Prognostic Value of Vitamin D Receptor in Patients with Metastatic Colorectal Cancer Received Irinotecan-Based Systemic Treatment as a Second Line

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Abstract

Background: Metastatic colorectal cancer (mCRC) constitutes one of the fatal worldwide neoplasms. Despite different modalities utilized in treatment, some patients progressed, raising the search for new predictive and prognostic markers. Vitamin D receptor (VDR) has different expression degrees in many cancers including those of colorectum and has a crucial role in the pathogenesis of intestinal neoplasm through different signaling pathways.

Objectives: The study aims to analyze the relation between VDR expression in mCRC patients receiving irinotecan-based systemic therapy, clinicopathological features, therapy response, and patient survival.

Patients and Methods: This study included 53 mCRC patients, their demographic data, clinicopathological features of their tumors, therapy response, and survival outcome with different IHC VDR expressions were analyzed.

Results: We noticed that the patients' mean age is 42(±14.6) years with 28/53 patients (52.8%) younger than 45 years**.** Of the studied patients 32/53 (60.4%) were diagnosed with stage IV and the other 21/53 patients with stage II and III before developing secondary metastases. Wild KRAS was more common in our patients 37/53 (69.8%).VDR expression was positive in 34/53 (64.2%) and negative in 19/53 (35.8%). Patients with positive VDR expression are associated with a significant reduction in duration of response by 8 months and progression-free survival by 5 months than those with negative expression, but no correlation with overall survival.

Conclusion: Positive VDR expression could be a poor predictive factor in mCRC patients treated with irinotecan-based therapy and may be incorporated into the predictive and prognostic mCRC panel. This mandate further studies with large sample size.

Keywords: VDR; mCRC; Irinotecan; Predictive.
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Introduction

Colorectal cancer (CRC) continues to be one of the considerable health burdens, it is the third most common cancer worldwide and the second leading cause of death after lung cancer **(Siegel et al.,2023).** It constitutes 7.4% of total cancers diagnosed in North Africa and the Middle East **(Kassem et al., 2019).** About 15-30% of the newly diagnosed cases had synchronous metastases and 25-50% of those diagnosed with locally advanced disease will develop metachronous metastases **(Cervantes et al., 2023).** It was reported that the relative 5 years overall survival is about 15% in metastatic colorectal cancer (mCRC) **(CDC,2022).**

Factors involved in the pathogenesis of CRC are miscellaneous and heterogeneous including lifestyle and dietary factors as well as in conjunction with inherited and acquired genetic mutation **(Fearon et al., 2011).** This results in the development of heterogeneous disease with distinct tumor behavior, pathologic features, and therapy response **(Dienstmann et al., 2017).**

Studying the treatment option in mCRC is very complex due to distinct patient populations regarding various molecular markers and location of the primary tumor. Therapy of mCRC includes classical chemotherapy as 5-fluorouracil (5-FU) which is the main drug in many therapy protocols and can be used alone or in conjunction with others as irinotecan or oxaliplatin and targeted therapy according to patient profile **(Baran et al.,2018)**.

Doublet chemotherapy with FOLFIRI (irinotecan+5-FU+leucovorin) constitutes one of the essential treatment protocols for mCRC that prolong survival in both first- and second-line settings as was reported in many trials **(Gil-Delgado et al., 2001).** The addition of biological therapy such as anti-epidermal growth factor receptors (EGFR) monoclonal antibodies as panitumumab or cetuximab or anti-vascular endothelial growth factors (VEGF) monoclonal antibodies as bevacizumab or aflibercept to chemotherapy resulted in the improvement of the patient's survival according to the molecular profile of the patients and tumor sidedness as was reported by many trials. The phase III VELOUR study reported prolongation of overall survival (OS) and progression-free survival (PFS) in those treated with anti-VEGF as a second line in combination with FOLFIRI **(Fernández et al.,2019).** Another phase III trial showed improvement in response rate to treatment with panitumumab in addition to FOLFIRI and also improvement of PFS in mCRC patients receiving anti-EGFR with chemotherapy as second-line **(Peeters et al.,2010).**

Vitamin D receptor is present in normal tissue as well as cancerous tissue, and there are many studies explaining the role of VDR in many cancers such as breast, pancreatic, brain cancer, and melanoma, with higher concentrations of VDR in intestinal epithelial cells. Growing evidence about the association between the altered VDR expression (either overexpression or repression) in tumor tissue and tissue-type dependent variation in the signaling of calcitriol **(Friedrich et al.,2003).**

Some studies reported that CRC is the most frequent cancer related to vitamin D deficiency **(Ferrer-Mayorga et al.,2017).** VDR is one of the nuclear superfamily receptors that is displayed in epithelial and mesenchymal cells and transfers the biological function of calcitriol (an active form of vitamin D) such as cellular differentiation, proliferation, metastases formation, angiogenesis as well as cancer signaling pathways **(Bandera et al.,2017).**

Many studies illustrated that calcitriol impedes the proliferation and induces the

differentiation of VDR-expressing intestinal epithelial cells through multilevel inhibition of Wnt/ β-catenin signaling pathways (**Klampfer, 2014).**

However, studies about the role of VDR in mCRC are controversial as study by **Wang et al . (2019)** noted that low VDR expression was closely related to chemotherapy sensitivity**,** while another study reported by **Ferrer-Mayorga et al. (2016)** reported that high VDR expression in tumoral tissue is linked to prolongation of patients survival (PFS and OS)**.**

The current study intended to analyze the role of VDR expression in patients with mCRC treated with irinotecan-based systemic therapy and assessed its association with clinicopathological features, therapy response, and patients survival.

Patients and methods

This study was authorized by the Institutional Review Board and Ethical Committee on 20th June 2021, under IRB approval No: 544. Inclusion criteria were patients age were more than 18 years old, both sexes involved, patients who had established histopathological diagnosis of CRC, diagnosis of metastases either radiologically or proven biopsy, patients treated with irinotecan-based systemic therapy as a second line for at least 2 cycles and assessment of the disease was done for at least once. Exclusion criteria are patients diagnosed with double malignancy and those who received only one cycle or missed the assessment. Applying these criteria, a total of 53 patients admitted at South Egypt Cancer Institute, Assiut University were incorporated into the study. Demographic and clinical data, and pathological features of the tumors, therapy responses, and survival were collected. The cut-off date for our data collection is February 28, 2023.

Treatment protocols

It was found that all patients in the study were treated with irinotecan-based chemotherapy as a second line and only thirty-one of them received biological therapy. Twenty-one patients received anti-EGFR and ten patients received anti-VEGF when applicable and available. All patients continued to receive the treatment protocol until the progression of the disease or un-tolerated toxicity. These protocols were used. FOLFIRI protocol (irinotecan 180 mg/m2 IV over 30–90 minutes on day 1, leucovorin 400 mg/m2 IV infusion on day 1, 5-FU 400 mg/m2 IV bolus on day 1, followed by 1200 mg/m2/day for 2 days by continuous infusion and repeated every 2 weeks). CAPIRI protocol (irinotecan 200 mg/m2 IV over 30–90 minutes on day 1 and capecitabine 1000 mg/m2 twice daily PO for 14 days, to be repeated every 3 weeks) **(André et al.,1999).** Cetuximab 500 mg/m2 IV over 2 hours, day 1, every 2 weeks. Panitumumab 6 mg/kg IV over 60 minutes, day 1, repeat every 2 weeks . Bevacizumab 5 mg/kg IV, day 1, repeat every 2 weeks **(Heinemann et al.,2014)**.

Immunohistochemistry (IHC)

IHC for formalin-fixed paraffin-embedded tissue (FFPET) was done utilizing USA Bioss Inc protocol, where they were sliced into sections of 3-micron thickness and then deparaffinized and rehydrated via diluted alcohol and distilled water. Then tissue sections were immersed into Coplin jars filled with Tris EDTA in the heating water bath at 90 for 45 minutes, after application tissue section were incubated into hydrogen peroxide for 10-15 minutes . Lastly, Ultra V block was added for 5 minutes and incubated at room temperature **(Dettmeyer. 2011).**

Primary rabbit polyclonal anti-VDR antibody (Catalog bs-2987R, Bioss Inc, USA) was added at 1:200 and incubated overnight at 4˚C in a humid chamber then washed for two or three times in phosphate buffer solution. Then IHC was done using secondary antibody of universal staining kit

"Ultra Vision Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use) (BIOCYC Gesellschaft für Biotechnologie, Kosmetik und Recyclingverfahren mbH & Co. Entwicklungs KG Am Mühlenberg 11, 14476 Potsdam, Germany) using the instructions of manufacturer.

Anti-mouse/rabbit polymer-HRP was applied in enough amount to completely cover the sections for 30 minutes, then tissue sections were rinsed and washed in PBS for two times. Diaminobenzidine (DAB solution) chromogen was added for 5 minutes and then tissue section were washed in distilled water. Tissue sections were then counterstained using Mayer's hematoxylin, washed in tap water, dehydrated in ascending grades of alcohols , cleared in Xylene, and dried in air. DPX is then added to tissue section with slippage of the cover.

Skin adnexal tissue sections were utilized as a positive control for discovering of the VDR on the nucleus or cytoplasm. The positive expression was defined as brown staining of the acinar cells cytoplasm, while sections of tissue-specific positive controls were stained using the same protocol but with omitting of the primary antibody. Evaluation of VDR expression was done by pathologists without previous knowledge of the clinicopathologic features of the lesions. The immune-stained section was examined histologically at a lower magnification (X4 and X10) to detect the positive stained cells and percentage of positive cells. The VDR positivity was recognized as brown cytoplasmic staining **(Chen et al.2016).**

Allred Score was utilized to assess the VDR expression. It combines the percentage of positive cells that takes score (A) where score 0=0%, 1<1%, 2=1-10%, 3=11-33%, 4=34-66%, and 5≥67% and the intensity of the stain in most of the examined field takes score B where no reaction=0, weak reaction=1, intermediate reaction=2, and strong reaction=3; both scores were added together (A+B) for a final score of 0-8 points **(Allred DC, et al.1998).** The optimal cutoff value of our study is classified into the following: scores of '0 to 3 were considered 'negative' while scores of '4 to 8' were considered 'positive' **(Ilić, et al.2019).**

Study endpoints : The primary endpoints were response to treatment including overall response rate, duration of response (DoR), duration of clinical benefit (DoCB), and progression-free survival (PFS). The secondary endpoint was overall survival.

Statistical analysis

Kolmogorov-Smirnov's normality test was used for assessing the normal distribution of data. Qualitative variables were illustrated as frequencies (percentages) and compared when appropriate using the chi-square test or Fisher's test, while quantitative variables were reported as mean ±SD (standard deviation or 95% confidence interval (95%CI) or median (range) according to the normality of distribution. Quantitative variables were evaluated with the use of a parametric Student's t-test or a nonparametric (Mann-Whitney U test) test, accordingly.

The reversed Kaplan–Meier method was used to calculate the median follow-up time. The survival curve was evaluated with the Kaplan-Meier methods and compared using the log-rank test. P-value (two-sided) < 0.05 was considered to have a statistically significant value. All statistical analyses were performed using SPSS version 22.

Results

VDR expression shows no correlation to the demographic and clinicopathological features of the studied patients

Our median age of the studied patients is 44 years old with a range from 19 to 80 years old, with more than half of them 28/53 (52.8%) being diagnosed younger than 45

years old while the other 25/53 (47.2%) patients are older than 45 years old. Thirty patients were females, and twenty-three patients were males. Regarding primary tumor location, colon cancer was diagnosed in twenty-five patients while the other twenty-eight patients had a rectal tumor. Synchronous metastases were diagnosed in 32/53 of the patients (60.4%), while metachronous were presented in 21/53 (39.6%) of them. Wild KRAS was predominant in the patients of the study 37/53 (69.8%) while mutated KRAS in 16/53 (30.2%) of them. VDR expression was positive in 34/53 (64.2%) of the patients, while the other nineteen patients 19/53 (35.8%) had a negative expression. There is no statistical significant correlation between VDR expression and any of the above-mentioned data. The rest of the data is listed in (**Table .1**).

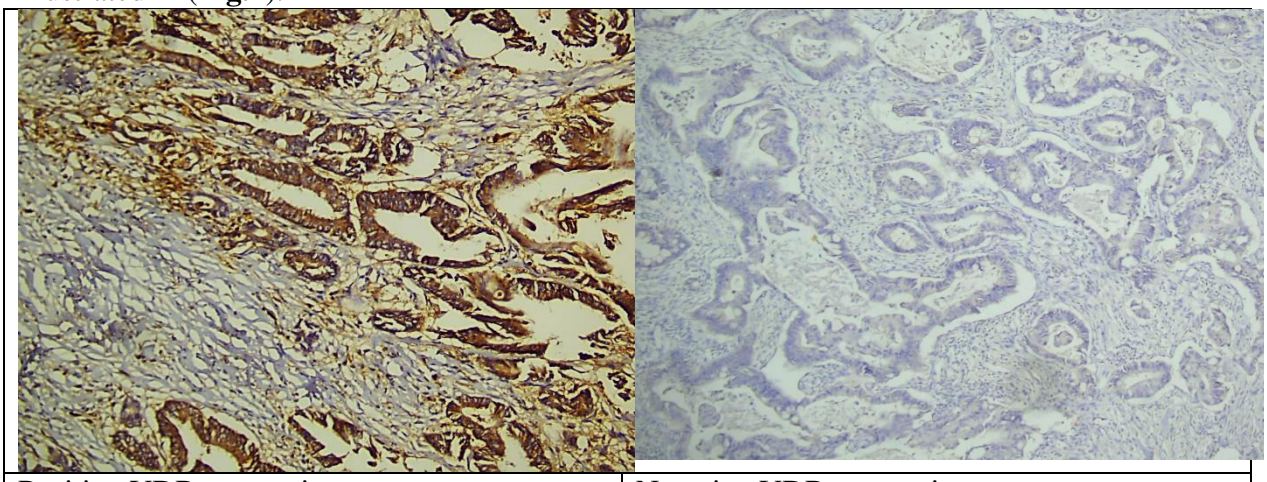
Table 1. Demographic and clinicopathological data of the studied patients (n=53)

Characteristics	Number	Percentage (%)
Age		
Mean (±SD)	42.6 (±14.6)	
95% CI	38.52-46.57	
≤45 years	28	52.8
>45years	25	47.2
Gender		
Male	23	43.4
Female	30	56.6
BMI		
Mean (±SD)	25.41 (±4.96)	
Obese	12	22.6
Non-obese	41	77.4
Tumor location		
Right colon	13	24.5
Left colon	12	22.6
Rectum	28	52.8
Metastatic status at presentation		
Synchronous metastases	32	60.4
Metachronous metastases	21	39.6
Number of metastatic sites		
≤ two sites	37	69.8
> two sites	16	30.2
Site of metastases		
Liver	20	37.7
Nodal	28	52.8
Peritoneal	27	50.9
Other sites such as lung, bone, ascites ,ovaries or local recurrence	26	49.1
Histology of the tumor		
Adenocarcinoma	33	62.3
Mucinous carcinoma	17	32.1
Signet ring carcinoma	3	5.7

Status of KRAS		
Wild	37	69.8
Mutated	16	30.2
VDR expression		
Positive	34	64.2
Negative	19	35.8
Initial staging		
Stage II	6	11.3
Stage III	15	28.3
Stage IV	32	60.4
Degree of differentiation		
Well/ moderate differentiated.	45	84.9
Poorly differentiated	8	15.1

Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation.

Positive and negative VDR expression in the studied patients is illustrated in (**Fig.1**).



Positive VDR expression | Negative VDR expression

Fig.1. Different expression VDR in the studied patients.

VDR expression and its relation to response to treatment

We have noticed that negative expression of VDR in the patients was related to a better overall response rate than those with positive expression (21.5% versus 8.8%), and those with negative expression had also a better disease control rate (52.6%) than those with positive expression (29.4%), although it is statistically insignificant (P value >0.05). Along with the previous data, the stationary course of the disease was observed more in negative expression (31.6%) than in positive expression (20.6%). Also, disease progression occurs less commonly in patients with negative expression (47.4%) than those with positive VDR expression (70.6%). In spite of these findings, it wasn't also of statistical significant. The rest of data is displayed in (**Table. 2**).

Table 2. VDR expression and response to therapy

Category	Negative VDR expression		Positive VDR expression		P value
Overall response	**No**	**%**	**No**	**%**	
Complete response	2	10.5	1	2.9	
Partial response	2	10.5	2	5.9	
Stationary disease	6	31.6	7	20.6	0.167
Progressive disease	9	47.4	24	70.6	
Total	19	35.9	34	64.1	
Objective response rate	**4**	**21.5**	**3**	**8.8**	**0.234**
Disease control rate	**10**	**52.6**	**10**	**29.4**	**0.140**

Negative VDR expression is associated with a statistically significant prolongation of duration of response around 8.4 months more than patients with positive expression with a log-rank P value of 0.01. These data are shown in (**Table .3** and **Fig.2**).

Table 3. VDR expression and Duration of response (DoR)

Category	No.of patients	No.of events	Median DoR (95%CI), months	P value
Negative VDR expression	4	3	14.0(0.0-60.5)	
Positive VDR expression	3	3	5.6(4.2-6.9)	**0.01**
Total	7	6	11.4(10.3-12.5)	

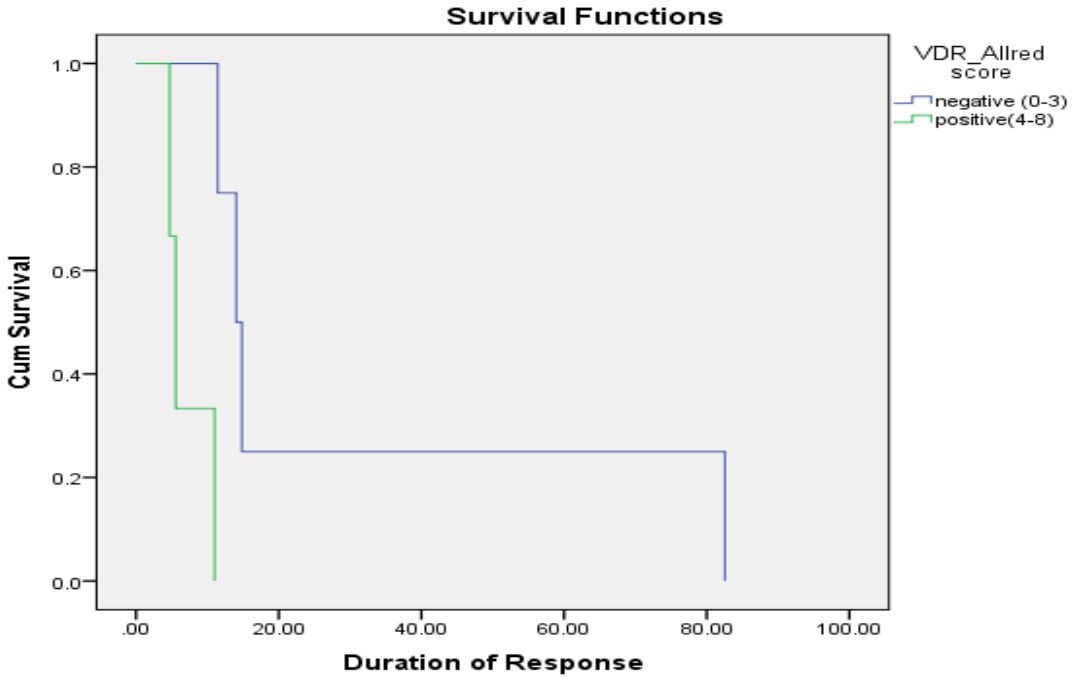


Fig.2. VDR expression and Duration of response (DoR)

The median duration of clinical benefit was 11.9 months, which was also prolonged in those with negative expression (14 months) while it was 7.1 months in those with positive expression, though it is of clinical significance, the log-rank P value is 0.32 making it statistically insignificant, as illustrated in (**Table .4**).

Table 4. VDR expression and Duration of clinical benefit (DoCB)

Category	No. of patients	No of events	Median DoCB (95%CI), months	P value
Negative VDR expression	9	6	14.02(10.91-17.15)	
Positive VDR expression	6	5	7.13(1.58-12.73)	**0.324**
Total	15	11	11.93(10.29-13.56)	

Survival data and VDR expression

The improvement in response rate in patients with negative VDR expression was reflected in significant prolongation of progression-free survival approximately 5.2 months longer than those with positive expression, log-rank P value 0.045. as described in (**Table. 5** and **Fig.3**).

Table 5. VDR expression and progression-free survival (PFS)

Category	No. of patients	No of events	Median PFS (95%CI), months	P value
Negative VDR expression	19	15	9.27(2.66-15.87)	
Positive VDR expression	34	33	4.07(3.18-4.97)	**0.045**
Total	53	48	4.73(3.85-5.61)	

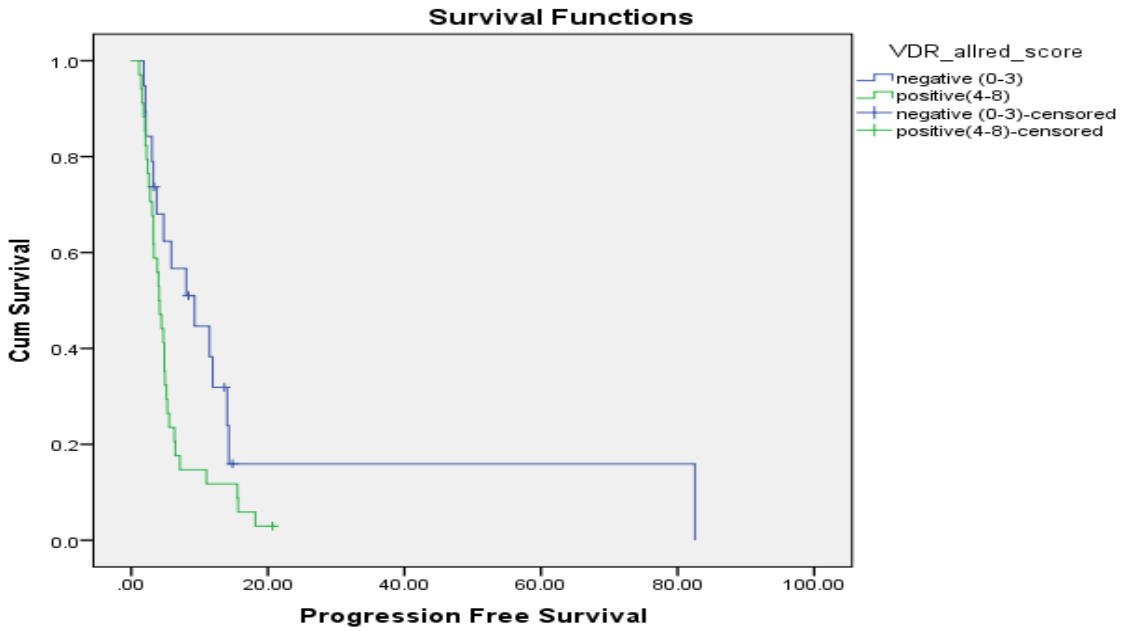


Fig. 3. VDR expression and Progression-free survival

Despite the enhanced effect seen in therapy response and progression-free survival seen in negative VDR expression patients that was matched with clinically longer overall survival, but it wasn't of statistically significance with log-rank P value 0.098. Median OS was 17.7 months, 95%CI (11.9-23.6) in patients with negative VDR expression while it was only 7 months, 95%CI (0.7-13.4). This is shown in (**Table. 6** and **Fig.4**).

Table 6. VDR expression and Overall survival

Category	No. of patients	No of events	Median OS (95%CI), months	P value
Negative VDR expression	19	13	17.74(11.90-23.58)	
Positive VDR expression	34	25	7.03(0.74-13.37)	**0.098**
Total	53	38	11.49(5.59-17.40)	

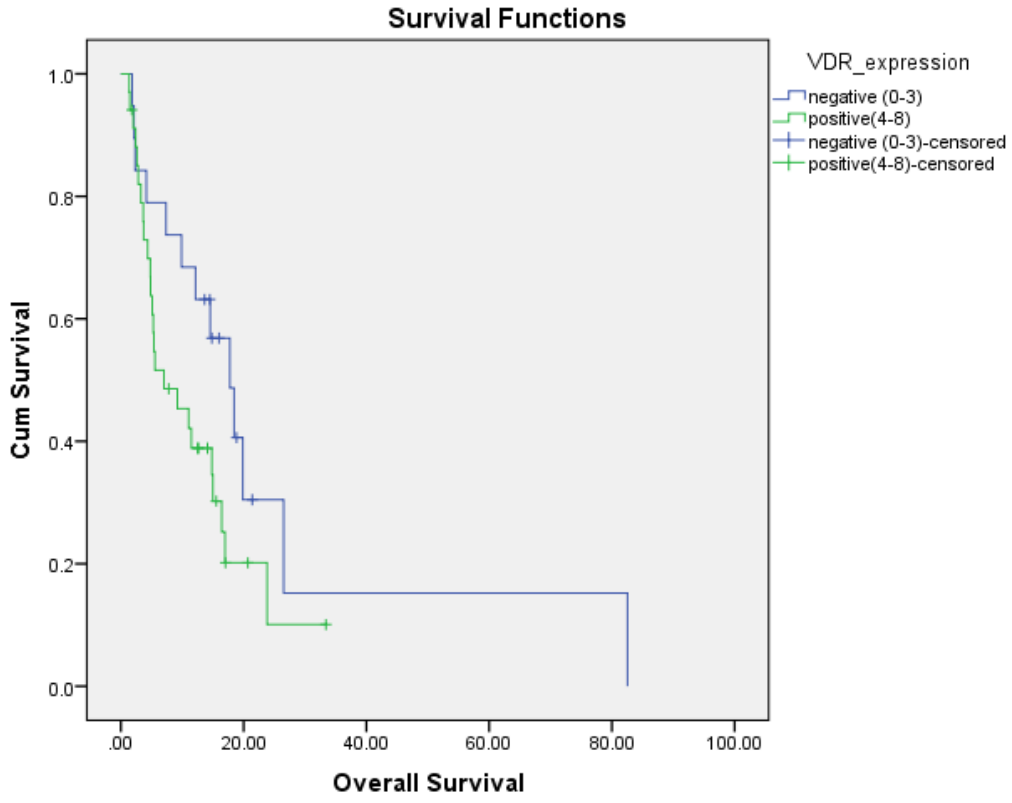


Fig.4. VDR expression and overall survival.

Discussion

Metastatic colorectal cancer stands as one of the most devastating worldwide neoplasms, with discouraging outcomes and 5 year-survival is almost 14%. Whilst early CRC can be completely cured, mCRC cannot be cured completely because of the heavy load of the metastatic cells that might include therapy-resistant cells **(Shin et al.,2023)**. Several research had illustrated the role of VDR in several cancer type including those of CRC and its incorporation in intestinal

tumor pathogenesis **(Ferrer-Mayorga et al.,2017)**, though the predictive or prognostic role of VDR in CRC is still a controversial matter.

VDR expression is positive in thirty-four patients (64.2%) and negative in nineteen patients (35.8%). There was no statistically significant relation between age, sex, BMI, primary tumor location or site of metastases, stage of the disease, degree of differentiation or KRAS status, and VDR expression. Our result is paralleled to the result of research by **Kure et al. (2009)** who described that VDR expression had no association with any of clinicopathological features except for KRAS **.** Whilst it is diverse from study result by **Yu et al. (2023)** who describes that high VDR expression is more common in early stage, low depth of invasion, or number of lymph node metastases**.** This may be explained by different population studies, methods of IHC of VDR expression, or another genetic factor.

In the aspect of therapy response, we have detected that negative VDR expression is linked with clinical increase in objective response rate (ORR) and disease control rate (DCR) rate (21.5% and 52.6% respectively) than those with positive expression which showed ORR of 8.82% and DCR of 29.4%, this isn't statistically significant with log-rank P value more than 0.05. However, this translated into a statistically significant benefit in duration of response (DoR) that was prolonged in those with negative VDR expression by around 8 months than in those with positive expression, with log-rank P value 0.01. Additionally, progression-free survival (PFS) displayed significant correlation with VDR expression with a log-rank P value of 0.045, as patients having negative expression had PFS of 9.3 months, while those with positive expression have PFS of 4 months. Our results are coincident with study results done by Wang H, et al.,(2019) who reported that low VDR expression might be associated with chemo-sensitivity due to different genetic alteration**.**

Lastly, this change in DOR or PFS reflects a clinical overall survival benefit but is not statistically significant with a log-rank P value of 0.098. There is clinical prolongation in OS in those with negative expression with approximately 10 months longer than those with positive expression. Our results regarding survival were different from those of **Shi, et al. (2020)** who reported that low VDR expression was associated with poor survival outcomes . Our results were matched with that of **Kure et al.(2009)** who showed that VDR expression wasn't associate with survival outcome of the patients **.** This difference in survival outcome and therapy response may be explained by different mutations in VDR genes in different population studies **(Wang et al., 2019).**

One limitation to our study is small sample size so we recommend a further study with large sample size.

Conclusion

Positive VDR expression could be a poor predictive factor in mCRC patients treated with irinotecan-based systemic therapy, and it may be incorporated into the predictive and prognostic Pannel of mCRC. This mandate further studies with large sample size.

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