reduce recruitment of infected donors.

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