

Incidence of NESTROFT-Positives and Haemoglobin S among the Jats and Brahmins of Sampla, Haryana

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KEYWORDS NESTROFT. Sickle Cell Trait. Jats. Brahmins. Haryana

ABSTRACT Nestroft is an uncumbersome and inexpensive test for the detection of haemoglobinopathies and thalassaemias. Here we report the incidence of positives and negatives with respect to this test as well as sickle cell trait among the Jats and Brahmins of Sampla, Haryana. Low percentages of Nestroft-positives and sickle cell trait suggest a low frequency of haemoglobin abnormalities, including thalassaemia, among them.

INTRODUCTION

Haemoglobinopathies are the common genetic disorders worldwide. The incidence of genetic disorders can be reduced remarkably by genetic counselling and prenatal diagnosis. For effective counselling the population at risk needs to be identified. Determination of the red cell indices, haemoglobin electrophoresis and HB F and HB A2 level estimation can be used for the identification of α -thalassaemia heterozygotes and other haemoglobinopathies like sickle cell anaemia. However, these techniques are time consuming and expensive for population screening. Kattamis et al. (1981) detected individuals with heterozygous α -thalassaemia on the basis of red cell osmotic fragility. This simple and inexpensive test, NESTROFT (Naked Eye Single Tube Red Cell osmotic fragility test) has been used for population surveys (Kattamis et al., 1981; Mahadik et al., 1986; Mehta et al., 1988; Gorakshaker et al., 1990; Raghavan et al., 1991; Suri et al., 2001; Saraswathy et al., 2001; Murry et al., 2003) and its sensitivity and specificity has been evaluated against electrophoretic methods. Kattamis et al., (1981) and Raghavan et al., (1991) reported that common haemoglobinopathies like HB E and HB S could be detected through NESTROFT.

Thomas et al. (1996) evaluated the NESTROFT against a high performance liquid chromatographic (HPLC) method for its usefulness in screening for β -thalassaemia and the common haemoglobinopathies and found it a reliable test. According to them, it is easy to perform, simple, inexpensive and does not require sophisticated equipment. It has a sensitivity ranging from 94 to 99 per cent (Kattamis, et al., 1981; Mehta et al., 1991; Manglani et al., 1997).

MATERIALS AND METHODS

Blood samples were collected in micro tubes containing EDTA from 225 individuals of either sex in case of Jats and 223 those of Brahmins. Three to four drops of blood were taken from the fingertip of each individual by pricking with a sterilized dispensable lancet. The samples collected on each day were kept in the icebox and transported on the same day to the field laboratory. Test was performed as per the method given by Mehta and Ramakrishna (1994) as follows. When 20 micro liter of blood is put in 2ml of 0.36% buffered saline solution, the cells which have normal discoid cell shape rupture and the solution becomes transparent which can be verified, by holding the tube against dark black lines drawn on a piece of white paper, the lines would be clearly visible. The abnormal cells fail to show this result as they have altered osmotic fragility and do not rupture and remain suspended in the solution thus making the solution translucent to almost opaque such that the lines are either invisible or are blurred.

The result of NESTROFT can be conveniently divided into three categories:

Those which show clear lines when the tube is held against the lines on the paper are referred to as NESTROFT negative, while those in which the lines are not seen at all or the solution turns opaque, are referred to as NESTROFT positive, and those in which the partial blurring is encountered are said to be NESTROFT doubtful.

Haemoglobin S was screened by sodium metabisulphite test (Daland and Castle, 1948) and confirmed by agar gel electrophoresis using sodium citrate buffer, pH 6.2 (Garrick et al., 1973).

RESULTS AND DISCUSSION

NESTROFT test was carried out to find out the abnormal osmotic fragility of red cell that could occur due to variety of reasons giving rise to altered shape and functioning of red cell. The red cells whose shape has been altered due to defective genes or whose functioning has been altered due to production of certain protein in less than normal amount show the positive test.

While the NESTROFT gives us picture of abnormal red cell functioning we cannot yet, ascertain as to what is the reason for the abnormal functioning of red cells as it may happen due to various reasons. NESTROFT positive individuals may have Sick cell anemia or Thalassemia and other hemoglobin variants such as HB D that also give positive test with this technique. G-6 PD deficient individuals also give positive result with this test. Therefore further confirmatory tests have to be performed if the status of the individual abnormality is to be identified.

Table 1 lists the distribution of NESTROFT observations among the Jats and Brahmins of Sampla. It shows that among the Jats 2.2 percent were NESTROFT –positive and 4 percent were doubtful, whereas among the Brahmins, the corresponding figures were 1.79 percent and 3.59 percent, respectively.

Accordingly when we compare the data for Chi-square test to assess the level of significance to measure genetic similarity or dissimilarity it is found that it is producing a value of 0.16, which when plotted against 2 degrees of freedom give a nonsignificant result with corresponding

probability value of 0.9226, indicating that these two populations are genetically close with respect to this parameter. Such commonality can be assigned to the environmental influence, as no occurrence of intermixture has been found among these populations so that to influence the genetic structures of each other.

Table 2 shows a comparative account for HB*S gene distribution among Brahmins along with its neighboring Jat community. Data shows that among 223 samples of Brahmins only one individual is found to have heterozygous AS status and rest 222 individuals are found to possess normal hemoglobin Hb AA. Among 225 Jats individuals tested, there is 100% occurrence of normal hemoglobin, HB AA. There is not a single incidence of either HB SS or any other kind of hemoglobin variants HB (D, E, C etc.) in either of the two caste populations studied here from Haryana.

Among the Indian population groups the frequency of sickle cell trait varies from complete absence to 0.410. Chahal and Bansal (2005) reported the frequency of Haemoglobin variant to be 0.7% in Himachal Pradesh, 0.9% in Uttaranchal, 1% in Punjab and 1.1% in Haryana, with an overall figure of 0.9% for people of North India. It is present in high frequency among the Scheduled Tribes (0.054) as compared to other ethnic groups. It is present in negligible percentage among castes of so called high status, though it is present in Scheduled Castes with a frequency of 0.024. The trait is reported to be present among those Scheduled Castes and communities who are living in close proximity with tribal populations. This may be explained by certain amount of genetic admixture (Bhasin

Table 1: Distribution of NESTROFT results among Jats and Brahmins of Sampla

Population	No. of samples	NESTROFT observations					
		Positive		Doubtful		Negative	
		No.	%	No.	%	No.	%
Jats	225	5	2.22	9	4.00	211	93.77
Brahmins	223	4	1.79	8	3.59	211	94.62

(Figures in parantheses are percentages.)

Table 2: Distribution of Haemoglobin Types among Jats and Brahmins of Sampla

Population	No. of samples	Haemoglobin (HB) Phenotypes						Allele Frequencies	
		AA		AS		S S		HB*A	HB*S
		No.	%	No.	%	No.	%		
Brahmins	(223)	222	0.9955	1	0.004	0	0	0.9978	0.0022
Jats	(225)	225	100	0	0	0	0	1.0000	0.0000

et al., 1992, 1994; Bhasin and Walter, 2001). Our findings are in agreement with the observations made by Bhasin and Walter (2001).

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