Chapter-4: Results

Chapter-4.1: Clinical characteristic, biochemical parameter, epidemiological and life style factors reported in patients with esophageal squamous cell carcinoma

4.1.1 Reporting of esophageal squamous cell carcinoma patient's clinical characteristics:

Summary of clinicopathological characteristics is provided in Table 5. Median age at initial diagnosis of ESCC was 55 years (range 33-77 years). 67.7% patients were male and 32.4% females. At presentation, a majority of patients, were in performance status (PS) 1 based on Eastern Cooperative Oncology Group (ECOG) scale in 66.7% and normal (PS-0) in 15.7% patients.

Majority (96.3%) of ESCC patients presented with dysphagia of duration of less than one to three months. 99% of the esophageal squamous cell cancer was found in the thoracic region in our studied patients. Mid-thoracic esophagus was found to be the common location (68 out of 108, 62.9%). 69.4% of ESCC patients had moderately differentiated squamous cell carcinoma. A representative image depicting different pathological grading of esophageal disease is given in the figure 12.

Factors	Number	Percent (%)
Age (Years)		
≤45	24	22.2
>45	84	77.8
Sex		
Male	73	67.6
Female	35	32.4
ECOG (Eastern Cooperative Oncology Group) **Performance Status**		
0	17	15.7
1	72	66.7
2	17	15.7
3	2	1.9
4	0	0
Initial Symptom		
Dysphagia	104	96.3
Abdominal pain	1	0.9
Voice hoarseness with dysphagia	2	1.9
Pain in supraclavicular	1	0.9
Duration of Initial Symptom (Months)		
≤1	48	44.4
>1 ≤ 3	47	43.5
> 3	13	12
Disease Site		
Cervical	1	0.9
Upper thoracic	19	17.6
Mid thoracic	68	63
Lower thoracic	20	18.5
Pathological Grading		
Well differentiated	28	25.9
Moderately differentiated	75	69.4
Poorly differentiated	5	4.6
Outcome after NAC (Neo adjuvant chemotherapy)		
Responder	22	20.4
Non responder	86	79.6

Table 5: Clinicopathological data of the squamous cell carcinoma of the esophagus.

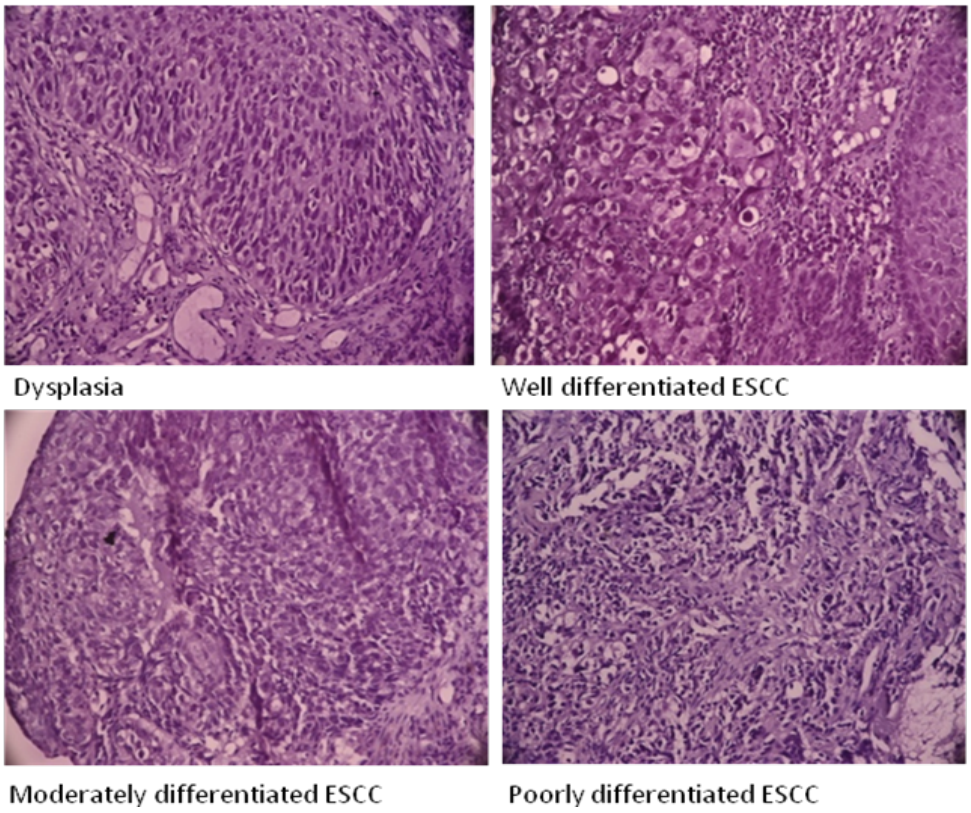


Figure 12. Different pathological grading of esophageal disease. Hematoxylin and eosin stained slides. ESCC: Esophageal squamous cell carcinoma

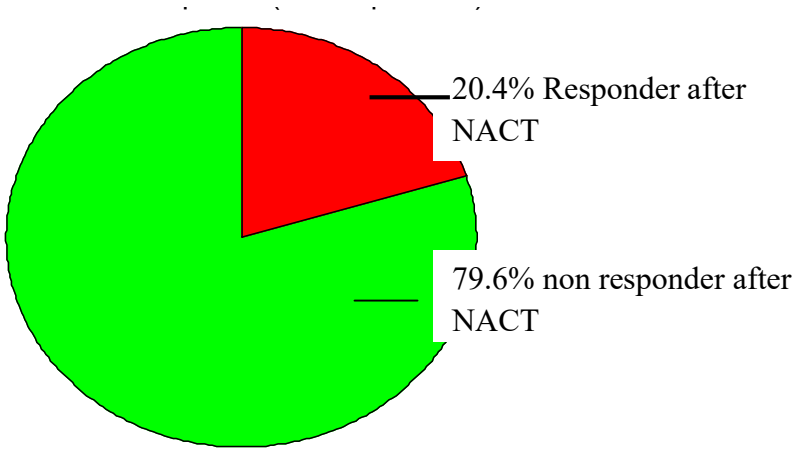


Figure 13. Percentage of ESCC patients who were reported responder and non responder after neo adjuvant chemotherapy (NACT).

Among 108 patients who received platinum and taxane based neo adjuvant chemotherapy, 22 patients (20.4%) achieved tumor remission, and considered as responders and rest 86 (79.6%) had residual disease (considered as non responders). Figure 13 represented the percentage of responders and non responders.

4.1.2 Reporting of epidemiological factors in esophageal squamous cell carcinoma patients:

91% of patients who were reported in this study were from Barak valley of the Southern Assam. Patients from Tripura and Halflong constitutes each 3% whereas patients from Kamrup reported 2% and Tinuskia reported 1%. It was shown in figure 14.

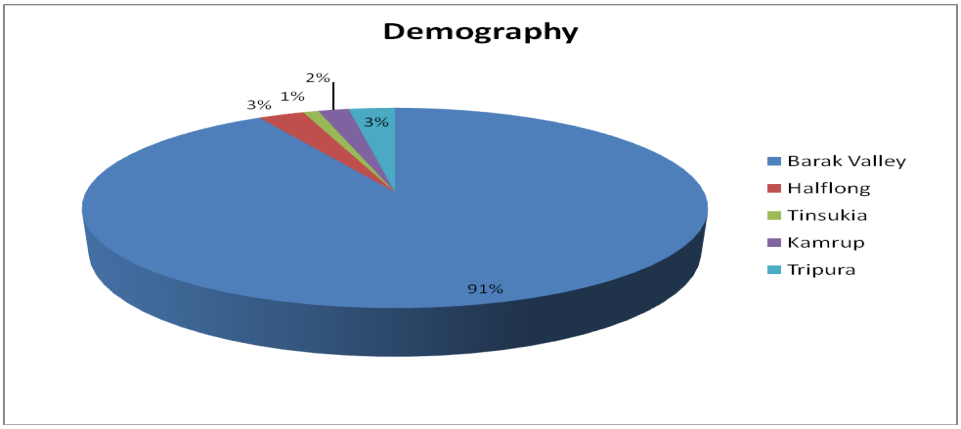
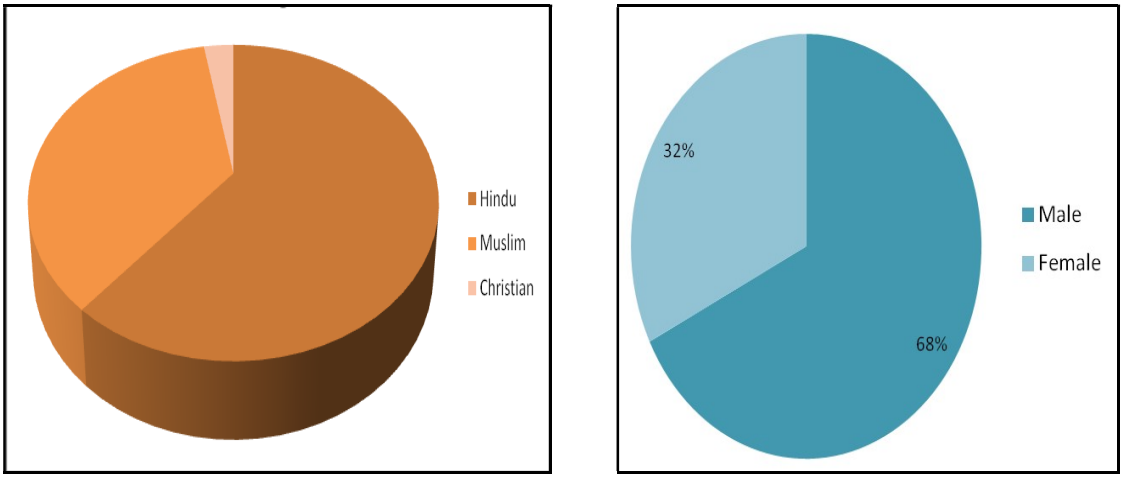


Figure 14 . Demographic distribution of the studied esophageal cancer patients.

Majority 61% among the studied ESCC patients were reported followers of the Hindu religion, 36% reported following Islam and rest 3% were follower of Christian religion. The percentage of male and female was 68% and 32% respectively among the 108 ESCC patients. Figure 15 showed religion and gender of the ESCC studied patients.Females reported better survival in Kaplan-Meier overall survival analysis as represented in the figure 16.



A B

Figure 15: A:Religion of studied esophageal cancer patients. B: Gender of studied esophageal cancer patients.

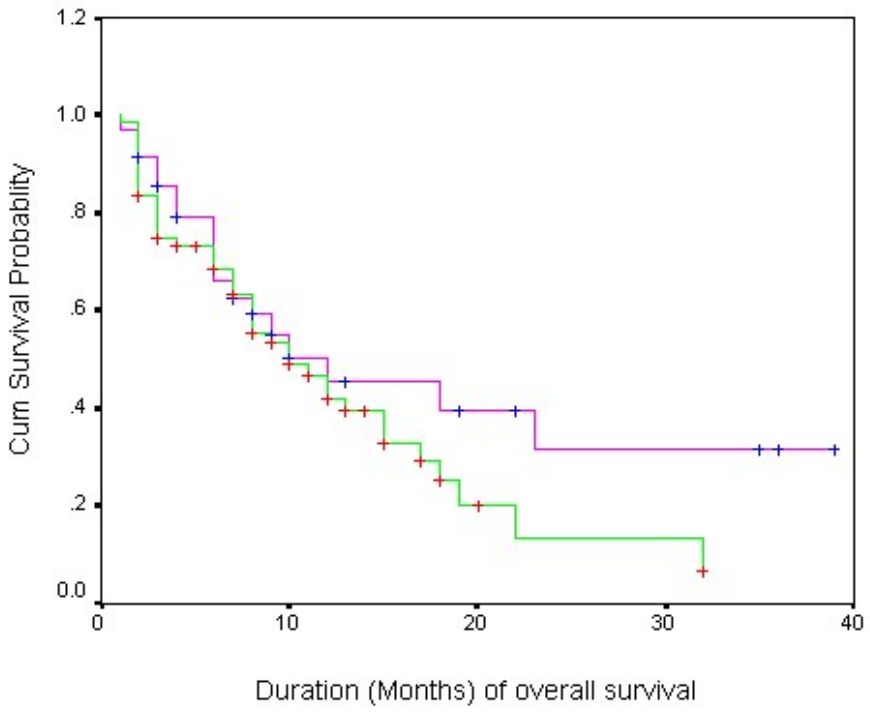


Figure 16: Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients who were female (n=35; pink line) and male (n=73; green line).

Out of 108 esophageal squamous cell carcinoma patients, we found 24 patients (22.2%) among those who were 45 years of age or less than that and rest 84 patients (77.8) were reported in the age group of those who were more than 45 years of age at the time of presentation (figure 17).

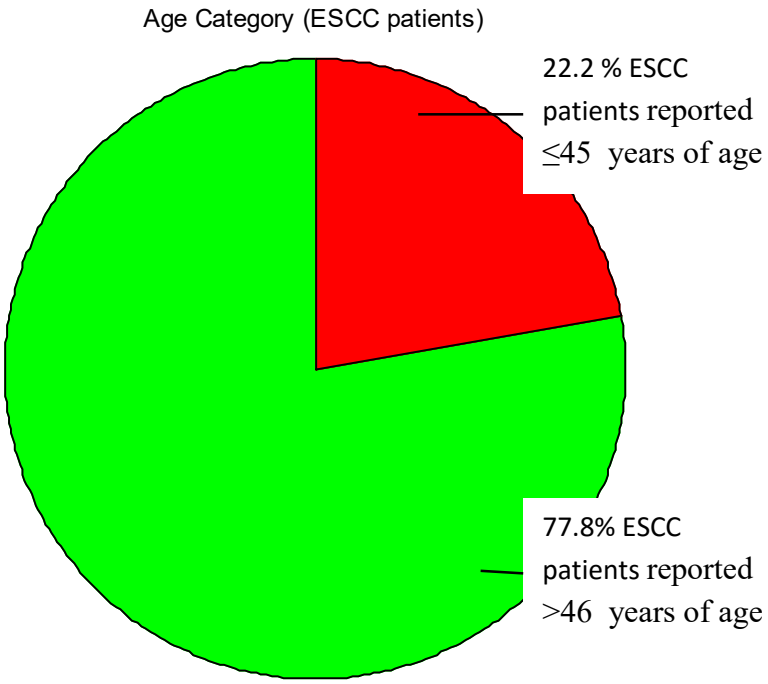
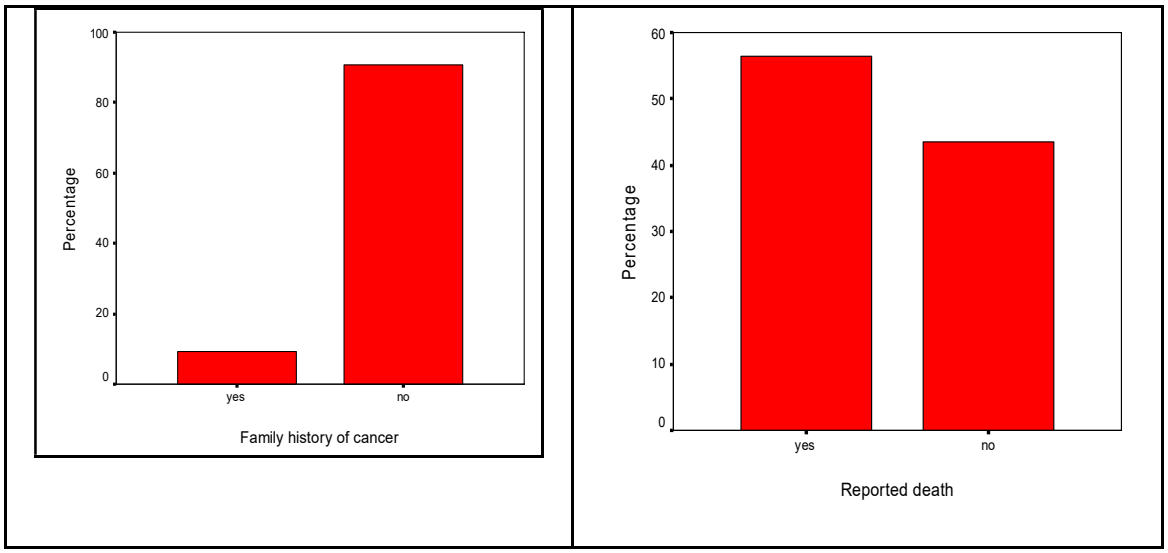


Figure 17: Age category of reported ESCC patients.

We found 9.3% of the total 108 patients having family history of cancer. The rest 90.7% were not found with any family history of cancer. In predominantly 96% of the total reported 108 esophageal squamous cell carcinoma patients, dysphagia (difficulty in swallowing) was the primary symptom. Few esophaeal squamous cell carcinoma patients were also reported abdominal pain, voice hoarseness with dysphagia, and pain in supraclavicular as listed in Table.



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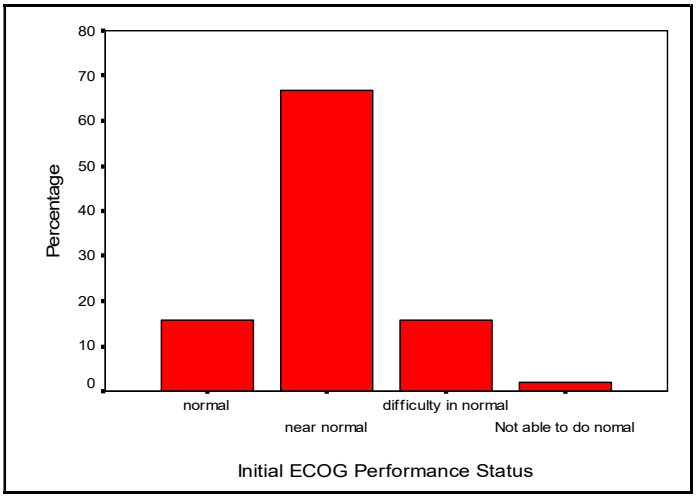


C

Figure 18: ESCC patients with A. Family history of cancer. B. Reported deaths on follow up. C.Initial ECOG performance status at the time of first visit to clinic

ESCC patients who visited the cancer hospital for the very first time was assessed through Eastern Cooperative Oncology Group performance status (ECOG PS) scale to determine their overall physical strength and well being. ECOG PS 0 was assigned to those ESCC patients who can do activities like a normal individual. ECOG PS 1 was

assigned to those patients who can work like near normal individuals. Those patients who had difficulty in performing their normal activities were assigned ECOG PS score 2 and those patients who weren't able to perform their normal activity at the time of hospital visit were assigned ECOG PS 3. ECOG PS 4 score represents bed ridden patients at the time of presentation.

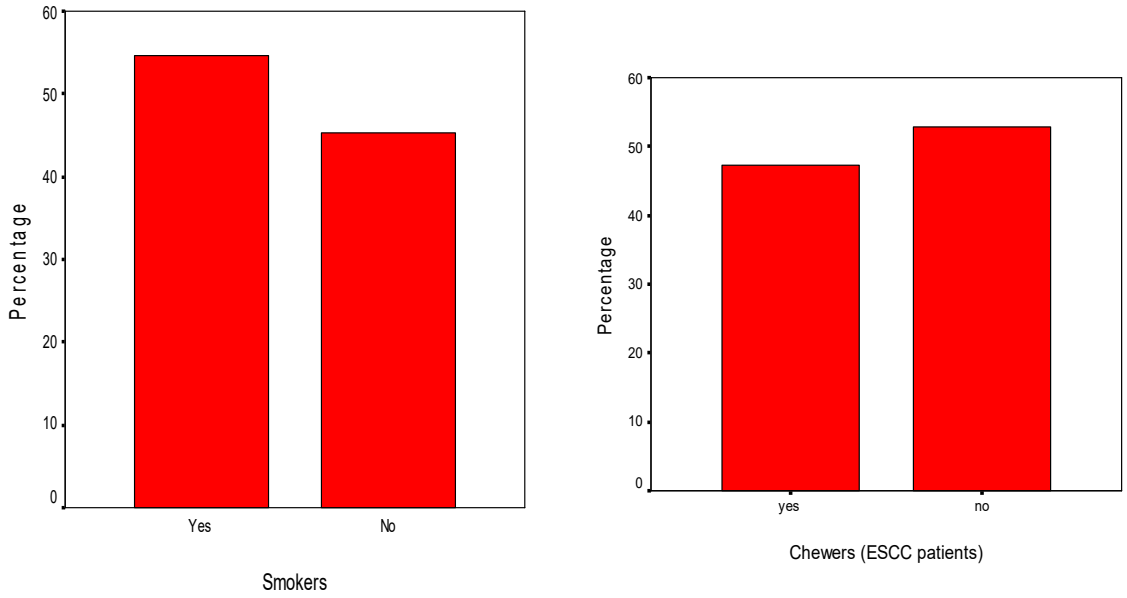
15.7% of the ESCC patients were able to do their normal activities at the initial presentation in the hospital (ECOG PS 0 score), 66.7% were able to do near normal activities (ECOG PS 1 score), 15.7% were having difficulty in performing their normal activity (ECOG PS 2 score) and 1.9% were not able to do their normal activity (ECOG PS 3 score). We didn't reported any ESCC patients with ECOG PS score 4.

During the course of follow up of the studied ESCC patients, we reported 61 (56.5%) deaths and rest 47 (43.5%) were alive till our last date of follow up. Figure 18 depicted the reported death, family history of cancer and initial ECOG performance status in ESCC patients.

4.1.3 Reporting of lifestyle factors in esophageal squamous cell carcinoma patients:

In the current study of life style factors on esophageal squamous cell carcinoma patients, we found that 77.7% of the patients were using some form of tobacco either through smoking or chewing. These two are predominant categories for using tobacco in this region.

54.6% of the 108 ESCC patients were found tobacco smokers whereas rest 45.4% of the same category of the patients didn't reported tobacco use through smoking as represented by the figure 19.

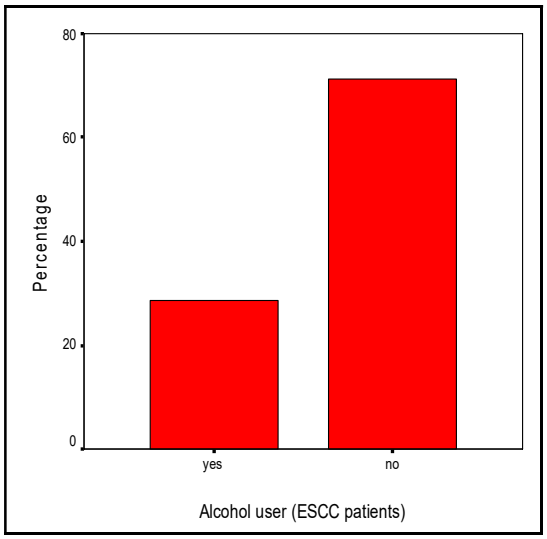


A B

Figure 19: A. Comparison of tobacco smokers VS non smokers B. Comparison of percentage of tobacco chewers VS non chewers in the ESCC patients

Among 108 ESCC patients studied, 47.2% reported tobacco chewing whereas 52.8% were not using tobacco in chewing form.

Alcohol use among ESCC studied patient was evident in 28.7% of the cases whereas majority 71.3% didn't reported use of alcohol. Alcohol use among the ESCC patients unlike tobacco and areca nut use was not widespread.



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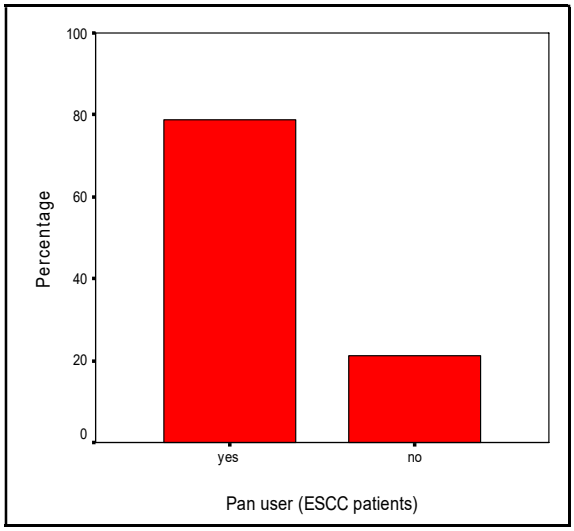

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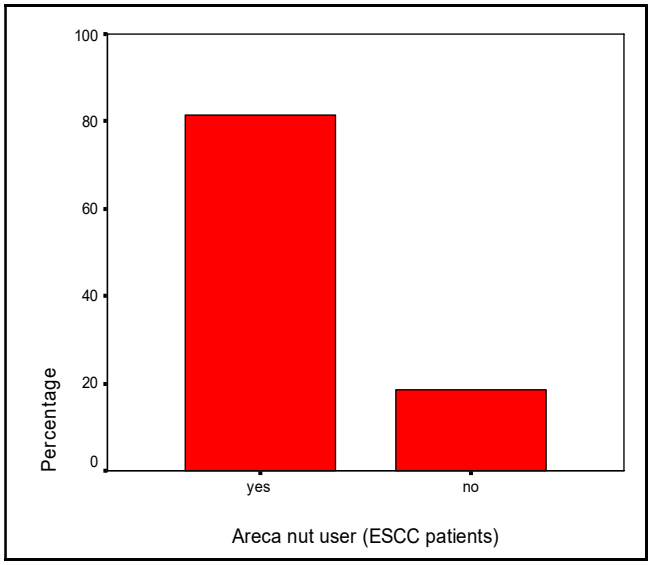


C

Figure 20: Life style habits of ESCC patients: A. Percentage of alcohol user VS non alcohol user in the ESCC patients. B. Percentage of pan user VS non pan user in the ESCC patients. C. Percentage of areca nut user VS non areca nut user in the ESCC patients.

Areca nut use was found rampant among the studied ESCC patients. 81.5% of the

ESCC patients reported using areca nut whereas 18.5% didn't reported areca nut use. Among the 108 ESCC cancer patients, 85 (78.7%) patients had documented using pan and the rest 23 (21.3%) patients didn't documented pan use. Figure 20 represented the percentage use of alcohol, pan, and areca nut among ESCC studied patients.

4.1.4 Reporting of biochemical factors in esophageal squamous cell carcinoma patients:

Biochemical parameters like glucose, urea nitrogen, creatinine, total protein, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatise and total bilirubin based on the analysis of the ESCC patients serum during the first visit of the patient were documented and analysed.

We had reported the biochemical value of only those patients who didn't received any prior cancer directed treatment to analyse the characteristic features of various biochemical parameter in the ESCC patients. The normal range of all the biochemical parameters was taken from the Johnson & Johnson Automated Biochemical Analyzer, USA recommended normal range.

The normal range of blood glucose is 74-106 mg/dL. In the ESCC patients, 24% of them reported ≥ 106 mg/dL of blood glucose whereas rest 76% reported < 106 mg/dL of blood glucose level.

In case of initial urea nitrogen level whose normal range is 9-20 mg/dL, the ESCC patients predominantly (97%) reported value < 20 mg/dL and rest 3% reported ≥ 20 mg/dL. Figure 21 represented the percentage of ESCC patients with normal and more than normal blood glucose, urea nitrogen and creatinine level.

The normal range of serum creatinine is 0.80-1.50 mg/dL. Among the 108 ESCC patients whose blood was analyzed for serum creatinine, 98% reported to have < 1.50 mg/dL value and rest 2% were reported to have ≥ 1.50 mg/dL of serum creatinine.

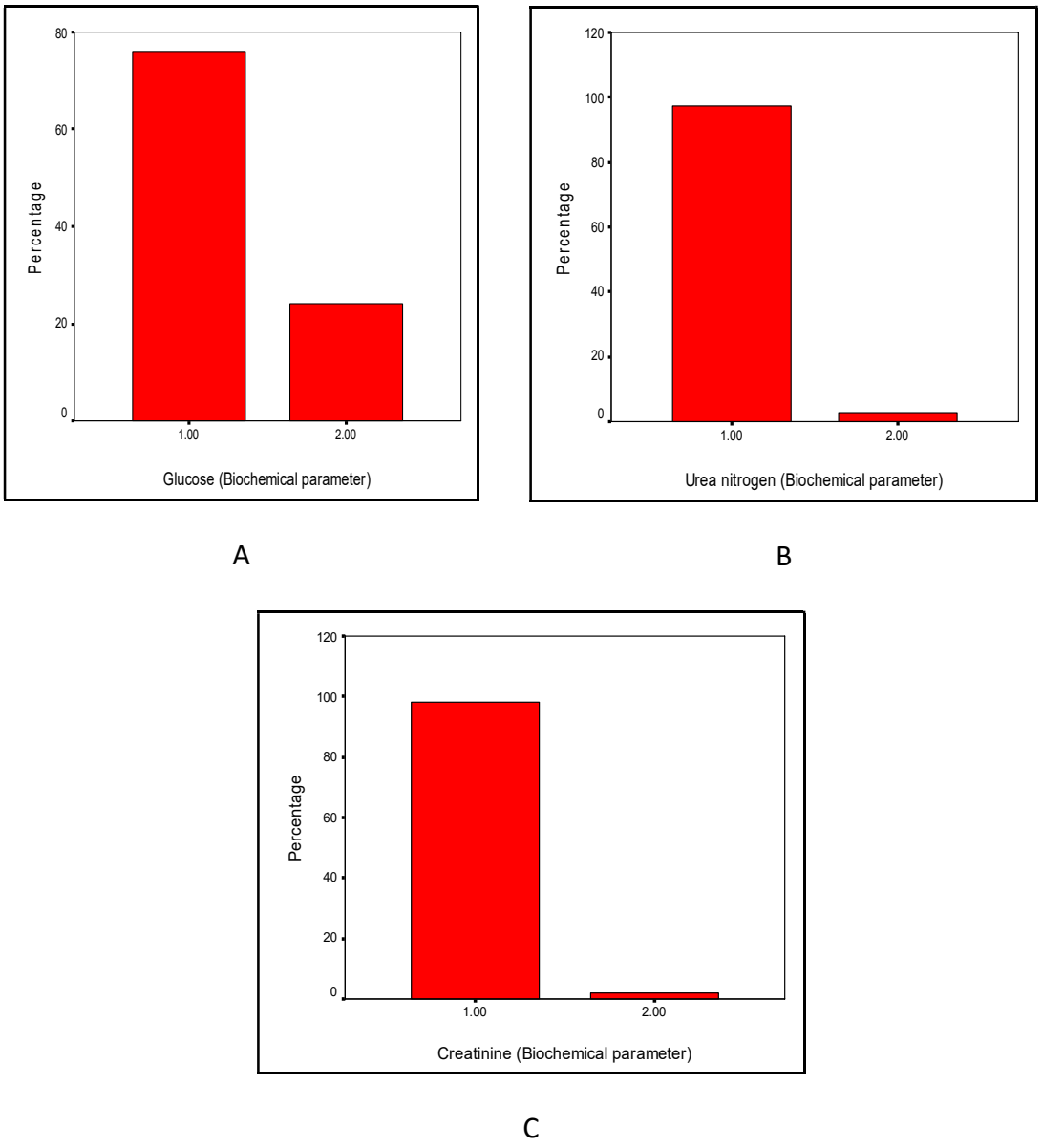


Figure 21: Biochemical profile of ESCC patients. A Percentage of ESCC patients having blood glucose level where 1.00 = <106 mg/dL and 2.00 = ≥ 106 mg/dL. B. Percentage of ESCC patients having where 1.00 = < 20 mg/dL and 2.00 = ≥ 20 mg/dL. C. percentage of ESCC patents having serum creatinine level where. 1.00 = < 1.50 mg/dL and 2.00 = ≥ 1.50 mg/dL

Serum total protein normal value ranges from 6.30-8.20 g/dL. In our study where we had documented biochemical test of serum total protein on 108 ESCC patients, we found 90% of the ESCC patient's serum total protein level < 8.20 g/dL and the rest 10% had the value ≥ 8.20 g/dL.

Normal serum albumin level ranges from 3.5-5 g/dL. We reported 22% of the 108 ESCC patients with serum albumin value < 3.5 g/dL and rest 78% of the ESCC patients reported serum albumin level ≥ 3.5 g/dL.

The normal range of aspartate transaminase (AST) is 17-59 U/L. In our study, we reported that 95% of the ESCC patients showed the serum AST value < 59 U/L whereas 5% of the ESCC patients reported ≥ 59 U/L of serum AST.

Normal serum alanine transaminase (ALT) range is 21-72 U/L. Out of 108 ESCC patients, predominantly 99% showed serum ALT level as < 72 U/L and the rest 1% showed ≥ 72 U/L.

In case of normal serum alkaline phosphatase (ALKP), the normal value ranges from 38-126 U/L. In the present study, we reported 16% of the ESCC patients having ALKP value as < 80 U/L, 82% of the same patients having ALKP value in the range of 80-280 U/L and rest 2% having the ALKP value as > 280 U/L. The normal range of serum total bilirubin ranges from 0.2-1.30 mg/dL. Among the 108 ESCC, we reported 93% having serum total bilirubin level at < 1.30 mg/dL whereas the rest 7% of it showed the serum total bilirubin level at ≥ 1.30 mg/dL. Figure 22 represented the biochemical profile of serum ALT, AST, ALKP, albumin, total protein and bilirubin.

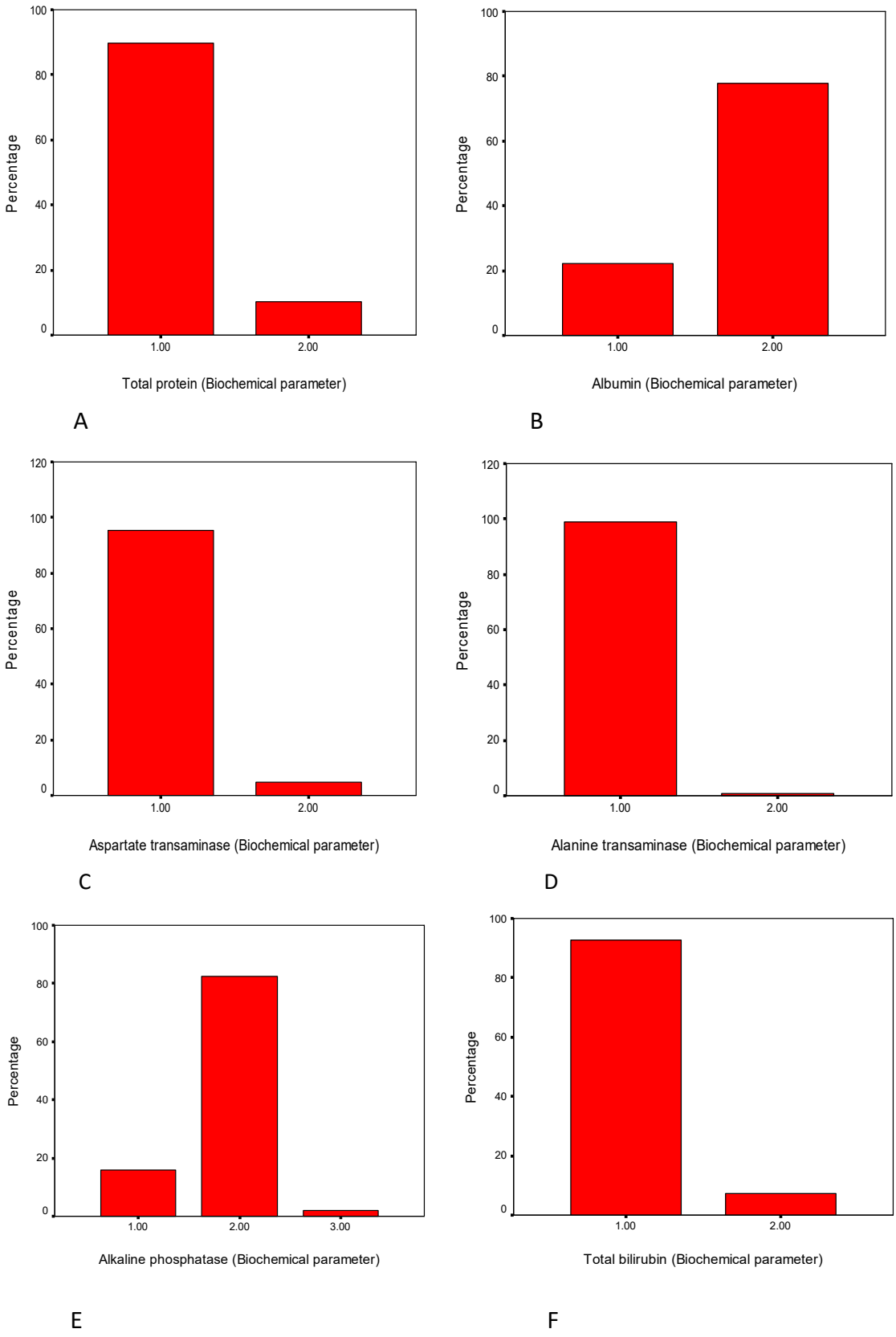


Figure 22: Biochemical profile of ESCC patients.

A. Percentage of ESCC patients having serum total protein level where 1.00 = < 8.20 g/dL and 2.00 = ≥ 8.20 g/dL. B. Percentage of ESCC patients having albumin level where 1.00 = < 3.5 g/dL and 2.00 = ≥ 3.5g/dL. C. Percentage of patients having serum aspartate transaminase (AST) level where 1.00 = < 17 U/L and 2.00 = ≥ 59 U/L. D Patients having serum alanine transaminase biochemical level where 1.00 = < 72 U/L and 2.00 = ≥ 72 U/L. E Patients having serum alkaline phosphatase level where1.00 = < 80 U/L, 2.00 = 80=280 U/L and 3.00 = > 280 U/L. F represent percentage of patients with serum total bilirubin level where 1.00 = < 1.30 mg/dL and 2.00 = ≥ 1.30 mg/dL.

4.1.5 Biochemical parameter correlation with gender status in esophageal squamous cell carcinoma patients

23% of male and 25% of females out of their total number showed elevated level of glucose whereas those who had showed elevated glucose level in their peripheral blood during initial screening. The correlation was tabulated in table 6. 65% were males and 35% were females (*P*=0.78). Minority proportion (3%) of the patients showed higher urea nitrogen level and among them 67% were males and 33% were females. Even lesser number of patients (2%) showed spiked level of creatinine and all of them were males (P=0.32). 10% of the patients showed increase in total protein level, out of which 55% and 45% were reported males and females respectively. 22% of the patients showed lower albumin level in their serum during initial presentation, among those 79% were males and rest 21% were females. In case of Aspartate transaminase (AST) and Alanine transaminase (ALT), elevated level more than the normal were in found in 5% and 1% of cases, out of them 80% and 100% were males. 16% of the patients showed alkaline phosphatase (ALKP) level less than 80U/L whereas 2% showed ALKP level more than

280 U/L. Among males, 18% and 3% and among females, 11% and 0% showed ALKP level less than 80U/L and more than 280U/L respectively (*P*=0.40). 7% of the patient showed higher total bilirubin level, out of them 63% were reported males and 37% were females (*P*=0.74). None of the biochemical parameter showed any statistical significance with the gender status of the studied patients.

Biochemical parameter	**Male (73)**	**Female (35)**	***P*-value**
Glucose			0.78
<106 mg/dL	56	26	
≥106 mg/dL	17	9	
Urea nitrogen			0.97
< 20 mg/dL	71	34	
≥ 20 mg/dL	2	1	
Creatinine			0.32
< 1.50 mg/dL	71	35	
≥ 1.50 mg/dL	2	0	
Total protein			0.32
< 8.20 g/dL	67	30	
≥ 8.20 g/dL	6	5	
Albumin			0.17
< 3.5 g/dL	19	5	
≥ 3.5 g/dL	54	30	
Aspartate transaminase			0.54
< 59 U/L	69	34	
≥ 59 U/L	4	1	
Alanine transaminase			0.48
(ALT)	72	35	
< 72 U/L	1	0	
≥ 72 U/L			
Alkaline phosphatase			0.40
< 80 U/L	13	4	
80-280 U/L	58	31	
≥ 280 U/L	2	0	
Total Bilirubin			0.74
< 1.30 mg/dL	68	32	
≥ 1.30 mg/dL	5	3	

Table 6: Biochemical parameter correlation with the reported gender of the ESCC patients

4.1.6 Biochemical parameter correlation with reported age group in esophageal squamous cell carcinoma patients

Table 7 depicts the analysis of age group with the reported biochemical parameter in the enrolled patients. 84% of the patients who showed increased glucose level of ≥106 mg/dL and 77% of patients who showed <106 mg/dL glucose level were of more than 46 years of age (*P*=0.67). 100% of the patients who showed elevated urea nitrogen and creatinine level had fall into more than 46 years of age group.

91% of those patients who showed elevated level of protein were of more than 46 years (*P*=0.26). 22% of the total patients reported less albumin level in their serum and out of that 71% belonged to >46 year of age group and rest 29% with ≤45 years of age group (*P*=0.35). 60% of the patients whose AST serum level were found elevated belonged to more than 46 years of age. Whereas for ALT, 100% of the patients in high ALT level were reported in the age group of patients with 45 years of age or less (*P*=0.060).

65% of the patients with ALKP level less than 80U/L were reported in the age group of more than 46 years whereas equal number of patients having more than 280U/L ALKP level were reported in both age group (*P*=0.21). 75% of the patients with high total bilirubin level were of more than 46 years of age (*P*=0.84). No statistical significance was found between any of the biochemical parameter and age group.

Biochemical parameter	Age: ≤45 year (24)	Age: >46 year (84)	*P*-value
Glucose			0.67
<106 mg/dL	19	63	
≥106 mg/dL	5	21	
Urea nitrogen			0.34
< 20 mg/dL	24	81	
≥ 20 mg/dL	0	3	
Creatinine			0.44
< 1.50 mg/dL	24	82	
≥ 1.50 mg/dL	0	2	
Total protein			0.26
< 8.20 g/dL	23	74	
≥ 8.20 g/dL	1	10	
Albumin			0.35
< 3.5 g/dL	7	17	
≥ 3.5 g/dL	17	67	
Aspartate transaminase			0.32
< 59 U/L	22	81	
≥ 59 U/L	2	3	
Alanine transaminase			0.060
< 72 U/L	23	84	
≥ 72 U/L	1	0	
Alkaline phosphatase			0.21
< 80 U/L	6	11	
80-280 U/L	17	72	
≥ 280 U/L	1	1	
Total Bilirubin			0.84
< 1.30 mg/dL	22	78	
≥ 1.30 mg/dL	2	6	

Table 7: Biochemical data correlation with the reported age group in the ESCC patients.

4.1.7 Biochemical parameter correlation with reported initial performance status in esophageal squamous cell carcinoma patients

Performance status of the patients was reported during initial presentation following Eastern Cooperative Oncology Group (ECOG) scale and table 8 described the correlation of biochemical parameter with performance status. In this study, it was found that the majority (73%) of patients who were having elevated blood glucose level, presented initially at performance status 1 (P=0.74). 100 % patients with higher urea nitrogen level in their blood were confined to performance status 1 whereas 4% out of total patients who reported initial performance status 1 where having high creatinine level (P=0.62). 10% of the reported patients showed elevated level of serum total protein and amongst them 55% were in the performance status 1 (P=0.28). 22% of the patients showed lower level of albumin and among them majority 75% were reported to have performance status 1.

In context of AST and ALT 5% and 1% of the reported patients showed higher level and amongst them 80% and 100% were in performance status 1 respectively. 18%, 76% and 6% of the those patients who reported performance status 2 whereas those who represented in the performance status 1 category, 18%, 81% and 1% were having ALKP level <80 U/L, 80-280 U/L and ≥ 280 U/L respectively (P=0.28). 63% of those patients who showed higher total bilirubin level were 1 whereas 7% of the total performance status 1 patients were having high bilirubin level in their serum (P=0.067). Except serum creatinine level none of the other biochemical parameter showed any statistical correlation with the performance status in the ESCC patients.

Biochemical parameter	Performance				*P*-value
	status: 0	status: 1	status: 2	status: 3	
Glucose					
<106 mg/dL	13	53	14	2	0.74
≥106 mg/dL	4	19	3	0	
Urea nitrogen					
< 20 mg/dL	17	69	17	2	0.62
≥ 20 mg/dL	0	3	0	0	
Creatinine					
< 1.50 mg/dL	15	72	17	2	0.012
≥ 1.50 mg/dL	2	0	0	0	
Total protein					
< 8.20 g/dL	15	66	15	1	0.28
≥ 8.20 g/dL	2	6	2	1	
Albumin					
< 3.5 g/dL	2	18	4	0	0.57
≥ 3.5 g/dL	15	54	13	2	
Aspartate transaminase					
< 59 U/L	17	68	16	2	0.77
≥ 59 U/L	0	4	1	0	
Alanine transaminase					
< 72 U/L	17	71	17	2	0.91
≥ 72 U/L	0	1	0	0	
Alkaline phosphatase					
< 80 U/L	0	13	3	1	0.28
80-280 U/L	17	58	13	1	
≥ 280 U/L	0	1	1	0	
Total Bilirubin					
< 1.30 mg/dL	15	67	17	1	0.067
≥ 1.30 mg/dL	2	5	0	1	

Table 8: Biochemical data correlation in the ESCC patients with their performance status.

4.1.8 Biochemical parameter correlation with pathological grading in esophageal squamous cell carcinoma patients

Pathological grading in ESCC patients was correlated with the routine biochemical parameter and the result was summarized in the Table 9. 60%, 20% and 29% of the patients with high blood glucose level was pathologically graded poorly, moderately and well differentiated squamous cell carcinoma respectively (P=0.10).

Only moderately differentiated squamous cell carcinoma reported elevated urea nitrogen level in their blood (P=0.50). Similarly, high creatinine level was also reported only in moderately differentiated squamous cell carcinoma (P=0.63).

11% of the well differentiated SCC, 9% of the moderately differentiated SCC and 20% of the poorly differentiated SCC had showed high total protein level in their blood (P=0.74). 29%, and 20% each in well differentiated SCC, moderately differentiated SCC and poorly differentiated SCC respectively showed low albumin level in their blood (P=0.64).

None of the patients with poorly differentiated SCC were having high AST, ALT or ALKP level. 4% of the well differentiated SCC, 8% of the moderately differentiated SCC and 20% of the poorly differentiated SCC reported high serum total bilirubin level (P=0.40). None of the parameters correlated statistically with the pathological grading in the studied ESCC patients.

Biochemical parameter	Well Diff. SCC	Mod. Diff. SCC	Poorly Diff. SCC	*P*-value
Glucose				0.10
<106 mg/dL	20	60	2	
≥106 mg/dL	8	15	3	
Urea nitrogen				0.50
< 20 mg/dL	28	72	5	
≥ 20 mg/dL	0	3	0	
Creatinine				0.63
< 1.50 mg/dL	28	73	5	
≥ 1.50 mg/dL	0	2	0	
Total protein				0.74
< 8.20 g/dL	25	68	4	
≥ 8.20 g/dL	3	7	1	
Albumin				0.64
< 3.5 g/dL	8	15	1	
≥ 3.5 g/dL	20	60	4	
Aspartate transaminase				0.70
< 59 U/L	26	72	5	
≥ 59 U/L	2	3	0	
Alanine transaminase				0.80
< 72 U/L	28	74	5	
≥ 72 U/L	0	1	0	
Alkaline phosphatase				0.79
< 80 U/L	4	10	3	
80-280 U/L	23	64	2	
≥ 280 U/L	1	1	0	
Total Bilirubin				0.40
< 1.30 mg/dL	27	69	4	
≥ 1.30 mg/dL	1	6	1	

Table 9: Biochemical data correlation in the ESCC patients with their pathological grading.

4.1.9 Correlation of reported death with biochemical parameter in esophageal squamous cell carcinoma patients

Routine biochemical parameter was correlated with the reported death. Table 10 showed the relevant correlation data. Among the ESCC patients who had elevated blood glucose level, 54% were reported alive and rest 46% were dead (*P*=0.22). Those who had increase blood urea nitrogen level in their blood reported more deaths (67%). And in case of high creatinine level, 100% of them reported death (*P*=0.21). Among those who died, 10% showed increased blood total protein level whereas those patients who were alive 11% showed elevated total protein (*P*=0.89). 21% of those who died were having low serum albumin level whereas 23% of were alive had also the similar albumin level. High AST, ALT and ALKP level was found elevated in those patients who died during follow up. 13% of the patients who were alive and 3% of the patients who died had high total serum bilirubin level (*P*=0.062).

Biochemical parameter	Death: Yes	Death: No	*P*-value
Glucose			0.22
<106 mg/dL	49	33	
≥106 mg/dL	12	14	
Urea nitrogen			0.71
< 20 mg/dL	59	46	
≥ 20 mg/dL	2	1	
Creatinine			0.21
< 1.50 mg/dL	59	47	
≥ 1.50 mg/dL	2	0	
Total protein			0.89
< 8.20 g/dL	55	42	
≥ 8.20 g/dL	6	5	
Albumin			0.79
< 3.5 g/dL	13	11	
≥ 3.5 g/dL	48	36	
Aspartate transaminase (AST)			0.87

< 59 U/L	58	45	
≥ 59 U/L	3	2	
Alanine transaminase (ALT)			0.37
< 72 U/L	60	47	
≥ 72 U/L	1	0	
Alkaline phosphatase (ALKP)			0.43
< 80 U/L	10	7	
80-280 U/L	49	40	
≥ 280 U/L	2	0	
Total Bilirubin			0.062
< 1.30 mg/dL	59	41	
≥ 1.30 mg/dL	2	6	

Table 10: Biochemical data correlation in the ESCC patients with reported death status.

4.1.10 Correlation of circumferential disease status with biochemical parameter in esophageal squamous cell carcinoma patients

Routine biochemical parameter was correlated with the circumferential spread of the disease and shown in the Table 11. 23% of the patients who had whole circumferential disease, 17% of the patients who had 3/4 circumferential disease, 31% who had 2/3 of the circumferential disease and 26% who had 1/2 circumferential disease were reported to have high blood glucose level in their blood. High blood urea nitrogen was seen only in the patients with the whole circumferential disease (P=0.58). Likewise, serum creatinine was also found elevated only in those patients who were having whole circumferential disease (P=0.73). ESCC patients with 1/2 circumferential disease and whole circumferential disease showed high total serum protein level in 17% and 11% of the total patients respectively (P=0.32). 29% patients who had shown whole circumferential disease were reported to had low serum albumin than 13% of patients with ½ circumferential disease (P=0.21). Among the patients who had shown high AST

level in the blood, 20% reported in ESCC patients with 2/3 of the circumferential disease and 80% in patients with the whole circumferential disease (P=0.57). 100% of the patients with high ALT found in the category of those who had 2/3 circumferential disease (P=0.061). High level of ALKP was found only in patients with whole circumferential disease (P=0.80). 9% of the patients with 1/2 circumferential disease, 8% with 2/3 circumferential disease, 17% with 3/4 circumferential disease and 6% with the whole circumferential disease were found with high elevated level of total bilirubin in their blood (P=0.80). None showed any statistical correlation.

Biochemical parameter	**Circumferential disease**				***P*-value**
	1/2	2/3	3/4	Whole	
Glucose					
<106 mg/dL	17	9	5	51	0.89
≥106 mg/dL	6	4	1	15	
Urea nitrogen					
< 20 mg/dL	23	13	6	63	0.58
≥ 20 mg/dL	0	0	0	3	
Creatinine					
< 1.50 g/dL	23	13	6	64	0.73
≥ 1.50 mg/dL	0	0	0	2	
Total protein					
< 8.20 g/dL	19	13	6	59	0.32
≥ 8.20 g/dL	4	0	0	7	
Albumin					
< 3.5 g/dL	3	1	1	19	0.21
≥ 3.5 g/dL	20	12	5	47	
Aspartate transaminase					
< 59 U/L	23	12	6	62	0.57
≥ 59 U/L	0	1	0	4	
Alanine transaminase					
< 72 U/L	23	12	6	66	0.061
≥ 72 U/L	0	1	0	0	
Alkaline phosphatase					
< 80 U/L	5	2	0	10	0.80
80-280 U/L	18	11	6	54	
≥ 280 U/L	0	0	0	2	
Total Bilirubin					
< 1.30mg/dL	21	12	5	62	0.80
≥ 1.30 mg/dL	2	1	1	4	

Table 11: Biochemical data correlation in the ESCC patients with circumferential disease

4.1.11 Correlation of family history of cancer with biochemical parameter in esophageal squamous cell carcinoma patients

In certain cancer cases like breast and ovarian, family history of cancer has been found quite significant. In esophageal squamous cell cancer, this study reported 9% of the patients with family history of cancer. Table 12 showed the correlation of routine biochemical parameter with the family history of cancer. Among those who reported family history of cancer, 30% were found having high blood glucose level whereas among those who didn't had family history of cancer, 23% reported increase in their blood glucose level (P=0.64). There was no patients with family history of cancer who were having high blood urea nitrogen and 3% of those patients who didn't reported family history of cancer were having elevated level of urea nitrogen (P=0.57). Similarly, none of the patients with family history of cancer were found with high creatinine level. 30% of the patients with family history of cancer were found having elevated total protein level whereas the same was found in 8% of the patients without having family history of cancer (P=0.030).

Among the patients with family history of cancer, 10% reported low serum albumin level whereas 23% reported low serum albumin level in patients without family history of cancer (P=0.32). In case of serum levels of AST, ALT and ALKP, it was reported at higher than normal in only those ESCC patients who didn't had family history of cancer. Serum level of total bilirubin was found elevated only in patients without family history of cancer whereas in the same patient group, serum bilirubin level was found high in 8% of subjects (P=0.34). Total protein level showed statistical correlation.

Biochemical parameter	Family history of cancer: Yes	Family history of cancer: No	*P*-value
Glucose			
<106 mg/dL	7	75	0.64
≥106 mg/dL	3	23	
Urea nitrogen			
< 20 mg/dL	10	95	0.57
≥ 20 mg/dL	0	3	
Creatinine			
< 1.50 mg/dL	10	96	0.64
≥ 1.50 mg/dL	0	2	
Total protein			
< 8.20 g/dL	7	90	0.030
≥ 8.20 g/dL	3	8	
Albumin			
< 3.5 g/dL	1	23	0.32
≥ 3.5 g/dL	9	75	
Aspartate transaminase (AST)			
< 59 U/L	10	93	0.46
≥ 59 U/L	0	5	
Alanine transaminase (ALT)			
< 72 U/L	10	97	0.74
≥ 72 U/L	0	1	
Alkaline phosphatase (ALKP)			
< 80 U/L	3	14	0.40
80-280 U/L	7	82	
≥ 280 U/L	0	2	
Total Bilirubin			
< 1.30 mg/dL	10	90	0.34
≥ 1.30 mg/dL	0	8	

Table 12: Biochemical data correlation in the ESCC patients with family history of cancer.

4.1.12 Correlation of site of tumor with the biochemical parameter in esophageal squamous cell carcinoma patients

Growth in esophagus was found at different sites called cervical, upper thoracic, mid thoracic and lower thoracic. Routine biochemical parameter was correlated with all these different disease sites as depicted in table 13. Increase in the level of blood glucose was found in 0%, 15%, 70% and 15% of patients who reported esophageal cancer at cervical, upper thoracic, mid thoracic and lower thoracic sites. 21% of the patients with cancer at upper thoracic site and 26% of those having cancer in mid thoracic reported high blood glucose level (P=0.84). Urea nitrogen level was found high only in patients with esophageal cancer in upper (67%) and lower (33%) thoracic regions. 3% of the patient with cancer at mid thoracic whereas 5% of the patient with cancer at lower thoracic were found having high blood urea nitrogen level (P=0.81). High creatinine level was reported equally in patients with disease at upper and mid thoracic regions of the esophagus. 5% and 1% of the patients with esophageal cancer at upper and mid thoracic level respectively were reported high serum creatinine level (P=0.64). 0%, 16%, 7% and 15% of the patients having esophageal cancer at cervical, upper thoracic, mid thoracic and lower thoracic site respectively reported elevated total protein level in their blood (P=0.60). Low level of albumin was reported in 0%, 12.5%, 62.5% and 25% of the patients who reported esophageal cancer at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively. Serum AST elevated level was reported in 20% and 80% of the patients with esophageal cancer at the upper and mid thoracic sites respectively and among patients who reported esophageal cancer at upper and mid thoracic level, 5% and 6% were reported having elevated serum AST level respectively (P=0.73). ALT serum level was found high only in patients with tumor at the mid thoracic site. Similarly, high ALKP level was also reported high in patients with cancer at

their mid thoracic site. Elevated level of total bilirubin in blood was found in 0%, 11%, 4% and 15% of the patients with esophageal cancer at the cervical, upper thoracic, mid thoracic and lower thoracic sites (P=0.40).

Biochemical parameter	Cervical	Upper thoracic	Mid thoracic	Lower thoracic	*P*-value
Glucose					
<106 mg/dL	1	15	50	16	0.84
≥106 mg/dL	0	4	18	4	
Urea nitrogen					
< 20 mg/dL	1	19	66	19	0.81
≥ 20 mg/dL	0	0	2	1	
Creatinine					
< 1.50 mg/dL	1	18	67	20	0.64
≥ 1.50 mg/dL	0	1	1	0	
Total protein					
< 8.20 g/dL	1	16	63	17	0.60
≥ 8.20 g/dL	0	3	5	3	
Albumin					
< 3.5 g/dL	0	3	15	6	0.69
≥ 3.5 g/dL	1	16	53	14	
Aspartate transaminase					
< 59 U/L	1	18	64	20	0.73
≥ 59 U/L	0	1	4	0	
Alanine transaminase					
< 72 U/L	1	19	67	20	0.89
≥ 72 U/L	0	0	1	0	
Alkaline phosphatase					
< 80 U/L	0	0	12	5	0.36
80-280 U/L	1	19	54	15	
≥ 280 U/L	0	0	2	0	
Total Bilirubin					0.40
< 1.30 mg/dL	1	17	65	17	
≥ 1.30 mg/dL	0	2	3	3	

Table 13: Biochemical data correlation with the site of the disease in the ESCC patients.

4.1.13 Correlation of follow up duration with the biochemical parameter in esophageal squamous cell carcinoma patients

Table 14 reported the correlation between follow up duration of the esophageal cancer patients with their reported initial routine biochemical parameter. It was reported that 21%, 26%, 29%, 17%, 40% and 0% patients who reported follow up duration that ranged from 0-6, 7-12, 13-18, 19-24, 25-36 and more than 37 months were having high blood glucose level (P=0.88). ESCC patients who reported less than 18 months follow up were only showed high blood urea nitrogen in their serum. 2% of the patients who reported 6 months or less of follow up whereas 20% of the patients who reported follow up duration between 25-36 months reported elevated level of serum creatinine (P=0.072). Patients with follow up duration 0-6 months, 7-12 months, 13-18 months, 19-24 months, 25-36 months and more than 37 months had reported high total protein level in their blood in 6%, 12%, 14%, 0%, 40% and 0% of those patients respectively (P=0.23).

35% of the patients with 0-6 months of follow up reported low albumin level in their blood whereas 9% of the patients with more than 19 months of follow up reported low albumin level (P=0.053). AST level in blood was found elevated in 8% and 17% of the patients with 0-6 and 19-24 month follow up respectively (P=0.29). Serum ALT was found elevated only in patients with 0-6 month duration. 2% of the patients with high ALKP level in their serum were found having 0-6 months of follow up in comparison to 17% of the patients with 19-24 months of follow up respectively (P=0.079). 6%, 6% and 60% of the patients with 0-6, 7-12 and 25-36 month follow up respectively reported high blood total bilirubin level (P=0.001). None showed statistical correlation except total bilirubin.

Biochemical parameter	Follow up: 0-6	Follow up: 7-12	Follow up: 13-18	Follow up: 19-24	Follow up: 25-36	Follow up: ≥ 37	*P*-value
Glucose							
<106 mg/dL	38	25	10	5	3	1	0.88
≥106 mg/dL	10	9	4	1	2	0	
Urea nitrogen							
< 20 mg/dL	47	33	13	6	5	1	0.92
≥ 20 mg/dL	1	1	1	0	0	0	
Creatinine							
< 1.50 mg/dL	47	34	14	6	4	1	0.072
≥ 1.50 mg/dL	1	0	0	0	1	0	
Total protein							
< 8.20 g/dL	45	30	12	6	3	1	0.23
≥ 8.20 g/dL	3	4	2	0	2	0	
Albumin							
< 3.5 g/dL	17	4	2	0	1	0	0.053
≥ 3.5 g/dL	31	30	12	6	4	1	
Aspartate transaminase							0.29
< 59 U/L	44	34	14	5	5	1	
≥ 59 U/L	4	0	0	1	0	0	
Alanine transaminase							0.93
< 72 U/L	47	34	14	6	5	1	
≥ 72 U/L	1	0	0	0	0	0	
Alkaline phosphatase							
< 80 U/L	8	7	1	0	0	1	0.079
80-280 U/L	39	27	13	5	5	0	
≥ 280 U/L	1	0	0	1	0	0	
Total Bilirubin							
< 1.30 mg/dL	45	32	14	6	2	1	0.001
≥ 1.30 mg/dL	3	2	0	0	3	0	

Table 14: Biochemical data correlation with follow up duration of the ESCC patients.

Chapter-4.2: Treatment response and prognostic factor analysis based on molecular markers ALDH1, HER2 and p16 in esophageal squamous cell carcinoma

4.2.1 Expression of ALDH1, HER2 in pre-treated ESCC tumor

ALDH1 over expression was found in 65.7% (71 out of 108) of ESCC patient. Out of 108, only 8 (7.4%) ESCC tumor tissue were stained positive for HER2. Figure 23 depicts the staining pattern of ALDH1 and HER2 in ESCC. All 8 positive HER2 cases were correlated with high ALDH1 expression (*P*=0.034) and majority (87.5%) were in non responder category (*P*=.0.56) as shown in Table 15 and 16 respectively. Majority of ALDH1 positive ESCC patients were insignificantly associated with more than 45 years of age (*P*=0.38), moderately differentiated squamous cell carcinoma (*P*=0.20), mid thoracic location (*P*=0.63) and more reported deaths (*P*=0.71) . Response to NACT was poor in patients with ALDH1 positive ESCC tumors (*P*<0.001).

	ALDH1 Positive (n=71)	**ALDH1 Negative (n=37)**	***P*-value**
Age (in Years)			
≤45	14	10	0.38
>45	57	27	
Gender			
Male	48	25	0.99
Female	23	12	
Initial performance status			
Performance status: 0	9	8	
Performance status: 1	48	24	0.50
Performance status: 2	13	4	
Performance status: 3	1	1	
Pathological Grade			0.20

Well differentiated	16	12	
Moderately differentiated	53	22	
Poorly differentiated	2	3	
Cancer Site			
Cervical	1	0	
Upper thoracic	14	5	0.63
Mid thoracic	42	26	
Lower thoracic	14	6	
Disease spread			
Circumference: 1/2	12	11	
Circumference: 2/3	9	4	0.49
Circumference: 3/4	46	20	
Circumference: Whole	4	2	
Neo adjuvant chemotherapy			
Responders	7	15	<0.001
Non Responders	64	22	
HER2 status			
Positive	8	0	0.034
Negative	63	37	
Family history of cancer			
Yes	8	2	0.31
No	63	35	
Death			
Yes	41	20	0.71
No	30	17	

Table 15: Correlation analysis of clinicopathological factors with ALDH1 expression in ESCC patients.

	HER2 Positive (n=8)	**HER2 Negative (n=100)**	***P*-value**
Age (in Years)			
≤45	2	6	0.84
>45	6	78	
Gender			
Male	7	66	0.21
Female	1	34	

Initial performance status			
Performance status: 0	1	16	
Performance status: 1	7	65	0.54
Performance status: 2	0	17	
Performance status: 3	0	2	
Pathological Grade			
Well differentiated	1	27	0.40
Moderately differentiated	6	69	
Poorly differentiated	1	4	
Cancer Site			
Cervical	0	1	
Upper thoracic	1	18	0.93
Mid thoracic	5	63	
Lower thoracic	2	18	
Disease spread			
Circumference: 1/2	3	20	
Circumference: 2/3	1	12	0.45
Circumference: 3/4	3	63	
Circumference: Whole	1	5	
Neo adjuvant chemotherapy			
Responders	1	21	0.56
Non Responders	7	79	
Family history of cancer			
Yes	1	9	0.74
No	7	91	
Death			
Yes	3	58	0.26
No	5	42	

Table 16: Correlation analysis of clinicopathological factors with HER2 expression in ESCC patients.

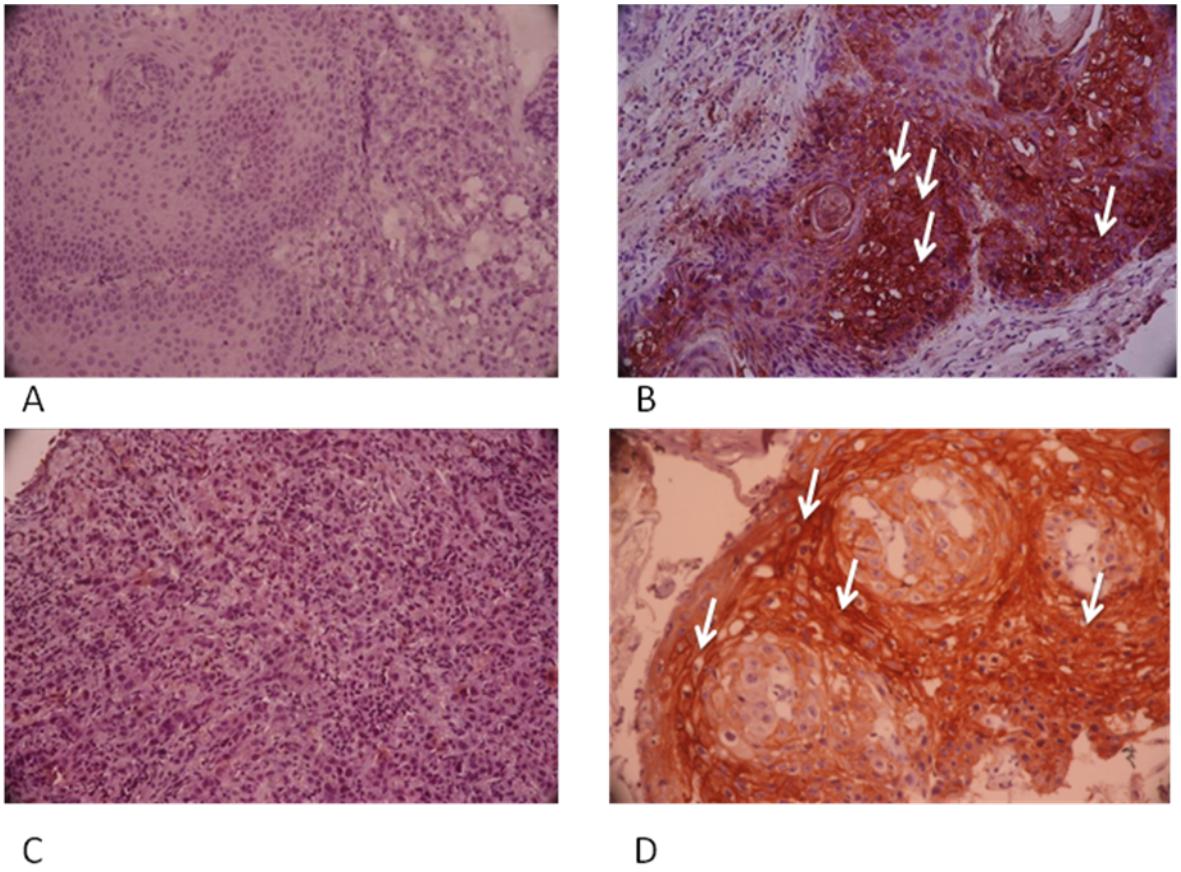


Figure 23. ALDH1 and HER2 immunohistochemistry in esophageal squamous cell carcinoma. (A) ALDH1 negative staining; (B) ALDH1 positive staining (> 10% of tumor cells); (C) HER2 negative staining; (D) HER2 positive staining.

Kaplan-Meier probability distribution of overall survival for esophageal squamous cell carcinoma patients who were reported positive for ALDH1 and HER2 was represented in the figure 24 and 25 respectively.

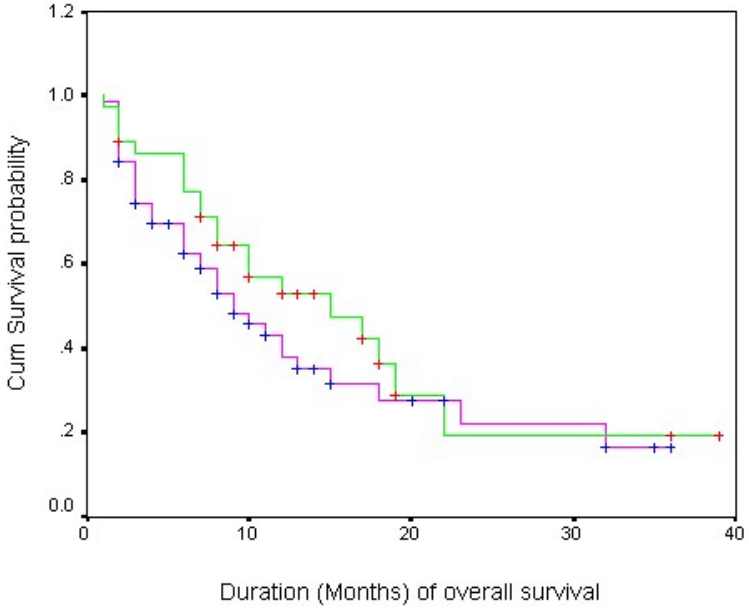


Figure 24. Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients with (n=71; pink line) and without (n=37; green line) ALDH1 expression.

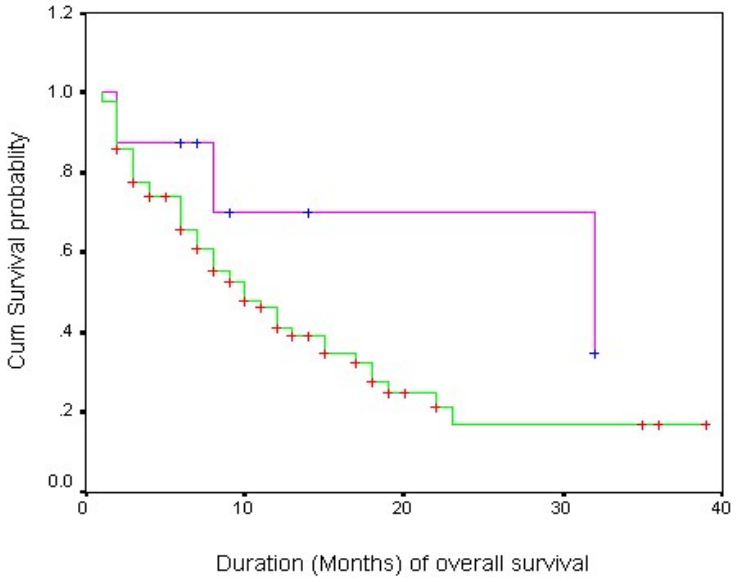


Figure 25. Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients with (n=8; pink line) and without (n=100; green line) HER2 expression.

4.2.2 Expression of molecular marker p16 in pre-treated ESCC tumor

Correlation analysis of clinicopathological factors with p16 expression in ESCC patients is shown in Table 17. Over expression of p16 was reported in 22% of ESCC (24 out of 108) patients. Those who had shown p16 over expression, majority (92%) were more than 45 years old (*P*=0.044). 62% of p16 positive patients were males (15 out of 24) (*P*=0.54).

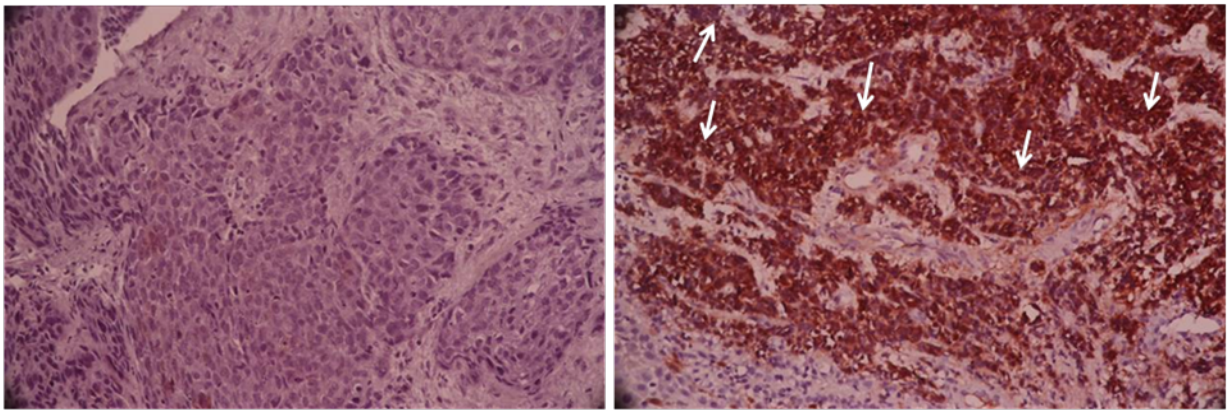
Most of the ESCC patients presented with dysphagia as an initial symptom. Initial Eastern Cooperative Oncology Group (ECOG) performance status was reported either 0 (normal) or 1 (near normal) or 2 (difficulty in normal activity) in 16%, 67% and 16% patients respectively. p16 positive tumors appeared more prominently in upper and mid thoracic region (83%) and tended to expressed more in moderately differentiated ESCC (58%).

54% of the patient's who had shown p16 positivity were found having three fourth circumferential disease. However, ESCC tumors that were found positive for p16 expression appeared to fall into responders group rather than non responders (*P*=<0.001) and reported with less mortality (*P*=0.097) that couldn't attain statistical significance.

ALDH1 and HER2 expression couldn't correlate statistically with p16 expression in this study. 46% p16 positive were found responders whereas 50% responders were observed p16 positive. Figure 26 represented the p16 positive and negative ESCC tumor section whereas figure 27 represented the Kaplan-Meier probability distribution of overall survival in p16 positive and negative ESCC patients whereas figure 28 showed the Kaplan-Meier probability distribution of overall survival in responder and non responder group of ESCC patients.

	p16 Positive (n=24)	p16 Negative (n=84)	*P*-value
Age (in Years)			
≤45	2	22	0.044
>45	22	62	
Gender			
Male	15	58	0.54
Female	9	26	
Initial performance status			
Performance status: 0	1	16	
Performance status: 1	17	55	0.17
Performance status: 2	6	11	
Performance status: 3	0	2	
Pathological Grade			
Well differentiated	8	20	0.34
Moderately differentiated	14	61	
Poorly differentiated	2	3	
Cancer Site			
Cervical	0	1	
Upper thoracic	7	12	0.38
Mid thoracic	13	55	
Lower thoracic	4	16	
Disease spread			
Circumference: 1/2	6	17	
Circumference: 2/3	4	9	0.77
Circumference: 3/4	13	53	
Circumference: Whole	1	5	
Neo adjuvant chemotherapy			
Responders	11	11	<0.001
Non Responders	13	73	
ALDH1			
Negative	9	28	0.70
Positive	15	56	
HER2			
Negative	22	78	0.84
Positive	2	6	
Family history of cancer			
Yes	1	9	0.32
No	23	75	
Death			
Yes	10	51	0.097
No	14	33	

Table 17. Correlation analysis of clinicopathological factors with p16 expression in ESCC patients.



A B

Figure 26: p16 expression in ESCC. **A.** p16 negative ESCC tumor; **B.** p16 positive ESCC tumor

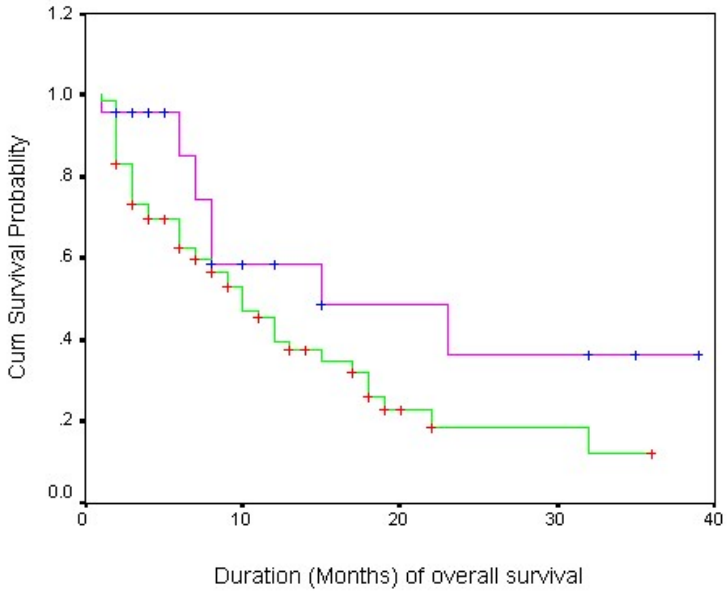


Figure 27: Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients with (n=24; pink line) and without (n=84; green line) p16 expression.

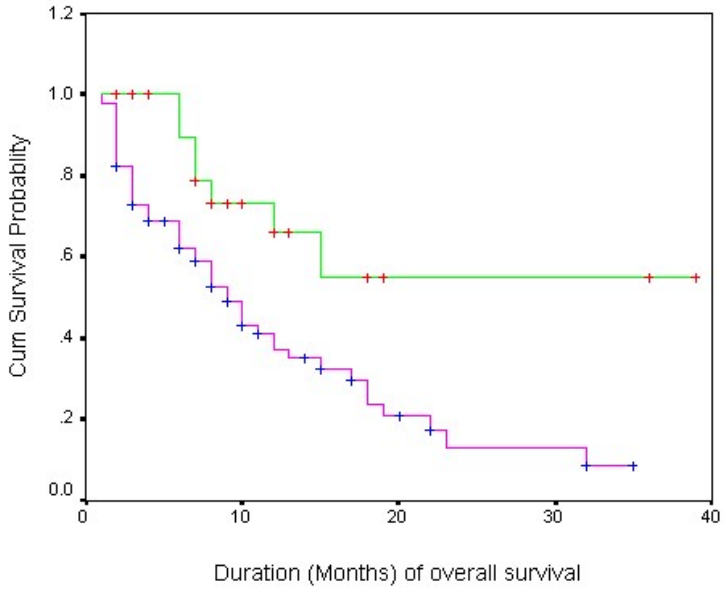


Figure 28: Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients who not responded (n=86; pink line) and those who responded (n=22; green line) to neo adjuvant chemotherapy.

Chapter-4.3: Treatment response and prognostic factor analysis based on cell cycle parameter (Ploidy estimation and S phase fraction) in esophageal squamous cell carcinoma

4.3.1 Cell cycle analysis through ploidy estimation in esophageal squamous cell carcinoma patients

Patients with esophageal squamous cell cancer displayed various ploidy levels that ranged from hypodiploidy (49%), hyperploidy (35%), tetraploidy (2%), polyploidy (7%) and diploid (7%). In all these cases, the variation was reported more in the higher age group of more than 46 years of age like 83% of the patients who displayed hypoploidy tumor were more than 45 years old. 79% and 66% of hyperploidy and hypoploidy groups

were males respectively (*P*=0.11). Majority of the patients (68%) irrespective of ploidy status were initially reported in the performance status 1 (*P*=0.15) and 69% were of moderately differentiated tumor grade (*P*=0.31). 26% patients with hyperdiploidy tumor, 23% with hypodiplody tumor and 14% with diploid tumor showed one half of the circumferential disease spread whereas in the same group of patients 66%, 57% and 88% showed the whole circumferential disease spread respectively (*P*=0.078). 57% of the patients with diploid tumor, 28% with hypoploid tumor and 8% with hyperploid tumor responded to neo-adjuvant chemotherapy (*P*=0.007). Family history of cancer and reported death were not found statistically significant in this group. The correlation was depicted in the table 18.

	Hypoploid Tumor (53)	**Hyperploid Tumor (38)**	**Tetraploid Tumor (02)**	**Polyploid Tumor (08)**	**Diploid Tumor (07)**	***P*-value**
Age (in Years)						
≤45	9	12	0	2	1	0.45
>45	44	26	2	6	6	
Gender						
Male	35	30	1	5	2	0.11
Female	18	8	1	3	5	
Initial performance status						
Performance status: 0	5	9	0	1	2	0.15
Performance status: 1	37	25	1	6	3	
Performance status: 2	11	3	1	0	2	
Performance status: 3	0	1	0	1	0	
Pathological Grade						
Well differentiated	14	7	1	2	4	
Moderately differentiated	38	27	1	6	3	0.31
Poorly differentiated	1	4	0	0	0	
Cancer Site						
Cervical	1	0	0	0	0	
Upper thoracic	9	8	2	0	0	0.087
Mid thoracic	34	21	0	6	7	
Lower thoracic	9	9	0	2	0	

Disease spread						
Circumference: 1/2	12	10	0	0	1	0.078
Circumference: 2/3	7	3	0	3	0	
Circumference: 3/4	4	0	0	2	0	
Circumference: Whole	30	25	2	3	6	
Neo adjuvant chemotherapy						0.007
Responders	15	3	0	0	4	
Non Responders	38	35	2	8	3	
Family history of cancer						0.60
Yes	7	2	0	1	0	
No	46	36	2	7	7	
Death						
Yes	28	24	1	5	3	0.79
No	25	14	1	3	4	

Table 18: Clinicopathological data correlation of the squamous cell carcinoma of the esophagus: diploid and different types of aneuploid cases.

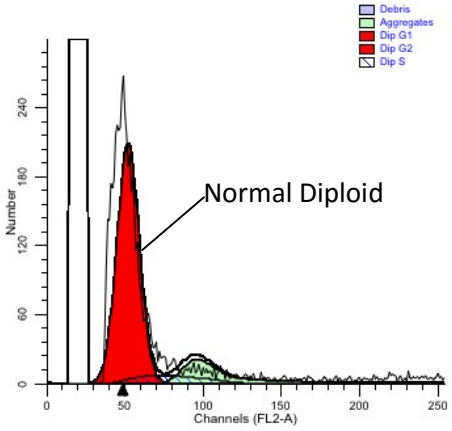
Table 19 represented the correlation between ploidy status of the tumor and different markers. 41% of tumors were found in the range of 51-75% S-phase fraction and 19% were found having more than 75% cells in S-phase whereas 30% of the tumor cells were reported in the S-phase fraction range of 0-25% (P=0.003). In other words, 8% and 92% of hypoploid and hyperploid tumors were having 0% S-phase fraction respectively. 71% of diploid tumors cells fall in the S-phase range of 51-75% in comparison to 25% in polyploid tumors, 50% in tetraploid tumor, 29% in hyperploid tumor and 47% in hypoploid tumors. 76% of hyperploids tumor and 43% of diploid tumor were found ALDH1 positive whereas 41% of ALDH1 positive tumor were reported hperploidy and 4% of ALDH1 positive tumor showed diploid ploidy status (P=0.059). 50% of the HER2 positive tumor cases showed hyperploidy status whereas

10% of cases with hyperploidy status were HER2 positive (*P*=0.64). No tetraploid tumor expressed HER2 and p16. Majority of p16 positive tumor (50%) showed hypodiploidy status and 23% of tumors with hypodiploid status were found p16 positive (P=0.61).

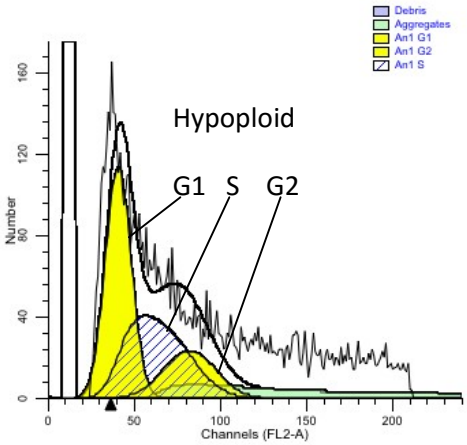
	Hypoploid Tumor (53)	**Hyperploid Tumor (38)**	**Tetraploid Tumor (02)**	**Polyploid Tumor (08)**	**Diploid Tumor (07)**	***P*-value**
S-Phase fraction						
0%	1	12	0	0	0	
1-25%	9	7	0	3	0	0.003
26-50%	4	4	1	2	0	
51-75%	25	11	1	2	5	
>75%	14	4	0	1	2	
ALDH1						
Positive	30	29	2	7	3	0.059
Negative	23	9	0	1	4	
HER2						
Positive	2	4	0	1	1	0.64
Negative	51	34	2	7	6	
p16						
Positive	12	7	0	2	3	0.61
Negative	41	31	2	6	4	

Table 19: Correlation between ploidy status with various molecular markers.

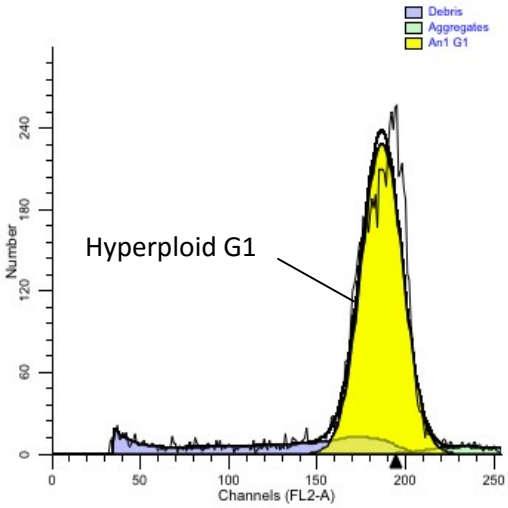
Figure 29 and 30 represented the different ploidy status that was found in the ESCC patients. Majority of the ploidy status that we found in this study were of hypodiploid and hyperdiploid with tetraploid and polyploid tumor also in some cases.



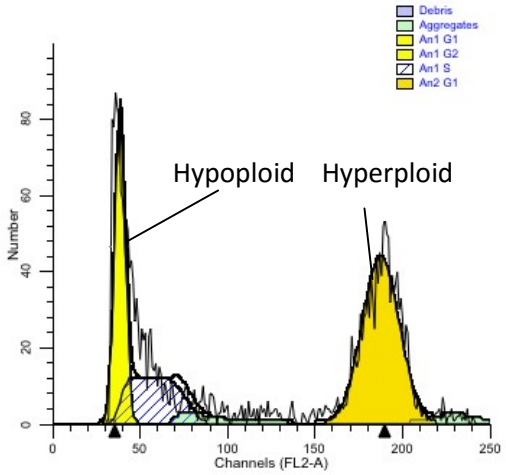
A. Normal Diploid



B. Hypodiploid

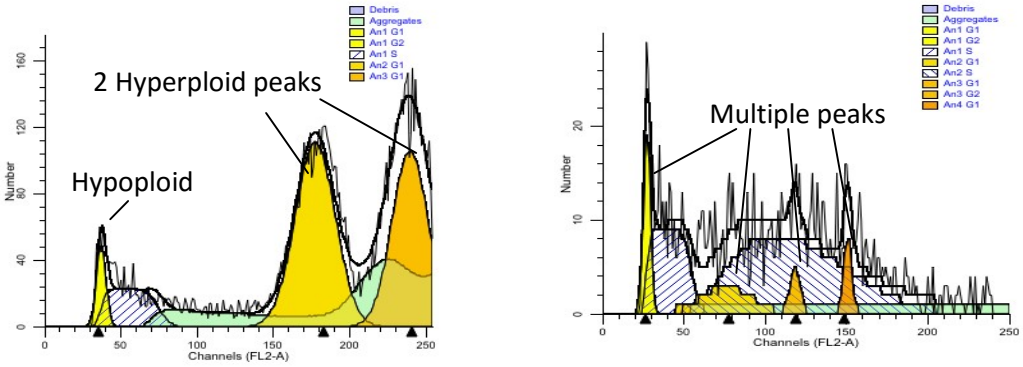


C. Hyperdiplod

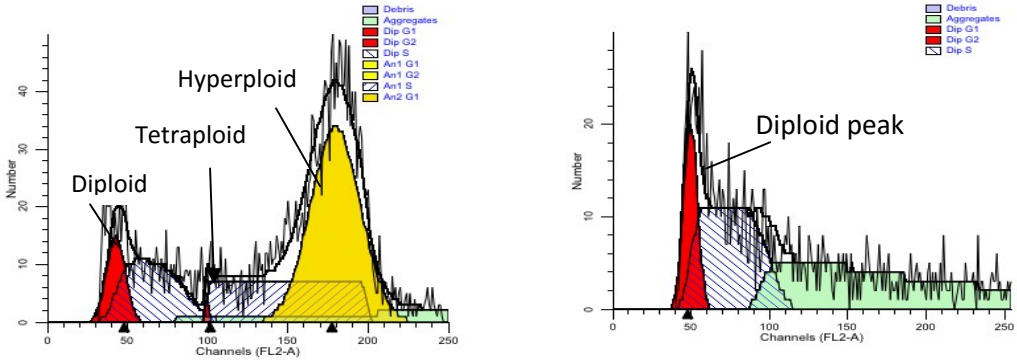


D. Mix population of hypodiploid and hyperdiplod

Figure 29: Different nuclear (DNA) composition of cells in esophageal squamous cell carcinoma (B,C&D) in comparison to normal esophageal squamous cells (A).

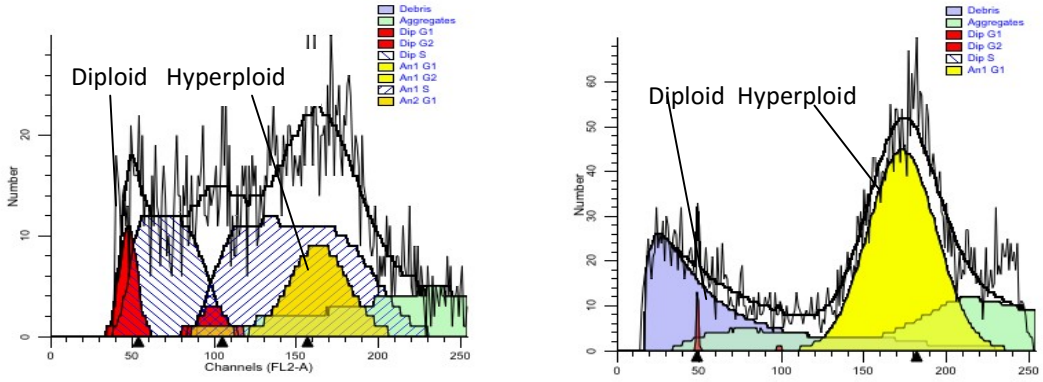


A. Polyploid tumors



B. Diploid + tetraploid + aneuploid

C. Diploid tumor



D. Diploid + aneuploid tumors

Figure 30: Various types of ploidy reported in esophageal squamous cell carcinoma. (Aneuploid = Hypoploid or Hyperploid or Tetraploid or Polyploid)

4.3.2 Cell cycle analysis through S phase fraction estimation in esophageal squamous cell carcinoma patients

ESCC patients were divided into 5 categories of S phase fraction dependent on its value and correlated with the clinic pathological factors as tabulated in the Table 20. More than 50% of the S phase fraction was reported more in patients who were more than 45 years of age (85%) than those who were less than 45 years of age. Similar results came when we compare low S phase fraction with age group. 85% of those patients who showed 0% S phase fraction were males and rest15% were females whereas among the patients who reported more than 75% S phase fraction, it was found that 62% were male and rest were female (P=0.30).

No statistical correlation of S phase fraction could be established with pathological grading, cancer site, circumferential disease spread, family history of cancer and reported death in the studied ESCC patients. Among those patients who responded to neo adjuvant chemotherapy, 14% were reported to have less than 50% and 86% were reported to have more than 50% S phase fraction respectively whereas among non responders 47% and 53% were reported to have less than and more than 50% of S phase fraction (P=0.041).

	S Phase fraction					***P*-value**
	0%	**1-25%**	**26-50%**	**51-75%**	**>75%**	
Age (in Years)						
≤45	3	7	4	5	5	0.15
>45	10	12	7	39	16	
Gender						
Male	11	14	9	26	13	0.30
Female	2	5	2	18	8	
Initial performance status						
0	4	2	2	7	2	
1	8	14	7	28	15	0.75
2	1	3	1	8	4	
3	0	0	1	1	0	

Pathological Grade						
Well differentiated	2	7	3	12	4	0.38
Moderately differentiated	9	12	8	29	17	
Poorly differentiated	2	0	0	3	0	
Cancer Site						
Cervical	0	1	0	0	0	
Upper thoracic	4	2	3	6	4	0.65
Mid thoracic	7	12	6	28	15	
Lower thoracic	2	4	2	10	2	
Disease spread						
Circumference: 1/2	4	2	2	10	5	
Circumference: 2/3	1	1	2	7	2	0.57
Circumference: 3/4	0	3	0	3	0	
Circumference: Whole	8	13	7	24	14	
Neo adjuvant chemotherapy						0.041
Responders	0	2	1	11	8	
Non Responders	13	17	10	33	13	
Family history of cancer						
Yes	1	1	0	6	2	0.64
No	12	18	11	38	19	
Death						
Yes	9	13	7	24	8	0.27
No	4	6	4	20	13	

Table 20: Clinicopathological data correlation with the S phase fraction in the squamous cell carcinoma of the esophagus.

Table 21 depicts the correlation between different levels of S phase fraction and molecular marker along with tumor ploidy status. Among those patients who reported hyperploids tumor status, 61% showed less than 50% S phase fraction whereas rest 39% showed tumor with more than 50% S phase fraction. On the contrary, patients with diploid tumor showed 100% S phase fraction in the range of more than 50% (P=0.003). Those patients who were having 0% S phase fraction, 100% of them were ALDH1 positive whereas patients who were showed more than 75% S phase fraction reported to have 57% ALDH1 positivity. In other words, among all the ALDH1 positive tumors, 45% of them showed S phase fraction less than 50% whereas rest 55% showed more than

50% S phase fraction and among ALDH1 negative group of ESCC patients, 30% showed less than 50% S phase fraction whereas 70% tumor showed more than 50% S phase fraction (P=0.034). No statistical correlation was found between different values of S phase fraction and HER2 and p16 expression status. Figure 31 showed a tumor population with high and low S phase fraction.

	S Phase fraction					*P*-value
	0%	**1-25%**	**26-50%**	**51-75%**	**>75%**	
Ploidy status						
Hypoploid	1	9	4	25	14	0.003
Hyperploid	12	7	4	11	4	
Tetraploid	0	0	1	1	0	
Polyploid	0	3	2	2	1	
Diploid	0	0	0	5	2	
ALDH1						
Negative	0	5	6	17	9	0.034
Positive	13	14	5	27	12	
HER2						
Negative	11	17	10	41	21	0.52
Positive	2	2	1	3	0	
p16						
Negative	10	14	19	34	16	0.85
Positive	3	5	1	10	5	

Table 21: Correlation between cell cycle S phase fraction with various molecular markers.

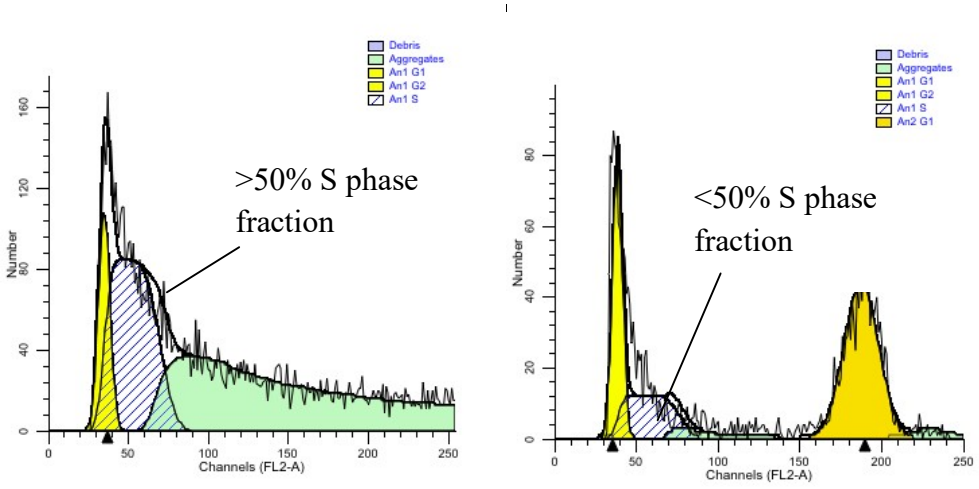


Figure 31: ESCC patients with different percentage of S phase fraction.

Chapter-4.4: Analysis of inter-relationship among biochemical, epidemiological and molecular factors with their impact on clinical outcome in esophageal squamous cell carcinoma patients

4.4.1 Biochemical parameter correlation with ALDH1 expression in esophageal squamous cell carcinoma patients

Biochemical parameter was correlated with the ALDH1 expression in the ESCC patients as given in table 22. 62% of the patients with high glucose level were ALDH1 positive whereas 23% among the ALDH1 positive cases were reported increased blood glucose level, and among ALDH1 negative patients, 27% were having high blood glucose level (*P*=0.60). Amongst the ALDH1 positive cases, merely 1% was having high urea nitrogen level whereas in ALDH1 negative group 5% were having more urea nitrogen level than normal (*P*=0.23). 100% of the patients having high creatinine level showed ALDH1 positivity (*P*=0.30).

Among the patients with high serum total protein, 64% and 36% showed ALDH1 positive and negative status respectively whereas 10% among ALDH1 positive cases showed elevated serum total protein level (*P*=0.87). 25% of ALDH1 positive patients and 19% of the ALDH1 negative patients showed low level of albumin in their blood (*P*=0.27). ALT and AST high levels was reported in majority patients with ALDH1 positive status viz. 80% and 100% respectively. Although ALKP levels were found equally high in both ALDH1 positive and negative status patients. Serum total bilirubin level was found elevated more in ALDH1 positive tumor (75%) than ALDH1 negative tumors whereas among ALDH1 positive tumors, merely 8% were having high bilirubin

leveal and in ALDH1 negative tumor groups, 5% were having high bilirubin level in their serum (*P*=0.56). However, none of the biochemical parameter showed any statistical significance when compared with the ALDH1 expression status in ESCC patients.

Biochemical parameter	**ALDH1 positive**	**ALDH1 negative**	***P*-value**
Glucose			0.60
<106 mg/dL	55	27	
≥106 mg/dL	16	10	
Urea nitrogen			0.23
< 20 mg/dL	70	35	
≥ 20 mg/dL	1	2	
Creatinine			0.30
< 1.50 mg/dL	69	37	
≥ 1.50 mg/dL	2	0	
Total protein			0.87
< 8.20 g/dL	64	33	
≥ 8.20 g/dL	7	4	
Albumin			0.27
< 3.5 g/dL	18	6	
≥ 3.5 g/dL	53	31	
Aspartate transaminase (AST)			0.49
< 59 U/L	67	36	
≥ 59 U/L	4	1	
Alanine transaminase (ALT)			0.46
< 72 U/L	70	37	
≥ 72 U/L	1	0	
Alkaline phosphatase (ALKP)			0.70
< 80 U/L	10	7	
80-280 U/L	60	29	
≥ 280 U/L	1	1	
Total Bilirubin			0.56
< 1.30 mg/dL	65	35	
≥ 1.30 mg/dL	6	2	

Table 22: ALDH1 expression correlation with the biochemical parameter in the ESCC patients.

4.4.2 Biochemical parameter correlation with HER2 positive status in esophageal squamous cell carcinoma patients

HER2 status was correlated with the biochemical parameters in ESCC patients as depicted in table 23. 88% of HER2 positive cases and 75% of HER2 negative were having blood glucose level <106 mg/dL whereas among patients with elevated blood glucose level, majority 96% belongs to HER2 negative patients (*P*=0.42). 100% of the patients with elevated urea nitrogen were in HER2 negative group (*P*=0.61).

Among HER2 positive cases, 12.5% of the patients were having high creatinine level whereas it was only 1% in case of HER2 negative cases. Therefore, HER2 positive status appears to indicate high serum creatinine level (*P*=0.020). 9% of HER2 negative group of patients showed high level of total protein whereas 25% of HER2 positive cases showed high level of total protein in their serum (*P*=0.15).

12.5% of the HER2 positive cases and 23% of the HER2 negative cases showed low albumin serum level (*P*=0.49). High serum levels of AST, ALT and ALKP were reported in only HER2 negative cases. 25% of the HER2 positive and 6% of the HER2 negative status were documented in patients with high total bilirubin level, where it appears that HER2 positive status correlates with high total bilirubin level in serum (P=0.048).

Biochemical parameter	HER2 positive	HER2 negative	*P*-value
Glucose			0.42
<106 mg/dL	7	75	
≥106 mg/dL	1	25	
Urea nitrogen			0.61
< 20 mg/dL	8	97	
≥ 20 mg/dL	0	3	
Creatinine			0.020
< 1.50 mg/dL	7	99	
≥ 1.50 mg/dL	1	1	
Total protein			0.15
< 8.20 g/dL	6	91	
≥ 8.20 g/dL	2	9	
Albumin			0.49
< 3.5 g/dL	1	23	
≥ 3.5 g/dL	7	77	
Aspartate transaminase (AST)			0.51
< 59 U/L	8	95	
≥ 59 U/L	0	5	
Alanine transaminase (ALT)			0.77
< 72 U/L	8	99	
≥ 72 U/L	0	1	
Alkaline phosphatase (ALKP)			0.70
< 80 U/L	2	15	
80-280 U/L	6	83	
≥ 280 U/L	0	2	
Total Bilirubin			0.048
< 1.30 mg/dL	6	94	
≥ 1.30 mg/dL	2	6	

Table 23: Routine biochemical data correlation in the ESCC patients with the HER2 expression status.

4.4.3 Biochemical parameter correlation with p16 expression status in esophageal squamous cell carcinoma patients

P16 expression status was correlated with the routine biochemical parameter in the ESCC patients and presented in the Table 24. 33% and 21% of ESCC patients with p16 positive and negative expressing tumor respectively were having elevated blood glucose level (*P*=0.22). Among the patients with high urea nitrogen level, 67% were fall into the p16 negative group and the rest 33% into p16 positive tumor category (*P*=0.63). Serum creatinine level was found elevated only in p16 negative tumor (P=0.44). Among the p16 positive group, 17% were having high total protein level whereas among the p16 negative group, 8% were having elevated total protein in their blood (*P*=0.23). 25% of the p16 negative patients showed low serum albumin level whereas 12.5% of the p16 positive tumor showed the similar albumin level in their blood (*P*=0.19). None of the p16 positive ESCC patients showed high AST, ALT or ALKP level in their blood on the contrary p16 negative tumor reflected all such instances. 37.5% of the total patients with elevated serum total bilirubin level were p16 positive and rest were negative whereas out of total p16 positive cases 12.5% were represented high total bilirubin level in their blood (P=0.28).

Biochemical parameter	p16 positive	p16 negative	*P*-value
Glucose			
<106 mg/dL	16	66	0.22
≥106 mg/dL	8	18	
Urea nitrogen			
< 20 mg/dL	23	82	0.63
≥ 20 mg/dL	1	2	
Creatinine			
< 1.50 mg/dL	24	82	0.44
≥ 1.50 mg/dL	0	2	
Total protein			0.23

< 8.20 g/dL ≥ 8.20 g/dL	20 4	77 7	
Albumin < 3.5 g/dL ≥ 3.5 g/dL	 3 21	 21 63	0.19
Aspartate transaminase (AST) < 59 U/L ≥ 59 U/L	 24 0	 79 5	0.22
Alanine transaminase (ALT) < 72 U/L ≥ 72 U/L	 24 0	 83 1	0.59
Alkaline phosphatase (ALKP) < 80 U/L 80-280 U/L ≥ 280 U/L	 4 20 0	 13 69 2	0.74
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	 21 3	 79 5	0.28

Table 24: p16 expression correlation with biochemical parameter in the ESCC patients.

4.4.4 Biochemical parameter correlation with reported ploidy status in esophageal squamous cell carcinoma patients

In the following table 25, ploidy status was correlated with the routine biochemical parameter of the ESCC patients. 22% of the patients with aneuploidy were having high blood glucose level in comparison to 57% of the patients with diploid tumors (*P*=0.034). High urea nitrogen level was seen only in patients with aneuploid tumors whereas 3% of the aneuploid tumor associated patients reported high urea nitrogen level (*P*=0.64). 2% of the patients among the aneuploid cases showed high serum creatinine level and none of the patients with diploid tumor had showed the same (*P*=0.70). 91% of patients with high total protein serum level were having aneuploid tumors whereas 10%

of the patients with aneuploid tumors showed elevated serum protein level (*P*=0.71). Low level of serum albumin was reported in 22% and 29% of the patients with aneploid and diploid tumors respectively (*P*=0.67). High serum level of AST, ALT and ALKP were predominantly reported in patients with aneuploid status. In similar way, total serum bilirubin was also found elevated only among patients with aneuploid status and among aneuploid group 8% were having high total serum bilirubin (*P*=0.43).

Biochemical parameter	Aneuploid	Diploid	*P*-value
Glucose			
<106 mg/dL	79	3	0.034
≥106 mg/dL	22	4	
Urea nitrogen			
< 20 mg/dL	98	7	0.64
≥ 20 mg/dL	3	0	
Creatinine			
< 1.50 mg/dL	99	7	0.70
≥ 1.50 mg/dL	2	0	
Total protein			
< 8.20 g/dL	91	6	0.71
≥ 8.20 g/dL	10	1	
Albumin			
< 3.5 g/dL	22	2	0.67
≥ 3.5 g/dL	79	5	
Aspartate transaminase (AST)			
< 59 U/L	97	6	0.20
≥ 59 U/L	4	1	
Alanine transaminase (ALT)			
< 72 U/L	100	7	0.79
≥ 72 U/L	1	0	
Alkaline phosphatase (ALKP)			
< 80 U/L	16	1	0.92
80-280 U/L	83	6	
≥ 280 U/L	2	0	
Total Bilirubin			
< 1.30 mg/dL	93	7	0.43
≥ 1.30 mg/dL	8	0	

Table 25: Ploidy status correlation with biochemical parameter in the ESCC patients.

4.4.5 Correlation with reported different variation of ploidy status with biochemical parameter in esophageal squamous cell carcinoma patients

In the Table 26, we further differentiated the aneuploidy into different groups to see how it was correlating with the various routine biochemical parameters. High level of blood glucose was seen in 28%, 19%, 0%, 0% and 57% of patients with hypoploidy, hyperploidy, tetraploidy, polyploidy and diploid status respectively (*P*=0.037). 4% and 3% of the patients with hypoploidy and hyperploidy tumors respectively showed increase in their serum urea nitrogen level whereas other ploidy groups like tetraploidy, polyploidy and diploid showed nil percentage (*P*=0.95). High level of serum creatinine was reported only in patients with hyperploid tumors whereas merely 5% of the patients of this group showed high blood creatinine level (*P*=0.44).

Elevated total protein level was found in 45% each in patients with hypodiploid and hyperdiploid status respectively and in diploid tumor it was nearly 10% (*P*=0.79). 19% of the hypodiploid tumor group patients represented low serum albumin level whereas it was 24% in hyperploidy group, 50% in tetraploidy group, 25% in polyploidy group and 29% in diploid group (*P*=0.82). Patients with tetraploidy tumor didn't showed high AST level whereas 2%, 5%, 12.5% and 14% of hypoploid, hyperploid, polyploidy and diploid tumor respectively showed high AST level (*P*=0.45). In case of ALT, only patients with hypodiploid tumor showed its increased level in serum whereas its percentage was 2% among those patients who reported high ALT serum (*P*=0.90). 50% of patients with high ALKP level was reported each in hyperploids and polyploidy groups (*P*=0.54). High total bilirubin level appears to suggest the hyperploidy or tetraploidy status in the ESCC patients (*P*=0.013).

Biochemical parameter	Hypo-ploidy	Hyper-ploidy	Tetra ploidy	Poly ploidy	Diploid	*P*-value
Glucose						
<106 mg/dL	38	31	2	8	3	0.037
≥106 mg/dL	15	7	0	0	4	
Urea nitrogen						
< 20 mg/dL	51	37	2	8	7	0.95
≥ 20 mg/dL	2	1	0	0	0	
Creatinine						
< 1.50 mg/dL	53	36	2	8	7	0.44
≥ 1.50 mg/dL	0	2	0	0	0	
Total protein						
< 8.20 g/dL	48	33	2	8	6	0.79
≥ 8.20 g/dL	5	5	0	0	1	
Albumin						
< 3.5 g/dL	10	9	1	2	2	0.82
≥ 3.5 g/dL	43	29	1	6	5	
Aspartate transaminase						
< 59 U/L	52	36	2	7	6	0.45
≥ 59 U/L	1	2	0	1	1	
Alanine transaminase						
< 72 U/L	52	38	2	8	7	0.90
≥ 72 U/L	1	0	0	0	0	
Alkaline phosphatase						
< 80 U/L	8	7	0	1	1	0.54
80-280 U/L	45	30	2	6	6	
≥ 280 U/L	0	1	0	1	0	
Total Bilirubin						
< 1.30 mg/dL	52	32	1	8	7	0.013
≥ 1.30 mg/dL	1	6	1	0	0	

Table 26: Routine biochemical data correlation in the ESCC patients with their different indicator's of ploidy status.

4.4.6 S phase fraction status correlation with the biochemical in esophageal squamous cell carcinoma patients

S-phase fraction an indication of cellular proliferation was correlated with the routine biochemical parameters in the studied patients and listed in the Table 27. Predominantly 73% of the patients with elevated level of blood glucose were reported more than 51% S-phase fraction (*P*=0.50). Nearly 3% reported high urea nitrogen in their blood and they are distributed equally in patients with 0%, 26-50% and >75% S-phase fraction respectively (*P*=0.29). Serum creatinine level was found elevated in patients with 1-25% and 51-75% S-phase fraction status (P=0.71). 18% of the patients with 51-75% of S-phase fraction reported high total protein level in their blood in comparison to nearly 10% of the patients with more than 75% of S-phase fraction (*P*=0.16). 22% of the patients out of total showed low level of serum albumin, among them the highest percentage of the patients (26%) reported was in with those having S-phase fraction 1-25% (*P*=0.85). Minor percentage of the patients showing different S-phase fraction reported high AST, ALT and ALKP serum level. 18% of the patients who reported their S-phase fraction as 26-50% were found having high serum bilirubin level in comparison to 15% and 5% who had 0% and more than 75% S-phase fraction (*P*=0.30).

Biochemical parameter	S-phase fraction: 0	S-phase fraction: 1-25%	S-phase fraction: 26-50%	S-phase fraction: 51-75%	S-phase fraction: >75%	*P*-value
Glucose						
<106 mg/dL	10	17	9	32	14	0.50
≥106 mg/dL	3	2	2	12	7	
Urea nitrogen						
< 20 mg/dL	12	19	10	44	20	0.29
≥ 20 mg/dL	1	0	1	0	1	

Creatinine < 1.50 mg/dL ≥ 1.50 mg/dL	13 0	18 1	11 0	43 1	21 0	0.71
Total protein < 8.20 g/dL ≥ 8.20 g/dL	12 1	19 0	11 0	36 8	19 2	0.16
Albumin < 3.5 g/dL ≥ 3.5 g/dL	3 10	5 14	1 10	10 34	5 16	0.85
Aspartate transaminase < 59 U/L ≥ 59 U/L	13 0	18 1	9 2	42 2	21 0	0.18
Alanine transaminase < 72 U/L ≥ 72 U/L	13 0	19 0	10 1	44 0	21 0	0.064
Alkaline phosphatase < 80 U/L 80-280 U/L ≥ 280 U/L	1 12 0	2 16 1	0 10 1	11 33 0	3 18 0	0.18
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	11 2	19 0	9 2	41 3	20 1	0.30

Table 27: S phase status correlation with biochemical parameter in the ESCC patients.

4.4.7 Treatment response to chemotherapy correlation with the biochemical parameter in esophageal squamous cell carcinoma patients

Response to chemotherapy was correlated with the routine biochemical parameter of the ESCC patients as depicted in the Table 28. 36% and 21% of the patients with high blood glucose level were reported as responders and non responders respectively (P=0.13). Among those patients with elevated urea nitrogen, 67% were non responders

and rest 33% were responders (P=0.57). Only non responders showed high level of creatinine in their blood whereas among non responders, merely 2% were having high serum creatinine level (P=0.47). 9% and 10% of the patients with response and non response status against neoadjuvatant chemotherapy showed high total protein level in their blood respectively (*P*=0.84). 24% of the non responder and 14% of the responder showed low albumin level in their blood (*P*=0.27).

Patients with non responder status against NACT only showed elevated level of AST, ALT and ALKP in their blood. 8% and 7% of the patients with responder and non responder status respectively reported high total bilirubin level in their blood (P=0.73).

Biochemical parameter	**Responder**	**Non responder**	***P*-value**
Glucose			
<106 mg/dL	14	68	0.13
≥106 mg/dL	8	18	
Urea nitrogen			
< 20 mg/dL	21	84	0.57
≥ 20 mg/dL	1	2	
Creatinine			
< 1.50 mg/dL	22	84	0.47
≥ 1.50 mg/dL	0	2	
Total protein			
< 8.20 g/dL	20	77	0.84
≥ 8.20 g/dL	2	9	
Albumin			
< 3.5 g/dL	3	21	0.27
≥ 3.5 g/dL	19	65	
Aspartate transaminase			
< 59 U/L	22	81	0.24
≥ 59 U/L	0	5	
Alanine transaminase			
< 72 U/L	22	85	0.61
≥ 72 U/L	0	1	

Alkaline phosphatase < 80 U/L 80-280 U/L ≥ 280 U/L	 4 18 0	 13 71 2	0.73
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	 20 2	 80 6	0.73

Table 28: Response to chemotherapy status correlation with biochemical parameter in the ESCC patients.

4.4.8 Inter-relationship between biochemical parameter in esophageal squamous cell carcinoma patients

Different routine biochemical parameter was correlated with each other expression in ESCC patients and the correlation listed in the Table 29. Blood glucose level in ESCC patients couldn't find any statistical correlation with either urea nitrogen or creatinine or total protein or albumin or AST or ALT or ALKP or total bilirubin level. Similarly, urea nitrogen blood level was not found correlated with any other biochemical parameter in the ESCC patients. Serum creatinine level was found correlated with the blood total bilirubin level in the patients (P=0.020). In case of total serum protein, it was found to correlate with the blood level of albumin (P=0.015) and total bilirubin (P=0.002). Albumin serum level couldn't found any significance with the levels of any other biochemical parameters. Serum level of AST were found correlated with ALT (P=0.012) and ALKP (0.001) in the ESCC patients. Other parameters related to routine biochemical were not found any statistical significance with each-other in the current subject study.

	Glucose	Urea nitrogen	Creatinine	Total protein	Albumin	AST	ALT	ALKP	Total bilirubin
Glucose		0.70	0.42	0.63	0.50	0.39	0.074	0.29	0.42
Urea nitrogen	0.70		0.80	0.17	0.060	0.69	0.86	0.71	0.61
Creatinine	0.42	0.80		0.060	0.44	0.75	0.89	0.80	0.020
Total protein	0.63	0.17	0.060		0.002	0.45	0.73	0.71	0.002
Albumin	0.50	0.060	0.44	0.015		0.32	0.59	0.069	0.11
AST	0.39	0.69	0.75	0.45	0.32		0.012	0.001	0.51
ALT	0.074	0.86	0.89	0.73	0.59	0.012		0.89	0.77
ALKP	0.29	0.71	0.80	0.71	0.069	0.001	0.89		0.88
Total Bilirubin	0.42	0.61	0.020	0.001	0.11	0.51	0.77	0.88	

Table 29: Inter-relationship between biochemical data correlation in the ESCC patients. All the quoted values were p value.

4.4.9 Correlation of smoking habit with the reported routine biochemical parameter.

Among the studied 108 ESCC patients, 55% were smokers and rest 45% were non smokers. On the analysis of biochemical factor with smoking habit in Table 30, we found following observations. Among the smokers, 24% were having high blood glucose level whereas among those patients who reported high blood glucose level in their blood, 54% were smokers and rest 46% were non smokers (P=0.92). Only 3% out of 108 ESCC patients reported high urea nitrogen level in their blood. The percentage among smokers was 2% who reported high serum urea nitrogen whereas among non smokers it was 4% (P=0.45). 2% of the smokers reported high serum creatinine level whereas same percentage of non smokers also reported high creatinine level in their blood (P=0.89). 10% of the smokers and non smokers reported high blood protein level (P=0.99). Low level of albumin was reported 62.5% of the smokers whereas it was 37.5% in non smokers. 25% among smokers were having low serum albumin, on the contrary 18%

among non smokers were having similar low value of albumin (P=0.38). 5% among smokers reported high serum AST level whereas it was 4% among non smokers (P=0.80). Serum ALT and ALKP were found elevated in only those patients who were smokers. Among smokers, 10% were reported high serum total bilirubin level whereas 4% among non smokers reported high serum total bilirubin level (P=0.22).

Biochemical parameter	Smokers	Non smokers	*P*-value
Glucose			
<106 mg/dL	45	37	0.92
≥106 mg/dL	14	12	
Urea nitrogen			
< 20 mg/dL	58	47	0.45
≥ 20 mg/dL	1	2	
Creatinine			
< 1.50 mg/dL	58	48	0.89
≥ 1.50 mg/dL	1	1	
Total protein			
< 8.20 g/dL	53	44	0.99
≥ 8.20 g/dL	6	5	
Albumin			
< 3.5 g/dL	15	9	0.38
≥ 3.5 g/dL	44	40	
Aspartate transaminase (AST)			
< 59 U/L	56	47	0.80
≥ 59 U/L	3	2	
Alanine transaminase (ALT)			
< 72 U/L	58	49	0.36
≥ 72 U/L	1	0	
Alkaline phosphatase (ALKP)			
< 80 U/L	10	7	0.38
80-280 U/L	47	42	
≥ 280 U/L	2	0	
Total Bilirubin			
< 1.30 mg/dL	53	47	0.22
≥ 1.30 mg/dL	6	2	

Table 30: Smoking habit correlation with the biochemical parameter in the ESCC patients.

4.4.10 Correlation of tobacco chewing habit status with the reported routine biochemical parameter.

Table 31 depicted the correlation between tobacco chewing and biochemical parameter. 47% of the studied ESCC patients reported tobacco chewing. The percentage of tobacco chewers was 27% who reported high blood glucose level whereas 21% of non tobacco chewers showed elevated blood glucose level (P=0.43). The serum level of blood urea nitrogen was found elevated only in non chewers. Among chewers, 0% reported high serum urea nitrogen whereas among non chewers 5% reported high serum urea nitrogen level in their blood (P=0.048). Only non chewers reported high serum creatinine level where 3.5% reported the same (P=0.10). Among tobacco chewers, 10% were reported having high serum total protein level whereas 11% reported the same among the non tobacco chewers group (P=0.90).

The percentage was 22% who reported low serum albumin level among tobacco chewers on the contrary it was 23% in those who were non tobacco chewers (P=0.87). 8% among tobacco chewers reported high AST level in their blood in comparison to 2% among non tobacco chewers (P=0.13). High level of serum ALT was reported only in those patients who were non chewers. In case of ALKP, elevated level in the blood was reported only among chewers. The percentage was 2% among chewers who were having elevated total bilirubin level in their blood whereas it was 12% among the non chewers group (P=0.030).

Biochemical parameter	Tobacco Chewers	Tobacco Non chewers	*P*-value
Glucose <106 mg/dL ≥106 mg/dL	 37 14	 45 12	0.43
Urea nitrogen < 20 mg/dL ≥ 20 mg/dL	 51 0	 54 3	0.048
Creatinine < 1.50 mg/dL ≥ 1.50 mg/dL	 51 0	 55 2	0.10
Total protein < 8.20 g/dL ≥ 8.20 g/dL	 46 5	 51 6	0.90
Albumin < 3.5 g/dL ≥ 3.5 g/dL	 11 40	 13 44	0.87
Aspartate transaminase (AST) < 59 U/L ≥ 59 U/L	 47 4	 56 1	0.13
Alanine transaminase (ALT) < 72 U/L ≥ 72 U/L	 51 0	 56 1	0.34
Alkaline phosphatase (ALKP) < 80 U/L 80-280 U/L ≥ 280 U/L	 11 38 2	 6 51 0	0.054
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	 50 1	 50 7	0.030

Table 31: Tobacco chewing habit correlation with the biochemical parameter in the ESCC patients.

4.4.11 Correlation of alcohol use status with the reported biochemical parameter.

The association between alcohol use and the biochemical parameter was described in table 32. Among the studied ESCC patients, 29% reported alcohol use as among one of their habits. In case of alcohol user, 35% were reported having high blood glucose level whereas among non alcohol user patients it was 19% of the patients who had elevated blood glucose level (P=0.078). 3% alcohol user reported high serum urea nitrogen level whereas similar percentage of group reported elevated level in patients who didn't use alcohol (P=0.85). No one reported high serum creatinine in alcohol user group whereas 3% reported it in patients without any habit of alcohol use (P=0.36). The percentage was found 3% in alcohol users who showed elevated total protein level in their blood on the contrary it was 13% among the patients who didn't use alcohol (P=0.12).

Alcohol users reported low level of serum albumin in 32% whereas among non alcohol user, it was reported 18% (P=0.11). High AST level was reported in 6% of the alcohol users whereas it was 4% in patients who were non alcohol user (P=0.56). High ALT and ALKP was reported only in non alcohol user patients. Furthermore, 10% of the non alcohol user reported high serum total bilirubin level on contrary of 0% (P=0.017). Except total bilirubin level in the serum of the reported patients, non showed any statistical correlation with the alcohol habit.

Biochemical parameter	Alcohol user	Non alcohol user	*P*-value
Glucose			
<106 mg/dL	20	62	0.078
≥106 mg/dL	11	15	
Urea nitrogen			
< 20 mg/dL	30	75	0.85
≥ 20 mg/dL	1	2	
Creatinine			
< 1.50 mg/dL	31	75	0.36
≥ 1.50 mg/dL	0	2	
Total protein			
< 8.20 g/dL	30	67	0.12
≥ 8.20 g/dL	1	10	
Albumin			
< 3.5 g/dL	10	14	0.11
≥ 3.5 g/dL	21	63	
Aspartate transaminase			
< 59 U/L	29	74	0.56
≥ 59 U/L	2	3	
Alanine transaminase			
< 72 U/L	30	77	0.11
≥ 72 U/L	1	0	
Alkaline phosphatase			
< 80 U/L	7	10	0.32
80-280 U/L	24	65	
≥ 280 U/L	0	2	
Total Bilirubin			
< 1.30 mg/dL	31	69	0.017
≥ 1.30 mg/dL	0	8	

Table 32: Alcohol use correlation with the biochemical parameter in the ESCC patients.

4.4.12 Correlation of areca nut chewing status with the reported routine biochemical parameter.

Majority 81% of the reported patients were found using areca nut. Their analysis with routine biochemical parameter was depicted in the Table 33. Among areca nut users, 24% were reported having high blood glucose level whereas it was 25% in those who were not areca nut users (P=0.91). 1% of the areca nut users reported high level of blood urea nitrogen whereas in case of patients who did not consumed areca nut, the reported percentage was 10% (P=0.029). In case of areca nut users, 2% showed elevated serum creatinine level whereas it was 0% in those who were non areca nut user (P=0.49). Areca nut users reported elevated level of total protein in 11% whereas 5% of the non areca nut users reported high level of total protein (P=0.39). 24% of the areca nut users reported low level of serum albumin in comparison to 15% that were reported in non areca nut users (P=0.38).

The serum level of AST, ALT and ALKP were reported at elevated level only in those patients who used areca nut. 7% of the areca nut users had shown elevated level of total bilirubin in their blood whereas it was reported 10% in patients who were no user of areca nut (P=0.62). None of the biochemical factors except urea nitrogen showed statistical significance when compared with the areca nut user status of the ESCC patients.

Biochemical parameter	**Areca nut chewers**	**Areca nut non chewers**	***P*-value**
Glucose			
<106 mg/dL	67	15	0.91
≥106 mg/dL	21	5	
Urea nitrogen			
< 20 mg/dL	87	18	0.029
≥ 20 mg/dL	1	2	

Creatinine < 1.50 mg/dL ≥ 1.50 mg/dL	 86 2	 20 0	0.49
Total protein < 8.20 g/dL ≥ 8.20 g/dL	 78 10	 19 1	0.39
Albumin < 3.5 g/dL ≥ 3.5 g/dL	 21 67	 3 17	0.38
Aspartate transaminase (AST) < 59 U/L ≥ 59 U/L	 83 5	 20 0	0.27
Alanine transaminase (ALT) < 72 U/L ≥ 72 U/L	 87 1	 20 0	0.63
Alkaline phosphatase (ALKP) < 80 U/L 80-280 U/L ≥ 280 U/L	 16 70 2	 1 19 0	0.25
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	 82 6	 18 2	0.62

Table 33: Areca nut user status correlation with the routine biochemical parameter in the ESCC patients.

4.4.13 Correlation of pan chewing status with the reported routine biochemical parameter.

Use of pan among the studied ESCC patients was reported in 79% of the cases as depicted in table 34. Patients who were taking pan showed 24% amongst them as having high blood glucose level in comparison to 26% of those who were non pan users (P=0.79). Among the pan users, 1% showed elevated serum urea nitrogen level in comparison to 9% in case of non pan user ESCC patients (P=0.052). Increase in the level

of serum creatinine was reported only in patients who were non pan user. The percentage was 0% among pan users whereas it was 9% among non pan users who reported high serum creatinine level (P=0.006). Pan users reported elevated level of serum total protein in 7% whereas it was reported 22% in patients who were no pan users. Among the patients who were found using pan, 24% showed low level of serum albumin whereas among the patients who were non pan users, 17% showed low serum albumin (P=0.53).

The percentage of pan user patients with high AST level were 6% whereas it was 0% among non pan users (P=0.23). Similarly, ALT and ALKP serum level was found reported higher in patients who were pan users. Among the patients who were pan user, it was found that 6% were having elevated serum total bilirubin level whereas it was 13% among non pan user patients (P=0.24). Serum creatinine and total protein level was found statistical significant whereas other biochemical parameters could not found any statistical correlation with status of pan use.

Biochemical parameter	**Pan users**	**Pan non users**	***P*-value**
Glucose			
<106 mg/dL	65	17	0.79
≥106 mg/dL	20	6	
Urea nitrogen			
< 20 mg/dL	84	21	0.052
≥ 20 mg/dL	1	2	
Creatinine			
< 1.50 mg/dL	85	21	0.006
≥ 1.50 mg/dL	0	2	
Total protein			
< 8.20 g/dL	79	18	0.039
≥ 8.20 g/dL	6	5	
Albumin			
< 3.5 g/dL	20	4	0.53
≥ 3.5 g/dL	65	19	

Aspartate transaminase (AST) < 59 U/L ≥ 59 U/L	 80 5	 23 0	0.23
Alanine transaminase (ALT) < 72 U/L ≥ 72 U/L	 84 1	 23 0	0.60
Alkaline phosphatase (ALKP) < 80 U/L 80-280 U/L ≥ 280 U/L	 16 67 2	 1 22 0	0.16
Total Bilirubin < 1.30 mg/dL ≥ 1.30 mg/dL	 80 5	 20 3	0.24

Table 34: Pan user status correlation with the routine biochemical parameter in the ESCC patients.

4.4.14 Correlation of follow up status with the reported habits among the ESCC patients.

Table 35 listed out the correlation between habits history of the ESCC patients with the follow up duration. ESCC patients who reported 0-6, 7-12, 13-18, 19-24, 25-36 and ≥ 37 months of follow up, amongst them it was found that 65%, 41%, 64%, 33%, 60% and 0% were tobacco smokers whereas rest were non smokers respectively (P=0.20). In those patients who reported 0-12 months of follow up, 49% were tobacco chewers whereas those who reported 13-24 months of follow up, 45% were found among tobacco chewers and those ESCC patients who were in the follow up for 25-36 months, 20% were tobacco chewers (P=0.49). Patients who showed 0-6 months of follow up, 21% were found alcohol user amongst them whereas 44% were found using alcohol in those patients who showed 7-12 months of follow up in comparison to 20% of the patients who reported 25-36 months of follow up (P=0.12).

Most of the studied patients (81%) were areca nut users and couldn't got any statistical significance when compared with different follow up duration like other habits. The percentage was found 82% in the patients who reported 0-12 month of the follow up who were pan users in comparison to 45% among the patients who were on the follow up for 19-36 months (P=0.036).

Habits	Follow up: 0-6	Follow up: 7-12	Follow up: 13-18	Follow up: 19-24	Follow up: 25-36	Follow up: ≥ 37	*P*-value
Smokers	31	14	9	2	3	0	0.20
Non smokers	17	20	5	4	2	1	
Chewers	21	19	7	2	1	1	0.49
Non chewers	27	15	7	4	4	0	
Alcohol user	10	15	5	0	1	0	0.12
Non alcohol user	38	19	9	6	4	1	
Areca nut user	39	27	12	4	5	1	0.77
Non Areca nut user	9	7	2	2	0	0	
Pan user	39	28	12	4	1	1	0.036
Pan non user	9	6	2	2	4	0	

Table 35: Different habits status correlation with the follow up in the ESCC patients.

4.4.15 Correlation of smoking status with the molecular and clinico- pathological parameter in the studied ESCC patients.

Molecular and clinicopathological factors were correlated with smoking status of the ESCC patients as illustrated in the Table 36. Among tobacco smokers 71% were found ALDH1 positive whereas among non smokers, the percentage of ALDH1 positive was 59% (P=0.19). 8% of both smokers and non smokers reported HER2 positivity.

Smokers who were found positive to p16 expression was 20% whereas 24% on non smokers tested positive to p16 (P=0.60). ESCC patients who were smokers had reported 49% hypoploidy, 41% hyperploidy, 0%tetraploid, 8% polyploidy and 2% diploid tumor status on the contrary those patients who were non smokers reported 49% hypoploidy, 29% hyperploidy, 4%tetraploid, 6% polyploidy and 12% diploid tumor status (P=0.051). It was near to the statistical significance.

The percentage was found 17%, 22%, 12%, 32% and 17% among the ESCC patients who smokes in various categories of S phase fraction: 0%, 1-25%, 26-50%, 51-75% and more than 75% respectively whereas among non smokers within the same listed categories of S phase fraction it was found 6%, 12%, 8%, 51%, 23% respectively (P=0.12). 10% of the smokers were found responder against neoadjuvatant chemotherapy (NACT) whereas 33% of the non smokers were reported responder against NACT (P=0.004). Among smokers 20%, 61%, 17% and 2% of them reported initial performance status as 0, 1, 2, 3 respectively on the contrary among non smokers the percentage of the patients was 10%, 74%, 14% and 2% who were at the initial performance status 0, 1, 2, 3 respectively (P=0.47). Majority 63% of the ESCC patients reported tumor at the mid thoracic site of the esophagus. There wasn't much difference between status of the tumor site in the smoker and non smoker group. In ESCC patients who smoked, 2%, 15%, 59% and 24% reported tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively in contrast to patients who were non smoker, 0%, 20%, 68% and 12% showed tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively (P=0.33).

Among smoker group, circumferential disease spread at half, two third, three

fourth and whole was found in 27%, 15%, 2% and 56% of the patients which contrast with the finding of disease spread in the non smoker group where it was reported 14%, 8%, 10% and 68% of the patients (P=0.056). 69% of the 108 patients reported moderately differentiated squamous cell carcinoma. 27%, 68% and 5% of the ESCC tumors who were smokers tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively whereas 25%, 71% and 4% among non smokers tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively (P=0.91). 81% of the smokers were found more than 45 years of age whereas 73% of the non smokers were reported in more than 45 year of age (P=0.32). Non smokers reported death in 47% whereas smokers reported death in 64% of the patients among their respective groups (P=0.068).

Molecular and clinico-pathological parameter	**Smokers**	**Non smokers**	***P*-value**
ALDH1 status			
Positive	42	29	0.19
Negative	17	20	
HER2 status			
Positive	4	4	0.78
Negative	55	45	
p16 status			
Positive	12	12	0.60
Negative	47	37	
Ploidy status			
Hypoploid	29	24	
Hyperploid	24	14	
Tetraploid	0	2	0.051
Polyploid	5	3	
Diploid	1	6	
S-phase fraction status			
S-phase fraction: 0%	10	3	0.12

S-phase fraction: 1-25% S-phase fraction: 26-50% S-phase fraction: 51-75% S-phase fraction: ≥ 76%	13 7 19 10	6 4 25 11	
Neo adjuvant response status Responder Non responder	 6 53	 16 33	0.004
Initial performance status Performance status: 0 Performance status: 1 Performance status: 2 Performance status: 3	 12 36 10 1	 5 36 7 1	0.47
Disease site Cervical Upper thoracic Mid thoracic Lower thoracic	 1 9 35 14	 0 10 33 6	0.33
Disease spread Circumference: 1/2 Circumference: 2/3 Circumference: 3/4 Circumference: Whole	 16 9 1 33	 7 4 5 33	0.056
Pathological report Well differentiated SCC Moderately differentiated SCC Poorly differentiated SCC	 16 40 3	 12 35 2	0.91
Age ≤ 45 years > 45 years	 11 48	 13 36	0.32
Death Yes No	 38 21	 23 26	0.068

Table 36: Molecular and clinico- pathological parameter correlation with the smoking status of the patients.

4.4.16 Correlation of tobacco chewing status with the molecular and clinico-pathological parameter in the studied ESCC patients.

The data related to molecular and clinico- pathological parameter correlation with tobacco chewing status of the studied patients is list in the Table 37. ALDH1 positivity was found in 44% of the tobacco chewers and rest in non tobacco chewers. 61% of the tobacco chewers were ALDH1 positive whereas 70% of the non chewers were ALDH1 positive (P=0.30). Equal percentage of the chewers and non chewers were found HER2 positive. Among chewers group, 8% were tested HER2 positive and in non chewer group 7% were tested HER2 positive (P=0.87). 20% of the chewers reported p16 expression against 25% of non chewers (P=0.53).

Patients who were tobacco chewers had reported 51% hypoploidy, 35% hyperploidy, 2% tetraploid, 8% polyploidy and 4% diploid tumor status whereas those patients who were non chewers reported 47% hypoploidy, 35% hyperploidy, 2% tetraploid, 7% polyploidy and 9% diploid tumor status (P=0.89). 8%, 12%, 10%, 55% and 15% of the ESCC patients who were chewers reported various categories of S phase fraction: 0%, 1-25%, 26-50%, 51-75% and more than 75% respectively whereas among non chewers within the same categories of S phase fraction, the percentage of the patient was found 16%, 23%, 10%, 28%, and 23% respectively (P=0.068). 20% of the patients having history of tobacco chewing were found responder against neoadjuvatant chemotherapy (NACT) whereas 21% of the non chewers were found responder against NACT (P=0.85).

Among tobacco chewer patients 15%, 69%, 14% and 2% of them reported initial performance status as 0, 1, 2, 3 respectively on the contrary among non chewer group of the patients, percentage of the patients was 16%, 65%, 17% and 2% (P=0.95). In ESCC patients who were chewers 0%, 16%, 65% and 19% reported tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively in contrast to patients who

were non chewers, the percentage of the patients reported was 2%, 19%, 61% and 18% who showed tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively (P=0.75).

Among tobacco chewers group, circumferential disease spread at half, two third, three fourth and whole was found in 18%, 16%, 4% and 62% of the patients whereas among non chewer group, it was reported 24%, 9%, 7% and 60% of the patients (P=0.53). 31%, 63% and 6% of the ESCC tumors who were reported tobacco chewing as one of their habit were tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively whereas 21%, 75% and 4% among non chewers tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively (P=0.35). More than 45 years of age was reported among 73% of the patients who were tobacco chewers contrary to 82% of the patients who were non chewers reported more than 45 years of age (P=0.21). 51% of the tobacco chewers reported death on the follow up in comparison to 61% in the patients who were non chewers (P=0.27). None of the parameters could be found statistical significant when correlated with the tobacco chewing habit of the ESCC patients.

Molecular and clinico- pathological parameter	**Tobacco Chewers**	**Tobacco Non chewers**	***P*-value**
ALDH1 status			
Positive	31	40	0.30
Negative	20	17	
HER2 status			
Positive	4	4	0.87
Negative	47	53	
p16 status			
Positive	10	14	0.53
Negative	41	43	

Ploidy status			
Hypoploid	26	27	0.89
Hyperploid	18	20	
Tetraploid	1	1	
Polyploid	4	4	
Diploid	2	5	
S-phase fraction status			
S-phase fraction: 0%	4	9	0.068
S-phase fraction: 1-25%	6	13	
S-phase fraction: 26-50%	5	6	
S-phase fraction: 51-75%	28	16	
S-phase fraction: ≥ 76%	8	13	
Neo adjuvant response status			
Responder	10	12	0.85
Non responder	41	45	
Initial performance status			
Performance status: 0	8	9	0.95
Performance status: 1	35	37	
Performance status: 2	7	10	
Performance status: 3	1	1	
Disease site			
Cervical	0	1	0.75
Upper thoracic	8	11	
Mid thoracic	33	35	
Lower thoracic	10	10	
Disease spread			
Circumference: 1/2	9	14	0.53
Circumference: 2/3	8	5	
Circumference: 3/4	2	4	
Circumference: Whole	32	34	
Pathological report			
Well differentiated SCC	16	12	0.35
Moderately differentiated SCC	32	43	
Poorly differentiated SCC	3	2	
Age			
≤ 45 years	14	10	0.21
> 45 years	37	47	
Death			
Yes	26	35	0.27
No	25	22	

Table 37: Molecular and clinico- pathological parameter correlation with the tobacco chewing status of the patients.

4.4.17 Correlation of alcohol use status with the molecular and clinico- pathological parameter in the studied ESCC patients.

Table 38 depicts the analysis between molecular and clinico- pathological parameter and alcohol use among the studied patients. ALDH1 positivity was found in 65% of the patients who were alcohol users whereas among non alcohol users the ALDH1 positivity was reported 66% (P=0.86). Among patients who were HER2 positive, 37.5% were alcohol users whereas 62.5% were non alcohol user. Among alcohol user group, 10% were tested HER2 positive and in non alcohol user group, 6% were tested HER2 positive (P=0.56). Patients who were using alcohol reported p16 positivity in 19% of the cases whereas those who were non alcohol users, they reported p16 positivity in 23% of the case (P=0.64).

Patients who were alcohol user had reported 55% hypoploidy, 36% hyperploidy, 0% tetraploid, 6% polyploidy and 3% diploid tumor status whereas those patients who were non alcohol users reported 47% hypoploidy, 35% hyperploidy, 2% tetraploid, 8% polyploidy and 8% diploid tumor status (P=0.76). 13%, 13%, 19%, 26% and 29% of the ESCC patients who were found using alcohol reported various categories of S phase fraction: 0%, 1-25%, 26-50%, 51-75% and more than 75% respectively on the contrary, those patients who were not user of alcohol within the same categories of S phase fraction, the percentage of the patient was found 12%, 19%, 6%, 47%, and 16% respectively (P=0.072). In case of patients who responded against neoadjuvatant chemotherapy (NACT), 36% were alcohol users and rest 64% were non alcohol user. 26% of the patients with history of alcohol use were found responder against NACT whereas 18% of the non alcohol user were found responder against NACT (P=0.37).

Among alcohol user patients 16%, 71%, 13% and 0% of them reported initial performance status as 0, 1, 2, 3 respectively whereas among the non alcohol user group, percentage of the patients was 16%, 65%, 17% and 2% (P=0.76). In ESCC patients who were alcohol users 3%, 13%, 74% and 10% reported tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively, on the contrary patients who were non alcohol users, the percentage of the patients reported was 0%, 19%, 58% and 22% who showed tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively (P=0.12).

Among alcohol user group, circumferential disease spread at half, two third, three fourth and whole was found in 22%, 13%, 0% and 65% of the patients whereas among non alcohol user group, it was reported 20%, 12%, 8% and 60% of the patients respectively (P=0.46). 23%, 74% and 3% of the ESCC tumors who were reported alcohol user were found tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively whereas 27%, 68% and 5% among non alcohol user tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively (P=0.77). Age was found statistically correlation with the alcohol use status in the ESCC patients. The percentage was 35% among alcohol using group who were either 45 years of age or less than that whereas it was 17% in those patients who were non alcohol users (P=0.035). It reflects that the age of drinking alcohol was less in the current study. Among alcohol user patients, the reported death on follow up was 48% whereas among non alcohol user patients the reported death on follow up was 60% (P=0.28). There was a trend towards less number of deaths in patients who were non alcohol user. None of the

parameters except age category could be found statistical significant when correlated with the alcohol use in the ESCC patients.

Molecular and clinico-pathological parameter	Alcohol user	Non Alcohol user	*P*-value
ALDH1 status			
Positive	20	51	0.86
Negative	11	26	
HER2 status			
Positive	3	5	0.56
Negative	28	72	
p16 status			
Positive	6	18	0.64
Negative	26	59	
Ploidy status			
Hypoploid	17	36	
Hyperploid	11	27	0.76
Tetraploid	0	2	
Polyploid	2	6	
Diploid	1	6	
S-phase fraction status			
S-phase fraction: 0%	4	9	
S-phase fraction: 1-25%	4	15	0.072
S-phase fraction: 26-50%	6	5	
S-phase fraction: 51-75%	8	36	
S-phase fraction: ≥ 76%	9	12	
Neo adjuvant response status			
Responder	8	14	0.37
Non responder	23	63	
Initial performance status			
Performance status: 0	5	12	
Performance status: 1	22	50	0.76
Performance status: 2	4	13	
Performance status: 3	0	2	
Disease site			
Cervical	1	0	
Upper thoracic	4	15	0.12
Mid thoracic	23	45	
Lower thoracic	3	17	

Disease spread			
Circumference: 1/2	7	16	0.46
Circumference: 2/3	4	9	
Circumference: 3/4	0	6	
Circumference: Whole	20	46	
Pathological report			
Well differentiated SCC	7	21	0.77
Moderately differentiated SCC	23	52	
Poorly differentiated SCC	1	4	
Age			
≤ 45 years	11	13	0.035
> 45 years	20	64	
Death			
Yes	15	46	0.28
No	16	31	

Table 38: Molecular and clinico- pathological parameter correlation with the alcohol use status of the ESCC patients.

4.4.18 Correlation of areca nut use status with the molecular and clinico-pathological parameter in the studied ESCC patients.

The correlative analysis between molecular and clinico- pathological parameter and areca nut use among the studied patients is shown in Table 39. 81% of the studied patients were found areca nut user. ALDH1 positivity was found in 67% of the patients who were areca nut users whereas those who were non areca nut users, the ALDH1 positivity was reported 60% (P=0.54). Among the patients who were reported HER2 positive, 75% were areca nut users whereas rest 25% were non areca nut user. Among areca nut user group, 7% were tested HER2 positive and in non areca nut user group, 10% were tested HER2 positive (P=0.62). Patients who were using areca nut with p16 positivity reported in 23% of the cases whereas those who were non areca nut users, they

reported p16 positivity in 20% of the case (P=0.79).

Those patients who were areca nut user had reported 49% hypoploidy, 36% hyperploidy, 1% tetraploid, 7% polyploidy and 7% diploid tumor status whereas those patients who were non areca nut users were found with 50% hypoploidy, 30% hyperploidy, 5% tetraploid, 10% polyploidy and 5% diploid tumor status (P=0.77). 12.5%, 17%, 8%, 45% and 17% of the ESCC patients who were found using areca nut as habit reported various categories of S phase fraction: 0%, 1-25%, 26-50%, 51-75% and more than 75% on the cell cycle analysis of their tumor respectively on the contrary, those patients who were not user of areca nut within the same categories of S phase fraction, the percentage of the ESCC patient was found 10%, 20%, 20%, 20%, and 30% respectively (P=0.16). Those patients who responded against neoadjuvatant chemotherapy (NACT), 95% were areca nut users and the rest 5% were non areca nut user. Among the patients who had the habit of areca nut use, 24% of such patients were found responder against NACT whereas 5% of the non areca nut user were found responder against NACT (P=0.033).

Among the areca nut user patients, 14%, 69%, 15% and 2% of them reported initial performance status as 0, 1, 2, 3 respectively on the contrary those patients who were from the non areca nut user group, the percentage of the patients was 25%, 55%, 20% and 0% (P=0.46). Within the studied ESCC patients who were areca nut users 0%, 16%, 64% and 20% reported tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively, `whereas patients who were non areca nut users, the percentage of the ESCC patients reported among them was 5%, 25%, 60% and 10% who showed tumor at cervical, upper thoracic, mid thoracic and lower thoracic sites respectively (P=0.10).

Among the areca nut user group, circumferential disease spread at half, two third, three fourth and whole was found in 23%, 11%, 5% and 61% respectively of the patients in contrast to 15%, 15%, 10% and 60% of the patients respectively, who belonged to the non areca nut user group (P=0.67). 25%, 70% and 5% of the ESCC tumors who reported areca nut use as one of their habit were found tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively whereas 30%, 65% and 5% among non areca nut user tested pathologically well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively (P=0.88). The percentage was 77% among the patients with areca nut user habit who were more than 45 years of age in comparison to 80% of the non areca nut users who reported more than 45 years of age (P=0.79). Among the areca nut user patients, the reported death on follow up was 53% whereas among those who were not using areca nut, the reported death on follow up was 70% (P=0.17).

Molecular and clinico- pathological parameter	**Areca nut user**	**Non areca nut user**	***P*-value**
ALDH1 status			
Positive	59	12	0.54
Negative	29	8	
HER2 status			
Positive	6	2	0.62
Negative	82	18	
p16 status			
Positive	20	4	0.79
Negative	68	16	
Ploidy status			
Hypoploid	43	10	
Hyperploid	32	6	0.77
Tetraploid	1	1	
Polyploid	6	2	
Diploid	6	1	
S-phase fraction status			0.16

S-phase fraction: 0%	11	2	
S-phase fraction: 1-25%	15	4	
S-phase fraction: 26-50%	7	4	
S-phase fraction: 51-75%	40	4	
S-phase fraction: ≥ 76%	15	6	
Neo adjuvant response status			
Responder	21	1	0.033
Non responder	67	19	
Initial performance status			
Performance status: 0	12	5	
Performance status: 1	61	11	0.46
Performance status: 2	13	4	
Performance status: 3	2	0	
Disease site			
Cervical	0	1	
Upper thoracic	14	5	0.10
Mid thoracic	56	12	
Lower thoracic	18	2	
Disease spread			
Circumference: 1/2	20	3	
Circumference: 2/3	10	3	0.67
Circumference: 3/4	4	2	
Circumference: Whole	54	12	
Pathological report			
Well differentiated SCC	22	6	0.88
Moderately differentiated SCC	62	13	
Poorly differentiated SCC	4	1	
Age			
≤ 45 years	20	4	0.79
> 45 years	68	16	
Death			
Yes	47	14	0.17
No	41	6	

Table 39: Molecular and clinico- pathological parameter correlation with the areca nut user status of the ESCC patients.