

Department of Biotechnology Hargobind Khurana School of Life Sciences ASSAM UNIVERSITY, SILCHAR Silchar-788011, Assam, India

# **DECLARATION**

I, Rajeev Kumar, bearing Registration Number, Ph.D. 1706/2011 dated 15.09.11, and Assam University Registration Number 16-100010545 do hereby declare that the subject matter of the thesis entitled "Epidemiological, Biochemical and Molecular factors Associated with Clinical outcome of Esophageal Squamous Cell Carcinoma in the North Eastern India" is the record of work done by me and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. The thesis has not been submitted in any other University or Institute.

Place: Silchar Rajeev Kumar

Date:

Acknowledgement

I would like to take this opportunity while documenting and compiling my research findings in

the present form of a doctoral thesis, to express my sincere thanks and gratitude to my

supervisor Prof. (Dr.) Sankar Kumar Ghosh, Dean, School of Life Sciences and Professor,

Department of Biotechnology, Assam University, and to my Co- Supervisor Dr. R. Ravi

Kannan, Director, Cachar Cancer Hospital & Research Centre, for giving me this opportunity

to work on the field of cancer research, helping, mentoring and encouraging me throughout my

work.

I convey my sincere thanks to the Honourable Vice Chancellor, Assam University, Silchar

for felicitating my research endeavour. I am thankful to HOD and all the honourable faculty

members, technical and admin staffs of the Department of Biotechnology, Assam University,

Silchar for their generous support. I thank all the colleagues and friends of Department of

Biotechnology, Assam University for their cordial cooperation.

I am thankful to the colleagues of the Cachar Cancer Hospital & Research Centre for their

support. I would like to convey my sincere thanks to Ms. Seetha RaviKannan, Dr. Anuradha

Talukdar, Dr. Monoj Deka, and Dr. Ritesh Tapkire for their encouragement. I am grateful to

the Department of Biotechnology, Government of India for supporting my research work.

I am grateful to my close friends Santosh, Jayanand, Kundan, Kunal, Abhishek, Saurabh,

Manish for their unparallel continuous motivation. My family members have been the huge

source of motivation, especially my elder brother Shri Rajesh Kumar, who had inspired and

motivated me the most.

I would like to dedicate my doctoral research to my friend Major Ashish Kumar Roy, Indian

Army who became the martyr in 2012 while in action.

Place: Silchar

Rajeev Kumar

Date

## **CONTENTS**

Sl No.	Chapter	Page No.
1	Chapter-1: Introduction	1-17
1.1	Cancer	1
1.2	A brief History of cancer research	2
1.3	Hallmarks of cancer	6
1.4	Esophageal cancer	7
1.5	Distribution of esophageal cancer	11
1.6	Risk factors for esophageal cancer development	13
1.7	Esophageal squamous cell carcinoma (ESCC)	14
1.8	Treatment Modality in ESCC	15
1.9	Research question in relevance to the better understanding of treatment outcome and prognosis in ESCC	16
1.10	Objectives	17
2	Chapter-2: Review of Literature	18-26
2.1	Geographical distribution, life style and epidemiology of esophageal cancer	18
2.2	Molecular Markers in Esophageal squamous cell carcinoma	21
2.3	Cell cycle parameters in esophageal cancer	25
3	Chapter-3: Materials and Methods	27-35
3.1	Materials	
3.1.1	ESCC Patient Selection	27
3.1.2	Ethical approval and consideration	27
3.1.3	Inclusion and Exclusion criteria	28
3.1.4	Chemicals and reagents	29
3.2	Methods	
3.2.1	Clinical, epidemiological, biochemical and lifestyle information documentation	30
3.2.2	Treatment response assessment	31
3.2.3	Immunohistochemistry of molecular markers (ALDH1, HER2, p16):	31
3.2.4	Immunohistochemical scoring of molecular markers (ALDH1, HER2, p16)	33

	3.2.5	Cell Cycle analysis (Ploidy and S phase fraction estimation) protocol	34
	3.2.6	Statistical analysis	35
	4	Chapter-4: Results	36-66
4.1		Clinical characteristic, biochemical parameter, epidemiological and life style factors reported in patients with esophageal squamous cell carcinoma	36
	4.1.1	Reporting of esophageal squamous cell carcinoma patient's clinical characteristics	36
	4.1.2	Reporting of epidemiological factors in esophageal squamous cell carcinoma patients	39
	4.1.3	Reporting of lifestyle factors in esophageal squamous cell carcinoma patients	43
	4.1.4	Reporting of biochemical factors in esophageal squamous cell carcinoma patients	46
	4.1.5	Biochemical parameter correlation with gender status in esophageal squamous cell carcinoma patients	50
	4.1.6	Biochemical parameter correlation with reported age group in esophageal squamous cell carcinoma patients	52
	4.1.7	Biochemical parameter correlation with reported initial performance status in esophageal squamous cell carcinoma patients	54
	4.1.8	Biochemical parameter correlation with pathological grading in esophageal squamous cell carcinoma patients	56
	4.1.9	Correlation of reported death with biochemical parameter in esophageal squamous cell carcinoma patients	58
	4.1.10	Correlation of circumferential disease status with biochemical parameter in esophageal squamous cell carcinoma patients	59
	4.1.11	Correlation of family history of cancer with biochemical parameter in esophageal squamous cell carcinoma patients	61
	4.1.12	Correlation of site of tumor with the biochemical parameter in esophageal squamous cell carcinoma patients	63
	4.1.13	Correlation of follow up duration with the biochemical parameter in esophageal squamous cell carcinoma patients	65
4.2		Treatment response and prognostic factor analysis based on molecular markers ALDH1, HER2 and p16 in esophageal squamous cell carcinoma	67-74

4.2	1 Expression of ALDH1, HER2 in pre-treated ESCC tumor	67
4.2	2 Expression of molecular marker p16 in pre-treated ESCC tumor	72
4.3	Treatment response and prognostic factor analysis based on cell cycle parameter (Ploidy estimation and S phase fraction) in esophageal squamous cell carcinoma	75-83
4.3.	Cell cycle analysis through ploidy estimation in esophageal squamous cell carcinoma patients	75
4.3	2 Cell cycle analysis through S phase fraction estimation in esophageal squamous cell carcinoma patients	81
4.4	Analysis of inter-relationship among biochemical, epidemiological and molecular factors with their impact on clinical outcome in esophageal squamous cell carcinoma patients	84-120
4.4.1	Biochemical parameter correlation with ALDH1 expression in esophageal squamous cell carcinoma patients	84
4.4.2	Biochemical parameter correlation with HER2 positive status in esophageal squamous cell carcinoma patients	86
4.4.3	Biochemical parameter correlation with p16 expression status in esophageal squamous cell carcinoma patients	88
4.4.4	Biochemical parameter correlation with reported ploidy status in esophageal squamous cell carcinoma patients	89
4.4.	Correlation with reported different variation of ploidy status with biochemical parameter in esophageal squamous cell carcinoma patients	91
4.4.	S phase fraction status correlation with the biochemical in esophageal squamous cell carcinoma patients	93
4.4.	Treatment response to chemotherapy correlation with the biochemical parameter in esophageal squamous cell carcinoma patients	94
4.4.	Inter-relationship between biochemical parameter in esophageal squamous cell carcinoma patients	96
4.4	9 Correlation of smoking habit with the reported routine biochemical parameter.	97
4.4.1	O Correlation of tobacco chewing habit status with the reported routine biochemical parameter	99
4.4.1	1 Correlation of alcohol use status with the reported biochemical parameter	101

	7	Chapter-7: References	130-143
	6	Chapter-6: Summary	128-129
5.4		A proposed model for treatment response assessment in ESCC	126
5.3		Impact of cell cycle parameter on clinical course of ESCC	126
5.2		Molecular markers ALDH1, HER2 & p16 impact on treatment response and prognosis in ESCC	122
5.1		Inference of epidemiological, lifestyle and biochemical factors on ESCC	121
	5	Chapter-5: Discussion	121-127
	4.4.18	Correlation of areca nut use status with the molecular and clinico- pathological parameter in the studied ESCC patients.	117
	4.4.17	Correlation of alcohol use status with the molecular and clinico- pathological parameter in the studied ESCC patients.	114
	4.4.16	Correlation of tobacco chewing status with the molecular and clinico-pathological parameter in the studied ESCC patients.	110
	4.4.15	Correlation of smoking status with the molecular and clinico- pathological parameter in the studied ESCC patients.	107
	4.4.14	Correlation of follow up status with the reported habits among the ESCC patients.	106
	4.4.13	Correlation of pan chewing status with the reported routine biochemical parameter	104
	4.4.12	Correlation of areca nut chewing status with the reported routine biochemical parameter	102

i-viii

Publication

### LIST OF TABLES

Table No	Title	Page No
1	Milestone discoveries and significant events in the cancer field and changing relative survival rates for cancer patients	3
2	American Joint Commission on Cancer (AJCC) Staging for Esophageal Cancer	10
3	Percentage of reported esophageal cancer patients in Cachar Cancer Hospital and Research Centre has seen from the last 5 year	16
4	Description of the scoring system in ECOG performance status	30
5	Clinicopathological data of the squamous cell carcinoma of the esophagus.	37
6	Biochemical parameter correlation with the reported gender of the ESCC patients	51
7	Biochemical data correlation with the reported age group in the ESCC patients	53
8	Biochemical data correlation in the ESCC patients with their performance status	55
9	Biochemical data correlation in the ESCC patients with their pathological grading.	57
10	Biochemical data correlation in the ESCC patients with reported death status.	59
11	Biochemical data correlation in the ESCC patients with circumferential disease	60
12	Biochemical data correlation in the ESCC patients with family history of cancer.	62
13	Biochemical data correlation with the site of the disease in the ESCC patients	64
14	Biochemical data correlation with follow up duration of the ESCC patients	66
15	Correlation analysis of clinicopathological factors with ALDH1 expression in ESCC patients.	68
16	Correlation analysis of clinicopathological factors with HER2 expression in ESCC patients.	69
17	Correlation analysis of clinicopathological factors with p16 expression in ESCC patients	73
18	Clinicopathological data correlation of the squamous cell carcinoma of the esophagus: diploid and different types of aneuploid cases.	77
19	Correlation between ploidy status with various molecular markers	78
20	Clinicopathological data correlation with the S phase fraction in the squamous cell	82

carcinoma of the esophagus.

21	Correlation between cell cycle S phase fraction with various molecular markers.	83
22	ALDH1 expression correlation with the biochemical parameter in the ESCC patients	85
23	HER2 status correlation with the biochemical parameters in ESCC patients	87
24	p16 expression correlation with biochemical parameter in the ESCC patients	89
25	Ploidy status correlation with biochemical parameter in the ESCC patients	90
26	Biochemical data correlation in the ESCC patients with their different indicator's of ploidy status	92
27	S phase status correlation with biochemical parameter in the ESCC patients	94
28	Response to chemotherapy status correlation with biochemical parameter in the ESCC patients	96
29	Inter-relationship between biochemical data correlation in the ESCC patients.	97
30	Smoking habit correlation with the biochemical parameter in the ESCC patients	99
31	Tobacco chewing habit correlation with the biochemical parameter in the ESCC patients	100
32	Alcohol use correlation with the biochemical parameter in the ESCC patients	102
33	Areca nut user status correlation with the routine biochemical parameter in the ESCC patients.	104
34	Pan user status correlation with the routine biochemical parameter in the ESCC patients.	106
35	Different habits status correlation with the follow up in the ESCC patients.	107
36	Molecular and clinico- pathological parameter correlation with the smoking status of the patients.	110
37	Molecular and clinico- pathological parameter correlation with the tobacco chewing status of the patients.	113
38	Molecular and clinico- pathological parameter correlation with the alcohol use status of the ESCC patients.	117
39	Molecular and clinico- pathological parameter correlation with the areca nut user status of the ESCC patients.	120
40	A proposed model for response assessment against neo adjuvant chemotherapy for esophageal squamous cell carcinoma patients	127

### LIST OF FIGURES

Figure No	Title	Page No
1	Individuals suffering from cancer	2
2	Timeline of pivotal events in cancer treatment.	5
3	Timeline of pivotal events in cancer prevention.	6
4	Four distinct region of esophagus	8
5	A pictorial representation of different stages in esophageal cancer	9
6	A representation of squamous cell carcinoma progression	11
7	Comparison of estimated age-standardized incidence and mortality (Both Sexes) of esophageal cancer in major countries and regions in 2012. (GLOBOCAN 2012)	12
8	Esophageal cancer belt of central Asia	19
9	Overview of Flow Cytometer based cell cycle analysis which can provide information related to ploidy estimation and S phase fraction.	26
10	Schematic representation of a basic immunohistochemistry experiment.	32
11	A schematic diagram of cell cycle experiment.	34
12	Different pathological grading of esophageal disease. Hematoxylin and eosin stained slides. ESCC: Esophageal squamous cell carcinoma	38
13	Percentage of ESCC patients who were reported responder and non responder after neo adjuvant chemotherapy (NACT)	38
14	Demographic distribution of the studied esophageal cancer patients.	39
15	A: Religion of studied esophageal cancer patients. B: Gender of studied esophageal cancer patients.	40
16	Kaplan-Meier probability distribution of overall survival. Results are shown for esophageal squamous cell carcinoma patients	40
17	Age category of reported ESCC patients.	41
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	42
19	A. Comparison of tobacco smokers VS non smokers B. Comparison of percentage of tobacco chewers VS non chewers in the ESCC patients	44

20	Life style habits of ESCC patients:	45
21	Biochemical profile of ESCC patients.	47
22	Biochemical profile of ESCC patients.	49
23	ALDH1 and HER2 immunohistochemistry in esophageal squamous cell carcinoma.	70
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.	71
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	71
26	p16 expression in ESCC. <b>A.</b> p16 negative ESCC tumor; <b>B.</b> p16 positive ESCC tumor	74
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	74
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.	75
29	Different nuclear (DNA) composition of cells in esophageal squamous cell carcinoma in comparison to normal esophageal squamous cells	79
30	Various types of ploidy reported in esophageal squamous cell carcinoma	80
31	ESCC patients with different percentage of S phase fraction.	83

#### **List of Abbreviations**

ESCC : Esophageal squamous cell carcinoma

PS : Performance Status

NACT : Neo adjuvant chemotherapy

NCI : National Cancer Institute

USA : United States of America

NEGM : New England Journal of Medicine

HPV : Human papilloma virus

FDA : Food and Drug Administration

GE : Gastro-esophageal

TNM : Tumor- node-metastasis

AJCC : American Joint Commission on Cancer

AAR : Age adjusted rate

ALDH1 : Aldehyde dehydrogenase 1

HER2 : Human epidermal growth factor receptor-2

GPS : Glasgow Prognostic Score

ALT : Alalanine transaminase

AST : Aspartate transaminase

ALKP : Alkaline phosphatase

NCCN : National Cancer Care Network

 $\pi g$ : Microgram

dL : decilitre

EDTA : Ethylene diamine tetra acetic acid

PBS : Phosphate buffered saline

IHC : Immunohistochemistry

CT : Computed tomography