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RESEARCH ARTICLE

Validation of Osmotic Fragility Test using a modified new Garden Angelica Reagent in Healthy Individuals Blood, Saudia Arabia

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ABSTRACT

Background: Recently, there has been a global trend toward the use of natural substances present in fruits, vegetable, oilseeds and herbs as medicine and functional food.. Taken together, administration of Angelica sinensis extract may have beneficial effects in the treatment of hereditary sphryocytosis by measuring the osmotic fragility effects.

Objective: To Validation of Osmotic Fragility Test using a modified new Garden Angelica Reagent

Methodology: The human red blood cells were collected in heparin Anticoagulant and Osmotic fragility test reagent was prepared according to Daci and Lewis techniques.100 gram of dried root parts of *Angelica* were purchased from Sakaka herbal medicine farmer's cooperative, Aljouf. The botanical identification was made by herbalist, Aljouf University, Saudia Arabia. The roots were ground with a Wiley mill to pass a 1 mm screen and were extracted with Distilled water at 40°C for 12 h. The extract was then filtered through Advantec No. 1 filter paper. The collected filtrate suspension concentration was 40 g/dl then was diluted in different concentrations using normal saline. After evaporation, the concentrated extract was stored in a refrigerator at 2°C until used. Osmotic fragility test was done for heparinized whole blood samples of 30 healthy males using both the osmotic fragility(phosphate buffer saline) and the new modified angelica reagents(AR).The range of values represented the percentage of erythrocyte lysis at each corresponding phosphate buffer saline concentration (PBS) and angelica reagents(AR) were calculated. The corresponding concentration of PBS solution that caused 50% lysis of erythrocytes defined the MCF index (Dewey et al., 1982; Krogmeier et al., 1993). The erythrocyte osmotic fragility curve; the plot of percentage of erythrocyte lysis versus concentrations of PBS solution was used to obtain the MCF values. The relative capacity of the chemical stabilize or destabilize erythrocyte membrane was evaluated as percentage of the quotient of the difference between MCF values of test and control samples to the control sample (Parpart et al., 1947; Chikezie, 2007).

Results: The mean (±SD) MCF values of the MCF for PBS was 4.38±2.04 and for AR was 3.71±0.98 of blood samples obtained from donors. In addition, the erythrocytes exhibited significantly ($p<0.05$) decreased MCF values when AR was used. This was an obvious reflection of higher fragility index of these erythrocytes.

Conclusion: One of the properties of an ideal the new modified angelica reagent applicable for in vitro blood processing procedure for the osmotic fragility test is one that exhibit minimum or insignificant destabilizing effect on erythrocyte membrane. The present study showed the critical concentrations of angelica reagent that engendered membrane destabilization of human erythrocyte.

Key word: Osmotic Fragility Test, Garden Angelica, Reagent.

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1. INTRODUCTION

Osmosis is defined as the diffusion of water from areas of low solvent concentration to areas of high solvent concentration across a semi-permeable membrane. We call the liquid with more dissolved solutes in it a *hypertonic* solution and the liquid with less dissoved solutes is called a *hypotonic* solution. If the solutions on both sides of the membrane have the same concentration, it is called *isotonic*[1]. Mammalian red blood cells have a biconcave (doughnut-like) shape. If red blood cells are placed in a 0.3 M NaCl solution, there is little net osmotic movement of water, the size and shape of the cells stay the same; the NaCl solution is isotonic to the cell. If red blood cells are placed in a solution with a lower solute concentration than is found in the cells, water moves into the cells by osmosis, causing the cells to swell; such a solution is hypotonic to the cells. When red blood cells are placed in pure water, water rapidly enters the cells by osmosis and causes the cells to burst, a phenomenon known as hemolysis. If the red blood cells are placed in a solution with a higher solute concentration, water moves out of the cell by osmosis, the cell becomes smaller and crenated in shape; such a solution is hypertonic to the cells[2] [3]. These observations have several important practical implications. First, hospitals must store red blood cells in a plasma solution which has the correct proportions of salts and proteins. The plasma solution is made to be slightly hypertonic to the red cells so that the integrity of the cells is preserved and hemolysis is prevented. Second, when doctors inject a drug intravenously into a patient, the drug is suspended in a saline solution which is slightly hypertonic to red blood cells. Intravenous injection of a drug in pure water will cause some of the patient's red blood cells to hemolyze because water is hypotonic to the red blood cells. Therefore, there is a need for a simple, low cost, rapid and reliable reagent for osmotic fragility technique for the screening of the red cell hemolysis[4] [5]. The present study evaluates the efficacy of a new angelica reagent for naked eye single tube red cell osmotic fragility test and its effects in the treatment of hereditary sphyrocytosis

2. MATERIAL AND METHODS

The human red blood cells were collected in heparin Anticoagulant and Osmotic fragility test reagent was prepared according to Daci and Lewis techniques[6].100 gram of dried root parts of *Angelica* were purchased from Sakaka herbal medicine farmer's cooperative, Aljouf. The botanical identification was made by herbalist, Aljouf University, Saudia Arabia. The roots were ground with a Wiley mill to pass a 1 mm screen and were extracted with Distilled water at 40°C for 12 h. The extract was then filtered through Advantec No. 1 filter paper. The collected filtrate suspension concentration was 40 g/dl then was diluted in different concentrations using normal saline. After evaporation, the concentrated extract was stored in a refrigerator at 2°C until used. Osmotic fragility test was done for heparinized whole blood samples of 30 healthy males using both the osmotic fragility (phosphate buffer saline) and the new modified angelica reagents (AR).

2.1. Determination of erythrocyte osmotic fragility:

Osmotic fragility of human erythrocyte was determined by a measure of haemoglobin released from erythrocytes when placed in an environment containing serial dilutions of Phosphate Buffer Saline (PBS) and the new modified angelica reagents (AR) solutions as described by Oyewale [7] with minor modifications Mafuvadze *et al*[8].

2.2. Evaluation of percentage haemolysis and stabilization of erythrocytes: The quotient of absorbance at 540 nm of the content of each test tube (1st-6th) and the seventh test tube was multiplied by a factor of 100. The range of values represented the percentage of erythrocyte lysis at each corresponding phosphate buffer saline concentration (PBS).

Percent of hemolysis (%) = 0.DA/0.DB x 100

Where:

0.DA = Absorbance of test tube (1st-6th) supernatant

0.DB = Absorbance of 7th test tube supernatant

The corresponding concentration of PBS solution that caused 50% lysis of erythrocytes defined the MCF index. [9][10]. The erythrocyte osmotic fragility curve; the plot of percentage of erythrocyte lysis versus concentrations of PBS solution was used to obtain the MCF values.

The relative capacity of the chemical stabilize or destabilize erythrocyte membrane was evaluated as percentage of the quotient of the difference between MCF values of test and control samples to the control sample. [11]. Thus:

Relative Stability (%) = MCF Control – MCF Test/ MCF Control x 100

3. RESULTS

The MCF index represented and interpreted level of erythrocyte membrane stability. The mean (±SD) MCF values for PBS and AR of the erythrocyte of blood samples obtained from blood donors is presented in Table 1. The capacities of the new AR at the specified concentrations to stabilized the erythrocytes was not significantly different ($p>0.05$) from the control/reference PBS. Corresponding higher concentrations of these drugs engendered membrane destabilization. However, Table 1. showed that the MCF bor both reagents used for osmotic fragility tests

did not significantly (p>0.05) promote erythrocyte membrane destabilization Fig.1.

	N	Mean MCF	Std. Deviation	p. value
PBS	30	4.38	2.04	0.000
AR	30	3.71	0.98	

Table 1. The mean MCF index of phosphate buffer saline (PBS) and angelica reagent (AR) of red cells

	N	Minimum	Maximum	Mean	Std. Deviation
Relative	30	1.20	9.00	2.99	2.00
	30				

Table 2. Relative Stability (%) of red cell membrane

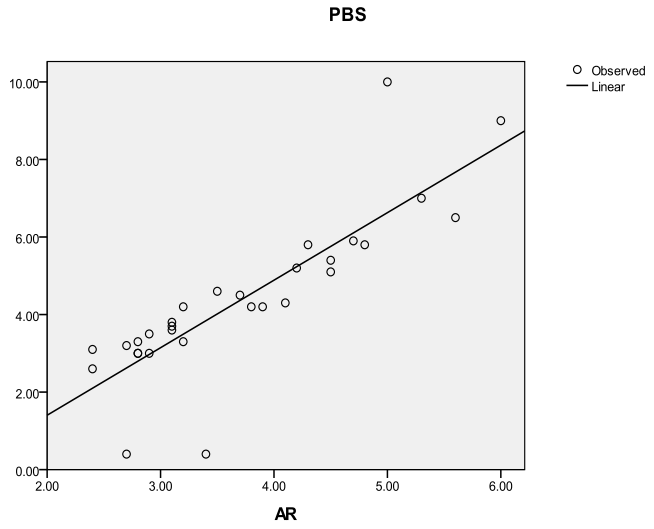

Figure 1 The relationship between MCF fir both PBS an AR reagents

4. DISCUSSION

From comparative investigations, the results presented in Table 1 and 2 showed that human erythrocyte of exhibited the least stability. In agreement with these results,[9]asserted that differences in erythrocyte osmotic fragility are under the control of the individual genotype of the erythrocytes. Thus, during erythrocyte formation, any of the number of erythrocyte properties such as membrane structure, cell shape or internal salt balance, responsible for variant erythrocyte behavior occurred according to the dictate of genetic makeup of corresponding erythrocytes.[9] From a similar perspective, it is probable that variations in some physicochemical properties and oxidant levels of the erythrocyte contributed to the differences in mechanical stabilities and capacities of the erythrocytes to withstand osmotic stress.[12][13] Erythrocytes and their membranes are susceptible to endogenous free radical-mediated oxidative damage that correlates with the proportion of irreversibly cell. [14] In agreement with these lines of reasoning,[14] reported that higher superoxide generation in human erythrocytes was associated with increased tendency of diminished mechanical and osmotic stability compared with human RA erythrocytes. Furthermore, erythrocytes generate superoxide species under normal physiological conditions, but drastically increase in PBS. Unstable hemoglobin produced under this condition generates free radicals and further induce erythrocyte hemolysis.[15] Therefore, accumulation of oxidant contributes to accelerated damage of erythrocyte membranes and senescence of these cells. [15] From another perspective, comparative osmotic stability of human erythrocytes showed connection with the relative tendency of the cells to retain more sodium ion (Na+) intracellular with a concomitant loss of potassium ion (K+).[16] The similarity in the pattern of membrane stabilization/destabilization in the presence of the two Reagents suggested common mode of action on erythrocyte integrity.

At the beginning, each laboratory prepared their own reagents and the reliability of test results varied depending on the quality of reagents especially AR solution which is very sensitive to pH drift. In 12004, Fucharoen and coworkers developed.[17] the dichlorophenol indophenol precipitation (DCIP) Clear reagents. Combinations of these 2 reagents have been reported to be an effective preliminary screening for diagnosis of hereditary sphyrocytosis. Thus the AR and DCIP tests can be used in a screening strategy provided to a wide population to support the control.

Numerous membrane destabilizing agents may act by direct interaction with architectural membrane proteins and enzymes, thereby modifying their structure/function relationship that is necessary and required for membrane integrity.[18] Angelica reagent may be useful for detection and treatment of hereditary sphryocytosis.

5. CONCLUSION

One of the properties of the new modified Angelica Reagent, applicable for in vitro blood osmotic fragility processing procedure for the prevention of erythrocyte induced hemoloysis is one that exhibit minimum or insignificant destabilizing effect on erythrocyte membrane. The present study showed the critical concentrations of AR and PBS that engendered membrane destabilization of human erythrocyte. However, further investigation is necessary to ascertain whether concentrations below the corresponding critical values of the two reagents are capable to eradicate the hereditary sphyrocytosis. Finally, the erythrocyte was exhibit stability within the range of experimental concentrations of the two Reagents. Therefore, the Angelica reagent may be

suitable for in vitro blood osmotic fragility processing procedure for diagnosis of red cells hemolysis or destrucyion in hereditary sphyrocytosis.

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Conflict of Interest: None Declared

