# AN ANALYSIS OF GENOTYPE FREQUENCIES OF BLOOD GROUP ANTIGENS FROM BANNU REGION (NWFP) IN PAKISTAN

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## ABSTRACT

**Objective:** A community-based study was carried out on a random population sample from urban and rural areas of Bannu region, North West Frontier Province (NWFP) of Pakistan. The objective was to identify the genotypic frequency of blood groups in the region, in order to comprehend the allelic diversity.

**Material & Methods:** Blood grouping was carried out over a period of 16 month from January 2002 to April 2003, and encompassed 2581 subjects, in which 57.09% were male and 42.09% female. These were categorized according to ABO/Rh system. Allele frequency was computed according to Hardy-Weinberg law.

**Results:** The distribution of phenotypes in the total sample were 0.3623, 0.3103, 0.2507 and 0.0767 for group B, A, O and AB, respectively, with 0.672 Rh positive ® and 0.328 Rh negative (r). B group was dominant in both the genders, and AB was rare in both males as well as females. The distribution of the alleles in the total sample was 0.345, 0.378 and 0.277 for $I^A$, $I^B$ and i, respectively.

**Conclusion:** The studied population exhibited a predominance of group B, in the order of B>A>O>AB, as well as Rh positive antigen for both male (90.26%) and female (87.98%) subjects within the population, with Rh negative men and women being 9.74% and 12.02% respectively. Allele frequency recorded was in the order of $I^B > I^A >$ i, and R > r,

**Key words:** Alleles; Gene frequency; Blood groups; Rh factor; Transfusion; Bannu; Pakistan.

## INTRODUCTION

Circulation of important nutrients, enzymes, hormones and oxygen occurs through blood. Antigens present in the blood impart different characteristics, which its reactivity, and blind mixing of blood can initiate an immune reaction. Only the blood samples, which share the same antigenic identity, do not initiate an immune response, and hence are termed as compatible.

Almost half a century ago, Tijio and Leven demonstrated that there are 46 chromosomes in humans, arranged in twenty-three pairs, of which 22 pairs are autosomal and one pair contains sex chromosomes[(1)]. Some cells contain diploid number of chromosomes (46), while germs cells after undergoing meiosis have the haploid number of chromosomes (23). Hence there is only one respective of each pair (somatic as well as germ), in both the egg and sperm cells. Zygote formation leads to the pairing of these chromosomes so that each individual is controlled by 2 genes, one from each parent. This pair of genes which control one character, are called alleles[(2)] and they can either be identical (Homozygous) or different (Heterozygous). The true genetic make up of an individual for any specific character, including blood groups, is termed as a genotype and effect of the genes can be observed by the apparent outcome determined through the dominance of a specific allele, known as the phenotype[(3)].

Several blood group system, based upon different antigens, have been proposed so far, however, the ABO system put forward in 1900 by Kerl Landsteiner has been established as the only medically established system. In combination with the Rhesus system, the ABO system has become the recognized system for determining blood group compatibility for safe transfusions.

Genes responsible for the ABO antigens appear to be located on the long arm of chromosomes number nine[(2)]. A set of three possible alleles at an autosomal locus are responsible for four blood types. The gene symbols i or $I^0$, $I^A$ and $I^B$, are often used to denote these alleles, where I stands for isoimmunogglutinin. The superscript indicates the specifics antigen. One of these (i=O), is recessive to the other two ($I^A$ =A and $I^B$ , produce any antigen. Allele $I^A$ produces antigen A and is co-dominate with the third allele $I^B$, with produces antigen B. These three alleles combine to yield six genotypes and four phenotypes. Two alleles, R and r, are responsible for the inheritance for rhesus blood groups, with R denoting $Rh^{+ve}$, and r implying $Rh^{-ve}$ blood group allele.

Gene frequency is simply the proportion of different alleles for a gene that are present in the population. This proportion is computed by taking into consideration the number of various genotypes in the population, in order to estimate the relative allele frequencies by application of the Hardy-Weinberg law[3].

Muller was the first investigator in year 1927 who made the examination of the ABO antigens among Yourba people. He has determine the frequency of alleles as 0 = 0.07271, A = 0.1320 and B = 0.1409[4]. It was clear that in Africans O blood group is more prevalent following over the group in sequence A,B and AB. However in Asian and Caucasians the prevalence of blood group B is more followed by the sequence O, A, and AB[5].

The present study has been carried out to record the genotypic frequency of various alleles in the blood group among the population of Bannu, North West Frontier Province (NWFP), Pakistan, with a view to generate data with multipurpose future utilities for the health.

## MATERIALS AND METHODS

**Subject:** A total of 2581 subjects, comprising 1123 females and 1458 males, were screened for their blood groups. The subjects belonged to both rural and urban areas of Bannu, NWFP, Pakistan.

**Collection of blood samples:** A 2.0 ml samples of blood was drawn from the antecubital vein of each subject in a disposable syringe, and transferred immediately to a tube containing Ethylene diamine tetra acetic acid (EDTA).

**Determination of blood group:** Blood grouping (ABO) and Rhesus factors (Rh), was done by the antigen antibody agglutination test. The antisera used were obtained from Plasmatic (Kent, UK). Plasmatic ABO monoclonal reagent are in vitro culture supernatants of hybridized immunoglobulins secreting mouse cell-line. For determination of Rh factor, plasmatic anti D (1gm) Lo.-Du and LO-Du2 monoclonal reagent prepared from different antibody producing human B-lymphocyte cell lines were used.

**Computation of allele frequencies:** Allele frequency for the antigens was computed by application of the Hardy-Weinberg law[3], on the basis of the number of subjects with blood groups, ABO and Rhesus factor (Rh).

## RESULTS

Table 1 shows the phenotypic frequency of ABO blood groups in the studied population, with gender distribution. The distribution of phenotypes in the total sample were 0.3623, 0.3103, 0.2507 and 0.0767 for groups B, A, and AB, respectively, with 0.672 Rh positive and 0.328 Rh negative. B group was dominate in both the genders, and AB was rare in both males and females. Table 2 depicts the distribution of allele frequencies of ABO antigens in the studied population, in comparison with certain earlier studies. The distribution of the alleles in the total samples was 0.345, 0.378 and 0.277 for $I^A$, $I^B$ and i, respectively. Table 3 compares the distribution of allele frequencies of Rh factor antigens, in the studied population with earlier studies on different populations.

## DISCUSSIONS

A survey conducted by Wagner in South Western Germany designated the ABO allele frequency as O = 0.0640, A = 0.279, B = 0.081[6]. Estimate of the gene frequency for ABO system in Hungary has been reported as i = 0.5593, $I^A$ = 0.2989 and $I^B$ = 0.1418[7]. In Nairobi (Kenya), the percentage distribution of blood groups has been reported as 0 = 47.4%, A = 26.2%, B = 22.0%, AB = 4.4%, with the relative gene frequencies being i = 0.690, $I^A$ = 0.168 and $I^B$ = 0.142 and 9601% Rh positive having gene fre-

**Table 1: Phenotypic frequencies of various blood groups (ABO and Rh) in the studied population.**

| Blood | Total Subjects | | | Male Subjects | | | Female Subjects | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | Complete | $Rh^+$ | $Rh^-$ | Complete | $Rh^+$ | $Rh^-$ | Complete | $Rh^+$ | $Rh^-$ |
| A | 0.3103 | 0.3108 | 0.3069 | 0.3484 | 0.3496 | 0.3380 | 0.2609 | 0.2591 | 0.2741 |
| B | 0.3623 | 0.3637 | 0.3502 | 0.3608 | 0.3670 | 0.3028 | 0.3642 | 0.3591 | 0.4000 |
| AB | 0.0767 | 0.0686 | 0.1444 | 0.0480 | 0.0357 | 0.1620 | 0.1140 | 0.1124 | 0.1259 |
| O | 0.2507 | 0.2569 | 0.1985 | 0.2428 | 0.2477 | 0.1972 | 0.2609 | 0.2692 | 0.2000 |
| **Total** | **1.0000** | **1.0000** | **1.0000** | **1.0000** | **1.0000** | **1.0000** | **1.0000** | **1.0000** | **1.0000** |

**Table 2: Frequency of blood groups (ABO) in different populations.**

| | Allele Frequency | | | |
|---|---|---|---|---|
| **Population** | **$I^A$** | **$I^B$** | **i** | **Reference** |
| Britain | 0.496 | 0.331 | 0.173 | (30) |
| India | 0.318 | 0.452 | 0.230 | (30) |
| Nigeria | 0.356 | 0.479 | 0.165 | (4) |
| Kenya | 0.343 | 0.447 | 0.210 | (5) |
| Hazara (Pakistan) | 0.260 | 0.408 | 0.332 | (9) |
| Swabi (Pakistan) | 0.307 | 0.396 | 0.279 | (10) |
| Peshawar (Pakistan) | 0.327 | 0.408 | 0.265 | (31) |
| Bahawalpur (Pakistan) | 0.275 | 0.480 | 0.245 | (12) |
| Bannu (Pakistan) | 0.345 | 0.378 | 0.277 | Present Study |

**Table 3: Frequency of Rh antigens in different populations.**

| | Allele Frequency | | |
|---|---|---|---|
| **Population** | **R** | **r** | **Reference** |
| Lahore (Pakistan) | 0.717 | 0.283 | (32) |
| Nigeria | 0.934 | 0.057 | (4) |
| Kenya | 0.803 | 0.197 | (5) |
| Azad Jammu & Kashmir | 0.848 | 0.152 | (33) |
| Islamabad | 0.729 | 0.271 | (34) |
| Peshawar (Pakistan) | 0.768 | 0.232 | (29) |
| Bannu (Pakistan) | 0.675 | 0.328 | Present study |

quency = 0.804 ( R ) and Rh (D) negative were only 3.9% gene frequency ( r ) = 0.0196[5]. In the United States 85% of the Caucasians and 92% Negroids are $Rh^{+ve}$, while the Japanese, Chinese and pure American Indians are 99% $Rh^{+ve}$[8]. Prevalence of $Rh^{+ve}$, is reported as 100% in China, 84% in Europe and 94.6% in W. America[9].

India and Pakistan both have a higher frequency of group B as a compared to Europe and U.S.A.[1,9,10]. In Pakistan work has been done on blood group distribution in all provinces. In Wah cantonment (Punjab) gene frequency distribution is as $I^A = 0.203$, $I^B = 0.255$, $i = 0.542$, similarly for alleles $R = 0.730$ and $r = 0.271$[11]. In Bahawalpur City the percentage of blood group O was 37%. A was 21%, B was 36% and AB was 6%[12], while in Bannu (NWFP) it was 31.03%, 36.23%, 7.67% and 25.07%, for A,B,AB and O blood group, respectively[13].

In the study under discussion, the relative frequency of the various blood groups does not seem to deviate from those which have been recorded from studies on various segments of the Pakistan population (Table-2). However, comparison with the

data from the British and African populations, presented in the same table, reveals that there is an equal dominance of group B and O in the Indo-Pak sub-continent, in contrast to only O group for the British and African populations. The least reported group, in all the populations, has been AB.

It has been reported[14-16], that in the population of the United State, Asian, Syrian Arabs and Palestinians, group O is dominant, with AB being the rarest. Racial (genetic) and environmental factors have been reported to influence the frequency of various blood groups in studied carried in various societies, including Bangladesh and Latin America[17-19]. The genetic and environmental factors responsible for varying frequency of the blood groups among the Pakistan populace needs to be probed further.

I terms of presence of Rh antibodies, the data from several studies on Pakistan as well as certain African population is compared in Table-3, along with allele frequency of R and r. The present study has shown comparatively the highest percentage of Rh negative cases, however, it is till very low and follows the global trend of being significantly rarer than Rh positive individuals. The finding of the present study are in consistence with the results obtained in an earlier study carried out in Wah Cantt. (Pakistan), in which allele frequencies of R and r were found to be 0.730 and 0.271, respectively[11].

An association with the blood groups with several diseases, specially cardiovascular diseases, which has been reported over the years[21-29], would make the data generated by the study, to be useful for health planners as it will provide an in depth information of the relative distribution of various alleles in the population and help making efforts to face the future health challenges for the region.

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