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Full Length Research Paper

Pulsed high voltage discharge induce hematologic changes

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The aim of this work to examine the effect of the gas-liquid hybrid discharge treatment system on some hematological parameters. The gas-liquid hybrid discharge (HD) reactor consists of high voltage point discharge electrode above blood surface and cylinderical ground copper electrode containing the blood (in the same time act as the vessel reactor). The HD could produce both arc discharge in gas phase and liquid phase. The high energy plasma arc produces a pressure shock wave, electromagnetic radiations, ozone and free radicals. For basic hematology we determined the hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). For differential blood count the number of red blood cells (RBC) and white blood cells (WBC), which were further differentiated into lymphocytes, monocytes, granulocytes, and platelets, were measured.

Key words: Hematological parameters, hybrid discharge, shock wave, leukocytic count, hemoglobin.

INTRODUCTION

Pulsed hybrid gas-liquid electrical discharge (PHD) is one of the hopeful candidates in various industrial liquid treatments. In this type of discharge, cavitated bubbles are generated by the pulsed arc discharge, and induced shock-like pressure rises are induced in a cavitation zone near the discharge electrodes. These cavitated bubbles are a major source of UV emission and radical species such as ozone, OH, H_2O_2, etc. These chemical and physical reactions occur simultaneously in the cavitation zone (Shah et al., 1999; Casadonte, 2000; Suslick et al., 1999). These reactions are expected to affect the liquid treatment. But, details of this mechanism are not understood.

There are two types of pulsed discharge. One is a pulsed corona discharge, which has a feature of only light radiation without a large current and a strong pressure rise. The other is a pulsed arc discharge, which has a light radiation, a large current and a strong pressure rise. The pressure increases as the input power and the gap distance increase.

Pulsed hybrid discharges are produced during the rapid release of stored electrical charge across electrodes, the resulting formation of an electrical arc across the spark gap produces a localized plasma region (i.e., ionized gas) that emits UV radiation and generates pressure and thermal shocks (Ching et al., 2001). These phenomena can also produce radical species and ionic reactions. The sonochemical effects are due to the electrohydraulic cavitation in liquid that causes the formation of cavitation bubbles, which can grow and implode under the periodic variations of the pressure field of the ultrasonic waves. The rapid implosion (which occurs within 10^{-11} s) of the eventually instable gas bubbles causes adiabatic heating of the bubble vapour phase. In this way, localized and transient high temperatures of several 1000 Kelvin and pressures of some 10 MPa exist in the final stage of the compression phase of the oscillating or collapsing cavitation bubbles.

UV irradiation of blood-platelet concentrates is used in transfusion practice to prevent the development of post-transfusion alloimmunization and inactivate viruses and bacteria in the concentrates. UV radiation may affect the blood-platelet metabolism and function. The exposure of blood platelets to UV radiation induces the generation of free radicals. These free radicals generated in blood platelets after stimulation by UV radiation are involved in platelet activation and metabolism of platelet polyphosphoinositides (Bednarska et al., 2000).

Sterilization appears to be the best way to ensure a very high level of safety in transfusion of blood and its

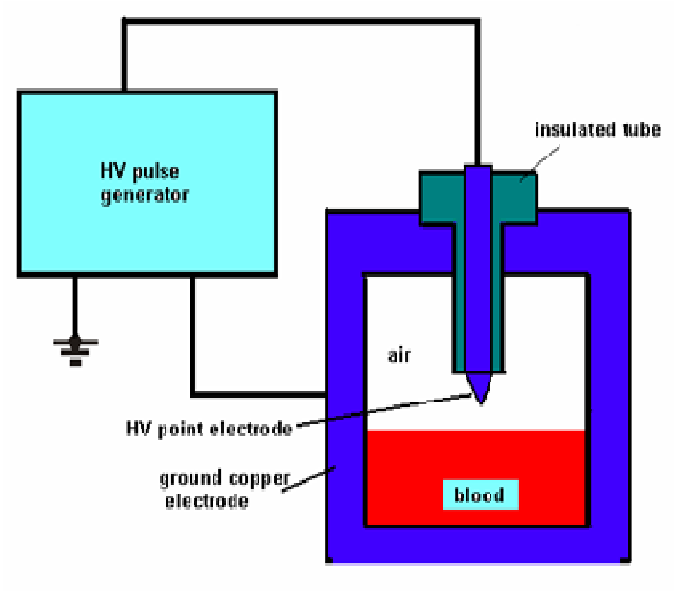

Figure 1. Experimental set-up.

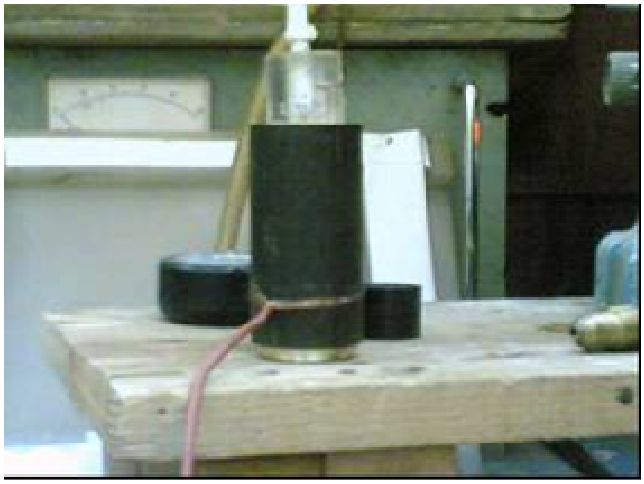

Figure 2. A photo of experimental set-up.

components. Currently, all blood products are available in sterilized forms with the exception of red cell and platelet concentrates. Sterilization of cellular blood components presents a unique challenge because cell structure and function are disrupted more easily than those of individual proteins. Various approaches have been taken for virus sterilization of RBC and platelets (Horowitz and Valinsky, 1991; Matthews et al., 1988), however, favorable results were obtained only with photodynamic treatment (PDT) (Smetana et al., 1994; Rywkin et al.,1994). As a result, almost all the efforts are now focused on this approach.

Objective of this study was to determine the effect of pulsed hybrid gas-liquid electrical discharge (PHD) after exposure to different number of pulses and to study the variations which may occur on some hematological parameters following exposure.

MATERIALS AND METHODS

Experimental set-up

Schematic diagram of the apparatus for generation of the Pulsed hybrid gas-liquid electrical discharge (PHD) is shown in Figure 1. The point electrode was made from a sharpened copper rod, which was almost totally insulated from surrounding water and ground copper electrode (reactor vessel) by an insulated tube.

A pulsed high voltage applied to the point electrode was provided by a pulse power supply (HV). It consists of a variable voltage 0 - 40 kV DC source, a low inductance storage capacitor and a rotating spark gap. The temporal evolution of the gap voltage and electrical current through the reactor were measured by a home-made resistive voltage divider and a Rogowski coil, respectively, and recorded simultaneously by the four channel Lecroy digital oscilloscope with a sampling rate up to 200 MSa s-1.

The sample (blood) was injected into the treatment chamber after cleaning and sterile it for the purpose of sterilization. One of the electrodes was connected to the high voltage pulse generator and the other electrode connected to the ground. The high voltage was adjusted pulse generator parameters as follows: output voltage 20 kV, discharging frequency 25 Hz and action time 120 s. After treatment, the sample was taken out from treatment chamber and put it in sterile tube after the voltage drops to zero voltage. The blood samples are divided into two groups; one unexposed control group and tested group which was exposed to number of pulses. At the end of exposure time, the blood samples collected were analyzed for laboratory assessment.

The circuit of the pulse voltage generator used in this study consists of the pulse-forming capacitor (20 nF) was charged through a 50 kilo-ohm resistor by a negative dc high-voltage power supply and rotating spark gap switch. As the voltage on the capacitor reached the spark-over voltage of the spark gap electrode, the capacitor was discharged, producing narrow positive high-voltage pulse. A photo of experimental set-up shown in Figure 2.

Assays

For hematological parameters, and differential blood counts, fresh blood was obtained from healthy donors before each experiment and the samples were collected on anticoagulant (EDTA). The blood samples were divided equally into four groups: one unexposed control group and three tested groups which were exposed to different number of pulses (function of exposure time) of high E-field intensities, ultraviolet radiation, shock wave, and radical species such as O_3 , H_2O_2 , O● and OH● by intense spark discharge. At the end of the exposure time, the blood samples collected were analyzed for laboratory assessment of hematological and biochemical parameters. The first, second and third experimental groups were exposed to one, two and three minutes (25 pulse per second), respectively.

The blood sample was injected into the tank of the exposure system (PHD) for the purpose of treatment. One of the electrodes was connected to the high voltage pulse generator and the other electrode to the ground. The high voltage pulse generator parameters were adjusted as follows; output voltage 20 kV, discharging frequency 25 Hz and the action time up to 240 s. After exposure (treatment), the blood sample were taken out from the treatment tank by syringe and placed in sterile tube after the voltage drops to zero voltage.

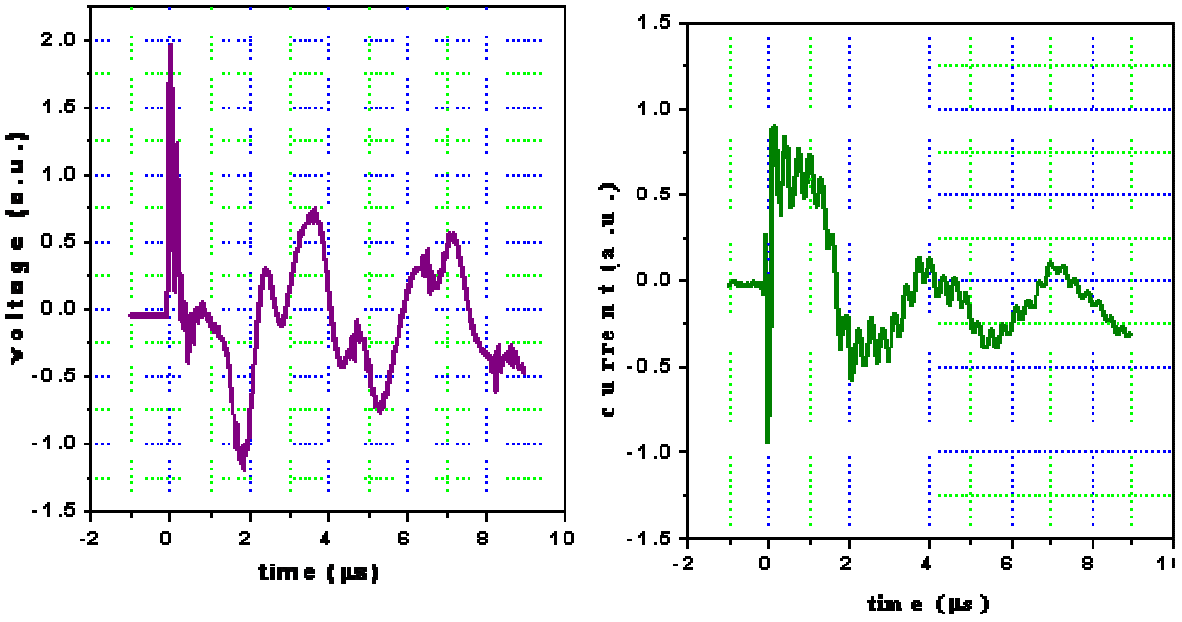

Figure 3. The current and the voltage waveform of the device.

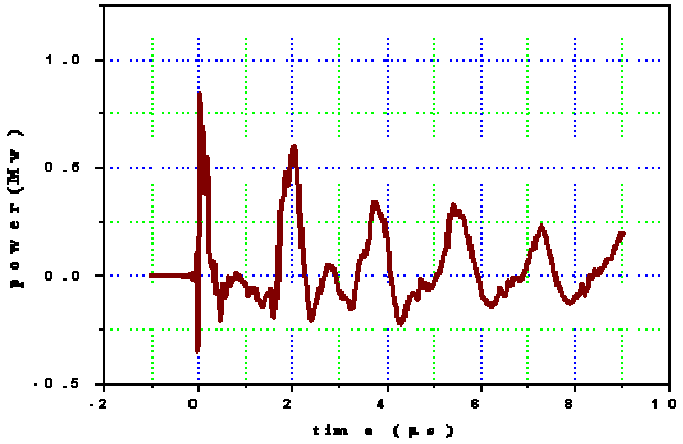

Figure 4. The power waveform of the device.

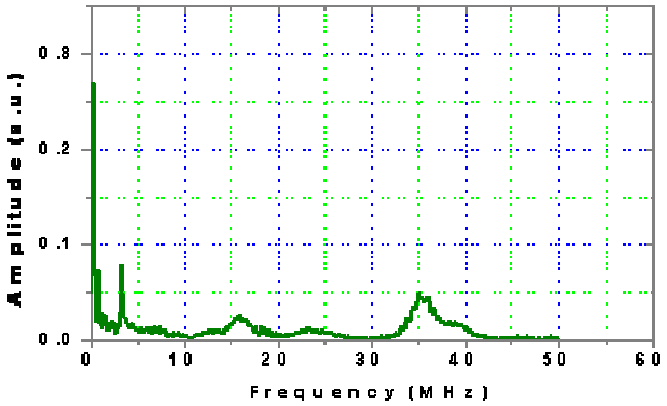

Figure 5. Fourier transformation of the discharge current pulse.

For basic hematology we determined the hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). For differential blood count the number of red blood cells (RBC) and white blood cells (WBC), which were further differentiated into lymphocytes, monocytes, basophils, eosinophils, staff, segmented, and platelets, were measured.

RESULTS AND DISCUSSION

The peak value of the discharge current was measured is approximately 450 A during the pulse. Figure 3 shows the current and voltage waveforms that characterized the HD device. Current and voltage were measured as a function of time at an input energy of 9 J (maximum applied voltage 30 kV) and air pressure of 1 bar. From these measurements the power transfer to the reactor vessel was obtained as a function of time as shown in Figure 4.

Fourier transformation shows the amplitude spectrum of the discharge current that is displays the different frequency components in the pulse (Figure 5). It can be seen that the most of the energy in the discharge current is between 100 kHz and 50 MHz. We see that the spectrum have several peaks at different frequencies the maximum component at 0.29 MHz (fundamental frequency) and the other components at 3.32 MHz, 16.0 MHz and 35.0 MHz (harmonic frequencies). It is clear that the energy is spread over a very large frequency range; this is a characteristic feature of a short pulse.

The effect of pulsed hybrid gas-liquid electrical dis-

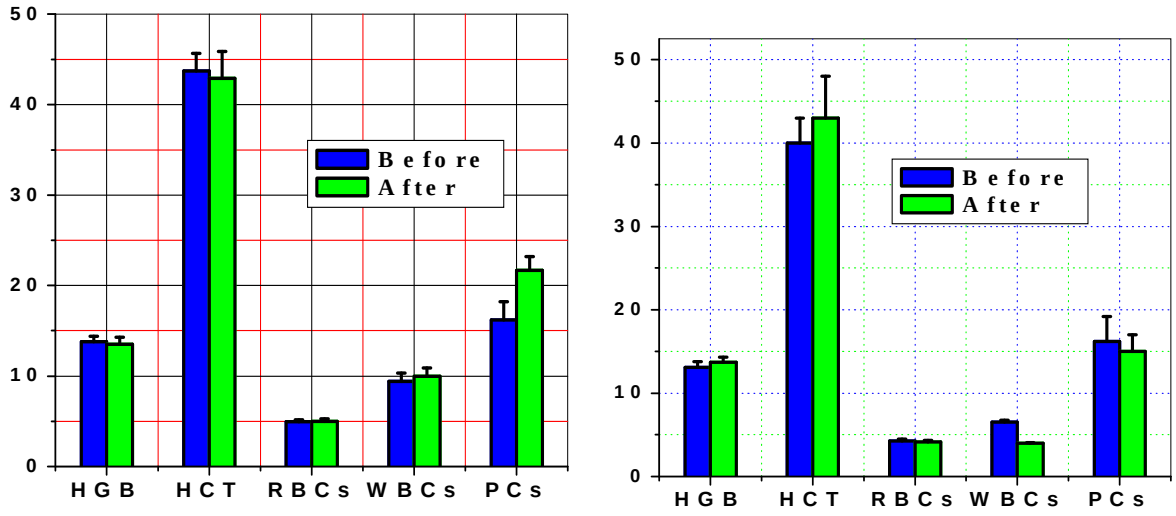

Figure 6. Effect of plasma products (electric field, ozone, uv, shock waves and free radicals) at different values of current (450 A for the left figure and 350 for the right one), with exposure time of 120 s and charging voltage about 20 Kv. Abbreviations: RBC, red blood cells (10^6 /mm^3); WBC, white blood cells (10^3 /mm^3); HGB, Haemoglobin (g /dl); HCT, Haematocrit (%); PC, Platelet Count (10^4/ mm^3). The blue bar indicate contol sample and green bar indicate exposed sample. Error bars indicate mean ± S.D (standard deviation) for ***n*** ≥ 3.

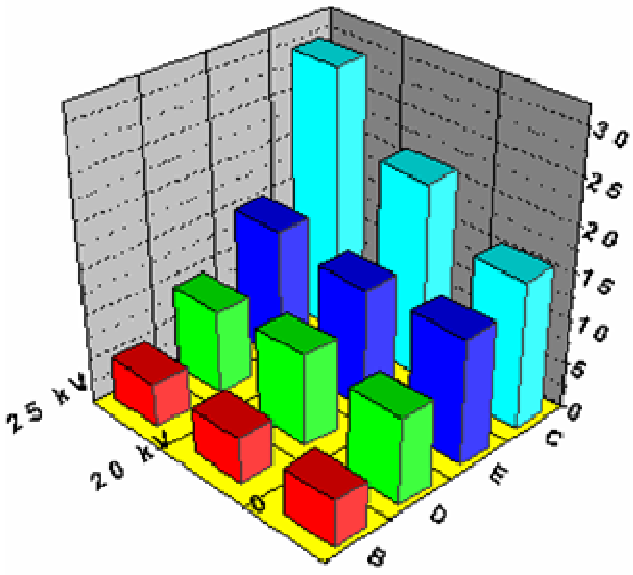

Figure 7. Effect of plasma products (electric field, ozone, uv, shock waves and free radicals) on complete blood count at different values of charging voltage (20 kV and 25 kV), with exposure time of 120 s . Abbreviations: B, red blood cells (10^6 /mm^3); D, white blood cells (10^3/mm^3); E, Haemoglobin (g /dl); C, Platelet Count (10^4/ mm^3).

charge (shock wave, electromagnetic radiations, ozone and free radicals) on hematologic parameters in the four exposed groups (one, two and three minutes without magnetic field and fourth group exposed to one minute with magnetic field) as compared to the control group were studied. Our investigations revealed that there was increase in the mean values of red blood cells (RBC) count in all exposed groups in comparison with the control group (Figures 6 and 10). Data showed that there was a significant increase in mean values of the hematocrit (HCT, %) and hemoglobin (HGB) concentration of the first group and non-significant difference of the second and third group as compared to the control group at different experimental conditions (Figures 6 and 10). On the other hand non-significant differences in the mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in all exposed groups as compared to control group (Figure 8).

Mean values of total white blood cells (WBC) were only increased in the second exposed group (2 min) and the other groups were decreased as compared with the control group. On the other hand a significant difference was found in mean values of lymphocytic count in the first group only as compared to the control group, while non-significant difference was found in the other exposed groups as compared to the control group (Figure 9). Data of the present study showed that mean values of platelet count were significantly increased in all exposed groups as compared to the control group (Figure 10). It was found that the platelets are mildly increased with magnetic field than without magnetic field.

The effect of different values of charging voltage (20 and 25 kV) on complete blood count (CBC) is shown in Figure 7. All the exposed groups were increased as compared to the control group by increasing charging voltage at the exposure time about 120 s. Results of different values of discharging current (450 and 350 A)

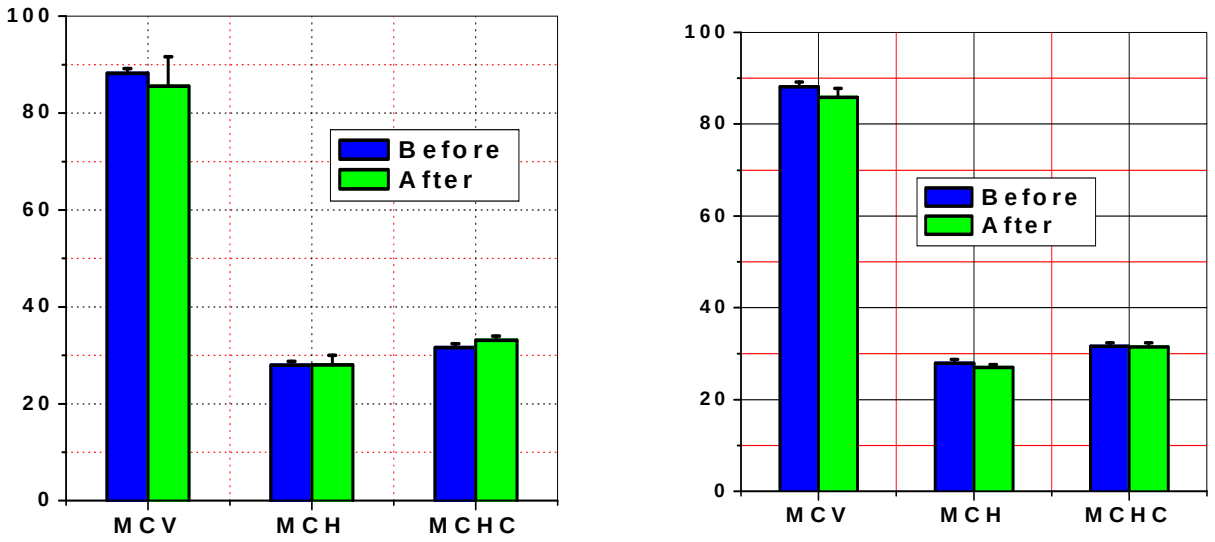

Figure 8. Effect of plasma products (electric field, ozone, uv, shock waves and free radicals) on red cell indices at different values of the exposure time (60 s for the left figure and 120 s for the right one), and charging voltage of 20 kV. Abbreviations: MCH, mean cell hemoglobin (pg); MCV, mean cell volume (fL) and MCHC, mean cell hemoglobin concentration (g/dl). The blue bar indicate contol sample and green bar indicate exposed sample. Error bars indicate mean ± S.D (standard deviation) for $n \geq 3$.

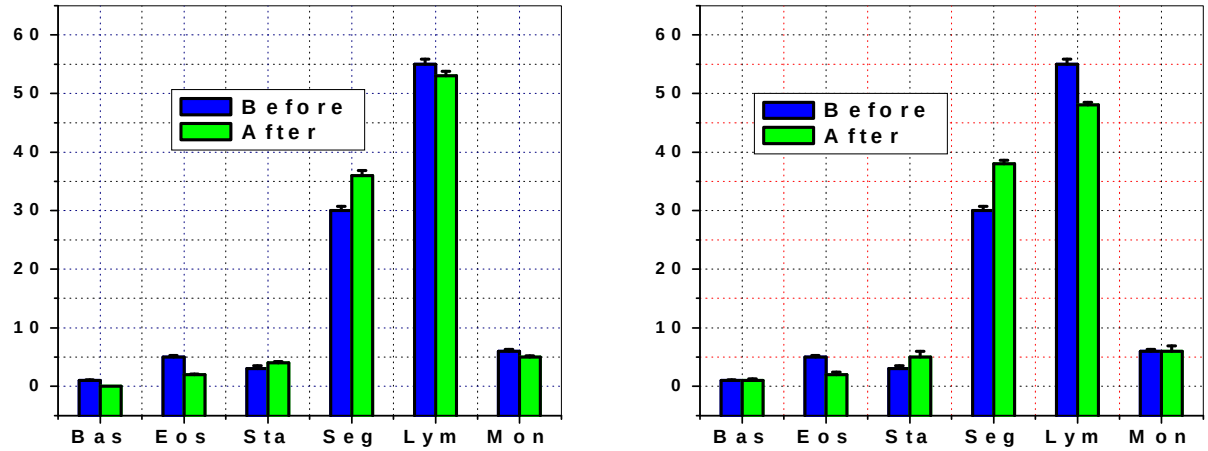

Figure 9. Effect of plasma products (electric field, ozone, uv, shock waves and free radicals) on Leucocytic Count at different values of the exposure time (120 s for the left figure and 180 s for the right one), and charging voltage of 20 kV. Abbreviations: Bas, Basophils (%); Eos, Eosinophils (%); Sat, Staff (%); Seg, Segmented (%); Lym, Lymphocytes (%); Mon, Monocytes (%). The blue bar indicate contol sample and green bar indicate exposed sample. Error bars indicate mean ± S.D (standard deviation) for $n \geq 3$.

on CBC at the exposure time about 120 s is shown in Figure 6. It was found that the platelet count was decreased in the exposure sample as compared to control group at low value of current (350 A) while it was increased in the case of high value of current (450 A).

When human blood is exposed to O_3, this gas dissolves in the plasmatic water and reacts immediately with several biomolecules, mainly polyunsaturated fatty acids generating H_2O_2 and an array of lipid oxidation products (LOP) (Pryor, 1994; Pryor et al., 1995). Both H_2O_2 and LOP can elicit a number of biological effects on blood cells which, by displaying widely different functions, can also have different medical effects. Ozone autohemotherapy (O_3-AHT) is a form of therapy where the blood of a patient is exposed to predetermined ozone concentration for a given time and then reinfused back into the patient. The ozone dissolves in the plasma and reacts with organic molecules generating substances

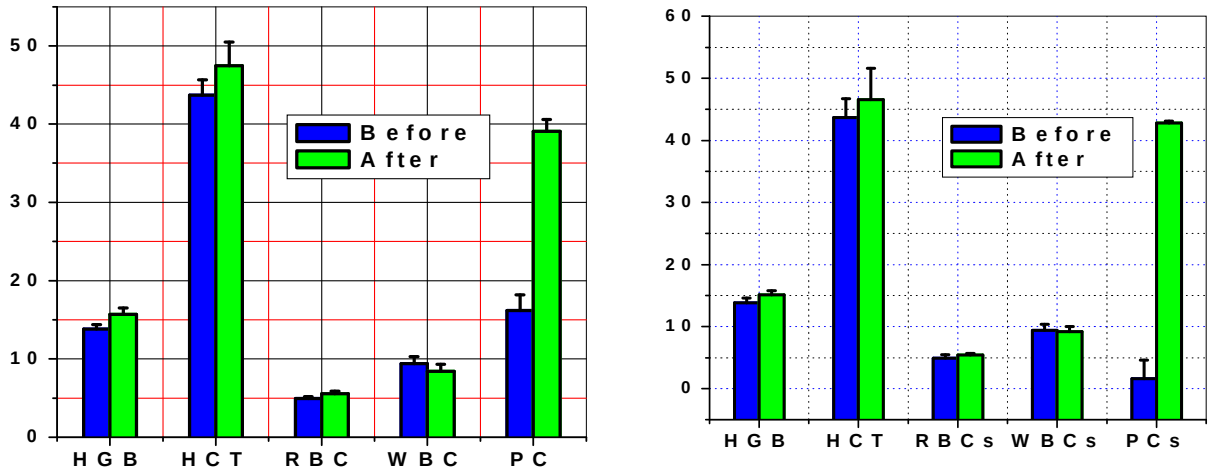

Figure 10. Effect of plasma products (electric field, ozone, uv, shock waves and free radicals) without magnetic field (left figure) and with magnetic field (right figure), the exposure time about 60 s and charging voltage of 20 kV. Abbreviations: RBC, red blood cells (106 /mm3); WBC, white blood cells (103/mm3); HGB, Haemoglobin (g /dl); HCT, Haematocrit (%) ; PC, Platelet Count (104/ mm3). The blue bar indicate contol sample and green bar indicate exposed sample. Error bars indicate mean ± S.D (standard deviation) for n ≥ 3.

including lipid ozonation products, free radicals (mainly H_2O_2) and ozonides Ozone reacts selectively with the haem groups of haemoglobin, because it is recognized as oxygen (Cataldo and Gentilini, 2005). At the haemoglobin sites, it causes the oxidation of Fe (II) to Fe (III) and this is followed by a complete breakdown of the porphyrin rings of haemoglobin.

Poliachik et al. (2004) Shows that high intensity ultrasonic-induced cavitation, which is responsible for platelet rupture that leads to platelet aggregation in samples of platelet rich plasma (PRP) alone. Ultrasonic induced bulk fluid flow is necessary to mix platelet-activating factors and to allow platelet-platelet interactions. An optimal range of cavitation dose exists for achieving platelet activation and aggregation without causing extensive platelet damage.

UV blood irradiation that leads to biochemical effects are generated by the activation of molecular oxygen to singlet oxygen. This active species initiates a cascade of molecular reactions. The improvement in oxidation, rise in red blood cells, and increase in red cell may provide a significant boost to the body.

The sonolytic effectiveness of a given ultrasound exposure on a blood cell population support the observation that larger cells such as lymphocytes are more susceptible to mechanically induced forces than are smaller cells such as erythrocytes.

Conclusion

The effect of pulsed hybrid gas-liquid electrical discharge (shock wave, electromagnetic radiations, ozone and free radicals) leads to hematological changes; increase in erythrocytes, increase in hemoglobin, increase in white blood cells, decrease in lymphocytes and increasing of thrombocytes.

Ultrasound cavitation, may cause irreversible cell damage and modify the membrane structure and functional properties of the cell.

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