International Journal of Scientific Research and Engineering Development-– Volume 4 Issue 5, Sep- Oct 2021

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Acoustic Impacts of Ultrasonic Waveson Haemoglobin, White Blood Cells and Platelets of Human Blood at Various Frequencies

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Abstract:

An acoustic impact of Ultrasonic waveson human blood contents at 5, 7, 9 & 11 MHz frequenciesare reported. Ultrasound machine is used for production of ultrasonic waves andlinear transducer PLM – 805AT usedto check exposure of ultrasound, this transducercomprisesall ranges of frequency from5MHz to 11 MHz.In this vitro study, three major blood contents i.eHaemoglobin, Leucocytes and Thrombocytes are considered to see ultrasonic effects in human blood. These effects are discovered duringultrasound of blood containing tube as sound waves absorbs in blood due to which weak echo received and that cause to give shadow in sonogram. Human blood collected from twelve number of healthy volunteers having mean aged 40.36±10.64 years in K2 EDTA BD Vacutainer (3ml).Haematology analyser (Huma count 80^{TS}) is used for CBC analysis and Toshiba Nemio XG iStyle Ultrasound Scannerfor applying ultrasound on tube.The ultrasonic waves having frequencies 5, 7, 9 and 11 megahertz (03 x blood samples scanned at same frequency) is passed on K2 EDTA tube for 10 minutes viatransducer. Before and after application of ultrasound, CBC reports are compared and assessthe ultrasonic effects.By increasingfrequency, the acoustic attenuation increases in blood,it is the basic phenomenon of this article to see the impacts of ultrasonic waves in human blood and to create awareness of superfluous use of ultrasound during pregnancy as well. One way ANOVA is used to measure variance in CBC reports. The assessment of acoustic impacts in blood contents is also discussed in this article.

Keywords: K2 EDTA, Linear Transducer, Absorption, attenuation,Blood Contents

1. INTRODUCTION

Ultrasound is a diagnostic technique that works atvery high range of frequency (in megahertz). Waves play an important role in ultrasound technology. When the frequency of sound waves decreasesthenwavelength will be increased as $f = v/\lambda$, by this relation frequency is inversely proportional to the wavelength and vice versa. To decrease in wavelength, the resolution improved due to which well clear and furnish image be attained. Ultrasound waves having frequency 2-15 MHz[1] is better enough to take well- furnish image of human organs, that's why for medical diagnostic purposes, ultrasound waves being used up to 20 MHz [2]. A part of ultrasound machine called transducer is used to generate waves. The frequency ofultrasonic waves in ultrasound machineis adjusted manually. Every transducer is designed up to a particularrange of frequency, according to requirement frequency tuned i.e for abdominal examinations 3MHz, for children neck 5 MHz, for children examination 5-7 MHz and for adults5 MHz needed [3]. Acoustic attenuation is the loss of sound signal during propagation. The reduction in blood cells i.ehaemoglobin, white blood cells& platelets by ultrasoundis due to absorption and scattering of sound wave in blood proteins and blood contents.It has been experimentally proved that sound waves drop its intensity and magnitude by absorption and scattering through any tissue or material i.e blood, polypropylene, water, and other muscle tissues. Absorption and scattering are the major factors of classical reduction of blood contents [4]. Attenuation increases when frequency and concentration of proteins in human blood increases [5]. It also depends upon temperature when temperature gets low attenuation increases[6]. When ultrasound waves propagated through blood, some blood contents reduced i.e White blood cell, Haemoglobin and Platelets [7]. This was due to absorption of sound wave in blood. Absorption is a mechanism occurred by sound energy, it is the conversion of sound energy into heat by the elastic motion of particles and large viscosity of tissues. Attenuation due to absorption of ultrasound wave in human blood is 60 % of the total attenuation [8]. The absorption of ultrasound wave in human tissues depends upon the composition of proteins, lipids, and carbohydrates. The absorption coefficient increases with increasing condensed matter in tissues [8]. In blood, absorption relaxation phenomenon is used to clarify absorption in proteins i.e albumin, globulin, and fibrinogen [9]. Absorption of sound energy is different in different media, in proteins absorption is larger than amino acids and polypeptides [10], its value different in red blood cells than nearby plasma [11].

2. MATERIALS AND METHODS

To evaluate the ultrasonicdisruption in human blood, there were 12 xof freshblood specimens were taken from healthy volunteershaving mean aged 40.36±10.64 years. The blood specimenwaspreserved inBD Vacutainer of polypropylene (3ml) containing K2EDTA (Di-potassium ethylenediaminetetraaceticacid), 13mm x 75mm (Becton, BD, 1 Becton Drive, Franklin Lakes, NJ 07417, USA) [12]. BD Vacutainer K2 EDTA isa purple colour tube which is used for haematological investigations (Complete Blood Count) whereas EDTA is an anti-coagulate acid that preserve blood from clotting [13].CBC Test provides complete picture and information about number of blood contents, haemoglobin concentration and other blood parameters that are important for of diagnose disease and infections in human body [14]. Complete blood count (CBC) test was performed by Haematology Analyser (Huma count 80^{TS}) inhaematologicallaboratory whereas for ultrasound of blood Toshiba Nemio XG iStyle Ultrasound Scanner having Linear Transducer Probe PLM – 805AT, (3.0- to 13.0 MHz)was used as shown in Figure 1. The main purpose of this study was to evaluate the impacts of ultrasonic waves inblood as blood is the main constituent of human blood which can't be synthesized in any laboratory. The concentration of albumin, globulin and fibrinogen and various proteins,absorbs ultrasound waves that causes to reduce blood contents [15].To remove the air gap and best transmission of ultrasonic signal a couplent material Water Soluble Ultrasound Gel (gel GelMatik, Lahore Pakistan)was used homogeneously between transducer probe and blood-filled BD vacutainer (K2-EDTA Tube). At 0.05 level the population means are significantly different

For statistical analysis, CBC ofblood specimen before applying ultrasound was conducted in haematological lab. The ultrasound applied on same blood specimen for 10 minutes by linear transducer as shown in Figure1and got its CBC again. All CBC reports before and after applying ultrasoundcompared and One way analysis of variance (ANOVA) test was used to measure the variability of CBC of blood samples. A p-value ($P = 0.05$) was statistically significant in data. The same procedure was applied on all blood samples/specimens.For better justification, 03 x blood samples collected at same time and temperature (37 □C) and processed all samples within an hour at same ultrasound machine at same frequency i.e at 5 MHz three blood samples inspected and taking average, this shows the reduction by applying ultrasound.

Figure 1 Ultrasonography of K2 EDTA Tube containing human blood

To see the ultrasonic effects with and without blood, the blood containing tube (K2-EDTA) was put on transducer as shown in Figure1 and observed sonogram on monitor of ultrasound machine. It was found that the sonogram of blood containing tube was shadowed due to much absorption of sound waves through blood as shown in Figure2. Due to absorption, weak echo was received at same transducer whereas without blood containing tube sonogram was found brighter due to less absorption (strong echo) as shown in Figure 3.

Figure 3 *Figure 2*weak echo signal *strong echoes signal*

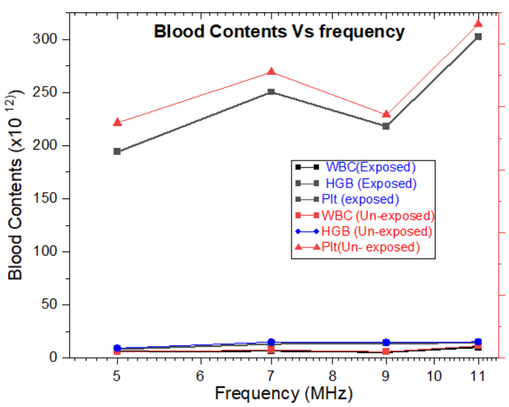

Figure 4 comparison of unexposed to exposed ultrasound of blood sample

3. RESULTS

Human Blood Contentsi.e haemoglobin, leucocytes (white blood cells)& thrombocytes (platelets)were reduced significantly by applying ultrasound as shown in Table1 and Figure 5. When frequency kept low, the difference was also low and by increasing frequency large effect was observed as shown in Table 2 & Figure 6. Platelets were averaged decreased upto 12.72 % whereas at 5 MHz was 3.44 % as shown in Table 2 &Figure 5by comparing unexposed to exposed through ultrasound, this was due to absorption of sound wave in blood. It was also derived that amount of Haemoglobin declined by applying ultrasound wavesupto 4-10.75 % as shown in Table 2.Leucocytes (white blood cells) was seen less as compared to normal rangeupto 3.90- 16.76 %, it was due to high concentration of mineral elements in blood. The value of haemoglobin cells is decreased atthe rate of increasing frequency. The average was calculated to clear the difference in unexposed toan exposed blood sample through ultrasound. The difference was realistic, clear, calculated which satisfy the previous results [15,16,17].

*Table 1*EFFECTS OF ULTRASOUND IN HUMAN BLOOD AT 5, 7, 9 & 11 MHZ

f (MHz)	Blood Sample	White Blood Cells (x 10^{12})		Hemoglobin (g/dL)		Platelet Count (x 10^{12})	
		Un-exp	exp	Un-exp	exp	Un-exp	exp
5	S1	9.5	9.4	10	10	218	215
	S2	5.5	5.2	16	15.2	262	251
	S3	5.7	5.3	14.1	13.3	102	96
7	S4	9.6	9.4	9.9	9.2	340	310
	S5	13.35	12.4	7.5	7.4	194	166
	S6	8.22	7.8	8	7.3	217	207
9	S7	7.2	6.3	14.5	13.2	309	290
	S8	4.8	4.6	14.2	12.8	187	171
	S9	5	4.7	14.5	13.2	158	120
11	S10	6.8	5.2	12.3	11.3	288	246
	S11	6	5	14.9	14	232	190
	S12	5.6	5.3	14.8	12.2	387	361
	Average	7.27	6.71	12.59	11.59	241.16	218.58

*Table 2*PERCENTAGE DECLINE IN BLOOD CONTENTS BY INCREASING FREQUENCY

Frequency//blood contents	White Blood Cells (%)	Hemoglobin (%)	Platelet Count (%)

5 MHz	3.91	4	3.44
7MHz	5	5.9	9.5
9MHz	8.23	9.26	11.16
11MHz	16.76	10.71	12.72

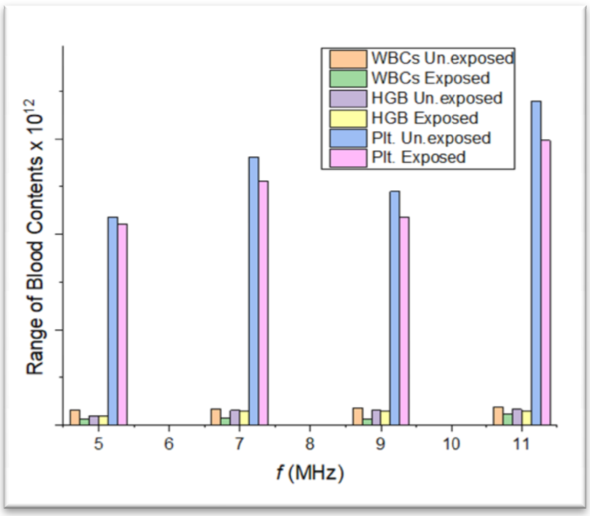

Figure 5 Ultrasonic Effects Un-exposed to an Exposed Blood Sample

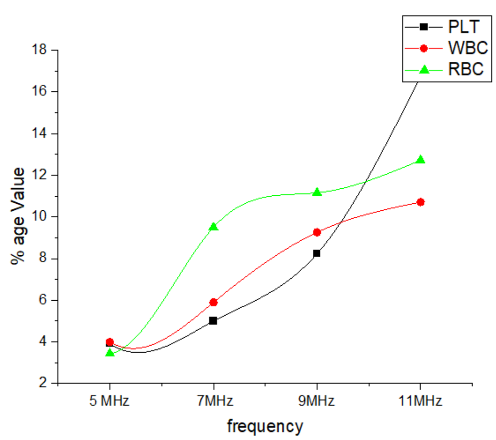

Figure 6Percentage in reduction in Blood Contents Vs frequency

4. DISCUSSION

Blood is the main constituent of human body, life existence is impossible without it. It contained leucocytes (WBCs), Thrombocytes (Platelets), Erythrocytes (RBCs) and Plasma, these cells cover near about 45 % by volume of whole blood.Plasma is also in liquid form having yellowish colour containing 55% volume of whole blood [17]. All blood cells suspended in plasma. If blood cells removed from blood by using source of centrifugation, remaining fluid (plasma) contains 91 % water and three major proteins albumin, globulin, and fibrinogen [18]. In the CBC profile the normal ranges of Thrombocytes 165-396 x 10^9 cells/L [19], Leucocytes 3.7 – 11 x 10^9 cells whereas Haemoglobin is 11.8 – 16.7 g/dL [20]. When ultrasonic waves applied on blood via K2 EDTA BD Vacutainer through transducer then various blood contents decreased by means of absorption like other tissue of the body [21]. The phenomenon behind absorption is the relaxation absorption due to which energy dissipates in blood. When ultrasound waves travel out of phase then relaxation phenomenon be significant [22]. The thermal effect of ultrasound that comes from absorptions of sound energy and production of heat, depends upon ultrasound beam. The formation and oscillation of gas bubbles is known as ultrasound cavitation it also effects on the propagation of sound wave [23-24]. Cavitation effects are noted higher at higher frequency, at lower frequency these effects are not prominent [25 - 26]. Thermal effects caused by ultrasound waves also disturb the blood cell counts [27]. Temperature also affect CBC, all test performed at same temperature and encouraged same results without any change in temperature. Acoustic Attenuation is the loss of sound signal which depends upon various factors i.e concentration of blood cells, structure of molecule, intermolecular forces,density,viscosity, and thermal molecular properties in human blood [28]. Density plays a key role in absorption,larger density means large number of concentrations in blood [29]whichcause to increase attenuation. Due to change in density of blood cells, the viscosity of plasma change [30]. In human body tissues, by increasing attenuation tissue density increases due to which fluid in tissue reduced [31], likewise blood cells or contents decreasedas shown in Table 1-5. This was due to thermal effect produced by sound waves, causing evaporation of interstitial, intravascular and intracellular water by increasing temperature [32] and mechanical consequencei.e cavitation (creation of gas bubbles by ultrasonic waves) [33-34]it was confirmed during high intensity focused ultrasound (HIFU) treatment [35]. Cavitation and formation of bubbles in blood during ultrasonography was noted, at low frequency this effect was low whereas at higher frequency this effect was prominent. The formation of bubbles size in blood was calculated 3.9 µm in diameter (resonance size) at 2 MHz[36].In this experimental setup, the quantity of platelets reduced by the application of ultrasound which was proved by W. L. Nyborg, and C. C. Whitcomb paper (1979) according to article not only quantity of platelets reduced although entity fades [37-38].At high intensity and frequency, the platelets reduced due to adhesion of acoustic forces. The quantity of platelet cells reduced in high intensity focused ultrasound (HIFU)during the therapy of different tissues [39]. The reduction in platelets in this experiment as measured 10 % which can be calculated from table 5. All blood contents decline from 5 MHz to 11 MHz frequency observed and clearly shown in Figure 6.Haemoglobinfunction is to carry oxygen to whole body cells and tissues and carbon dioxide back from lungs. Oxyhaemoglobin in dilute aqueous solution changed into methaemoglobin by ultrasonic waves that destroyed gradually [40-41]. In this study the attenuation (difference in unexposed to exposed blood sample) of HGB (g/dL) was increased upto 7.7 %. as shown in table 2, however attenuation coefficient of HGB increases directly with increase of frequencies to see Figure 6. This study verified by Schneider et al.1969 at 10 MHz he found 04% increase in the attenuation coefficient in deoxygenated haemoglobin solution as compared to oxygenated haemoglobin solutions [42].Haemoglobin carries lot of proteins [43-44] that absorbs ultrasonic waves due to which attenuation increased [45], this was verified in this paper as shown in table 2 & Figure 5.

5.ACKNOWLEDGEMENT

I would like to thankDr. Muhammad Arshad Javid for continuous advice, guidance, and assistance in this research. Especially lot of thanks to the princely help of Head of Radiology Department for Ultrasonography of Blood Samples inHospital, Taxila, Pakistan and offering me calm prospect in experiments. I am very thankful to my sweetheart parents specially and specifically with bundle of respect my father Muhammad Anwar whom encouraging me to research study in the field ofBasic Sciences (Physics).

CONCLUSION

In this vitro study, ultrasound waves are not completely safe, it decreases the blood contents. Acoustic waves cause to reduce White Blood Cells more than the other contents of human blood. It isconcluded that the excessive use of ultrasound is dangerous and harmful for humans. It is the exclusive articles on vitro study, in this kind of paper not published before whereas the vivo study explained ultrasonography well. Furthermore, developments are being encouraged to evaluate this kind of research to estimate the attenuation of ultrasound waves in human blood as well on the other organs of the body. Ultrasonic exposure may produce more effects on extra high frequency and scanning of body for more time. Previous study emphasized that the safe range of ultrasonic scanning is upto 15 MHz, this study was also limited upto this range of frequency. The main purpose of this research was to create awareness regarding excessive use ultrasound. It is also cleared that blood contents affected same by ultrasound, there was no variance found in respect of age category.

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