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Osmotic Fragility of Normal and Ultrasound Irradiated Human Blood

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Abstract

Ultrasound is commonly employed in clinical practice as a part of physical therapy. The present paper reports the data on the effect of ultrasound on osmotic fragility of Human blood. Blood samples of volume 6 ml were obtained from healthy donors with their consent, each time an experiment was performed. To prevent the blood from coagulation, it was preserved in a heparin anticoagulant tube. The sample was then divided into two parts, that is, control sample and sample for irradiation. The human blood sample was irradiated to ultrasonic standing waves using an Ultrasonic interferometer of 3 MHz frequency for a duration of 1 h. Osmotic fragility of normal and ultrasonically irradiated human blood was determined. The study reveals variation in the osmotic fragility of ultrasonically irradiated human blood compared with that of a normal sample.

Keywords: Human blood, Osmotic fragility, Ultrasonic interferometer, Ultrasound

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Introduction

The association of acoustic waves with living structures contributes to a special branch of physics called ultrasonic biophysics. Ultrasound wave propagation properties of biological tissues are important to have an insight into cellular assembly and molecular architecture involved in different systems to understand the normal and abnormal living processes. Hence, ultrasonic wave propagation properties of biological tissues have drawn the interest of a variety of scientists from diverse research areas. The investigation into how ultrasound impacts biological materials can be regarded as bioeffects studies that can contribute to clinical applications and risk analysis for diagnostic ultrasound applications. Very little proof regarding the biological effects of ultrasound is available. Further studies are needed to understand its effect on human blood. The effects of ultrasound can be determined in a variety of cell components. Hemolysis is one of the bioeffects of cavitation that has been observed due to exposure to ultrasound.[1-3] Ultrasound exposure to mice lungs leads to blood vessel damage, further cell death appeared to be lytic[4] and DNA damage.[5,6] The tendency of ultrasound to lyse cells is well-known. The main reason is commonly thought to be related to the production of gas bubbles. Cell lysis occurs quickly as a result of gas body activation. Hemolysis in dilute suspensions is useful as a cavitation indicator in studies of nucleation and evolution of cavitation.[7] Changes in pH, conductivity were observed in human blood irradiated to ultrasound.[8]

In general, the osmotic fragility test of red blood cells is used to assess the sensitivity of erythrocytes toward a hypotonic solution. The capability of regular Red blood cells to survive hypotonicity causes the cell to expand in its volume until the surface membrane ruptures. When the limit is reached, lysis happens.[9] As a result, measuring Osmotic fragility is a useful predictor of whether or not the irradiated cell is normal or damaged. In the osmotic fragility test, whole blood is added to varying concentrations of NaCl solution and allowed to incubate at room temperature.[10-12]

The osmotic fragility test helps in the diagnosis of anemia, in which the physical properties of red blood cells are altered. This test detects whether or not the red blood cells can be easily hemolyzed.

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The red cell membrane allows water to flow through thus confining the solutes. It is considered an osmosis. Red cells shrink due to exosmosis when put in a solution that is more condensed than the concentration of the solute within. On the other side, the red cells absorb water by endosmosis when contained in a hypotonic solution like water, which results in hemolysis leading to swelling and rupture of cells. In an isotonic solution, that is, a solution of equal concentration as the red cell content, the red cell stays intact.

Considering the explosion of new knowledge on the potential effects of ultrasound on human blood, it is with this sense an attempt has been made to study the effect of ultrasound on the osmotic fragility of human blood.

Materials and Methods

Human Use and Regulatory Status

The blood samples were taken from Normal Healthy Human subjects with their consent every time an experiment was performed. This study was performed following the procedures formulated by National Ethical Guidelines for Biomedical and Health Research by ICMR (Indian Council of Medical Research), New Delhi, and certify that the studies on Human Blood *in vitro* were carried out in accordance with the principles of the declaration as laid down in the 1964 Declaration of Helsinki.

Blood Sample Collection

Human blood samples of volume 6 ml each were obtained from 8 healthy donors each time an experiment was performed. To prevent the blood from coagulation, it was preserved in a heparin anticoagulant tube. The sample was then separated into two parts.

Exposure of Ultrasound

A variable path ultrasonic interferometer (Figure 1Mittal Enterprises, Model M-81) of frequency 3 MHz has been utilized for irradiation. The blood sample to be irradiated to ultrasonic standing waves is filled into the measuring cell. The high-frequency generator excites the quartz crystal present at the bottom of the measuring cell at its resonant frequency which generates ultrasonic waves of 3 MHz frequency in the Human Blood sample. The sample was irradiated for a duration of 1 h.[8]

Specifications of Ultrasonic Interferometer

Mains Voltage: 220V, 50Hz; Measuring Frequency: 3 MHz.

Analysis of Human Blood Sample

To analyze the effect of ultrasound on the percentage lysis of RBC, an osmotic fragility test was performed.

In the osmotic fragility test, Nacl solution of various concentrations was prepared using 0.9% NaCl solution and diluting it with distilled water as shown in Table 1.

Tubes were arranged in the rack and then numbered. To each of the tubes containing a dilute NaCl solution, 0.05 ml of heparinized blood was added using a micropipette and mixed well. After 30 min the sample was centrifuged for 4 min. The optical density of the supernatant was measured at 540 nm wavelength using a spectro-colorimeter. About 0.1% saline tube serves as 100% hemolysis. The same procedure was followed for ultrasound irradiated blood. The percentage of lysis of normal and ultrasound irradiated human blood was calculated using the formula

$$\%lysis = \frac{O.D\,of\ each\,tube}{O.D\,of\ 0.1\%\,tube} * 100$$

If no hemolysis occurs, the RBC would be formed at the lower end of the tubes with a transparent salt solution above. If there is hemolysis, the salt solution will be tinged red with hemoglobin. If the hemolysis is complete, the fluid will be uniform in color throughout and RBC will not be visible at the bottom end of the tube. The experimental arrangement for performing osmotic fragility test is shown in Figure 2.

RESULTS

Osmotic fragility curves are shown in the figures. Figures 3-10 summarize the results (for 8 Different Blood samples). Hemolysis usually start at 0.40%–0.45% NaCl for healthy blood samples which is in good agreement with the results obtained in our study for Normal Blood samples. Whereas in case of ultrasound irradiated human blood, it was observed that hemolysis was starting at 0.50%–0.55% of NaCl Concentration.

OriginPro 8.5 software was utilized for plotting Osmotic fragility curves. NaCl solution concentration is plotted on the X-axis and the percentage of hemolysis produced is plotted on the Y-axis. Normal blood sample in the osmotic fragility curve is plotted in black Color whereas irradiated blood is plotted in red color. It can be observed from the plots that in the case of ultrasound irradiated human blood osmotic fragility is increased and the hemolysis curve shift toward the right.

Figure 1: Ultrasonic interferometer

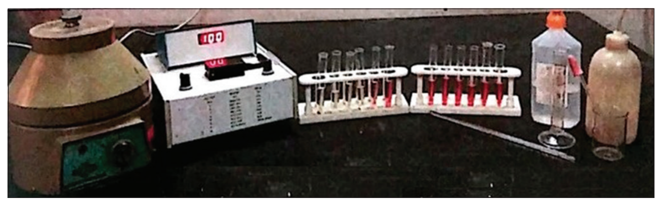

Figure 2: Experimental setup of osmotic fragility test

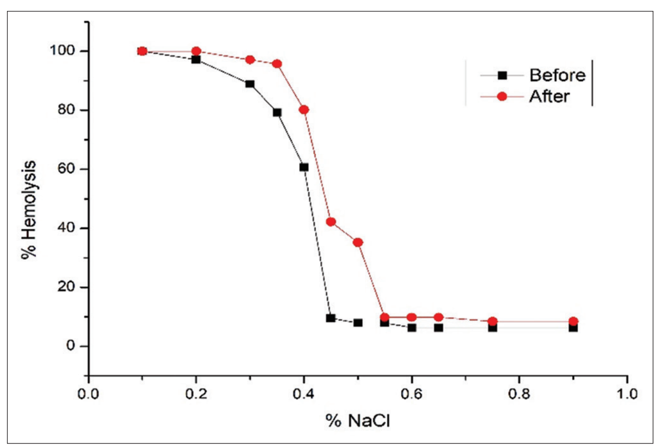

Figure 3: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 1

Table 1: Preparation of saline (0.9% NaCl) solution

Tube No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0.9%Nacl	5.00	4.2	3.6	3.3	3.1	2.8	2.5	2.2	1.9	1.7	1.1	0.6
D.W (ml)	0.00	0.8	1.4	1.7	1.9	2.2	2.5	2.8	3.1	3.3	3.9	4.4
%Nacl	0.90	0.75	0.65	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.20	0.10

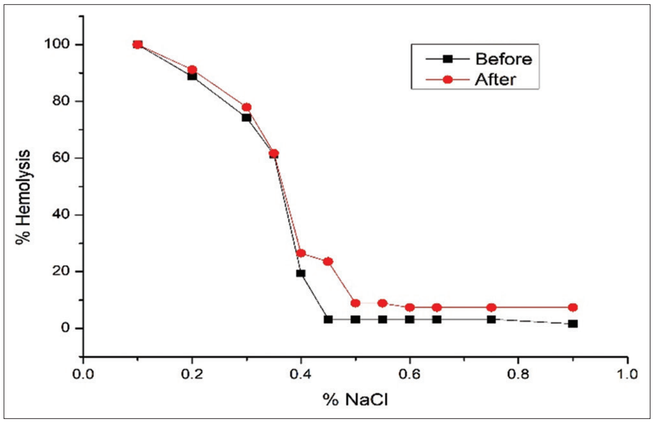

Figure 4: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 2

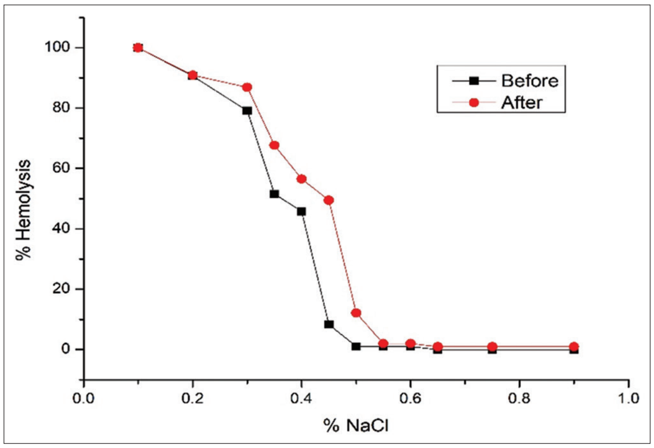

Figure 5: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 3

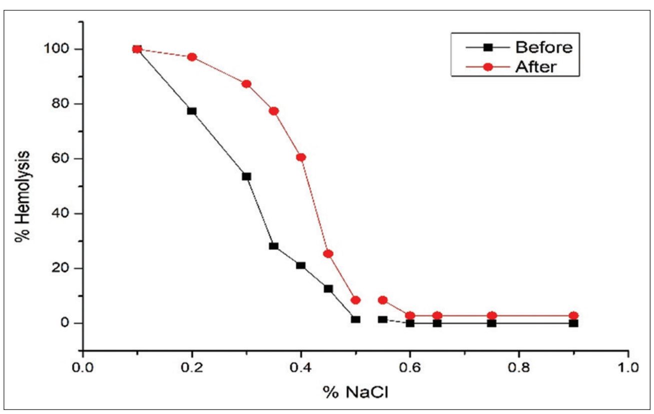

Figure 6: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 4

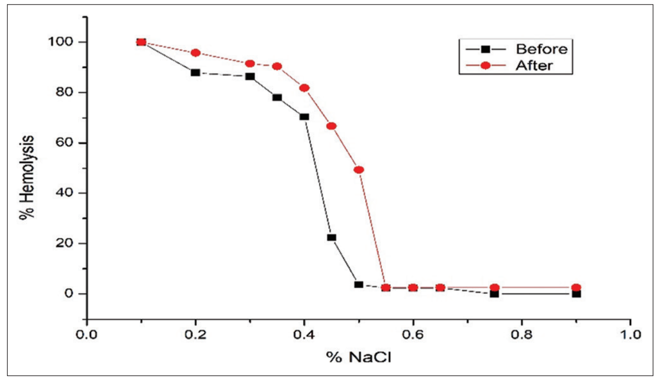

Figure 7: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 5

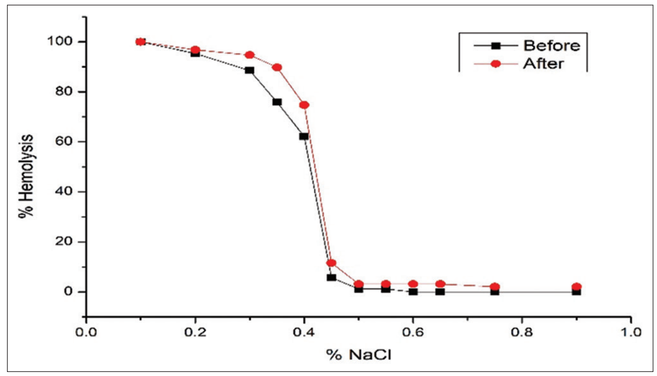

Figure 8: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 6

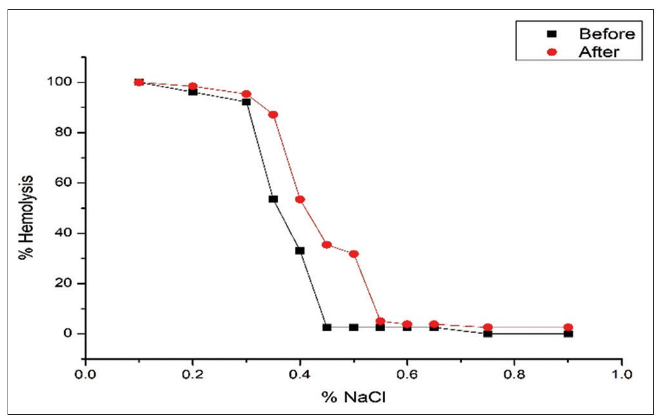

Figure 9: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 7

Discussion and Conclusion

Blood is valuable to mankind because without it life cannot exist. It is the most important constituents of the circulating system which helps in the normal functioning and growth of different parts of the human body. So any changes in human blood will effects the entire human body.[8]

Osmotic fragility test describes the status of RBC, which determines the stability and strength of RBC in various osmotic gradients. It is a quick and straightforward evaluation that reflects changes in the membrane physiology.[13,14] It is a well-known fact that RBC membranes are semi-permeable. Always there will be an Osmotic equilibrium across the cell membrane, such that there will be no influx or efflux of water due to intracellular and extracellular fluids. There will be an influx and outflow of water due to the extracellular and intracellular fluids when there exists an osmotic gradient. The proteins, hemoglobin, solutions of salts,

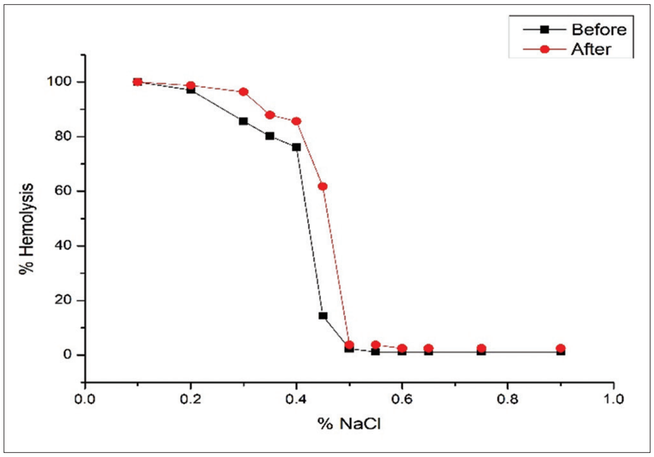

Figure 10: Plot between % Hemolysis and % NaCl of Normal and Ultrasound Irradiated Blood for Sample 8

and glucose are the components of the intracellular fluid of the erythrocyte. Blood cells when suspended in a prepared isotonic solution of 0.9% NaCl, then there exists an osmotic equilibrium across the membrane. If the blood cells are suspended in a lower concentration of NaCl solution, that is, in a hypotonic solution, then there will be an influx of water into the cell which causes the cell to swell, increase in the size of the cell and finally after some point it gets burst to release the hemoglobin. Similarly when blood cells are suspended in a higher concentration of NaCl solution, that is, in a hypertonic solution, then the water from the cell goes out in the medium causing the shrinkage of the cell.

The reaction of any biological tissue to ultrasound irradiation changes, depending on their biological properties and also with respect to their function. A huge number of experiments concerning the effects of ultrasound have been done on animals.[15] The osmotic fragility test can be used to determine whether or not the ultrasound irradiated erythrocytes are normal.

In the present investigation, the osmotic fragility of normal and ultrasound irradiated human blood for a duration of 1 h using an ultrasonic interferometer of 3 MHz frequency has been determined. The present study reveals that the osmotic fragility of human blood before ultrasound irradiation is less when compared to ultrasound irradiated human blood. Further, the plot between %NaCl and % Hemolysis reveals that the osmotic fragility curve shifts towards the right indicating an increase in osmotic fragility; this represents the lower resistance of ultrasound irradiated blood to hypotonicity. Therefore, the percent hemolysis of ultrasonically irradiated human blood is more than that of normal human blood. World Federation for Ultrasound in Medicine and Biology in the year 1997 reported that an *in vitro* analysis has shown an increase in cell lysis due to 1 MHz continuous Ultrasound which is in agreement with the results of our present study. The hemolysis of the erythrocyte membrane causes the loss of integrity of the cells which leads to the release of hemoglobin as reported by Schon *et al*. in the year 1994.[16] Further Esper Mortensen[17] in his paper "Studies on the Osmotic fragility of Normal human erythrocytes," reported that the pH of the surrounding medium affects the Osmotic fragility of Human erythrocyte. Buchan *et al*.[18] in the year 1980 reported that the size and shape of Red Blood Cells are important factors that affect the deformability and fragility of the RBCs. So this effect could be explained concerning the size and shape of the erythrocyte. The ultrasound irradiation might have caused the erythrocyte membrane to swell, causing hemolysis. Thus due to the exposure to ultrasound, the erythrocyte membrane might have become more fragile than the normal erythrocyte membrane.

Ultrasound is being extensively used in the medical field. In the present study, variations were observed in the osmotic fragility of ultrasound irradiated human blood. The findings of the present study regarding the ultrasound effects along with additional research may help us to reconsider its overuse in the future.

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