Change in Blood Coagulation Parameters After Total Body Irradiation

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ABSTRACT

We aim to test the changes of coagulation parameters after the TBI by thromboelastography. Eleven patients who underwent TBI between march 2005 and march 2006 in our department were included in this study. Blood samples were taken from all patients immediately before the delivery of TBI and 12 hours after the completion of TBI. Complete blood counts, partial thromboplastin time (aPTT), prothrombin time (PT), and the fibrinogen levels were measured. FIB-EXTEM, extrinsic (TEG-EXTEM), intrinsic (TEG-INTEM) clotting pathways, and platelet functions were measured by rotational thromboelastogram (ROTEM). All measured parameters were compared by using wilcoxon-paired test.

There was no difference in the PT, aPTT, thrombin and thrombocyte counts before and after TBI. However, maximum clot firmness was decreased after TBI in FIBTEM measurement (18.6 vs 15.5, $p<0.05$). The clot formation time was also significantly prolonged (115.8 vs 121.7 seconds, $p<0.05$) and maximum clot firmness was decreased in EXTEM measurements. The fibrinogen levels were significantly decreased after TBI.

Our results demonstrated that the clot formation was significaltly decreased in the early period after TBI. Prolongation of clot formation time and decrease in maximum clot firmness in extrinsic pathway after TBI indicates disturbance in platelet functions.

Key Words: Total body irradiation, Coagulation system, Thromboelastography, Platelet function, Clot formation

ÖZET

Tüm Vücut Işınlaması Sonrasında Kan Koagulasyon Parametrelerindeki Değişimler

Bu çalışmanın amacı TBI uygulanan hastalarda koagülasyon parametrelerindeki değişiklileri tromboelastografi yöntemi ile araştırmaktır. Mart 2005 ve Mart 2006 tarihleri arasında bölümümüzde TBI uygulanan 11 hasta çalışmaya dâhil edilmiştir. Kan örnekleri TBI'dan hemen önce ve TBI'dan 12 saat sonra alınmıştır. Tam kan sayımı, parsiyel tromboplastin zamanı (aPTT), protrombin zamanı (PT) ve fibrinojen seviyeleri ölçülmüştür. FIB-EXTEM, ekstrinsik (TEG-EXTEM), intrinsik (TEG-INTEM) pıhtılaşma yolakları ve trombosit fonksiyonları rotasyonal tromboelastogram (ROTEM) ile değerlendirilmiştir. Bütün ölçümler wilcoxon-paired test kullanılarak karşılaştırılmıştır.

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PT, aPTT, trombin ve trombosit değerlerinde TBI'dan önce ve sonra yapılan ölçümlerde bir fark saptanmamıştır, ancak FIBTEM ölçümlerinde maksimum pıhtı sağlamlığının TBI'dan sonra azaldığı gözlenmiştir (18.6 vs15.5, p<0.05). Pıhtı oluşum zamanı belirgin uzamıştır (115.8 vs 121.7 saniye, p<0.05) ve masimum pıhtı sağlamlığı EXTEM ölçümlerinde azalmıştır. TBI'dan sonra fibrinojen düzeyleri belirgin azalmıştır.
Sonuçlarımız TBI'dan sonraki erken dönemde pıhtı oluşumunun belirgin azaldığını göstermektedir. Pıhtı oluşma zamanının uzaması ve ekstrinsik yolakta maksimum pıhtı sağlamlığının azalması TBI'dan sonra trombosit fonksiyonlarının bozulduğunu göstermektedir.

Anahtar Kelimeler: Total vücut ışınlaması, Koagülasyon sistemi, Tromboelastografi, Trombosit fonksiyonu, Pıhtı oluşumu

INTRODUCTION

Bone marrow transplantation (BMT) is the intravenous infusion of hematopoietic progenitor cells for re-establishing marrow function in a patient with damaged or defective bone marrow (1). Over the past 3 decades, BMT has become a prominent procedure in the management of most hematological malignancies. Total body irradiation (TBI) is a well-established approach for the conditioning of the patients with hematological malignancies before transplantation and is particularly employed in allogeneic BMT, since both immunosupression and tumor eradication are intended (1,2).

The dose range of TBI given in most conditioning regimens varies between 7.5-15 Gy (3, 4). Even with hematopoietic rescue and optimal supportive care, the treatment dose window of TBI is narrow. Both adequate immunosupression and significant leukemia cell kill require a TBI dose that may cause serious pulmonary, hepatic, and gastrointestinal damage. The thin therapeutic index was demonstrated in a prospectively randomized study from the Seattle group which showed that increasing TBI dose from 1200 to 1575 cGy in patients with CML in the first remission resulted in a decrease in leukemia relapse at 3 years from 35% to 12% (p=0.06). Unfortunately, transplant-related mortality increased from 12% to 32% (p=0.04), leading to an overall survival that was nearly identical in the two groups owing to the radiation-related toxicity and mortality (5). There is obvious evidence that tolerance dose of radiation for critical organs is lower during TBI than whole organ irradiation. A possible explanation for the difference in organ tolerance doses between TBI and conventional radiotherapy regimens for kidney disease is reported to be the contribution of the intensive chemotherapy regimens used before and as a part of the conditioning for BMT, as well as that the patient is immunocomprised, temporarily in poor condition and susceptible to graft vs. host reactions. Furthermore, many patients also receive high dose of antibiotics and antifungal drugs during the course of transplantation (6,7). The majority of the patients being irradiated to partial volumes also receive intensive chemotherapy and many other potentially toxic regimens.

We have previously demonstrated changes in the trace element contents of kidney after TBI. The change in the trace element concentration in kidney has been shown after TBI and this might have a role in the development of toxicity (8). It has also been shown that there are changes in cytokines and redox-active metal composition of blood after TBI (9). TBI, changes the blood content of trace elements (8), probably acute phase response proteins (10) and many other inflammatory proteins and cytokines (11-13) within the blood. The change in blood chemistry might lead to the blood clotting tendency which has been one of the proposed mechanisms of late injury. We have proposed that organ dysfunction after TBI might be due to the damage to parenchymal cells and blood vessels (14). In order to test this hypothesis, we have investigated if there is any change in the blood coagulation parameters shortly after TBI.

PATIENTS AND METHODS

Eleven patients (10 men, 1 woman) who underwent TBI for bone marrow transplantation at the Hacettepe University, Department of Radiation Oncology in March 2005 and March 2006 were included in the study.

Table 1. Blood cell counts before and after TBI

Blood cell	Before TBI	After TBI	P
Red blood cells	4.01 (3.17-5.05)x106	3.78 (2.95-4.96)x106	0.01
White blood cells	5540 (400-8800)	3820 (300-8900)	0.01
Thrombocytes	156.6 (98-351)x 103	159 (80-321) x103	0.87
Neutrophils	3560 (200-8000)	3140 (100-8400)	0.33
Lymphocytes	1420 (100-3200)	400 (100-1400)	0.01
Monocytes	360 (0-900)	240 (0-700)	0.03
Basophils	20 (0-100)	20 (0-100)	1
Eosinophils	60 (0-300)	20 (0-100)	0.16

The diagnosis of the patients were 6 with leukemia (4 with acute lymphoblastic lymphoma, 2 with chronic myeloid leukemia), non-Hodgkin lymphoma (4 patients) and multiple myeloma (1 patient). TBI was delivered in every case after conditioning chemotherapy. Six patients received 1200 cGy TBI in 6 equally divided doses over 3 days, and remaining 5 patients received 600 cGy TBI in 3 divided doses, twice daily, with a minimum 8-h interval between fractions by using Cobalt 60 teletherapy unit. The instantaneous dose rate was between 0.06 and 0.07 Gy/min. The prescribed dose to central axis (abdomen) was 200 cGy/ fraction in all patients. The lung dose was limited to a maximum of 10 Gy by customized blockings in those patients who received 12 Gy. Patients were set 4 m from the source and tissue equivalent compensators were put around the thin body regions, such as head, neck and legs.

Blood samples were driven from all patients just before the start of TBI and 12 hours after TBI. Complete blood counts, partial thromboplastin time (aPTT), prothrombin time (PT), prothrombin time activity, PT INR, thrombin time and fibrinogen levels were studied. In addition, a sample of blood was also taken for rotational thromboelastography (ROTEG®, Pentapharm, and GmbH, Germany). ROTEG is a relatively new method for global and quick measurement for extrinsic (TEG-EXTEM), and intrinsic coagulation (TEG-INTEM) pathways, and additionally, for the assessment of the contribution of plasma factors (FIB-EXTEM) and thrombocyte functions. Coagulation time (CT), clot formation time (CFT), α-angle, maximum clot firmness (MCF) were also measured.

Table 2. Coagulation parameters before and after TBI

	Before TBI	After TBI	P
Prothrombin Time Activity	93.8 (79-114) %	92.6 (79-103) %	0.67
Prothrombin Time	13.3 (11.6-14.6) sec.	13.3 (12.2-14.6) sec.	0.67
PT INR	1.05 (0.91-1.19)	1.06 (0.98-1.19)	0.67
APTT	28.2 (25.5-30.3) sec.	35.6 (20.9-80.3) sec.	0.38
Fibrinogen	378.2 (283-471)	307.2 (190-475)	0.01
Thrombin Time	20.2 (15.3-31.7) sec.	39.8 (18.4-120) sec.	0.33
Ferritin	1073 (43-2331) ng/ml	1988 (37-5751) ng/ml	0.07

Table 3. ROTEG findings

	Before TBI	After TBI	P
Teg-Extem			
Coagulation Time	55.7 (40-129)	179.9 (42-1506)	0.91
Clot formation time	115.8 (51-273)	121.7 (59-231)	0.01
Alpha angle	74.8 (55-81)	72 (54-81)	0.08
Maximum clott firmness	56.6 (38-70)	51.7 (4-67)	0.01
Teg-Intem			
Coagulation Time	183.2 (118-503)	389.2 (111-2883)	0.10
Clot formation time	133.6 (45-407)	89.7 (47-170)	0.76
Alpha angle	72 (55-81)	74.8 (61-80)	0.87
Maximum clott firmness	53 (31-69)	54.9 (43-66)	0.53
Fib-extem			
Coagulation Time	55.4 (41-143)	188.6 (40-1599)	0.68
Clot formation time	934.6 (80-2022)	979.5 (476-1544)	1.00
Alpha angle	72.7 (47-80)	72.4 (49-79)	0.17
Maximum clott firmness	18.6 (10-29)	15.5 (5-22)	0.03

The studied parameters before and after the TBI were compared using the Wilcoxon-paired test. The data were analyzed by SPSS 9.0 software (SPSS inc., Chicago,IL.).

RESULTS

The patients median age was 26 (range between 18 and 62). All of the patients did well during TBI. All patients received various conditioning chemotherapy regimens.

There were significant decreases in the red and white blood cell counts (especially, lymphocytes and monocytes). Platelet, eosinophil, basophil and neutrophil counts did not show any significant change after TBI (Table 1).

The mean fibrinogen level decreased only significantly 12 hours after TBI. The mean fibrinogen level was 378.2 mg/dL vs. 307.2 mg/dL, before and after TBI, respectively. The rest of the parameters; PT, aPTT, PTZ, thrombin time, PT INR did not change significantly after TBI (Table 2).

Thromboleastographic measurements of the blood coagulation parameters and pathways showed that the maximum clot firmness was significantly less after TBI in FIB-EXTEM measurements (18.6 vs 15.5 mm $p< 0.05$). The clot formation time was also significantly prolonged (115.8 vs. 121.7 seconds, $p < 0.05$) and the maximum clot firmness was decreased in TEG-EXTEM measurements. There was no change in the TEG-INTEM measurements (Table 3).

DISCUSSION

To the best of our knowledge, the present study is the first to report blood coagulation parameters shortly after TBI. Our findings revealed that, there is a decrease in the fibrinogen level together with the prolongation of the clot formation in extrinsic pathway and a decrease in the maximum clot firmness in both extrinsic pathway and FIB-EXTEM measurements. Altogether, these findings suggest that the prolongation of clotting time is probably due to the platelet dysfunction.

The coagulation panels studied before and after the TBI just yielded a decrease in fibrinogen levels. The thromboplastin time (aPTT), prothrombin time (PT), prothrombin time activity, PT INR, thrombin time levels did not show any significant change after TBI.

Fibrinogen and most of the coagulation parameters are synthesized in liver (15). In our previous study, we have shown that there is a rise in acute phase response proteins after pelvic radiotherapy (10). Being an acute phase protein we expected an increase in the level of fibrinogen. However shortly after TBI, there is a decrease in fibrinogen levels. One of the mechanisms of this could be related to the organ toxicity. TBI is such a toxic regimen that liver and body may not respond well enough to produce inflammatory response in early phase after TBI. Liver and body systems may respond differently to local irradiation and TBI.

We could speculate that different biological effects of TBI and partial body irradiations are responsible for the increased toxicity after TBI. Although the biological effects of TBI are extensively studied, there is still no clear data regarding the differences of mechanisms of TBI and partial body irradiation. A possible explanation for the differences in organ tolerance doses between TBI and conventional radiotherapy regimens is reported to be the contribution of the intensive chemotherapy regimens used before and as part of the conditioning for BMT. The patient is immunocomprised, temporarily in poor condition and susceptible to graft vs. host reactions. Moreover many patients also receive high doses of antibiotics and antifungal drugs during the course of transplantation (16,17). However, the majority of the patients being irradiated to partial volumes also receive intensive chemotherapy and many other potentially toxic regimens.

Radiobiologically, damage to two groups of the cells, parenchymal and vascular cells is accused to be responsible for the development of organ dysfunctions after irradiation. Beside damage to target cells, distribution of function (homogenous versus heterogeneous) in the organ and structural organization of the functional subunits (parallel or redundant versus in series or independent) may play a major role (18). Nonetheless, functional distribution and structural organizations should not be important during TBI or partial body irradiation if it includes the whole organ, since whole units of the organ receive the same radiation dose. Since the tolerances of target parenchymal and vascular cells, on the other hand, are not same during TBI and conventional whole organ irradiation. There should be other underlying factor (or factors) that might influence the organ tolerances. The differences in the acute phase responses might be a factor.

The decrease in red blood cell count 12 hours after completion of TBI is an unexpected finding. Red blood cell (RBC) levels are reported to be the latest blood parameter that is affected from the radiation because of the long life span of the red blood cells (19). However, in our study, there is a significant loss of RBC, shortly after the initiation of TBI. Drop in RBC counts might be due to the combined effect of previously initiated conditioning chemotherapy and TBI. Significant decrease in lymphocyte counts is an expected finding, since lymphocytes are the most radiosensitive cells in the blood. Not only the precursors of lymphocytes, but also mature lymphocytes are quite radiosensitive which decrease sharply immediately after TBI. The granulocyte counts are reported to fall 1-2 days after TBI, however, in our study blood samples are drawn 12 hours after TBI and the interval is not long enough to find any change in the granulocyte counts. There is a significant drop in the monocyte counts. There is no change in eosinophil or basophil counts after TBI. We could not detect any change in the platelets counts after TBI due to the short time interval between TBI and blood cell counts.

This study has some limitations, since the patient number is small, the diagnosis and previous chemotherapy regimens, as well as the conditioning chemotherapy regimens, are not similar for all the patients. TBI doses are also not uniform, since two different regimens have been used. The coagulation parameters only at the 12th hour after the TBI have been studied and this may not reflect long term change in blood homeostasis. In conclusion, these findings reflect some changes in the blood coagulation parameters after total body irradiation may give some insight and promote further research on this issue.

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