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Osmotic Fragility And Hemolysis% Of Human Erythrocytes Β-Thalassemia Major And Anemia Patients Compared To Healthy Subjects

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ABSTRACT

Contemplating the osmotic conduct and hemolysis% of human erythrocytes isn't just significant clinically, yet it is additionally considerable in comprehension of material transfer across biological membranes. The fragile gram piece of the spot of hemolysis were viewed as an element of extra-cellular osmolarity. The object of this field is to assess the (hemolysis% and osmotic fragility) tests of "β – thalassemia major and anemia" patients erythrocytes compared with healthy subjects and to assess the chance of utilizing any of them as a fundamental screening instrument for "β – thalassemia major and frailty" patients. Hemolysis % and osmotic fragility tests were examined for (40 β – thalassemia major and 25 anemia) patients contrasted with 20 healthy subjects. There was an expansion in the hemolysis % for "β-thalassemia major and anemia" patients by (176 and 273.5) % individually contrasted with the typical erythrocytes. The fragility curve by utilizing NaCl on "β-thalassemia and anemia" patients erythrocytes demonstrated move towards lower NaCl concentration showing that the normal osmotic fragility H50 (the NaCl fixation delivering half homolysis) to be (0.1 and 0.2)% of "β – thalassemia major and anemia" patients Erythrocytes separately contrasted with (0.45 %) healthy subjects. In addition to use different concentrations from saline (NaCl) solution, this study tested the osmotic behavior of human RBCs for all studied groups using H2O2 as another osmolytes. The data are clearly different from to that spotted with sodium chloride. Fragility grams of human RBCs showed that with increase the concentration of H2O2 the H% were increased. Finally, H50 equal to 0.5%, 0.45% for "β – thalassemia major and anemia" patients and 0.5% of healthy subjects.

Keywords: β-thalassemia major, sodium chloride, H2O2, osmotic fragility and red blood cells.

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INTRODUCTION

Every racial group & geographic area around the world have been faced from thalassemia's; while, the most common areas for these diseases expanded from sub-Saharan in Africa, out of the Mediterranean region, Middle East & Arabian Peninsula to the Indian distinguishable part , India and South-eastern Asia , also detected a hights extent in areas historically afflicted with malaria (Weatherall DJ., 2018; Taher AT, *et al*., 2018). The global and most common monogenic human diseases are thalassemia's. Thalassemia's also known as a hereditary anemia's, occur due to mutations in the gene clusters of hemoglobin (Hb) which damage, the rate of synthesis of one or more of the globin chain subunits of the Hbs tetramer(Cappellini MD, *et al*.,2018), so thalassemia is characterized by a defect in the formation of Hb, which results in the improper O_2 transport and devastation of erythrocytes. Adult Hb (HbA), normally, contain (two α- and two β-) globin chains arranged into a hetero- tetramer, but a person with thalassemia may classified as α- thalassemia "result in an decreased α-globin production " or β- thalassemia " result in an excess of β-chains in adults and excess γ-chains in newborns " (Yan Su., *et al*., 2015). Erythrocytes are a nucleated biconcave and containing Hb molecules(Salvagno GL, *et al*., 2015 ; Maurya PK., *et al*., 2015), deliverers O_2 with a high concentration of polyunsaturated fatty acids in cell membranes which made them highly susceptible to oxidative stress that is implicated in the pathogenesis of diseases(Petibois C and Déléris G.,2005; Habif S., *et al.,*2001). A very useful screening test for β - thalassemia trait is osmotic fragility (Bobhate SK, *et al*., 2002) .There is still question needs answer if a changed erythrocytes membrane conductance to understand this disease and possibly a more effective treatment(Badens, C., and Guizouarn, H.,2016). Haemolytic diseases and hereditary spherocytosis were diagnosed by conducting a fragility test which determines the erythrocyte osmotic fragility (Kolanjippan K, *et al*., 2002). Haemolysis estimation under hypo - osmotic stress by measurable quantities in haematology were related to osmotic stability and fragility of RBCs (Igbokwe NA.,2016). Measuring the intensity of light which transmitted through a Hb solution resulted in suspension of RBCs in a hypotonic media using λ = 540 nm where Hb as a major protein of the RBCs suspension absorbed known as osmotic fragility test(S. O. Sowemimo-Coker,2002), while, osmotic fragility acquainted as a shift occurred in the haemolysis curve which is plotting absorbance versus sodium chloride concentration and estimated by (50) % of the haemolysis points(Tomasz Walski,2014).

This study was carried out in order to evaluate the influence of NaCl and H_2O_2 on hemolysis of human RBCs in patients with "β – thalassemia major and anemia" compared to healthy subjects , which reflects a modification in the RBCs membrane integrity; osmotic fragility, which reflects the capacity of RBCs to resist hemolysis.

MATERIALS AND METHODS

Chemicals

All common laboratory chemicals and reagents were obtained from the ALDRICH, Germany Company.

production of washed human RBC suspension

On the day of each experiment, (5 ml) whole blood was drawn from the antecubital vein of (20) normal volunteers who were free from any medication for at least two weeks, (25) patients with anemia as pathological control and (40) β-thalassemia major patients with age ranges from (19 to 48) years to all studied groups, with an average hemoglobin concentration between (7 to 10) g/dl for anemia patients (4 to 6.7) g/dl for thalassemia patients. The patients were under the conduct of clinical particlrist in Azadi Teaching Hospital, during the period from "October to April 2018". Samples were collected by vein puncture into EDTA tubes. Whole blood was separated with centrifugation at (1500 xg for 12 min) and the (plasma, buffy coating, and top layer of cells) were discarded. The remaining packed RBC was washed three times with saline. Then cells were stored at 4C∘, no more than 3 days. On the day of experiment (500) µl of packed RBCs were diluted to (1.5) ml with phosphate buffer saline (PBS).

Hemolysis examines (Desouky, O. ,2005)

Erythrocytes hemolysis was assessed by estimation of Hb delivered from the cell's comparative with the total cellular Hb content. Ten micro litter of whole fresh blood for each thoughtful group were incubated in (5) ml normal saline (0.9% NaCl) for 30 min. The samples were centrifuged at 1500 xg for 10 min, and the supernatant absorbency was estimated calorimetrically at 540 nm. The % of hemolysis was taken against complete blood hemolysis:

$$\%H = A_{sample} / A_{100\%lysis} \times 100$$

Where A_{sample} and $A_{100\%\ lysis}$ is the absorbance of the Hb released from RBCs in normal saline and after complete hemolysis, respectively.

Osmotic Fragility Test

The osmosis procedure was elaborated by mix tiny volumes of blood with solutions of buffer saline, with pH = 7.4 and /or H2O2 with various tonicity within the extent of (1–100). The reaction was completed at RT. The erythrocytes osmotic lysis is identified through liberation of Hb to the extra-cellular fluid. The measure of Hb bringing to light within the media was resolved calorimetrically as per the strategy detailed by Dacie and Lewis (Plummer, D. T.,1987). The fragility curve is drawn by plotting % hemolysis versus [NaCl and / or H_2O_2] concentration using the above information:

$$\text{Percent hemolysis (H\%)} = (A_{test}/A_{distilled\ water}) \times 100.$$

The H% curve was obtained by plotting percent hemolysis against the saline concentrations.

RESULTS

The rate of hemolysis can be successfully managed from the hemolysis test, where the erythrocytes hemolysis rate for (β-thalassemia major and anemia) patients outperforms the standard rate. In this examination, the rate of hemolysis of patient's groups was around (2 to 3-folds) the rate of erythrocytes hemolysis were display in figure 1:

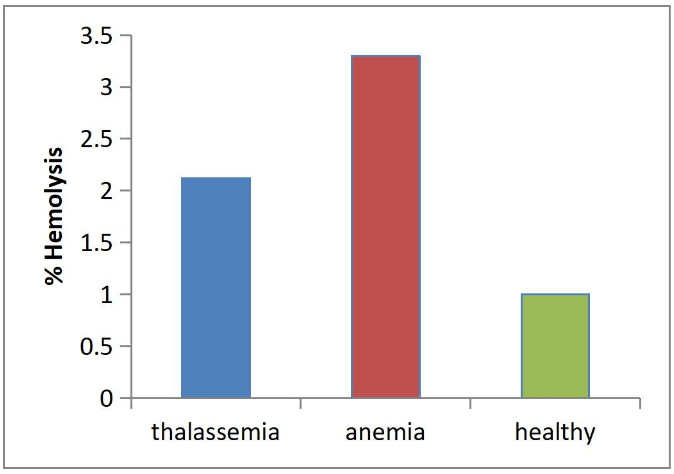

Figure 1. Normal hemolysis for healthy, β- -thalassemia major and anemia erythrocytes.

The hemolysis % of (β-thalassemia major and anemia) patients were found_equal to (176 and 273.5) % respectively compared to the normal erythrocytes.

The average osmotic fragility H50(the sodium chloride concentration creating half % hemolysis), can be assessed by using the fragility curve which demonstrated a move to lower sodium chloride concentration, showing a decline in average of H50 by (22.2 and 44) %, respectively compared to the normal cells, (Figure 2: A, B and C).

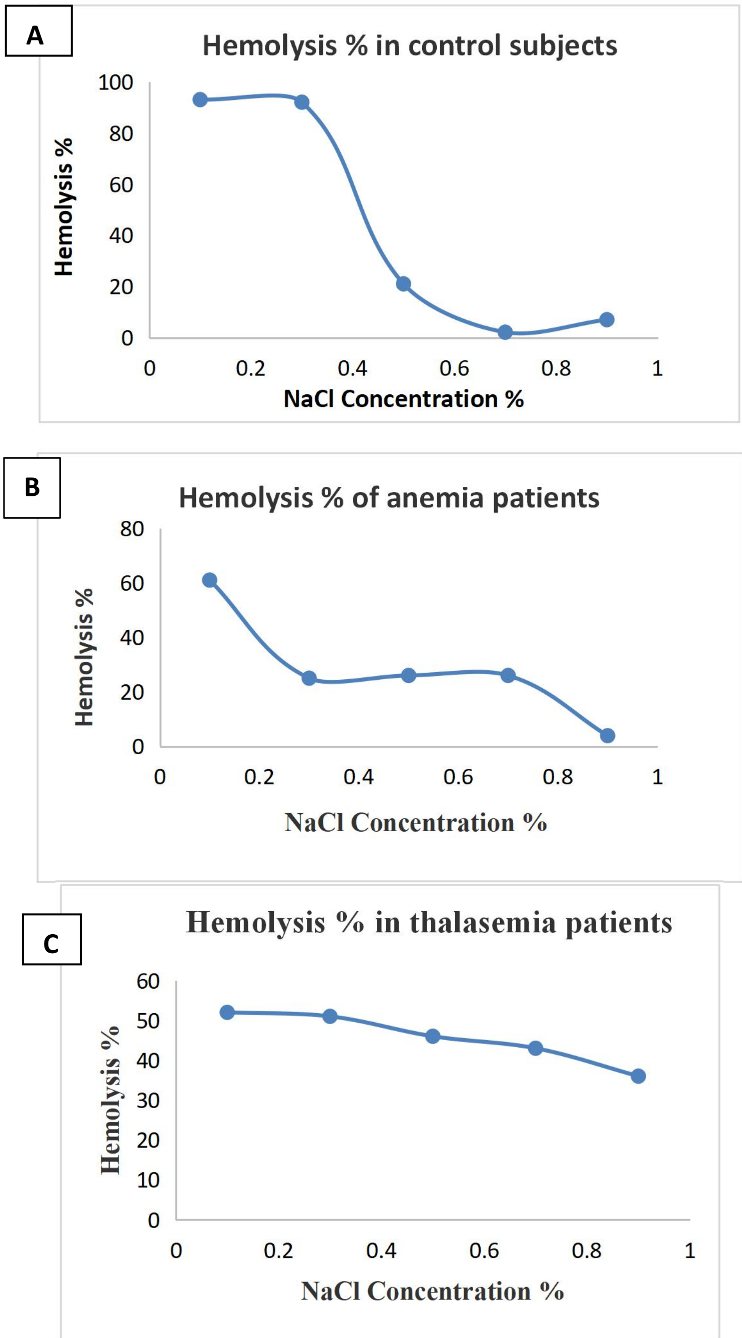

Figure 2. Osmotic fragility of normal, β – thalassemia major & anemia, H50(the NaCl concentration producing 50% hemolysis).

In addition to use different concentrations from saline (NaCl) solution, this study tested the osmotic behavior of human RBCs for all studied groups using other osmolytes. The resulting of monotonic sigmoidal fragile grams had been expected earlier. Figure 3 (A, B and C) shows fragile grams obtained for human RBCs using different concentrations of H_2O_2 as another osmolyte.

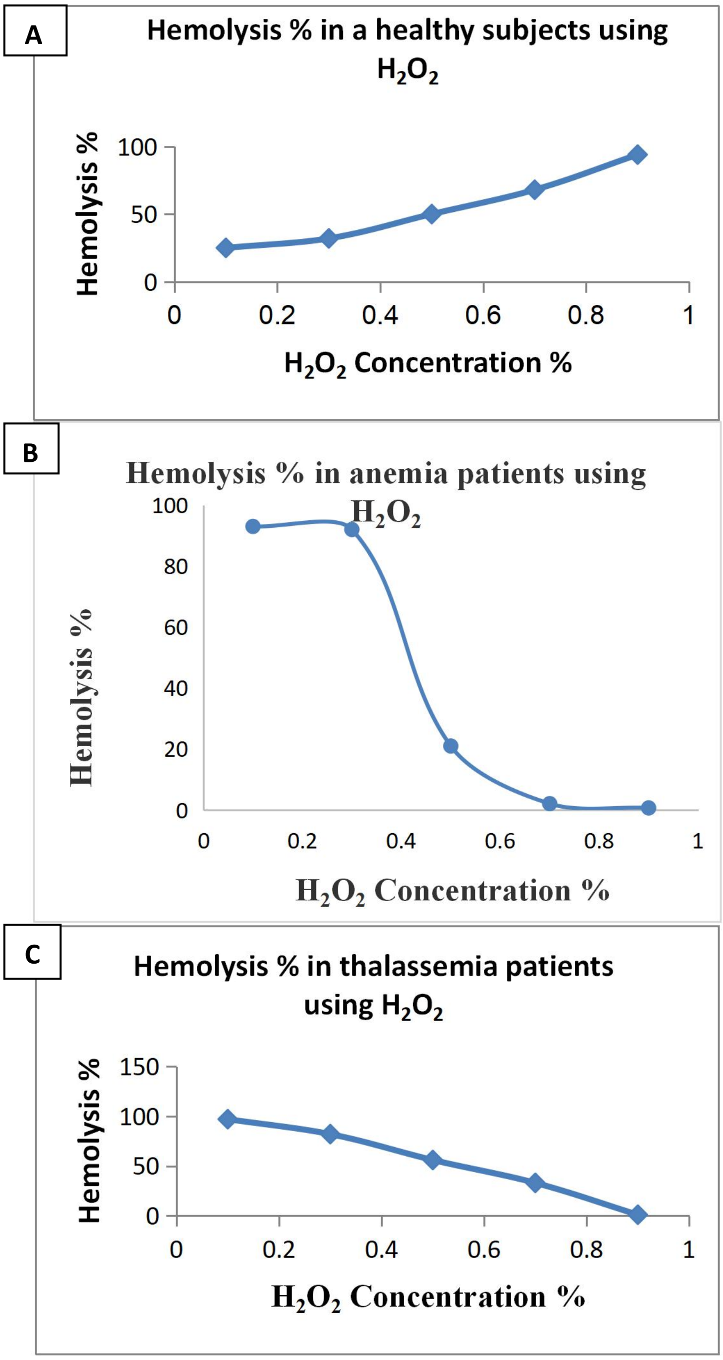

Figure 3. Fragili grams for human RBCs to all studied groups using H_2O_2.

The data are clearly different from to that found with sodium chloride. Fragili grams of human erythrocytes showed that with increase the concentration of H_2O_2 the H% were decreased and for healthy subject group H_{50} equal to 0.5% , while, "β – thalassemia major and anemia" patients H_{50} equal to (0.5 and 0.45)% respectively.

DISCUSSION

The disclosure of non-monotonic osmotic behavior of erythrocytes of patients with (β - thalassemia major and anemia) utilizing ionic osmolytes is of essential significance in this study. This opens the access to the sensational avenues for further detailed studies on term of different channels and/or pumps connected with proper morphological characterizations of human erythrocytes with different diseases. This study would be considerable from the perspective of not only human physiology, but also towards earning mechanistic insights into varying transport phenomena across biological membranes broadly. Nations experiencing high predominance of thalassemia, and restricted monetary wellbeing plan need to build up the successful and financial demonstrative strategies for thalassemia that is significant key preceding treatment. The present study focuses about the biological attribute to (β- thalassemic major and anemia) patients' erythrocytes, including (hemolysis & osmotic fragility) tests. The feature of human erythrocytes in β- thalassemia major and anemia patients can aid in the precise and prompt examination of these diseases established on the study of the erythrocyte's properties rather than the Hb molecule. The results of this study specified that there was difference between patients' groups in compression to the natural erythrocytes samples. The results of this study for the hemolysis tests indicated that there was an expansion in the hemolysis % for (β-thalassemia major and paleness) patients contrasted with healthy subjects , this can be attributed to the exitance of single Hb chain in plenty inside the erythrocytes which bring about oxidative denaturation of its membrane proteins (Schrier, S. L., et al.,1989). The outcomes acquired by utilizing various concentrations of NaCl that there were moving across diminished NaCl concentration, demonstrating an expansion in normal osmotic fragility H50 by (22.2 and 44) % for (β-thalassemic major and anemia) patients contrasted with the typical cells, which might be credited to the higher osmotic opposition of β-thalassemia major and anemia erythrocytes started by the presence of abundance α-globin chains, hence, they have more slow crack rate (Silvestroni, E., and Bianco, I. ,1983). This unusual increment in the α- globin chains brings about oxidative (denaturation and precipitation) as Heinz bodies, with the resulting increment in the surface region to volume proportion, increment in layer inflexibility and abatement in the capacity to go through cell distortion under hypotonic stress(Schrier, S. L., et al., 1989).The results of using different concentrations from H_2O_2 may be due to that the uptake of H_2O gets coupled with the H2O2 uptake as a sustaining resulting in "normal" the fragile grams (i.e. in hypotonic conditions, H_2O found to be able to enter the cells along with H_2O_2 leading to osmotic release of hb but not osmotic rupture) (Snigdha Singh, et al.,2019). Finally, the findings of this study may provide powerful proof towards a motivation aspect of human physiology.

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