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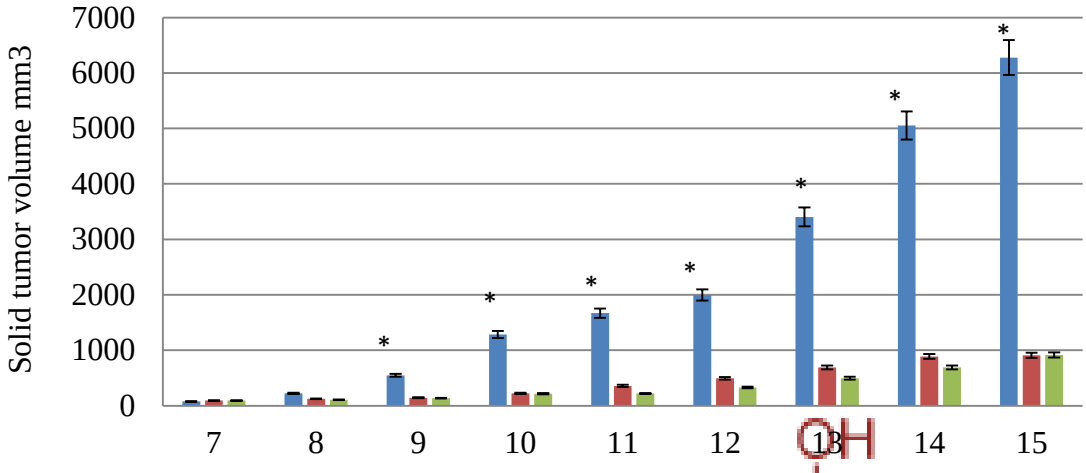

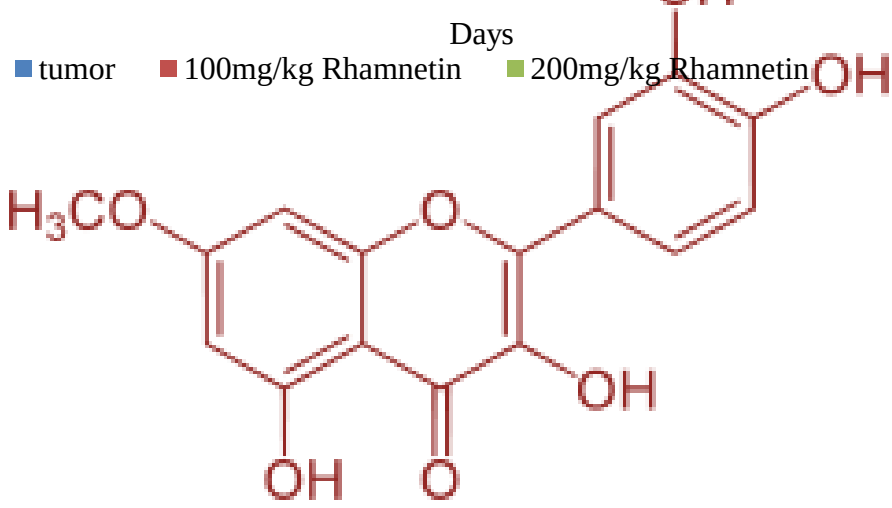

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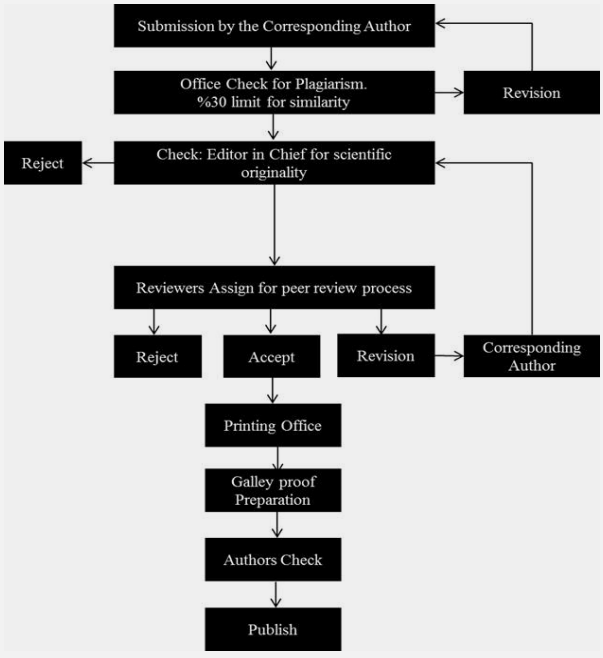

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Prognostic role of inflammatory markers in hepatocellular cancer patients receiving sorafenib therapy

Ali Oğul[1]*, Mahmut Büyükşimşek[1]

Abstract

Objective: Systemic inflammatory markers have been shown to have prognostic value in many types of cancers. Although the prognostic role of the systemic immune-inflammation index (SII), derived neutrophil-lymphocyte ratio (dNLR) and platelet lymphocyte ratio (PLR) has been shown in hepatocellular cancer (HCC) patients who underwent transplantation, its prognostic value has not been investigated in HCC patients under sorafenib treatment. We investigated the prognostic value of inflammatory indices in patients with HCC under sorafenib treatment.

Materials and Methods: The data of 46 patients with stage III unresectable and stage IV HCC were evaluated retrospectively. SII and dNLR were dichotomized based on receiver operating characteristic (ROC) curve analysis (cut-off values: 355 and 1.8). No cut-off value could be determined for PLR; therefore, the median value was defined as the cut-off for PLR. At the time of diagnosis, values of these three inflammatory markers were analyzed to determine their association with clinicopathologic characteristics and to assess their prognostic values via the Kaplan-Meier method and multivariate Cox regression analysis.

Results: Overall survival (OS) was significantly shorter in patients with a high SII, dNLR, or PLR. In univariate analyses, tumor stage, tumor focus count, and presence of extrahepatic lesions seemed to affect survival. Multivariate analysis revealed SII and the presence of extrahepatic lesions as independent risk factors for survival.

Conclusion: The findings of the present study suggest that a high SII is an independent risk factor for survival in patients with HCC under sorafenib treatment.

Keywords: Sorafenib, hepatocellular cancer, systemic immune-inflammation index, derived neutrophil-lymphocyte ratio, platelet lymphocyte ratio

Introduction

Hepatocellular cancer (HCC) is the fourth leading cause of cancer-related death and leads to an annual number of fatalities around 800,000 worldwide (1). It is the ninth most common cancer in women and the fifth most common cancer in men (2). Treatment options include surgery, local ablative treatments, and systemic therapies for HCC. Sorafenib is an oral tyrosine kinase inhibitor that inhibits the rapidly accelerated fibrosarcoma (RAF) kinase and vascular endothelial growth factor (VEGFR) kinase pathways (3). Sorafenib was shown to improve overall survival (OS) and slow radiological progression of the disease compared with placebo in patients with Child Plough class A cirrhosis and inoperable HCC in the multicenter SHARP trial (4). Whereas adverse events related to treatment and decreases in baseline plasma biomarkers such as AST, ALT, and AFP have been proposed as prognostic markers of response to sorafenib, no marker has been widely accepted in this regard (5-7).

The cellular immune system is known to be related to cancer and a marker of the systemic inflammatory response (8). Recently, inflammatory markers such as platelet lymphocyte ratio (PLR), derived neutrophil-lymphocyte ratio (dNLR), and systemic immune-inflammation index (SII) which are calculated from blood count parameters have been proposed to predict survival in various disorders (9-11). In 2012, Hu et al. (12) developed SII as a marker of prognosis in patients with HCC who underwent curative surgery. SII is calculated by multiplying NLR per liter by platelet count (12). Afterward, SII has been shown to predict postoperative prognosis and response to treatment in various types of cancers (13,14). Similarly, dNLR and PLR have been reported to be associated with survival and response to treatment in patients with malignancies such as colorectal and prostate cancers (10,11). Shao et al. (15) reported that a decrease of more than 20% in alpha-fetoprotein levels could be used to predict response to

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treatment in patients with advanced HCC under anti-antiandrogen treatment. Based on all these findings, we aimed to investigate the role of SII, PLR, and dNLR in predicting survival in patients with HCC under sorafenib treatment.

Materials and Methods

Study population and definition

Patient data have been collected from their files and electronic records. A total of 46 patients with stage 4 or stage 3 inoperable HCC with respect to the eighth edition of American Joint Committee on Cancer (AJCC) TNM classification and also Child-Pugh class A cirrhosis and an AFP level above 20 ng/ml who were diagnosed radiologically or pathologically and were under sorafenib (Nexavar) treatment for a minimum of eight weeks were enrolled to this retrospective study.

Before the initiation of sorafenib treatment, all of the patients underwent an evaluation including complete blood count analysis; AFP; albumin, INR, and bilirubin (for Child-Pugh classification); and ultrasound to scan for the presence of ascites. For inclusion to the study, the patients had to have an Eastern Cooperative Oncology Group (ECOG) performance score of lower than two.

The age, sex, ECOG, PS, underlying liver disease, TNM stage, number of tumor foci in liver parenchyma on imaging, extrahepatic metastases, and pre-treatment laboratory values of the patients were recorded.

The initial dose of sorafenib was 400 mg bid. Sorafenib was discontinued only in case of disease progression or grade III unmanageable or grade IV toxicity. In the case of grade III manageable toxicity, sorafenib dose was reduced to 200 mg bid.

Sorafenib was discontinued in these patients only if grade III toxicity recurred under the reduced dose regimen. A decrease of more than 20% in AFP level after eight weeks of sorafenib treatment was defined as a response to treatment. In order to calculate pre-treatment SII, dNLR, and PLR values, respectively, platelet count X(neutrophil count/lymphocyte count), absolute neutrophil count/(white blood cell count-absolute neutrophil count) and platelet count/lymphocyte count formulas were used. The overall survival (OS) was calculated for all patients as the duration between the initiation of treatment and death or the last follow-up time for that patient.

Statistical analysis

To determine the cut-off values for SII and dNLR, ROC curve analysis was used. The log-rank test was used in univariate analyses, and Cox-regression model was used in multivariate analysis. Kaplan-Meier analysis was used to calculate OS duration, and survival graphics were presented. In the comparison of survival between groups, the log-rank test was used.

The variables consisted of age, sex, ECOG, PS, etiology, tumor stage, number of tumor foci, extrahepatic metastasis, albumin, SII, dNLR, PLR, the need for sorafenib dose modification, development of adverse events, response to sorafenib, and OS. No cut-off could be determined for PLR using ROC curve analysis, so its median value was used as the cut-off for PLR. Prognostic significance of SII, dNLR, and PLR were evaluated using univariate and multivariate analyses.

The odds ratio and 95% confidence interval were determined by cox regression analysis.

SPSS version 21.0 (IBM Inc., Chicago, IL, ABD) was used for statistical analyses. Statistical significance was set at $p<0.05$.

Results

Patient characteristics

A total of 46 patients with HCC who used sorafenib treatment were included. The most sensitive and specific values for study variables were determined using receiver-operating characteristics (ROC) curve analysis: cut-off values were 355 for SII and 1.8 for dNLR (Figure 1). The baseline characteristics of the patients are summarized in Table 1.

The median age was 58 (39-82), and 8.6% of the study population were females. Most of the patients (78.2%) had an ECOG PS of 0. The etiology of liver disease was HBV in 71.7% and HCV in 21.8%, and 4.3% of the study population reported alcohol use.

Survival outcomes

The median OS was 10.2 months, and the median duration of follow up was 9.8 months. In univariate analyses, the median OS was 10.8 months in stage III patients and 8.7 months in stage IV patients (p=0.024).

While patients with a single tumor focus had a median OS of 10.5 months, those with multiple tumor foci had a median OS of 9.6 months (p=0.047).

The patients with extrahepatic lesions had a median OS of 7.3 months, and those without extrahepatic lesions had a median OS of 10.4 months (p=0.011).

While the median OS was 11.7 months in the patients with an SII $\leqslant$355, it was 7.9 months in those with an SII >355 (p=0.008).

The median OS was 10.5 months in the patients with a dNLR $\leqslant$1.8, and it was 8.2 months in those with a dNLR >1.8 (p=0.025). The median OS was 10.2 months in the patients with a PLR $\leqslant$184 while it was 8.9 months in those with a PLR >184 (p=0.038). The patients with and without response to sorafenib had a median OS of 11.2 and 9.5 months, respectively (p=0.032).

There was no significant association between OS and age, sex, ECOG PS, etiology, albumin, need for sorafenib dose modification, and development of adverse events (p=0.117, p=0.145, p=0.684, p=1.125, p=1.187, p=0.251, and p=0.148, respectively). Multivariate analysis revealed that OS was independently associated with the presence of extrahepatic lesions and a high SII (p=0.014 and p=0.016, respectively, Table 2). Figure 2 demonstrates the survival graphs.

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Table 1. Baseline characteristics in hepatocellular carcinoma patients treated with sorafenib (n:46)

		Number of patients n (%)
Age	≤65	24 (52,1%)
	>65	22 (47,9%)
Gender	Female	4 (8,6%)
	Male	42 (91,4%)
ECOG PS	0	36 (78,2%)
	1	10 (21,8%)
Etiology	HBV	33 (71,7%)
	HCV	10 (21,8%)
	Alcohol	2 (4,3%)
	Unknown	1 (2,2%)
TNM stage	Stage III inoperabl	9 (19,5%)
	Stage IV	37 (80,5%)
Tumor focus count	Single	10 (21,8%)
	Multiple	36 (78,2%)
Extrahepatic lesion	Yes	5 (10,8%)
	No	41 (89,2%)
Albumin <3.5 (g/dL)	Yes	18 (39%)
	No	28 (61%)
WBC counts ($x10^9$/L), median(min-max)		7,25 (2,58-22,52)
Neutrophil counts ($x10^9$/L), median(min-max)		2,37 (1,58-3,87)
Lymphocyte counts ($x10^9$/L), median(min-max)		1,12 (0,68-1,76)
Platelet counts ($x10^9$/L), median(min-max)		102.0 (65.2-278.0)
SII	≤355	26 (56,5%)
	>355	20 (43,5%)
dNLR	≤1,8	18 (39,1%)
	>1,8	28 (60,9%)
PLR	≤184	25 (54,3%)
	>184	21 (45,7%)
Sorafenib dose modification	Yes	8 (17,3%)
	No	38 (82,7%)
Adverse events	Grade III	5 (10,8%)
	Grade IV	1 (2,2%)
OS (months), median (min,max)		10,20 (3,02-39,08)
OS (months)	≤10.2	24 (52,1%)
	>10.2	22 (47,9%)
Response to sorafenib	Yes	27 (58,6%)
	No	19 (41,4%)

ECOG PS: Eastern Cooperative Oncology Group Performance Score, TNM: Tumor Node Metastasis, WBC: White Blood Cell, SII: Systemic immune-inflammation index, dNLR: Derived neutrophil-lymphocyte ratio, PLR: platelet lymphocyte ratio, OS: Overall survival

Table 2. Univariable and multivariable analyses to predict overall survival in hepatocellular carcinoma patients

Variables	Univariable		Multivariable	
	HR(95%Cl)	*P* value	HR(95%Cl)	*P* value
Age greater than ≤65	1.419 (0.945-2.317)	0.117		
Sex (Female vs male)	1.434 (0.914-2.436)	0.145		
ECOG PS 0 vs 1	1.386 (0.748-2.187)	0.684		
Etiology HBV vs HCV	1.681 (0.581-2.168)	1.125		
TNM stage Stage III inoperable vs Stage IV	2.214 (1.108-3.847)	**0.024**	0.912 (0.625-1.098)	0.242
Tumor focus count Single vs multiple	1.194 (0.856-1.452)	**0.047**	1.069 (0.875-1.365)	0.358
Extrahepatic lesion Yes vs no	1.321 (0.682-2.654)	**0.011**	1.258 (1.068-1.785)	**0.014**
Albumin <3.5 g/Dl Yes vs no	1.654 (0.548-3.457)	1.187		
SII ≤355 vs >355	2.914 (1.425-5.129)	**0.008**	1.312 (1.057-1.456)	**0.016**
dNLR ≤1,8 vs >1,8	2.458 (1.015-3.587)	**0.025**	1.275 (1.014-1.502)	0.125
PLR ≤184 vs 184	2.147 (1.915-5.478)	**0.038**	1.354 (0.981-1.681)	0.087
Sorafenib dose modification Yes vs no	1.528 (0.756-2.112)	0.251		
Adverse events Yes vs no	1.451 (0.825-2.237)	0.148		
Response to sorafenib Yes vs no	2.328 (1.741-3.458)	**0.032**	1.181 (0.764-1.385)	0.343

Statistically significant p-values (<0.05). HR: Hazard ratio, CI: Confidence interval, ECOG PS: Eastern Cooperative Oncology Group Performance Score, TNM: Tumor Node Metastasis, SII: systemic immune-inflammation index, dNLR: derived neutrophil lymhocyte ratio, PLR: platelet lymphocyte ratio

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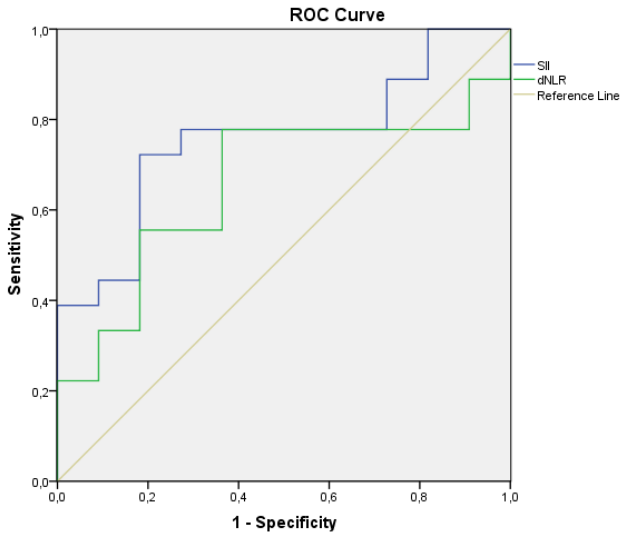

Fig. 1. ROC analysis and AUC for sensitivity and specificity of inflammatory parameters: SII: systemic immune-inflammation index, dNLR: derived neutrophil lymphocyte ratio. SII-AUC:0.758 and dNLR-AUC:0.657

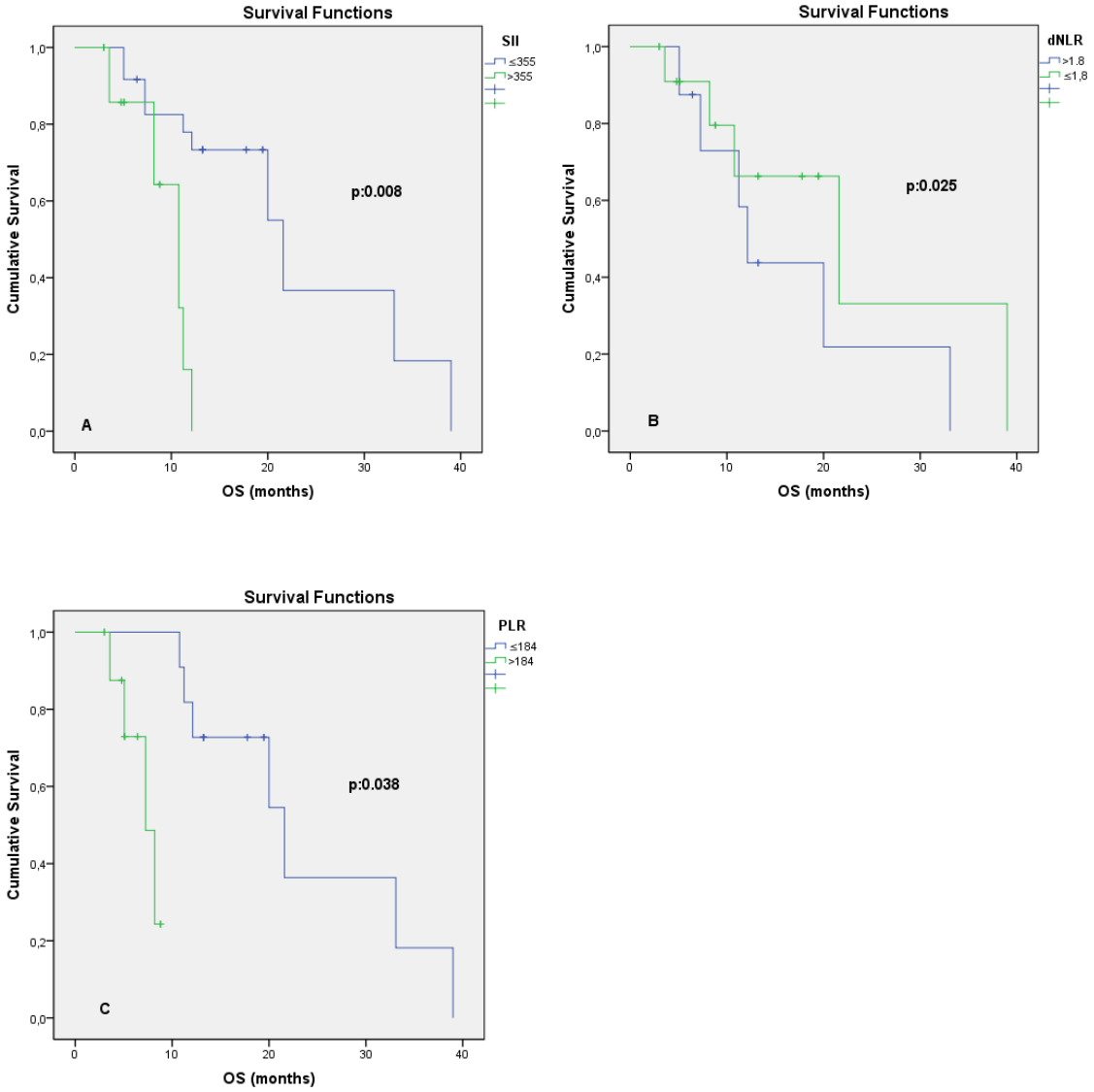

Fig. 2. Overall survival times according to inflammatory markers. SII: systemic immune-inflammation index (A), dNLR: neutrophil lymphocyte ratio (B), PLR: platelet lymphocyte ratio (C)

Discussion

CR is described as having a <5% BMPC ratio in addition to Hepatocellular cancer is a highly angiogenic tumor that develops in the setting of chronic inflammation and cirrhosis. The contributory role of inflammation in the development of cancer has long been studied, and recent studies focus on the effect of surrogate inflammatory markers on tumor dissemination and survival. Blood count parameter ratios have been used as markers predictive of tumor biology, aggressiveness, and other adverse outcomes associated with cancer (16). The vascular endothelial growth factor is released by platelets and neutrophils and plays an essential role in angiogenesis and tumor progression (17). Whereas the role of neutrophils in cancer pathogenesis is debated, they are known to take place in the microenvironment of various tumors such as HCC, renal cell carcinoma, and glioblastoma (18-20). On the other hand, lymphocytes release cytokines and prevent tumor progression by contributing to cytotoxic cell death (21). Sorafenib is an expensive anti-angiogenic multiple tyrosine kinase inhibitor that has been used in the treatment of HCC. In the present study, we aimed to evaluate the role of inflammatory indices, which are derived from complete blood count parameters, in predicting response to sorafenib treatment in patients with HCC. For this purpose, we investigated the predictive role of SII, dNLR, and PLR and found SII as an independent predictor of survival in these patients.

Inflammation is an essential step in the development of cancer and also a contributor to tumor progression. Inflammatory markers have been reported to be associated with poor survival and unresponsiveness to treatment in several solid organ cancers. In addition to thrombocytosis and neutrophilia, dNLR and PLR have been investigated in numerous studies in this regard (22). In 2012, Proctor et al. (23) reported that dNLR provided more prognostic information than NLR among more than ten thousand people with all cancer types (23). Li et al. (24) reported that a high dNLR was an independent prognostic factor in terms of survival and microvascular invasion in patients with HCC. In the present study, a high dNLR seemed to be associated with short survival time, but this association was not confirmed in the multivariate analysis.

Recently, the prognostic role of PLR has been investigated in patients with HCC who underwent hepatectomy or liver transplantation, and a high PLR before surgery has been found to be associated with HCC recurrence (25). To the best of our knowledge, the role of PLR in patients with HCC under sorafenib treatment has not been investigated in detail, and the findings of the present study may shed light on its role in this regard. Although it was not independently associated with survival in multivariate analysis, a high PLR was related to shorter survival in patients with HCC under sorafenib treatment in the present study.

It is not clear which physiological process affects SII. Neutrophils are associated with chemokines and proteases, which have a regulatory role in angiogenesis. These processes may affect blood circulation in tumors and tumor growth rate (26,27).

The platelets are able to release chemokines and cytokines. These cytokines may induce the proliferation of tumor cells (28). Lymphocytes play an important role in immune defense. A higher intensity of lymphocytes in the tumor microenvironment has been reported to be associated with better clinical outcomes and that these lymphocytes are related to circulating lymphocytes (29). A high lymphocyte ratio has also been reported to be associated with a better prognosis in several cancer types, and circulating lymphocytes may inhibit tumor metastasis and proliferation (30,31).

The systemic immune-inflammation index has been reported as an excellent prognostic index in patients with small-cell lung cancer, esophageal cancer, and renal cell carcinoma (13,32,33). In the present study, we investigated the prognostic value of SII in patients with HCC under sorafenib treatment, and our findings suggest that it is an independent predictor of survival.

The present study has several limitations. Firstly, it was a retrospective study with small sample size. Secondly, SII levels may be influenced by conditions such as acute infections and chronic viral infections. Nonetheless, it is not easy to determine an optimal cut-off for inflammatory markers because of the individual variation in the immune response. Therefore, the change in baseline SII level may be a better predictor than a constant cut-off. It may be rational to compare the prognostic value of the change in SII levels after treatment rather than a constant cut-off in further prospective studies.

Conclusion

The findings of the present study are important in terms of the independent association between SII levels and survival in patients with HCC under sorafenib treatment. We suggest that using SII to predict survival in HCC patients under sorafenib treatment may prove beneficial provided that our findings are confirmed in prospective studies.

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Conceptualization: Ali Ogul, Methodology: Mahmut Buyuksimsek; Formal analysis and investigation: Mahmut Buyuksimsek; Writing - original draft preparation: Ali Ogul; Writing - review and editing: Mahmut Buyuksimsek; Supervision: Mahmut Buyuksimsek.

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Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Evaluation of Neutrophil, Lymphocyte, Platelet, Mean Platelet Volume, Neutrophil-Lymphocyte Ratio, and Platelet-Lymphocyte Ratio in Prostate Cancer Patients Treated with Radiotherapy

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Abstract

Objective: Radiotherapy is one of the treatment methods for prostate cancer. Ionizing radiation can cause inflammation of tissues in and around the irradiated sites. But it is also suggested that low-dose radiation has anti-inflammatory effects. The present study was aimed at investigating the effects of radiotherapy on some inflammatory markers in prostate cancer patients who received radiotherapy.

Material and Methods: A total of 42 patients with prostate cancer and 30 healthy subjects were included in the present study. Venous blood samples of subjects were collected the day prior to radiotherapy (pre radiotherapy group), and on the last day last of radiotherapy (post radiotherapy group). Neutrophil, lymphocyte, platelet, mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) levels were measured and calculated. Also, the control group venous blood samples were used for comparison.

Results: Neutrophil values of the pre radiotherapy group were higher than the control group ($p<0.05$), and values of the post radiotherapy group were lower than the pre radiotherapy group ($p<0.001$). In addition, lymphocyte values of the post radiotherapy group were lower than the control and the pre radiotherapy groups ($p <0.001$ for both). Platelet values were decreased in the post radiotherapy group compared to the pre radiotherapy group ($p <0.01$). MPV values of the pre radiotherapy group were higher than the control and post radiotherapy groups ($p<0.05$, and $p<0.001$, respectively). NLR and PLR values were increased in the post radiotherapy group compared to the control and the pre radiotherapy groups ($p <0.001$ for all).

Conclusion: Our findings showed that neutrophil, and MPV were increased in the pre radiotherapy group compared to the control group. Neutrophil, lymphocyte, platelet, and MPV were decreased, NLR and PLR were increased in the post radiotherapy group compared to the pre radiotherapy group. However, further molecular studies are needed to clarify the mechanism related to this process.

Keywords: prostate cancer, radiotherapy, neutrophil, lymphocyte, platelet

Introduction

Prostate cancer is the most common cancer affecting males in developed countries (1). There are many studies in the literature about prostate cancer, but its underlying etiology still remains unclear. In some studies, it is reported that inflammation's role in multiple stages of prostate cancer development (1-3). Also, it has been suggested that neutrophils, T and B lymphocytes, and platelets play a prominent role in cancer inflammation and immunology (4, 5). Neutrophils, lymphocytes, and platelets, which are recognized as inflammation markers, can be easily obtained by a simple complete blood count test. Also, as different inflammation markers, the neutrophil/lymphocyte ratio (NLR), as well as the platelet/lymphocyte ratio (PLR) can be determined by dividing the sum of the neutrophil and platelet counts by the lymphocyte count (4). Neutrophils are immature phagocytes with a short half-life. They are the first cell to migrate in the early stage of inflammation regulated by macrophages and mast cells in the tissues. They have proteolytic enzymes and oxygen free radicals actively contributing to the damage produced during inflammatory processes (6, 7). In inflammation the different leucocyte types, macrophages, and lymphocytes are activated and recruited to the inflammatory site at a later stage (7). The platelets and mean platelet volume (MPV) are other inflammatory biomarkers, which are examined in our study.

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It is known that platelets are necessary for homeostasis and coagulation function. As a platelet function marker, MPV is an indicator of the average thrombocyte volume, recognized as a hallmark of platelet production rate, and stimulation. Larger platelets are more metabolically and enzymatically active than smaller platelets (4, 8).

When we reviewed studies associated with cancer and inflammation we saw that various inflammatory markers are studied in various cancer types. But in these studies, these markers were mostly examined on disease prognosis and survival (9-13). Prostate cancer patients frequently are treated with radiotherapy (14). It is also known that radiotherapy can initiate a pro-inflammatory immune response within the cancer microenvironment (15). Considering the possible effect of radiation on the inflammatory process, in the present study, we aimed to examine the association between values of neutrophils, lymphocytes, platelets, MPV, NLR, and PLR in prostate cancer patients before and after radiotherapy, and to compare these values with healthy subjects.

Material and Methods

Case Selection

A total of 42 male patients (mean age 65.19, range 55-76 years) diagnosed with histologically confirmed adenocarcinoma of the prostate and treated with radiotherapy at the Istanbul Training and Research Hospital, Department of Radiation Oncology between April 2019 and March 2020 were included in the study. A total of 30 healthy male volunteers of similar age (mean age 60.82, range 45-71 years), who did not receive any medication constituted the control group. Medical history, including inflammatory diseases, infectious diseases, autoimmune diseases, diabetes, hypertension, distant metastases, or other malignant diseases were designated as exclusion criteria for patients with prostate cancer. Known history of chronic, inflammatory, or malignant diseases were the exclusion criteria for the healthy control subjects. Patients with prostate cancer were treated with VMAT (Varian Trilogy Rapid Arc Radiotherapy Device; Varian Medical Systems, Inc., Palo Alto, CA, USA) (Total dose range 66-78 Gy) with a 1.8 Gy to 2.0 Gy per fraction. The present study was approved by the Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Ethics Committee, and was performed in accordance with The Declaration of Helsinki. All patients gave written informed consent.

Sample collection and analysis

The day prior to radiotherapy (pre radiotherapy group) and the day radiotherapy was completed (post radiotherapy group) venous blood samples were collected into tubes containing EDTA from patients with prostate cancer. The same volume of blood was collected into tubes from the healthy control subjects. Blood samples were analyzed using Cell-DYN C1600 (Abbott Pharmacuetical Co., Ltd., Lake Bluff, IL, USA) blood count device using blood collected as aforementioned.

Statistical analysis

Data are presented as mean ± the standard deviation (SD). Statistical analysis was performed using the Wilcoxon, Paired t - test and Mann - Whitney U test. 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. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Patient data

Demographic data of patient and control groups are presented in Table 1. Neutrophil, lymphocyte, platelet, MPV, NLR, and PLR values are presented in Table 2 as the mean ± SD.

Neutrophil, lymphocyte, platelet, MPV, NLR, and PLR results of all studied groups

Neutrophil values of the pre radiotherapy group were higher than the control group ($p<0.05$). The values of the post radiotherapy group were lower than the pre radiotherapy group ($p<0.001$). There was no significant change in the comparison of the post radiotherapy group with the control group ($p>0.05$) (Table 2).

Lymphocyte values were decreased in the post radiotherapy group compared to the control group ($p <0.001$). The values of the post radiotherapy group were also lower than the pre radiotherapy group ($p<0.001$). There was no significant change in the comparison of the pre radiotherapy group with the control group ($p>0.05$) (Table 2).

Platelet values were decreased in the post radiotherapy group compared to the pre radiotherapy group ($p <0.01$). There were no significant differences between other groups (Table 2).

MPV values of the pre radiotherapy group were higher than the control group ($p<0.05$). MPV values were decreased in the post radiotherapy group compared to the pre radiotherapy group ($p <0.001$). There was no significant change with comparison of other groups ($p>0.05$) (Table 2).

NLR values were increased in the post radiotherapy group compared to the control group ($p <0.001$). The values of the post radiotherapy group were higher than the pre radiotherapy group ($p<0.001$). There was no significant change in the comparison of the pre radiotherapy group with the control group ($p>0.05$) (Table 2).

Similarly, PLR values were increased in the post radiotherapy group compared to the control group ($p <0.001$). The values of the post radiotherapy group were higher than the pre radiotherapy group ($p<0.001$). There was no significant change in the comparison of the pre radiotherapy group with the control group ($p>0.05$) (Table 2).

Correlation results of all studied markers in the pre radiotherapy group

In the pre radiotherapy group, PLR values were positively correlated with NLR and platelet values (r=0.516, and r=0.786, respectively). But, PLR values were negatively correlated with MPV and lymphocyte values (r=-0.675, and r=-0.331, respectively). Also, there were positive correlations between NRL and neutrophil (r=0.649), and negative correlations between NLR and lymphocyte (r=-0.587), and between platelet and MPV (r=-0.449) (Table 3), (Figure 1 A-B).

Correlation results of all studied markers in the post radiotherapy group

In the post radiotherapy group, PLR values were positively correlated with NLR and platelet values (r=0.586, and r=0.380, respectively). But, PLR values were negatively correlated with lymphocyte values (r=-0.704). Also, there were negative correlations between NLR and lymphocyte (r=-0.647), and a positive correlation between NLR and neutrophil (r=0.491). Moreover, platelet values negatively correlated with MPV (r=-0.343) (Table 4), (Figure 2 A-B).

Table 1. Demographic data of prostate cancer and control groups.

	Control (n:30)	Prostate Cancer (n:42)	p- value
Age (Year)	60.82±6.70[a]	65.19±7.84[a]	0.114
Histology			___
Adenocarcinoma, n (%)	NA	42 (100%)	
Total Radiation Dose (Gy)	NA	66 -78.0	___
Radiation Dose per fraction (Gy)	NA	1.8–2.0	___
Tumor Stage			
T2b, n (%)		15 (35.7%)	___
T2c, n (%)	NA	17 (40.4%)	
T3b, n (%)		10 (23.8%)	

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

Table 2. Comparison of the inflammatory markers of prostate cancer and control groups

	Control	Pre Radiotherapy	Post Radiotherapy
Neutrophil (x10^3/ml)	3.61±1.01	4.19±1.12[a*]	3.55±1.16[c***]
Lymphocyte (x10^3/ml)	1.86±0.49	1.94±0.52	0.92±0.33[b***,c***]
Platelet (x10^3/ml)	218.20±36.10	232.000±45.98	212.90±46.10[c**]
MPV (fl)	8.37±1.20	8.93±0.96[a*]	8.44±1.01[c***]
NLR	2.14±0.96	2.26±0.71	4.24±1.74[b***,c***]
PLR	145.30±42.09	121.70±38.81	256.60±47.88[b***,c***]

All values are presented as the mean ± standard deviation; MPV, mean platelet volumes; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; [a]Control vs. Pre Radiotherapy, [b]Control vs. Post Radiotherapy, [c]Pre Radiotherapy vs. Post Radiotherapy, *p<0.05, *p<0.01, ***p<0.001.

Table 3. Correlation of the inflammatory markers of prostate cancer pre radiotherapy

	Neutrophil	Lymphocyte	Platelet	MPV	NLR
Lymphocyte	p=0.233 r=0.187				
Platelet	p=0.451 r=0.119	p=0.385 r=-0.137			
MPV	p=0.751 r=-0.051	p=0.886 r=-0.022	**p=0.002** **r=-0.449**		
NLR	**p<0.001** **r=0.649**	**p<0.001** **r=-0.587**	p=0.184 r=0.209	p=0.848 r=-0.031	
PLR	p=0.825 r=-0.035	**p<0.001** **r=-0.675**	**p<0.001** **r=0.786**	**p=0.043** **r=-0.331**	**p<0.001** **r=0.516**

MPV, mean platelet volumes; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; p, significance; r, correlation coefficient; significant values were presented in bold.

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Table 4. Correlation of the inflammatory markers of prostate cancer post radiotherapy

	Neutrophil	Lymphocyte	Platelet	MPV	NLR
Lymphocyte	p=0.104 r=0.254				
Platelet	p=0.567 r=0.091	p=0.146 r=0.228			
MPV	p=0.296 r=0.164	p=0.683 r=-0.064	**p=0.029** **r=-0.343**		
NLR	**p<0.001** **r=0.491**	**p<0.001** **r=-0.647**	p=0.657 r=-0.070	p=0.368 r=0.142	
PLR	p=0.295 r=-0.165	**p<0.001** **r=-0.704**	**p=0.013** **r=0.380**	p=0.281 r=-0.170	**p<0.001** **r=0.586**

MPV, mean platelet volumes; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; p, significance; r, correlation coefficient; significant values were presented in bold.

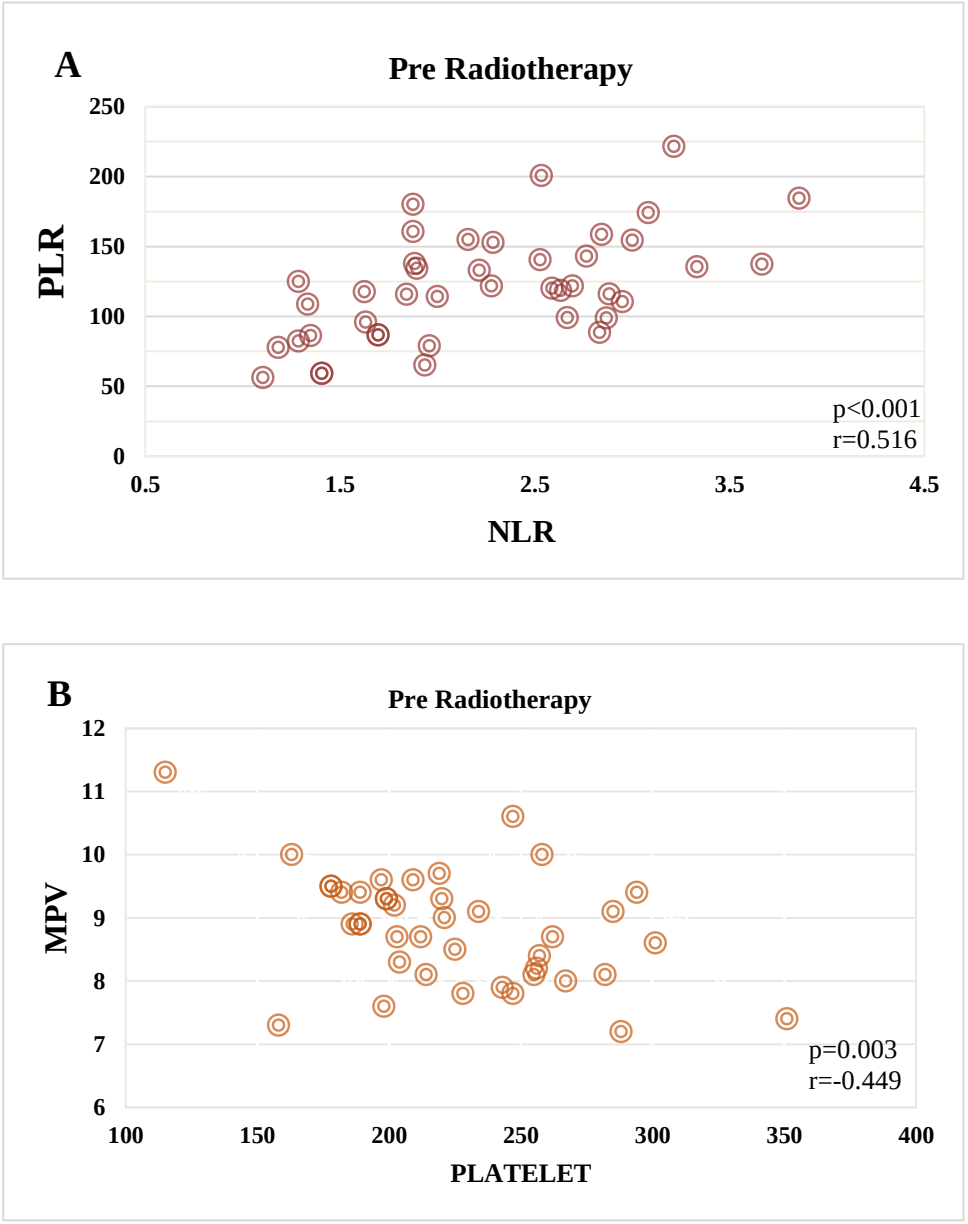

Figure 1 Correlations between NLR and PLR (**A**), platelet and MPV (**B**) in the pre radiotherapy group. NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; MPV, mean platelet volumes; p, significance; r, correlation coefficient.

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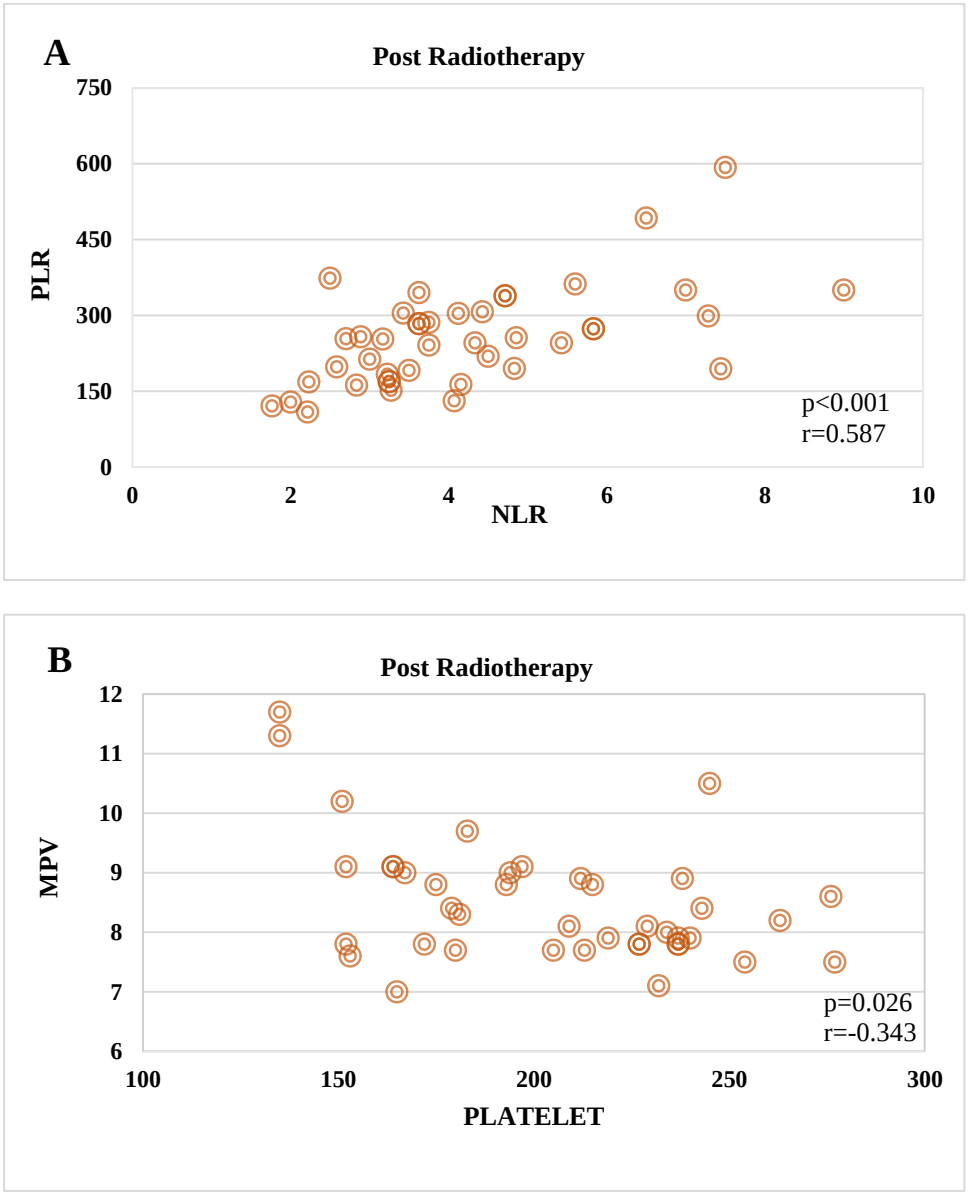

Figure 2. Correlations between NLR and PLR (A), platelet and MPV (B) in the post radiotherapy group. NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; MPV, mean platelet volumes; p, significance; r, correlation coefficient.

Discussion

The inflammatory process plays a role in cancer development. The effect of radiotherapy on the inflammatory process may be an important role in both the pathogenesis and prognosis of the disease (1-3). The present study was performed to examine the role of radiotherapy on inflammatory markers such as neutrophils, lymphocytes, platelets, MPV, NLR, and PLR in prostate cancer patients.

Neutrophils contribute substantially to cancer progression both by direct effects on the cancer cells, and indirect effects on the cancer microenvironment. While in many cases neutrophils have been shown to promote cancer progression, there are also protective effects, particularly when antibody immunotherapy is performed (16).

Lymphocytes are the cells that prevent cancer cell proliferation. It is reported that increased infiltration of cancers with lymphocytes has been associated with better prognosis in cancer patients. The elevated circulating lymphocyte counts have been associated with prolonged survival. Also, normalization of initial lymphocytopenia has been associated with an improved clinical outcome (3).

In the last years, it has been verified that activated platelets are involved in cancer development, and metastasis. Interaction between platelets and cancer cells is not dependent on the platelet quantity but on the volume and size as larger platelets have more granules and receptors (17).

Also, NLR and PLR have been frequently used as markers for the determination of prognosis and survival in many cancer types (3, 9, 10, 13). NLR represents the state of balance between neutrophils and lymphocytes. The elevated NLR values may reflect both an elevated neutrophil-dependent inflammatory reaction and a lower lymphocyte-mediated antitumor immune response (3). The values of PLR have a similar effect with NLR in predicting the prognosis of cancer patients. The mechanism of poor prognosis caused by elevated PLR may be related to cancer metastasis or lymphocyte reduction associated with increased platelet count in cancer patients (18).

In literature, there are studies examining these inflammatory markers in different types of cancers. For example, Kiliçalp et al. reported that the MPV level was significantly higher in pre-operative gastric cancer patients compared to healthy subjects (10). Kemal et al. noted that NLR and PLR values were significantly higher in lung cancer patients compared to healthy subjects (4). But Yaylaci et al. informed that no significant differences between papillary thyroid cancer and benign goiter groups were apparent in the NLR, MPV, platelet, neutrophil, and lymphocyte levels ($p>0.05$) (19).

Our results showed that neutrophil, lymphocyte, platelet, MPV, and NLR values in the pre radiotherapy group were higher than the control group. However, only neutrophils and MPV levels were increased statistically significantly in the pre radiotherapy group as compared to the control group ($p<0.05$ for both). The results of our study may indicate the role of neutrophils in cancer progression and communication of increased volume of platelet with cancer cells.

When we reviewed studies associated with inflammation in cancer patients treated with radiotherapy, it was seen that the lymphocyte and NLP values in non-small cell lung cancer patients were increased in the post radiotherapy group compared to the pre radiotherapy group (20). Son et al. demonstrated that a low NLR in pre radiotherapy was significantly associated with better progression-free survival, and overall survival in patients with locally advanced hepatocellular carcinoma (21).

dos Santos et al. reported that a significant decrease in the total leukocytes, neutrophils, lymphocytes, monocytes, and platelets counts were seen from the first week of treatment with conventional external beam radiation therapy (22). Wu et al. informed that a decreased circulating lymphocyte count during neoadjuvant therapy for locally advanced rectal cancer was associated with better cancer regression. It was noted that lymphocytes may be involved in the immune response provoked by radiotherapy and chemotherapy (23).

The results of our study indicated that neutrophil, lymphocyte, platelet, and MPV were decreased, but NLR and PLR were increased in the post radiotherapy group compared to the pre radiotherapy. Increased NLR and PLR values in our findings support the effect of radiotherapy on the inflammatory process.

Conclusion

Our findings showed that neutrophils and MPV values were increased in the pre radiotherapy group compared to the control group. Moreover, neutrophils, lymphocytes, platelets, and MPV values were decreased, and NLR and PLR values were increased in the post radiotherapy group compared to the pre radiotherapy group. These data may indicate effects of radiotherapy on inflammatory markers. However, further molecular studies are needed to clarify the significance and underlying mechanisms of these markers in prostate cancer radiotherapy.

Ethical approval: The study was approved by the Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Ethics Committee.

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Author's contributions: NB, ÖM; Study design and patient examinations, Biochemical analyzes, **NB;** Revisions

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Estimating how many flebotomists are required in the flebotomy unit: an artificial intelligence study

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Abstract

Objective: In this study, after the examination, most patients apply to phlebotomy units to perform the necessary examinations. Sufficient plebotomists should be taken to the phlebotomy unit to serve a large number of patients. The aim of this study is to determine the reguired number of phlebotomists in blood center using the artificial intelligence.

Material and Methods: This study was conducted in the Health Sciences University Tepecik Training and Research Hospital Blood center between the September-November 2019 . The required number of phlebotomists in the unit was determined with an artificial intelligence-based method. With this system, the number of patients coming to the phlebotomy unit is estimated in real time and considering the past performance of the working phlebotomists, how many phlebotomists are needed in real time is calculated.

Results: The number of phlebotomists who both serve patients as quickly as possible and use the personnel resources of hospital efficiently needs to be optimized. In order to solve this problem, an AI-based system has been developed. With this system, the number of patients coming to phlebotomy unit is estimated in real time and considering the past performances of the working phlebotomists, it calculates how many phlebotomists are needed in real time

Conclusion: The suggestions made by this AI-based system have made a great contribution to the management of the phlebotomy unit. Managers used hospital staff resources in the most efficient way and at the same time, they were able to ensure that patients receive phlebotomy service by following the system's recommendations.

Keywords: Phlebotomy, Phlebotomist count prediction, Artificial intelligence, Health

Introduction

The human factor is the center of healthcare; however, healthcare intuitions have shortcomings in managing this human resource. In a study, the strategies of human resources management of a university and a public hospital were examined and as a result it was reported that these two hospitals did not have clear strategic goals and there is no consistency and direction in human resources management (1). Distribution of hospital resources to various units of the hospital in accordance with the flow of different patient groups is defined as a complex problem that is difficult to manage. This problem is expressed as a dynamic problem due to the fact that patient applications and treatment processes are stochastic (2).Hospitals are searching solutions that improve both the quality of service and lower costs, such as reducing patients' waiting time (2). It is reported in the surveys that waiting time is the most important factor affecting patient satisfaction (3).

In a study, it has been stated that issues such as the role of human resources management in the health sector, its impact on employee welfare, and its contribution to improved health care outcomes have become important in recent years (4). Studies are carried out to enhance phlebotomy times for inpatients. Without using extra staff and eliminating only the defects in the study, there was a good recovery in phlebotomy time (5). The improvement achieved by regulating the model in the phlebotomy processes of inpatient also shows that lower waiting times are able to be achieved with personnel without extra staff in phlebotomy processes of outpatients. With s heuristic algorithm developed in a doctoral dissertation study, workhours of the day are divided into 15 time zones and weekly work plans of phlebotomists were in time intervals. With this study, it has been reported that the workload is able to be met with fewer phlebotomists.

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At the same time, the decrease in service time is presented in the results of the study (6). In another study, by determining the number of patients at 14 different phlebotomy units from Monday to Friday every 30 minutes and how many phlebotomists were required for these patients. After the study, phlebotomy units that employ phlebotomists more or less than needed were determined. It has been reported in the in the results of the study that the waiting times of the patient also decreased. Patient satisfaction was noted with a questionnaire made to patients because of waiting time (7). We see that human resource management is vital in phlebotomy units when we examine the studies and results in this field. There are positive gains for both hospitals and patients when the work plans of phlebotomists are done well. We understand that different solutions have been tried for these achievements and with these studies, they have carried the service quality to a better point than their current status. In our study, by using the latest technologies, we have determined the number of phlebotomists needed in real time depending on the change in workload, using the data held in phlebotomy units and modern machine learning algorithms. Therefore, by estimating the patient load which will occur in the coming hours, we are able to suggest the number of phlebotomists at which the phlebotomy unit needs before the patient density is formed.

Material and Methods

It was carried out by using the data of patients applying to the phlebotomy unit of Health Sciences University Tepecik Training and Research Hospital and data of phlebotomists serving in the phlebotomy unit between September 2019 and November 2019. Test requests of patients, coming time to phlebotomy units, waiting time in phlebotomy unit, the blood test done by the polyclinic, patient priority information and individual information were anonymized. Database of phlebotomists were matched with unique id information, average phlebotomy times, the working hours they started and stopped were used. Phlerobo: Artificial Intelligence and Blood Collection Unit Management System (8) used in the phlebotomy unit of the Health Sciences University Tepecik Training and Research Hospital was used in collecting these data. This system is important in keeping reliable health records and conducting the study with reliable data.

Phlerobo has the characteristics of recording the data of the phlebotomy process with all details. The first step of the bloodletting process starts with the physician requesting the laboratory tests to the patient. The physician directs the patient to the phlebotomy unit to give blood, after he completes the test requests. The patient coming to he phlebotomy unit has stated that he came to the phlebotomy unit by using the kiosks at the entrance of the phlebotomy unit. Then, the patient is announced to give blood and the patient goes to phlebotomist. When the patient sits in the seat of the phlebotomist, the phlebotomist verifies the ID of the patient. Later, bloodletting is performed, and barcodes of the blood-drawn tubes are read and recorded. All steps of this process are instantly recorded by Phlerobo system. These data with high resolution and reliability are vital for the success of the study.

All factors which affect the process must be determined well in order to clarify the number of phlebotomists required for hospitals to achieve their service quality targets. Each institution tries to determine the number of phlebotomists to be assigned in the phlebotomy unit by making some calculations. In these calculations, all the patients who applying to the phlebotomy unit is taken into consideration. The additional number of phlebotomists is tried to be determined by adding extra personnel if the waiting time in phlebotomy unit begins to be above hospital targets. It is rarely seen that more than one phlebotomist has been directed and the quality targets have been achieved however the staff is not used effectively. The total number of patients admitted to the phlebotomy unit is undisputedly the most important factor in determining the required number of phlebotomists. It is easily calculated the average number of patients have applied to the phlebotomy unit from patient records. However, the distribution of arrival hours of patients to the phlebotomy unit during the day is as vital as the total number of patients applying. It is usually seen that in the morning when the polyclinic services are more intense, the patients applying to the phlebotomy units are more and this density decreases greatly in the afternoon. It suggests that instead of assigning a fixed number of phlebotomists, assigning varying number of phlebotomists in the phlebotomy unit depending on the workload will be more appropriate. Another difficulty in determining the number of phlebotomists correctly is the variability among the performance of each employee. Without doubt, every phlebotomist is not able to complete blood withdraw in the same period. Even the same phlebotomist is able to perform differently on different days. It clearly shows that individual performances must be considered when calculating the number of phlebotomists needed. Determining the changing need for phlebotomist during the day is not an easy task. By following the need at all times and making predictions enough phlebotomists should be started before the crisis or the qualify targets are exceeded. In this study, the information required to estimate the number of phlebotomists are needed have been evaluated under two main headings. The first is the number of the patients applying to the phlebotomy unit within a certain period and the other is how long it will be possible to serve the waiting and incoming patients with the current number of nurses. In order to determine the number of incoming patients stated in the first main title, calculations and estimations should be made in many subtitles, for instance, the number of patients requested for testing, the past behavior of the patients, the time of transportation of the patient from the polyclinic to phlebotomy unit, revisiting of patient the next day or certain day when the test is studied. In the second main title, by learning the bloodletting performance of the working staff based on past and current behaviors, the calculation of waiting time can be provided to the current patient flow. The calculations and estimations under these two main titles were made and the number of phlebotomists were needed based on the hospital quality targets was instantly estimated.

The process of patients which come to the phlebotomy unit is started by the physicians making the laboratory test

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request. Knowing the patients requested to test means knowing the patients applying to the phlebotomy unit. However, the problem is how long the patients will apply to the phlebotomy unit. At this stage, we usually encounter two different processes. In the first one, the patient whose test request is made by physician, goes directly to the phlebotomy unit. In this process, the patients apply to the phlebotomy unit after a certain period of time, depending on the distance between the clinic and the phlebotomy unit. In the second process, it is a kind of process that patients apply to the phlebotomy unit the next day or the following days although a laboratory test request is made, due to reasons such as the necessity of the patient to be hungry and the working hours of the test. The number of patients in this group increase especially at noon. Ratio of patients applied to the phlebotomy unit with a polyclinic examination in the afternoon is high because these tests generally require 8 hours of fasting. With the developed algorithm, the potential patient flow is calculated by estimating how many of the patients who undergo a laboratory test but have not applied to the blood collection unit within a certain period of time. The algorithm calculates how many new applications will be available in the next at time period by estimating the time to reach the phlebotomy unit and the number of patients to arrive in the following days. Especially in the first minutes of the phlebotomy unit, it is seen that the patients from previous days applied to the phlebotomy unit. The algorithm also considers the estimation of these patients from the previous day and calculates the number of phlebotomists needed. In Fig 1 and Fig 2, the points below zero show the patients from previous days. Especially in the first hours of the day, we see that a lot of patients from previous day applied to the phlebotomy unit. The present load of the phlebotomy unit is calculated by summing the estimated potential patient load and the number of patients waiting in the hall. Serving this patient burden is a necessity in a time that complies with the quality targets of the hospital. However, in order to calculate how long this patient load can be served, it is necessary to know the performance of working phlebotomists. It is known that the period of bloodletting differs depending on the experience of phlebotomists (9). It can be learned from past records. Therefore, the differences among the phlebotomists are also taken into account. Since the same person may have a performance difference day-by-day, the algorithm also takes into consideration the working performance of the phlebotomist that day. Thus, it is able to be calculated how long the current patient flow can be served with the current staff. Other factors affecting the speed of phlebotomists are the demographic characteristics, diagnosis and the number of tests requested by the patients. It takes longer for an elderly patient to sit at the phlebotomy table, prepare for bloodletting and get off the table than a young patient. It may take longer to find the vascular access of a patient receiving treatment in the oncology unit than other patients. As the number of tubes to be taken from the patient increases the phlebotomy duration increases. Parameters varying from patient to patient must be taken into account as well as the number of patients.

The chart below illustrates the variation in phlebotomy duration and mean phlebotomy duration among patients. As seen in the Fig 3, phlebotomy duration varies between 0.5 minutes and 7 minutes, while the average phlebotomy duration varies between 1.5-2.0 minutes in a much narrower duration.

Statistical Analysis: The data were stored in the MongoDB database in JSON (Javascript Object Notation) format. Algorithms and calculations are made using Javascript programming language

Results

We have compared the values of the average waiting times, the maximum number of waiting patients and the number of working nurses in Sağlık Bilimleri University Tepecik Training and Research Hospital phlebotomy unit between September-November 2019 when the suggestion was made and was not made. We have observed how feasible the number of phlebotomists recommended by the one, responsible for phlebotomy unit. We have compared the average waiting time and the maximum number of waiting patients in the days when the person who is responsible for phlebotomy unit, was able to implement the recommendations of the system and in the days when he or she was not.

The number of phlebotomists suggested by the system should be evaluated in two ways. The first one is to make instant resource planning based on the recommendations made by the system in real time. However, it is not easy to apply this instantaneous variability in the field with same sensitivity.

The other form of evaluation should be the spread of real time suggestions to the general. For instance, the system recommends that 2 or 3 phlebotomists should work until 9:00 every morning. With this proposal not varying greatly among days, a general conclusion can be made about the time of personnel to begin to work and the number of phlebotomists which should work. Similarly, it is seen from the recommendations that 1 or 2 phlebotomists are enough for the afternoon. This suggestion can be transformed into a general practice and more staff can be evaluated more efficiently in another unit.

In Fig 4, the average waiting time in the last column with the number of working and suggested phlebotomists throughout September is presented. In the phlebotomy unit in which the study was conducted, 8 minutes for routine patients and 3 minutes for priority patients were determined as the quality aim. In accordance with these aims, the number of phlebotomists was proposed to be less.

When we look at the mean waiting time column, it can be seen that the number of phlebotomists recruited, and the quality aims are successful. According to these figures, we can review the quality aims and we can aim shorter waiting times, or we can conclude that with less phlebotomists serving, we achieve the quality aims.

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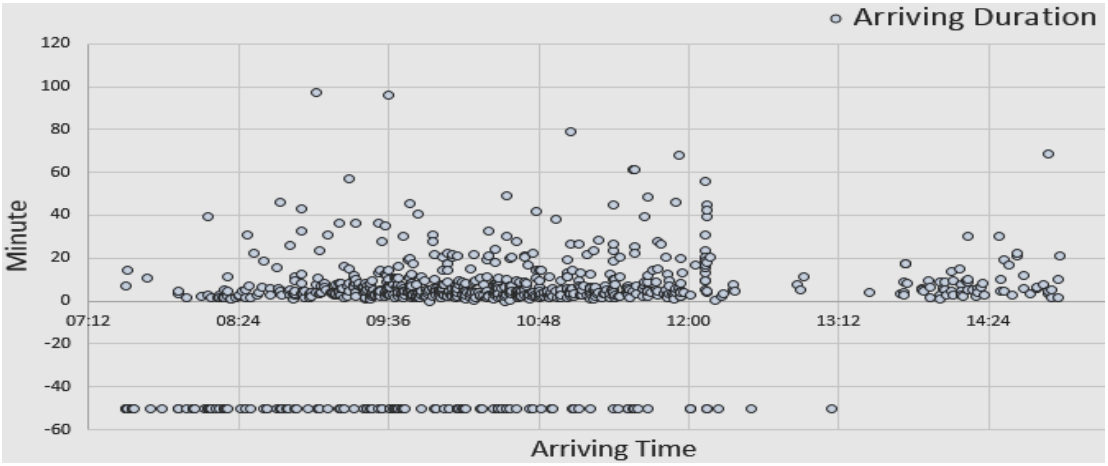

Figure 1: Reaching time to the phlebotomy unit (02.09.2019)

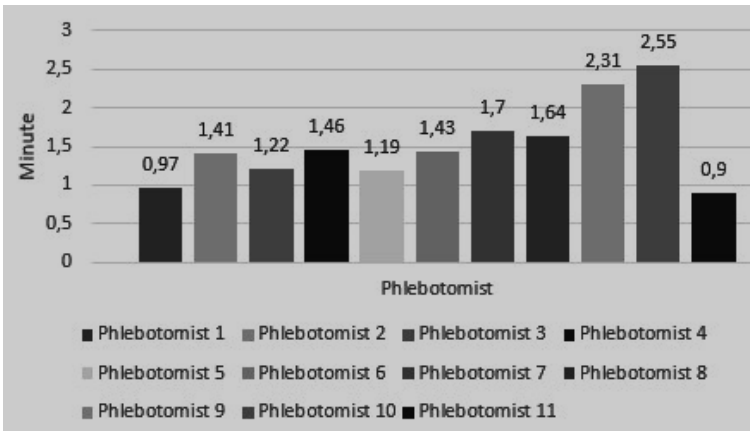

Figure 2: Average bloodletting period (02.09.2019)

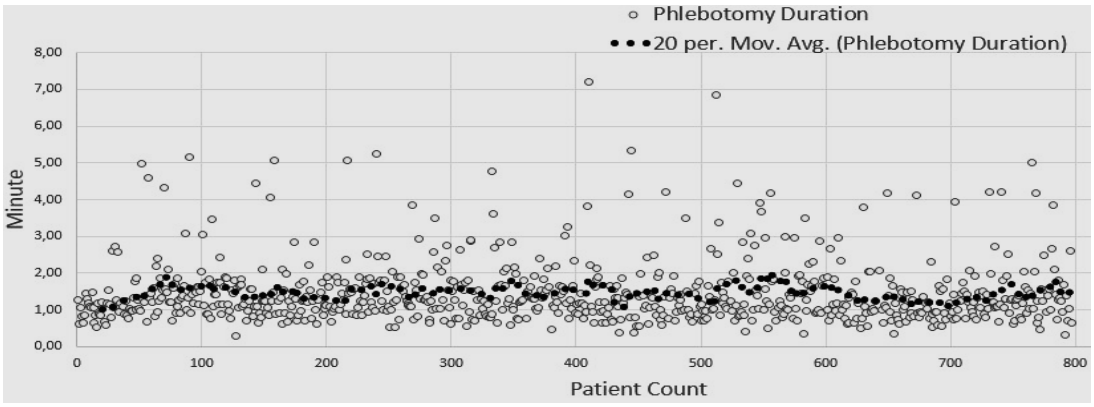

Figure 3: Phlebotomy duration (02.09.2019)

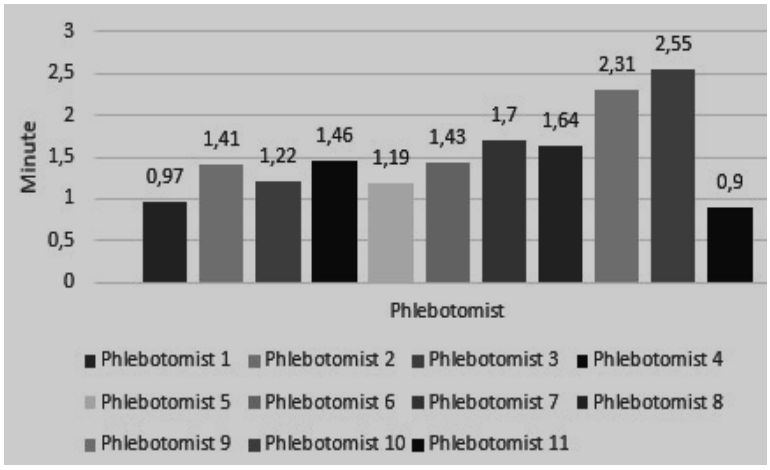

Figure 4: September 2019 Actual/Predicted Phlebotomist Count

Discussion

First, the responsible staff must follow the recommendations during the day for the number of phlebotomists suggested by the system to be implemented in the field. It is not always possible to reach the needed staff although the suggested number of phlebotomists is regularly monitored. This kind of problems were evaluated as administrative problems, not systemic. However, the system triggers the emergence of administrative problems and necessary actions to be taken for its solution. Taking instant actions is not that easy in practice. However, a general working pattern must be created from instant recommendations. After the general order is provided, it will be easier to implement instant recommendations. In the later stages of the study, when the resources available to the system is introduced, it can be controlled completely which staff will start working at the phlebotomy unit at what time, when and how long take a break and what time to complete the work by the intelligent system (8). When the number of phlebotomists is not enough, a call message can be sent to the phlebotomists chosen by the system and the next phlebotomists can be determined. If the phlebotomist expected to be next one does not start within the expected time, the system is able to invite new phlebotomists automatically (9). When the number of phlebotomists is high, it can be suggested by the system that they will take a break, or they have completed their work for that day. Thus, we are able to create phlebotomy units at which the entire process is managed by artificial intelligence and human errors are minimized.

Conclusion

In conclusion, this AI-based system have made a great contribution to the management of the phlebotomy unit. Managers used hospital staff resources in the most efficient way and at the same time, they were able to ensure that patients receive phlebotomy service by following the system's recommendations.

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Rhamnetin improves antioxidant status in the liver of Ehrlich solid tumor bearing mice

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Abstract

Objective: Rhamnetin, a flavanol, is in the subclasses of the flavonoids existing in plants. The antioxidant properties of several plants containing flavonoids have been extensively studied in several diseases including cancer. This study investigated the effects of rhamnetin on tumor masses, oxidant and antioxidant status in the livers of mice bearing Ehrlich solid tumor.

Material and Methods: Fifty male Balb/C mice weighing 25-30 g were used in the study. Ten mice were kept for Ehrlich ascites tumor (EAT) cells production and the remaining mice were randomly assigned to four groups containing 10 mice in each as healthy control and treatments receiving 1x106 EAT cells and EAT cells plus 100 µg/kg/day or 200 µg/kg/day rhamnetin via subcutaneous route. The tumor inhibition rates of rhamnetin treatments were calculated. The livers were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels.

Results: Compare to tumor control, both levels of rhamnetin suppressed tumor masses throughout the experiment. The MDA levels were increased whereas SOD and CAT activities were reduced by EAT cells injection in the liver of mice. The 100 µg/kg/day rhamnetin treatment decreased MDA level but 200 µg/kg/day rhamnetin had no significant effect on increased MDA level. The reduced liver SOD ($p<0.001$) and CAT ($p<0.01$) activities were elevated by both levels of rhamnetin injection.

Conclusions: The results of this study have revealed that rhamnetin suppresses tumor progression and improves antioxidant status in the livers of solid tumor-bearing mice.

Key words: Ehrlich solid tumor, lipid peroxidation, antioxidant enzymes, rhamnetin

Introduction

Cancer, which DNA damage based malformation, is a major public health problem worldwide. Cancer which is accepted among chronic diseases is frequent and is the second leading cause of mortality after cardiovascular diseases (1). Surgical removal of the tumor masses, chemotherapy and radiotherapy or their combinations are common applications for the treatment of cancer cases. Unfortunately, chemotherapeutic agents may have many side effects and chemotherapy treatments takes very long time. Therefore, in recent years, there is a growing intention to use the plant products along with the chemotherapeutic agents or radiotherapy or as a possible alternative in cancer therapy (2-4). The most commonly used alternative methods in cancer cases are herbal therapies.

Nowadays, phytotherapy is defined as a complementary and alternative treatment method (3,4). Several natural products have been investigated for their anticancer activities. Flavonoids have attracted considerable interest in recent years due to their various pharmacological properties, including their protective effects against cytotoxicity and cancer. Most of the flavonoids are considered to be safe and have limited side effects or toxicities (5,6). Flavonols are the subclasses of the flavonoids (7). Flavonols, plant-derived polyphenolic compounds, are commonly consumed in the diet . Rhamnetin [2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-methoxychromen-4-one], O-methylated flavonol, can be extracted particularly from cloves and many other plants species such as fruit, vegetables, tea and coffee (7-9).

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Although antioxidant (6,10-12), reactive oxygen species (ROS) scavenging (12-14) and anti-inflammatory (15-17) properties of rhamnetin have been shown in previous studies, most of these studies have been conducted at in vitro conditions.

Park et al.(6) have investigated the protective effect of rhamnetin on cell viability, apoptosis and ROS production and found that rhamnetin protected the H9c2 cardiomyoblast cells against H_2O_2 induced cell death. These authors also determined that rhamnetin increased CAT and Mn-SOD expression and inhibits intracellular ROS production. Oak et al. (11) demonstrated in vitro anticancer activity, antioxidant and anti-proliferative ability of rhamnetin by determining increases in the expression of caspase-3 and caspase-9, which induce the apoptosis as well as reduce the intracellular ROS levels in prostate cancer cells.

Although, flavonoids, found in many food items, play a beneficial role in disease prevention, further studies are needed to investigate whether pure forms have similar beneficial effects at in vivo conditions (5,18).

Therefore, in the present study, the effects of different doses of the rhamnetin on MDA levels, which is the indicator of lipid peroxidation, and antioxidant enzymes, SOD and CAT, were determined in the liver of Balb/C mice bearing Ehrlich solid tumor.

Materials and Methods

Animals, management and experimental design

Fifty, 8 week old, male Balb/C mice weighing 25-30 g were used in the study. The mice were provided by Erciyes University Experimental and Clinical Research Center (DEKAM).

Animals (five mice per cage) were maintained in polycarbonate cages sized 42x26x15 cmand 21±2^{o}C room temperature, 50±5 % humidity, environmental ventilation system with air flow rotation of 12 per hour and 12 hours of light/dark cycle were provided for the highest welfare conditions throughout the study. Water and commercially available pellet diet that met or over the daily nutritional requirement of the mice were provided ad libitum during the study.

Ten mice were kept as cancer stock to obtain Ehrlich ascites tumor (EAT) cells. The remaining 40 animals were evenly distributed into four experimental groups as healthy control, tumor control and rhamnetin treatments.

On the first day of the experiment, mice in all groups except the mice kept as healthy control were inoculated with 0.1 ml of ascites fluid containing 1x10^{6} EAT cells via subcutaneus (s.c.) route through nape skinfor solid tumor developmentandthe mice in healthy control group received 0.1 ml of sterile physiologic saline solution via s.c. route.

A 24 hour later, a daily dose of either 100 µg/kg or 200 µg/kg rhamnetin (in 0.1 ml) was injected to each mouse in treatment groups via intra peritoneal (i.p.) route for 15 days. A 0.1 ml of sterile physiologic saline solution was administered via i.p. route to each mouse in healthy and tumor control groups every day throughout the experiment.

Preparation of Ehrlich Ascites Tumor Cell and Stock Mice

The EAT cells, previously used in the studies conducted in our laboratory and preserved in cryovials at -80^{o}C, were thawed at room temperature and 0.1 ml of EAT cell suspension was inoculated into the peritoneal cavity of a mouse.

Following EAT inoculation, the mouse was controlled every day for abdominal ascitic fluid volume for 11 days. On day 11, the ascitic fluid was collected by an injector and 0.1 ml of this ascitic fluid was inoculated into the peritoneal cavity of another mouse to provide more aggressive EAT cells.

This animal was also observed for 11 days and on day 11, approximately 3 ml of ascitic fluid was collected and preserved in cryovials. The EAT cell count was performed. For this purpose ascitic fluid was diluted with PBS (1/1 v/v) and inoculated onto cell culture then 100 µl of cell culture fluid was stained with the trypan blue staining technique for determination of cell viability. The cells were counted undera light microscope (Olympus CX31, Tokyo, Japan) and the inoculum size was determined.

Preparation of rhamnetin solution

Rhamnetin was purchased from Sigma Aldrich (Cat no: 17799, Sigma-Aldrich). A five mg of rhamnetin was dissolved in 0.5 ml of 1% methanol and then filled up to 10 ml with distilled water. The rhamnetin solution was sterilized by filtering through a 0.45 micrometer filter.

Measurements of body weights, tumor volumes and tumor inhibition rate

Mice were weighed and body weightswere recorded daily. Animals were palpated every day for the solid tumors development. The tumor sizes were measured by a digital caliper with 0.01 mm sensitivity (A Brand Digital Caliper 300 mm, China). Tumor sizes were recorded every day (Figure 1).

Tumor volumes were determined with the following formula: Tumor volume (mm^3)=width²xlengthx0.52 (19). For determination of the efficacy of rhamnetin levels, the tumor inhibition rates (TIR) were calculated with the following formula: Tumor inhibition rate=(mean tumor volume value of control group-mean tumor volume value of treatment group)/(mean tumor volume value of control group)x100 (20).

Sample collection and preparations

At the end of the experiment (on day 16), animals were sacrificed with 50 mg/kg ketamine/15 mg/kg xylazine mixture under general anesthesia. The tumor masses were removed and their sizes were measured. The livers of animals were collected into sterile plastic bags and they were transferred immediately to the laboratory under cold chain and stored at -80^{o}C until biochemical analyses.

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Figure 1. Measurements of tumor sizes and appearance of Ehrlich solid tumor

Homogenization of the livers

The 500 mg of liver samples were homogenized in a glass-glass homogenizer with physiological saline solution (p H=7.4) (1/10, w/v). The homogenates were centrifuged at 12 000 rpm for 20 minutes at 4 °C and used for MDA, SOD and CAT analyses.

Biochemical analysis

Determination of malondialdehyde concentration

Malondialdehyde levels of the livers were determined with the method described by Ohkawa et al. (21). Freshly prepared 10, 20, 40, 60, 80 and 100 nMol/ml of 1,1,3,3-tetramethoxypropane (density: 0.99 g/mL) solutions were used as standards. The method was briefly as follow: A 100 µl of liver homogenate was mixed with 8.1% of sodium dodecyl sulfate (SDS), 20% of acetic acid (pH 3.5) and 0.8% of thiobarbituric acid (TBA) (pH 3.5) and incubated at 95°C for 30 minutes. Then cooled and n-butanol-pyridine solution and distilled water were added and strongly vortex mixed. The supernatant was separated following the centrifugation at 4000 rpm for 10 minutes. The absorbance of the complex developed after heat treatment at 95°C was measured at 532 nm by a UV-Visible spectrophotometer (Shimadzu, UV1601, USA). The result was expressed as nMol/mg protein.

Determination of superoxide dismutase activity

The liver was homogenized with 1/10 of distilled water. The sample was mixed with the chloroform/ethanol mixture 1/1 (v/v) and centrifuged at 12000 rpm for 2 hours at +4 ºC. Supernatant was separated to determine SOD activity. The activity of SOD was measured spectrophotometrically according to the method described by Sun et al. (22). This method was briefly as follows: A 50 µl of tissue supernatant and 50 µl XO in 2 M ammonium sulfate solution (1/100, v/v) were added to 2.9 ml of the reagent mixture consisting of xanthine solution+ NBT+ Na2CO3+ BSA.

After incubation at 25 °C for 20 minutes, 1 ml of 0.8 mM CuCl2 was added to the tube and the optical density of the sample was read at 560 nm. The SOD activity was expressed as Unit/mg protein (1 unit=50% inhibition of NBT reduction) and % inhibition was calculated with the following formula: % inhibition = [(blank abs-tissue abs)/blank abs] x100.

Determination of catalase activity

The CAT activity was determined with the method previously described by Aebi (23). The CAT assay was briefly as follows: Liver homogenate was mixed with H2O2 solution (30 mM) and freshly prepared phosphate buffer (50 mM, pH=7.0) then the absorbance was measured spectrophotometrically at 240 nm.

The extinction coefficient was 0.004 (0.0039) mM-1mm-1. The CAT activity was expressed as U/mg protein/min for tissue.

Statistical analysis of the data

Statistical analyses of the data were performed with IBM SPSS Statistics 22.0 (IBM Corp., Armonk, New York, USA) program. The normality of the data was evaluated byhistogram, q-q graphs and Shapiro-Wilk test. The variance homogeneity was tested by the Levene test. One way ANOVA and Kruskal Wallis test were used in the group comparisons where appropriate.

When the F values were significant, Tukey and Dunn-Bonferroni tests were applied for multiple comparisons. The data were evaluated using the R 3.2.3 program. Data were presented as means ± standard deviation of the means and median (25%-75% percentiles) where appropriate. Significance level was accepted as $p < 0.05$.

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Results

Body weight changes, solid tumor development, tumor volumes and tumor inhibition rates

The body weights of all animals increased during the study. The body weight changes of the mice in rhamnetin treated groups were close to the ones in healthy controlgroup (Table 1). Tumor development started only in tumor control group on the 5th day of the experiment, which could not be measured until day 7. On day 7, measurable solid tumor was developed in 5 mice in tumor control group whereas in rhamnetin treated groups 3 mice exhibited tumor masses. Statistically significant differences were determined between tumor control and rhamnetin treated groups after day 9. Tumor volumes were significantly lower in rhamnetin injected mice than the tumor control mice from day 8 to the end of the study.

There was no significant difference between 100 and 200 µg/kg rhamnetin treated groups (Table 2, Figure 2). However, the tumor inhibition rate of 200 µg/kg rhamnetin was higher than 100 µg/kg rhamnetin treatment (Table 3).

Liver MDA levels, SOD and CAT activities

Compare to healthy control mice, a significant increase was determined in MDA level ($p<0.001$) of the tumor control mice. The SOD ($p<0.001$) and CAT ($p<0.01$) activities were lower in tumor control mice than healthy controls.

The injection of 100 µg/kg rhamnetin decreased the elevated MDA level but 200 µg/kg rhamnetin had no significant effect. Both levels of rhamnetin increased the reduced SOD and CAT activities (Table 4, Figure 3).

Table 1. Body weight (g) changes of controls and rhamnetin treatedmice bearing Ehrlich solid tumor

Days	Healthy control	Tumor control	Rhamnetin		p
	n:10	n:10	100 µg/kg n:10	200 µg/kg n:10	
1- 15	8.31±1.50^{ab}	3.53±2.56^{c}	8.43±2.78^{a}	5.61±1.98^{cb}	0.000

$^{a-c}$ The values within the same row with different superscript differ significantly.

Table 2. Solid tumor volumes (mm^3) of tumor control and rhamnetin treated mice bearing Ehrlich solid tumor

Days	Tumor Control		Rhamnetin 100 µg/kg /day		Rhamnetin 200 µg/kg/day		p
	n		n		n		
7	5	76.09±35.96	3	92.89±22.11	3	93.06±19.03	0.651
8	8	221.14±168.25	6	121.80±48.84	6	108.25±71.70	0.170
9	10	546.92±470.47^{a}	9	143.40±91.28^{b}	7	135.20±88.86^{b}	0.011
10	10	1283.78±933.85^{a}	10	224.47±164.14^{b}	7	216.05±46.64^{b}	0.001
11	10	1668.38±1198.44^{a}	10	357.70±356.45^{b}	8	219.72±148.78^{b}	0.000
12	10	1996.09±1510.30^{a}	10	494.62±422.62^{b}	9	328.83±213.58^{b}	0.001
13	10	3404.43±2899.53^{a}	10	690.71±611.12^{b}	10	495.89±442.46^{b}	0.001
14	10	5052.48±4541.40^{a}	8	887.61±655.41^{b}	10	687.79±592.44^{b}	0.003
15	10	6278.52±5015.10^{a}	7	909.55±275.24^{b}	10	913.67±731.32^{b}	0.001

^{a,b} The values within the same row with different superscript differ significantly.

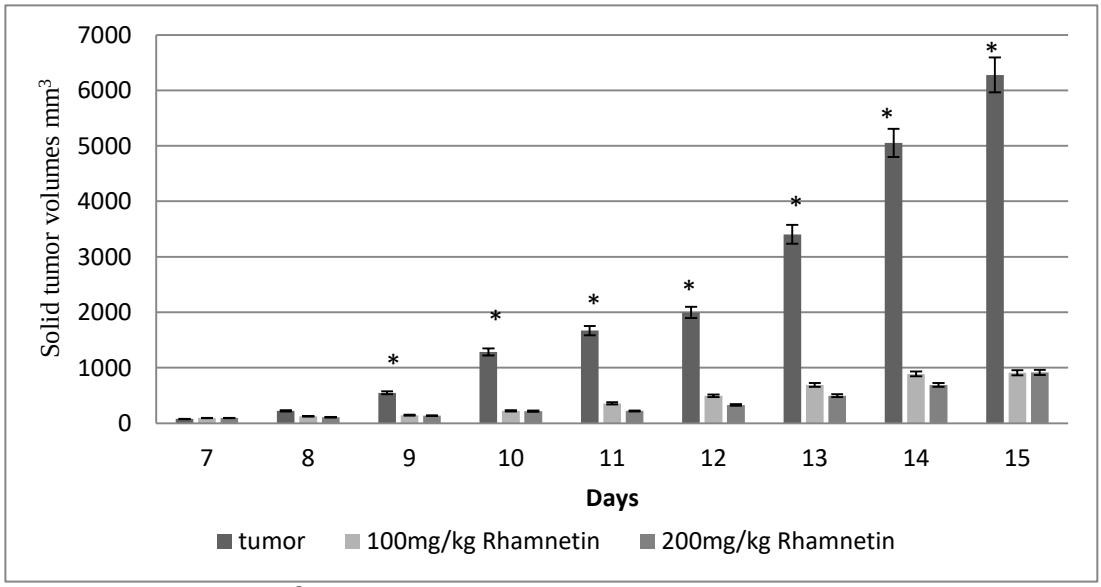

Figure 2. Solid tumor volumes (mm^3) of all groups

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Liver MDA levels, SOD and CAT activities

Compare to healthy control mice, a significant increase was determined in MDA level ($p<0.001$) of the tumor control mice. The SOD ($p<0.001$) and CAT ($p<0.01$) activities were lower in tumor control mice than healthy controls.

The injection of 100 µg/kg rhamnetin decreased the elevated MDA level but 200 µg/kg rhamnetin had no significant effect. Both levels of rhamnetin increased the reduced SOD and CAT activities (Table 4, Figure 3).

Table 3. Tumor inhibition rates of 100 µg/kg and 200 µg/kg rhamnetin levels in the mice bearing Ehrlich solid tumor

Days	Rhamnetin	
	100 µg/kg /day	200 µg/kg/day
7	-22.08	-22.30
8	44.92	51.05
9	73.78	75.28
10	82.51	83.17
11	78.56	86.83
12	75.22	83.53
13	79.71	85.43
14	82.43	86.38
15	85.51	85.44

Table 4. Liver MDA levels, SOD and CAT activities in rhamnetin treated mice bearing Ehrlich solid tumor

Parameters	Healthy Control	Tumor Control	Rhamnetin 100 µg/kg/day	Rhamnetin 200 µg/kg/day	p
MDA	12.06±0.53^{a}	15.81±0.94^{c}	14.30±0.90^{b}	16.42±1.00^{c}	0.000
SOD	6.60±0.26^{c}	4.44±0.16^{a}	5.57±0.22^{b}	6.94±1.00^{c}	0.000
CAT	47.96±3.10^{b}	41.76±5.96^{a}	47.27±3.07^{ab}	50.16±2.73^{b}	0.003

$^{a-c}$The values within the same row with different superscript differ significantly.

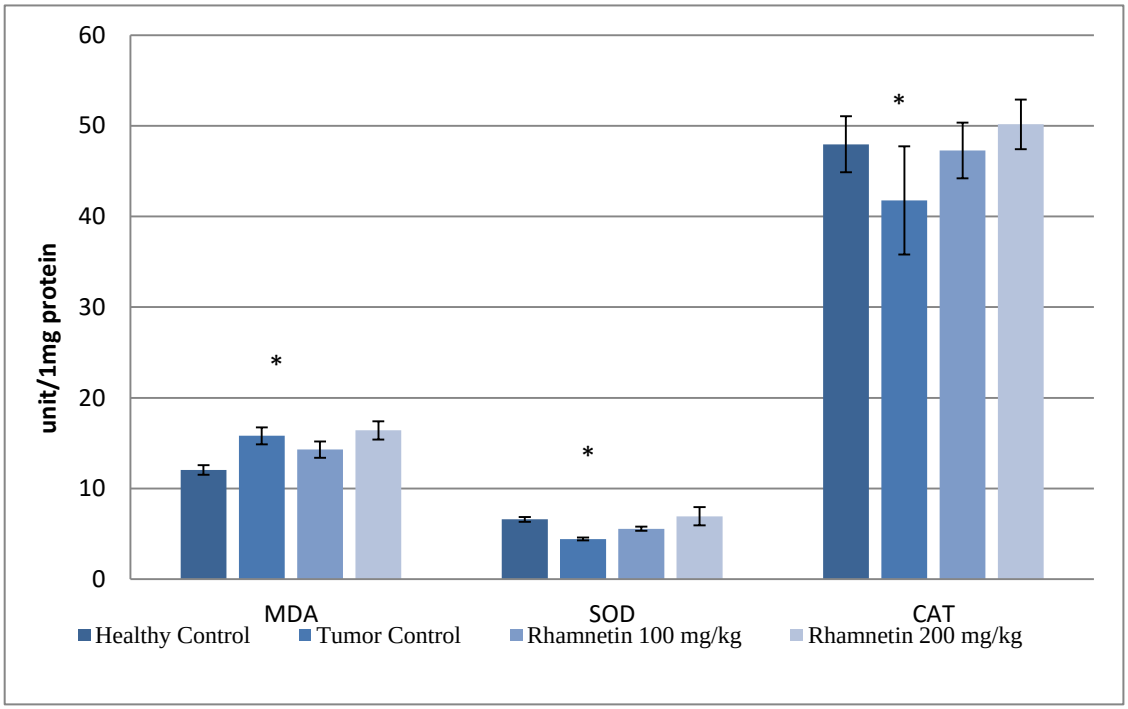

Figure 3. Liver MDA levels, SOD and CAT activities all of the groups

Discussion

Cancer cells increase production of ROS compare to normal cells and it is speculated that tumorigenic signaling also increases expression of antioxidant proteins to balance the high ROS production to maintain redox homeostasis (24,25). Reuter et al. (26) have reported that oxidative stress, chronic inflammation and cancer are closely related. Ehrlich ascites carcinoma, a spontaneous murine mammary adenocarcinoma, is adapted to ascites form by serial intraperitoneal passages (27). Because Ehrlich ascites tumor (EAT) cells do not contain H-2 histocompatibility antigens, they rapidly proliferate in almost all mouse species (28). Ehrlich tumor cells cause morphological and metabolic changes including alterations in oxidant and antioxidant status in the animals (29). Therefore, Ehrlich solid tumor model was chosen to investigate the effect of rhamnetin on lipid peroxidation and antioxidant status in the liver tissue of tumor bearing mice in the present study.

The efficacy of anti-carcinogenic agents can be determined via body weight, tumor volumes or tumor inhibition rate (20). The body weights of mice in all groups increased during the study but the changes in body weights in healthy and rhamnetin treated mice, particularly in 100 µg/kg rhamnetin injected group, were very close to each other. The least weight changes were observed in mice in tumor control group ($p<0.001$) (Table 1). In the present study, the tumor masses showed rapid growth rate and reached the palpable size on day 5 following the SC injection of 0.1 ml of ascitic fluid containing $1x10^6$ EAT cells. The volume of tumor masses became measurable on day 7 in mice kept as tumor control and rhamnetin injected groups. The solid tumor was developed in all animals in tumor control after day 9 whereas tumor masses reached to measurable size in all animals on day 10 in 100 µg/kg rhamnetin treated group. In 200 µg/kg rhamnetin injected group, all animals exhibited solid tumor after day 13.Rapid development of solid tumor in all EAT injected mice is due to aggressive behavior andthe rapid proliferation of EAT cells because of the lack of H-2 histocompatibility antigens (28). During the experimental period, the tumor volumes increased in all groups but both levels of rhamnetin treatments reduced the elevated tumor volumes significantly ($p<0.05$- $p<0.001$) (Table 2). The decrease in tumor volume was more pronounced with 200µg/kg rhamnetin. The decreases in tumor volumesdue to rhamnetin treatments may result from the decreased rRNA gene expression capacity which indicates the suppression of tumor formation (30) and induced apoptosis (11). In the present study, tumor inhibition rates of both levels of rhamnetin increased with the increasing length of their use. However, 200 µg/kg rhamnetin treatment was found to be more efficient throughout the experiment (Table 3).

Many previous studies have emphasized the antioxidant capacities of flavonoids which are found in various fruits, vegetables, seeds, tea and red wine (6,11). The antioxidant property is due to the hydroxylation status of the aromatic ring of the flavonoids. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) exert beneficial and deleterious effects. ROS act as secondary messenger in intracellular signaling cascade (31) thus ROS are involved in the initiation, progression and malignancy of tumors (11,32).

Malondialdehyde, a secondary product of lipid peroxidation, is a uniqueindicator of lipid peroxidation. In the present study, liver MDA concentration was increased by tumor development as previously indicated by Kabel et al. (29) who also subcutaneously implanted EAT cells into mice and found increases in MDA levels in tumor tissue. In human prostate cancer cells, 5-80 µM rhamnetin reduced the ROS production in dose dependent manner in the study of Oak et al (11). In a rat study of Igarashi and Ohmuma (33), TBARS content of the liver of rats receiving cholesterol free diet was reduced by feeding with 0.01% and 0.2% rhamnetin without any significant difference between the rhamnetin levels and rhamnetin treatment had no effect on liver SOD and CAT activities. However, in the present study, the IP injection of 100 µg/kg rhamnetin decreased the elevated MDA level but interestingly 200 µg/kg rhamnetin had no significant effect. In the present study, injection of EAT cells resulted in significant decreases in liver antioxidant enzymes. Kabel (29) has reported that subcutaneous implantation of Ehrlich tumor cells into mice decreases tumor tissue CAT activity. Similarly, in the present study, tumor formation significantly reduced liver SOD ($p<0.001$) and CAT ($p<0.01$) activities and the reduced liver SOD activity was increased by both levels of rhamnetin whereas a significant increase was achieved in CAT activity with 200 µg/kg rhamnetin treatment (Table 4). Antioxidants alleviate the oxidative damage directly by reacting with free radicals or indirectly by suppressing radical generating enzyme or enhancing the activity and/or synthesis of antioxidant enzymes (32). Rhamnetin enhanced the expression of catalase and Mn-SOD, thus inhibits production of intracellular ROS in the study of Park et al. (6). Majewska et al. (12) have also shown radical scavenging property and antioxidant activity of rhamnetin at in vitro test conditions. On the other hand, in the present study, rhamnetin elevated antioxidant enzymes without affecting lipid peroxidation which indicates that the protective effect of rhamnetin against oxidative stress is not mediated by direct radical scavenging (5).

Conclusions

This study has shown that rhamnetin suppresses tumor progression and improves antioxidant status in the livers of solid tumor-bearing mice.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in this study were in accordance with the ethical standards of the Erciyes University Experimental Animals Local Ethics Committee (approval no: 14/30, date: 12.02.2014).

Author Contributions: MS, OB, TE, DC, Nİ, ÖA, HG, EU; study design and concept, **MS;** Biochemical analysis, statistic, writing, revisions

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Evaluation of seasonal changes in the diagnosis of acute leukemia in Turkey

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Abstract

Objective: The etiology of acute leukemia (AL) has been under investigation for decades but the exact cause is still unknown. There are studies suggesting that infection plays a critical role in the development of AL in conjunction with other risk factors. In some studies, it has been shown that the incidence of AL increases after influenza endemics. This shows that viruses may play a role in the etiology. The theory that viruses might have a role in the etiopathogenesis created the idea that AL frequency may peak during some specific months; therefore, in this study, we aimed to research the relationship between AL diagnosis frequency and seasons in Turkey.

Method: The 186 patients who were diagnosed with de novo acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) diagnosis at our center were included in the study.

Results: The frequency of ALL diagnoses were as follows: 25 (34.3%) in winter, 19 (26%) in spring, 15 (20.5%) in autumn, and 14 (19.2%) in summer. The frequency of AML diagnose was as follows: 24 (21.2%) in winter, 30 (26.6%) in spring, 27 (23.8%) in autumn and 32 (28.4%) in summer. In our study, we did not find a statistically significant relationship between AL diagnosis frequency and seasons.

Conclusion: According to our literature review, there are two studies including our study, searching for a relationship between AL diagnosis frequency and seasons in Turkey. Neither of the studies found a relationship between AL and seasons. According to our analysis the numbers of the patient in studies are limited; therefore the studies with high number of patients are needed to find out a relation between seasons and diagnosis time of AL.

Keywords: Acute leukemia, seasonality, acute myeloid leukemia, acute lymphoblastic leukemia

Introduction

Acute leukemias (AL) are hematological malignancies characterized by the abnormal proliferation of the blasts caused by hematopoietic myeloid or lymphoid precursors or both. They become symptomatic in a short time due to their aggressive nature. The most frequently seen AL type in adults is acute myeloid leukemia (AML) and it has an incidence of 5-8 /100.000 (1,2). On the other hand, acute lymphoblastic leukemia (ALL) has an incidence of 1.28/ 100.000 and it is less commonly observed in adults compared to AML (3).

The identification of causes and prevention from AL is the main goal. For this reason, the etiology of AL has been under investigation for decades, but the exact cause is still unknown. Benzene, radiation, toxic gases, chemicals, hereditary diseases, benign hematologic diseases, and viruses have been researched in the etiology of AL. Ionizing radiation and congenital genetic syndromes such as Down's, neurofibromatosis, Fanconi's anemia, and Bloom's Syndrome, all of them together explain less than 10% of cases (4). There are studies suggesting that infection plays a critical role in the development of AL in conjunction with other risk factors. However, until today no specific microorganism has been definitively associated with AL. On the other hand, in some studies, it has been shown that the incidence of AL increases after influenza endemics (5,6). This may show that viruses play a role in the etiology. In addition to these, there are studies indicating that the incidence of AL increases in some periods in a year.

Turkey is located between 36° - 42° north latitudes and 26° - 45° east longitudes. December, January, and February are winter months; March, April, and May are spring months; June, July, and August are summer months; September, October, and November are autumn months. In this study, we aimed to research the relationship between AL diagnosis frequency and season in Turkey.

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Material and Methods

186 patients diagnosed with de novo AML and ALL at our center between December 2009 and March 2019 were included in the study. The data regarding the gender, age, leukemia type, and diagnosis date were retrospectively analyzed. The patients whose AL were diagnosed with the examination of the morphological findings of bone marrow aspirates and flow cytometry or immunohistochemical analysis, and who were over the age of 18 were included in the study. The patients diagnosed at a different center and whose diagnosis date could not be reached were not included in the study.

The statistical analyses were performed by using IBM SPSS Statistics v21 software. Descriptive statistics were applied for numerical data, and the Chi-square test was used for the evaluation of categorical data and comparisons among the groups.

Results

The AL patients included in the study consisted of 73 (39.2%) ALL and 113 (60.8%) AML patients. While the median age among ALL patients was 30 (range 16-58), it was 38 (range 18-64) among AML patients; the median age for all AL patients was 36 (range 16-64). 25 (34.2%) out of 73 ALL patients were female, and 48 (65.8%) of them were male whereas 39 (34.5%) out of 113 AML patients were female, and 74 (65.5%) of them were male.

The frequency of ALL diagnosis was the most common in December and for AML it was the most common in June, however, no statistically significant relationship was found between the frequency of AL diagnosis and months (Table 1).

The frequency of ALL diagnosis was the most common in winter and for AML it was the most common in spring, however, no statistically significant relationship was found between the frequency of AL diagnosis and seasons (Table 2).

Discussion

The pathogenesis of AL patients involves a complex chain of events that alter the proliferation and differentiation of hematopoietic precursor cells, chromosomal translocations, inversions, or point mutations (1-3). The factor triggering these events has not yet been clearly specified. It has been suspected that viruses could be one of the potential triggering factors associated with AL pathogenesis. The role of John Cunningham (JC) virus, Epstein-Barr virus, cytomegalovirus, and parvovirus B19 has been researched as an etiological factor in AL and parvovirus B19 has been found to play a potential role in the etiology of AL (7-11). The theory that viruses might have a role in the etiopathogenesis created the idea that AL frequency may peak during some specific months; therefore, studies have been conducted to search a relationship between AL diagnosis frequency and seasons in various countries.

In the study conducted by Gao et al., in Sweden, ALL diagnosis was the most common in winter although in Singapore and the United States of America a seasonal relationship in the diagnosis of ALL was not found (12). Similar to results in Sweden, in a previous study, it was reported that, in Finland, the diagnosis of ALL was the most common in winter (13). Contrary to the previous studies, Badrinath et al. indicated that ALL diagnosis was the most common in summer in England (14).

Table 1: The distribution of acute leukemia diagnosis times

Months	ALL (n, %)		AML (n, %)		p-value
January	9	(12.3%)	11	(9.7%)	
February	6	(8.2%)	6	(5.3%)	
March	8	(11%)	12	(10.6%)	
April	7	(9.6%)	11	(9.7%)	
May	4	(5.5%)	7	(6.2%)	
June	6	(8.2%)	13	(11.5%)	p=0,9
July	4	(5.5%)	9	(8%)	
August	4	(5.5%)	10	(8.8%)	
September	5	(6.8%)	8	(7.1%)	
October	2	(2.7%)	7	(6.2%)	
November	8	(11%)	12	(10.6%)	
December	10	(13.7%)	7	(6.2%)	
Total	73	(%100)	113	(%100)	

Table 2: The distribution of acute leukemia diagnosis times

Seasons	ALL (n, %)		AML (n, %)		p-value
Winter	25	(34.3%)	24	(21.2%)	
Spring	19	(26%)	30	(26.6%)	
Summer	14	(19.2%)	32	(24.8%)	p=0,2
Autumn	15	(20.5%)	27	(22.6%)	
Total	73	(%100)	113	(100%)	

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Ross et al. showed that ALL diagnosis is the most common in summer in the United States of America (15). In addition to the relationship between AL diagnosis frequency and season, the relationship between influenza epidemic and AL diagnosis was researched. It was shown that there were more ALL diagnosis during influenza epidemics. However, an increase in AML diagnosis was not found during the influenza epidemic. Eatough et al. found out that AML diagnosis is the most common in February and March in England (16). Calip et al. found that AML diagnosis was the most common in winter in various regions of the United States of America (17). In a study, it was reported that AML was diagnosed with the most common in September and October in Pakistan (18). Drapkin et al showed that the incidence of de novo AML increased between October and November (19). In a study including 833 AL patients in Mexico, no relationship between AL diagnosis frequency and the season was found (20). In the study conducted by Eren et al. in Istanbul, the frequency of acute leukemia diagnosis was the most common (13%) in August and the least in June (3.7%). In their study, all ALs were diagnosed with the following percentages with respect to seasons: 24.1% in winter, 24.7% in spring, 24.7% in summer, and 26.5% in autumn. They found no statistically significant relationship between the diagnosis of AL and seasons (21).

In our study, we found that ALL patients had their diagnoses in the following numbers, percentages and seasons: 25 (34.3%) in winter, 19 (26%) in spring, 15 (20.5%) in autumn, and 14 (19.2%) in summer. We also found that AML patients had their diagnoses in the following numbers, percentages and seasons: 24 (21.2%) in winter, 30 (26.6%) in spring, 27 (23.8%) in autumn, and 32 (28.4%) in summer. No relationship was observed between seasons and both ALL and AML.

Conclusion

In conclusion, among the studies examining the relationship between AL and seasons in various geographical regions of the world, some studies found a relationship between AL diagnosis frequency and seasons whereas some other did not reveal such a relationship. According to our literature review, there are two studies including our study, searching for a relationship between AL diagnosis frequency and seasons in Turkey. Neither of the studies found a relationship between AL and seasons. The number of the patients in this study is limited therefore studies with high number of patients are needed to find out a relation between seasons and diagnosis time of AL.

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Conflict of interest: The authors declare that they have no conflict of interest.

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