CDK4/6 Inhibitor-Associated Mean Corpuscular Volume Change: A Potential Parameter for Predicting Survival in Metastatic Breast Cancer?

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ABSTRACT

Objective: To evaluate the impact of CDK4/6 inhibitors on erythrocyte mean corpuscular volume (MCV) change and its possible correlation with progression-free survival (PFS) and overall survival (OS).

Study Design: Observational study.

Place and Duration of the Study: Department of Medical Oncology, Kahramanmaras Necip Fazil City Hospital, Kahramanmaras, Turkiye, between January 2020 and 2023.

Methodology: The data of 74 patients with HR (+) HER2 (-) metastatic breast cancer were analysed retrospectively. MCV and other complete blood count metrics were noted before and after the treatment. The first post-treatment evaluation was performed at three months. The median ΔMCV values at the third month after treatment-baseline were calculated.

Results: The patients were all females, with a median age of 55 years (between 35 and 80). Prior to the therapy, the baseline median MCV level was 90.4 (min-max: 77.3-113.2). After three months, the median MCV level was 95 (min-max: 84.3-115.3). Moreover, 7.15 was the median ΔMCV level. Regarding PFS (16.53 *vs.* 15.26 months) (p = 0.13) and OS (21.46 *vs*. 17.83 months) (p = 0.08), there was no statistically significant difference seen between the group with ΔMCV ≥7.15 and the group with ΔMCV <7.15.

Conclusion: CDK4/6 inhibitors led to an increase in MCV but there was no significant difference between PFS or OS and the increase in MCV. To figure out whether the rise in MCV represents a prognostic or predictive marker, further research is required.

Key Words: *Breast cancer, CDK4/6 inhibitors, Mean corpuscular volume, Prognosis.*

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INTRODUCTION

Globally, breast cancer continues to be the primary cause of cancer-related fatalities. Pharmacological treatment of metastatic breast cancer is typically selected based on the molecular characteristics of the tumour. Among the agents used are hormone therapy, chemotherapy, HER2-targeted therapy, antidrug conjugates, tyrosine kinase inhibitors, cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors, mTOR inhibitors, phosphatidylinositol 3-kinase (PI3K) inhibitors.[1] Overall survival (OS) and progression-free survival (PFS) have improved with the introduction of new-generation medicines in metastatic breast cancer.[2] Predictive factors are also needed to identify patients who may benefit from this class of drugs.

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CDK4/6 inhibitors are next-generation anti-cancer agents that show their anti-tumour effects by preventing the cell cycle transition from the G1 to the S phase. There are three internationally-approved drugs in this group with a similar mechanism of action: Abemaciclib, palbociclib, and ribociclib. In conjunction with hormone treatment, these drugs have demonstrated activity against hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2-) in advanced or metastatic breast cancer.[3] Although the efficacy of these drugs appears to be similar, there are differences in safety and adverse effects. Palbociclib and ribociclib cause more myelosuppression, but abemaciclib causes more gastrointestinal damage.[4] Neutropenia is a known side effect of CDK4/6 inhibitors. The incidence of all-grade neutropenia varied from 41.3 to 80%, all-grade anaemia from 18.6 to 81.8%, and all-grade thrombocytopenia from 9 to 36.2%.[5]

Studies have shown that the use of tyrosine kinase inhibitors such as imatinib, sunitinib induces macrocytosis, and macrocytosis is predictive for OS and PFS.[6] The study also showed that macrocytosis is predictive during pemetrexed therapy for individuals with advanced non-small cell lung cancer.[7] In another study, it was determined that erythrocyte mean corpuscular volume (MCV) change after capecitabine treatment was not

associated with treatment response, PFS, and OS.[8] Studies conducted with a limited number of patients have shown that CDK4/6 inhibitors cause reversible macrocytosis.[9] However, it is not known whether it has clinical significance. This study was designed to determine the changes in MCV values during the treatment with CDK4/6 inhibitors and their possible role as predictive and prognostic factors.

METHODOLOGY

In the present study, 74 individuals' records who were monitored in the Medical Oncology Department of Kahramanmaras Necip Fazil City Hospital, Kahramanmaras, Turkiye, between January 2020 and 2023 were retrospectively examined. Patients who were pathologically diagnosed with breast carcinoma and treated with CDK4/6 inhibitors in the metastatic stage were incorporated into the research. Patients with pretreatment vitamin B12 and folic acid deficiency, hypothyroidism, and other malignancies were excluded. The patients' demographic data and molecular subtypes, pre- and post-treatment evaluation data, data related to progression, and, if the patient died, death dates were recorded. Whole blood values were recorded, including MCV, haematocrit, haemoglobin, thrombocyte count, lymphocyte and neutrophil count measured before the treatment and during the evaluation. Patient responses to the treatment were obtained from archive screening, clinical examination, and laboratory and imaging records, PFS, and OS durations of patients were calculated. PFS is the time from the start of the treatment until death or disease progression, while OS is the time from the start of the treatment until the date of death or last follow-up. HER2-low is known as an HER2 immunohistochemical expression of 1+ or 2+ without amplification by *in situ* hybridisation. Response evaluation was made in the 3rd month. ΔMCV values were calculated as (MCV values in the 3rd month of the treatment) - (initial MCV values).

Ethics Committee of the University's Medical Faculty gave its approval to the project (Approval no: 2023/193, Dated: 04.12.2023). Ethical principles laid down in the Declaration of Helsinki were observed. Statistical analysis was performed using SPSS 24 Programme. The study data were summarised using descriptive statistics. In the case of descriptive statistics, frequency and number were displayed. Quantitative data were expressed as mean and standard deviation. In quantitative data analysis, when comparing regularly distributed data between groups, the Student's t-test was employed. Furthermore, when comparing data that did not exhibit normal distribution between groups, measurement of skewness and kurtosis was used. OS and PFS were computed using the Kaplan-Meier technique. A 95% confidence interval and a $p < 0.05$ significance level were used to assess the results.

RESULTS

All patients were females, with a median age of 55 years varying between 35 and 80 years. Distribution of clinical data of the patients is summarised in Table I. Fifty-one (68.9%) of the patients had luminal A type and 81.1% were using ribociclib as a CDK4/6 inhibitor, while 73% were using letrozole as a hormone drug. The majority of the patients were postmenopausal (81.1%). Moreover, 64.9% of the patients were using CDK4/6 inhibitors in the first stage. While the baseline median MCV level was 90.4 (min-max: 77.3-113.2) before the treatment, the median MCV level in the 3rd month was determined to be 95 (min-max: 84.3-115.3). While the pretreatment-baseline median Hb level was 12.45 (min-max: 8.3-15.9), this value was 11.55 (min-max: 8.9-14.4) in the 3rd month. While pretreatment-baseline platelets were $230x10^3$ (min-max: 103×10^3 - 656×10^3), platelets examined in the 3rd month were determined as 211×10^3 (min-max: 44×10^3-436×10^3).

Table I: Distribution of clinical findings for the patients.

		n	%
Bone metastases at diagnosis	Absent	21	28.4
	Present	53	71.6
Visceral metastases at diagnosis	Absent	35	47.3
	Present	39	52.7
Brain metastases at diagnosis	Absent	70	94.5
	Present	4	5.5
Histopathology	IC-no special type	67	90.5
	Others	7	9.5
PR	Negative	2	2.8
	Positive	72	97.2
HER-2	Negative	52	70.2
	Low Positive	22	32.8
Luminal	A	51	68.9
	B	23	31.1
Metastasis status	*De novo*	43	58.1
	Recurrent	31	41.9
Treatment line for metastatic disease	1st	48	64.9
	2nd	21	28.4
	3rd ve more	5	6.8
Menopausal status	Pre	14	18.9
	Post	60	81.1
CDK4/6 inhibitor agent	Ribociclib	60	81.1
	Palbociclib	14	18.9
Hormone-drug name	Letrozole	54	73.0
	Fulvestrant	19	25.7
	Anastrozole	1	1.4
Progression	Absent	46	62.2
	Present	28	37.8
Death	Absent	61	82.4
	Present	13	17.6

IC, Invasive carcinoma; PR, Progesterone receptor; HER-2, Human epidermal growth factor receptor-2.

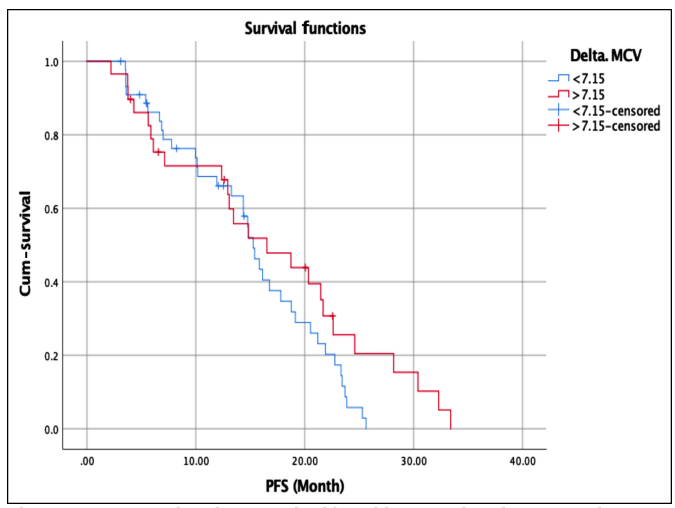

Figure 1: Progression-free survival (PFS) in ΔMCV level ≥7.15 *vs.* in ΔMCV level <7.15.

Table II: Evaluation of clinical measurements according to ΔMCV classification.

	ΔMCV				[b]p-value
	<7.15		>7.15		
	Mean ± SD	Min-Max (median)	Mean ± SD	Min-Max (median)	
Age	55.33 ± 13.05	35 - 80 (54)	54.45 ± 7.26	41 - 69 (56)	0.740
Basal HGB	12.23 ± 1.5	8.3 - 15.9 (12.3)	12.33 ± 1.44	9 - 15.6 (12.6)	0.780
Basal PLT	235.89 ± 88.13	103 - 656 (221)	258.14 ± 72.11	157 - 419 (239)	0.260
3rd-month HGB	11.73 ± 1.48	9.1 - 14.4 (11.7)	11.34 ± 1.27	8.9 - 14.2 (11.2)	0.225
3rd-month PLT	224.62 ± 83.44	44 - 436 (213)	228.31 ± 74.59	109 - 387 (210)	0.847

[b]*Independent-sample T; MCV, Mean corpuscular volume; HGB, Haemoglobin; PLT, Platelet.*

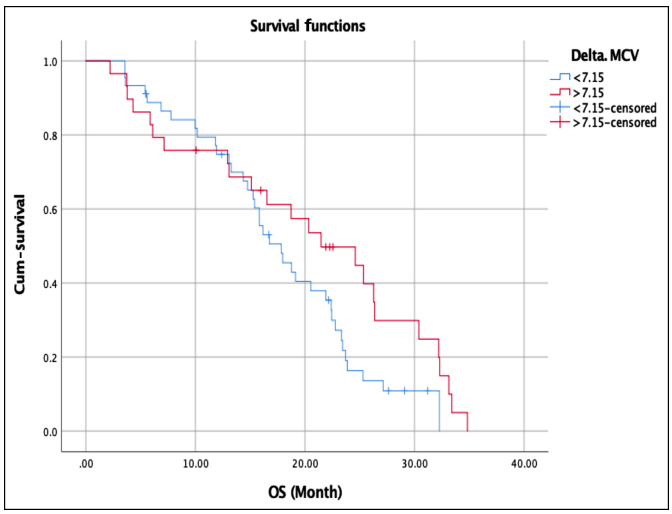

Figure 2: Overall survival (OS) in ΔMCV level ≥7.15 *vs.* in ΔMCV level <7.15.

Evaluation of clinical measurements according to ΔMCV classification is presented in Table II. The median ΔMCV level (values at the third month after treatment-baseline) was 7.15. The group with ΔMCV level <7.15 had a PFS of 15.26 months (95% CI, 3.1-25.63), whereas the group with ΔMCV level ≥7.15 had a PFS of 16.53 months (95% CI, 2.2-34.83) (p = 0.13, Figure 1). While OS was determined to be 17.83 months (95% CI, 3.57-32.27) in the group with ΔMCV level <7.15, it was 21.46 months (95% CI, 2.2-33.4) in ΔMCV level ≥7.15 group (p = 0.08, Figure 2). According to ΔMCV classification (<7.15 and ≥7.15), no statistically significant differences were determined between bone metastasis, visceral metastasis, and brain metastasis incidence rates at diagnosis, between histology results, between progesterone receptor status, between at what stage it is used, and between drug types (p >0.05).

DISCUSSION

In this study, it was determined whether MCV values changed during the treatment in patients with HR (+) HER2 (-) metastatic breast cancer treated with CDK4/6 inhibitors, and to find out whether this would be predictive or prognostic. An increase was determined in the MCV values of all the patients during the treatment. However, no significant relationship was determined between this increase and OS and PFS.

Breast cancers are divided into various groups molecularly according to the hormone receptor and HER2 status.[10] HR-positive / HR-negative is the most prevalent cancer type, and endocrine treatment forms the therapeutic backbone of the treatment of this cancer. While anti-oestrogen treatments are effective in the beginning, approximately 50% of oestrogen receptor (ER) positive patients develop resistance against endocrine treatment throughout their lives, and in the end, the disease recurs, and limited clinical benefits are achieved.[11]

Using CDK4/6 inhibitors is now integrated with the care of ER-positive / HER2-negative breast cancers,[12] and their addition to ET has significantly improved the results of ER-positive progressed breast cancer patients compared to antioestrogens alone.[13] CDK4/6 kinases link cyclin proteins during the change in cell cycle phases from G1 to S. Retinoblastoma proteins (RB) are phosphorylated by the cyclin D-CDK4/6 complex, which also sequesters them from E2F transcription factors that drive the cell cycle forward.[14-16] CDK4/6 inhibitors selectively inhibit downflow CDK4/6 mediated Rb phosphorylation, and cause cell cycle to stop in the G0 / G1 phase.[17,18]

The literature only contains a small number of research assessing the connection between MCV alteration and CDK4/6 inhibitors. In one study, 81 patients with metastatic breast cancer who were taking CDK4/6 inhibitor were examined, and time-related MCV increase with CDK4/6 inhibitor treatment was demonstrated. It was proposed that there might be a relationship between MCV increase and time-to-treatment failure (TTF). In that study, evaluation was made by calculating DMCV.[19] In the present study, baseline MCV, 3rd month MCV, and DMCV were calculated. The median ΔMCV level in the study was 7.15. The patients were split into two groups based on ΔMCV ≥7.15 and MCV <7.15, and there was no statistically significant difference in OS or PFS between the two groups (p = 0.08 and p = 0.13, respectively). In the second study, Anampa *et al*. evaluated three palbociclib-using patients with metastatic breast cancer, and as a result of palbociclib use, reversible macrocytosis and dysplastic haematopoiesis that mimicked MSD were reported.[20] In that study, B12 and folate deficiency, liver failure, or hypothyroidism which are prevalent causes of macrocytosis were not determined. MCV-increase observed in all patients was interpreted as a potential *in vivo* pharmacodynamic biomarker of CDK4/6 inhibition in metastatic breast cancer patients who used palbociclib.[20] Kamboj *et al.* assessed six patients who were receiving hormone therapy together with a CDK4/6 inhibitor.[9]

Dates of initiation of CDK4/6 inhibitor, beginning of macrocytosis, maximum mean corpuscular volume, and MCV normalisation in the absence of CDK4/6 inhibitor (reversibility of macrocytosis) were noted. There was increased MCV observed in all patients 4-6 weeks after the start of CDK4/6 inhibitor. In three patients in whom CDK4/6 inhibitor treatment was suspended due to health-related problems, MCV was reversible. Taking into account that reversible macrocytic anaemia with unclear clinical relevance was caused by CDK4/6 inhibition, and there was an increase in MCV in all patients along with radiological remission, it was interpreted that there was a potential causal relationship between high MCV and treatment response.[9] No significant correlation was determined between MCV elevation observed in each patient, PFS and OS.

The two main causes of macrocytosis are deficiencies in the folic acid and vitamin B12. The levels of these two vitamins were found to be in normal ranges before and during the treatment. This study's patients did not have any of the other causes of macrocytosis, such as alcohol consumption, chronic liver disease, aplastic anaemia, or myelodysplastic syndrome.

The study had certain limitations. First, the data were collected retrospectively, and there was a heterogeneous patient group with a small number of patients in the subgroups. The difference in the order of CDK4/6 inhibitors' use, some being used in the 1st stage and others being used in the later stages, limited the generalisability of the results. Nevertheless, it is thought that the results obtained will guide future studies.

CONCLUSION

It was determined that all patients' MCV values increased during the treatment with CDK4/6 inhibitors. Nevertheless, no statistically significant correlation was found between the changes in MCV, PFS, and OS. To use changes in MCV induced by CDK4/6 inhibitor as a marker for improvement in PFS and OS, larger prospective studies are needed.

ETHICAL APPROVAL:

Ethics Committee of the University's Medical Faculty gave its approval to the project (Approval no: 2023/193, Dated: 04.12.2023), and ethical principles laid down in the Declaration of Helsinki were observed.

PATIENTS' CONSENT:

Due to the retrospective nature of the study, informed consent of the patients was not required.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

AY: Study design, data acquisition, statistical analysis, and interpretation of the data.
MED: Draft of the work and critical revision for the important intellectual content.
EF: Final approval of the manuscript.
All authors approved the final version of the manuscript to be published.

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