Original Article

The Effects of Ultraviolet Light Irradiation on Hematological and Morphological Characteristics and Potassium Level of Human Blood for Transfusion Associated Graft Versus Host Disease Prevention

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ABSTRACT

Purpose- To investigate whether the whole blood and blood products UV irradiation is safe with a minimum dose which is needed for Transfusion Associated Graft Versus Host Disease (TAGVHD) prevention. The safety of 5 new inventions is also investigated. Ultraviolet irradiation has been shown to be effective for prevention. However, ionizing irradiation has not yet been replaced by ultraviolet irradiation for blood irradiation at some blood banks because there are still questions about the safety of this technique.

Methods/Materials- In this research, the hematological, morphological characteristics and potassium level of the irradiated blood, irradiated with an equivalent dose of 4 J/cm2 of UVC (254nm) for 3 min, which is the minimum dose shown to be effective for TAGVHD prevention according to literature available, has been studied. The data was analyzed with SPSS software.

Results- The results showed that UV irradiation does not change the blood potassium level and hence does not damage the RBC membrane i.e. the correlation coefficient (cc) was 0.9879 with a p-value<0.005. Furthermore, the hematological tests showed no significant hematological change after ultraviolet exposure i.e. the cc for all of them were >0.5 with p<0.005. Moreover, the morphology of RBCs and PLTs after ultraviolet irradiation was normal (cc>0.7 with p<0.005).

Conclusions- According to the results, the ultraviolet irradiation is a safe and suitable way for blood and blood component irradiation for TAGVHD prevention and other applications with an equivalent dose of up to this UV irradiation dose. Accordingly, the new proposed machines and techniques are safe to be built for TAGVHD prevention; besides a new facility in the hematology and blood banking unit may be opened for patients that have autoimmune diseases.

Key words:
Blood Irradiation,
Ultraviolet Light (UV or UVC),
Blood Transfusion,
Transfusion Associated Graft Versus Host Disease (TAGVHD) Prevention,
UV Blood Irradiator Machine.

1. Introduction

The need for blood transfusion has been well known. There are some specific diseases like Thalassemia, Hemophilia and other chronic diseases that the patient is in need of continuous blood transfusion. On the other hand, blood transfusion from a donor to a patient in some cases has many risks such as infection and TAGVHD (Transfusion associated graft versus host disease). TAGVHD happens by the transfusion of untreated blood to immunocompromised recipients or when the blood of the donor is homozygous and the recipient is heterozygous for an HLA (Human Leukocyte Antigen) haplotype. The clinical manifestations of

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TAGVHD are similar to that of GVHD but due to the involvement of bone marrow lymphoid tissue, it is associated with a higher mortality (80-90%).

Currently, the blood products that are produced in the transfusion department of the hospitals are whole blood, Fresh Frozen Plasma (FFP), platelet rich plasma, cryoprecipitate, cryosupernatant, Red Blood Cells (PRBC) in the form of packed cells or red cell concentrate, and platelet concentrate.

Transfusion associated graft versus host disease (TAGVHD) is caused by the replication-competent donor lymphocytes in cellular blood products that engraft and lead to immune-mediated destruction of host tissues. The development of TAGVHD reflects the inability of the recipient's immune system to reject the transfused, and thus foreign, lymphocytes.

Blood recipients with acquired or congenital immunodeficiencies, leukemia, lymphoma, aplastic anaemia, solid organ transplants, allogeneic and autologous hematopoietic progenitor cell transplantation, pregnant women, Hodgkin's disease, immature immune systems, or those receiving closely HLA-matched platelet transfusions especially from first-degree family members are at risk of developing TAGVHD. There is no effective treatment for TAGVHD and its mortality rate is greater than 90% with blood transfusion in these cases. Hence, its prevention is of great importance. TAGVHD causes severe problems such as fever, rash, diarrhea and liver injury; it also results in severe marrow aplasia, with bleeding because of thrombocytopenia and infection due to neutropenia.

Currently, TAGVHD is prevented by ionizing a radiation e.g. gamma irradiation or x-ray irradiation. TAGVHD is effectively prevented by exposing blood components to a minimum of 25 Gy of gamma or x-ray radiation prior to transfusion to vulnerable patients. However, this technique has some side effects on the blood product irradiated [1].

TAGVHD is more common in immunocompromised patients, immunosuppressant, immunodeficient patients e.g. getting treated with chemotherapy or immunosuppressive drugs (for diseases such as multiple sclerosis or rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosus, focal segmental glomerulosclerosis, Crohn's disease, Behcet's disease, pemphigus, and ulcerative colitis), low-birth-weight neonates, intrauterine transfusions, bone marrow transplant patients, patients with congenital immunodeficiency syndromes, patients with Hodgkin's or non-Hodgkin's lymphoma, patients undergoing intensive treatment, patients being treated for acute leukemia and units donated by a blood relative. The aforementioned patient groups are the higher mortality patient groups [Handbook of Blood Banking and Transfusion Medicine].

Ionizing irradiation exposed to blood bags prevents the occurrence of TAGVHD by altering the DNA in the WBCs present in the blood such that they cannot divide and therefore cannot engraft in the recipient's blood after transfusion. The ionizing irradiated blood products are advised for more vulnerable patients with the probability of TAGVHD because of the side effects of ionizing irradiation. For example, ionizing irradiation increases the blood potassium, hence ionizing irradiated blood products are not transfused to patient with high potassium levels. Another problem caused by ionizing irradiation is the decrease in the pH of blood. In addition, platelets are more damaged by ionizing irradiation and hence irradiation must be done after storage. Therefore, storage is a problem for ionizing irradiated blood products. Moreover, there is the dose homogeneity and error problem.

Several studies have been undertaken on the suitability of ultraviolet radiation on platelet concentrates both for the disinfection [2-6] and TAGVHD prevention [7-14]. On the other hand, in some other studies it has been mentioned that the platelets are activated upon UV irradiation [15, 16]. Also, the UV irradiation may be done using photopheresis technique, and the safety of the photopheresis without the blood separation is questionable.

In addition, the available UV blood irradiator machines have disadvantages which leaves the controversy whether to choose non-ionizing UV radiation as a replacement for ionizing radiation. Besides, the suitability and safety of ultraviolet radiation on PRBC and whole blood for the TAGVHD prevention (in the higher mortality patient groups) [Handbook of Blood Banking and Transfusion Medicine]) still needs to be investigated.

In this study, the suitability of blood and blood products by non-ionizing ultraviolet irradiation has been investigated for the TAGVHD prevention.

2. Materials and Methods

In this study, the hematological and morphological characteristics of a total of 30 blood samples were examined before and after UV irradiation of 30 blood samples which is the lowest amount that can statistically give significant results using the T-test. These blood samples were human blood samples from the hematology laboratory of University Science Malaysia's health clinic which had K2EDTA anticoagulant.

The samples were separated into two vacuum plastic test tubes. An amount of 1 ml of blood were irradiated and 1 ml were kept as the control of each blood sample which were separated into vacuum plastic test tubes.

The second 30 samples were irradiated with an equivalent dose of 4 J/cm^2 of UVC light for 3 min (with a wavelength of 254 nm) which is the minimum dose, shown to be effective for the TAGVHD prevention according to the available literature. The instrument by which the second 30 samples were irradiated with was an ultraviolet fluorescence analysis cabinet, Spectroline model CX-20 with a shortwave UVC light source. The test tubes used for this purpose were vacuum plastic test tubes and the absorbance of the UV for these test tubes were analyzed with a spectrometer. The transparency of these plastic test tubes to UV light was insured.

The blood samples were analyzed with a Sysmex Hematoanalyzer before and after the irradiation. The blood cell counts, blood cell volumes, hematocrit and hemoglobin of the 60 samples were analyzed.

In addition, blood slides were prepared using a blood smear technique for microscope investigation prepared from a single blood drop before and after the UV irradiation. The red blood cells and platelets morphologies were examined with a microscope.

Furthermore, another 30 blood samples with sodium citrate anticoagulant were separated into two vacuum plastic test tubes, 0.6 ml each 30 were irradiated with the aforementioned dose and the other 30 were kept as the control. The potassium level of the 60 samples were achieved using an Olympus AU640 biochemistry analyzer.

In addition, in this study, the SPSS software was used for the statistical analysis of the data.

3. Results

In this study, almost an equal percentage of female and male blood samples were considered. In other words, 12 Male and 18 Female blood samples were considered out of the 30 total blood samples, i.e. 40.7% Male and 59.3% Female.

In addition, about more adult samples (16/30 or 53.3%) were considered in comparison to old samples (11/30 or 36.6%) as is the case normally for blood donators (the age of donator of 3 out of 30 blood samples were not available).

The hematological and potassium level results of the blood samples are displayed in Table 1. Before, observing this table, one must take into account that the 9 parameters are of different order and unit so they should not be compared with each other.

Table 1. The correlation coefficient and p-value and also mean and std and mean difference of each parameter before and after UVC irradiation.

	Correlation Coefficient	p-value	Mean of non-Irradiated Blood	Mean of Irradiated Blood	Mean Difference	Std of non-Irradiated Blood	Std of Irradiated Blood
RBC	0.5206	0.0032	4.8767	5.2963	0.4197	0.5799	0.7359
MCV	0.9969	0.0000	84.0900	84.0533	-0.0367	5.8834	5.8079
HGB	0.6010	0.0004	13.5333	14.8600	1.3267	1.7876	2.3057
HCT	0.5235	0.0030	40.9033	44.4133	3.5100	4.7157	6.2285
MCH	0.9732	0.0000	27.8267	28.1267	0.3000	2.5461	2.5955
MCHC	0.7810	0.0000	33.1000	33.4100	0.3100	1.0939	1.1330

PLT	0.7600	0.0000	260.9667	243.7333	-17.2333	61.4118	71.9382
MPV	0.7719	0.0000	9.6833	10.2700	0.5867	0.8983	0.7910
K	0.9879	0.0000	4.5933	4.5900	-0.0033	0.5433	0.4978

According to this table all the 9 parameters have a correlation before and after irradiation with a p-value less than 0.005. All the 9 parameters have a mean difference before and after the irradiation much less than the standard deviation of the control and irradiated groups; i.e. all 9 parameters did not have statistically significant change after the irradiation. In other words, according to Table 1 there is no statistically significant difference between the values of RBC, HGB, HCT, MCH, MCHC, MCV and PLT and MPV before and after the UV irradiation. In addition, the potassium level after UV irradiation is similar to the potassium level before the UV irradiation statistically.

Furthermore, the UV irradiation has no impact on the red blood cells or platelet morphology as was checked in the blood slides prepared under the microscope. No abnormal red blood cells (e.g. spherical, sickle shaped, etc) or abnormal platelets (large platelets, etc) were observed after the irradiation.

4. Discussion

According to the results, the UV irradiation of a dose equivalent to 4 J/cm^2 of UVC light for 3 min (with a wavelength of 254 nm) does not change the RBC count and volume and also the hemoglobin capacity of the RBC and potassium level of the blood. In other words, it has no impact on the RBC of the blood and its features and also on the RBC membrane, which leads to a longer storage life. Therefore, this dose can be used for the RBC irradiation for the TAGVHD prevention.

This could be simply done by the present UV irradiating machines available for the RBC irradiation which are not used that routinely in the blood banks instead of ionizing radiation due to low penetration depth. In other words, the present available RBC UV irradiators' additional consumables' cost are too much and they are difficult to work with and need special care, attention and accuracy when doing the process.

Alternatively, according to this safety approval, the invention of a more simpler machine that works with a separate transparent RBC bag that can be connected to the RBC bags with a vent with a standard speed and a line that can be inserted to the blood bag and a cylindrical UV lamp with a standard dose, would also be safe and can give the desirable effect considering the flow speed. The advantage of this invention is an automatic accuracy (built in accuracy).

In addition, this UV irradiation does not change the PLT count and volume. In other words, it has no impact on the PLT of the blood and its features. This could be simply done by the present UV irradiating machines available for platelet irradiation.

Likewise, this could be done using a 2nd invention that is much simpler with a separated transparent platelet bag that can be connected to the platelet concentrate bags with a vent with a standard speed and a line and a cylindrical UV lamp with a standard dose that can give the desirable effect by adjusting the flow speed.

In conclusion, the results of this study prove that this UV irradiation does not affect the RBC nor the PLT. Hence, UV irradiation with this dose is safe for the whole blood irradiation.

Thus, this proves the safety of a third invention for automatically making TAGVHD free blood or blood products automatically by a built in prototype inside the apheresis machine that is consisted of two main components: a UV transparent tube and a cylindrical UV lamp that together by calculating the flow speed and the tube length and the lamp intensity equalizes to the study's dose.

Of course, a manual version of this invention makes the 4th invention which does exactly the same job except that can be operated manually and by hematology and blood bank personal. This machine is made of a cylindrical UV lamp that a UV transparent tube can be inserted inside is axis which is connected to a separate blood or blood component storage bag and the connected tube can be inserted by a needle to the blood bag and also is consisted of a vent which the blood flow speed

can be adjusted by it. Likewise, the dose must be calculated by the inventor or at least a formula must be proposed by the inventor that can give the desirable result.

Furthermore, this can also be done using the third invention, which may open a new facility in the hematology and blood banking unit for patients that have autoimmune diseases.

Finally, the whole blood TAGVHD prevention can also be done straight away from the patient into a blood donation bag by the 5th invention, which consists of the blood donation bag having a line or a tube that is UV transparent and a filter and a cylindrical UV lamp which the dose must be adjusted. However, for this last proposed UV blood irradiator machine, the safety of the process for the blood donator should also be assured after the flow rate calculation.

5. Conclusions

According to this research, UV irradiation does not change the hematological or morphological characteristics of the blood. Moreover, UV irradiation does not damage the RBC membrane of the blood which will result in longer life. This is one of the main advantages in comparison to ionizing radiation blood irradiation. This makes UV irradiation of red blood cells a better option for the TAGVHD prevention in comparison to ionizing X-ray or gamma-ray blood irradiation techniques.

Hence, UV irradiation of a dose equivalent to 4 J/cm^2 of UVC light for 3 min (with a wavelength of 254 nm) maybe a better method for the TAGVHD prevention of RBC, platelet or whole blood collected or treated either by apheresis technique or donated routinely in the blood banks. In addition, this advantage increases the transfusion option for more patients at risk of TAGHVD like neonates.

In conclusion, this UV light irradiation is a safe technique for whole blood or blood components irradiation with the dose adequate for the TAGVHD prevention.

Finally, considering the fact that this UV irradiation does not change the potassium level and that the RBC's membrane is not damaged means a longer RBC life. This is the main feature of UV irradiation comparing to ionized irradiation and also other risks that ionized irradiated may have on the blood bank personnel.

Finally, UV irradiation is safe to be included as a new additional prototype for Apheresis Machines or in the blood donating process.

Furthermore, this study proves the safety of the 5 new inventions mentioned in the discussion, which can be used for easier or less costly or safer or faster or automatic TAGVHD free whole blood or blood component production; and also may open a new facility in the hematology and blood banking unit for patients that have autoimmune diseases.

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