Research article

Upregulation of anaphase promoting complex (APC) 7 as a prognostic marker for esophageal squamous cell carcinoma: A hospital based study

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ABSTRACT

Esophageal cancer is the sixth leading cause of cancer death, and esophageal squamous cell carcinoma (ESCC) is the most prevalent type worldwide, with a poor prognosis due to late diagnosis. The search for new molecular prognostic biomarkers revealed that dysregulation of anaphase promoting complex/cyclosome (APC/C) activation due to altered expression of APC molecules might lead to perturbed mitotic progression leading to malignancy. We analyzed the expression of the four different subunits of the APC/C complex—APC3, APC4, APC5 and APC7—by Real Time Polymerase Chain Reaction (RT–PCR). The findings were then correlated with clinico-pathological parameters and different lifestyle factors. Significant upregulation of APC7 (tissue and blood: N = 50; 3.72 ± 1.21 and 4.45 ± 1.18, respectively) and APC3 (tissue and blood: N = 52 and 55 and 4.50 ± 1.41 and 4.58 ± 1.06, respectively) suggests their role in uncontrolled cell proliferation. In addition to their association with increasing age, their significant association with tumor size, node stage (only APC7 ($p < 0.05$)), and dysphagia grade supports a potential role in tumorigenic transformation in ESCC. Furthermore, several exclusive lifestyle-associated factors play a crucial supporting role in the development of ESCC in the Northeast Indian population. Various lifestyle factors, such as the duration of smoking, tobacco and betel nut consumption, and the duration of alcohol consumption, are significantly associated with the expression of APC. Analysis based on Pearson's correlation coefficient indicated a positive correlation among the gene expression levels of APC3 (both blood and tissue), APC5 (tissue) and APC3 (tissue), APC7 (tissue) and APC3 (tissue), and APC7 (tissue) and APC3 (blood). Additionally, a positive correlation was found between APC7 expression in blood and tissue samples. However, no significant correlation was found between APC 7 expression and APC4 and APC5 expression in either blood or tissue samples.

1. Introduction

Cell cycle progression through mitosis is mediated by the spatial and temporal degradation of several cell cycle checkpoint proteins through ubiquitin-mediated 26S proteasomal degradation. Protein ubiquitination is a multistep process involving three distinct enzymatic steps [1] involving E1, E2 and E3 enzymes. E3 ubiquitin ligases are key regulators of cell cycle progression and comprise mainly two subtypes—the SCF (Skp1/Cullin/F-box) and anaphase-promoting complex/cyclosome (APC/C). APC/C is primarily involved in mitotic exit via the ubiquitin-mediated degradation of Pds1/securin and cyclin B (M-phase cyclin) to maintain negligible cdk activity during late metaphase and anaphase. However, during G1 phase, a low level of mitotic cdk activity is maintained by Cdc20-and Cdh1-mediated temporal regulation of APC/C activity [2, 3, 4].

Apc3/cdc27, a part of the conserved canonical tetratricopeptide residue (TPR) subunits of APC/C, regulates chromosomal segregation during mitosis by controlling activity following cyclin degradation. In particular, the interaction of APC3 with some proteins inhibits substrate binding to APC/C, promoting mitotic checkpoint activity. APC3 mediates

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Table 1. Comparison of APC3, APC4, APC5 & APC7 expression (Mean ± SD) between blood and tissue samples of ESCC patients (P value <0.05 is considered as significant).

APC3 (Tissue) Mean ± SD	APC3 (Blood) Mean ± SD	P-Value	APC4 (Tissue) Mean ± SD	APC4 (Blood) Mean ± SD	P-Value	APC5 (Tissue) Mean ± SD	APC5 (Blood) Mean ± SD	P-Value	APC7 (Tissue) Mean ± SD	APC7 (Blood) Mean ± SD	P-Value
3.53 ± 2.06	3.76 ± 1.85	0.000*	3.66 ± 9.55	6.74 ± 3.07	0.595	5.10 ± 4.80	4.25 ± 1.36	0.620	2.81 ± 1.71	3.40 ± 1.93	0.000*

Total (N = 70)	APC3 (Tissue) Up regulation	Down regulation	P-Value	APC3 (Blood) Up regulation	Down regulation	P-Value	APC4 (Tissue) Up regulation	Down regulation	P-Value	APC4 (Blood) Up regulation	Down regulation	P-Value	APC5 (Tissue) Up regulation	Down regulation	P-Value	APC5 (Blood) Up regulation	Down regulation	P-Value	APC7 (Tissue) Up regulation	Down regulation	P-Value	APC7 (Blood) Up regulation	Down regulation	P-Value
	4.5 ± 1.41	0.72 ± 0.23	**0.001***	4.58 ± 1.06	0.73 ± 0.12	**0.006***	7.75 ± 4.56	0.41 ± 0.12	0.184	9.27 ± 8.6	027 ± 0.06	0.244	5.69 ± 4.1	0.55 ± 0.2	**0.004***	4.92 ± 3.36	0.22 ± 0.12	0.314	3.75 ± 1.21	0.737 ± 0.22	**0.001***	4.45 ± 1.18	0.8 ± 0.15	**0.001***

Table 2. Association of APC3, APC4, APC5 & APC7 expression (Mean ± SD) with different clinicopathological parameters in ESCC patients.

	N	APC3 (tissue) mRNA expression	P-value	APC3 (Blood) mRNA expression	P-value	APC4 (tissue) mRNA expression	P-value	APC 4 (Blood) mRNA expression	P-value	APC5 (tissue) mRNA expression	P-value	APC5 (Blood) mRNA expression	P-value	APC7 (tissue) mRNA expression	P-value	APC7 (Blood) mRNA expression	P-value
Duration of age			**0.037***		**0.010***		0.831		0.586	3.16			**0.023***		**0.025***		**0.033***
30–39 years	7	1.91 ± 0.74		1.79 ± 1,16		3.16 ± 3.33		7.93 ± 4.72		3.52 ± 3.17		9.16 ± 2.17		1.31 ± 0.69		1.69 ± 0.72	
40–49 years	7	2.64 ± 1.41		3.13 ± 1.22		0.99 ± 0.73		2.44 ± 3.93		5.44 ± 2.49		4.05 ± 1.81		1.86 ± 1.19		2.24 ± 1.52	
50–59 years	26	3.36 ± 1.86		3.71 ± 1.55		15.09 ± 13.93		18.73 ± 13.59		4.64 ± 4.50		3.49 ± 2.25		3.03 ± 1.48		3.57 ± 1.62	
60–69 years	18	4.07 ± 2,13		4.22 ± 1.83		2.39 ± 3.45		7.06 ± 4.17		7.07 ± 7.08		3.20 ± 1.84		3.07 ± 1.85		3.69 ± 2.18	
70–79 years	12	4.53 ± 2.55		4.67 ± 1.02		4.33 ± 9.69		7.06 ± 5.85		3.91 ± 1.63		4.75 ± 1.16		3.61 ± 2.13		4.29 ± 2.64	
Gender			0.355		0.253		0.859		0.34		0.471		0.327		0.538		0.562
Male	43	3.34 ± 2.08		3.55 ± 1.99		3.50 ± 10.88		6.23 ± 5.00		5.44 ± 5.76		3.84 ± 1.61		2.75 ± 1.78		3.29 ± 2.13	
Female	27	3.82 ± 2.03		4.08 ± 1.58		3.92 ± 7.12		8.39 ± 13.35		4.58 ± 2.71		4.90 ± 6.76		3.01 ± 1.67		3.58 ± 1.86	
Dysphasia			**0.011***		**0.001***		0.076		**0.038***		0.663		**0.001***		**0.029***		**0.025***
Solid	40	3.56 ± 2.08		3.65 ± 1.91		1.44 ± 1.52		4.80 ± 5.13		5.50 ± 4.95		4.44 ± 1.35		2.82 ± 1.79		3.46 ± 2.11	
Solid + liquid	26	3.92 ± 1.86		4.36 ± 1.42		16.55 ± 14.31		15.34 ± 12.24		4.66 ± 3.67		2.88 ± 2.29		3.22 ± 1.56		371 ± 1.77	
No dysphasia	4	0.67 ± 0.24		0.842 ± 0.192		8.82 ± 0		2.83 ± 0		2.39 ± 0		36.50 ± 0		0.77 ± 0.165		0.82 ± 1.77	
Duration of dysphasia			0.483		0.113		0.815		0.873		0.599		0.684		0.111		0.192
1 month	17	3.1 ± 2.2		3.35 ± 2.07		4.57 ± 8.23		18.87 ± 15.71		6.03 ± 7.04		5.58 ± 4.24		2.26 ± 1.84		2.68 ± 1.87	
2 month	15	3.38 ± 1.92		3.63 ± 1.59		1.35 ± 1.24		5.75 ± 5.01		6.53 ± 5.61		4.28 ± 1.22		2.83 ± 1.46		3.3 ± 2.1	
3 month	18	4.1 ± 2.25		4.40 ± 1.75		5.73 ± 16.52		6.85 ± 5.96		4.46 ± 2.08		3.33 ± 2.08		3.25 ± 1.75		4.09 ± 2.16	
4 month	6	4.23 ± 1.91		4.27 ± 1.76		6.98 ± 5.31		6.88 ± 6.08		3.69 ± 3.83		4.26 ± 2.12		4.13 ± 195		3.85 ± 2.40	
5 month	6	2.46 ± 1./61		2.12 ± 1.82		1.16 ± 1.04		9.27 ± 5.56		3.50 ± 2.65		4.89 ± 1.83		1.75 ± 0.87		2.28 ± 1.23	
6 month	8	3.69 ± 1.86		4.26 ± 1.60		6.78 ± 5.22		4.66 ± 4.42		4.20 ± 3.61		2.98 ± 2.20		3.13 ± 1.15		4.14 ± 1.54	
Chemotherapy			0.569		0.443		0.914		0.901		0.247		0.228		0.405		0.765
yes	**31**	3.68 ± 1.94		3.95 ± 1.76		3.52 ± 6.90		6.91 ± 6.12		4.36 ± 2.59		3.54 ± 2.21		3.05 ± 1.61		3.32 ± 2.04	
no	39	3.40 ± 2.16		3.60 ± 1.93		3.77 ± 11.31		7.19 ± 11.04		5.70 ± 5.99		4.82 ± 5.50		2.70 ± 1.82		3.47 ± 2.03	
Radiation Therapy			0.569		0.443		0.914		0.901		0.247		0.228		0.405		0.765
yes	**31**	3.68 ± 1.94		3.95 ± 1.76		3.52 ± 2.90		6.91 ± 6.12		4.36 ± 2.59		3.50 ± 2.12		3.05 ± 1.61		3.32 ± 2.04	
no	39	3.40 ± 2.16		3.60 ± 1.98		3.77 ± 2.31		7.19 ± 6.04		6.70 ± 5.99		4.82 ± 5.50		2.703.05 ± 1.611.82		3.47 ± 2.03	
Both Therapy (Chemo + Radiation)					0.275		0.488		0.853		0.944		0.422		0.482	0.890 ± 0	0.370
No	1	0.720 ± 0	0.396	0.88 ± 0		3.86 ± 2.62		7.14 ± 11.19		5.55 ± 4.13		4.90 ± 3.62		0.830 ± 0		3.31 ± 1.86	
Yes	33	3.57 ± 1.94		3.89 ± 1.72		3.42 ± 2.69		6.95 ± 5.25		4.61 ± 2.67		3.53 ± 2.12		2.95 ± 1.61			

(*continued on next page*)

Table 2 (continued)

	N	APC3 (tissue) mRNA expression	P-value	APC3 (Blood) mRNA expression	P-value	APC4 (tissue) mRNA expression	P-value	APC 4 (Blood) mRNA expression	P-value	APC5 (tissue) mRNA expression	P-value	APC5 (Blood) mRNA expression	P-value	APC7 (tissue) mRNA expression	P-value	APC7 (Blood) mRNA expression	P-value
Tumor Size			0.007*		0.004*		0.496		0.793		0.037*		0.047*		0.028*		0.016*
T2	20	2.42 ± 1.08		2.71 ± 1.82		4.99 ± 3.35		6.31 ± 5.80		6.52 ± 3.75		3.14 ± 1.97		2.08 ± 1.84		2.56 ± 1.93	
T3	30	3.69 ± 1.94		3.89 ± 1.82		1.85 ± 1.22		8.06 ± 2.76		5.17 ± 2.70		3.99 ± 1.87		2.93 ± 1.52		3.33 ± 2.09	
T4a/T4b	20	4.39 ± 1.79		4.60 ± 1.47		4.01 ± 3.10		6.92 ± 5.15		2.83 ± 1.68		6.33 ± 4.74		3.51 ± 1.69		4.36 ± 1.63	
Tumor node			0.906		0.309		0.466		0.599		0.523		0.027*		0.262		0.079
N0	9	3.88 ± 2.30		4.32 ± 2.00		2.31 ± 2.87		4.25 ± 3.96		5.93 ± 4.34		7.98 ± 10.08		3.59 ± 2.09		4.19 ± 2.03	
N1	16	3.21 ± 2.10		3.25 ± 2.01		4.24 ± 3.86		7.02 ± 5.26		3.55 ± 2.46		4.14 ± 2.20		2.33 ± 1.42		2.48 ± 1.99	
N2	18	3.40 ± 1.80		4.22 ± 1.51		1.12 ± .94		9.15 ± 3.43		5.32 ± 5.31		3.71 ± 1.57		3.12 ± 1.64		4.01 ± 1.62	
N3	26	3.58 ± 2.22		3.50 ± 1.90		5.59 ± 4.84		6.74 ± 4.30		5.61 ± 4.42		3.27 ± 2.15		2.62 ± 1.72		3.18 ± 2.11	
Metastasis			0.351		0.669		0.400		0.054*		0.608		0.273		0.150		0.090
No	39	3.32 ± 2.22		3.67 ± 1.96		4.19 ± 10.80		5.93 ± 5.80		5.27 ± 5.31		4.57 ± 4.83		2.58 ± 1.74		3.04 ± 2.10	
Yes	31	3.74 ± 1.83		3.86 ± 1.73		1.88 ± 1.76		10.92 ± 15.63		4.56 ± 2.52		3.20 ± 1.94		3.19 ± 1.68		3.86 ± 1.85	
Differentiation			0.493				0.801		0.435		0.313		0.03*		0.232		0.135
Poorly differentiate	16	2.98 ± 2.39		3.35 ± 2.07		3.96 ± 3.65		11.41 ± 10.24		6.42 ± 9.30		8.71 ± 3.50		2.32 ± 1.76		3.06 ± 2.15	
Moderately differentiate	28	3.67 ± 2.09		3.80 ± 1.89		2.93 ± 1.12		7.12 ± 1.39		5.71 ± 5.32		3.80 ± 2.16		2.73 ± 1.66		3.01 ± 2.05	
Well differentiate	26	3.71 ± 1.82		3.96 ± 1.69		4.54 ± 3.43		6.07 ± 4.51		4.05 ± 2.11		3.88 ± 1.69		3.25 ± 1.75		4.03 ± 1.82	

the linking of Emi 1 (early mitotic inhibitor-1) to APC/C and subsequent prevention of chromosome missegregation during mitosis [5]. Thus, APC3 plays a crucial role in activating APC/C to recognize and degrade target substrates based on its preferential binding to either the Cdc20 or Cdh1 coactivator molecule at different stages of the cell cycle. During the G2/M transition, APC3 binds to Cdc20 and regulates UPS (ubiquitin proteasome system)-mediated degradation of securin and cyclin B to allow chromosome segregation. During the M/G1 transition, APC3-Cdh1 maintains low Cdk activity by degrading mitotic cyclins [2], Cdc25A [6], Skp2 [7] and Cks1. Additionally, it regulates the G1/S transition by degrading Geminin [8], Cdc6 [9] and its own Cyclin-A stabilizer E2, UbcH10, which inhibits the inactivation of APC/CCdh1.

Altered expression of APC3, a core component of APC/C, leads to either inadequate or incessant ubiquitination of securin and CyclinA1/2, CyclinB and Cyclin D1, altering the protein expression level [10]. This event results in either constitutive or delayed G0/G1 phase transition. Altered expression of APC3 has been reported in most cancers, including gastric colorectal cancer, thyroid cancer, testis cancer and lung adeno-carcinoma, suggesting the role of APC3 in oncogenic pathway activation [11, 12]. Thus, APC3 upregulation may enhance tumorigenesis. Over-expression of APC3 leads to proliferation, tumor formation, migration and invasion [13]. Furthermore, its association with tumor size, tumor nodes (N), TNM grade and metastasis stage suggests its correlation with tumor progression and poor patient survival [14].

Apc4 and APC5, in association with APC1, form the APC/C platform sub complex [15]. Crystal structure analysis of APC4 [16] has revealed its bidomain architecture comprising a 360 amino acid residue containing seven bladed WD40β propeller domains (APC4^{WD40}) and a helix bundle domain (APC4^{HBD}) containing four α-helices. The APC4^{HBD} domain is inserted between strands C and D of blade 4 within the extended lower wider surface of the propeller domain APC4 WD40, generating an L-shaped APC4 molecule. Additionally, another interaction between the two domains occurs through contact between β-strands A and B of blade 3 with APC4^{HBD} and an intradomain insert of β-strand D from blade 5 between β-strands C and D of blade 6 within APC4^{WD40}, leading to the formation of an edge β-strand (βE5). These extensive bottom surface cum strand surface contacts between the APC4^{WD40} and APC4^{HBD} domains confer tertiary structural stability to APC4.

The APC5 crystal structure reveals an N-terminal 7 α helical subunit APC5^{N} and Apc5^{TPR} subunit containing seven TPR motifs per TPR su-perhelical turn [17]. The N-terminus of the Apc5^{TPR} TPR superhelix is stabilized by interaction with the N-terminus of APC15, and the C-ter-minal 13 amino acid residues containing the APC1 cap fold back on the superhelix, enclosing a cavity within the APC5^{TPR} C-terminus [16]. However, APC5^{N} and APC5^{TPR} interact via the involvement of an extensive APC4 interface, facilitating conformational changes in the APC/C platform required for Cdh1 binding-mediated stimulation of APC/C activity [18]. Furthermore, the secondary E2, UBE2S, binds to the APC2-APC4 groove and accelerates the activity of UBE2C, maintaining a controlled E2-E3 cascade and substrate polyubiquitination.

APC7 is a part of the tetratricopeptide repeat subunit (TPR) complex, which is the major constituent of the "arc lamp" of APC/C [19]. Studies have revealed that APC3 forms a complex with APC7 and is stabilized by APC16, forming an APC3/APC7/APC16 subcomplex [20]. Although APC16 plays a role in providing substrate stability and mitotic progres-sion during mitosis, it is not involved in APC/C assembly. In vitro dele-tion analysis involving APC7 and APC16 reduces ubiquitination of the target substrates securin and cyclin-B1 [21]. The literature suggests that positive expression of APC7 is a good prognostic factor in various types of cancer and has a prime role in pathogenesis in human colorectal carci-noma and AML [22, 23]. However, some cancers have downregulated APC7; therefore, evaluating the expression of APC7 in ESCC is crucial [22].

APC is involved in the cell cycle and controls various other substrates in regulating the cell cycle. The role of APC in cancer is evident; there-fore, the role of APC in cancer progression must be investigated.
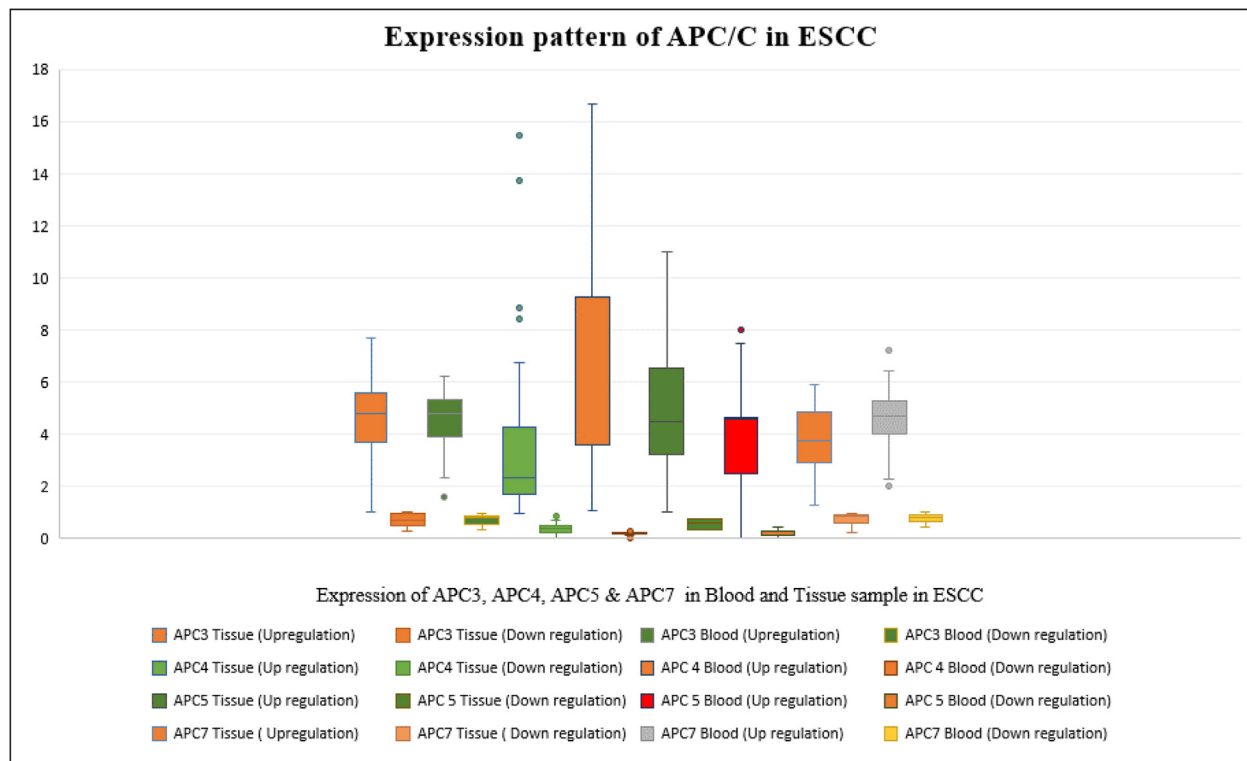
Figure 1. Box plot depicting expression of APC.

Therefore, we analyzed and compared the expression of selected APC/C subunits between blood and biopsy samples of esophageal squamous cell carcinoma by qualitative real-time PCR. Next, we assessed whether the data were correlated with various lifestyle factors (smoking, betel nut chewing, alcohol consumption and tobacco consumption), clinicopathological characteristics (tumor size, tumor node and metastasis, cell differentiation) and other physiological parameters, such as the age and sex of patients. Thus, this study will provide insight into APC/C dysregulation via altered expression of its different subunits and its association with various clinicopathological parameters in human esophageal squamous cell carcinoma (ESCC).

2. Methodology

2.1. Sample size and sample collection

A total of 70 ESCC patients (male and female) with equal numbers of age- and sex-matched healthy individuals were enrolled in our study after obtaining informed consent. The sample size was determined as previously described in Suresh et al. 2012 [24].

The diagnostic procedure included upper gastrointestinal endoscopy and pathological evaluation of tumor biopsy samples. The tumor samples and adjacent samples were obtained via biopsy from all the study participants. Standard venopuncture was used to collect blood samples. All the esophageal cancer patient samples were collected from Gauhati Medical College and Hospital, Guwahati and North East Cancer Hospital, Jorabat, Guwahati after obtaining approval from the Ethics Committee. The patients were followed up, and detailed information concerning food habits, physical activity, medical history, and lifestyle habits was collected. The data on lifestyle factors and clinicopathological parameters were divided into different groups.

2.2. RNA isolation and complementary DNA (cDNA) preparation

Total RNA was isolated manually from blood and homogenized tissue samples using TRIzol Reagent (Invitrogen, Inc., Carlsbad, CA). cDNA was prepared using an iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA) and stored at -20 °C.

2.3. mRNA expression analysis by real-time PCR

mRNA expression analysis of tissue samples was performed using a Roter-Gene Q Real-time PCR detection system (QIAGEN, Valencia, CA). The housekeeping gene β-actin was used as the reference gene to analyze gene expression. PCR amplification was performed using the SYBR Green method according to the manufacturer's instructions. The comparative Ct ($2^{-\Delta\Delta Ct}$) method was used to manually estimate the expression level of the studied genes.

2.4. Statistical analysis

The expression of the APC3, 4, 5 and 7 genes was analyzed with the different lifestyle factors and clinicopathological parameters using SPSS 18.0 software. All the data were expressed as means ± standard deviation. Independent-samples T test was performed for two levels, and multilevel samples were analyzed by one-way ANOVA. All the tests were 2-tailed and considered significant when the P value < 0.05. Kaplan–Meier survival analysis was performed using the log rank test, and univariate hazard analysis was performed using Cox's regression model. Furthermore, Pearson correlation analysis was performed to determine the relationship between the study variables. The correlation coefficient values were between 1.0 and +1.0. A value of 1.0 indicates a perfect positive correlation. For a perfect negative correlation, the two

Table 3. Association of APC3, APC4, APC5 & APC7 expression (Mean ± SD) with different life style factor in ESCC patients.

Life style factors	N	APC3 (tissue) mRNA expression	P-value	APC3 (blood) mRNA expression	P-value	APC4 (tissue) mRNA expression	P-value	APC4 (blood) mRNA expression	P-value	APC5(tissue) mRNA expression	P-value	APC5 (blood) mRNA expression	P-value	APC7(tissue) mRNA expression	P-value	APC7 (blood) mRNA expression	P-value
Smoking frequency			0.054*		0.217		0.72		0.446		0.49		0.39		0.372		0.731
1-5 times/day	15	4.39 ± 1.96		4.38 ± 1.79		7.07 ± 6.17		4.45 ± 3.08		5.02 ± 4.80		4.25 ± 1.22		3.30 ± 1.92		3.51 ± 2.31	
6-10 times/day	5	1.81 ± 1.78		2.52 ± 1.49		1.70 ± 0.66		7.62 ± 6.40		3.57 ± 1.43		3.38 ± 1.93		1.90 ± 0.82		2.60 ± 1.46	
16-20 times/day	3	3.96 ± 1.91		3.29 ± 1.75		1.44 ± 0.08		4.73 ± 3.86		1.40 ± 0.13		3.20 ± 2.41		3.13 ± 1.81		3.71 ± 1.55	
Duration of smoking			0.151		0.073		0.718		0.375		0.613		0.855		**0.040***		**0.028***
1–10 years	5	4.10 ± 1.56		4.31 ± 1.23		5.16 ± 4.44		7.40 ± 3.24		2.20 ± 1.83		3.41 ± 2.80		2.70 ± 1.12		3.30 ± 2.46	
11–20 years	13	3.57 ± 1.56		3.69 ± 1.95		9.56 ± 2.31		3.63 ± 2.77		5.58 ± 4.49		4.13 ± 1.45		2.67 ± 1.65		2.79 ± 1.68	
21–30 years	10	4.81 ± 0.66		4.71 ± 0.68		1.85 ± 1.12		4.63 ± 3.41		2.66 ± 1.37		3.65 ± 1.72		3.49 ± 1.11		3.90 ± 1.81	
31–40 years	4	5.32 ± 2.28		5.85 ± 0.61		5.16 ± 4.44		7.64 ± 3.69		4.92 ± 1.26		4.21 ± 0.84		5.18 ± 0.41		6.07 ± 1.07	
Smoking category			**0.016***		**0.027***		0.365		0.248		0.291		0.665		0.241		0.634
No	36	2.95 ± 1.26		3.28 ± 1.00		2.93 ± 1.58		7.95 ± 6.61		5.53 ± 4.78		4.41 ± 3.23		2.61 ± 1.75		3.29 ± 1.05	
Yes	34	4.13 ± 1.65		4.26 ± 1.56		5.15 ± 4.75		5.25 ± 4.59		4.23 ± 3.85		3.93 ± 1.54		3.10 ± 1.70		3.52 ± 2.01	
Duration of alcohol consumption			0.323		**0.015***		0.575		0.601		0.723		0.88		0.215		**0.017***
1–10 years	5	2.50 ± 1.95		1.85 ± 1.88		1.21 ± 0.58		8.33 ± 1.25		2.52 ± 1.82		3.65 ± 1.81		1.95 ± 1.27		2.12 ± 1.89	
11–20 years	7	4.11 ± 2.03		4.27 ± 1.79		2.34 ± 1.81		5.60 ± 4.14		6.18 ± 3.32		4.14 ± 1.75		2.65 ± 1.78		2.59 ± 1.81	
21–30 years	3	2.76 ± 1.89		2.82 ± 1.75		1.45 ± 0.07		9.35 ± 8.37		3.75 ± 0.78		4.64 ± 0.02		2.52 ± 2.39		2.45 ± 2.21	
31–40 years	4	5.18 ± 2.29		5.93 ± 0,.44		1.28 ± 1.07		4.98 ± 3.96		3.63 ± 2.57		4.19 ± 0.85		4.64 ± 1.24		6.26 ± 0.75	
Category of alcohol consumption			0.570		0.806		0.43		0.924		0.564		0.725		0.846		0.779
No	51	3.44 ± 2.04	0.570	3.72 ± 1.75		3.72 ± 1.75		7.13 ± 2.45		5.38 ± 4.70		4.37 ± 2.09		2.83 ± 1.75		3.45 ± 1.97	
Yes	19	3.76 ± 2.15		3.85 ± 2.14		3.85 ± 2.14		6.90 ± 4.63		4.58 ± 5.14		3.96 ± 1.50		2.92 ± 1.75		3.29 ± 2.21	
Duration of betel nut consumption			0.319		0.160		0.864		0.743		0.924		0.746		0.263		0.111
10 years	15	2.95 ± 1.04		2.96 ± 1.99		2.81 ± 1.92		5.40 ± 0.12		4.40 ± 3.08		5.66 ± 4.83		2.24 ± 2.02		2.55 ± 2.11	
20 years	9	3.15 ± 1.89		3.56 ± 1.65		5.42 ± 4.13		10.07 ± 8.87		5.51 ± 4.22		3.53 ± 2.26		2.72 ± 1.54		3.48 ± 1.67	
30 years	6	4.13 ± 1.78		4.20 ± 1.74		1.67 ± 0.83		6.46 ± 5.98		4.05 ± 2.65		4.60 ± 1.03		3.38 ± 1.54		3.38 ± 2.12	
40 years	12	3.59 ± 2.25		4.62 ± 1.94		2.2 ± 1.24		7.17 ± 4.18		6.01 ± 5.55		3.70 ± 1.72		3.21 ± 1.86		4.53 ± 1.89	
50 years	3	5.65 ± 0.80		5.24 ± 1.57		5.82 ± 4..35		6.26 ± 5.26		4.97 ± 0.81		3.14 ± 2.49		4.70 ± 0.50		4.92 ± 0.84	
Category of betel nut chewing			**0.011***		**0.012***		0.365		0.289		0.877		0.807		**0.005***		**0.004***
No	22	2.61 ± 1.27		2.93 ± 1.16		1.44 ± 0..24		4.63 ± 3.60		5.30 ± 1.85		3.98 ± 1.21		2.00 ± 1.62		2.39 ± 2.14	
Yes	48	3.95 ± 1.83		4.14 ± 1.57		4.17 ± 3..52		7.62 ± 6.83		5.06 ± 4.27		4.31 ± 2.81		3.24 ± 1.65		3.87 ± 1.80	
Tobacco consumption frequency			**0.002***		**0.001***		0.846		0.852		0.449		**0.027***		**0.003***		**0.019***
1-3 times/day	10	3.27 ± 2.07		3.80 ± 1.81		0.40 ± 0.06		8.10 ± 1.18		7.17 ± 1.90		4.94 ± 0.43		2.31 ± 1.71		3.20 ± 2.05	
4-6times/day	24	4.46 ± 1.72		4.69 ± 1.27		0.45 ± 0		0.35 ± 0		0.310 ± 0		2.92 ± 0		3.76 ± 1.42		4.30 ± 1.84	
7-10 times/day	6	4.83 ± 0.69		4.89 ± 0.50		2.27 ± 1.37		6.11 ± 5.83		7.73 ± 6.29		3.92 ± 1.37		3.49 ± 1.56		3.97 ± 1.82	
Duration of tobacco consumption			**0.003***		**0.001***		0.808		0.192		0.738		0.844		**0.002***		**0.002***
0–10 years	11	3.77 ± 1.56		4.17 ± 1.46		2.72 ± 1.33		2.72 ± 1.78		5.16 ± 4.40		3.13 ± 1.96		2.83 ± 1.59		3.23 ± 1.92	
11–20 years	12	3.44 ± 2.17		4.09 ± 1.72		6.77 ± 5.13		7.87 ± 6.51		3.82 ± 2.21		5.50 ± 2.76		2.78 ± 1.69		3.61 ± 1.87	
21–30 years	9	4.64 ± 0.62		4.51 ± 0.71		2.83 ± 1.39		5.06 ± 2.16		5.99 ± 4.44		4.22 ± 1.50		3.34 ± 1.07		3.6 ± 1.64	
31–40 years	6	5.42 ± 1.82		5.69 ± 0.63		2.58 ± 1.03		6.12 ± 3.97		7.17 ± 6.91		3.70 ± 1.73		4.82 ± 1.01		6.08 ± 0.87	
41- 40 years	2	5.83 ± 0.55		5.41 ± 0.65		1.28 ± 0.52		9.27 ± 0.06		4.68 ± 0.49		4.52 ± 0.13		5.44 ± 0.60		5.44 ± 1.11	
Tobacco consumption category			**0.002***		**0.001***		0.735		0.142		0.817		0.81		**0.004***		**0.005***
no	30	2.61 ± 2.09		2.77 ± 1.96		3.12 ± 2.96		9.29 ± 8.16		4.92 ± 2.80		4.08 ± 2.33		2.18 ± 1.69		2.64 ± 1.95	
yes	40	4.22 ± 1.76		4.50 ± 1.38		3.94 ± 2.72		5.90 ± 4.95		5.20 ± 4.61		4.34 ± 3.14		3.36 ± 1.60		3.98 ± 1.90	

Table 4. Association of expression of APC3, APC4, APC5 & APC7 between relatively younger and older age group of individuals among ESCC patients.

SmokingcateGorigoryCAT	Age group	APC3 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P -Value	APC4 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P- Value	APC5 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P- Value	APC7 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P -Value
Yes	Older Age group	2.94 ± 1.93	3.67	3.51 ± 1.79	0.922	2.25 ± 1.7	0.504	4.40 ± 3.11	0.153	6.47 ± 4.04	0.301	4.62 ± 0.01	0.663	2.10 ± 0.85	0.763	3.37 ± 1.60	0.746
	Younger age group	3.59 ± 1.43		3.59 ± 1.85		6.69 ± 2.24		7.95 ± 6.02		3.60 ± 2.13		6.39 ± 3.61	0.962	2.23 ± 1.09		3.14 ± 1.51	
No	Older Age group	3.43 ± 1.57	.224	3.09 ± 1.67	0.369	6.69 ± 3.38	0.227	8.41 ± 2.6	0.258	6.47 ± 1.27	0.531	3.38 ± 1.69		2.31 ± 0.94	0.628	3.06 ± 1.24	0.789
	Younger age group	3.93 ± 1.17		3.52 ± 1.61		2.0 ± 3.17		5.13 ± 3.61		5.09 ± 2.42		3.42 ± 2.18		2.43 ± 0.82		3.15 ± 1.11	
Smoking Frequency		**APC3**				**APC4**				**APC5**				**APC7**			
Frequency	**Age group**	**Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value	**Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value	**Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value
1-5 times	Older Age group	3.04 ± 2.00	0.757	2.78 ± 1.78	0.669	2.76 ± 1.97	0.539	3.57 ± 8.99	0.111	9.34 ± 8.99	0.110	3.70 ± 2.04	0.987	1.83 ± 0.85	0.612	2.83 ± 1.04	0.986
	Younger age group	3.32 ± 1.47		3.23 ± 1.93		5.63 ± 3.51		6.64 ± 2.88		4.25 ± 2.54		3.71 ± 1.92		2.16 ± 1.26		2.84 ± 1.31	
6-10 times	Older Age group	5.34 ± 0.23	0.575	5.78 ± 0.32	0.275	1.95 ± 0.212	0.568	8.57 ± 00	0.495	3.85 ± 0.636	0.254	4.64 ± 0.014	0.065	2.31 ± 0.933	0.485	3.87 ± 2.96	0.774
	Younger age group	3.27 ± 2.10		3.05 ± 1.02		1.53 ± 0.86		14.64 ± 10.61		4.66 ± 0.636		4.61 ± 0.058		2.76 ± 0.398		4.49 ± 1.63	
16-20 times	Older Age group	1.49 ± 0.41	**0.001***	4.20 ± 1.11	0.514	0.290 ± 0.1	0.683	4.46 ± 3.72	0.664	0.50 ± 0.12	0.714	4.63 ± 0.34	0.666	3.01 ± 0.20	0.475	5.07 ± 0.23	0.522
	Younger age group	4.89 ± 0.211		5.14 ± 1.52		2.01 ± 2.58		4.46 ± 5.72		1.36 ± 1.45		20.57 ± 22.52		1.81 ± 0.90		2.66 ± 2.10	
Duration of beetle nut consumption																	
Duration	**Age group**	**APC3** **Mean exp (Tissue)**	**P-Value**	**Mean exp (Blood)**	**P -Value**	**APC4** **Mean exp (Tissue)**	**P-Value**	**Mean exp (Blood)**	**P -Value**	**APC5** **Mean exp (Tissue)**	**P-Value**	**Mean exp (Blood)**	**P- Value**	**APC7** **Mean exp (Tissue)**	**P-Value**	**Mean exp (Tissue)**	**P -Value**
10 years	Older Age group	2.60 ± 1.20	0.198	2.45 ± 1.51	0.172	10.62 ± 13.89	0.127	16.18 ± 2.58	0.333	7.68 ± 5.99	0.145	2.43 ± 1.04	0.335	2.09 ± 0.68	0.575	2.19 ± 0.737	0.155
	Younger age group	3.60 ± 1.41		3.68 ± 1.56		3.05 ± 4.42		6.18 ± 3.61		2.75 ± 2.05		7.27 ± 4.52		2.40 ± 1.10		3.30 ± 1.54	
20 years	Older Age group	3.60 ± 1.32	0.492	3.27 ± 1.44	0.717	2.36 ± 4.02	0.650	6.47 ± 4.07	0.699	4.49 ± 2.95	0.672	3.48 ± 2.29	0.969	234 ± 0.55	0.693	3.14 ± 1.09	0.742
	Younger age group	3.92 ± 0.72		3.51 ± 1.27		6.70 ± 18.33		5.68 ± 3.46		5.78 ± 5.73		3.53 ± 2.32		2.43 ± 0.33		3.00 ± 80	
30 years	Older Age group	5.62 ± 0.05	0.138	3.68 ± 1.20	0.986	1.82 ± 1.24	0.876	3.29 ± 2.60	0.166	2.8 ± 1.7	0.190	4.20 ± 0.85	0.169	3.60 ± 2.31	0.322	3.23 ± 0.933	0.870
	Younger age group	3.88 ± 1.27		3.78 ± 2.18		2.04 ± 1.91		14.27 ± 10.94		4.56 ± 2.07		4.84 ± 0.59		2.34 ± 0.66		3.48 ± 1.89	
40 years	Older Age group	4.06 ± 1.83	0.348	4.98 ± 0.54	0.7	2.65 ± 1.22	0.263	5.79 ± 4.07	0.658	6.74 ± 2.48	0.295	4.01 ± 1.36	0.586	3.01 ± 0.43	0.931	4.32 ± 1.09	0.599
	Younger age group	4.79 ± 0.64		5.16 ± 0.877		1.01 ± .21		6.96 ± 2.68		3.05 ± 2.30		3.48 ± 2.23		3.05 ± 0.99		3.97 ± 1.10	
50 years	Older Age group	5.09 ± 0.91		5.37 ± 0.59		1.65 ± 1.69	0.642	6.41 ± 2.96	0.355	5.38 ± 2.26	0.499	4.57 ± 2.10	0.132	2.78 ± 0.287	0.885	4.96 ± 1.29	0.382
	Younger age group	5.21 ± 0		2.99 ± 0		0.340 ± 0		0.60 ± 0		6.24 ± 0.92		2.37 ± 1.89		2.85 ± 0.23		2.65 ± 0	
Duration of smoking																	
Duration	**Age group**	**APC3** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC4** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC5** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value	**APC7** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value
1–10 Years	Older age group																
	Younger age group	2.88 ± 0.72		3.15 ± 1.77		5.16 ± 7.43		6.00 ± 3.12		3.89 ± 1.27		3.45 ± 2.01		2.78 ± 1.87		3.44 ± 2.07	
11–20 years	Older age group	3.60 ± 2.32	0.594	3.41 ± 2.04	0.620	2.82 ± 2.34	0.475	2.33 ± 1.40	**0.004***	9.27 ± 8.86	0.251	4.61 ± 0.01	0.540	2.01 ± 1.08	0.744	3.15 ± 1.57	0.215
	Younger age group	2.90 ± 1.43		2.82 ± 1.32		14.95 ± 10.62		8.51 ± .00		3.24 ± 1.33		9.56 ± 8.17		1.82 ± 0.62		2.05 ± 0.81	
21–30 years	Older age group	1.78 ± 0	0.940	3.42 ± 0		1.80 ± 0	0.968	8.51 ± 0	0.989	3.40 ± 0	0.804	4.65 ± 0	0.717	1.65 ± 0	0.747	1.77 ± 0	0..357
	Younger age group	3.96 ± 1.36		3.60 ± 2.15		1.85 ± 0.255		8.33 ± 7.89		4.35 ± 3.29		4.94 ± 0.69		2.08 ± 1.15		3.63 ± 1.63	
31–40 years	Older Age group	2.43 ± 1.75	0.116	3.68 ± 2.22	0.237	1.64 ± 0.975	0.214	5.79 ± 4.69	0.495	4.81 ± 2.42	0.993	3.10 ± 2.64	0.498	2.37 ± 0.72	0.475	4.19 ± 1.70	0.985
	Younger age group	5.44 ± 0.44		6.13 ± 0.134		0.470 ± 0.311		8.51 ± 00		4.80 ± 0.707		4.61 ± 0.02		2.84 ± 0.40		4.23 ± 0.63	
Category of alcohol consumption																	
Category	**Age group**	**APC3** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC4** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC5** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value	**APC7** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value
Yes	Older age group	2.39 ± 1.95	**0.043***	2.79 ± 1.79	0.246	3.08 ± 1.62	0.723	3.57 ± 2.04	0.222	8.71 ± 7.26	0.129	4.61 ± .008	0.703	1.9 ± 0.954	0.287	3.21 ± 1.73	0.829
	Younger age group	3.97 ± 1.15		3.76 ± 1.48		6.18 ± 7.83		7.53 ± 6.49		4.59 ± 2.39		4.59 ± 2.39		2.28 ± 0.505		3.38 ± 1.46	
No	Older age group	3.48 ± 1.57	0.580	3.28 ± 1.69	0.761	3.61 ± 2.98	0.467	8.27 ± 7.83	0.322	5.21 ± 4.82	0.633	3.65 ± 1.89	0.924	2.32 ± 0.90	0.770	3.13 ± 1.27	0.794
	Younger age group	3.71 ± 1.34		3.43 ± 1.80		2.46 ± 1.86		5.47 ± 3.52		4.58 ± 3.66		3.71 ± 2.31		2.4 ± 1.09		3.02 ± 1.15	
Duration of Alcohol consumption																	
Duration	**Age grouping**	**APC3** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC4** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value	**APC5** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P- Value	**APC7** **Mean exp (Tissue)**	P-Value	**Mean exp(Blood)**	P -Value
1–10 years	Older age group																
	Younger age group	3.51 ± 1.24		3.34 ± 1.27		1.21 ± 1.57		5.15 ± 3.97		4.55 ± 3.097		9.622 ± 5.10		2.20 ± 0.61		3.17 ± 2.11	
11–20 years	Older age group	3.03 ± 2.46	.0.616	2.62 ± 1.59		3.67 ± 1.99	0.087	3.99 ± 2.85	0.179	12.196 ± 7.21	0.183	4.61 ± .00	0.475	1.68 ± 1.05	0.572	2.51 ± 1.11	0.293
	Younger age group	3.77 ± 1.18		2.93 ± 0.70		1.33 ± 0.898		7.00 ± 3.00		3.72 ± 1.49		4.54 ± 0.153		2.04 ± 0.51		3.27 ± 0.61	
21–30 years	Older age group																
	Younger age group	4.26 ± 0.64		4.07 ± 2.74		1.45±-0.070		13.63 ± 4.75		5.42 ± 1.58		4.62 ± 0.007		2.51 ± 0.44		4..52 ± 2.12	
31–40 years	Older age group	1.43 ± 0.33	**0.022***	3.05 ± 2.70	0.307	2.20 ± 0.14	**0.006***	4.44 ± 3.75	0.482	3.49 ± 1.138	0.198	4.62 ± 0.007	0.420	2.26 ± 0.99	0.593	4.26 ± 2.41	0.735
	Younger age group	5.43 ± 0.791		5.76 ± 0.664		0.350 ± 0.141		7.96 ± 0.777		6.90 ± 2.26		5.41 ± 1.10		2.72 ± 0.22		3.60 ± 0.240	
Category of betel nut chewing																	

(continued on next page)

Table 4 (*continued*)

SmokingcateGoriGoryCAT	Age group	APC3 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P-Value	APC4 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P-Value	APC5 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P-Value	APC7 Mean exp (Tissue)	P-Value	Mean exp (Blood)	P-Value
Category	Age grouping																
yes	Older age group	1.68 ± 0.35	0.392	1.51 ± 0.84	0.526	4.32 ± 3.30	0.928	7.87 ± 6.88	0.526	6.40 ± 5.52	0.275	3.51 ± 1.69	0.334	2.57 ± 0.83	0.938	3.49 ± 1.29	0.630
	Younger age group	2.16 ± 1.48		1.06 ± 0.32		4.06 ± 1.2		6.12 ± 5.08		4.84 ± 3.19		4.75 ± 3.90		2.55 ± 0.80		3.23 ± 1.23	
No	Older age group	3.89 ± 1.54	0.673	3.81 ± 1.49	0.904	1.31 ± 1.1	0.679	6.44 ± 3.51	0.904	4.14 ± 2.02	0.186	4.63 ± 1.77	0.203	1.39 ± 0.42	0.246	2.18 ± 0.93	0.60
	Younger age group	4.04 ± 1.06		3.90 ± 1.48		1.62 ± 1.6		6.65 ± 3.51		2.80 ± 0.73		3.17 ± 2.08		1.04 ± 0.62		1.93 ± 0.61	
Category of tobacco consumption	Age grouping																
Yes	Older age group	3.26 ± 1.71	0.165	3.28 ± 1.76	0.488	2.84 ± 0.8	0.564	9.02 ± 1.82	0.516	5.77 ± 4.23	0.612	4.12 ± 1.8	0.580	2.34 ± 0.72	0.911	3.18 ± 1.9	0.915
	Younger age group	3.89 ± 1.28		3.65 ± 1.80		4.72 ± 14		2.84 ± 1.02		5.06 ± 4.11		4.98 ± 2.58		2.31 ± 0.90		3.22 ± 1.24	
No	Older age group	3.36 ± 1.63	0.669	3.07 ± 1.62	0.675	4.70 ± 2.09	0.314	4.84 ± 3.51	0.951	5.85 ± 3.77	0.307	3.29 ± 1.61	0.617	2.10 ± 1.18	0.432	3.08 ± 1.45	0.901
	Younger age group	3.62 ± 1.27		3.33 ± 1.44		1.77 ± .90		4.74 ± 3.76		3.58 ± 2.63		3.73 ± 2.45		2.46 ± 1.18		3.01 ± 1.34	
Duration of Tobacco consumption	Age grouping																
0–10 years	Older age group	2.80 ± 1.39	0.641	2.66 ± 1.87	0.664	1.72 ± 2.54	0.412	7.17 ± 3.32	0.292	4.97 ± 3.39	0.225	3.87 ± 1.76	0.757	2.29 ± 1.02	0.665	2.76 ± 1.10	0.265
	Younger age group	3.22 ± 1.20		3.25 ± 2.18		4.18 ± 6.37		4.84 ± 2.88		2.65 ± 0.760		3.46 ± 2.29		2.66 ± 1.59		3.70 ± 1.39	
11–20 years	Older age group	2.56 ± 1.72	0.074	2.78 ± 1.89	0.273	4.0 ± 4.28	0.70	19.46 ± 15.84	0.176	4.22 ± 3.10	0.684	5.29 ± 1.51	0.788	2.10 ± 0.71	0.558	2.69 ± 1.51	0.872
	Younger age group	4.01 ± 1.17		3.77 ± 1.42		8.10 ± 2.29		6.27 ± 3.63		4.77 ± 2.03		6.63 ± 0.67		2.30 ± 0.54		2.80 ± 0.99	
21–30 years	Older age group	5.23 ± 0.60	0.169	3.19 ± 0.516	0.777	2.77 ± 0.1	0.986	2.34 ± 0.79	0.282	5.50 ± 1.41	0.508	4.59 ± 06	0.730	2.41 ± 0.62	0.575	2.95 ± 0.53	0.999
	Younger age group	3.59 ± 1.47		2.79 ± 1.83		2.84 ± 1.9		8.67 ± 7.43		4.40 ± 2.086		3.99 ± 2.29		1.46 ± 1.01		2.95 ± 1.37	
31–40 years	Older age group	3.51 ± 2.09	0.281	4.21 ± 1.8	0.218	0.52 ± 0	0.029*	8.51 ± 0	0.425	7.46 ± 0	0.729	0.050 ± 0	0.002*	2.57 ± 0.57	0.577	4.08 ± 1.34	0.902
	Younger age group	5.44 ± 0.77		6.13 ± 0.13		1.53 ± 0.2		3.40 ± 0.45		12.01 ± 9.89		4.53 ± 0.172		2.84 ± 0.40		4.22 ± 0.63	
41–50 years	Older age group	4.44 ± 0	0.888	4.95 ± 0	0.404	0.52 ± 0	0.029*	8.51 ± 0	0.425	7.47 ± 0	0.729	0.05 ± 0	0.002*	2.58 ± 0.81		4.05 ± 0	0.517
	Younger age group	4.50 ± 0.36		5.29 ± 0.28		1.53 ± 0.2		3.40 ± 0.45		12.01 ± 9.89		4.53 ± 0.17		2.77 ± 0.11		5.08 ± 1.43	
Tobacco consumption frequency	Age grouping																
1–3 times/day	Older age group	3.00 ± 1.96	0.440	2.69 ± 1.60	0.172	2.02 ± 0.49	0.245	6.76 ± 3.90	0.680	6.93 ± 4.69	0.574	4.48 ± 1.88	0.395	2.22 ± 0.99	0.883	2.61 ± 0.97	0.580
	Younger age group	3.87 ± 1.96		4.14 ± 1.99		0.74 ± 0.74		6.01 ± 3.51		5.26 ± 2.78		8.34 ± 7.02		2.30 ± 0.91		3.01 ± 1.50	
4–6 times/day	Older age group	3.25 ± 1.75	0.171	3.20 ± 1.90	0.510	3.63 ± 2.16	0.657	12.93 ± 10.36	0.284	4.62 ± 2.98	0.766	4.04 ± 1.52	0.892	2.25 ± 0.53	0.776	3.44 ± 1.48	0.998
	Younger age group	3.97 ± 1.12		3.66 ± 1.85		6.21 ± 5.92		6.71 ± 5.82		5.15 ± 4.59		4.14 ± 1.63		2.34 ± 0.95		3.44 ± 1.37	
7–10 times/day	Older age group	3.62 ± 0.20	0.708	4.89 ± 0.311	0.017*	2.50 ± 1.90	0.366	4.39 ± 3.82	0.423	4.38 ± 0.16	0.924	2.81 ± 1.41	0.921	3.00 ± 0.32	0.123	3.24 ± 0.01	0.605
	Younger age group	3.34 ± 0.162		2.90 ± 0.197		0.12 ± 0.05		8.51 ± 1.55		4.50 ± 1.55		3.07 ± 2.19		2.33 ± 0.16		3.10 ± 0.32	

variables move in opposite directions, while a zero correlation implies no linear relationship.

2.5. Ethical declarations

This study was approved by the institutional review board of the Gauhati Medical College and Hospital, Guwahati, Assam, and all procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration. All patients provided written informed consent.

3. Results

3.1. Expression of the APC3, APC4, APC5 and APC7 genes in ESCC

Expression analysis of APC3, APC4, APC5 and APC7 among the 70 ESCC cases exhibited overall positive expression in both blood and tissue samples (Table 1). However, a significant difference was observed in the mean expression level (mean $\pm$ SD) between the groups (i.e., tissue and blood) for the expression of APC3 and APC7 only. A higher level of significance was obtained after analysis of the expression regulation within each group for both APC3 and APC7. Of 70 samples, 52 showed APC3 gene up regulation, with a mean fold change value of 4.5 $\pm$ 1.41, and 18 showed downregulation, with a mean value of 0.72 $\pm$ 0.23 (p = 0.001 < 0.05) in the tissue samples. APC3 gene expression in blood samples was upregulated in 55 samples, with a mean fold change value of 4.58 $\pm$ 1.06, and downregulated in 15 samples, with a mean value of 0.73 $\pm$ 0.12 (p = 0.006 < 0.05). Expression analysis of the APC4 gene showed upregulation (N = 41), with a mean fold change value of 7.75 $\pm$ 4.56, and downregulation (N = 29), with a mean value of 0.41 $\pm$ 0.12, in the tissue samples. In blood samples, the APC4 gene showed upregulation in 53 samples, with a mean fold change value of 9.27 $\pm$ 6.52, and downregulation in 17 samples, with a mean value of 0.27 $\pm$ 0.06. Thus, no significant difference was found between the groups (p = 0.224 > 0.05). The APC5 gene showed upregulation (N = 62), with a mean fold change value of 5.69 $\pm$ 4.1, and downregulation (N = 8), with a mean value of 0.55 $\pm$ 0.2, in the tissue samples (p = 0.004 < 0.05). The APC5 gene showed upregulation (N = 60), with a mean fold change value of 4.92 $\pm$ 3.36, and downregulation (N = 10), with a mean value of 0.22 $\pm$ 0.12, in the blood samples (p = 0.314 > 0.05). The APC7 gene was upregulated in 50 samples, with a mean fold change value of 3.75 $\pm$ 1.21, and downregulated in 20 samples, with a mean value of 0.737 $\pm$ 0.22 (p = 0.001 < 0.05), in the tissue samples. Further expression analysis of the APC7 gene in blood samples showed upregulation in 50 samples, with a mean fold change value of 4.45 $\pm$ 1.18, and downregulation in 20 samples, with a mean value of 0.8 $\pm$ 0.15 (p = 0.001 < 0.05) (Table 2; Figure 1).

3.2. Association of the gene expression of APC3, APC4, APC5 and APC7 in blood samples and tissue samples with clinicopathological parameters in ESCC

The association of altered expression of APC3, APC4, APC5 and APC7 in tissue and blood samples with different clinicopathological parameters, such as age, sex, dysphasia, duration of dysphasia, tumor size, tumor node, metastasis, effect of therapy, and differentiation level, in ESCC cases was analyzed (Table 2).

Age-based stratification of the ESCC cases exhibited gradual upregulation of APC3 and APC7 expression in both blood and tissue samples with increasing age, in addition to APC5 expression in blood (p = 0.023 < 0.05) (Table 2). A significant mean difference was observed in APC3 expression among the different age groups for blood (p = 0.010 < 0.05) and tissue (p = 0.037 < 0.05) samples. Similarly, the expression of APC7 also exhibited a significant mean difference in both the blood (p = 0.033 < 0.05) and tissue (p = 0.025 < 0.05) of ESCC cases from different age groups. Analysis of the association of gene expression with different dysphasia types showed significant differences in the mean expression of

APC3 and APC7 in blood (p = 0.001, p = 0.025 < 0.05, respectively) and tissue (p = 0.011, p = 0.029 < 0.05) samples in addition to APC4 (p = 0.038) and APC5 (p = 0.001) expression in blood samples only. Tumor size, as a clinicopathological parameter, showed a significant association with altered expression of APC3, APC5 and APC7. The mean expression difference with advancing tumor size was significant for APC3 in tissue (p = 0.007) and blood (p = 0.004). A similar association was also observed in the expression of APC5 and APC 7 in different tumor size categories (Table 2). Tumor node stage was significantly associated with the mean expression difference in APC5 in blood samples (p = 0.027). However, the mean expression difference in APC7 in blood samples exhibited weak significance (p = 0.079) with advancing tumor node stage. Analysis of metastasis revealed a significant association with the mean expression difference of APC4 in blood (p = 0.054). Furthermore, differentiation was associated with the mean difference in APC5 in blood with advancing tumor differentiation from poorly differentiated to well-differentiated categories. However, clinicopathological parameters such as sex, dysphasia duration, and both chemotherapy and radiation therapy showed no significant association with the mean expression difference in APC/C subunits.

3.3. Association of the gene expression of the APC3, APC4, APC5 and APC7 genes in blood and tissue samples and lifestyle factors in ESCC

We also analyzed the association of APC3, APC4, APC5 and APC7 gene expression in blood and tissue samples with different lifestyle factors, such as smoking frequency, duration of smoking, smoking category, duration of alcohol consumption, category of alcohol consumption, duration of betel nut consumption, category of betel nut chewing, tobacco consumption frequency, duration of tobacco consumption, and tobacco consumption category, in ESCC patients.

Considering smoking as a lifestyle factor, a significant mean difference was observed in the expression of APC3 in the tissue (p = 0.016 < 0.05) and blood samples (p = 0.027) between smokers and nonsmokers. However, APC4, APC5, and APC7 showed no significant association with smoking category within the studied cases. Further analysis of the expression association among the smokers, considering the duration and frequency of smoking as a factor, exhibited a significant difference in the mean expression of APC3 and APC7 (Table 3) between the different groups within the factor.

No significant association was found between the expression of the evaluated genes and drinking alcohol; however, the duration of drinking alcohol showed a significant mean difference in the expression of APC7 in blood samples (p = 0.017 < 0.05) among drinkers with varied durations of drinking habits.

Betel nut chewing, as a lifestyle factor, exhibited a significant mean difference in the expression of APC3 and APC7 in both tissue and blood samples between chewers and nonchewers. The mean expression of APC3 in tissue samples was 3.95 $\pm$ 1.83 for chewers (N = 48) and 2.61 $\pm$ 2.27 for nonchewers (N = 22) (P = 0.011 < 0.05). APC3 expression in blood samples was 4.14 $\pm$ 1.57 for chewers and 2.93 $\pm$ 2.16 for nonchewers (p = 0.011). Similarly, the mean expression of APC7 in tissue was 3.24 $\pm$ 1.65 for chewers and 2.00 $\pm$ 1.62 for nonchewers (p = 0.005); that in blood samples was 3.87 $\pm$ 1.80 for chewers and 2.39 $\pm$ 2.14 for nonchewers (p = 0.004). The expression of APC4 and APC5 exhibited no significant association with this lifestyle factor. In this study, the duration of betel nut chewing showed no significant association with altered expression of the genes.

Analysis of tobacco consumption as a lifestyle factor revealed a significant difference in the expression of APC3 and APC7 in both tissue (p = 0.001 and p = 0.004, respectively) and blood (p = 0.001 and p = 0.005, respectively) samples between consumers and nonconsumers. Further detailed analysis of this factor considering the duration of tobacco consumption and its frequency also showed a highly significant association with altered expression of APC3 and APC7 in both tissue and blood samples and APC5 in blood samples only (Table 4).

Table 5. Survival analysis data of ESCC patients.

Parameters	Groups	Mean estimate	95% C.I Lower Bound	Upper bound	P value
Age group	30–39 years	30.71	18.36	43.06	0.536
	40–49 years	18.28	5.40	31.16	
	50–59 years	23.92	18.15	29.69	
	60–69 years	22.33	16.51	28.14	
	70–79 years	26.66	17.43	35.89	
Gender	Male	25.55	20.79	30.27	0.306
	Female	21.77	16.39	27.16	
Dysphasia	solid	29.31	18.32	42.32	0.431
	solid + liquid	18.20	6.40	3231	
	no dysphasia	23.90	20.11	29.31	
Duration of dysphasia	1 month	30.88	22.36	38.40	0.216
	2 month	17.60	11.99	23.20	
	3 month	24.33	17.80	30.85	
	4 month	19.16	4.54	33.79	
	5 month	22.83	10.06	35.59	
	6 month	26.19	15.52	36.47	
Chemotherapy	Yes	20.19	14.90	25.48	0.066
	No	27,20	22.48	31.92	
Radiation Therapy	Yes	20.08	16.012	24.155	**0.003***
	No	32.86	27.061	38.667	
Both Therapy	Yes	20.30	16.30	24.30	**0.003***
	No	32.95	26.82	39.08	
Metastasis	Yes	19.58	14.56	24.60	**0.027***
	No	27.69	22.87	32.51	
Alcohol category	Non alcoholic	21.72	17.69	25.75	**0.040***
	Alcoholic	30.47	23.48	37.46	
Duration of betel nut consumption	1–10yrs	25.13	16.47	33.78	0.655
	11–20 yrs	27.78	20.88	34.69	
	21–30yrs	25.83	10.43	41.23	
	31–40yrs	22.08	13.72	30.44	
	41–50yrs	19.33	9.06	29.60	
Betel nut category	Non chewers	32.72	26.78	38.67	**0.003***
	chewers	20.14	16.10	24.19	
APC3 expression in tissue	High	20.19	16.42	23.96	**0.001***
	Low	35.38	28.88	41.89	
APC3 expression in blood	High	21.00	17.20	24.79	**0.003***
	Low	35.46	28.52	42.41	
APC4 expression in tissue	High	26.59	21.71	31.47	0.079
	Low	20.35	15.37	25.34	
APC4 expression in blood	High	25.83	21.63	30.03	0.068
	Low	18.70	12.29	25.11	

Parameters	Groups	Mean estimate	95% C.I Lower Bound	Upper bound	P value
Tumor Size	T2	28.20	21.13	35.26	**0.023***
	T3	26.10	20.31	31.88	
	T4a/4b	17.00	12.45	21.54	
Tumor node	N0	32.11	22.11	42.54	0.512
	N1	24.06	16.63	31.49	
	N2	22.05	15.47	28.64	
	N3	23.50	17.57	29.42	
Differentiation	Poorly	38.46	32.84	44.08	**.000***
	Moderately	26.15	19.14	33.17	
	well	17.02	13.10	20.95	
Smoking Frequency	1-5 times/day	29.40	20.64	38.15	0.787
	6-10 times/day	34.80	23.56	46.03	
	16-20 times/day	25.00	8.97	41.03	
Duration of smoking	1–10yrs	17.60	4.16	31.03	0.866
	11–20 yrs	18..00	9.78	26.21	
	21–30yrs	13.70	6.85	20.54	
	31–40yrs	18..75	11.53	25.96	
Smoking category	Non smokers	29.52	24.87	34.17	**0.004***
	Smokers	18.35	13.47	23.23	
Duration of alcohol consumption	1–10yrs	18.02	5.31	13.03	0.784
	11–20 yrs	17.04	10.34	27.41	
	21–30yrs	28.45	17.34	2940	
	31–40yrs	20.34	14.43	21.00	
Frequency of tobacco consumption	1-3ttimestimes/day	31.40	20.72	42.07	**0.007***
	4-6 times/day	17.79	12.84	22.74	
	7-10 times/day	15.66	9.41	21.92	
Duration of tobacco consumption	1–10yrs	29.00	18.36	39.64	**0.014***
	11–20 yrs	22.66	16.17	29.15	
	21–30yrs	13.77	6.12	21.43	
	31–40yrs	14.50	7.51	21.48	
	41–50yrs	16.50	.000	35.12	
Tobacco category	Non chewers	28.40	22.84	33.35	0.050
	chewers	20.87	16.37	25.37	
APC5 expression in tissue	High	24.93	21.17	28.70	0.187
	Low	17.62	6.45	28.79	
APC5 expression in blood	High	24.26	20.37	28.15	0.827
	Low	23.10	13.42	32.78	
APC7 expression in tissue	High	19.93	16.12	23.75	**0.001***
	Low	33.81	27.39	40.22	
APC7 expression in blood	High	19.91	16.05	23.78	**0.001***
	Low	33.22	26.92	39.53	

Note: *statistically significant p value < 0.05.
Note: *statistically significant p value < 0.05.
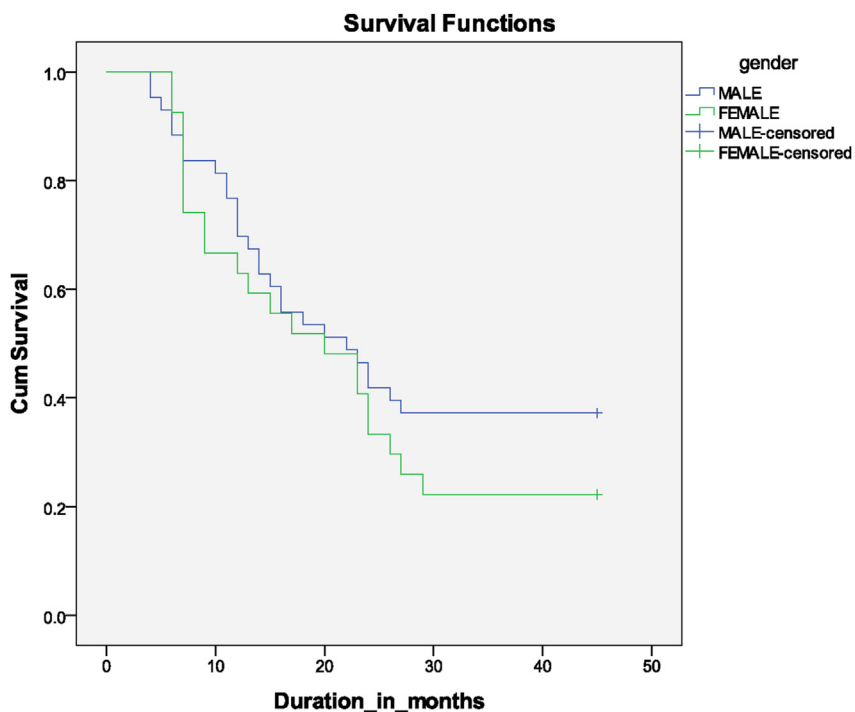
Figure 2. Plot of Kaplan-Meier analysis for age group.
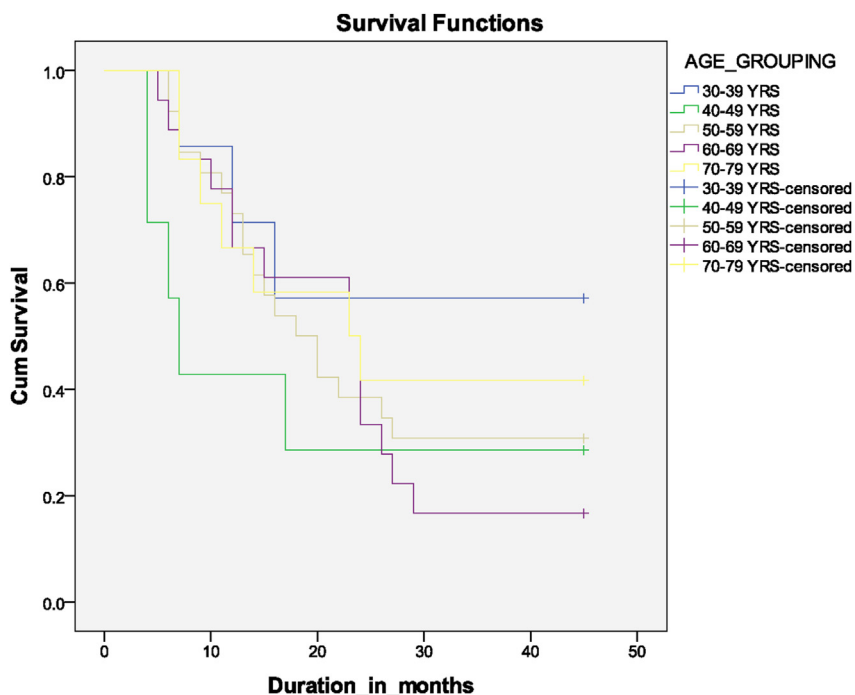
Figure 3. Plot of Kaplan-Meier analysis for age grouping.

3.4. Association of APC3, APC4, APC5 and APC7 expression with age considering different lifestyle factors

The expression association study with different clinicopathological parameters revealed that age was significantly associated with the altered expression of APC/C subunit genes. Further stratification based on the age of individuals as younger and older age groups demonstrated a significant association of the expression of APC3 with smoking frequency

($p = 0.001$), alcohol consumption category ($p = 0.043$) and its duration ($p = 0.02$) in tissue samples. For APC7, no significant association was found with any of the lifestyle factors after age-based separation into younger and older groups. However, the loss of a higher degree of significance between the expression level of the genes and lifestyle factors after age-based stratification may be due to the considerably lower number of patients in the younger age group category (N = 14) than in the older age group category (N = 56) (Table 4).
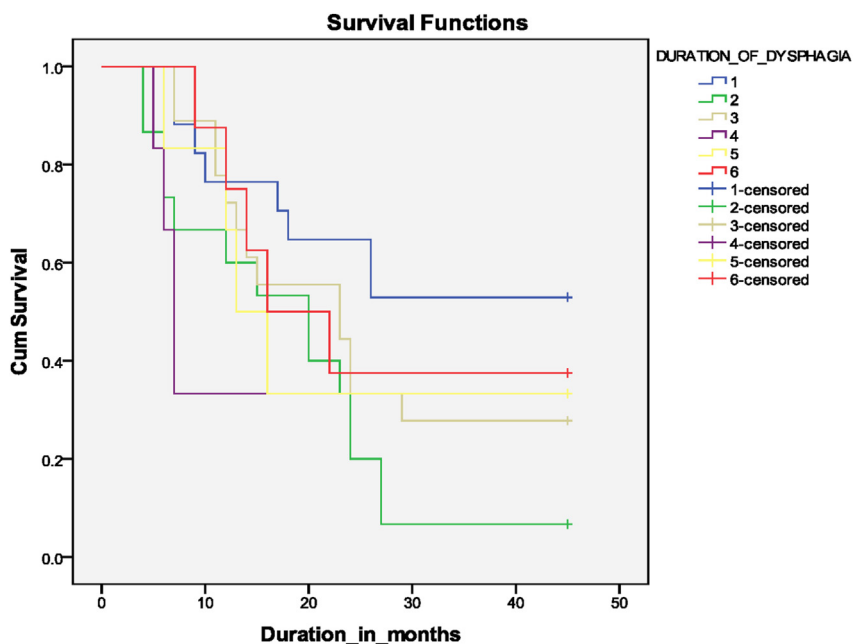
Figure 4. Plot of Kaplan-Meier analysis for duration of dysphasia.
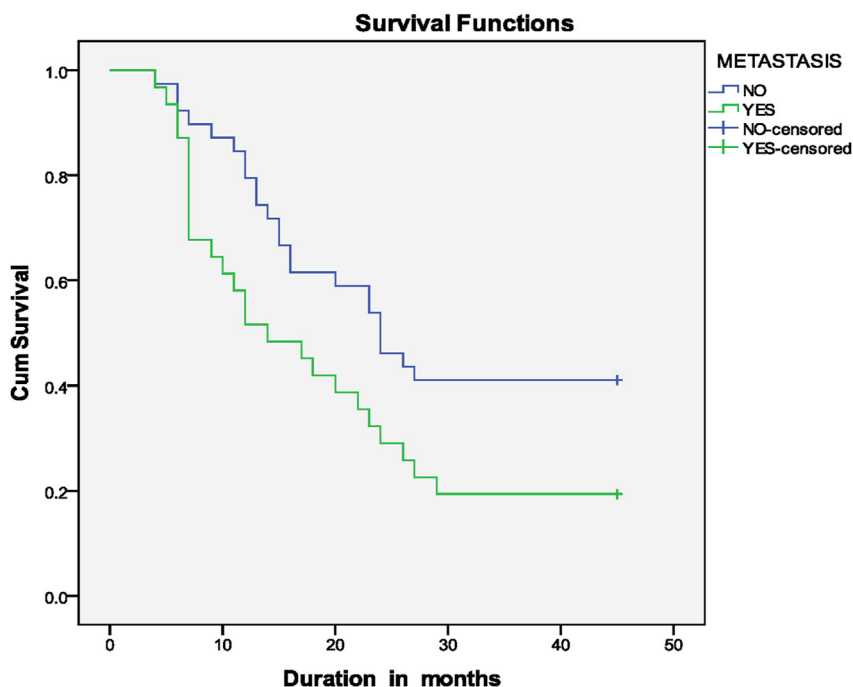
Figure 5. Plot of Kaplan-Meier analysis for Metastasis.

3.5. Survival analysis

All 70 ESCC patients were followed up until death or up to 45 months from the date of sampling, whichever was earlier, and Kaplan–Meier survival analysis was performed with different lifestyle and clinico-pathological factors to check their role in ESCC. Among the clinico-pathological parameters, tumor stage, metastasis, chemotherapy, radiation therapy, both chemotherapy and radiation therapy and tumor differentiation category exhibited significant associations with patient survival. Furthermore, the mean survival time for patients with different tumor stages T2, T3 and T4a/T4b was 28 months, 26 months and 17 months, respectively ($p = 0.023 < 0.05$). The presence or absence of

metastasis also demonstrated a significant association with patient survival. Patients without metastasis showed better survival, with a mean survival time of 27 months ($p = 0.027 < 0.05$). Additionally, the tumor differentiation level showed higher significance ($p = 0.000$), with the mean survival time of patients categorized as having poor, moderate and well-differentiated tumors (38 months, 26 months, and 17 months, respectively). Interestingly, survival analysis also showed that patients receiving chemotherapy, radiation therapy or both had better survival times ($p = 0.066$, $p = 0.003$, and $p = 0.003$, respectively) (Table 5).

Considering smoking as a lifestyle factor, a significant difference was observed in the mean survival time between smokers and nonsmokers (18 months and 29 months, respectively; $p = 0.004$). Similarly, a
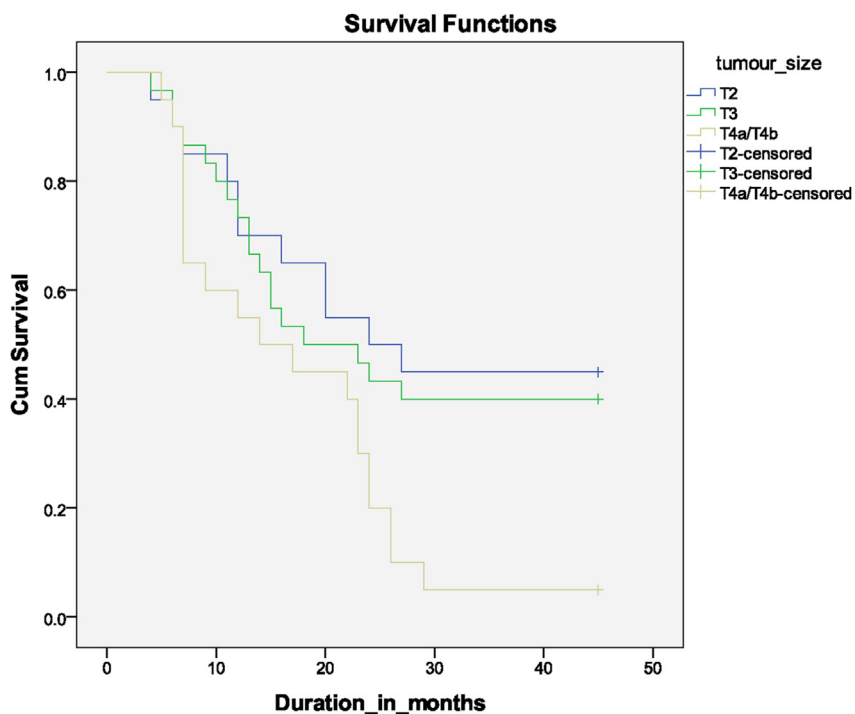
Figure 6. Plot of Kaplan-Meier analysis for tumor size.
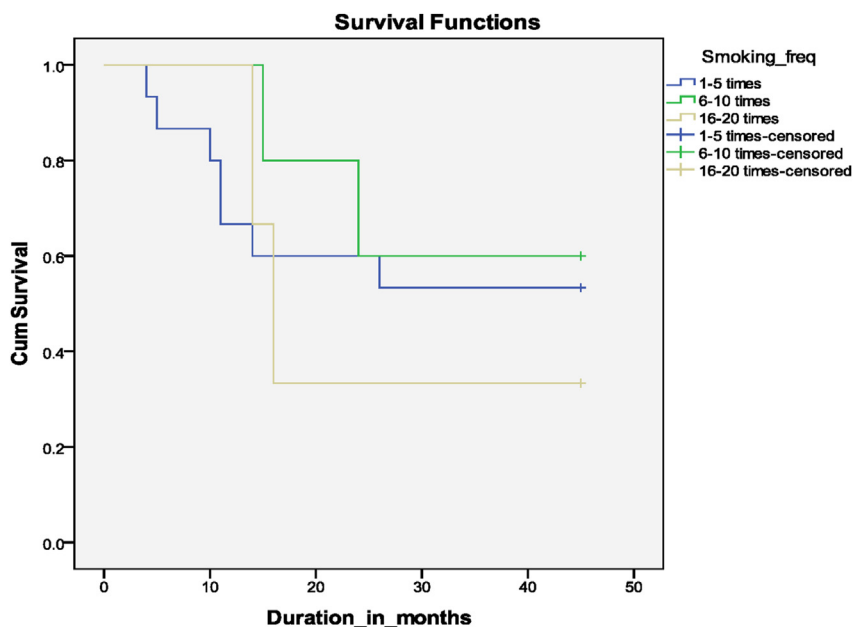
Figure 7. Plot of Kaplan-Meier analysis for smoking frequency.

significant difference was also observed between tobacco chewers and nonchewers (mean survival times of 20 months and 28 months, respectively; p = 0.050). Further analysis among the tobacco chewers considering tobacco chewing duration (p = 0.014) and frequency (p = 0.007) as factors revealed a significant difference in the mean survival time (Table 5) among the groups. The mean survival time for betel nut chewers was 20.14 months, and that for nonchewers was 32.72 months, indicating a significant association with this factor (p = 0.003).

Further analysis of the association between the expression of APC/C genes and survival time of patients showed a significant association with the mean expression of APC3 and APC7 only in tissue and blood samples. APC3 upregulation in tissue showed a mean survival time of 20 months, and that for blood was 21 months, indicating a significant difference (p = 0.001 and p = 0.003 < 0.05, respectively) with APC3 downregulation. APC7 upregulation in blood and tissue samples was also associated with the survival duration of patients (p = 0.001 < 0.05). Altered expression
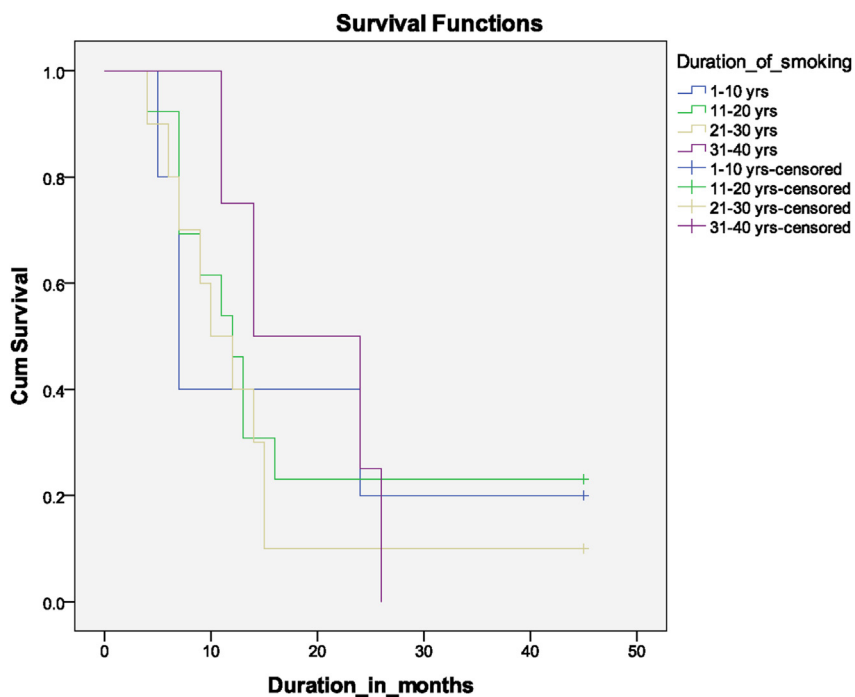
Figure 8. Plot of Kaplan-Meier analysis for duration of smoking.
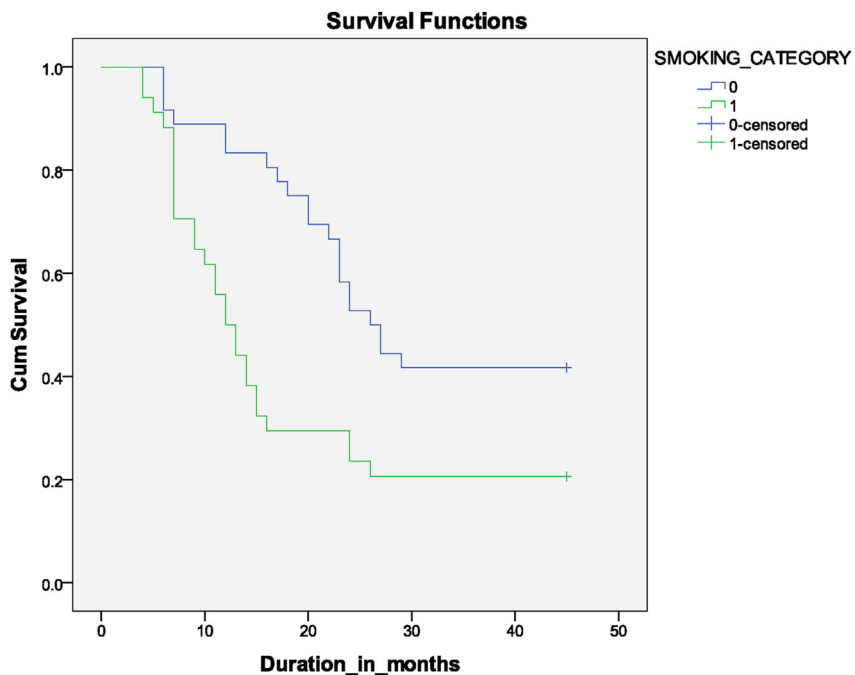
Figure 9. Plot of Kaplan-Meier analysis for smoking frequency.

of APC4 and APC5 was not significantly associated with patient survival (Figures 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23).

3.6. Cox regression analyses and hazard outcomes

According to the univariate model of Cox regression, the hazard ratio (HR) represents the ratio of the hazard outcomes corresponding to the conditions represented by 2 groups of a variable. An HR of 1 indicates no survival difference between the 2 groups, and an HR of less than or equal to 1 indicates that one group possesses better survival than the other. According to this model, the hazard (mortality) ratio for metastatic patients was 0.54 times that of nonmetastatic patients ($p = 0.033 < 0.05$). The HR obtained for patients receiving radiation therapy was 2.71 times that of patients without radiation therapy ($p = 0.005$). Patients receiving both radiation and chemotherapy also exhibited a hazard ratio of 0.60 ($p = 0.006$) compared with nonreceivers. Among the different lifestyle factors, alcoholic patients showed an HR of 2.06 times that of the nonalcoholic group ($p = 0.05$). Further duration of alcohol consumption
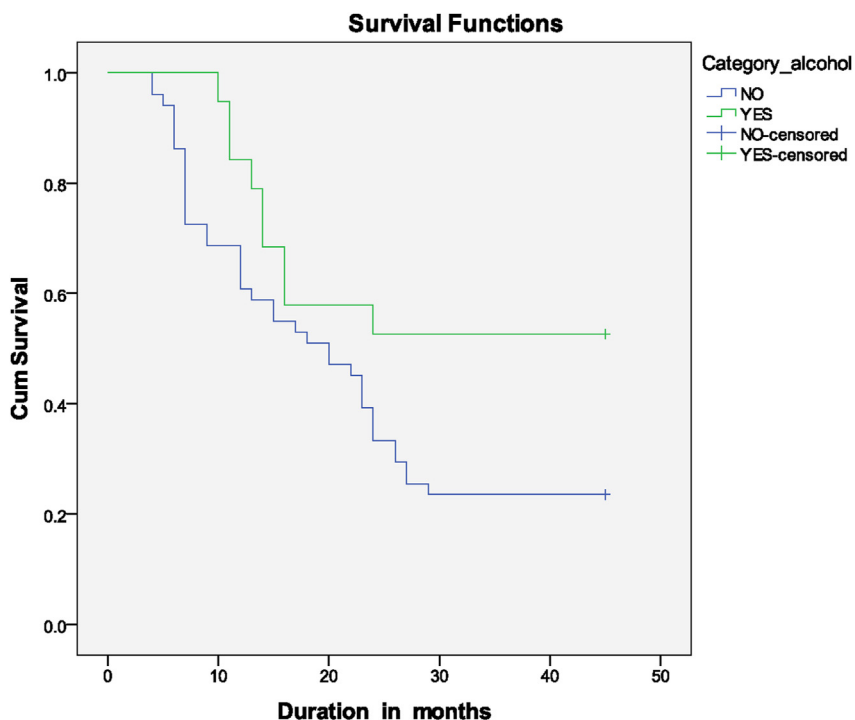
Figure 10. Plot of Kaplan-Meier analysis for category of alcohol consumption.
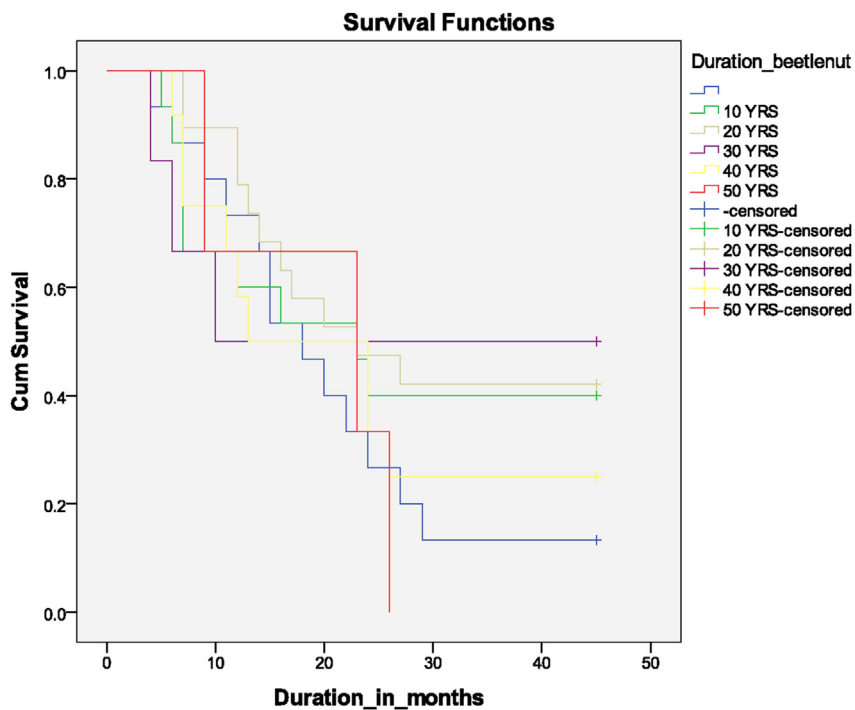
Figure 11. Plot of Kaplan-Meier analysis for duration of betel nut.

also exhibited a significant (p = 0.043) hazard ratio. Similarly, the hazard ratio was 0.61 for betel nut chewers and 0.75 for tobacco chewers compared with nonchewers (p = 0.006 and p = 0.053, respectively). In addition to the lifestyle and clinicopathological parameters, altered expression of APC3 and APC7 exhibited a significant hazard ratio between the groups (p < 0.05) (Table 6).

3.7. Pearson correlation analysis of APC3, APC4, APC5 & APC7 expression in blood and tissue in ESCC

Pearson correlation analysis of the association of expression for APC3, APC4, APC5 and APC7 in tissue and blood samples revealed a significant association between APC3 and APC7 expression in both tissue and blood,
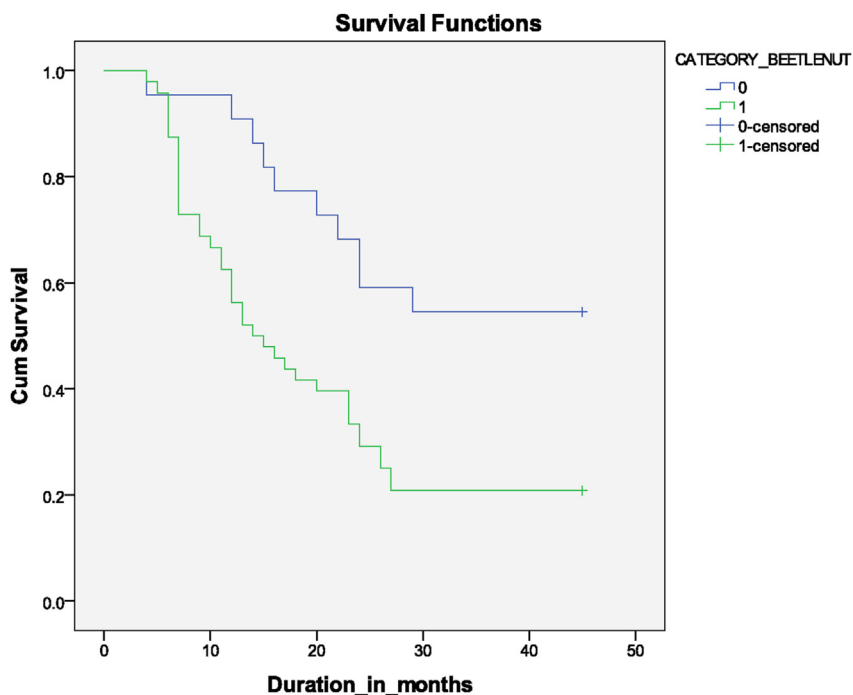
Figure 12. Plot of Kaplan-Meier analysis for category of betel nut.
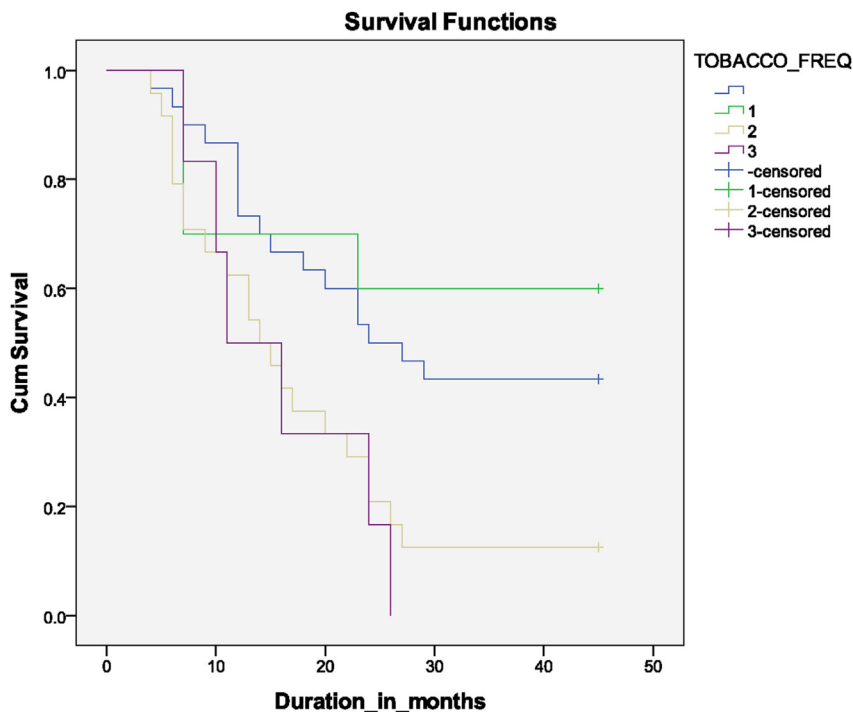
Figure 13. Plot of Kaplan-Meier analysis for tobacco frequency.

considering that the correlation was significant at the 0.01 level (2-tailed). Further survival and hazard analysis data also reflected a significant association of the expression of the gene pair (APC3 and APC7) with the mean survival duration of ESCC patients (Table 5 & Table 6). Thus, with a Pearson correlation coefficient (r) value greater than 0.7 and $p = 0.000 < 0.05$, the gene pair exhibits a higher degree of correlation and can be considered a potential prognostic gene pair for ESCC detection (Table 7 & Table 8).

4. Discussion

The present study analyzed the hypothesized association between altered APC/C subunit expression and tumorigenesis in esophageal carcinoma cases. Semiquantitative real-time PCR-based expression analysis showed considerable positive expression of the APC/C subunit under investigation (APC3, APC4, APC5, and APC7), specifically in blood samples compared with tissue samples.

Survival Functions
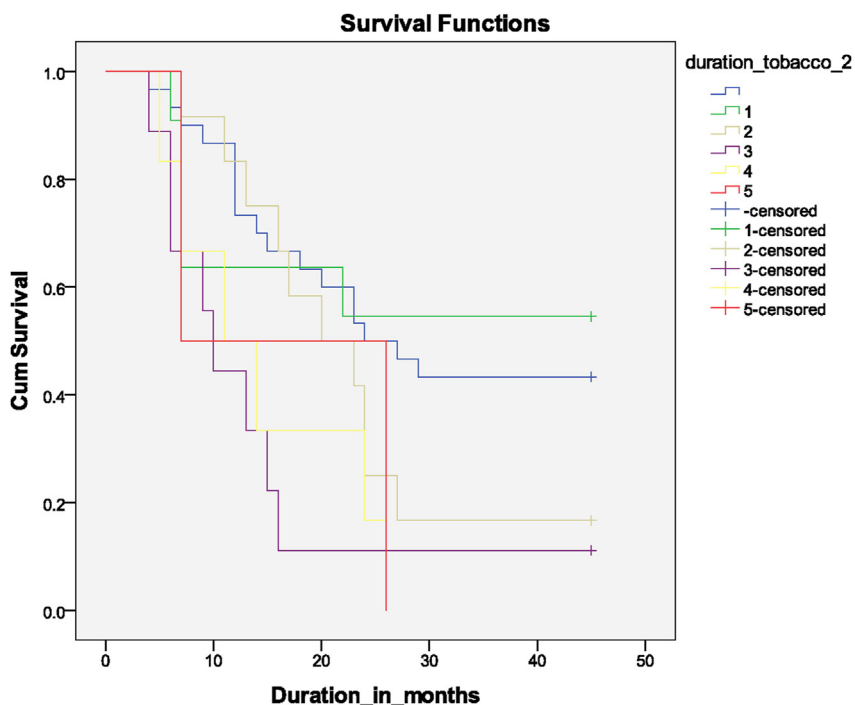
Figure 14. Plot of Kaplan-Meier analysis for duration of tobacco consumption.

Survival Functions
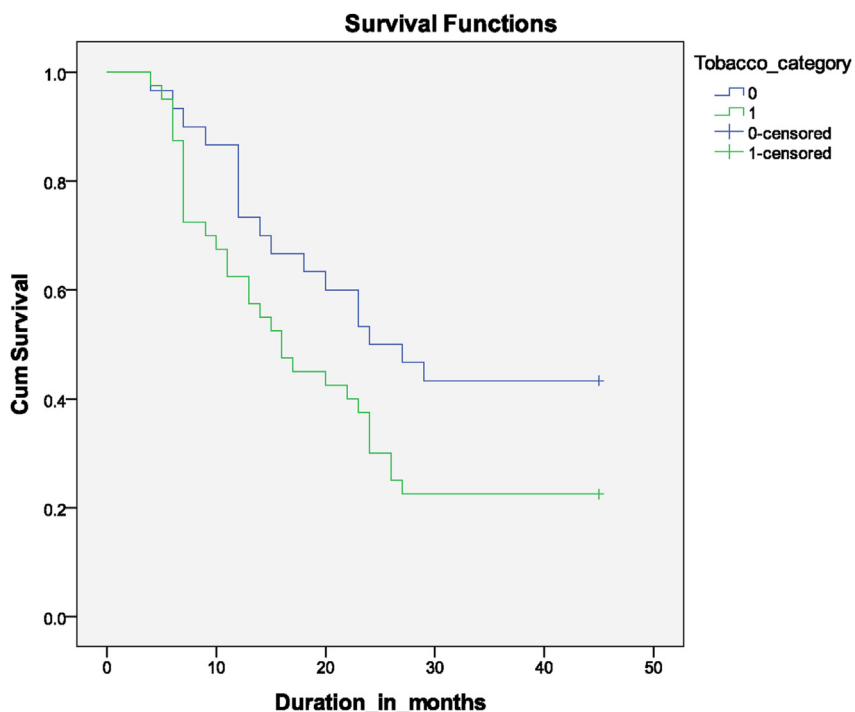
Figure 15. Plot of Kaplan-Meier analysis for tobacco category.

APC3/Cdc27 expression was found upregulated in most of the blood samples (N = 55), with a mean fold change value of 4.58 ± 1.06. Comparative statistical analysis of APC3 expression in blood using different clinicopathological parameters among the studied population revealed a significant correlation with the patient age, duration of dysphasia, and tumor size. Among the different lifestyle factors, the smoking category, betel nut chewing category, duration of alcohol consumption, tobacco consumption category, and consumption frequency and duration exhibited significant associations with altered expression of

APC3 in blood. An almost similar pattern of association was observed for APC3 expression in tissue. Survival analysis showed a significant impact of the abovementioned different lifestyle and clinicopathological parameters on the mean survival duration of patients (Table 5). Furthermore, survival and hazard analysis of altered APC3 expression in blood showed a mean survival time of 20 months (approx.) with upregulation of expression and a hazard ratio of 0.27 (APC3 blood expression low/high; p = 0.007 < 0.05). Thus, APC3 upregulation possesses a potentially hazardous association with ESCC in addition to significantly affecting
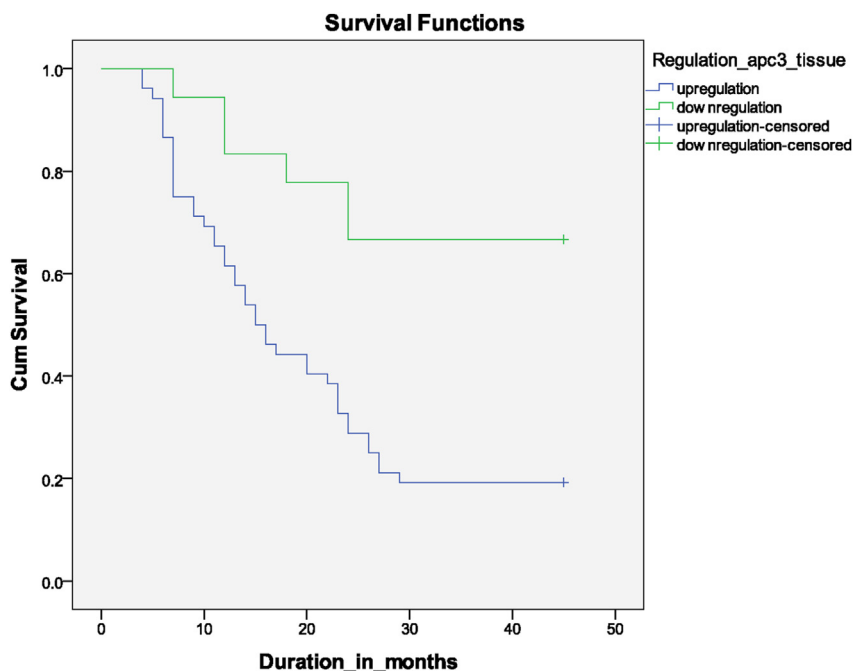
Figure 16. Plot of Kaplan-Meier analysis for expression of APC3 in tissue.
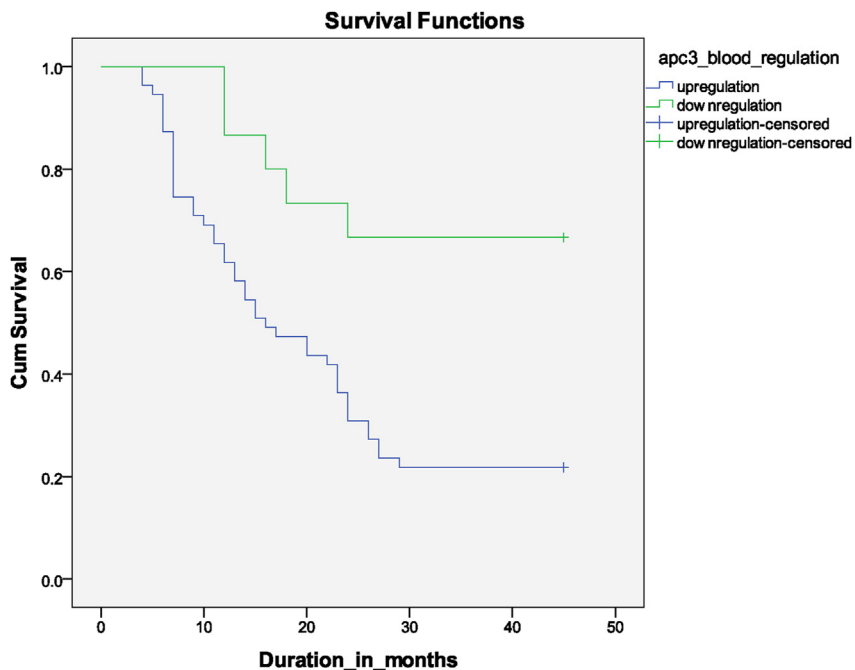
Figure 17. Plot of Kaplan-Meier analysis for APC3 in blood.

patient survival (p = 0.003). Furthermore, a significant mean expression difference in APC3 upregulated tissue and blood samples from patients with and without metastasis also supports APC3 upregulation-mediated advancement toward tumor metastasis. Thus, APC3 upregulation may suppress its inhibitory effect on tumorigenesis in addition to its association with a poor treatment response to radiation therapy [25]. In our study, the data obtained for APC3 expression were in good concordance when analyzed in blood and tissue samples.

APC4 expression showed a significant association with dysphasia type (p = 0.038 < 0.05) and metastasis (p = 0.054) only in the blood samples of ESCC cases. Further survival analysis data also showed no significant

impact on the mean survival duration of patients (p = 0.079 and 0.068 for tissue and blood, respectively). Hazard ratios of 0.611 in tissue and 1.761 in blood samples were obtained that were not significantly associated with patient survival.

APC5 expression analysis showed upregulation of expression in almost equal numbers of blood (N = 60) and tissue (N = 62) samples, with mean fold change values of 4.927 ± 3.367 and 5.694 ± 4.105, respectively. Comparative statistical analysis revealed that APC5 expression in blood was positively and directly correlated with the age of the individual in addition to the tumor size, tumor node stage and degree of differentiation (p < 0.05). APC 5 functions with APC7. Levine, K.N.
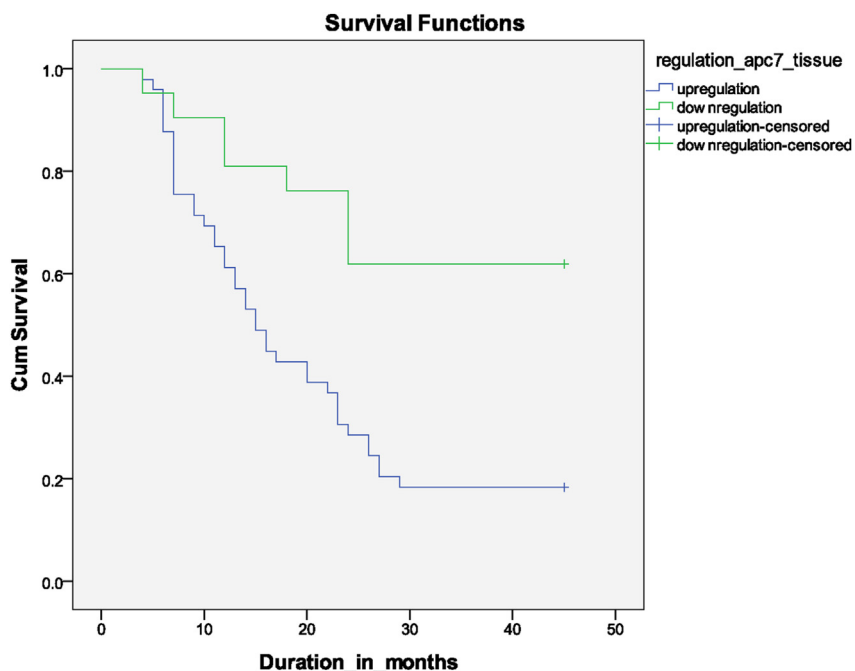
Figure 18. Plot of Kaplan-Meier analysis for expression of APC7 tissue.
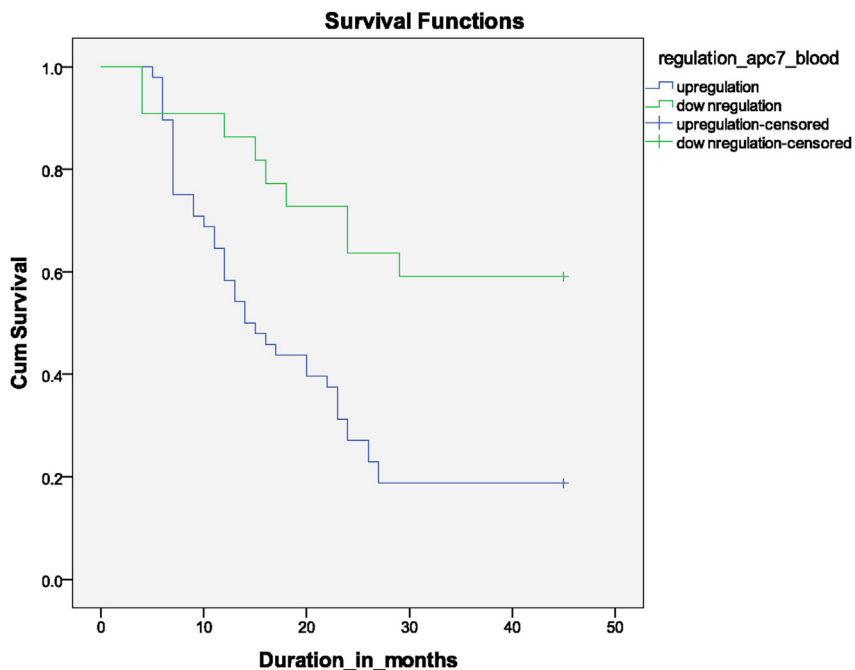

Figure 19. Plot of Kaplan-Meier analysis for expression of APC7 in blood.

et al., 2004 reported in a yeast three-hybrid study that APC5 and APC7 interact with the transcription activator CBP/p300, which has histone acetyltransferase activity and mediates transcriptional activation [26].

APC 7, which is a component of the tetratricopeptide repeat motif of E3 ubiquitin ligase, is generally expressed in normal tissue at a fairly constant level and interacts with cdc20 and cdh1 to regulate the cell cycle by promoting the transition from metaphase to anaphase [27]. Semi-quantitative real-time PCR-based expression analysis of APC7 showed considerable positive expression in both tissue and blood samples, with average fold changes of 2.81 ± 1.71 and 3.40 ± 1.93, respectively.

Furthermore, the findings were compared with clinicopathological parameters. With increasing age, the mean APC7 expression increased and the mean difference between the different age groups was significant. In our study, APC7 expression was significantly upregulated in both blood and tissue samples among the different age groups. While analyzing the expression pattern of APC7 among dysphasia types, significant alterations in the expression pattern were observed. Significant upregulation of APC7 expression with advancing tumor stage from T2 to T4a/T4b was also observed in both blood and tissue samples. However, a lower level of significance was observed for APC7 expression in blood
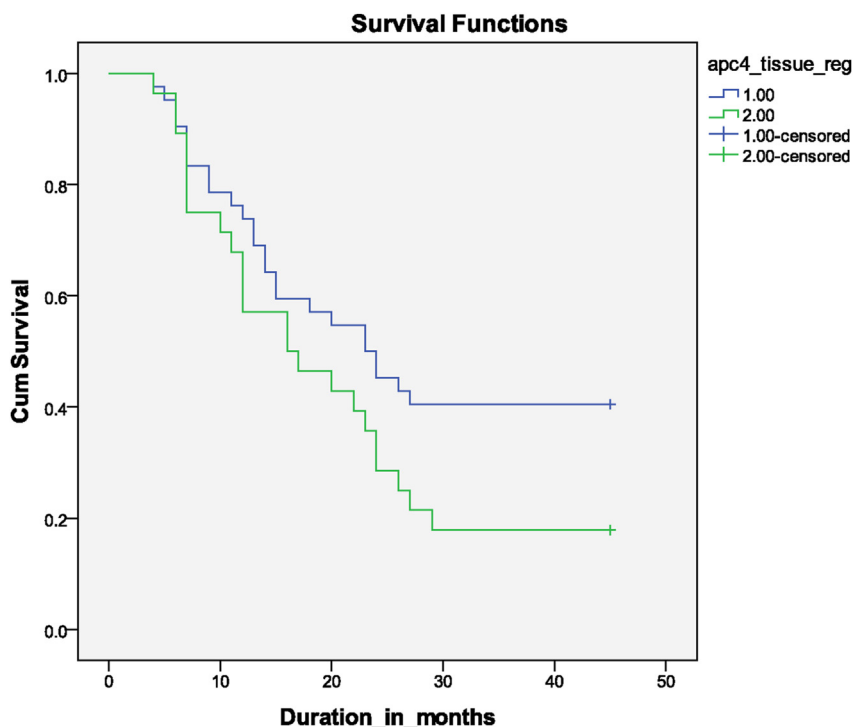
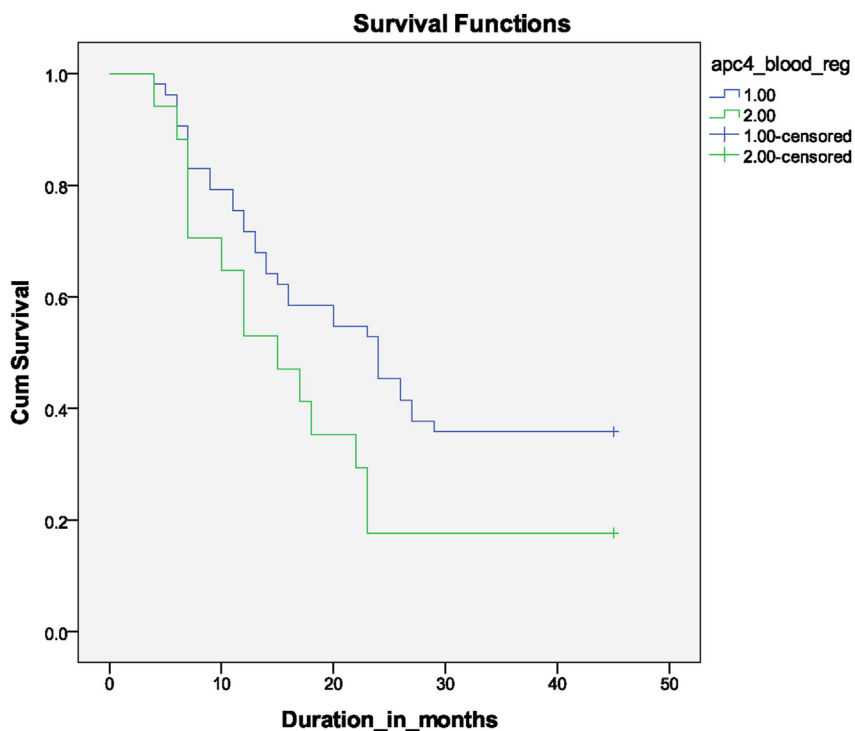**Figure 20.** Plot of Kaplan-Meier analysis expression of APC4 in tissue.

Figure 21. Plot of Kaplan-Meier analysis for expression APC4 in blood.

with advancing tumor nodes from N0 to N3. These findings are predictive of APC7 expression alteration and esophageal carcinoma tumorigenesis.

Various studies have reported that lifestyle factors, such as smoking, tobacco consumption, and betel nut chewing, are associated with different types of cancer [28, 29]. The present study also analyzed various lifestyle factors to determine their association with altered APC7 expression. A significant and strong correlation was suggested between

factors such as betel nut consumption, tobacco consumption category, duration of tobacco consumption and its frequency, duration of alcohol consumption and duration of smoking and APC7 expression [30]. Our study findings were also consistent because all 51 ESCC cases (72.85%) with a history of betel nut chewing showed positive APC7 expression with a mean fold change value of 3.23 ± 1.6 in blood. Furthermore, the mean fold change in APC7 expression between tobacco chewers and
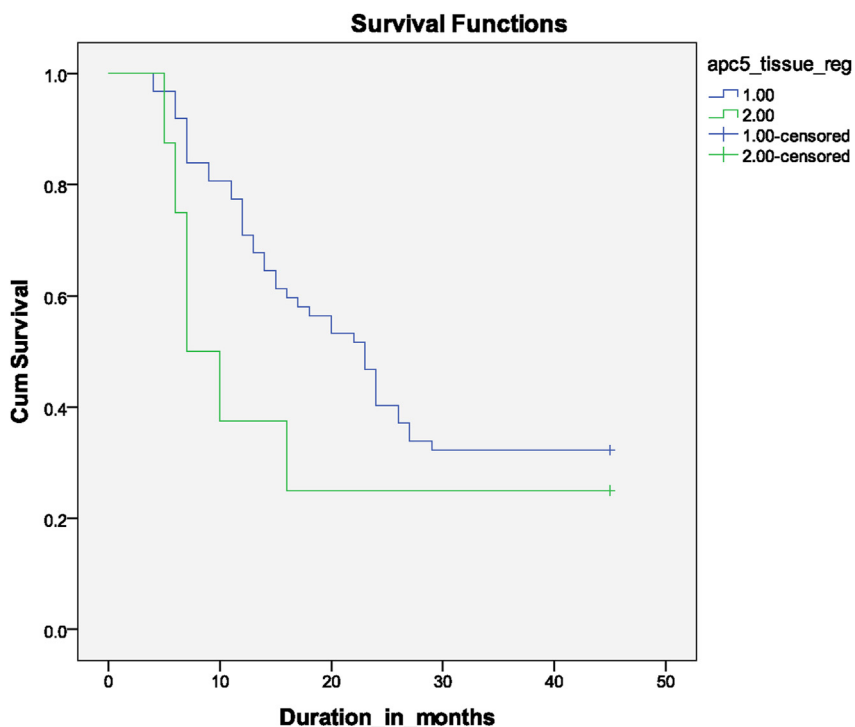
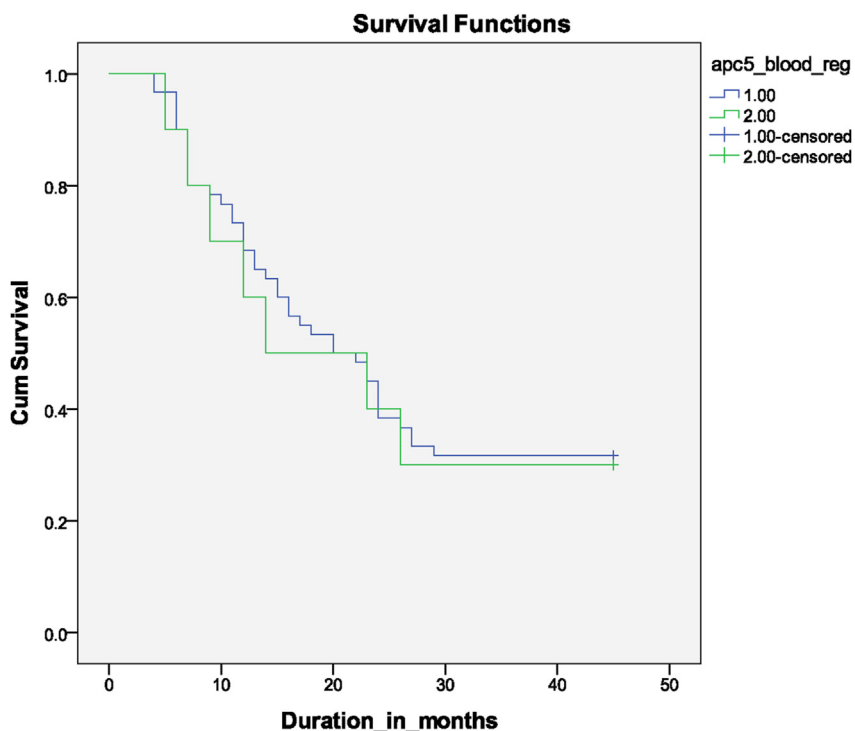**Figure 22.** Plot of Kaplan-Meier analysis for expression APC5 in tissue.

Figure 23. Plot of Kaplan-Meier analysis for expression of APC5 in blood.

nonchewers was 3.36 ± 1.60 and 2.18 ± 1.69, respectively, in tissue and 3.98 ± 1.9 and 2.64 ± 1.95, respectively, in blood samples. The difference in fold change obtained was significant; following stratification based on the duration and frequency of tobacco consumption, chewers showed significant upregulation of APC7 expression with increasing duration and frequency. No significant association of APC7 expression with the alcohol consumption category was found in our study

considering an alcohol consumption history up to 40 years in either the blood or tissue samples of ESCC patients. However, the duration of alcohol consumption showed a significant association in blood samples only. A significant association with the duration of smoking is another lifestyle-associated risk factor for ESCC.

Survival analysis and hazard analysis data of ESCC patients showed that the survival of esophageal cancer patients was significantly related

Table 6. Hazard analysis of ESCC patients.

Parameters	Group1/group2	p value	Hazard Ratio	95% Confidence interval Lower Bound	95% Confidence interval Upper Bound	Parameters	Group1/group2	p value	Hazard Ratio	95% Confidence interval Lower Bound	95% Confidence interval Upper bound
Age	40 yrs-49yrs/30 yrs–39 yrs	0.555	0.665	0.172	0.532	**Alcohol category**	Alcoholic/Non alcoholic	**0.050***	2.067	1.00	4.27
	50yrs–59yrs/30 yrs–39 yrs	0.253	1.954	2.573	3.056	**Duration of alcohol consumption**	1–10yrs/31-40yrs	0.271	1.371	0.781	2.407
	60yrs–69yrs/30 yrs–39 yrs	0.586	1.275	0.619	0.598		11–20 yrs/31-40yrs	**0.043***	0.372	0.193	0.968
	70yrs–79yrs/30 yrs–39 yrs	0.402	1.468	6.163	3.603		21–30yrs/31-40yrs	0.684	0.794	0.262	2.406
Gender	Female/Male	0.319	1.337	0.755	2.366	**Betel nut category**	Chewers/Non chewers	**0.006***	0.610	0.430	0.867
						Duration of betel nut consumption	1–10yrs	0.328	1.322	0.756	2.310
							11–20 yrs	0.628	0.856	0.455	1.608
Dysphasia	Solid/no dysphasia	0.542	1.942	2.351	3.034		21–30yrs	0.276	0.721	0.40	1.299
	solid + liquid/no dysphasia	0.345	1.232	0.625	0.845		31–40yrs	0.602	0.769	0.287	2.061
							41–50yrs	0.742	1.11	0.592	2.08
Duration of dysphasia	2 month/1month	0.437	0.641	0.209	1.965	**Tobacco category**	Chewers/Non chewers	0.053	0.751	0.558	1.01
	3 month/1month	0.231	1.869	0.672	5.20	**Frequency of tobacco consumption**	1-3 times per day	0.157	0.701	0.429	1.14
	4 month/1month	0.792	1.142	0.409	3.221		4-6 times per day	0.063	0.476	0.218	1.04
							6-10 times per day	**0.045***	1.614	1.011	2.577
Tumor Node	Stage 1/Stage 0	0.203	0.494	0.167	1.46	**Duration of tobacco consumption**	1–10yrs/41-50yrs	0.543	0.321	1.24	2.310
	Stage 2/Stage 0	0.907	0.956	0.452	2.02		11–20 yrs/41-50yrs	0.432	0.212	1.481	3.312
	Stage 3/Stage 0	0.758	1.116	0.55	2.24		21–30yrs/41-50yrs	0.452	0.312	0.31	0.821
Metastasis	Present/Absent	**0.033***	0.539	0.305	0.953		31–40yrs/41-50yrs	0.241	0.272	2.13	4.310
Smoking category	Smokers/Non smokers	**0.006***	0.666	0.499	0.889						
Smoking frequency	6-10/1-5 times per day	0.726	0.754	0.155	3.66	**APC3 expression in tissue**	Low/High	**0.002***	0.259	0.109	0.611
	16-20/1-5 times per day	0.502	0.510	0.071	3.64	**APC3 expression in blood**	Low/High	**0.007***	0.278	0.110	0.703
Duration of smoking	1–10yrs/31-40yrs	0.799	1.198	0.298	4.80	**APC7 expression in tissue**	Low/High	**0.003***	1.284	1.087	1.517
	11–20 yrs/31-40yrs	0.862	1.109	0.346	3.55	**APC7expression in blood**	Low/High	**0.003***	0.325	0.156	0.676
	21–30yrs/31-40yrs	0.492	1.51	0.462	4.96						
Tumor size	T2/T 4/4b	0.131	0.707	0.451	1.109	**APC4 expression in tissue**	Low/High	0.089	0.611	0.346	1.079
	T3/T 4a/4b	0.368	0.834	0.561	1.239	**APC4 expression in blood**	Low/High	0.078	1.761	0.939	3.303
Chemotherapy	Yes/No	0.075	0.773	0.582	1.027	**APC5 expression in tissue**	Low/High	0.203	1.746	0.740	4.121
Radiation Therapy	Yes/No	**0.005**	2.71	1.346	5.458	**APC5expression in blood**	Low/High	0.831	1.091	0.489	2.433
Both Therapy	Yes/No	**0.006**	0.600	0.417	0.864						

Table 7. Association of APC3, APC4, APC5 & APC7 expression in blood and tissue level in ESCC patients.

Parameters	p-value	Pearson correlation
Expression of APC3 (blood) and expression of APC3 (tissue)	**0.000***	0.746
Expression of APC4 (tissue) and expression of APC3 (tissue)	0.411	0.100
Expression of APC4 (tissue) and expression of APC3 (blood)	0.277	0.132
Expression of APC4 (blood) and expression of APC3(tissue)	0.680	0.050
Expression of APC4 (blood) and expression of APC3 (blood)	0.756	0.038
Expression of APC4 (blood) and expression of APC4 (tissue)	0.559	0.071
Expression of APC5 (tissue) and expression of APC3 (tissue)	**0.020**	0.279
Expression of APC5(tissue) and expression of APC3 (blood)	0.832	-0.026
Expression of APC5 (tissue) and expression of APC4 (tissue)	0.970	-0.005
Expression of APC5 (tissue) and expression of APC4 (blood)	0.222	-0.148
Expression of APC5 (blood) and expression of APC3 (tissue)	0.239	-0.143
Expression of APC5 (blood) and expression of APC3 (tissue)	0.192	-0.158
Expression of APC 5 (blood) and expression of APC4 (tissue)	0.542	0.074
Expression of APC5 (blood) and expression of APC4 (blood)	0.194	-0.157
Expression of APC5 (blood) and expression of APC5 (tissue)	0.620	-0.060
Expression of APC7(tissue) and expression of APC3 (tissue)	**.000***	0.750
Expression of APC7 (tissue) and expression of APC3(blood)	**.000***	0.726
Expression of APC7 (tissue) and expression of APC4 (tissue)	0.729	0.042
Expression of APC7 (tissue)and expression of APC4 (blood)	0.517	0.079
Expression of APC7 (tissue) and expression of APC5 (tissue)	0.648	-0.055
Expression of APC7 (tissue)and expression of APC5 (blood)	0.138	-0.179
Expression of APC7 (blood) and expression of APC3 (tissue)	**0.000***	0.592
Expression of APC7 (blood) and expression of APC3 (blood)	**0.000***	0.768
Expression of APC7 (blood) and expression of APC4 (tissue)	0.849	-0.023
Expression of APC7 (blood) and expression of APC4 (blood)	0.643	0.056
Expression of APC7 (blood) and expression of APC5 (tissue)	0.624	-0.060
Expression of APC7 (blood) and expression of APC5 (blood)	0.764	-0.036
Expression of APC7 (blood) and expression of APC7 (tissue)	**0.000***	0.790

Note: *statistically significant p value < 0.05.

to differences in tumor stage, presence or absence of metastasis, tumor differentiation, habit of chewing betel nut and tobacco, smoking habit, drinking habits and frequency of tobacco consumption per day. Additionally, hazard analysis data supported the effect of these parameters on the survival of esophageal cancer patients. During the present study, the hazard ratio was significant in patients with APC3 and APC7 upregulation in blood and tissue, metastasis, consuming betel nut and tobacco,

smoking habit, prolonged duration of drinking habit from 11 to 20 years or more and frequency of tobacco consumption 10 times per day. These data represent the individual influence of these parameters on the survival and mortality rate of esophageal cancer patients.

The overall heightened expression of APC3 and APC7 in most of the ESCC samples contradicts the study reported by Park et al. 2005 [31]. That study showed that APC7 is downregulated in breast carcinoma

Table 8. *Pearson correlation (r^2) of APC3, APC4, APC5& APC7 expression in blood and tissue in ESCC patients.*

		APC3 (tissue)	APC3 (blood)	APC4 (tissue)	APC4 (blood)	APC5 (tissue)	APC5 (blood)	APC7 (tissue)	APC7 (blood)
APC3 (tissue)	r^2	1	0.556	0.010	0.003	0.014	0.020	0.562	0.350
	p-value		**.000***	0.411	0.680	0.329	0.239	**0.000***	**0.000***
APC3 (blood)	r^2	0.556	1	0.017	0.001	0.001	0.024	0.527	0.589
	p-value	**0.000***		0.277	0.756	0.832	0.192	**0.000***	**0.000***
APC4 (tissue)	r^2	0.010	0.017	1	0.005	0.000	0.005	0.002	0.001
	p-value	0.411	0.277		0.559	0.970	0.542	0.729	0.849
APC4 (blood)	r^2	0.003	0.001	0.005	1	0.021	0.024	0.006	0.003
	p-value	0.680	0.756	0.559		0.222	0.194	0.517	0.643
APC5 (tissue)	r^2	0.013	0.001	0.000	0.021	1	0.004	0.003	0.004
	p-value	0.329	0.832	0.970	0.222		0.620	0.648	0.624
APC5 (blood)	r^2	0.020	0.024	0.005	0.024	0.004	1	0.003	0.001
	p-value	0.239	0.192	0.542	0.194	0.620		0.138	0.764
APC7 (tissue)	r^2	0.562	0.527	0.002	0.006	0.003	0.032	1	0.624
	p-value	**0.000***	**0.000***	0.729	0.517	0.648	0.138		**0.000***
APC7 (blood)	r^2	0.350	0.589	0.001	0.003	0.004	0.001	0.624	1
	p-value	**0.000***	**0.000***	0.849	0.643	0.624	0.764	**0.000***	

Note: *statistically significant p value < 0.05.

accompanied by the loss of another subunit, APC3, suggesting heterogeneous regulation of APC components in breast carcinomas. However, our data are consistent with other studies showing that the expression patterns of APC components are not simultaneously modulated [32] and that the components of APC can be individually modulated by environmental stimuli [30]. Differential modulation of APC subunits and dysregulation of APC activation can induce unscheduled mitotic progression [33] and lead to malignant transformation. Thus, the individual modulation of the expression of APC/C subunits 3, 4, 5 and 7 found in our study might explain the enhanced cell proliferation and tumorigenesis.

Our two-tailed correlation analysis of APC/C gene expression in blood and tumor tissue samples in ESCC patients demonstrated a more common expression pattern of APC 3, APC5 and APC7 in blood and tissue samples from ESCC patients. Thus, the APC3, APC5 and APC7 expression levels in blood may be prognostic markers.

Different clinicopathological and molecular parameters, such as tumor size, tumor node, degree of differentiation, lymph node metastases, histopathology grade, lymphovascular invasion, and alteration of oncogene expression, were reported to be good prognostic markers for various types of cancer detection [34]. In the present study, strong prognostic indicators of tumorigenesis, such as a high histopathologic grade, a high rate of proliferation, tumor node stage and probable degree of differentiation, were associated with significant positive expression of APC3, APC5 and APC7 in ESCC patients, suggesting poor survival. Furthermore, more significant APC3 and APC 7 positive expression in blood may support that positive expression of the APC3 and APC7 gene pair is a viable prognostic marker for a proper ESCC prognosis. More in-depth molecular epidemiological studies will help elucidate the role of the APC3 and APC7 genes and their interaction with the different lifestyle factors and clinicopathological relationships, contributing to the development of ESCC in Northeast India.

Declarations

Author contribution statement

Eyashin Ali: Conceived and designed the experiments.

Manash Jyoti Kalita: Analyzed and interpreted the data; Wrote the paper.

Simanta Kalita and Jasmin Sultana: Wrote the paper.

Jayasree Talukdar: Performed the experiments; Analyzed and interpreted the data.

Ankur Jyoti Deka, Md. Ghaznavi Idris and Sahana Bhattacharjee: Analyzed and interpreted the data.

Bikash Narayan Choudhury and Munindra Narayan Baruah: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Subhash Medhi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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