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Gene frequency reports of sickle cell trait among six human populations of Jammu and Kashmir, India

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ABSTRACT

Sickle cell disease (SCD) is the most commonly inherited blood disorder, causing high child mortality worldwide. The study aimed to determine the prevalence and gene frequencies of sickle cell trait (SCT) among six regional human populations from Jammu province of Northern India. The prevalence of SCT ranged from 4.41% to 16.36% among males and 6.67% to 20.83% among female children of six populations. The average prevalence of SCT was 9.30% observed among males and 11.16% among female children. The highest frequency of sickle cell trait was observed among Mughal population (male = 16.36%, female = 20.83% and combined group = 18.45%). The lowest frequency of sickle cell trait was found among Khan population (male = 4.41%, female = 6.67%, and combined group = 5.47%). The population-based chi-square values showed a significant difference ($p < 0.05$). We observed significant differences in the allele frequencies of SCT among six human populations. The results from this study provide information on the genetic variation of SCT among different human populations inhabiting Jammu and Kashmir. Understanding the epidemiology of SCT at population level would help in genetic counseling strategies to minimize its harmful consequences.

1. Introduction

Sickle cell disease (SCD) is the most common genetic disease, which affects the architecture, function or outturn of hemoglobin (Ohls and Christensen, 2004). The higher prevalence of SCD is in Africa, Mediterranean Europe, Middle East, Caribbean, South, Central America, and some regions of India. In Africa, about 230,000 new cases of SCD occur each year (Williams and Obaro, 2011). SCD is known to be associated with a very high rate of childhood mortality of about 50%–90% of the total child mortality in Africa (Grosse et al., 2011). In Nigeria there are about 100,000 infant sickle cell deaths each year. Population migration is the key factor for the increased incidence of SCD in other parts of the world. This makes SCD the most common and fastest-growing genetic disorder in different geographical regions. In the USA, SCD affects >70,000 African Americans including 1 in 375 newborns (Buchanan et al., 2010). In the UK, it affects 1 in 2400 live births across ethnic groups, and more than 15,000 individuals are living with SCD. The 20 million people of India are known to suffer from sickle cell disease (Ghai, 2002). The highest frequency of sickle cell gene in India is reported from Odisha, followed by Assam, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, and Gujarat (Balgir, 1996). The average frequency of sickle gene is 4.3%, while that in Odisha is 9.1% (Ambedkar et al., 2001). Higher allele frequency of sickle cell gene has been observed among female group (29.83%) in comparison with the male subjects (21.99%) of Manipur populations, Eastern India (Shah et al., 2012).

Sickle cell trait (SCT or sicklemia) is a heterozygous condition, which does not display the severe symptoms of SCD, which is inherited in the autosomal recessive fashion. The beta hemoglobin gene (β-globin) is responsible for sickle cell anemia present on the short arm of chromosome 11 (11p15). Identification of SCT prevalence and gene frequency analysis among different populations would add several advantages in transfusion medicine, transplantation, and disease risk. We aimed to estimate the prevalence and gene frequency of SCT among regional populations of Jammu. This study has never been carried out in these populations earlier, thus making it a preliminary effort toward genetic epidemiology of the trait.

2. Materials and methods

2.1. Ethics statement

The study was approved by Institutional Ethics Committee of Jawaharlal Nehru Medical College (JNMC), Aligarh Muslim University, India. We obtained the written informed consent from the parents, caretakers, or guardians on behalf of the minors/children participants involved in our study.

Abbreviations: H_o, Homozygosity; H_t, Heterozygosity; J&K, Jammu and Kashmir; SCD, Sickle cell disease; SCT, Sickle cell trait.

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Table 1
Characteristics of sample size.

Populations	Male	Female	Total (n)
P1	70 (51.47)	66 (48.53)	136
P2	55 (53.40)	48 (46.60)	103
P3	68 (53.12)	60 (46.88)	128
P4	52 (53.61)	45 (46.39)	97
P5	45 (47.37)	50 (52.63)	95
P6	63 (53.85)	54 (46.15)	117
Total	353 (52.22)	323 (47.78)	676

Values shown in the table indicate number (%).
Populations presented as P1 = Gujjar and Bakarwal, P2 = Mughal, P3 = Khan, P4 = Malik, P5 = Mir, P6 = Syed.
Total sample size 676, n = number of subjects.

2.2. Population and sample size

Jammu and Kashmir (J&K) is the northernmost state of India, situated between 32.17° and 36.58° North latitude and 37.26° and 80.30° East longitude (Fareed et al., 2012, 2015). According to the Indian census 2001, the human population of Jammu and Kashmir consists of 66.97% Muslims, 29.63% Hindus, 2.04% Sikhs, 1.12% Buddhists, 0.20% Christians, 0.024% Jains, and 0.012% others. Muslims of J&K belong to various castes and tribes. Gujjar and Bakarwal belong to Muslim population and is the major tribe of the state, densely populated in Rajouri and Poonch districts (Fareed et al., 2014). The survey was conducted in the duration of May 2013 through January 2014 and a total of 676 healthy children (6–15 years of age) were selected from six different Muslim populations during house-to-house visit. The details of sample size under study have been presented in Table 1.

2.3. Blood sample collection and slide preparation

The blood samples were collected by finger-prick method and were tested for sickle cell trait by wet sealed method using 2% freshly prepared sodium metabisulphite solution following the method of Daland and Castle (1948). The slides were prepared and then observed under microscope (40× and 100×).

2.4. Analysis of gene frequency for sickle cell trait

Gene frequency was calculated by using Hardy–Weinberg method ($p^2+q^2+2pq=1$).

Considering normal allele = p and mutation allele = q, then allele frequencies for sickle cell trait were calculated as follows:

$$p = \frac{\sqrt{\%\text{normal phenotype}}}{100} \quad (1)$$

Now, $q = 1 - p$.

2.5. Homozygosity and heterozygosity

The homozygosity (H_o) and heterozygosity (H_t) was determined using the formula:

$$H_o = \sum Pi^2 \quad (2)$$

where Pi represent the alleles.

Now, $H_t = 1 - \sum H_o$.

2.6. Statistical analysis

Statistical analysis was conducted using GraphPad InStat 3.0 (USA) and XLSTAT 2015 for windows version 17.1.02 (Addinsoft, Paris, France). Chi-square (χ^2) test was used to determine the significant differences.

$$\chi^2 = \sum \frac{(\text{Observed Number} - \text{Expected Number})^2}{\text{Expected Number}} \quad (3)$$

3. Results

3.1. Prevalence of SCT among six populations of Jammu region

Fig. 1 presents the slides of normal and sickle carrier (i.e., heterozygous condition) subjects. Table 2 shows the prevalence of SCT among male and female children of six human populations of Jammu region. The highest frequency of sickle cell trait was observed among Mughal population (male = 16.36%, female = 20.83%, and combined group = 18.45%). The lowest frequency of sickle cell trait was found among Khan population (male = 4.41%, female = 6.67%, and combined group = 5.47%). The population-based chi-square values for male ($\chi^2 = 8.064$, df = 5, p = 0.1527) and female ($\chi^2 = 6.432$, df = 5, p = 0.2665) were found to be non-significant, while combined group ($\chi^2 = 13.349$, df = 5, $p < 0.05$) showed a significant difference.

3.2. Allele frequency

Fig. 2 presents the differences in allele frequency of SCT among six human populations. The female group from all populations (except Mir population) showed higher allele frequencies of sickle cell gene than the male child group. The highest sickle carrier (i.e., q) frequency was observed among Mughal population (male = 0.0855, female = 0.1102) and the least was found among Khan (i.e., male = 0.0223, female = 0.0339).

3.3. Genotypic frequency

Table 3 presents the differences in genotypic frequencies of SCT among male and female children of six human populations. The homozygous recessive genotype (i.e., q^2), which is responsible for sickle cell

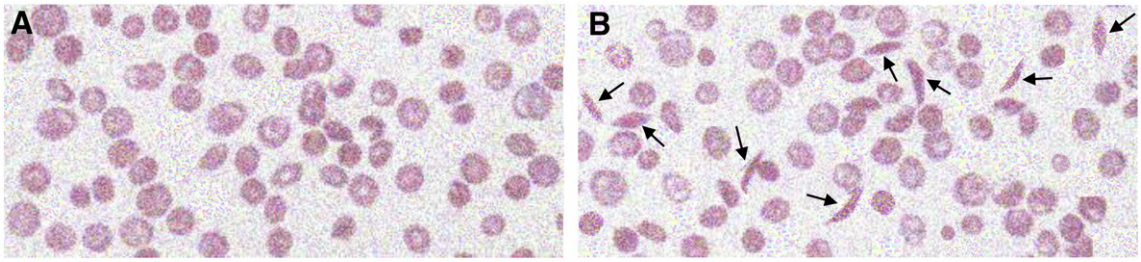

Fig. 1. Sickle cell trait. (A) Slide of normal red blood cells, (B) slide of carrier individual (heterozygous condition) having sickle-shaped cells (pointed with arrows).