BERKELEY PUBLICATION
AND RESEARCH INTERNATIONAL
12.31.2020
Journal of Med., Biological and Pharm. Sci. Vol. 14 No. 3

STUDY OF FRAGILITY OF ERYTHROCYTES IN VERTEBRATES (NORMAL HUMAN CELL, HUMAN SICKLE CELL, COW, SHEEP, GOAT AND CHICKEN) IN SOKOTO, NIGERIA

YUSUF SARKINGOBIR; UMMU TUKUR; & NAFISA ABDURRAHMAN ASHAFA
Department of Biology, Shehu Shagari College of Education, Sokoto, Sokoto state, Nigeria

ABSTRACT
Erythrocytes are important in the transport of oxygen to the tissues in the body. The need to preserve the integrity of erythrocytes played by their membranes is enormous.This work studied erythrocytes fragility in some vertebrates (normal human cell, human sickle cell, cow, sheep, goat and chicken), using the standard method. The results revealed that, normal human red blood cells (NHRCs) have lower fragility than sickle cells (SCs).Haemolysis (fragility) was higher from cow down to goat's red blood cells(RBCs).Chicken's RBCs possessed highest level of fragility, surpassing all. Conclusively, there are varying levels of fragility among vertebrates (erythrocytes).

Keywords: *Erythrocytes, erythrocytes fragility test, red cells, NaCl , osmosis, mean cell volume , surface-are - volume- ratio*

Introduction:

Erythrocytes are one of the vital components of blood in the biological system. They are composed of the haemoglobin (I.e oxygen carrying pigment that is parcel of the blood). Haemoglobin (situated in the red cells) is responsible for distribution of oxygen (the oxidizing agent in oxidative phosphorylation or ATP synthesis). At least, the organism cannot live without oxygen or cannot regain the complete ATP of the food fuels. Noteworthy, this inevitable function of haemoglobin of red cells is

ISSN: 1211-4401

Only feasible if it's integrity is intact [1] .This can be tested using a test known as "Fragility test".

The degree of resistance of red blood cells to a diminished salt content of their environment has long been utilized as a yardstick to measure their viability and in diagnostic characterization[2]. Basically, the principle is, when population of red cells reaches a certain volume (haemolytic volume), the haemoglobin will definitely diffuse to equilibrium inside and outside the cell, without rupture of the plasma membrane of the cell[3]. Any given population of red cells have individual cells that differ in the volume at which the haemoglobin will diffuse out. The osmotic resistance of that population of red cells follow a normal probability distribution. This is the assumption of " Fragility test " [4].In other words, by the time red blood cells are placed in hypotonic solution with reduced osmolarity the increase in it's water is both instant and quantitative. This behaviour is put to practical use in red blood cell (RBC) osmotic fragility test, which determines the movement (haemolysis) of haemoglobin from red blood cells in hypotonic sodium chloride solution. This make osmotic fragility index as a tool to measure the resistance of red cells to dissolution by osmotic stress [5].

Osmotic fragility is useful to quantify the level of stability and functionality of plasma membrane, erythrocyte mean cell volume (MCV), surface area-to- volume ratio (SAVR) and diagnosis of hereditary spherocytosis [5]. Some factors that affect osmotic fragility are: pH, temperature, rate of attainment of equilibrium, and chemical and environmental changes [3].Consequently, osmotic fragility is well known useful tool in diagnostic and research fashions in vertebrates but, its values are mostly considered in humans. Other vertebrates like birds are not been mostly considered.This trend may not be unconnected with the inaccuracy in the application of saline-solution technique use in [3] to the measurement of erythrocytes fragility of birds.Thus, [7] developed a minor modification of Parpart et al , (1947) technique for determining osmotic fragility curves, which they believed to be applicable to birds.While this test has been known since around 1920s, to date the data measuring the fragility of red cells in vertebrates is very limited [8]. This study will help in filling the knowledge gap.It main objective was to measure the specific fragility of some vatebrate cells (normal human cell, sickle human's cell, cow, sheep, goat and chicken .

MATERIALS AND METHODS

Sampling and Sample preparation

Suitable procedure have been used for sampling and sample preparation. Sample containers were disinfected using detergent for the equipments to be free from microorganisms and safe enough for laboratory use. All samples collected were stored at standard temperature. The samples collected for analysis were: blood of different vertebrates.

Blood samples

The blood samples used include :

1. Sickle cell blood was collected from Sickle cell patient in Gwadabawa, Sokoto state, Nigeria in EDTA container.
2. Normal human blood was collected in Gwadabawa, Sokoto state, Nigeria by venipuncture in EDTA container.
3. Blood sample from goat, sheep and cow were collected in Gwadabawa veterinary clinic (Sokoto state, Nigeria) through jugular vein in EDTA container.
4. Blood sample from chicken was collected after slaughtering and drived in EDTA container[9,10] .

Analytical Methods

Principle: Test and normal red cells were placed in a series of graded-strength sodium chloride solutions and any resultant haemolysis is compared with a 100%.

Procedure:

Twenty micro litre of fresh blood from normal human, sickler human , cow, sheep, goat and chicken each were placed in a set of three test tubes containing 5ml of salt concentration of 0.30, 0.40 and 0.50% respectively.Then mixed by inverting the tube and centrifuged at 1500 R.P.M.(Revolution Per Minute) for 10 minutes.The supernatants were measured spectrophotometrically at 540nm wavelength.The percentage haemolysis for each sample was calculated using the formular:

$$\text{Haemolysis}(\%) = \frac{\text{OD (optical density)of the test}}{\text{OD(optical density) of the standard}} * 100$$ [9,10] .

Statistical Analysis

The results obtained were analysed using one-way ANOVA by instat3 software (Version: San Diego, USA) and presented as mean ± Standard

Error of mean.Difference between means are considered significant at $p<0.05$.

RESULTS

Table1 : Fragility of the erythrocytes of some vertebrates

Percentage haemolysis in NaCl solution				
S/No.	**Organism category**	**0.30 NaCl Solution**	**0.40 NaCl Solution**	**0.50 NaCl Solution**
1	Human normal cell	40.00±0.01%	36.00±0.05%	34.26±0.04
2	Human sickle cell	78.00±1.28%	74.00±4.20%	72.00±0.80%
3	Cow	96.00±0.68%	94.00±0.68%	88.00±0.02%
4	Sheep	89.29±0.02%	85.29±1.70%	79.29±2.87%
5	Goat	80.00±1.02%	74.00±4.20%	72.00±0.80%
6	Chicken	132.00±2.28%	124.00±2.40%	116.00±1.45%

All results are expressed as mean ± standard error of mean, % = percentage haemolysis (fragility).

DISCUSSION

The erythrocyte is well-equipped with a specialized cytoskeleton providing membrane with adequate support.The essence of fragility test is to see at which concentration haemolysis start and at which it become complete.Osmotic fragility is a potent and cheap test of membrane stability in experimenal and diagnostic settings [11]. It is widely used to explain the mechanisms of the influence of different factors on the osmotic properties of red blood cell (RBC) membranes, such as shear stress ,mechanical stress ,temperature, ultrasound effects ,drugs , and irradiation.Similarly, it is useful in diagnosis of haematological diseases, such as, haemolytic anaemia, elliptocytosis ,glucose-6-phosphate dehydrogenase deficiency, sickle cell anaemia ,uremic and diabetic conditions[12].

The result of this study revealed a significant difference between human normal cell and human sickle cell.There was also, significant difference from cow down to goat, while chicken possessed very extreme value compared to others.In this result, the osmotic fragility of human's sickle cell is higher than that of normal human.This may not be unconnected with the extreme fragility of the sickle cells [8].Nevertheless, varying cells have

varying volumes of haemolysis [4].That is why the overall specific percentage fragility of the categories examined in this work differs. There was an increased trend in haemolysis from Cow, down to Goat, the vertebrates. But they are higher than both the normal and sickle cells human, and the chicken possessed very extreme value. This partially dispels the statement of [7] , which says " mammals have higher fragility than birds, except for camelidae due to different structural characteristics and elastic properties of their membranes [3,6,13,14].Samples examined in this study possessed varying degree of erythrocytes fragility. To a much surprise, the chicken possessed very extreme fragility value more than others examined in this study which partially deviate from the finding of [7].

The chicken possessed the highest fragility above all.This might be due to the spherical nature of their cells.Spherical cells have less ability to withstand stress or accommodate much water inflow to resist haemolysis than the biconcave cells [15,16,17]. Additionally,the chicken can have access to water easily,so there is no much need to allow much inflow into the cells like in camel(who had very high osmotic resistance and ability to accommodate much water influx). Whereas,normal human cell revealed more osmotic resistance than the sickle cell , because of deformation in the sickle cell [18].Cow,sheep,and goat might have elevated % fragility because,they need not to conserve much water compared to the camel ,who has much osmotic resistance in a bid suitable for specific water interaction behaviour [19,20,21] .

CONCLUSION

The vertebrates (human normal red cells, human sickle cells, cow, sheep, goat and chicken) examined in this study possessed varying degree of erythrocytes fragility.

REFERENCES

Greishmer, M.E. and Troyer, R.J.Physiology and Anatomy: with practical considerations. 7th edition. Lippincott company, USA;1955.

Jacobs MH.The complex nature of the effects of temperature on the rates of certain biological processes. The Am. Naturalist.1928; 62: 284-289.

Hunter FT. Photoelectric method for the quantitative determination of erythrocyte fragility .J. Clin. Invest. 1940; 19: 691-694.

Arthur, K. Parpart; et al, .The osmotic resistance (fragility) of human red cells. J. Clin. Invest.1947; 26(4):636–640.

Oyewale JO. Effect of storage of blood on the osmotic fragility of mammalian erythrocytes. J. Vet. Med.1993 A40:258-264.

Chikezie PC.Osmotic fragility index of HbAA red blood cells in the presence of aqueous extracts of three medicinal plants (Aframomum melegueta, Garina kola, and Cymbopogon Citracus). Global J. of Pure and Appli. Sci.2007; 13 (4): 496-499.

Viscor G, Palomeque, J.Method for determining the osmotic fragility curves of erythrocytes in birds. Lab. Ani.1982;16:48–50.

Harika, P.K., Asha, L.P, Praduya S. et al. Comparative study of erythrocyte fragility in diabetes mellitus and non-diabetes mellitus. Int. J. Med. Res. and H. Sci. 2015;4 (1): 182-185.

Olorunshola KV, Eze KO, Achie LN. Effect of road transport stress on erythrocytes osmotic fragility (EOF) of healthy young adult Nigerians during the harmattan season .Nig. J Physiol. Sci.,2012;27:157-164.

Charles AT, Bagudu UG, Osaro E, Tosan EC.Effect of storage on osmotic fragility in CPDA-1 stored blood in Sokoto, Northwestern Nigeria.Adv.Haema. Onco. Res,2018;1(1):1-6.

Wodu, E., Ewakwe, A.A. and Monamu MO. Osmotic fragility index and stability of human erythrocytes in the presence of four oral antiretroviral drugs .Scholars J. Appli. Medi. 2015;3(2E):884-887.

Okwushi IJ. Osmotic fragility in stored non-detergent wash human erythrocytes. Afri. J. Biomed. Red.2011;14(2):143-146.

Walski T, Chludzinska L, Komorowska M, Witkiewicz, W.Individual osmotic fragility distribution: a new parameter for determination of the osmotic properties of human red blood cells .Biomed. Res. Int.2014;1-7.

Adegoke, S. H. et al,(2014) . The effect of aqueous extract of allium sativum(Garlic) on erythrocyte osmotic fragility in human normal and sickle cells. Ameri. J. Life sci. 2014;2(5):278–281.

retrieved,31, January,2020.

Uzoigwe C. The human erythrocyte has developed the biconcave disc shape to optimize the flow properties of the blood in the large vessels.Med Hypotheses,67(5):1159-63.

Windberger U, Baskurt OK. Comparative haemorheology . Handbook of haemohaelogy and haemodynamics,2007;268-285.

Silva ID, Kuruwita VY. The osmotic fragility of erythrocytes of the Asian elephant (Elephas maximus) .GAJAH,1994;13:25-29.

Oyewale J, Dzenda T, Yaqub L, Aoanbi D, Ayo J, Owoyele O, Minka N, Dare TM. Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature,pH, and blood storage .Vet. Archiv.,2011;81(4):459-470.

Igbokwe NA.A review of factors that influence erythrocyte osmotic fragility.Sokoto J. of Vet. Sci.,2018;16(4):1-23.

Najim TM . Comparative evaluation of erythrocyte osmotic fragility between anaemic and chronically iron exposed sheep in Iraq.Int. J. Adv. Re. Biol. Sci.,2016;3(11):206-210.