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Distribution pattern of ABO and Rh blood groups and their allelic frequencies among different ethnic groups in Malaysia

Md. Ruhul Amin* Dwi Susanto and Laila Naher

Faculty of Agro Based Industry, University Malaysia Kelantan (Jeli campus), Locked bag 100, 17600 Jeli, Kelantan, Malaysia

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ABSTRACT

Background: The experiment involved 4 different racial populations such as Malay (M), Chinese (C), Indian Tamil (I) and confounded population termed as Others (O) from 13 states and 1 federal territory in Malaysia. **Methods**: A total of 1101 students in Universiti Malaysia Kelantan were surveyed for ABO blood type and Rh factor. **Results**: Phenotypic frequencies were 0.24, 0.27, 0.09 and 0.40 in M; 0.26, 0.21, 0.06 and 0.38 in C; 0.27, 0.31, 0.09 and 0.33 in I and 0.11, 0.29, 0.09 and 0.51 in O for A, B, AB and O blood group, respectively. Rh^+ve cases were found to be abundant and distributed as 0.92, 0.94, 0.89 and 0.79 among M, C, I and O race, respectively. Allele frequencies of I^A, I^B and I were estimated at 0.17, 0.20 and 0.63 in M; 0.19, 0.16 and 0.65 in C; 0.20, 0.23 and 0.57 in O, respectively. Insignificant $\chi^2(0.05, 2 = 5.991)$ interprets that ABO allele frequencies exist in Hardy-Weinberg equilibrium in all races. However, high magnitude of χ^2 in M (5.463) signifies continued admixture in the M genetic population or the population is less stable than C and I. C and I are in more stabilized condition in this regard that might be because of closed breeding within them.**Conclusion**: Results of this study might be useful to blood transfusion services in Malaysia apart from human population geneticists.

Key words: ABO Blood group, Race, Rh factor, Hardy-Weinberg Law, Malaysia

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INTRODUCTION

The world human population has been segmented into different racial categories or ethnic groups. They are categorized not only by anthropological attributes such as physical, cultural, lingual or behavioral differences but a great deal of genetic differences do exist between them.[1]Genetic variation in population arises from mutation, gene flow (migration between populations) and from recombination of genes via sexual reproduction.[12] Polymorphic alleles cause greater variation in the population than do biallelic ones. A number of blood group system have so far been discovered in man of which ABO blood type polymorphism and Rhesus (Rh) blood grouping received highest clinical, anthropological, immunological and biological attention.[3,5,7,4] Karl Landsteiner (1900) and his pupil Von Decastello and Sturli (1902) in Austria demonstrated that four blood group phenotypes (A, B, AB and O) exist in human being formed out of three polymorphic alleles (A,B and O). This happens according to the presence or absence of red blood cell (RBC) surface antigen viz. A, B and O sometimes referred to I^A, I^B and i respectively.The discoverer showed that an individual possessed antibodies against those antigens which lacked on his/her red cells (Eweidah *et al*, 2011).[5] Red cell surface antigens (marker) identifies the cell as belonging to 'self' or not. These cell surface antigens are characterized by a protein or lipid attached with a particular arrangement of sugars[4]. Human being having blood type A produce antibody B when exposed to antigen B and *vice versa*. AB phenotypes are incapable of producing antibodies B or A because both antigens are recognized as 'self'. Blood type O produces both the antibodies A and B. A, B and O antigens are identical, except that type A has an additional sugar: N-acetylgalactosamine,type B has an additional galactoseand type O possesses none of them.[4]Rhesus

***Address for Correspondence:**
Md. Ruhul Amin, Faculty of Agro Based Industry, University Malaysia Kelantan (Jeli Campus) Locked Bag 100, 17600 Jeli, Kelantan, Malaysia. **E-mail:** ruhulaminbau1961@gmail.com; **Phone** +609-9477160

typing is based on the presence (+ve) or absence (-ve) of Rhesus D antigen (Rh) on the RBC surface. Both ABO and Rh data are inevitably needed in blood transfusion and organ transplantation.[5,7]Apart from these, population data on ABO gene frequency can be used to estimate genetic distance between sub-populations[3], to study genetic and ethnic diversity of human population[5],to know genetic relationship among different populations by examining the geographical distribution of gene frequencies[10], to determine migration pattern and origin of human race[4], to manufacture and marketing of race-specific drug[12] which led to develop a subject like ethno-medicine and to resolve issues related to parentage.

Malaysia is a multiracial country. Three distinct races such as Malay (M), Chinese (C) and Indian Tamils (I) distributed over 13 states and 3 Federal Territories (Table 1), although unevenly, mainly constituted Malaysian population. Additionally, some migrants from Philippines (in Sabah), Portuguese (in Melaka) and Indian Sheikhs (distributed all over) and Orang Asli (aborigines) have incorporated in the Malaysian population in course of time. M represents original Malay (*bumiputra*) inhabitants and C and I were brought from China and Tamil Nadu in India during later part of British rule.

There are considerable number of reports regarding ABO blood groups and Rh factors and their allele frequencies in different ethnic populations of Bangladesh[1] Saudi Arabia[5], Nigeria[11,7], India[9,10]and many more.Till now distribution pattern of ABO and Rh blood types among races in Malaysia has not yet been made available in the literature. Current study, therefore, had been designed and carried out to document the phenotypic and allelic frequencies of these blood types among different races in Malaysia.

MATERIALS AND METHODS

A total of 1101 students of different academic years in Universiti Malaysia Kelantan (Jeli campus), Malaysia, were surveyed using a structured record sheet during 2012-2013. Individuals constituted the study population were randomly chosen from the university that encompassed students of both sexes, from 13 states and 1 Federal Territory (2 Federal territories such as Kuala Lumpur and Putrajaya were combined into 1 and called Kuala Lumpur) and from 4 racial categories (Malay, Chinese, Indian and "Others" which include Philippino, Portuguese, Sheikh or blend of Chinese and Indian). The detail distribution of the subjects distributed according to state of origin, sex and race are provided in Table 1.

Customarily students applying for admission into universities need to report their ABO and Rh blood type in the application. Therefore, it is known to every student that what blood group he/she possesses. Representativeness of the students from different state, sex and race were ensured because students were selected for the University by the government from amongst the applicants unbiasedly for these three fixed factors at least for these blood type traits.Data for this study were organized using Excel computer programme,and phenotypic frequencies for A, B, AB and O blood type and same for Rh^{+}ve and Rh^{-}ve were directly calculated.Allelic frequencies for I^A, I^B and i were determined using the statistical method based on the Hardy-Weinberg law of equilibrium[6,10]which states that

Let us consider
p = frequency of I^A
q = frequency of I^B
r = frequency of i

Table 1: Number of subjects in different sex and race under each state that constituted study population

State	Sex		Race			
	Male	Female	Malay	Chinese	Indian	Others
Terengganu	18	67	84	0	0	1
Kelantan	49	154	200	3	0	0
Perak	36	96	90	30	12	0
Selangor	34	92	85	20	19	2
Pahang	15	54	60	7	2	0
Johor	40	82	92	23	6	1
Kedah	24	82	94	7	5	0
Pulau Pinang	26	45	40	23	8	0
Kuala Lumpur	16	32	28	12	8	0
Negeri Sembilan	12	22	24	6	2	2
Melaka	6	19	18	6	1	0
Perlis	5	9	14	0	0	0
Sabah	10	24	10	2	1	21
Sarawak	17	15	8	16	0	8
Sub-total	308	793	847	155	64	35
Total	1101		1101			

For a gene with three alleles, the Hardy-Weinberg Law can written as

$$(p+q+r)^2 = p^2+q^2+r^2+2pq+2pr+2qr = 1$$

and

$$p + q + r = 1$$

Therefore, ABO blood type, genotypes and corresponding allele frequencies can be represented as in Table 2.

χ^2 test was performed to examine the difference (α=0.05) between observed and expected allele frequency separately for each races and in combination of races. [6]

RESULTS AND DISCUSSION

Phenotypic frequency distribution of ABO blood group: Table 3 represents the actual distribution of individuals belonging to A, B, AB and O blood type among different races across the states in Malaysia. It can be viewed that irrespective of race frequency of O blood type is higher than any other blood group phenotype which is close to 0.40 except among Indians (0.33) and 'Others' (0.51). In Chinese race,the frequency of B phenotype (0.21) is a little lower than that of A (0.26) but in Malay, Indians and Others,the frequency of B>the frequency of A.Thefrequency of AB phenotype ranged from 0.06 in Chinese to 0.09 in Indians and Malays. Eweidah *et al* (2011) in Saudi Arabia noticed that frequency distribution pattern of the blood types was O>A>B>AB which is in good agreement with Chinese in the present findings. Khan *et al* (2009) visualized ABO blood type phenotype to be distributed as B ≥O>A>AB in Azad Jammu and Kashmir. This pattern is inconsistent with any race in question in Malaysia. In Ibadan, Nigeria frequency distribution of O, A, B and AB phenotypes among the healthy infants were found to be distributed as 0.54, 0.22, 0.21 and 0.03[11] and it also followed the similar trend of the present findings. Same trend of frequencies were noted by Iyiola *et al* (2012) in Nigerian population where phenotype frequencies were O = 0.53, A = 0.22, B = 0.21 and AB = 0.04. Highest frequency of O phenotypes and lowest in AB were also reported in Egypt, Jordan, Nigeria, Kenya and Eastern region of Saudi Arabia which are in line with our results.[5] On the contrary, Syria, Lebanon, Israel and Jordan have different ABO phenotype distribution pattern where A was recorded as predominant phenotype.[5]Iyiola *et al* (2012) mentioned that a study among US Indians revealed that, there was none found with AB blood group and proportion of A, B, AB and O phenotypes were 0.4, 0.1, 0.0, 0.95 respectively. Begum *et al* (2011) conducted a study on tribal populations in Sylhet, Bangladesh. They noticed that blood group A was more frequent among Meiteis (40%) and Meitei-Pangans (42.41%) and blood group O was more frequent in Bishnupriyas (47.72%). However, frequency of AB blood type was least of all in all communities.This finding is inconsistent with our result indicating that population greatly differs in blood group variants.

Table 2: Blood types and their corresponding genotypes and allele frequencies

Blood type	Genotype	Allele frequency
A	I^AI^A and I^Ai	p^2+2pr
B	I^BI^B and I^Bi	q^2+2qr
AB	I^AI^B	$2pq$
O	ii	r^2

χ^2 test was performed to examine the difference (α=0.05) between observed and expected allele frequency separately for each races and in combination of races.[6]

Table 3: Blood type distribution among races across the states in Malaysia

Respondents	Malay				Chinese				Indian				Other				Total			
	A	B	AB	O	A	B	AB	O	A	B	AB	O	A	B	AB	O	A	B	AB	O
Terengganu	26	17	4	37	0	0	0	0	0	0	0	0	1	0	0	0	27	17	4	37
Kelantan	47	50	26	77	0	1	0	2	0	0	0	0	0	0	0	0	47	51	26	79
Perak	27	33	4	26	8	5	6	11	2	5	0	5	0	0	0	0	37	43	10	42
Selangor	15	30	4	36	11	7	0	2	6	8	2	3	0	0	1	1	32	45	7	42
Pahang	12	15	3	30	0	1	1	5	0	1	1	0	0	0	0	0	12	17	5	35
Johor	17	20	11	44	7	5	1	10	3	0	0	3	1	0	0	0	28	25	12	57
Kedah	26	26	5	37	1	4	1	1	2	1	0	2	0	0	0	0	29	31	6	40
P. Pinang	8	12	7	13	4	8	0	11	3	0	1	4	0	0	0	0	15	20	8	28
K. Lumpur	12	5	4	7	5	2	0	5	1	4	0	3	0	0	0	0	18	11	4	15
N. Sembilan	7	6	2	9	1	0	0	5	0	0	2	0	0	0	1	1	8	6	5	15
Melaka	4	7	2	5	4	0	0	2	0	1	0	0	0	0	0	0	8	8	2	7
Perlis	2	4	1	7	0	0	0	0	0	0	0	0	0	0	0	0	2	4	1	7
Sabah	1	3	0	6	0	0	0	2	0	0	0	1	1	5	0	15	2	8	0	24
Serawak	2	2	0	4	3	3	1	9	0	0	0	0	1	5	1	1	6	10	2	14
Total	206	230	73	338	44	36	10	65	17	20	6	21	4	10	3	18	271	296	92	442
Frequency	0.24	0.27	0.09	0.40	0.26	0.21	0.06	0.38	0.27	0.31	0.09	0.33	0.11	0.29	0.09	0.51	0.25	0.27	0.08	0.40

Frequency distribution of Rh factor: From Table 4, the Rh phenotypes in this study population show that Rh^+ are abundant and its frequency stands at close to 0.90 in all races (C>M>I>O) except in 'Others' (0.79). The overall Rh^-allele frequency was ranging from 0.24 to 0.33except in "Others" (0.46).Omotade *et al*. (1999) observed frequency of Rh^+ phenotypes among the healthy infants in a particular year in Nigeria to be two times than Rh^-. Rh phenotypes studied in South West Nigeria resulted in a ratio of 97:3 for positive and negative cases.[7] These two studies reflect difference in the phenotypic distribution in two different sites in the same country although Rh^+ cases are much higher than Rh^- in both cases. Eweidah *et al*. (2011) found that Rh^+ subjects were over 10 times more than Rh^- subjects in 4 cities of Al-Jouf province in Saudi Arabia. This is in line with current findings. Interestingly the authors illustrated that Rh^- incidence was recorded highest in B blood group subjects (4.5%), followed by O (2.0%), A (1.8%) and AB (0.5%) subjects. Begum *et al* (2011) found an overall 2.57% Rh^- cases in tribes of Sylhet of Bangladesh but the group difference was significant (χ^2=25.13; p<0.001) with 0% Rh^-ve in Khasia group in the same investigation. Begum *et al* (2011) reviewed that Rh^-ve possessor appeared in higher (7.7-10.9) frequency in some ethnic groups of Pakistan. They on the contrary found no existence of Rh^-ve individual in Khasia and Santal tribes in Bangladesh. Iyiola *et al* (2012) in their review on Rh factor in samples of humans in 15 countries in Asia, Europe, Africa and North America showed that Rh positivity only in Britain and USA are close to 0.85but it ranged between 0.90 to 0.97 in rest of the countries. In the current study Rh positivity in "Others" group was far below than values cited in literature.It hints that this population comprised of migrants from different ethnic groups or different geographical location of the world.

ABO allele frequency distribution: ABO blood group allele frequencies of this study have been shown in Table 5. This happens in the order of $i>I^B>I^A$in all the races except in Chinese in which frequency of I^A (0.19) is slightly higher than frequency of I^B (0.16). Pooled frequency of the alleles ordered as $i>I^B>I^A$. Distribution of the alleles in "Other" group showed greater allele frequency variation than in M, C and I. In this group of mixed race frequency of *i*(0.72) is much greater and frequency of I^A(0.08) is much less than in any other racial group. This might be happened because of admixture of blood from different origin. Apart, sampling error or natural selection might contribute in this variation. Their ancestors migrated from Philippines, Indonesia, Thailand, Portuguese and northern part of India (Sheikh) many years ago. Pattern of variation in allele frequency of this study differs from that of Iyiola *et al* (2012) in South-West Nigeria where they noticed this pattern to be ordered as $i>I^A>I^B$.On the other hand Khan *et al* (2009) studied the same problem in the Poonch district of Azad Jammu and Kashmir and Nagariya (2013) in Maharastra,India (Hindu and Muslim caste)demonstrated the order of human ABO allele frequencies as $i>I^B>I^A$.The frequency of I^A, I^B and *i* allele in Muslim and Hindu caste were 0.195, 0.267 and 0.542 and 0.175, 0.238 and 0.592, respectively. The trend is in agreement with C sub-population in Malaysia in the current investigation indicating that Chinese and Northern Indians might have some similarities in this regard. It is, however, universal that frequency of *i* allele was found to predominate in humans all over the globe but those forI^A and I^B are inconsistent.[5]

Allele frequencies in respect to Hardy-Weinberg equilibrium: Hardy-Weinberg equilibrium refers to balanceness of a population in terms of gene and genotype frequencies. The Hardy-Weinberg law states that frequency of recessive

Table 4: Rh+ and Rh- distribution among races across the states in Malaysia

Respondents	Malay		Chinese		Indian		Others		Total	
	Rh+	Rh-	Rh+	Rh-	Rh+	Rh-	Rh+	Rh-	Rh+	Rh-
Terengganu	52	6	0	0	0	0	0	0	52	6
Kelantan	123	12	3	0	0	0	0	0	126	12
Perak	58	2	22	2	8	2	0	0	88	6
Selangor	53	1	13	2	15	1	1	0	82	4
Pahang	31	2	7	0	1	0	0	0	39	2
Johor	58	4	18	1	4	1	1	0	81	6
Kedah	54	8	7	0	4	0	0	0	65	8
Pulau Pinang	25	1	23	0	7	1	0	0	55	2
Kuala Lumpur	0	0	0	0	0	0	0	0	0	0
Negeri Sembilan	12	3	3	0	1	0	1	0	17	3
Melaka	7	1	6	0	1	0	0	0	14	1
Perlis	11	0	1	0	0	0	1	0	13	0
Sabah	3	0	1	1	1	0	5	3	10	4
Serawak	5	0	10	1	0	0	6	1	21	2
Total	492	40	114	7	42	5	15	4	663	56
Phenotypic frequency	0.92	0.08	0.94	0.06	0.89	0.11	0.79	0.21	0.92	0.08
Allelic frequency	0.73	0.27	0.76	0.24	0.67	0.33	0.54	0.46	0.72	0.28

Table 5: Allelic frequencies of I^A, I^B, and i among different races in Malaysia

Allelic frequency	Malay (M)	Chinese (C)	Indian Tamil (I)	Others (O)	All (Malaysia)
r (i)=	0.63	0.65	0.57	0.72	0.63
p (I^A)=	0.17	0.19	0.20	0.08	0.17
q (I^B)=	0.20	0.16	0.23	0.21	0.20
Total=	1.00	1.00	1.00	1.00	1.00
χ^2=	5.463^{ns}	0.026^{ns}	0.008^{ns}	3.601^{ns}	5.689^{ns}

gene (i) is equal to the square root of the frequency of the homozygous recessive individuals. Significant departure of observed frequency from the expected one can be tested by using a non-parametric test called χ^2 test.[6]Chi-square test ($\chi^2_{0.05,}$ 2 = 5.991)revealed that overall gene frequencies for I^A, I^B and i are in equilibrium in Malaysia irrespective of races (Table 5). However, the smaller χ^2 values in Chinese (0.026) and Indian Tamils (0.008) indicate that very little deviation from balanced state exist in the gene frequencies in Chinese and Indian Tamils compared to rest of the races. Chinese and Indian Tamils have been maintaining relatively closed population circle being getting married within the race only.Chi-square values in Malay and Pooled population are very close to 5.991 at which null hypothesis (population is in equilibrium) could be rejected at $p<0.05$. They are rejectable at $p<0.0725$.[8]

CONCLUSION

Findings of the ABO and Rh phenotypic frequencies would be useful to blood transfusion services in Malaysia. Allele frequencies have potential for further studies in human genetics and their migration pattern in the region concerned.

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Authors Contribution:
MRA – designed the study, collected data in person, drafted manuscript & reviewed the manuscript; **DS** – collected data in person, analysed the data, reviewed the manuscript; **LN** – Data analysis, Editing the manuscript.

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