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FREQUENCY OF ABO BLOOD GROUPS AND SECRETOR STATUS IN THE RAJPUT COMMUNITY OF HIMACHAL PRADESH

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ABSTRACT

Blood and saliva samples from 170 subjects of both sexes were collected from the community, Rajputs of Himachal Pradesh and were examined to determine their ABO and Rhesus blood groups by standard conventional methods and the secretor status was determined with Absorption elution technique. Out of 170 subjects 148 were secretors. A frequency distribution of 16.2%, 43.2%, 8.1% and 32.4% was observed for blood groups A, B, AB and O, respectively. The allelic frequencies were calculated to be 0.2, 0.24 and 0.56 for A, B and O alleles. The allele frequency for the secretor gene was found to be Se =0.64, and for non-secretor, se = 0.36. The allelic frequencies of blood groups were found to be in Hardy-Weinberg equilibrium.

The Study of secretor status provides a useful tool in medico legal cases for detection of suspected crime. This finding of distribution of blood groups, secretors and non-secretors in normal healthy population would be beneficial for further study, protecting at least partially, from certain malignancies or allowing them to have less aggressive disease, and this finding might be useful in enhancing further studies and research in this direction.

KEY WORDS: Allelic Frequency, Blood Group, Ante-cubital Vein, Secretor.

INTRODUCTION:

The ABO and Rh blood groups are among the most important blood groups (Seeley *et al.,* 1998). Karl Landsteiner first described the ABO blood group in 1900, and it served the beginning of blood banking and transfusion medicine (Ali *et al.,* 2005). In the ABO blood group, individuals are divided into four major blood groups, A, B, AB and O, according to the presence of the antigens and agglutinins. Type A blood has type A antigens, type B blood has type B antigens, type AB blood has both types of antigens, and type O blood has neither A nor B antigens. In addition, plasma from type A blood contains type B antibodies, which act against type B antigens, whereas plasma from type B blood contains type A antibodies, which act against type A antigens. Type AB has neither type of antibody and type O blood has both A and B antibodies (Seeley *et al.,* 1998). Blood group antigens A and B are not only confined to red cells but can be detected in other tissue cells and in body fluids like saliva, sweat, semen, milk etc. It has been established that, secretion of group specific substances in body fluids is controlled by a pair of alleles Se and se. Thus, individuals can be homozygous [SeSe], heterozygous [Sese] or homozygous [sese]. The first 2 classes are called secretors and third class, non-secretor (Karpoor *et al.,* 2010). In Rh system blood groups are Rh-positive or Rh-negative on the basis of presence or absence of Rh antigens on red cell surface. Rh antigens are determined by three pairs of closely linked allelic genes located on chromosome 1. In clinical practice blood grouping is important because an antigen may, in certain circumstances, react with its corresponding antibody and cause harmful clinical effects like haemolytic transfusion reactions and haemolytic disease of newborn.

ABO and Rhesus (Rh) blood group antigens are hereditary characters and are useful in population genetic studies, researching population migration patterns, as well as resolving certain medicolegal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice. The need for blood group prevalence studies, is multipurpose, as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Platt *et al.,* 1985; Horby *et al.,* 1989; Meade *et al*, 1994; Green *et al.,* 1994). Estimates of gene's frequency provide very valuable information on the genetic similarity of different populations and to some extent on their ancestral genetic linkage, despite the cultural and religious differences of the two populations.

This study was carried out to determine the frequency of various ABO and Rh blood groups in the population of Rajputs of Himachal Pradesh and to compare our results with other studies conducted in India and elsewhere in the world and its multipurpose future utilities for the health planners.

MATERIALS AND METHODS:

Collection of blood and saliva samples:

170 subjects of both sexes were selected from the community, Rajputs of Himachal Pradesh. From each individual, 0.5 mL blood samples were collected under aseptic condition from ante-cubital vein for determination of blood groups in sterile appendorf. Subjects were informed to rinse their mouth with water prior to saliva collection. After few seconds, 0.5 mL of saliva was collected in sterile appendorf. Saliva samples were tested within 2 hours of collection.

ABO blood group test:

A drop of monoclonal anti-A, anti-B (Labkit, Barcelona, Spain) was added to a drop of finger prick blood on clean slide and mixed well. Results of agglutination were recorded immediately for ABO blood groups for standardization and confirmation. Blood group testing was done from saliva samples of the same subjects by the hemagglutination inhibition assay (Katsuji Nishi, 2005)

Determination of blood group from saliva sample:

The secretor status of an individual can be determined using any of the 3 methods

1. Absorption Inhibition technique. (Reference)
2. Absorption Elution technique.
3. Mixed Agglutination technique.

Of these techniques, the Absorption Elution technique was found to yield the most accurate results, and has been used.

Absorption Elution technique:

In this technique strong antiserum is used directly instead of its titre because it takes less time to determine the secretor status of the individual.

Four clean and dry eppendorfs (2 ml) were taken and marked as A, B, D and H respectively. A small piece of cloth containing a saliva sample was taken. A drop of anti- A serum, anti- B serum, anti- D serum and anti- H lectin was added to the eppendorfs respectively. They were incubated at 4^{0}C for two hours and were then washed with ice chilled normal saline to remove the anti serum from the cloth. A drop of fresh normal saline was added to each of the samples. The samples were then kept in a water bath at 56-60^{0}C for 15- 20 minutes. One drop (25µl) of 0.2% of A, B and O indicator cells were added to the respective eppendorfs and kept at 4^{0}C again for half an hour. After the final incubation, the contents were examined, both macroscopically as well as microscopically, for agglutination.

Agglutination in cavity				Blood group
Anti- A	Anti- B	Anti-D	Anti- H	
+	-	+/-	±	A +ve/-ve
-	+	+/-	±	B +ve/-ve
+	+	+/-	-	AB +ve/-ve
-	-	+/-	±	O +ve/-ve

Preparation of Anti- H Lectin:

2g seed of Ulex europaeus were soaked overnight in normal saline. The macerated seeds are agitated for half an hour to make a paste. It was then centrifuged at 3,000 rpm for 5 minutes and the sediment was discarded. As the supernatant was cloudy, it was centrifuged again at about 10,000 rpm for 15 minutes. The specificity of the lectin was titrated with group O cells. An Anti- H lectin sample with a titre of 32 was used for grouping reactions.

RESULT:

The result of the analysis of blood groups and the secretor status of Rajputs of Himachal Pradesh (Table1) shows that the frequency of blood group B is highest (43.2%) among the Rajputs followed by the frequency of blood group *O* (32.4%), *A* (16.2%), *AB* (8.1%). The allelic frequencies were calculated using formula:

$(p+q+r)^2=1$ i.e. $p^2+q^2+r^2+2pq+2pr+2qr=1$ where p is the frequency of A, q of B and r of O. Therefore, p^2+2pr represents the instance of the blood group A, q^2+2qr the instance of B, 2pq of AB and r^2 of the blood group O.

The allelic frequency for ABO blood group was found to be 0.2, 0.24 and 0.56 for A, B and O alleles. The frequency for the secretor allele (Se) was found to be 0.64, and for non-secretor (se) 0.36.

Results of present study is comparable with ABO allele frequencies reported in some earlier studies in which the frequency of allele *O* among the Rajputs (0.4151) of Kasauli is found to be highest as compared to allele *B* (0.3288), *A1*(0.2438), *A2*(0.0123) reported by Mukhopadhyaya and Kshatriya (2004) compared with the frequencies in Brahmin population of Kasauli of Solan with allele frequency, O(0.4906) and B(0.3106) and their observation are found to be similar to that observed for various population groups of Western Himalayan region (Bhalla *et al.,*1980; Bhasin *et al.,*1992)

The need for blood group prevalence studies in the particular area is not only important for blood transfusion, organ transplantation, evolution study and genetic research, but also beneficial in further study as a useful tool in medico legal cases for detection of suspected crime and in protecting, at least partially, from certain malignancies or allowing them to have less aggressive disease, and this finding might be useful in enhancing further studies and research in this direction.

Table 1: Percentage of Blood Groups

Blood group B	43.2% (64 samples)
Blood group A	16.2% (24 samples)
Blood group O	32.4% (48 samples)
Blood group AB	8.1% (12 samples)

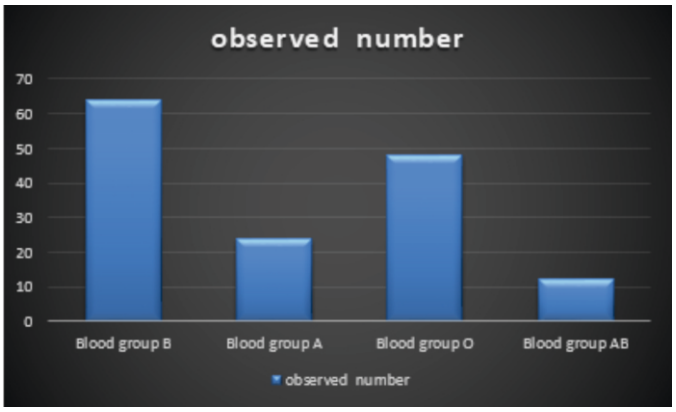

Figure1: Phenotypic number of different ABO groups observed

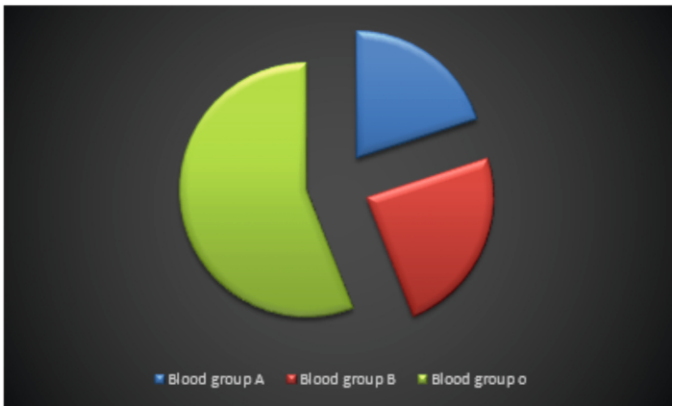

Figure 2: Analysis of allelic frequencies of A, B, and O blood groups in Rajputs

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