# Monitoring of platelet function parameters and microRNA expression levels in patients with prostate cancer treated with volumetric modulated arc radiotherapy

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**Abstract.** Radiotherapy (RT) may result in platelet activation and thrombosis development. To the best of our knowledge, the potential effect of volumetric-modulated arc therapy (VMAT), a novel radiotherapy technique, on platelet function and microRNA (miRNA/miR) expression has not been previously investigated. The present study aimed to determine the effect of VMAT on the alterations in platelet function parameters and miRNA expression levels. A total of 25 patients with prostate cancer and 25 healthy subjects were included in the present study. Blood samples were collected from the patient group on the day prior to RT (pre-RT), the day RT was completed (post-RT day 0), and 40 days following the end of therapy (post-RT day 40). Platelet count, mean platelet volume (MPV) value, platelet aggregation, plasma P-selectin, thrombospondin-1, platelet factor 4, plasma miR-223 and miR-126 expression levels were measured. A significant decrease in platelet count in the post-RT day 0 group was measured in comparison with the pre-RT and the post-RT day 40 groups. Pre-RT MPV values were higher than those of the post-RT day 0 and the post-RT day 40 groups. No significant differences were observed in the levels of platelet activation markers or miR-223 and miR-126 expression levels between the RT groups. Although RT may result in a reduction in platelet and MPV counts, the results of the present study indicate that platelet activation markers are not affected by VMAT. Therefore, it is possible that no platelet activation occurs during VMAT, owing to the conformal dose distributions, improved target volume coverage and the sparing of normal tissues from undesired radiation.

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## Introduction

Prostate cancer was a common global malignancy among men in 2012 (1,2). Ionizing radiation is used to treat various types of cancer (3), including localized prostate cancer (4). External beam radiotherapy (RT) has been demonstrated to be a curative method for localized prostate cancer (5) by the European Association of Urology guidelines (6).

In addition to killing cancerous cells, ionizing radiation damages healthy cells. The hematopoietic system is an important system in radiosensitive patients who are affected by the acute-phase effects of radiation (7). Ionizing radiation results in vascular damage through exertion of a cytotoxic effect on the vascular endothelium (8,9). Furthermore, homeostatic responses are activated due to oxidative damage that develops in the endothelium, including the activation of platelets in irradiated tissues, which results in platelet aggregation (9). The probability of thrombosis differs based on the type of cancer, with this probability varying between 6.9 and 11.4% in prostate cancer (10). Platelets are known to serve notable functions in the formation of thrombosis (11,12).

Since the mid-90s, developments in techniques used in RT have allowed researchers to increase control over the tumor while decreasing the side effects that result from the treatment. These developments have additionally benefitted radiation therapy used for prostate cancer (5). A previous study performed with three-dimensional conformal RT (3D-CRT) in prostate cancer revealed that toxicity may be decreased if the target and the RT volume are compatible (6). Intensity-modulated RT (IMRT), a technique used in 3D-CRT, is the most common method used to treat prostate cancer. This method provides a better distribution of conformal doses, thus increasing the targeted dose distribution and decreasing the toxicity effect on normal tissues (5). A recently developed technique named volumetric-modulated arc therapy (VMAT) delivers the radiation dose more efficiently with a dynamic modulated arc (5,13); however, there are a limited number of

studies focusing on the effect on platelet functions in acute and chronic phases of radiation therapy applied using the VMAT technique. Studies performed on the effects of RT on platelet functions usually focus on specific markers, including platelet count and mean platelet volume (MPV) (7,14-16). In addition to platelet aggregation (17), the levels of P-selectin (18,19), thrombospondin-1 (20) and platelet factor 4 (PF 4) (21) are less commonly used parameters as markers of platelet function.

Previous molecular studies have revealed an association between serum/plasma levels and platelet levels and a number of microRNAs (miRNA/miRs), which direct gene expression by targeting mRNA at the post-transcriptional level (22,23). As miRNA expression levels may be altered in various types of disease, these tissue-specific molecules may be used as disease markers by measuring their levels in circulation (24). It has been suggested that a number of miRNAs may additionally be used as platelet activation markers (25-27).

A number of miRNAs are associated with platelet function, miR-223 and miR-126, which is expressed in platelets, are among the most commonly studied molecules. One previous study revealed that miR-223 and miR-126 serve a critical function in regulating the expression of vascular cell adhesion protein 1 and P2Y12 receptors in platelets (25). It has been reported that the altered plasma levels of these two miRNAs in cardiovascular diseases is associated with platelet function (24,26,27). This change may be plausible in post-treatment serum/plasma levels as a result of miRNAs released from platelets due to the toxic effect of RT. However, to the best of our knowledge, no studies have investigated this type of change caused by treatment with RT, particularly VMAT, on patients with prostate or other types of cancer. In the light of the aforementioned information, the present study was conducted to analyze the effect of VMAT on platelet function in patients with prostate cancer using previously determined markers, and to evaluate the association of plasma levels of miR-223 and miR-126 with treatment.

## Materials and methods

*Case selection*. A total of 25 male patients (mean age 66.57, range 55-76 years) diagnosed with histologically confirmed adenocarcinoma of the prostate and treated with RT at the Istanbul University Oncology Institute, Department of Radiation Oncology (Istanbul, Turkey) between June 2013 and February 2014 were included in the study. A total of 25 healthy male volunteers of similar age (mean age 63.76, range 52-76 years), who did not receive any medication, constituted the control group. Medical history, including hematological disorders that affect platelet count or platelet function, distant metastases or other malignant diseases, diabetes, hypertension, chronic inflammatory diseases, infectious diseases and autoimmune diseases, were designated as exclusion criteria for the patients with prostate cancer. A known history of chronic, inflammatory or malignant diseases and the use of antithrombotic drugs were the exclusion criteria for the healthy control subjects. Patients with prostate cancer received therapy with VMAT (Varian Trilogy Rapid Arc Radiotherapy Device; Varian Medical Systems, Inc., Palo Alto, CA, USA) with a daily dose of 180-200 cGy fractions, 5 days a week for 30-37 days. The doses of the planned target volumes

for prostate and seminal vesicles were 66-74 and 50-54 Gy, respectively. The total postoperative dose administered to the prostatic fossa was 66 Gy. Whole pelvis irradiation was not given. All patients gave written informed consent, and the present study was approved by the Istanbul University Cerrahpasa Medical Faculty Ethics Committee (approval number: 28052/2012) and the present study was performed in accordance with The Declaration of Helsinki.

*Sample collection*. Venous blood was collected into tubes containing 3.8% sodium citrate and EDTA separately from each person the day prior to RT (pre-RT group), the day RT was completed (post-RT day 0 group) and 40 days following the end of RT (post-RT day 40 group) from patients with prostate cancer. The same volume of blood was collected into the tubes from healthy control subjects.

*Platelet count and MPV values*. Platelet counts and MPV values were determined using Cell-DYN C1600 (Abbott Pharmacuetical Co., Ltd., Lake Bluff, IL, USA) blood count device using blood collected as aforementioned.

*Platelet aggregation*. Platelet aggregation was determined according to the method of Angiolillo *et al* (28) using a light transmittance aggregometer (Chrono-log 500; Chrono Log Corp., Havertown, PA, USA). Venous blood samples were collected in tubes containing 3.8% sodium citrate. Platelet-rich plasma (PRP) was obtained as a supernatant subsequent to centrifugation of citrated blood at 160 x g for 10 min at 22°C. The remaining blood was centrifuged at 2,500 x g for 10 min at 22°C to obtain platelet poor plasma (PPP). PPP and PRP were incubated for 3 min at 37°C. Following incubation, 1 $\mu$M of adenosine diphosphate (Chrono Log Corp.) was added into PRP to induce the platelet aggregation. Platelet aggregation curves were recorded for 3 min (29). Results were expressed as the extent of maximal aggregation (% of maximal amplitude).

*Platelet activation*. Venous blood samples containing 3.8% sodium citrate were centrifuged for 15 min at 1,000 x g at 4°C, and the obtained plasma samples were stored at -80°C until plasma thrombospondin-1, P-selectin and PF 4 were measured using an ELISA. Subsequent to the thawing of each plasma sample at 4°C, the measurements of plasma thrombospondin-1 (EIAab Science Co. Ltd., Wuhan, Hubei, China; cat. no. E0611 h), P-selectin (Wuhan EIAab Science Co. Ltd.; cat. no. E0115 h) and PF 4 (Wuhan EIAab Science Co. Ltd.; cat. no. E0172 h) were performed using commercial kits according to the manufacturer's protocol.

*miRNA detection with reverse transcription-quantitative polymerase chain reaction (RT-qPCR)*. EDTA-plasma samples stored at -80°C were thawed at 4°C prior to use and miRNA was immediately isolated by using the Qiagen miRNeasy Serum/Plasma kit (Qiagen GmbH, Hilden, Germany) following manufacturer's protocol. cDNA synthesis was synthesized using ABM miRNA cDNA Synthesis kit with Poly(A) Polymerase Tailing (ABM Inc., Vancouver, Canada) according to manufacturer's protocol. Samples were stored at -80°C until further use. The expression levels of miRNAs were determined using the Eco™ Real-Time

PCR system (Illumina, Inc., San Diego, CA, USA). The primers used were as follows: *Homo sapiens* (hsa)-miR-223 forward, 5'-GTCCGCTGTCAGTTTGTCAAATAC-3' and reverse, 5'-GTGCGTGTCGTGGAGTC-3'; hsa-miR-126 forward, 5'-GTCCGCTCGTACCGTGAGTAATA-3' and reverse 5'-GTGCGTGTCGTGGAGTC-3'; U6-2 forward 5'-GCCCCTGCGCAAGGATGAC-3' and reverse, 5'-CCA GTGCAGGGTCCGAGGTA-3'. All miRNA primers were procured from ABM Inc. RT-qPCR was conducted by using the EvaGreen miRNA qPCR MasterMix (Applied Biological Materials, Inc., Richmond, BC, Canada). The reaction conditions were as follows: 1 cycle for 10 min at 95°C, 40 cycles for (10 sec at 95°C, 15 sec at 60°C and 5 sec at 72°C). Melting curve analysis was performed with a sensitivity of 0.1°C at temperatures between 55 and 90°C. PCR analyses were performed by calculating the standard curve and the number of duplicates. The Eco study software v5.0 (Illumina, Inc.) was used to calculate quantification cycle (Cq) expression values (30) for all genes. The U6-2 small nuclear RNA was used as an internal control to detect hsa-miR-223/miR-U6-2 and hsa-miR-126/miR-U6-2 ratios in the plasma.

*Statistical analysis.* Data are presented as mean ± the standard deviation (SD). Statistical analysis was performed using the Wilcoxon signed-rank and Mann-Whitney U-tests. Correlation analysis using Spearman's rank was used to study the association between markers. $P<0.05$ was considered to indicate a statistically significant difference. Variation coefficiency (% Cv) was used to calculate the scattering due to individual variations. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA). Cv values were calculated as: % Cv=(SD of measurement/measurement average) x100.

## Results

*Patient data.* Demographic data of patient and control groups are presented in Table I. Platelet count, MPV value and platelet activation markers are presented in Table II as the mean ± SD.

*Platelet count and MPV value.* The platelet count of the post-RT day 0 group was significantly reduced vs. the pre-RT and the post-RT day 40 groups (208.90±37.45 vs. 228.80±43.39 and 232.80±44.79; P=0.002 and P=0.001, respectively). There were no significant differences identified (P>0.05) between the platelet count of the post-RT day 40 group and the pre-RT group, and all RT groups compared with the control group. Platelet count values are presented as the mean ± SD in Table II.

The MPV values of the pre-RT group were identified to be higher than the post-RT day 0 and post-RT day 40 groups (8.81±0.81 vs. 8.34±0.79 and 8.19±0.81; P<0.001 and P<0.001, respectively). No significant difference in MPV values was identified between the post-RT day 0 and post-RT day 40 groups (P>0.05). Furthermore, there were no significant differences identified (P>0.05) in the control group compared with the post-RT day 0 and post-RT day 40 groups. However, a significant difference was identified between the pre-RT and control groups (P<0.05). MPV values are presented as the mean ± SD in Table II.

Table I. Demographic data of patient and control groups.

| Variables | Patients | Control | P-value |
|---|---|---|---|
| Age, years | 66.57±6.65[a] | 63.76±8.49[a] | 0.386 |
| Histology | | | |
| Adenocarcinoma, n (%) | 25 (100) | NA | |
| Tumor stage | | | |
| T2c, n (%) | 4 (16%) | NA | |
| T3b, n (%) | 12 (48%) | NA | |
| T3c, n (%) | 9 (36%) | NA | |
| Total radiation dose, cGy | 6,600-7,400 | NA | |
| Fractions, n | 30-37 | NA | |
| Family history | | | |
| Yes, n (%) | 3 (12%) | NA | |
| No, n (%) | 22 (88%) | NA | |

[a]Mean ± standard deviation; NA, not applicable.

*Platelet activation markers.* Platelet aggregation, plasma P-selectin, plasma thrombospondin-1 and plasma PF 4 were measured as platelet activation markers. There was no significant difference (P>0.05) between RT and control groups and among RT groups when platelet aggregation, plasma levels of P-selectin, thrombospondin-1 and PF 4 were measured (Table II). However, high values of plasma thrombospondin-1 and PF 4 were observed in the coefficient of variation calculations used to determine the scattering due to personal variations (Cv 51.6 to 63.4 and 41.8 to 54.5%, respectively; data not shown).

*miR-223 and miR-126 expression.* Plasma expression levels of miR-223 and miR-126 are given as Cq miR-223/Cq miR-U6-2 and Cq miR-126/Cq miR-U6-2 ratios (Fig. 1A and B, respectively). No significant difference (P>0.05) was identified between the plasma miR-223 expression levels of the RT-treated and control groups and amongst the RT groups. Similarly, the miR-126 expression levels did not vary significantly (P>0.05) between any of the groups (Table III). Fig. 2A-D shows the individual changes in Cq miR-223/Cq miR U6-2 and Cq miR-126/Cq miR-U6-2 ratios in RT-treated groups. Ct miR-223/Ct miR U6-2 and Ct miR-126/Ct miR U6-2 ratios in patient and control groups values are presented as the mean ± SD in Table III.

*Correlation between miRNA expression levels and platelet function markers.* A positive correlation was identified between Cq miR-223/Cq miR-U6-2 and Cq miR-126/Cq miR-U6-2 ratios in all RT-treated groups. Correlation between Cq miR-223/Cq miR-U6-2 and Cq miR-126/Cq miR-U6-2 ratios in all RT-treated groups are presented in Fig. 3A-C.

## Discussion

RT kills target cells by inducing DNA damage (31). However, RT may also harm the cells of healthy tissues and mitotically active bone marrow as collateral damage (14). Various studies have reported the existence of occlusions in the vascular system in the chronic phase of irradiation and aggregation of

Table II. Differences in the level of activation markers of platelets in patient and control groups.

| Patient group | Platelet counts, 10³/mm³ | Mean platelet volume, $\mu m^3$ | Platelet aggregation, % | Plasma P-selectin, ng/ml | Plasma thrombospondin-1, ng/ml | Plasma platelet factor 4, ng/ml |
|---|---|---|---|---|---|---|
| Pre-RT | 228.80±43.39 | 8.81±0.81[a] | 72.78±19.05 | 1.65±0.39 | 1.57±0.99 | 3.52±1.73 |
| Post-RT day 0 | 208.90±37.45[b] | 8.34±0.79[c] | 75.10±22.57 | 1.71±0.38 | 1.84±0.99 | 3.68±1.69 |
| Post-RT day 40 | 232.80±44.79[d] | 8.19±0.81[e] | 71.31±22.46 | 1.62±0.36 | 1.84±0.95 | 3.80±1.59 |
| Control | 231.60±40.89 | 7.97±1.17 | 77.25±16.27 | 1.62±0.26 | 1.68±0.98 | 3.26±1.77 |

All values are presented as the mean ± standard deviation. [a]Control vs. pre-RT, P<0.05; [b]pre-RT vs. post-RT day 0, P<0.01; [c]pre-RT vs. Post-RT day 0, P<0.001; [d]post-RT day 0 vs. post-RT day 40, P<0.001; [e]pre-RT vs. post-RT day 40, P<0.001. RT, radiotherapy.

Table III. Cq miR-223/Cq miR U6-2 ratios in patient and control groups.

| Patients (n=25 per group) | Cq miR-223/ Cq miR U6-2 | Cq miR-126/ Cq miR U6-2 |
|---|---|---|
| Pre-RT | 1.17±0.40 | 1.15±0.44 |
| Post-RT day 0 | 1.31±0.41 | 1.22±0.38 |
| Post-RT day 40 | 1.23±0.38 | 1.17±0.36 |
| Control | 1.19±0.23 | 1.21±0.20 |

Values are presented as the mean ± the standard deviation. RT, radiotherapy; miR, microRNA; Cq, quantification cycle.

platelets in the vascular lumen during the 2 h following irradiation (32-34). Platelet adhesion to vascular wall structures due to radiation results in radiation-associated thrombosis (32).

VMAT was developed as a novel form of arc therapy in 2007 to decrease the side effects of RT (35). There are a number of benefits of VMAT, including increased target specificity and improved healthy tissue avoidance, in addition to improving coverage of the target area by adjusting the gantry rotation and speed, better dose distribution and significantly reduced RT time (36).

Although previous studies have revealed that targeted dose distributions are better managed by VMAT (36,37), to the best of our knowledge there is no concrete data elucidating the effect of VMAT on platelet markers. Therefore, the present study aimed to measure the platelet parameters and activation markers prior to and following RT to determine the effects of radiation administered with VMAT on the platelet count, MPV value, platelet aggregation, levels of P-selectin, thrombospondin-1 and PF 4 markers. Furthermore, their association with miR-226 and miR-123 expression levels in patients with prostate cancer was also determined.

The results of the present study indicated the presence of a significant difference in platelet count and MPV value in pre- and post-RT with VMAT treatment groups of patients with prostate cancer. According to the results, the platelet count was decreased by 8.7% in the post-RT day 0 group in comparison with the pre-RT group and increased by 10% in the post-RT day 40 group compared with the post-RT day 0 group. Previous studies investigating conventional RT and IMRT techniques have reported a decrease in platelet count following treatment, thus corroborating the results of the present study (7,15,38,39). Furthermore, it has been demonstrated that sub-lethal radiation doses may result in abnormal hemostasis characteristics and coagulation biomarker values observed up to 21 days post-irradiation (40). Therefore, the post-RT decrease in platelet counts observed in the present study may be due to the suppressive effect of radiation on the hematopoietic system in the acute phase. The increase in the platelet count in the post-RT day 40 group may be associated with the removal of the suppressive effect of RT on bone marrow.

MPV is a marker of platelet function and activation. An increase in platelet volume may result in increased platelet activity. The changes in platelet volume may be used as a diagnostic marker for the prevention and follow-up of the
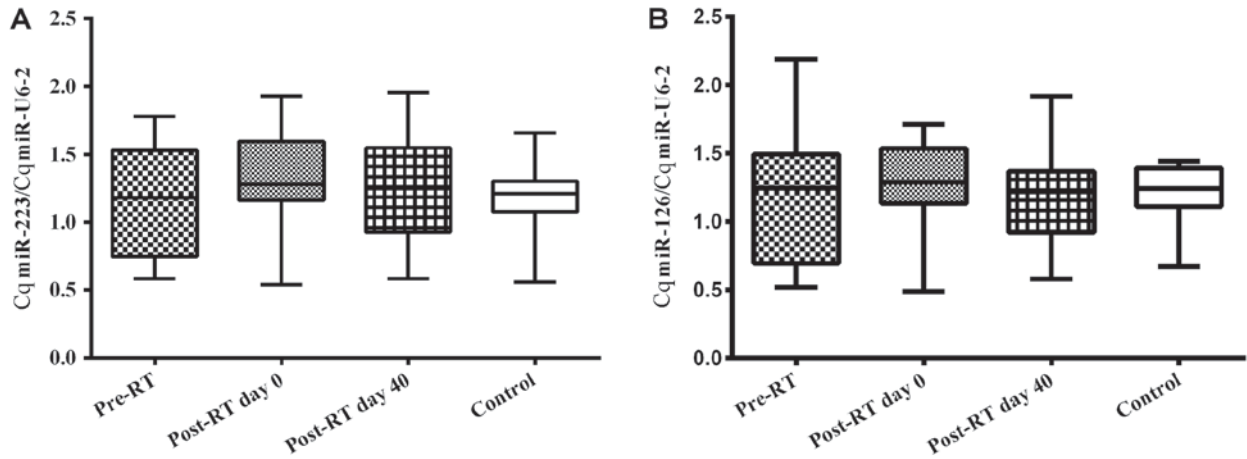
Figure 1. Ratio of miRNAs associated with platelet function in patients and controls. (A) Cq miR-223/Cq miR U6-2 ratios in patients prior to and following RT and control groups. (B) Cq miR-126/Cq miR U6-2 ratios in patients prior to and following RT and control groups. RT, radiotherapy; miR/miRNA, microRNA; Cq, quantification cycle.
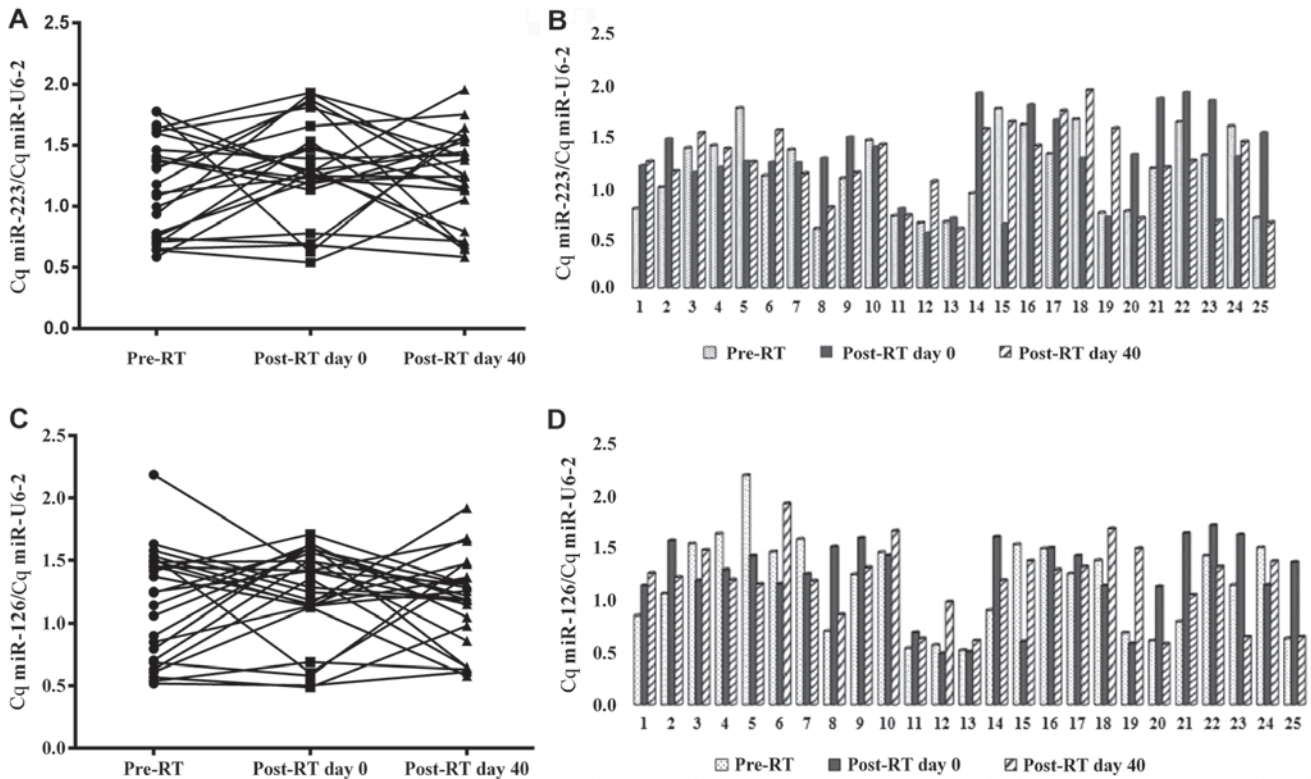


Figure 2. Individual changes to the Cq miR-223/Cq miR U6-2 and Cq miR-126/Cq miR-U6-2 ratios in RT-treated groups. (A and B) Individual changes to Cq miR-223/Cq miR U6-2 ratios in RT-treated groups. (C and D) Individual changes to Cq miR-126/Cq miR-U6-2 ratios in RT-treated groups. RT, radiotherapy; miR, microRNA; Cq, quantification cycle.

platelet-associated diseases (41). The formation and development of thrombi largely depends on platelet activation, in which a change in platelet parameters may affect the activation process (42). The MPV is the average measurement of platelet size, and as the platelet increases in size, the potential of thrombus formation increases (41).

The present study revealed that MPV values were increased by 10% in the pre-RT group in comparison with the control group and decreased by 6% in the pre-RT day 0 group and 10% in the pre-RT day 40 group comapred with the pre-RT

group. These results indicated that MPV values were increased owing to cancer and decreased owing to RT. The MPV values were investigated in various types of cancer, including prostate cancer (43,44); however, to the best of our knowledge, there are no studies investigating the change in MPV values associated with RT.

The platelet function markers investigated in the present study include platelet aggregation, a marker of platelet hyperactivity (45); P-selectin, an adhesion molecule, which is present in the alpha granules of platelets and released as a response to
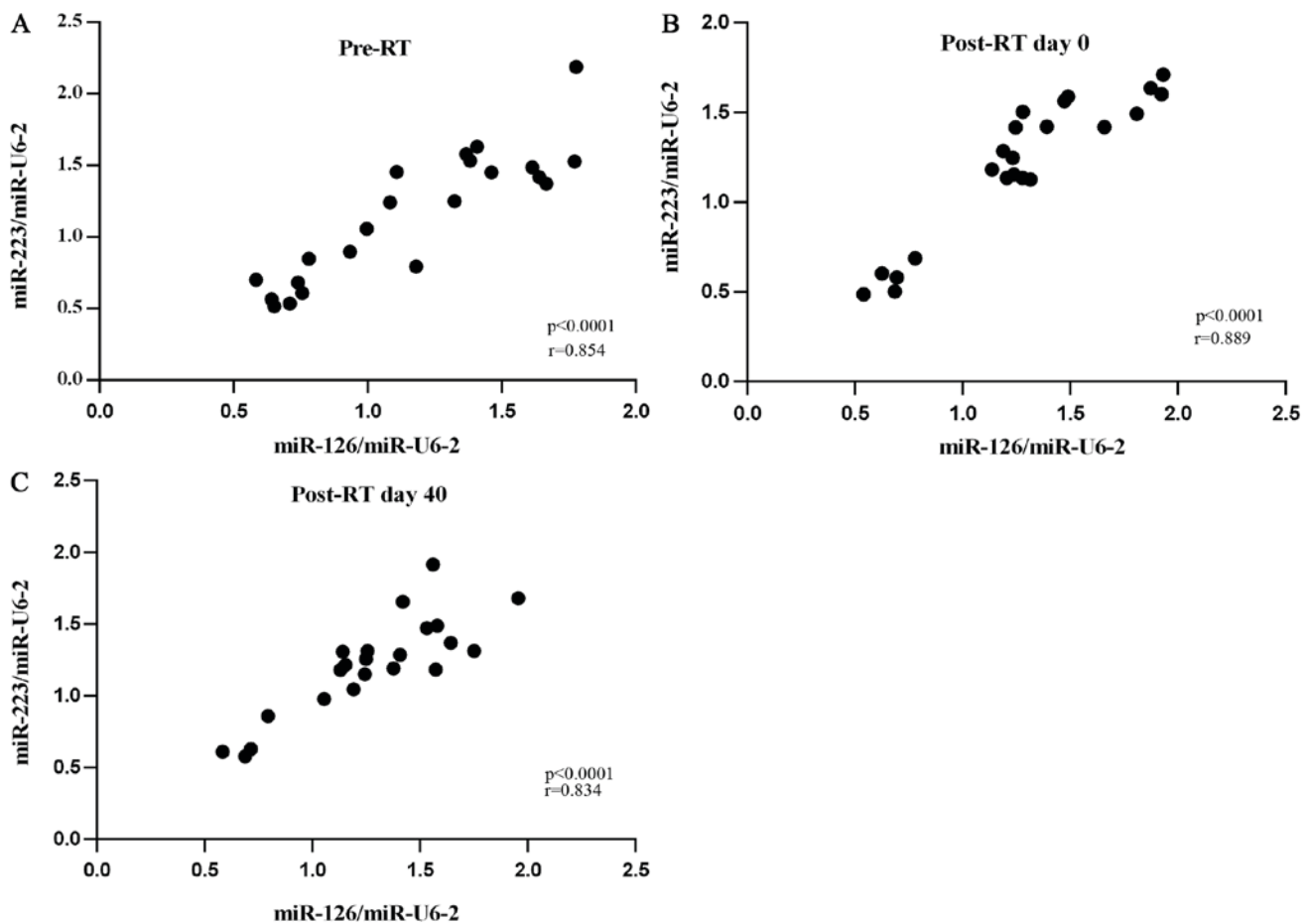
Figure 3. Correlation between Cq miR-223/Cq miR-U6-2 and Cq miR-126/Cq miR-U6-2 ratios in RT-treated groups. Correlation between Cq miR-223/Cq miR U6-2 and Cq miR-126/Cq miR U6-2 ratios in (A) the pre-RT group, (B) the post-RT day 0 group and (C) the post-RT day 40 group. RT, radiotherapy; miR, microRNA; Cq, quantification cycle.

cellular activities (46); thrombospondin-1, which is released during hemostatic plaque formation and an active player in thrombus formation (47), and PF 4, which serves a function in thrombosis and is released by platelet activation (48). When the data was evaluated, no significant change was identified in the levels of platelet aggregation, plasma P-selectin, thrombospondin-1 and PF 4 associated with RT between any of the RT groups.

Various studies reported a notable but unsignificant increase in platelet aggregation levels *in vitro* and in patients with metastatic prostate cancer compared with pre-RT conditions (17,49). It has been suggested that extracorporeal irradiation does not affect the platelet functions that are already in systemic circulation, however, it may affect bone marrow platelet stem cells (17).

Experimental molecular studies that determine the effects of ionizing radiation on P-selectin levels revealed an increase in the concentration of P-selectin in association with RT (18,33,50). However, Zekanowska *et al* (19) reported that the levels of soluble P-selectin do not change following RT in patients with prostate cancer. The results of the present study revealed that RT did not affect the plasma P-selectin concentration in patients with prostate cancer.

It has been revealed that the concentration of thrombospondin-1 changes owing to an increasing dose of

radiation over time (51,52). Lewinski *et al* (20) reported that thrombospondin-1 concentration did not change in association with the radioactive iodine treatment in patients with thyrotoxicosis.

Although contradictory results exist in clinical studies investigating PF 4 levels in various types of cancer (53-55), to the best of our knowledge no clinical studies have focused on the changes in plasma PF 4 levels associated with RT. A number of *in vitro* studies have provided evidence of the protective effect of PF 4 against the damaging effect of radiation (56,57).

At present, the basis for the lack of effect of VMAT on platelet function remains unknown, although VMAT has a partial effect on the platelet count and MPV value. A potential explanation for this is that the highly developed dose distribution of VMAT may result in platelet activation in the target area below detectable levels in systemic circulation.

miRNAs are small molecules that target specific genes and regulate their transcription (57). In previous years, it has been revealed that miRNAs serve functions in various pathological and physiological processes (57).

A previous study has reported alterations in miRNA expression in different diseases, and that platelet miRNA levels were biologically and clinically important markers of the following: i) Platelet protein translation and expression; ii) mature megakaryocyte

miRNA level; iii) hematological disease and platelet reactivity; and iv) basic mechanisms of megakaryocyte/platelets (58). Although the exact function of miRNAs in platelet function is not clearly understood, it is thought that miRNAs have notable functions in hemostasis and thrombosis (57).

Studies published in previous years provide evidence that a number of miRNAs are specific to cells and tissues and are differentially expressed (24,59,60). It has been demonstrated that miRNAs can enter into systemic circulation via certain mechanisms, including exosome secretion, cell death and blebbing, and that these circulating miRNAs could be used as potential markers for and in the follow-up of certain diseases (24). So far, studies have focused on the changes of miRNA expression levels associated with cancer (1,61). However, to the best of our knowledge there are no studies that have investigated the effects of radiation on platelet miRNA expression. In experimental and total body irradiation studies, it has been reported that the expression rates of a number of miRNAs, including miR-126 and miR-223, change in plasma and peripheral blood cells. miR-223 and miR-126 are miRNAs identified in platelets. It has been revealed that miR-223 targets the P2Y12 receptor that is present on platelets and inhibits it, thus suppressing platelet aggregation and activation (58). miR-126 is downregulated during megakaryocytopoiesis, indicating that it serves a function in differentiation (62).

Various studies have reported that changes in the plasma levels of miR-223 and miR-126 are associated with platelet function in cardiovascular diseases (24,26,27). Considering the effect of radiation on platelet functions from these previous studies, a change in the expression levels of miRNAs may be expected to exert an effect on platelet activation-associated RT. *In vitro* and experimental studies revealed that the expression levels of miR-223 and miR-126 changed over time with increasing radiation dose (63,64). Following RT, changes to RT-associated serum/plasma level changes can be expected as platelets release miRNAs as RT damages the cells. These changes may depend on the type and duration of treatment and the dose distribution. Thus, the present study additionally investigated the expression levels of miR-223 and miR-126 in the plasma and the changes in the expression of these miRNAs following VMAT application.

The results of the present study did not reveal a significant change in miR-223 and miR-126 expression levels in association with VMAT in patients with prostate cancer. The lack of significant differences in miRNA expression between pre-RT and control groups may indicate that these miRNAs are not tumor-specific in serum/plasma. The VMAT technique achieved highly conformal treatment plans and decreased the normal tissue toxicity compared with other conventional RT techniques (65). Therefore, no significant changes in the miRNA expression levels were observed. Dynamic changes to miRNA expression levels in response to antitumor therapy may have resulted in this non-significant difference (66). However, in the present study, there existed a clear inter-individual variability to radiation response in terms of the expression of miRNAs. Notably, this situation may not be easily explained by previous observations. The cells of patients with cancer respond differently to RT, even when they are treated with the same curative dose. Furthermore, numerous different factors, including genetic

variations, environmental stress, differences in nutritional state between patients and controls, and exposure to genotoxic chemical agents may affect individual responses to ionizing radiation (67-70). Therefore, further studies are required to understand the molecular events underlying the substantial inter-individual differences in miRNA expression in response to radiation.

There are a number of limitations to the present study, including its relatively small sample size, investigation of a limited number of miRNAs rather than all miRNAs associated with platelet activation and the focus on plasma samples. However, the levels of miRNA and activation markers in platelets may provide valuable data to evaluate the platelet function.

In summary, the present study demonstrated that there were no significant changes in platelet aggregation, plasma P-selectin, thrombospondin-1 and PF 4 levels and miR-226 and miR-123 expression in plasma associated with RT. However, a significant change in platelet count and MPV values were identified to be associated with RT. The results of the present study indicated that VMAT may not be a risk factor for platelet activation in patients with prostate cancer, despite the fact that it reduced the platelet count and MPV values, which may also be a result of the suppressive effect of radiation on the hematopoietic system.

## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

NB, İO and BA were responsible for study conception and design. NB, İO, BA, OB, ST, FYA and MCA performed data analysis and interpretation. FYA provided the patient specimens. All authors approved the final version of this manuscript.

## Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Cerrahpasa Medical Faculty of Istanbul University (Grant no. 28052/2012), and informed consent to participate in the study was obtained from all patients involved.

## Patient consent for publication

No identifying patient information is included in the published manuscript. Participants provided their consent for the publication of the data.

## Competing interests

All the authors declare that they have no competing interests.

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