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Race as Determinant of Red Blood Cell Osmotic Stress Haemolysis in South Indian and African Populations

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HTH and CJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EIO, OHS and KRN managed the analyses of the study. Authors NFO and ESG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was to compare the red blood cell osmotic fragility between South Indian and African ethnicities. A cross sectional study was performed in the Department of Physiology, Kasturba Medical College, Manipal. The study involved apparently healthy young adults between 18 to 40 years old. The whole study's total sample size was 50 healthy individuals, 25 South Indians (13 female and 12 male) and 25 Africans (13 female and 12 male). The results showed an increase in the percentage haemolysis that was statistically significant (p=0.0001) in South Indians (32.16

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±5.048%) compared to Africans (20.01 ± 3.151%), respectively. The present study has identified and quantified race's impact as one of the determinant factors of red blood cell osmotic stress haemolysis. Therefore, we conclude that the percentage of haemolysis is higher for the South Indian population than the African population.

Keywords: Ethnicity; determinants; red blood cell osmotic stress hemolysis; South Indian origins; African origins.

1. INTRODUCTION

Osmotic fragility (OF) can be defined as the percentage haemolysis obtained when RBCs are immersed in a non-isotonic solution under osmotic stress [1]. Osmotic stress haemolysis accounts for RBCs plasma membrane's ability to withstand rupturing when exposed to pressure difference created by placing the cells in a hypotonic solution [2]. Due to pressure differences, the water will continue to move into the cell until the pressure across the membrane becomes equal or the plasma membrane burst [3]. Haemolysis is usually recognized by free hemoglobin in the RBC-suspending media [4]. Free haemoglobin may be discharged to the surrounding media due to membrane rupture or when membrane bound haemoglobin is lost in macrovesicles [5]. The suspended free haemoglobin may consequently cause damage various tissues, such as injury to endothelium and kidney tubules [6]. Under the osmotic stress haemolysis, RBCs swell and become rounded; however, the cell's surface area doesn't change. This results in cell lysis, and consequently the haemoglobin leaks via haemolytic pores formed in the stretched membranes of swollen spherical cells in a hypotonic medium [6].

The osmotic fragility test is frequently employed to assess the integrity of the RBC membrane. The results obtained from this test are clinically significant as they may give diagnostic features for certain diseases. RBCs that are likely to burst in slightly hypotonic solution have increased osmotic fragility; this may indicate hereditary spherocytosis, chronic lymphocytic leukaemia, or transfusion acquired haemolytic anaemia. The ratio of intact RBCs in hypotonic solution is always an indicator of osmotic fragility. The acidic solution made erythrocytes less tolerant to hypotonic solution by inducing spherical shape change [7].

The osmotic fragility (OF) test accounts for the proportion of RBCs haemolytic change caused by osmotic stress [8]. Several authors have stipulated that the RBC cell ghost can be produced by dialyzing a sample of RBCs against non-isotonic solution or distilled water [9]. The aforesaid haemolytic procedure releases free haemoglobin with deficient ionic strength compared to RBCs' direct exposure to the hypotonic solution [10]. Therefore in the present study, the fragility test's underlying principle will solely depend on the degree of the membrane's resistance to rupture due to a decrease in salt concentration of the media. Experimentally, the conventional osmotic fragility test consists of measuring the intensity of light transmitted through hemoglobin solution produced by the suspension of erythrocytes in a hypotonic media [11]. The light wavelength commonly used is λ = 540 nm, where only hemoglobin, as a major protein of the RBC, contributes to light absorption [12]. Osmotic fragility is defined by shifts in the haemolysis curve, which relates absorbance versus NaCl concentration, and is often established by the determination of 50% of the haemolysis points [13]. Osmotic fragility is widely used to elucidate mechanisms of the influence of different factors on RBC membranes' osmotic properties, such as shear stress and mechanical haemolysis, temperature, ultrasound effects, drugs, and irradiation [8]. The osmotic fragility test is also useful for diagnosing certain haematological diseases, for example, haemolysis anaemia, hereditary spherocytosis, and elliptocytosis, glucose-6 phosphate dehydrogenase deficiency, and sickle cell anemia, as well as for RBCs from uremic or diabetic patients [14]. The osmotic fragility curve of red cells not only reflects the average membrane and cytoplasmic properties but may also provide information on the distribution of those properties within the sample [15].

Osmotic fragility may be influenced by ethnicity, sex, age, temperature, pH, transportation, inappropriate storage conditions, defects in the RBC membrane, pre-incubation in plasma, and proteolytic digestion [16]. Genetic variability is

anticipated to be high in donors with racial admixture from malaria endemic regions such as Africa and Asia [17]. A study conducted in Nigeria (African) male has stipulated that there are no significant differences in membrane's stability between fresh (non-stored) RBCs and RBCs stored for short period up to (24hrs) when subjected to osmotic stress haemolysis. In this study, the non-stored RBCs (0hr) and those stored up to (24hrs) haemolyzed in the range of 0.4-0.5% NaCl concentration, which accounts for about 10% haemolysis[18]. Haemolysis was nearly completed at NaC1 concentration of 0.2 - 0.1%, where more than 90% erythrocytes lysis had taken place. On the other hand, the blood samples stored up to 48 hours showed a significant difference in membrane stability than the samples above. They were found to start haemolysing at a 0.4-0.3% NaCl concentration gradient.

Several studies have focused on the RBC's haemolytic change during blood bank storage; however, the clear authentication of racial/ethnic, age, and gender influences the osmotic stress haemolysis non-stored RBCs is lacking. Therefore the present study aimed at determining the influence of racial/ethnic groups (South Indians and Africans) on osmotic stress haemolysis in non-stored RBCs.

The study was done to compare the red blood cell osmotic fragility between South Indian and African ethnicities.

2. MATERIALS AND METHODS

The study was a cross sectional study, performed in the Department of Physiology, Kasturba Medical College, Manipal. The study was conducted after getting approval from the institutional ethics committee of Kasturba Medical College, Manipal Academy of Higher Education, Manipal, and IEC number 102/2018 was obtained.

2.1 Human Subjects

The study involved apparently healthy young adults between 18 to 40 years study at Manipal of South Indian origin and African origin who are all students of various courses. The whole study's total sample size was 50 healthy individuals, 25 South Indians (13 female and 12 male) and 25 Africans (13 female and 12 male).

2.2 Inclusion Criteria

Healthy students, both male and female aged 18-40 years studying at Manipal of south Indian origin (native or residence of south Indian) & healthy students of African origin, were included in this study.

2.3 Exclusion Criteria

Patients with blood disorders such as anaemia, sickle cell anemia, and haemophilia were not part of the study.

3. METHODOLOGY

3.1 Preparation of Buffered Sodium Chloride Solution

3.1.1 Buffered sodium chloride

Stock solution pH - 7.4.

Dissolved: 22.5 gm NaCl, 3.41gm of Na_2HPO_4, and 0.59gm of NaH_2PO_4 in 100ml distilled Water.

3.1.2 Buffered NaCl solution 1%

Dilute 2 ml of stock solution to 20ml with distilled water.

3.2 Blood Sample Collection and Processing

The blood sample of 5ml was collected from the brachial vein using a syringe and transferred to a heparinized test tube according to standard operating procedures, and all sterile precautions of collecting blood were followed. Solutions of increasing hypo tonicity were prepared by mixing the 1% sodium chloride (NaCl) solution and distilled water in serially numbered test tubes from 1-18, to make the desired and relative concentrations of buffered 1% NaCl, the concentration of buffered NaCl %, distilled water to prepare NaCl solution of various strengths as shown in the Table 1.

These procedures for testing osmotic fragility tests are as per the standard operating procedures followed by Kasturba Medical College and Hospital in the Department of Haematology and Pathology.

- 5 ml of whole blood was placed into a test tube rack containing 12 glass beads.

- 0.03 ml heparinized blood sample was added to each test tube containing buffered saline solutions of varying concentrations. The procedures were repeated for all blood samples.
- Each tube was mixed gently and slowly by using Parafilm.
- The tubes were allowed to stand for about 30 minutes at room temperature.
- The tubes were remixed gently and centrifuged at 2000 rpm for 5 minutes.
- Each test tube's supernatant was carefully transferred to cuvettes, and readings were recorded on a spectrophotometer at a wavelength of 556 nm.
- The optical density was set at '0' using the distilled water.
- The controls for this experiment were isotonic solution (normal saline) and distilled water (tonicity nil), wherein tube number 1 represents the 0% haemolysis (show no haemolytic change) while test tube number 18 represents 100% haemolysis (show complete haemolysis).
- Finally, the observed degree of osmotic haemolytic change among ethnic groups (south Indians and Africans) and gender were compared, and inferences were drawn.

3.2.1 Outcome measures

Percentage osmotic hemolysis for each supernatant was calculated as hereunder.

> % of haemolysis = (Optical density of supernatant /Optical density of supernatant in tube with 100% hemolysis) x 100

The optical density of supernatant in a tube in which no haemolysis has occurred, RBCs were seen forming a red dot at the bottom of the tube with a clear supernatant saline solution.

The optical density of supernatant in a tube in which some hemolysis has occurred, the saline was tinged red with haemoglobin, and some RBCs were seen as a red dot at the bottom of the tube; this is called the onset of fragility.

The optical density of supernatant in which hemolysis complete (100% haemolysis) has occurred, saline solution was uniformly red with no red mass at the tube, called ending of fragility.

3.3 Statistical Analysis

The range of concentration of NaCl was expressed for commencement and end of haemolysis in both South Indian and African groups.

The statistical analysis of the independent sample t-test was performed to compare the mean percentage of haemolysis between the two ethnic groups with the value of significance at 0.05 level.

Table 1. Distilled water to prepare NaCl solution of various strengths

Tube number	Buffered 1% NaCl (ml)	Distilled water in (ml)	Conc. of buffered NaCl % (ml)
1	5.00	0.00	1.00
2	4.25	0.75	0.85
3	4.00	1.00	0.80
4	3.75	1.25	0.75
5	3.50	1.50	0.70
6	3.25	1.75	0.65
7	3.00	2.00	0.60
8	2.75	2.25	0.55
9	2.50	2.50	0.50
10	2.25	2.75	0.45
11	2.00	3.00	0.40
12	1.75	3.25	0.35
13	1.50	3.50	0.30
14	1.25	3.75	0.25
15	1.00	4.00	0.20
16	0.75	4.25	0.15
17	0.50	4.50	0.10
18	0.00	5.00	0.00

Table 2. Shows the summary of results for the % haemolysis for the south Indian and African races

Criteria	Starting of haemolysis		End of haemolysis	
	Conc. of NaCl % Max – Min Range =(Max-Min) Mean=	% Of haemolysis Max – Min Range =(Max-min) Mean of % haemolysis	Conc. of NaCl % Max – Min Range =(Max-Min) Mean =	% Of haemolysis Max – Min Range =(Max – Min) Mean of % haemolysis
Ethnicity for South Indian	0.50 – 0.35 Range = 0.15 Mean = 0.42	41.00 – 6.00 Range = 35 % Mean = 32.16%	0.10 – 0.00 Range = 0.10 Mean = 0.06	100 – 100 Range = 0% Mean =100%
Ethnicity for African	0.50 – 0.35 Range = 0.15 Mean = 0.41	26.00 – 5.00 Range = 21 % MeaN = 20.01%	0.10 – 0.00 Range = 0.10 Mean = 0.04	100 – 100 Range = 0% Mean =100%

Table 3. Shows the comparisons of results for the % haemolysis in the south Indian and African races

Group	Number of Subjects	Mean ± sd	T-value	P-value
South Indian Origin	25	32.16± 5.048	12.8955	0.0001
African Origin	25	20.01± 3.151		

Table 4. Shows the comparisons of the number of tubes with haemolysis among the South Indian and African populations. The South Indian population exhibited a significant number of haemolysis within the tubes. This shows a higher cumulative degree of haemolysis among the South Indians compared to the Africans

Group	Number of tubes with haemolysis (n)	Number of tubes without haemolysis (n)	Total
South Indian Origin	275 (245)[3.67]	175 (205)[4.39]	450
African Origin	215 (245)[3.67]	235 (205)[4.39]	450
Total	490	410	900

X^2= 16.13, DF=1, p- value = 0.00059

n - Represents the total number of test tubes where haemolysis occurred and where it did not occur. Chi Square was used to compare these numbers among the South Indian and African populations. The significant level was at 0.05

4. RESULTS

Most RBCs of the Indian population are completely haemolysed at relatively higher NaCl concentration compared to the African population.

The mean percentage haemolysis for South Indian origin subjects was significantly higher at 32.16% compared to 20.01% for subjects of African origin. This shows that the osmotic fragility of South Indians is more elevated than African.

5. DISCUSSION

This study aimed to compare red blood cell osmotic fragility based on ethnicity on subjects of South Indian and African origin. The results showed an increase in the percentage of haemolysis that was statistically significant (p=0.0001) in South Indians (32.16±5.048%) compared to Africans (20.01±3.151%), respectively. This indicates that the South Indian population's RBCs are more fragile as most RBCs are completely haemolysed at relatively higher NaCl concentration than the African population. Although the inheritance has been attributed to haemolysis, the clear justification is lacking as the correlation between the haemolysis and the decrease in the cell's energy's intracellular concentrations has not been established [19]. This could be attributed to stronger RBCs' resistance to osmotic fragility in the ethnic African population compared to the South Indian population. This could also be speculated about the genetically virgin/ pure human species that originated in the African subcontinent. When the people migrated to the Indian subcontinent through Europe and South East Asia, it is possible that the native genetic pool would have undergone multiple mutations

resulting in more fragile RBCs in the South Indian population [20]. Or the other possibility is since it is said that the South Indian population and African population have originated from two genetically different groups (African population derived from the South part of Africa and the South Indian population originated from Lakshadweep islands), these fundamental genetic differences could have contributed to this difference in osmotic fragility [20]. Studies have demonstrated the impact of donor characteristics on RBC predisposition to spontaneous and stress haemolysis after cold storage consistent with routine blood bank practice in large ethnically diverse blood donors' cohorts. In their study, the male sex, Asian and African race/ethnicity, and older age were reported to have the strongest associations with spontaneous or stress haemolysis [17].

The most profound association between race/ethnicity and osmotic haemolysis was observed in African individuals and osmotic stress haemolysis, the unique resistance of African individuals' RBCs to osmotic fragility may stem from the high prevalence of genetic traits for haemolytic diseases (example, sickle cell disease, and thalassaemia) in the blood of individuals for African descent that are known to reduce osmotic haemolysis [21].

The broader range of haemolysis in the south Indian population also confirms its heterogeneity. This can be attributed to population genetic studies' findings that indicate that the present South Indian population is genetically heterogeneous due to its population dynamics [22]. The influx and admixture of various genetically distinct migrant populations from the Middle East and Southeast Asian countries to South India would have contributed to the present south Indian population [23].

6. CONCLUSION

The present study has identified and quantified the impact of race/ethnicity as one of the determinant factors of red blood cell osmotic stress haemolysis. In conclusion, the percentage of haemolysis is higher for the South Indian population than the African population. This study could be an eye opener on the racial, ethnic, and even regional differences that could have an impact on the interpretation of laboratory results.

CONSENT

Informed consent was taken from healthy volunteers.

ETHICAL APPROVAL

The study was conducted after getting approval from the institutional ethics committee of Kasturba Medical College, Manipal Academy of Higher Education, Manipal, and IEC number 102/2018 was obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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