THE DISTRIBUTION OF THE ABO GENOTYPES AND PHENOTYPES IN SINGAPORE IN 1987

T C Mohan

ABSTRACT

The aims of this study are to determine for the 3 main ethnic groups in Singapore:
a) the ABO phenotype distribution in 1987
b) the A, B and O gene frequencies
c) the proportion of A and B group individuals who are homozygous using
(i) temporal studies (trial & error)
(ii) the derived A and B gene frequencies
This paper presents the method of study and the results obtained using a sample of 39,019 blood donors in 1987. The opportunity has also been taken to compare the data derived in this study with that quoted in 2 previous studies.

Key Words: ABO grouping, blood group antigens

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INTRODUCTION

Perhaps the earliest to recognize that the frequency of the blood groups varied from one population to another was Professor & Mrs. Hirszfeld (1). While working at Salonika, they had the opportunity to test large numbers of soldiers and civilians from may different countries during the First World War. Since then many millions of ABO tests have been undertaken and published all over the world.

ABO studies have been done in Singapore from time to time. The aims of this study are to determine for the 3 main ethnic groups in Singapore:

a) the ABO phenotype distribution in 1987
b) the A, B and O gene frequencies
c) the proportion of A and B group individuals who are homozygous using (i) temporal studies (trial & error)(ii) the derived A and B gene frequencies

This paper presents the method of study and the results obtained using a sample of 39,019 blood donors in 1987.

MATERIALS AND METHOD

1. LOGIC OF STUDY METHOD

The flowchart in Figure 1 depicts the logic flow of the present study.

2. SAMPLE SELECTION

As depicted in the flow-chart the raw-data was obtained from the records of the BLOOD TRANSFUSION SERVICE at the SINGAPORE GENERAL HOSPITAL. All blood donors who donated in 1987 were included in the study.

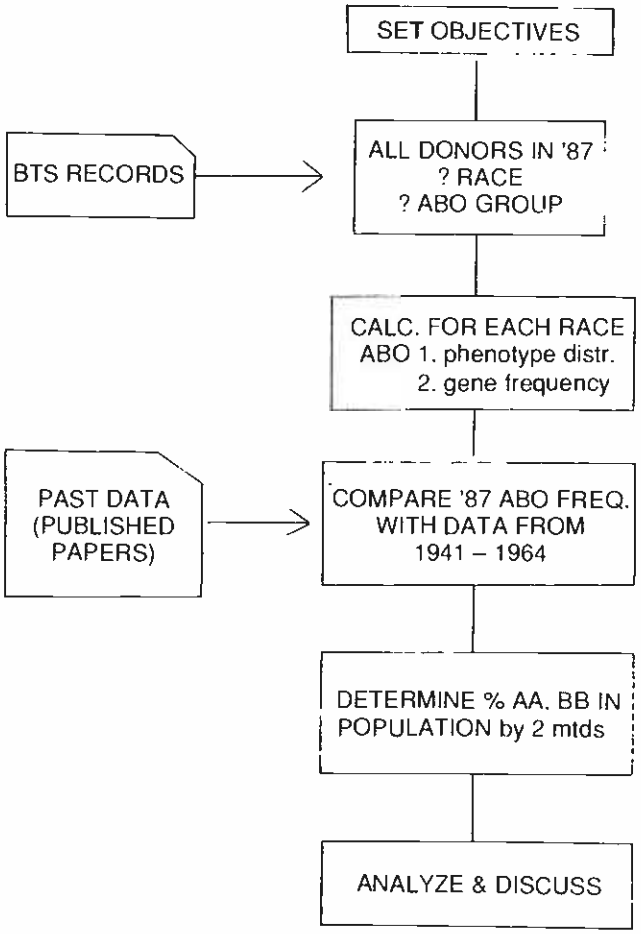

FIGURE I

Department of Haematology
Singapore General Hospital
Outram Road
Singapore 0316

TC Mohan, MBBS, Medical Officer

2.1 PARAMETERS RECORDED

For each donor the following parameters were recorded:

(i) ethnic group/race of donor
(ii) ABO group of donor

2.2 SAMPLE REPRESENTATIVENESS

As stated in the objective the aim of the study is to arrive at the ABO phenotypic and genotypic distribution that prevails in the healthy Singaporean adult population.

Blood donors are representative of healthy adult Singaporeans because of the stringent criteria set to determine potential donors' medical fitness for donation. Some of the relevant criteria include:

(i) Age to be between 18 & 60
(ii) Weight to be above 50 kg
(ii) Absence of any Cardiovascular, Respiratory or any other systemic illnesses
(iv) Hb level to be above 12.5 g/dl

However, since blood-donors with rarer blood-types (eg. type AB) are actively encouraged to donate blood, the sample for this study was defined as "all blood donors in 1987" rather than "all blood units collected in 1987". This would negate the bias that can be introduced by multiple donations. The ABO distribution observed in this donor sample can therefore be expected to approximate that prevalent in the Singaporean population in 1987.

2.3 CRITERIA FOR ETHNIC SUBDIVISION

The race recorded for each donor is identical to that recorded in his/her National Registration Identification Card (NRIC). The 3 major ethnic groups represented are the Chinese, Malays and the Indians.

2.4 CRITERIA FOR ABO GROUPING

The red blood cells of the donor were tested against standard Anti-A, Anti-B and Anti-A+B sera for agglutination. This grouping was cross-checked by testing the donor's serum against preparations of A,B & O cells.

2.5 SAMPLE SIZE

The total sample size was 39,019. This is sufficiently large to give small values for the standard error of proportions to be calculated.

3. DATA PROCESSING

The raw data was stored in a data-base while the summary data were stored and manipulated using a spreadsheet software. Whenever appropriate the distribution parameters have been calculated for each ethnic group.

4. CALCULATION OF GENE FREQUENCY (1,2)

The gene frequencies p,q and r representing the A, B and O genes respectively were calculated using the formulae of Fisher's method. The relevant formulae are:

$$p = (t-s)/v \quad q = (u-s)/v \quad r = s/v$$

where $s = O \quad t = O+A \quad u = O+B$

$$v = t + u - s$$

where O,A and B are the actual numbers of individuals of phenotype O, A and B respectively in the sample.

5. COMPARISON OF PRESENT DATA WITH PAST DATA

2 sources of past data were located:

(i) A study done in 1976 in Singapore by Loh, Ying & Ong (3) Study Sample : Patients registered during 1960-64 period.

(ii) A study done in 1961 in Singapore By K.T.Chan (4). Study Sample : Blood donors at BTS in 1947-61 period.

The 2 sets of past data were compared with the data obtained in the present study for the following:

(i) ABO phenotype distribution
(ii) A, B and O gene frequencies.

6. DETERMINATION OF AA AND BB GENOTYPE FREQUENCIES

In any population the individuals may belong to any one of the 6 common ABO genotypes : AO, AA, BB, BO, AB or OO. As long as no new genes are introduced into the population and the sampling errors are not too great, one would expect the proportions of these 6 genotypes to be constant from 1 generation to the next. The most straightforward method of deriving the proportions of AA and BB in the population is by making use of the gene frequuencies of the A and B genes (METHOD 2). It would, however, be interesting to derive, by a method of trial and error, (METHOD 1) the approximate proportions of AA and BB in the population by comparing 2 sets of ABO phenotype distribution figures of samples from populations one generation apart.

6.1 METHOD 1 : A TEMPORAL STUDY

Given the ABO genotypes of the parents one can calculate the probabilities of the different ABO genotypes expected in their offspring. For example when an AA male mates with a BO female the expected probabilities of AB and AO genotypes among the offspring are 0.5 each. Thus if the exact proportions 9 of AO, AA, BO, BB, AB and OO genotypes in a parent-sample is known it would be possible to calculate the expected proportions of these 6 ABO genotypes in the offspring population produced out of random matings in the initial parent-sample.

A spread-sheet program was written to calculate the expected ABO genotype proportions among the offspring population when fed with the ABO genotype frequencies in the parent sample. Figure 2 depicts the function of the program:

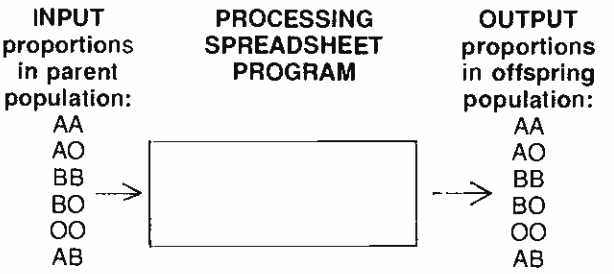

FIGURE 2. FUNCTION OF SPREADSHEET SOFTWARE

In a stable population, the population proportions of these 6 genotypes are related to each other in a fixed manner. However due to sampling error, the proportions of the 6 genotypes fed into the spreadsheet may not tally with the 6 output genotypes. The greater the sampling error the greater will this disparity be.

The sample used in the present study may be seen to represent the offspring population produced out of random matings in a parent-population that lived in Singapore 1 generation ago. The 2 samples cited in the reference papers above describe the ABO groupings of individuals in Singapore during 1947-61 and 1960-64 respectively. The samples provide 2 sets of values that may be taken to approximate the ABO distribution parameters in Singapore 1 generation ago.

From these 2 samples from the past we have 2 sets values for the proportions of genotypes AB, OO, (AA+AO)

and (BB+BO) for the previous generation of Singaporeans. Chosing one of the 2 sets of data at a time, one may input into the spread-sheet program described above the observed proportions of AB and OO and various combinations of proportions of AA, AO and BB, BO (such that they add up to the observed proportions of A and B). Different values input for the proportion of AA and BB (and therefore AO and BO) churned out different proportions for the 6 ABO genotypes in the resultant offspring.

It was noted that a small increase in the input value of proportion AA (and a concomitant decrease in the pro proportion of AO) resulted in a near-linear increase in the offspring proportion of A and AB and a near-linear decrease in the offspring proportion of group O. A parallel result was observed with increases in the input proportions of BB (i.e. increased B and AB; decreased O in the offspring).

By a method of trial and error and successive iterations attention was paid to the range of input values for the proportions AA and BB (and therefore AO and BO) that produced resultant offspring proportions of AB,), A (i.e AA + AO) and B (i.e. BB + BO) which were within 1.96 standard deviations of the ABO phenotypic proportions observed in the sample used for the present (1987) study. In other words, if and only if the proportions of AA and BB in the previous generation fall within this range, will it then be possible to account for the ABO phenotypic distribution observed in the present generation as represented by our present sample.

By this method the probable range of proportions of AA, AO, BB and BO in our present population was estimated for each of the 3 ethnic groups and later compared with those calculated by METHOD 2.

6.2 METHOD 2 : USING A & B GENE FREQUENCIES

A straight-forward method of confirming the AA and BB frequenciess obtained above is to make direct use of the A and B gene frequencies. Thus the expected probability of an individual being genotyped AA is the square of the probability of his having the A gene (i.e. the A gene frequency). Liikewise the proportion of the population that is of a genotype BB:

$$P[BB] = P[B] * P[B]$$

where P [B] ·s the gene frequency of B.

RESULTS

1. SUMMARY DATA

The sample size was 39,019. The data was recorded in a spread-sheet software. The absolute numbers and proportions of the ABO phenotypes is presented in TABLE 1.

TABLE 1.
DISTRIBUTION OF THE ABO PHENOTYPES AMONG THE RACES IN SINGAPORE, 1987

ABSOLUTE NOS.	CHINESE	MALAYS	INDIANS	OTHERS
GROUP A	7104	1309	1012	476
GROUP B	6628	1577	1669	449
GROUP O	11405	2313	1927	711
GROUP AB	1630	375	332	102
TOTAL	26767	5574	4940	1738

% PER BLD. GROUP FOR EACH RACE:	CHINESE	MALAYS	INDIANS	OTHERS
% A	26.54	23.48	20.49	27.39
% B	24.76	28.29	33.79	25.83
% O	42.61	41.50	39.01	40.91
% AB	6.09	6.73	6.72	5.87
TOTAL %	100.00	100.00	100.00	100.00

2. ANY DIFFERENCE BETWEEN THE RACES

First and foremost, a chi-squared test was done to confirm that the ABO phenotype distribution observed in each of the 3 main ethnic groups was significantly different from each other. TABLE 2 records this.

TABLE 2.
SIGNIFICANT DIFFERENCE NOTED IN ABO GENYOTYPE DISTRIBUTION BETWEEN THE RACES.

RACE	OBSERVED FREQUENCY				:	EXPECTED FREQUENCY				TOTAL
	O	A	B	AB	:	O	A	B	AB	
CHINESE	11405	7104	6628	1630	:	11233	6767	7089	1678	26767
MALAYS	2313	1309	1577	375	:	2339	1409	1476	349	5574
INDIANS	1927	1012	1669	332	:	2073	1249	1308	310	4940
TOTAL	15645	9425	9874	2337	:	15645	9425	9873	2337	37281

3. ABO GENE FREQUENCIES

The calculated A, B and O gene frequencies using the formulae of Fisher's method are shown in TABLE 3:

TABLE 3.
A, B & O GENE FREQUENCIES AMONG SINGAPOREANS, 1987.

1987 SAMPLE	A GENE FREQ. P	B GENE FREQ. Q	O GENE FREQ. R
CHINESE	0.178	0.168	0.653
MALAYS	0.162	0.191	0.645
INDIANS	0.147	0.229	0.625

4. COMPARISON WITH PAST DATA

The gene frequencies calculated from the previous 2 studies and the corresponding proportions of ABO phenotypes studied in the 1947-61 and 1960-64 samples cited earlier are presented in TABLE 4, together with present data:

TABLE 4.
ABO PHENOTYPE & GENOTYPE DISTRIBUTIONS (1947 – 1987)

SAMPLE	SIZE	% O	% A	% B	% AB	P	Q	R
RACE: CHINESE								
1947 – 61	15,262	43.53	25.99	24.99	5.48	17.36	16.74	65.90
1960 – 64	53,000	43.3	26.4	24.5	5.8	17.64	16.55	65.81
1987	26,767	42.61	26.54	24.76	6.09	17.8	16.8	65.3
RACE: MALAY								
1947 – 61	5,461	38.42	25.07	29.23	7.29	17.7	20.26	62.04
1960 – 64	8,594	40.1	24.6	28.7	6.6	17.08	19.60	63.32
1987	5,574	41.50	23.48	28.29	6.73	16.2	19.1	64.5
RACE: INDIANS								
1947 – 61	5,000	39.02	21.02	33.60	6.36	14.98	22.70	62.317
1960 – 64	7,099	36.7	22.5	33.6	7.2	16.36	23.21	60.43
1987	4,940	39.01	20.49	33.79	6.72	14.67	22.86	62.45

For each of the 3 gene proportions P,Q & R the standard error of proportion was calculated. The 3 sets of ABO gene freqency data obtained from the 3 study periods (1947 - 61, 1960 - 64 & 1987) were compared with each other for any significant difference. The result of these comparisons and the level of significance where a difference existed is presented in TABLE 5 :

TABLE 5.
COMPARISON OF ABO GENE FREQUENCIES

ETHNIC GROUP	SAMPLES COMPARED	ABO GENES COMPARED P	Q	R
CHINESE	1947 – 61 vs 1960 – 64	–	–	–
	1960 – 64 vs 1987	–	–	–
	1947 – 61 vs 1987	–	–	–
MALAYS	1947 – 61 vs 1960 – 64	–	–	–
	1960 – 64 vs 1987	–	–	–
	1947 – 61 vs 1987	P<0.05	–	P<0.01
INDIANS	1947 – 61 vs 1960-64	P<0.05	–	P<0.05
	1960 – 64 vs 1987	P<0.01	–	P<0.05
	1947 – 61 vs 1987	–	–	–

Thus among the Chinese there was no significant change in the ABO gene frequencies over the 4-decade composite study period (1947 - 1987). Among the Malays there appeared to be a slight increase in the O gene-frequency at the expense of A genes. Among the Indians the 1960-1964 sample had gene-frequencies significantly different from the other 2 samples in that it displayed more A genes at the expense of O genes.

5. DERIVATION OF AA AND BB FREQUENCIES

As described earlier the above frequencies were arrived at using 2 different methods.

5.1 METHOD 1: THE TEMPORAL STUDY

Using the spread-sheet program defined, the possible range of AA and BB frequencies in the past and present

populations were calculated so as to obtain an ABO phenotypic distribution which is not significantly different ($P < 0.05$) from that observed in the 1987 sample. 2 different sets of ranges were produced; one using the 61 sample and the other using the 1960-64 sample as possible parent generations of the present (offspring) population of Singaporeans, of which our current (1987) sample is a subset. TABLE 6 presents the results.

5.2 METHOD 2 : USING A and B gene-frequencies

Using the ABO gene-frequencies derived (TABLE 3) for each of the 3 main ethnic groups, the proportions of AA and BB in the population have been calculated and presented in TABLE 7. Note should be taken of the fact that these calculated figures are very similar to those derived by the previous method.

TABLE 6.
PREDICTED RANGE OF PROPORTIONS OF AA & BB GENOTYPES IN THE 1987 POPULATION.

using 1947 — 61 sample as parents
using 1960 — 64 sample as parents

Race	Sample	% AA in Population	% BB in Population
Chinese	1947-1961 Sample	3.05 -- 3.34	2.70 -- 2.96
	1960-1964 Sample	3.06 -- 3.34	2.68 -- 2.95
Malays	1947-1961 Sample	2.61 -- 2.91	3.33 -- 4.02
	1960-1964 Sample	2.43 -- 2.90	3.33 -- 4.02
Indians	1947-1961 Sample	2.58 -- 2.60	4.98 -- 5.05
	1960-1964 Sample	2.59 -- 2.62	4.99 -- 5.06

TABLE 7
CALCULATED FREQUENCIES OF AA & BB GENOTYPES (MEAN & 95% CONFIDENCE INTERVAL ARE SHOWN)

1989 SAMPLE	% AA		% BB	
ETHNICITY	MEAN	95% C.I.	MEAN	95% C.I.
CHINESE	3.17	2.99 — 3.41	2.82	2.62 — 3.02
MALAYS	2.62	2.2 — 3.04	3.65	3.16 — 4.15
INDIANS	2.15	1.75 — 2.56	5.23	4.61 — 5.85

DISCUSSION

The ABO phenotypic distribution for each of the 3 major ethnic groups in Singapore is quite distinctive. Among the Chinese and Malays about one half of the population belong to group A or group B, with a slight preponderance of A (26+%) among the Chinese and a preponderance of B among the Malays. Among the Indians group B individuals comprise almost 34% and group A individuals comprise only about 20%

The fact that these differences are indeed highly significant is borne out by the Chi-squared test, the result of which is presented in Table 2. In all 3 groups O and AB comprise about 40-42% and 6% respectively of the total.

The gene frequencies P, Q and R demonstrate a similar picture with a Q/P ratio of less than unity among the Chinese and more than unity among the Malays with the highest B gene frequency (0.2286) noted among the Indians.

The gene frequencies derived for this population may be compared with that arrived at by other workers. Mourant(1) for example notes that "the high B frequencies that characterizes all India and Pakistan reach their highest values in the north .... on the whole throughout most of China proper there are just over 20 per cent of A genes and just under 20 per cent of B, in contrast to India and southeastern Asia where B is generally in excess of A."

When the present data is compared with data gleaned from the past two studies, some interesting points were noted. Among the Chinese there was no significiant change in the ABO gene frequencies over the past four decades (1947 - 1987). This may be interpreted as there being no significant addition of any new ABO genes nor any loss of existing ABO genes over this period. Among the Malays though no significant difference was observed in the 1947 - 1961 vs 1960 - 1964 and 1960 - 1964 vs 1987 comparisons, a change was observed when the 1987 data was compared with that of the 1947 - 1961 sample. Over the past four decades there has been a small but significant increase in O genes at the expense of A and B genes. Among the Indians interpretation of the data was difficult as the 1960 - 1964 data generated a set of ABO gene frequencies which was significantly different from that of the earlier (1947 - 1961) and the present (1987) studies. One wonders if the Indians in the sample of 1961 - 1964 had a constitution of ethnic sub-groups different from that of

the other two samples. In this context it is worth noting that the 1960 - 1964 sample consisted of hospitalised patients whereas healthy blood donors formed the other two samples.

The vast majority of individuals of phenotypes A and B are noted to be heterozygous for the A or B alleles respectively. The AA and BB genotype frequencies derived by both methods 1 & 2 above are in agreement. This also serves to justify our assumption that the population from which the past two samples (1947 - 1961 and 1960 - 1964) were drawn is indeed the parent population of the present (offspring) population of Singapore. However to identity Group A and B individuals homozygous or otherwise at the ABO locus one would have to undertake family and/or transferase studies.

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REFERENCES

1. Mourant A E. The distribution of the human blood groups. Oxford : Blackwell Scientific Publications. 1954.
2. Race R R, Sanger. Blood groups in Man. 6th Ed, Oxford: Blackwell Scientific Publications 1975.
3. Loh SY, Ying CS, Ong YW : Distribution of ABO blood groups and of RH factor in Singapore. Mod Med Asia 1976; 12: 11
4. Chan KT : The ABO Blood group frequency distribution of Singapore based on a donor sample. Sing Med J 1962; 3: 1.