The Egyptian Journal of Hospital Medicine (October 2024) Vol. 97, Page 4283-4292

Prognostic Value of Erythropoietin-Producing Hepatocellular Receptor A2 (Epha2) Serum Marker in Colorectal Cancer

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ABSTRACT

Background: One of the biggest health issues in the globe is colorectal cancer (CRC). The third most common type of cancer to be diagnosed is colon cancer. Despite the many treatment approaches, there is a need to find novel prognostic and predictive indicators. EphA2, erythropoietin-producing hepatocellular receptor A2, is expressed to varying degrees in a variety of malignancies, including colorectal cancer, and may serve as a prognostic indicator and stage-specific marker.

Objectives: To analyze the serum marker EphA2 level expression by enzyme-linked immune-sorbent assay (ELISA) in patients with CRC and assess its association with clinicopathological features, and patient survival.

Patients and Methods: This study is a prospective study that included (80 cases) diagnosed with either cancer colon or rectal cancer, we selected patients who were diagnosed with primary colorectal cancer confirmed by histopathological diagnosis.

Results: The patients in our study, 44 / 80 (55%) were diagnosed at stage IV, while the remaining 36 patients (45%) were diagnosed at stages II and III. Wild-type KRAS was present in 24 /41 patients (58.8%). The median overall survival (OS) for our patients was 21.3 months. We found a significant positive correlation between EphA2 levels and carcinoembryonic antigen (CEA) levels, with a P-value of 0.001. Additionally, EphA2 showed a highly significant relationship with the disease stage; higher EphA2 levels were associated with more advanced stages of the disease. While KRAS did not correlate with varying levels of EphA2, a correlation was observed between EphA2 levels and OS. Specifically, high EphA2 levels were associated with worse survival compared to the low EphA2 levels, with a P-value of 0.034.

Conclusion: EphA2 serum level could be diagnostic and prognostic in CRC patients. It might be included in the CRC panel for prediction and prognosis. This needs more studies with larger sample numbers.

Keywords: EphA2; CRC; OS, Prognostic.

INTRODUCTION

CRC is a major public health problem, ranking as the third most frequent disease worldwide and the second biggest cause of cancer-related deaths after lung cancer [1]. It is responsible for around 7.4% of all cancer cases detected in North Africa and the Middle East [2]. In Egypt, the incidence of CRC represents around 6.1% of all cancer cases and contributes to 3.8% of cancer-related mortality [3].

The factors contributing to the pathogenesis of CRC are diverse and complex, including lifestyle and dietary choices, as well as inherited and acquired genetic mutations [4]. The treatment option for CRC is very complex due to distinct patient populations regarding the stage of disease, different molecular markers, and location of the primary tumor for metastatic CRC. EphA2 is one of the ephrin family and represents the largest group within the receptor tyrosine kinase (RTK) families. By interacting with the membrane-bound ephrin ligands, the Eph/ephrin system regulates various physiological and pathological processes, during development and after birth, including the immune response, angiogenesis, inflammation, atherosclerosis, and cancer through cell-to-cell communication and bidirectional signaling [5].

EphA2 is essential for the development of the brain and blood vessels during embryogenesis. Numerous malignancies and tumor cell lines frequently overexpress Eph and its ligands. It drives carcinogenesis in both traditional and non-classical ways. In the classical manner, EphA2 suppresses carcinogenesis by blocking ligand and RTK positive signaling. Cell motility and survival are impacted by inhibiting extracellular regulated protein kinases (ERK), protein kinase B (PKB), and focal adhesion kinase (FAK). EphA2 ligands and RTK non-dependent activation and phosphorylation are referred to as the non-classical pathway. Phosphorylation of EphA2 is induced by inflammatory cytokines and growth factors through ribosomal S6 kinase (RSK), serine/threonine protein kinase (AKT), and protein kinase A (PKA). EphA2 may be localized near the edge of moving cells as a result of this phosphorylation, which may promote and sustain certain cancer cell behaviors such cell motility and proliferation by causing the assembly of the actin cytoskeleton framework and the development of lamellipodia membranes [6].

Research has demonstrated that constitutive EphA2 tyrosine phosphorylation is low, the expression of this protein is high in cancer cells, and in some contexts, the pro-oncogenic MAPK/ERK and PI3K/Akt signaling pathways are attenuated when canonical signaling is stimulated [7]. Genetic studies, however, provide evidence for a pro-tumorigenic function of tyrosine phosphorylated EphA2 in certain cancer settings [8]. EphA2 and tumor cell apoptosis are tightly associated. Furthermore, it may be possible to successfully induce apoptosis, lower melanoma cell viability, and stop tumorigenic development by blocking the expression of the EphA2 gene.

Received: 03/07/2024
Accepted: 03/09/2024

Consequently, EphA2 may be a good target for cancers [9].

Recently, the involvement of EphA2 in CRC has been studied intensively. EphA2 is abnormally expressed in various malignant tumors, e.g. glioblastoma, CRC and breast cancer [10]. Several studies have observed high-level expression of EphA2 at different stages of CRC especially in stage II/III CRC, EphA2 expression is a marker of poor patient prognosis [11]. EphA2 signaling is often active in cancer cells and is reported to exert pro-tumorigenic functions, particularly increased invasiveness, and metastasis in cancer cells [12]. The current study intended to analyze the serum marker EphA2 level expression in patients with CRC and assess its association with clinicopathological features, and patient survival.

PATIENTS AND METHODS

This study is a prospective study that included (80 cases) diagnosed with either cancer colon or rectal cancer, we selected patients who were diagnosed with primary colorectal cancer confirmed by histopathological diagnosis. Our study started in May 2022 and continued until October 2022.

Inclusion criteria: Patients' ages were more than 18 years old, both sexes were involved, and patients had established histopathological diagnosis of CRC.

Exclusion criteria: Patients diagnosed with double malignancy, those with incomplete data.

Applying these criteria, 80 patients admitted at South Egypt Cancer Institute, Assiut University were incorporated into the study. Demographic, clinical data, pathological features of the tumors, therapy responses, and survival were recorded. These data were collected and analyzed to find out its relation to EphA2. The cut-off date for our data collection was May 31, 2024.

Sample Collection:

After informed consent, 3-mL blood samples from CRC patients were taken and centrifuged for 20 minutes at 1000g. the serum samples were kept at -80°C until usage.

Analysis Method:

All samples were centrifuged and kept at -80°C until testing. Serum levels of the EphA2 receptor were assessed utilizing a sandwich ELISA technique (Catalog No: EH3008, FineTest. Detection range: 78.125-5000 pg/mL, China).

Ethical approval:

The Institutional Review Board and Ethical Committee South Egypt Cancer Institute, Assiut University approved this study in July 2022, under IRB [Approval No: 544]. After receiving all of the information, all the participants signed their permission. The Helsinki Declaration was followed throughout the course of the investigation.

Survival analysis:

- Survival data of the patients were obtained by reviewing the files of rectosigmoid cancer patients attending to South Egypt Cancer Institute in the period between May 2022 - May 2024.
- Overall survival was determined from randomization to death due to any cause or last follow-up, whichever came first. Any patients who lost follow-up or were still alive at the time of study cut-off are censored [13].

Statistical analysis

The statistical software IBM SPSS version 20.0 was used for all calculations. The quantitative data were presented as mean± SD and the median (range), whilst the qualitative data were presented as percentages and frequencies. When comparing non-normally distributed quantitative variables between two groups, the Mann-Whitney test was employed; when comparing more than two groups, the Kruskal-Wallis test was employed. The X^2-test was used for categorical data, and Fisher's Exact test was used when anticipated frequencies were less than 5. While Cox regression assessed factors influencing OS. The ROC curve plotted sensitivity against specificity to evaluate diagnostic performance, with an area under the curve above 50% indicating acceptable performance. Survival curves for patients for calculation of overall survival (OS) were conducted by using the Kaplan-Meier method. A p-value was considered significant at 0.05.

RESULTS

36 cases were diagnosed with stages II and III. Stage II represented 21.3% of all cases. Among those 44 cases with metastatic disease, mutant KRAS was identified in 17/44 cases, and 24/44 of cases were wild type KRAS representing 54.5% of metastatic cases

The ROC curve was used to differentiate between early-stage and metastatic-stage cancer. An EphA2 level greater than 1534.82 ng/mL demonstrated high sensitivity and specificity. The AUC curve for EphA2 was 0.904. In comparison, carcinoembryonic antigen (CEA) with a cut-off greater than 3.9 ng/mL also showed strong sensitivity and specificity, with an AUC of 0.872. There was a positive correlation between EphA2 and CEA levels; as CEA levels increase, EphA2 levels also rise, which was statistically significant. This is demonstrated in **Table 1** and **Figure 1.**

Table (1): Diagnostic performance for CEA and EphA2 to discriminate metastatic stage (n = 44) from early stage (n = 36).

	AUC	P	95% C.I	Cut-off ng/mL	Sensitivity	Specificity	PPV	NPV
CEA	0.872	<0.001*	0.791 – 0.952	>3.9	84.09	75.0	80.4	79.4
EphA2	0.904	<0.001*	0.839 – 0.969	>1534.82	88.64	75.0	81.2	84.4

AUC: Area Under a Curve , p value: Probability value, CI: Confidence Intervals,
NPV: Negative predictive value, PPV: Positive predictive value,
*: Statistically significant at $p \leq 0.05$, #Cut off was choose according to Youden index.

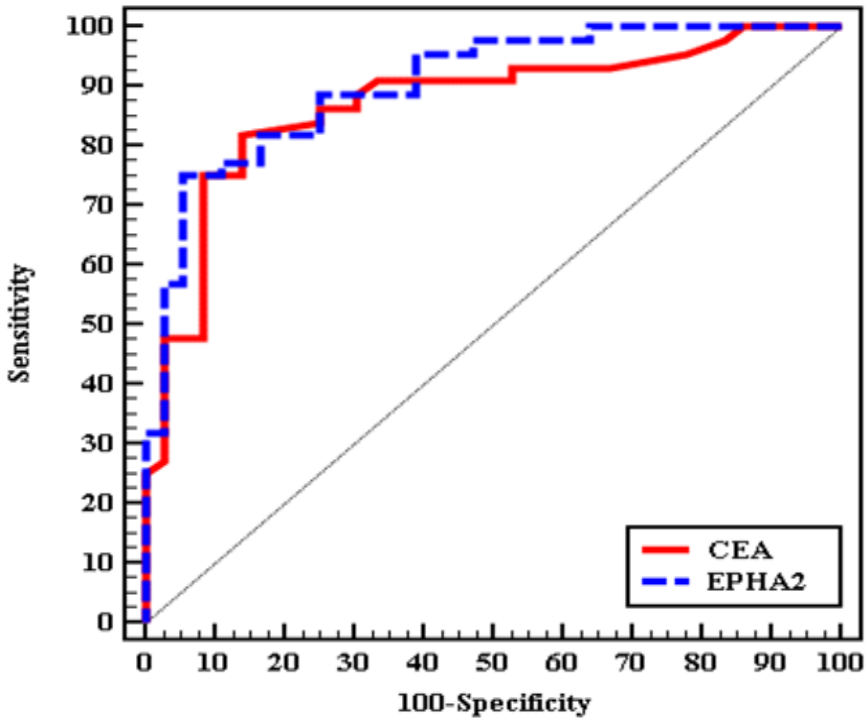

Figure (1): ROC curve for CEA and EphA2 to discriminate metastatic stage (n = 44) from early stage (n = 36).

Table 2 demonstrates the correlation between EphA2 and various parameters in the study. EphA2 exhibited a statistically significant correlation with the stage of the disease, showing an increase in EphA2 levels as the stage progressed to IV. Additionally, the site of metastasis was also significantly correlated with EphA2 levels.

Table (2): Relation between EphA2 and clinicopathological variables (n=80)

		N	EphA2 (ng/mL)		P
			Mean ± SD.	Median (Min. – Max.)	
Gender	Male	**40**	2902.4 ± 2388.1	2088.2 (314.0 – 7839.3)	0.124
	Female	**40**	3898.1 ± 2879.6	2425.6 (580.6 – 8732.1)	
Age (years)	<45	**33**	3511.2 ± 2850.5	2215.0 (405.2 – 8732.1)	0.729
	≥45	**47**	3322.3 ± 2574.7	2256.3 (314.0 – 7686.2)	
Tumor size	>3	**74**	3513.3 ± 2680.9	2269.1 (314.0 – 8732.1)	0.137
	<3	**6**	2005.2 ± 2381.8	1314.9 (453.2 – 6785.7)	
Stage	II	**17**	1547.4 ± 1572.5	1030.2 (453.2 – 6785.7)	<0.001*
	III	**19**	1606.4 ± 1229.9	1132.4 (314.0 – 5891.1)	
	IV	**44**	4983.5 ± 2437.6	6258.9 (864.4 – 8732.1)	
T stage (n = 36)	T1	**1#**	2001.0		0.864
	T2	**6**	1484.7 ± 974.4	1484.4 (453.2 – 2580.0)	
	T3	**24**	1602.3 ± 1701.9	973.1 (405.18 – 6785.7)	
	T4	**5**	1961.4 ± 2237.1	1241.2 (314.0 – 5891.1)	
N stage (n = 36)	N0	**17**	1573.8 ± 1502.2	1030.2 (453.2 – 6785.7)	0.998
	N1	**13**	1622.8 ± 1553.4	1128.0 (405.2 – 5891.1)	
	N2	**6**	2065.7 ± 2455.8	1060.1 (314.0 – 6784.7)	
Site of metastasis	No	**36**	1451.7 ± 1348.1	932.4 (314.0 – 6785.7)	<0.001*
	Liver	**21**	5226.9 ± 2215.8	6071.4 (1072.0 – 7686.2)	
	Peritoneal deposits	**12**	4564.8 ± 2699.8	4439.8 (1384.8 – 7839.3)	
	Liver/ Lung/ Peritoneal deposits	**10**	5270.3 ± 2581.3	6276.3 (1381.3 – 8732.1)	
	Lung	**1#**	6285.7		
Pathology	Adenocarcinoma	**63**	3445.7 ± 2595.5	2282.0 (314.0 – 7686.2)	0.401
	Mucinous	**12**	2774.5 ± 2828.9	1311.2 (650.0 – 7839.3)	
	Signet	**5**	4329.3 ± 3564.0	1902.7 (1512.5 – 8732.1)	
Grade	Well	**8**	4523.6 ± 2841.0	6112.5 (580.62 – 6964.3)	0.693
	Moderate	**58**	3328.2 ± 2651.6	2256.3 (314.0 – 8732.1)	
	Poor	**14**	3056.8 ± 2722.0	1951.8 (712.50 – 7678.6)	
LVI	Yes	**21**	3123.4 ± 2633.1	2580.0 (588.80 – 8732.1)	0.646
	No	**46**	2928.1 ± 2600.8	1662.8 (314.0 – 7839.3)	
	Not done	**13**	5518.0 ± 2090.0	6267.9 (1534.8 –7678.6)	
PNI	Yes	**19**	3069.2 ± 2282.4	2175.4 (580.62 – 6964.5)	0.341
	No	**48**	2957.7 ± 2727.6	1465.2 (314.0 – 8732.1)	
	Not done	**13**	5518.0 ± 2090.0	6267.9 (1534.8 –7678.6)	
Budding	3	**17**	2430.4 ± 2316.4	1639.3 (588.80 – 7839.3)	0.818
	1-2	**43**	3019.0 ± 2742.7	1512.5 (314.0 – 8732.1)	
	Not done	**20**	5044.3 ± 2090.0	6267.9 (1534.8 –7678.6)	
Obstruction	Yes	**14**	2393.7 ± 2543.4	1365.6 (314.0 – 7564.9)	0.070
	No	**66**	3613.8 ± 2672.8	2431.0 (405.18 – 8732.1)	
Perforation	Yes	**2**	1156.7 ± 413.5	1156.7 (864.37 – 1449.1)	0.279
	No	**78**	3457.8 ± 2685.1	2256.3 (314.0 – 8732.1)	
KRAS	Mutant	**17**	4740.0 ± 2389.4	5617.9 (879.37 – 7686)	0.721
	Wild	**24**	4474.7 ± 2875.3	6258.9 (588.80 – 8732)	
	Not done	**39**	2155.0 ± 2086.7	1346.5 (314.0 –7678.6)	

* Significant

Regarding EphA2 level expression was interpreted as high or low in **Table 3**, which shows the relation between EphA2 level expression and the clinicopathological details of the studied CRC cases. EphA2 level expression had statistically significant association with patients with size of tumor as larger tumor, >3 cm, was more likely to have high EphA2. The advanced stage was more likely to have a high level of EphA2. Higher CEA levels were significantly associated with high EphA2 levels. So high levels of EphA2 were associated with more advanced stages, larger tumor size, obstruction, and high CEA levels. These findings suggest that EphA2 could be a marker of aggressive disease and poor prognosis in CRC.

Table (3): Relation between EphA2 level and clinicopathological variables (n=80)

	EphA2				P
	Low (≤1534.82 ng/mL) (n = 32)		High (>1534.82 ng/mL) (n = 48)		
	No.	%	No.	%	
Gender					
Male	17	53.1	23	47.9	0.648
Female	15	46.9	25	52.1	
Age (years)					
<45	11	34.4	22	45.8	0.308
≥45	21	65.6	26	54.2	
Mean ± SD.	49.38 ± 12.95		44.56 ± 13.65		0.119
Median (Min. – Max.)	48.0 (28.0 – 76.0)		45.0 (19.0 – 73.0)		
Tumor size					
>3	27	84.4	47	97.9	0.035*
<3	5	15.6	1	2.1	
Stage					
II	12	37.5	5	10.4	<0.001*
III	15	46.9	4	8.3	
IV	5	15.6	39	81.3	
T stage (n = 36)					
T1	0	0.0	1	10.0	0.175
T2	3	11.5	3	30.0	
T3	19	73.1	5	50.0	
T4	4	15.4	1	10.0	
N stage (n = 36)					
N0	12	48.0	5	45.4	1.000
N1	9	36.0	4	36.4	
N2	4	16.0	2	18.2	
Site of metastasis					
No	26	83.9	10	20.4	<0.001*
Liver	2	6.4	19	38.8	
Peritoneal deposits	1	3.3	11	22.4	
Liver/ Lung/ Peritoneal deposits	2	6.4	8	16.4	
Lung	0	0.0	1	2	
Pathology					
Adenocarcinoma	24	75.0	39	81.3	0.291
Mucinous	7	21.9	5	10.4	
Signet	1	3.1	4	8.3	
Grade					
Well	3	9.4	5	10.4	1.000
Moderate	23	71.9	35	72.9	
Poor	6	18.8	8	16.7	
LVI					
Yes	8	25.0	13	27.1	0.365
No	23	71.9	23	47.9	
Not done	1	3.1	12	25.0	
PNI					
Yes	6	18.8	13	27.1	0.129
No	25	78.1	23	47.9	
Not done	1	3.1	12	25.0	
Budding					
3	8	25.0	9	18.8	0.774
1-2	22	68.8	21	43.8	
Not done	2	6.3	18	37.5	
Obstruction					
Yes	9	28.1	5	10.4	0.041*
No	23	71.9	43	89.6	
Perforation					
Yes	2	6.3	0	0.0	0.079

	EphA2				P
	Low (≤1534.82 ng/mL) (n = 32)		High (>1534.82 ng/mL) (n = 48)		
	No.	%	No.	%	
No	30	93.8	48	100.0	
KRAS					
Mutant	2	6.3	15	31.3	0.433
Wild	6	18.8	18	37.5	
Not done	24	75.0	15	31.3	
CEA					
Mean ± SD.	2.69 ± 1.41		9.81 ± 22.40		<0.001*
Median (Min. – Max.)	2.09 (1.0 – 7.0)		5.60 (1.70 – 158.0)		

* Significant

Concerning survival analysis, **Figure 2** below demonstrates the 2-year overall survival of all 80 patients. The median was 21.3 months in patients. The expression levels of EphA2 showed a statistically significant difference concerning the OS of patients, with a p-value of 0.034. As illustrated in **Figure 2** and **Table 4** the median survival for patients with low EphA2 levels was not reached, while those with high EphA2 levels had a shorter median survival of 19.26 months.

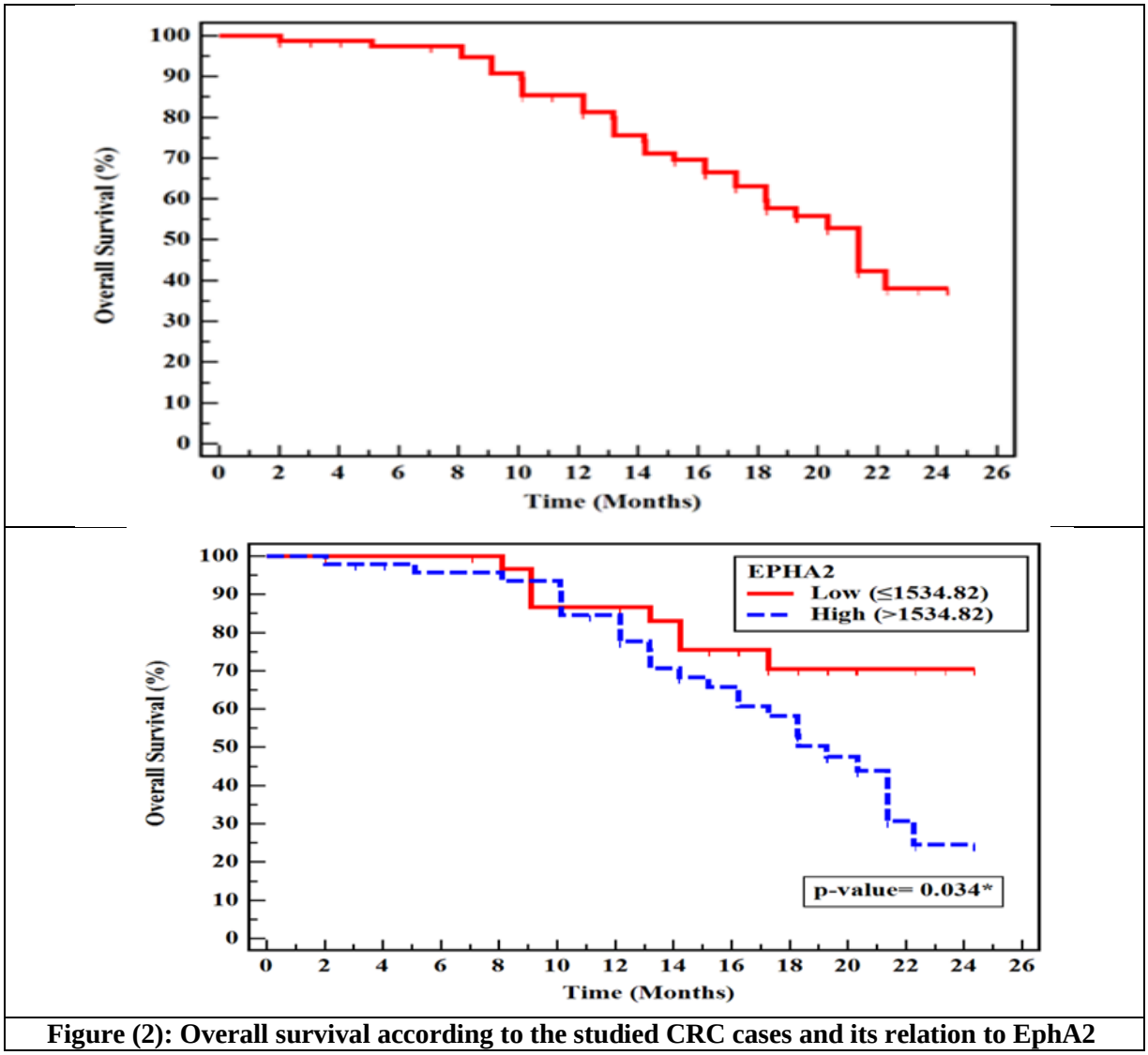

Figure (2): Overall survival according to the studied CRC cases and its relation to EphA2

Table (4): Kaplan-Meier survival curve for overall survival with EphA2 level

		Mean (months)	Median (months)	1 year %	2 years %	P
EphA2	Low (≤1534.82)	20.772	NR	86.7%	70.5%	0.034*
	High (>1534.82)	17.849	19.267	84.6%	24.6%	

* Significant.

In the multivariable analysis of OS, the following factors were identified as significant predictors: age ≥ 45 years, and KRAS mutation status. These factors emerged as the strongest predictors of OS. In contrast, other factors such as disease stage, budding, surgery, and EphA2 lost their statistical significance as predictors of OS in the multivariate analyses as illustrated in **Table 5.**

Table (5): Univariate and multivariate COX regression analysis for the parameters affecting overall survival

		Univariate		#Multivariate	
		P	**HR (LL – UL 95% C.I)**	**p**	**HR (LL – UL 95% C.I)**
Gender	Male	**Ref.**		**Ref.**	
	Female	0.428	0.762 (0.389 – 1.493)	0.291	0.509 (0.145 – 1.785)
Age (years)	<45	**Ref.**		**Ref.**	
	≥45	0.002*	0.334 (0.167 – 0.667)	0.002*	0.109 (0.028 – 0.429)
Diabetes	Yes	0.540	0.771 (0.336 – 1.769)	0.260	0.501 (0.150 – 1.669)
	No	**Ref.**		**Ref.**	
Tumor size	>3	0.337	2.648 (.362 – 19.372)	0.549	7.581 (0.010 – 5704.281)
	<3	**Ref.**		**Ref.**	
Stage	Early (II: III)	**Ref.**		**Ref.**	
	Metastatic (IV)	0.001*	3.907 (1.702 – 8.971)	0.419	0.046 (0.000 – 80.691)
LVI	Yes	0.551	1.272 (0.576 – 2.810)	0.886	0.909 (0.246 – 3.354)
	No	**Ref.**		**Ref.**	
PNI	Yes	0.153	1.789 (0.806 – 3.970)	0.804	1.199 (0.286 – 5.019)
	No	**Ref.**		**Ref.**	
Grade	Well	**Ref.**		**Ref.**	
	Moderate	0.403	1.845 (0.439 – 7.762)	0.877	1.167 (0.164 – 8.297)
	Poor	0.625	1.506 (0.292 – 7.775)	0.654	0.545 (0.039 – 7.699)
Budding	Yes	0.012*	3.067 (1.274 – 7.384)	0.845	1.148 (0.289 – 4.561)
	No	**Ref.**		**Ref.**	
Obstruction	Yes	0.396	1.409 (0.638 – 3.110)	0.148	3.648 (0.631 – 21.096)
	No	**Ref.**		**Ref.**	
Perforation	Yes	0.827	1.250 (0.169 – 9.238)	0.571	2.228 (0.140 – 35.534)
	No	**Ref.**		**Ref.**	
KRAS	Mutant	0.783	1.112 (0.524 – 2.359)	0.050*	0.121 (0.015 – 1.000)
	Wild	**Ref.**		**Ref.**	
CEA		0.209	1.007 (0.996 – 1.019)	0.245	0.986 (0.962 – 1.010)
EphA2	Low (≤1534.82)	**Ref.**		**Ref.**	
	High (>1534.82)	0.042*	2.268 (1.029 – 5.000)	0.568	1.721 (0.266 – 11.128)
Surgery	Yes	0.002*	0.344 (0.176 – 0.673)	0.209	0.217 (0.020 – 2.352)
	No	**Ref.**		**Ref.**	

* Significant

DISCUSSION

CRC is a worldwide health burden, accounting for the 3rd most diagnosed cancer around the world [14]. Even though considerable progress has been made in diagnostic and molecular approaches to CRC, patient response to treatment remains variable, raising the crucial need for new predictive and prognostic biomarkers to improve the outcome [15].

Poor patient outcomes are associated with high levels of EphA2 expression, underscoring the potential therapeutic benefits of EphA2 inhibition [16]. Several studies have observed high-level expression of EphA2 at different stages of CRC. In particular, EphA2 expression is a sign of a bad prognosis for patients with stage II/III CRC [17,18].

In this work, we examined EphA2's function as a prognostic indicator in patients with colorectal cancer. In our study stage IV was the most predominant in 55% of cases, which is in agreement with **Iliklerden and Kalayci**[19] with 90 colon cancer patients that stage IV was most dominant representing 66.6% of patients. The **Feller *et al.*** [20] study was conducted in Switzerland on 10,088 colorectal cancer cases diagnosed between 2000 and 2008. In this study stage IV represented 19.4% which was not the most predominant stage in cases. This may be due to a lack of awareness among Egyptian patients.

KRAS mutations are present in about 40% of

mCRCs, with exon 2 and codons 12 (which account for around 80% of all KRAS mutations), 13 and 61 having the highest frequency. However, 60% of mCRC patients had KRAS wild type. The first predicted positive biomarker for anti-EGFR therapy in mCRC was the presence of KRAS wild type [21]. Several investigations showed that KRAS exon 2 mutations cause constitutive activation of MAPK signaling by the use of therapeutic monoclonal antibodies like cetuximab or panitumumab to inhibit EGFR upstream. A poor prognosis and a reduced responsiveness to anticancer treatments are typically linked to RAS mutations [22].

Wild KRAS was predominant in most cases (58.5%) while mutated KRAS occurred in (41.5%), which is in agreement with results reported by **Oukkal *et al.*** [23], who reported that wild KRAS is more predominant in the North Africa and Middle East than mutational status that is more prevalent in western countries.

The cancer death rate can be reduced by early detection of colon cancer. As a result, finding novel biomarkers is crucial for early colon cancer detection. Tumor cells and cancer biomarkers in bodily fluids. Diagnoses, prognoses, and therapy efficacy predictions may all be made using these indicators. EphA2 has recently been shown to be useful in the detection of colon cancer [24]. As an RTK, EphA2 is essential for the development of the brain and blood vessels during embryogenesis. Numerous malignancies and tumor cell lines frequently overexpress Eph and its ligands [24].

One of the most promising cell membrane-associated tumor antigens is EphA2, a member of the Eph receptor family class A that is overexpressed in a number of human malignancies, including CRC [25]. EphA2 controls cellular features involved in tumor development, including migration and invasion, as well as those involved in carcinogenesis, like survival and proliferation [26].

Patients with CRC exhibited a poorer outcome when EphA2 expression was excessively high. EphA2 is also linked to intracellular reactive oxygen species clusters that reach deadly levels, the buildup of peroxidized lipids in the cell membrane, and ferroptosis, a kind of programmed cell death distinct from apoptosis and necrosis. The expression of common ferroptosis-related genes in CRC was significantly correlated with EphA2 expression. Therefore, EphA2 may regulate ferroptosis to affect the development of CRC. EphA2 is linked to the invasion of immune cells. The infiltration of myeloid dendritic cells, neutrophils, and macrophages was substantially and favorably connected with EphA2 expression. Consequently, EphA2 may affect the development and spread of CRC by infiltrating certain immune cells [27].

In this study EphA2 serum marker had a positive correlation with the CEA serum marker when the level of CEA increased the level of EphA2 increased with a significant $P < 0.001$, which can lead to EphA2 could be helpful as a prognostic tool in CRC. This contrasts with **Wang *et al.*** [18], who recruited 106 Chinese patients with CRC to examine the relationship between the pathophysiology of the disease and the blood levels of VEGF-A and EphA2, as well as the potential use of these molecules in the detection of CRC. There was no correlation between EphA2 and CEA. The ROC curve analysis found that the AUC of EphA2 was 0.622 and the AUC of CEA was 0.673. This may be explained as the cut-off of this study for EphA2 was > 297.92 ng/ml and the expression of EphA2 decreased with increasing tumor stage and CEA cut-off level was > 5.1 ng/ml [18]. Gender and age were not significantly correlated with EphA2 levels, which aligns with the findings of **Wang *et al.*** [18] who also reported no significant relationship between these factors and EphA2.

In our study, we observed a significant correlation between EphA2 levels and cancer stages II-III and IV, with EphA2 levels increasing as the disease progressed to stage IV ($P < 0.001$). This outcome is in line with the conclusions of **Iliklerden and Kalayci**[19] who showed that EphA2 levels increased from stage I to IV, with this difference being statistically significant ($P < 0.001$). In contrast, **Wang *et al.*** [18] reported that EphA2 was not significantly related to the cancer stage, possibly because they found that EphA2 expression decreased with increasing tumor stage.

Patients with late-stage pancreatic cancer (III and IV) had substantially greater EphA2 expression levels than patients with early-stage pancreatic cancer (I and II) ($P = 0.020$). Patients with late-stage illnesses (III and IV) had substantially greater EphA2 expression levels than patients with early-stage illnesses (I and II) ($P = 0.020$) [28].

KRAS expression in our study had no significance in correlation with EphA2 level. Several trials discussed the relationship between KRAS expression and EphA2. **Martini *et al.*** [29] study discussed EphA2's function as a possible therapeutic target expression and predictive biomarker of resistance in CRC tissue samples from patients receiving cetuximab and chemotherapy (FOLFIRI). With initial cetuximab resistance, a panel of several human CRC cell lines exhibits overexpression of EphA2. Two human CRC cell lines that had developed acquired resistance to cetuximab were shown to have elevated levels of phosphor-EphA2, indicating that EphA2 could be a major factor in the development of cetuximab resistance. Additionally, human CRC cell lines that were resistant to cetuximab showed dose-dependent suppression of cell growth and induction of apoptosis when treated with the small-molecule RTK inhibitor ALWII-41-27, which pharmacologically inhibits EphA2 activation and downstream signaling.

Furthermore, both AKT and MAPK-activated intracellular signaling were markedly suppressed by the combination of the anti-EphA2 ALW-II-41-27 and the

anti-EGFR cetuximab, indicating that this is a key mechanism via which EphA2 contributes to cetuximab cancer cell resistance [17]. Already in 2013, **Strimpakos *et al.*** [30], which discussed the prognostic role of EphA2 in patients with advanced CRC treated with cetuximab, revealed that a high expression level of the EphA2 receptor is associated with poor patient responses to cetuximab-based therapy, as patients with high EphA2 expression had a median survival of 20 months, whereas patients with low Epha2 expression had a median survival of 27 months, which was significantly longer than a high expression (P 0.015).

The relationship between 5-FU and EphA2 was discussed in 2022, by the research of **Yao *et al.*** [31], which was held in China by cell culture and RNA extraction. In the created RNA network, EphA2 was shown to be a hub gene. The expression and activity of EphA2 were examined, and it was discovered that chemoresistant cells had higher levels of EphA2 and its phosphorylation than chemosensitive cells. RNA-sequencing data indicated that EphA2 was increased in chemoresistant Fu and chemoresistant DDP cells. This clarifies EphA2, which controls chemoresistance in CRC.

In our study EphA2 level expression of high and low levels had statistical differences with significant with OS (P=0.034) that the patients with high-level EphA2 had worse median survival than patients with low levels of EphA2 and in univariate analysis of OS by COX regression EphA2 was a predictor of OS as patients with the high level associated with worse survival in agreement with **De Robertis *et al.*** [32], which was held in Italy, gene expression study was done in EphA2 cells derived from the mice model of CRC. Median OS was worse with EphA2 high level than low EphA2 with a significant difference P 0.0048. According to clinical outcome data, patients with EphA2 high had a significantly lower OS survival length than patients with EphA2 low, suggesting that an increased expression of the EphA2 gene is associated with a worse prognosis for CRC [32].

In contrast to **Martini *et al.*** [29] who discussed EphA2 as a predictive with patients who received, patients with high EphA2 had a lower median overall survival compared to those with low EphA2 after receiving first-line treatment with FOLFIRI plus cetuximab (28.4 months; 95% CI, 13.1–43.7 and 39.8 months; 95% CI, 30.2–49.4, respectively); however, this difference did not reach statistical significance [P 0.23].

Our study's limited sample size is one of its limitations, thus we advise doing more research with a larger sample size.

CONCLUSION

We concluded that our study revealed a significant statistical correlation between high serum EphA2 level and stage. Increased level of marker with advanced stage. EphA2 can help in the differentiation of colorectal cancer stages. Also, there was a significant statistical correlation between EphA2 level and overall survival. So, EphA2 could be considered as a bad prognostic factor that is associated with overall survival. We recommend further studies on serum EphA2 using a larger sample size and more effort to give a chance for the development of targeted therapy.

ACKNOWLEDGMENT

We acknowledge all patients who permitted us to use their blood samples in our study. We also acknowledge all employees who help us to obtain hard copies of patients' files.

Conflict of interest: None.

Financial disclosures: Our research had a grant from the South Egypt Cancer Institute Project at Assiut University through the Egyptian Ministry of Higher Education and Scientific Research.

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