Predictive potential role of *GSTT1* and *GSTM1* genes in the overall survival and treatment outcome of lung cancer patients treated with platinum-based doublet chemotherapy

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the award of degree of

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in

Biotechnology

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July-2017

DECLARATION

I hereby declare that work done in this seminar report entitled, "Predictive potential role of *GSTT1* and *GSTM1* in the treatment outcome of lung cancer patients treated with platinum-based doublet chemotherapy " submitted towards partial fulfilment of requirement for award of **Master of Science degree** in Biotechnology in **Biotechnology Department** of **Thapar University, Patiala,** is an authentic record of work carried out by me under the supervision and guidance of **Dr. Siddharth Sharma, Associate Professor** of **Biotechnology Department, Thapar University, Patiala**.

This matter embodied in this report has not been submitted in part or full to any other university or institute for the award of any degree.

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This is to certify that above declaration made by the student concerned is correct to the best of my knowledge and belief.

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CERTIFICATE

This is to certify that dissertation entitled, "Predictive potential role of *GSTT1* and *GSTM1* in the treatment outcome of lung cancer patients treated with platinum-based doublet chemotherapy'' submitted by **Ms. Ushina Verma** in partial fulfilment of the requirements for the award of M.Sc. in Biotechnology at Thapar University, Patiala is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in this dissertation has not been submitted to any other university/institute for award of any Degree or Diploma.

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ABSTRACT

Some studies have evaluated the association of genetic polymorphisms in GST genes and survival in lung cancer patients based on smoking status, histological sub-types and performance status and many others report the relationship between genetic distribution of *GSTT1* and *GSTM1* and treatment outcomes of patients receiving platinum based chemotherapy. However, the results have been contradicting and have yet not been evaluated in North Indian population. The focus of this study is to find an association between *GSTT1* and *GSTM1*, individually and in different combinations, with the overall survival and treatment outcome in patients receiving platinum-based chemotherapy. Methods: a total of 323 patients were evaluated for *GSTT1* and *GSTM1* polymorphisms. The genes were amplified using multiplex PCR. Results: the present study found no significant association of *GSTT1* and *GSTM1* with the overall survival in LC patients. Based on smoking status, a statistically significant association was observed between the *GSTT1* null genotype and overall survival in case of non-smokers (HR'=2.72; 95%CI=1.09-6.79; **p=0.03**). Also, it was found that *GSTT1* null genotype was associated with the performance status of patients under the KPS scale of 70-80, showing about 2.3 folds increased death risk as compared to the patients with wild *GSTT1* genotype (HR'=2.34; 95%CI=1.44-3.82; **p=0.0006**) but no such statistically significant association was found with *GSTM1*. The study also investigated on various clinic-pathological parameters contributing in determining the susceptibility in lung cancer patients and it was found that the patients in stage IV, with null *GSTT1* genotype had a 2.6 folds higher susceptibility to lung cancer as compared to the patients in clinical stage III (AOR=2.64; 95%CI=1.38-5.04; **p=0.003)**. It was also seen that the patients with null *GSTT1* genotype showed a 2-fold increased metastasis of lung cancer (AOR=2.12; 95%CI=1.13-3.98; **p=0.019**). On studying the combined effect of these genetic polymorphisms, it was seen that the SCLC patients with the genotypic combination of wild *GSTT1* and null *GSTM1* genotype had a higher death risk due to lung cancer (HR'=1.71; 95%CI=1.03-2.84; **p=0.035**). Further, the association of *GSTM1* and *GSTT1* polymorphisms and the outcomes of chemotherapy in patients with lung cancer were examined. The results suggested that null *GSTM1* genotype was associated with poor response to chemotherapy in patients with lung cancer (AOR=2.00; 95%CI=1.04-3.84; **p=0.018**).

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ABBREVIATIONS USED

NNK	Nicotine-derived nitrosamine ketone
PAHs	Polycyclic aromatic hydrocarbons
DNA	Deoxyribonucleic acid
GST	Glutathione S-transferase
ADRs	Adverse drug reaction
LC	Lung cancer
EFGR	Epidermal growth factor receptor
WHO	World health organization
CT	Computed tomography
LDCT	Low dose computed tomography
TNM	Tumor, node, metastasis
SCLC	Small cell lung carcinoma
ADCC	Adenocarcinoma
SQCC	Squamous cell carcinoma
NSCLC	Non-small cell lung carcinoma
SCC	Squamous cell carcinoma
LCC	Large cell carcinoma
CYP	Cytochrome P-450
GSH	Glutathione-SH(Glucocorticoid-Suppressible Hyperaldosteronism)
SNP	Single nuclear polymorphism
PGIMER	Post graduated institute of medical education and research
TE	Tris-EDTA
HCl	Hydrochrolic acid
EDTA	Ethylene diamine tetraacetic acid
SDS	Sodium dodecyl sulfate
NaCl	Sodium chloride
PCI	Phenol chloroform isoamyl alcohol
TAE	Tris acetate EDTA
TBE	Tris borate EDTA
PCR	Polymerase chain reaction
BSA	Bovine serum albumin
dNTPs	Deoxynucleotide triphosphate
Taq	Thermos aquaticus
OR	Odds ratio
HR	Hazard ratio
CR	Complete response
PR	Partial response
SD	Stable disease
PD	Progressive disease
KPS	Karnofsky performance status
ECOG	Eastern cooperative oncology group

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Chapter 1

Introduction

In the recent years, lung cancer has emerged as the most frequently occurring cancer globally accounting for 12.6% of the new cancers and 17.8% of cancer deaths. Most of the incidences of lung cancer are reported from developing nations, but its proportions are alarmingly high in developed countries (22% vs. 14.6% of cancer deaths respectively). According to the GLOBOCON statistics for 2012, it has been estimated that 14.1 million new cases were detected and the accounted deaths due to cancer were about 8.2 million. In India, as for 2012, the overall lung cancer mortality was 63,759, making it the third most common cause of cancer-related deaths after breast and cervical cancer [1].

Cancer has become the major cause of death owing to the poor lifestyle behaviours such as smoking, poor diet, lack of physical activity and also the ever-increasing pollution caused by rapid industrialisation and technology advancement, which increases the levels of environmental pollutants to a fatal rate. Based on lung cancer histology, it has been seen that small-cell lung cancer is extensively linked to smoking and accounts for approximately 20 percent of all lung cancer cases whereas non small cell lung cancer accounts for the rest 80 percent of all lung cancers. Due to the increase in use of filtered cigarettes, the recent decades have witnessed a decrease in squamous cell carcinoma, but an increase in adenocarcinoma [2].

As compared to the western population, the incidences of lung cancer have increased dramatically in India. Approximately 63,000 lung cancer patients are reported each year in India (Noronha et al., 2012) [3]. As assessed worldwide for 2000, 85% of lung cancer in men and 47% of lung cancer in women was found to be the consequence of smoking stating that the major factor behind this epidemic state has been the carcinogens present in tobacco smoking [4]. But various epidemiological evidences have shown that other factors such as occupational carcinogens (asbestos, radon, silica, etc.) have also contributed largely towards the development of lung cancer, mainly among non-smokers. Further, the carcinogenic effect of passive smoking has been attributed to an individual's exposure to nicotine-derived carcinogenic nitrosamines such as NNK,

albumin adducts of polycyclic aromatic hydrocarbons (PAHs) and to haemoglobin adducts of 4-amino-byphenyl, a carcinogen in tobacco smoke [5].

Various experimental and epidemiological evidences have highlighted the role of genetic polymorphisms and environment in lung carcinogenesis, also relating the effects of certain metabolic genes with overall survival and treatment outcomes in lung cancer patients [6]. Reduced or inability to metabolise the xenobiotic compounds by a host's inbuilt detoxification mechanism, renders the host susceptible to development of lung cancer and also influences their treatment outcome and survival after being diagnosed with this fatal disease.

This inbuilt xenobiotic detoxification system consists of various phase I and phase II enzymes which catalyze the reactions involved in excreting the harmful compounds from the body. These metabolic enzymes act by sequentially processing the carcinogens by different biotransformation reactions, thus aiding in their disposal. Initially, the pro-carcinogens are activated by phase I enzymes, predominantly by hydroxylating cytochrome-P450s. These enzymes catalyze many oxidation and dealkylation reactions, which transforms the xenobiotics to a less toxic form or converts them into more active form, to be metabolised by phase II enzymes. The activated carcinogens subsequently bind DNA, resulting in formation of DNA adducts and thereby inducing carcinogenesis. However, the phase II detoxification system metabolises these DNA adducts and facilitate their elimination from the cells [7]. The main class of phase II enzymes is the Glutathione S-transferase (GST) enzymes. The GST enzymes are encoded by a super-family of GST genes which detoxify the carcinogens, therapeutic drugs and other environmental toxins by catalysing the conjugation of glutathione with electrophilic carbon, sulphur or nitrogen atoms and convert them into non-polar substances, thereby inhibiting their interaction with crucial cellular proteins and nucleic acids [8]. The efficiency of this detoxification system is determined by the presence, amount and nature of GST isoenzymes. Among the 5 major classes of GSTs, polymorphism has been reported in genes coding for α, μ, π and θ class [9]. The impact of genetic polymorphisms in *GSTT1* and *GSTM1* on overall survival and prognosis of lung cancer have been comprehensively studied since these enzymes play a critical role in detoxification of major carcinogens which lead to lung cancer development.The genetic deletions in *GSTM1* and *GSTT1* among varied ethnical populations have

resulted in loss of corresponding catalytic activity [10]. It has been postulated that a reduction in GSTs activity is linked with higher incidences of cancer development, which is probably due to reduced elimination of electrophillic carcinogens [11]. In this study, the genetic polymorphisms of two GST variants, *GSTT1* and *GSTM1*, have been analysed for their potential role in determining the overall survival and treatment outcomes in lung cancer patients who received platinum-based chemotherapy. The polymorphic *GSTM1* gene is represented by two active alleles and a non-functional null allele which results from the entire *GSTM1* gene deletion mutation. It basically functions to deactivate the carcinogenic intermediates of polycyclic aromatic hydrocarbons. *GSTT1* is studied for its phase II bio-transforming enzymes which act upon various drugs and industrial chemicals, e.g., cytostatic drugs, hydrocarbons, and halogenated hydrocarbons [9].

Till date only a few molecular investigations have seen the potential of genetic variation in metabolic enzymes for the determination of overall survival in cancer patients. Thus, the aim of this study was to determine the effect of genetic polymorphisms on overall survival in lung cancer patients based on various parameters such as age, gender, smoking status, histological subtypes, performance status and also the patient's response to chemotherapy. Also various clinic-pathological parameters, such as tumor stage, lymph node invasion and tumor metastasis were taken into account for determining the patients' susceptibility to lung cancer.

Owing to the medical and molecular research advancements, the past decade has seen an increment of about 17.8% in the overall survival in lung cancer patients [12]. This is mainly credited to the concept of targeted therapy drugs and the appropriate selection of the patients. The most frequently prescribed treatment for advanced stage tumors is platinum-based chemotherapy. Platinum-based compounds (cisplatin or carboplatin) are found to be good electrophilic substrates, which are detoxified by the glutathione metabolic pathway through the catalytic activity of GST enzymes [13]. However, it has been suggested that tumor cells readily develop resistance to chemotherapeutic agents leading to poor survival outcomes. This is mainly caused due to elevated glutathione conjugation with active drug metabolites and reduced efflux of platinum complexes from the cells. Therefore it has been suggested that an imbalance in the host's detoxification system can result in loss of enzymatic activity, ultimately leading to

treatment failure and also adverse drug reactions (ADRs) [14]. Thus, by anticipating an individual's glutathione system activity, the patient's response to platinum drugs could be easily quantified and it could potentially provide clinicians with useful prognostic information. By following these findings and hypothesis, this study is designed to demonstrate the role of genotypic polymorphisms of GSTs and their association with the overall survival in lung cancer and the treatment response related to platinum-based chemotherapy.

Chapter 2

Review of literature

2.1 Lung cancer

The incidences of cancer are growing at an alarming rate globally due to the increase in population and aging factors mainly in the developed nations [15]. Cancer has become the major cause of death owing to the poor lifestyle behaviours such as smoking, poor diet, lack of physical activity and also the increasing pollution caused by rapid industrialisation and technology advancement [16]. Of all the fatal neoplasias, lung cancer (LC) has been the most important and frequent malign tumours in the last years and results in frequent death rates globally [17]. As this disease is not diagnosed in early stages due to late appearance of clinical signs, the treatment becomes difficult which ultimately cuts down the survival period of the suffering patients.

2.2 Causes and risk factors

Of all the studies conducted to enlist the major causes of lung cancer, it has been evident that the development of this disease is a multi-factorial process, means that there are lots of factors which contribute to cause of lung cancer. These factors include:

- Smoking

  It is estimated that smoke from tobacco products is the primary cause of risk of lung cancer which causes about 90% of overall deaths [18]. This relation between smoking and lung cancer development is due to the presence of various chemicals in tobacco smoke, which are mostly carcinogenic [19]. Age becomes a key factor in determining the risk of developing lung cancer as exposure of a human being to smoke of cigarette during his/her life cycle is mainly attributed to his/her susceptibility for the disease. Total exposure in an entire life period is usually expressed in pack-years. However, scientists have shown that not only from the tobacco smoke, but risk of lung cancer also increases with use of drugs like marijuana and crack-cocaine [20].

- Passive Smoking

  Passive smoking is referred to getting exposed to different kind of smokes at various places like home, at work, or in public areas. It can also be referred by various other terms as stated - second-hand smoking, environmental tobacco smoking and side-stream smoking.

- Environmental Carcinogens

  There are certain substances in the environment that contain several harmful substances which on exposure could lead to genetic damage that could ultimately result in disease called carcinogenesis. Such substances are called environmental carcinogens. For example, asbestos, radon, arsenic, chromium, nickel, polycyclic aromatic hydrocarbons (PAHs), etc [21].

- Some other carcinogens present in the environment

  These include chloromethyl methyl ether, ionizing radiations, gamma radiation, mustard gas, soots, tars, mineral oils, and vinyl chloride. Acrylonitrile, cadmium, beryllium, lead, and ferric oxide dust are some of the suspected lung carcinogens [22].

- Genetic Factors

  Risk of lung cancer accounts to be increased due to 8-14% of inherited factors [23]. The individuals having a family history of lung cancer are at an elevated risk of about 2.4 times. The Active oncogenes or the inactive tumor suppressing genes can lead to initiation of cancer [24]. For instance, 10-30% of lung adenocarcinomas are caused by mutations in the *K-ras* proto-oncogene [25]. Epidermal growth factor receptor (EGFR) regulates the proliferation, apoptosis, tumor invasion and also angiogenesis. Mutations in EGFR lead to non-small cell lung cancer [26].

- Age

  Various genetic defects accumulate in the cells before they turn cancerous. Therefore, with age, the probability of accumulating such genetic damage increases and the person has an elevated risk of cancer. Also, as a person ages, the immune system loses its efficiency which further enhances the chances of cancer cells to escape the natural surveillance system of cancer [27].

2.3 Epidemiology

➢ 2.3.1 Lung Cancer worldwide

It has been estimated by the WHO that cancer causes more deaths than all other cardio-vascular diseases [25]. The worldwide demographic and epidemiologic state shows a mounting burden of cancer in the coming years, particularly in less developed nations reaching to over 200 lakh new cases per year by 2025. [28] As estimated by the GLOBOCAN for 2012, 14.1 million new cases were detected and the accounted deaths due to cancer were about 8.2 million (shown in table 2.1). According to the statistics for diagnosing various cancers, lung cancer accounted for about 1.82 millions and is responsible for approximately 1.6 million deaths worldwide [29].

Table 2.1. Estimated Incidence, Mortality and Prevalence Worldwide in 2012 (GLOBOCAN, 2012) .

Estimated Numbers (thousands)	**Men**		**Women**	
World	**Cases**	**Deaths**	**Cases**	**Deaths**
	1242	1099	583	491
More developed regions	490	470	268	210
Less developed regions	751	682	315	281
WHO Africa region (AFRO)	12	11	6	6
WHO Americas region (PAHO)	178	149	146	113
WHO East Mediterranean region (EMRO)	26	23	7	6
WHO Europe region (EURO)	323	283	126	105
WHO South-East Asia region (SEARO)	116	104	46	42
WHO Western Pacific region (WPRO)	588	528	251	220
United States of America	112	92	102	76
China	459	422	193	175

India	54	49	17	15

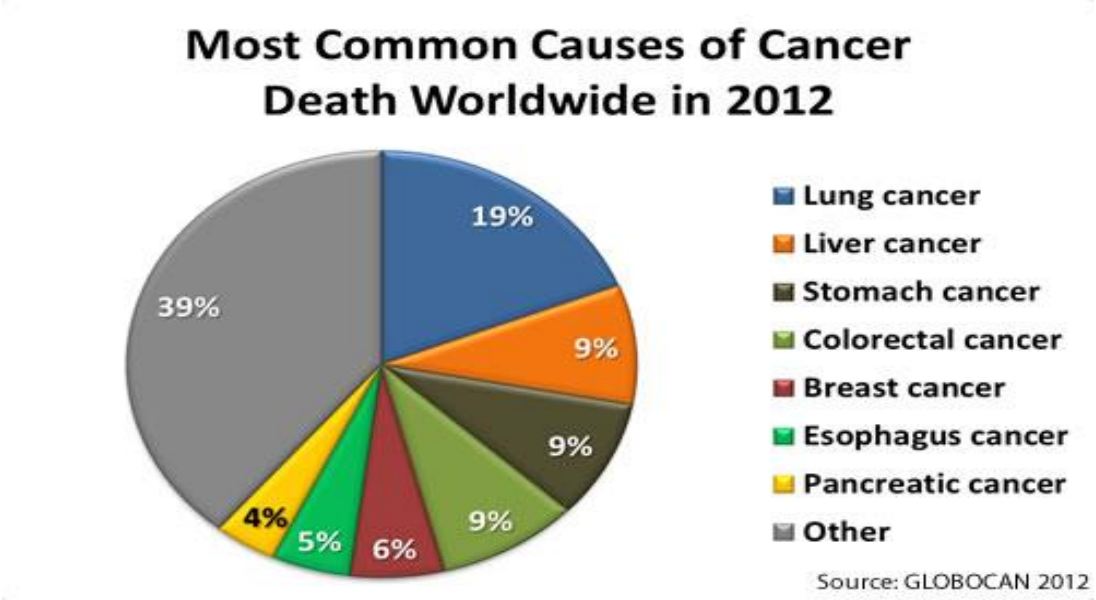


Figure 2.1 Depicting the most common causes of cancer death worldwide in 2012. (Source:http://3.bp.blogspot.com/-0HV6gJ6O-as/VmwD_eTWuAI/AAAAAAAAg1Q/M23-aNNQuzw/s1600/cm3.jpg)

➢ 2.3.2 Lung Cancer in India

As per the report by GLOBOCAN for 2012, the lung cancer incidence in India was 70,275 in all ages and both genders; crude incidence rate was 5.6 per 100,000, age-standardized rate was 6.9 per 100,000 and 0.85 was the cumulative risk [30]. Based upon the ranking, lung cancer stands on fourth position, top three on list were: breast, cervical and oral cavity cancer in terms of incidence rates, being second in males and sixth in females on the basis of incidence of cancer. In 2012, the overall lung cancer mortality in India was 63,759, ranking it the third most common cause of cancer-related deaths after breast and cervical cancer. [25]

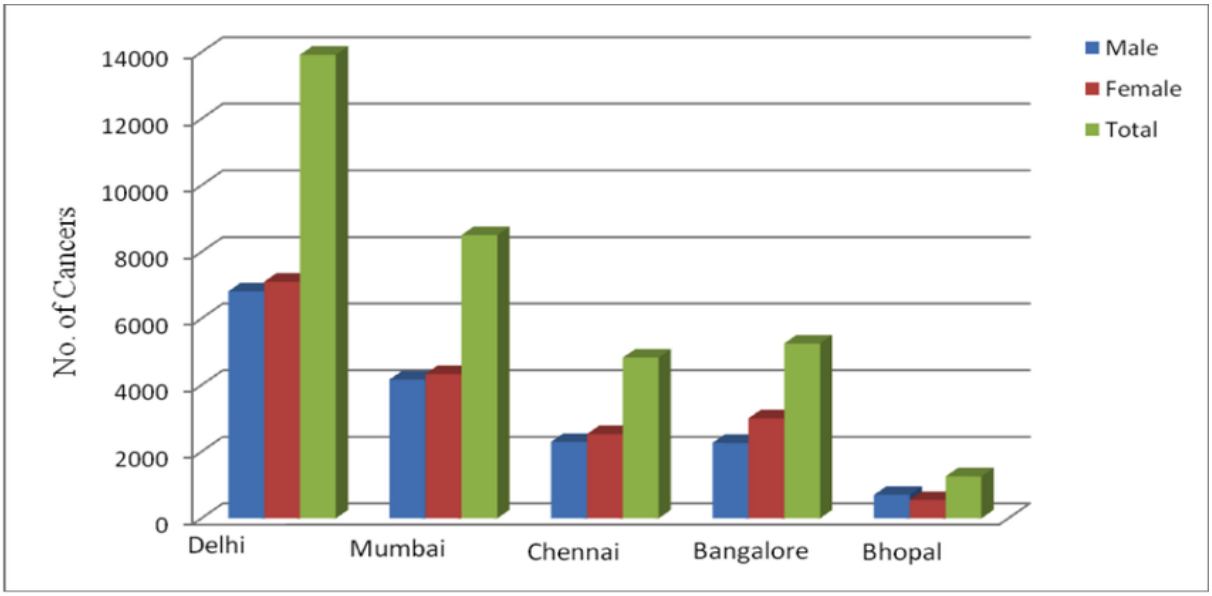


Figure 2.2 Lung cancer trend in 5 Indian metro cities. (Marimuthu, 2008)

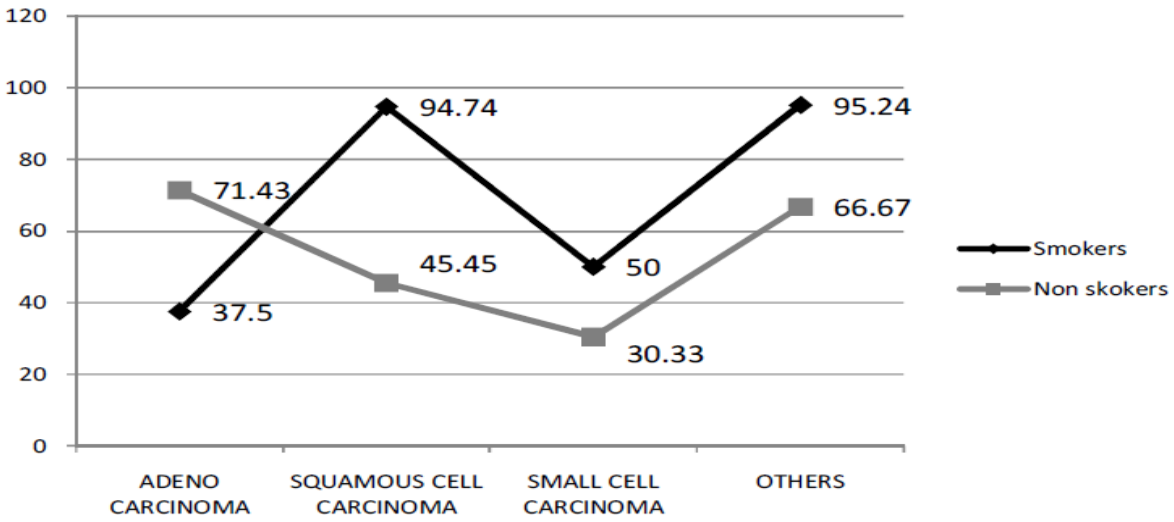


Figure 2.3 Relationship of various lung cancers with smoking in India.

(Kirmani, N *et al*., 2010).

➢ 2.3.3 Lung cancer scenario in India

As studied in the Asian sub-continent, it has been found that multiple factors have contributed to the development of lung cancer mainly in countries like India. For instance, on the basis of smoking status among males and females, it has been seen that females are not much associated with smoking as compared to males (1.4 vs. 33.4%). Also, the habit of smoking was found more prevalent in rural areas as compared to urban areas (31.3 vs. 21.5%). Various means of carcinogens have been known which

form their way into the respiratory tract mainly through consumption of tobacco. Though, there is a marginal difference but it has been reported that the beedi smokers are at an elevated risk of developing lung cancer as compared to cigarette smokers (2.64 vs. 2.23%). Further, the population studies have demonstrated that based on age standardization, the males have higher incidence rates as compared to females (10.9 vs. 2.5, per 100,000 populations). Also, owing to the difference in lifestyle behaviours, it has been observed that the metro-cities are emerging as a smoking hub, ultimately leading to increased risks of developing lung cancer as shown in figure 2.2 [30].

2.4 Clinical Features and diagnosis

Some initial symptoms of this disease are:

- A prolonged cough that worsens over time
- Hoarseness
- Problem in breathing and severe chest pain
- Loss of appetite and reduced weight
- Fatigue
- Shortness of breath
- Coughing up blood or phlegm [32].

2.5 Lung Cancer Screening

Lung cancer screening refers to observing for signs of lung cancer before the person is hit by the symptoms of the disease [33]. Low-dose computed tomography (also called a low-dose CT scan or LDCT) is the technique used for screening of lung cancer. Annual screening is recommended for the people who are at greater risk for lung cancer. The individuals belonging to any following categories are included in this criterion:

• The age of individual is between 55 and 80 years;

• Smoking history of person counts to 30 pack-years (A “pack year” is defined as an average of smoking 1 pack of cigarettes per day for 1 year. And

• Individuals who are current smokers or former smokers might have quit smoking completely within the last 14-15 years [34].

2.6 Staging

It is a method by which the severity of the disease can be classified which can aid in identifying appropriate treatment approaches and determining prognosis. All available factors, including clinical factors (physical exam, imaging and laboratory findings) and pathological finding (from tissue specimens obtained via bronchoscopy, mediastinoscopy or surgery) are used to determine stage [35]. The methods for staging differ based on cellular classification. The American Joint Committee on Cancer has designated staging by tumor, node and metastases (TNM) classification. This staging system takes into account the level of the tumor (T), the extent of involvement of regional lymph node (N) and the presence of metastases (M) [36].

2.7 Histology

There are two main categories of lung cancer on basis of its appearance under the microscope:

- Small cell lung cancer (SCLC) and
- Non-small cell lung cancer (NSCLC) [37]

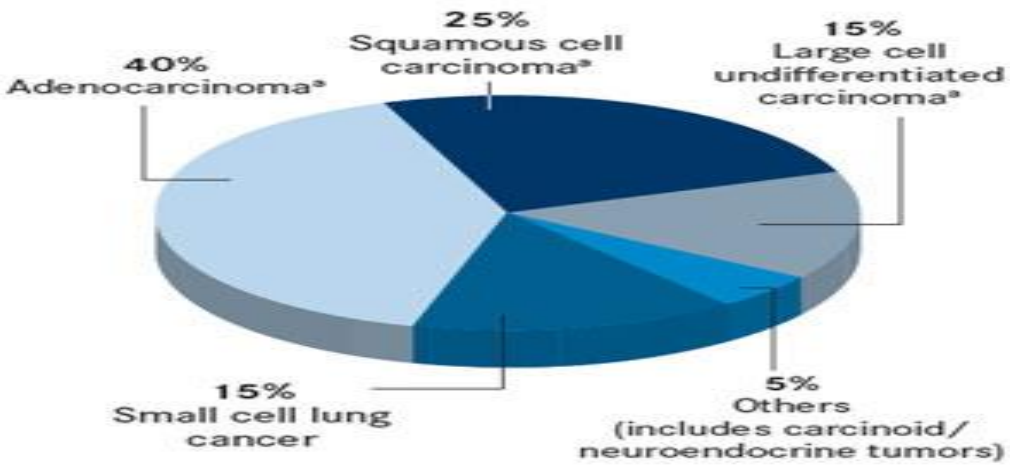


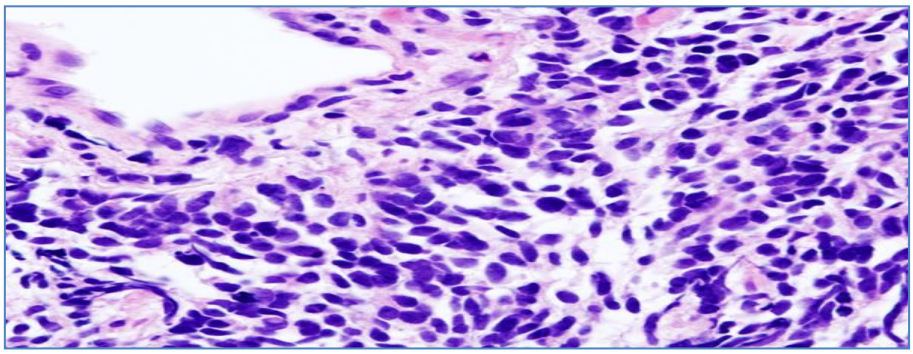
Figure 2.4 The various histological subtypes of lung cancer.
(Source:http://www.onclive.com/_media/_upload_image/TKI-figureure.jpg)

- Small Cell Lung Cancer

The epithelial cells of SCLC are abnormally small. The cells resemble oat grains which led to the term oat cell carcinoma. It is also termed as undifferentiated carcinoma.

Some characteristics of SCLC are:

- ✓ SCLC is strongly related to smoking but its chances are very rare among non-smokers.
- ✓ It grows faster as compared to NSCLC. Early in the disease it spreads to lymph nodes and is metastatic.
- ✓ SCLC readily responds to treatments such as chemotherapy and radiotherapy in early stages.
- ✓ The tumors are located near the center of the lung.
- ✓ Most patients with SCLC are detected with metastasis at an early stage [38].



Figureure 2.5 Microscopic view of SCLC cells. (Source:http://www.onclive.com/_media/_upload_image/TKI-figureure.jpg)

- ➢ Non-Small Cell Lung Cancer

NSCLC is divided into three types: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. They have almost similar growth patterns. Some characteristic features of these stated categories of NSCLC are:

- Adenocarcinoma

The appearance of cells is glandular type. Mucin (thick fluid) formation results from such tumors.

- ✓ It is responsible factor for about 40% of all lung cancers and constitutes almost 55% of NSCLCs.
- ✓ It stands out to be most observed lung cancer among females and non-smokers. Its variants include bronchioloalveolar, acinar and papillary adenocarinoma.
- ✓ It is mostly observed in people below the age of 50.
- ✓ The tumors are mostly located in outer regions of the lungs.
- ✓ It is mainly characterized by the scarring of lung tissue [39].

- Squamous Cell Carcinoma

  It is also called epidermoid carcinoma and is featured by large, flat cells. Keratin is a substance produced by tumors and can be easily observed under the microscope. Some characteristic properties are:

  - ✓ Papillary, clear cell, along with small cell, and basaloid SCC are listed as some of the variants.
  - ✓ Stated as one of most common type of cancer and usually found in individuals above 65 years of age.
  - ✓ The tumors are mostly located in central area of the lungs.
  - ✓ SCC metastasizes in later stages as compared to other forms of NSCLC. SCC have a tendency to metastasize somewhat later than other forms of NSCLC.
  - ✓ Tobacco smoking is mainly related to SCC [40].

- Large Cell Carcinoma

  It is characterized by the largest cells of all other NSCLCs. The cells appear to be largely undifferentiated. Some features of LCC are:

  - ✓ The variants include clear cell, basaloid and large cell neuroendocrine carcinoma.
  - ✓ It can affect all parts of the lung.
  - ✓ The prognosis of LCC is not so favorable as compared to other forms of NSCLC [41].

➢ Other type cancers in the lungs

The lungs form a frequent location for the metastasis of tumors from other parts of the body. Some other forms of lung cancer which are mainly non-epithelial in origin are carcinoid tumors, malignant pleural mesotheliomas, fibrosarcomas, and leiomyosarcomas [42].

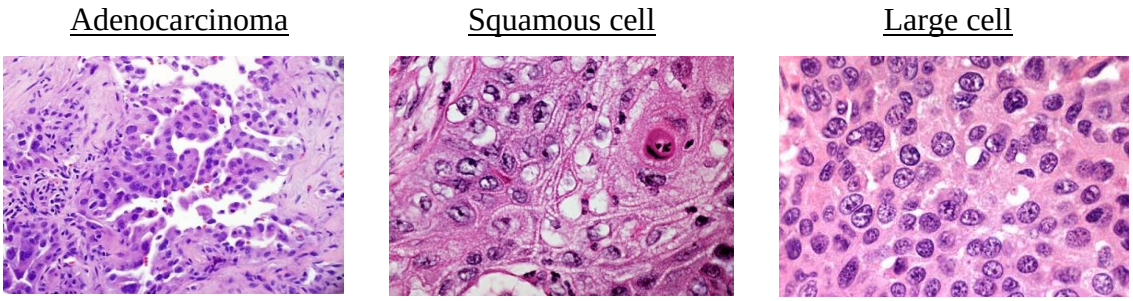


Figure 2.6 Microscopic views of different types of NSCLC. (Source: http://lookfordiagnosis.com/mesh_info.php?term=carcinoma,+large+cell&lang=1)

2.8 Treatment and Therapy

Three types of treatment can be given to the patient suffering from lung cancer. It is based on the type of lung cancer and its stage. It also largely depends on the patients' response to a particular given treatment [43].

As SCLC is characterized by rapid and early metastasis, the most favourable treatment is platinum-based chemotherapy. Long term survival can be achieved if the disease is detected at an early stage and chemotherapeutic treatment is combined with radiation therapy [44]. Chemotherapy basically aims at improving the life quality and prolonging the survival period, mainly when the disease has reached the extensive tumor stage [45]. The treatment for NSCLC largely depends on the stage. If the patient is medically fit, and is in stage I or II, the most preferable treatment is surgery else radiation therapy is the best alternative. But this is not usual as the detection of cancer at an early stage is very rare. If the patients are in tumor stage III or IV, surgical treatment is given first followed by adjuvant chemotherapy to lower the possibility of relapse [46].

In general, the main mode of treatment in stage III and stage IV tumors is chemotherapy and also small molecule kinase-inhibitor therapy. Presently, platinum-doublet therapy is the main form of chemotherapy for NSCLC [47]. In this a platinum compound, a DNA cross-linking agent (such as cisplatin) is combined with a nucleoside analogue such as gemcitabine, which acts as an anti-mitotic compound, paclitaxel, which is a microtubule inhibitor, etopside/ camptothecin acting as topoisomerase inhibitor or pemetrexed as thymidylate synthase inhibitor [48].

For many solid tumors, cisplatin acts as the most effective anti-cancer therapy which generally shows significant clinical activity. Cisplatin creates certain DNA lesions in the form of intra-strand DNA mono-adducts and also some inter-strand crosslinking. These inhibit DNA replication which results in cell death [49]. All inhibit and block DNA replication resulting in cell death. Side effects include damage to the kidney and nerves, hearing loss, nausea and vomiting but the latter can be controlled with other drugs.

Targeted therapies have been designed and synthesized based on oncogenes identified to be important in NSCLC carcinogenesis, such as EGFR. EGFR inhibitors developed include gefitinib and erlotinib [50]. Some NSCLC patients have intrinsically chemo-resistant disease while others become chemo-resistant during the treatment process, with disease that either progresses through or remains stable whilst receiving chemotherapy [51]. In particular the molecular mechanisms underlying platinum-resistance are still largely unknown. Two to 30% of all NSCLC patients are inherently platinum resistant and disease still progresses during early cycles of platinum chemotherapy. It is possible that the resistance is mediated by decreased accumulation of cisplatin at the intracellular region, enhanced inactivation of drugs by metallothionine and glutathione, enhancement of repair activity of DNA damage, decreased formation of DNA-platinum adducts, and altering of oncogene expression and regulatory proteins [48].

2.9 Biotransformation of Xenobiotics

With the rapid industrialization and enormous advancements in technology, the environment and hence the humans are constantly exposed to various harmful chemicals or xenobiotics including man-made chemicals, alkaloids, smoke from

cigarettes or tobacco smoke, industrial chemicals, pesticides etc. [52]. Being lipophilic in nature, these chemicals are readily absorbed by the skin, lungs as well as the gastrointestinal track, mainly through the process of passive diffusion. Due to their lipophilicity, these chemicals are not easily removed from the body and result in lethal effects for the human body [53]. To counter these harmful effects, there is an inbuilt metabolic system in the body which converts these lipophilic compounds into hydrophilic compounds, which are easily eliminated from the body. This process is called biotransformation or detoxification [54]. A series of biotransformation reactions are catalyzed by the enzymes present mainly in the liver and also other tissues such as lungs, which makes these compounds suitable for excretion by the body [55].

There are two stages of eliminating the non-favourable and harmful compounds of lipid-soluble compounds, performed by cellular proteins or enzymes in dual set configureuration, termed as phase I (transformation) and phase II (conjugation) enzymes [56].

Biochemical requirements and responses of phase I and II metabolisms vary with different metabolic signals and work in unison so that unwanted xenobiotics (such as toxins or drugs) or endobiotics (such as excess hormones) can be removed properly. The liver is the primary detoxification organ but intestine, kidney, lungs, and brain are also some of other organs which deal with detoxification, rest of the body being involved to a lesser extent in dealing with phase I and II reactions [57].

2.9.1 Phase I detoxification enzymes

Cytochrome P450s

Out of various compounds present in cigarette smoke, only 50 are known carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aromatic amines and N-nitroso compounds. Only few of these carcinogens are reactive in nature, rest are pro-carcinogens, which need Phase-I enzymes in order to get activated [58]. For example CYP supergene family helps in encoding and converting these pro-carcinogens into active carcinogens. All these reactive carcinogens generally bind the DNA and result in the formation of DNA adducts, which is capable of inducing mutations and causing carcinogenesis [59]. CYPs belong to a multi-gene super-family that has mono-oxygenases, which perform mixed functions. This super-family comprises of 10 sub-

families, CYP1/CYP10, on the basis of sequence homology. Subfamilies CYP1CYP2, CYP3 and CYP4 are mainly responsible for metabolism of drugs [60].

2.9.2 Phase II detoxification enzymes

The Glutathione S-transferase (GST) Gene Family

Numerous detoxification and toxification mechanisms are carried out the *GST* gene family, by conjugation reactions of reduced glutathione, using different pharmaceutical and environmental pollutants as substrates. This family also encodes various enzymes that are crucial for life processes [64]. The *GST* genes are highly expressed in various tumors and are up-regulated by cellular oxidative stress. This could lead to complications during chemotherapy [65]. The *GST* gene family comprises of 16 genes in six subfamilies namely, alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT) and zeta (GSTZ) [61-63, 66].

The Glutathione-S-transferases (GSTs) enzymes

The GST enzymes function to detoxify the carcinogenic and xenobiotic compounds by catalysing the conjugation of GSH with electrophillic substrates, which are capable of producing free radicals [67]. The deletion or polymorphism in either of such genes, which encode a metabolic enzyme, proves to cause an imbalance in the host's inbuilt ability to detoxify such harmful substances [68]. This may be attributed to a decreased or completely lost enzymatic activity, due to genetic polymorphisms. Therefore, the genetic polymorphisms which are associated with altered GST activity are of interest in studying the host's susceptibility, survival and response to a given treatment [69]. In the recent years, *GSTT1*, *GSTM1* and *GSTP1* have been extensively studied for their significant role in regulating an individual's susceptibility to environment-related diseases, such as lung cancer [70, 71].

Tissue distribution of *GSTT1* and *GSTM1*

- *GSTT1* is located on chromosome 22q11.2 and is mainly associated with Kidney, liver, small intestine, brain, spleen, prostate, pancreas, testis, heart and lung.
- *GSTM1* is located on chromosome 1p13.3 and is found in liver, testis, brain, adrenal, kidney, pancreas, lung and heart.

Preferred substrates for *GSTT1* and *GSTM1*

- Trans-stilbene oxide
- 1-chloro-2,4-dinitrobenzene
- 1,2-dichloro-4-nitrobenzene
- Cumene hydroperoxide
- 1, 2-epoxy-3-(*p*-nitro-phenoxy) propane [72].

Structure and function of glutathione

The primary function of GSTs is to detoxify xenobiotics by conjugation reactions between GSH and electrophillic carbon, sulphur or nitrogen atoms of various xenobiotic substrates, hence preventing their interaction with DNA and other important cellular proteins [73]. This catalytic activity of GSTs is dependent on the continuous supply of GSH from gamma-glutamylcysteine synthetase and glutathione synthetase. It also depends upon the action of certain transporter proteins which aid in removing the GSH conjugates from the cell [62].

Glutathione is a tri-peptide composed of g-glutamate, Cysteine and Glycine [74].The conjugation of GSH with xenobiotics or antineoplastic agents renders the compounds highly anionic, thus facilitating their excretion from the cells by ATP-dependent GS-X pump [75].

The general reaction catalysed by GSTs is: GSH + R-X → GSR + HX [76] as shown in figure 2.7.

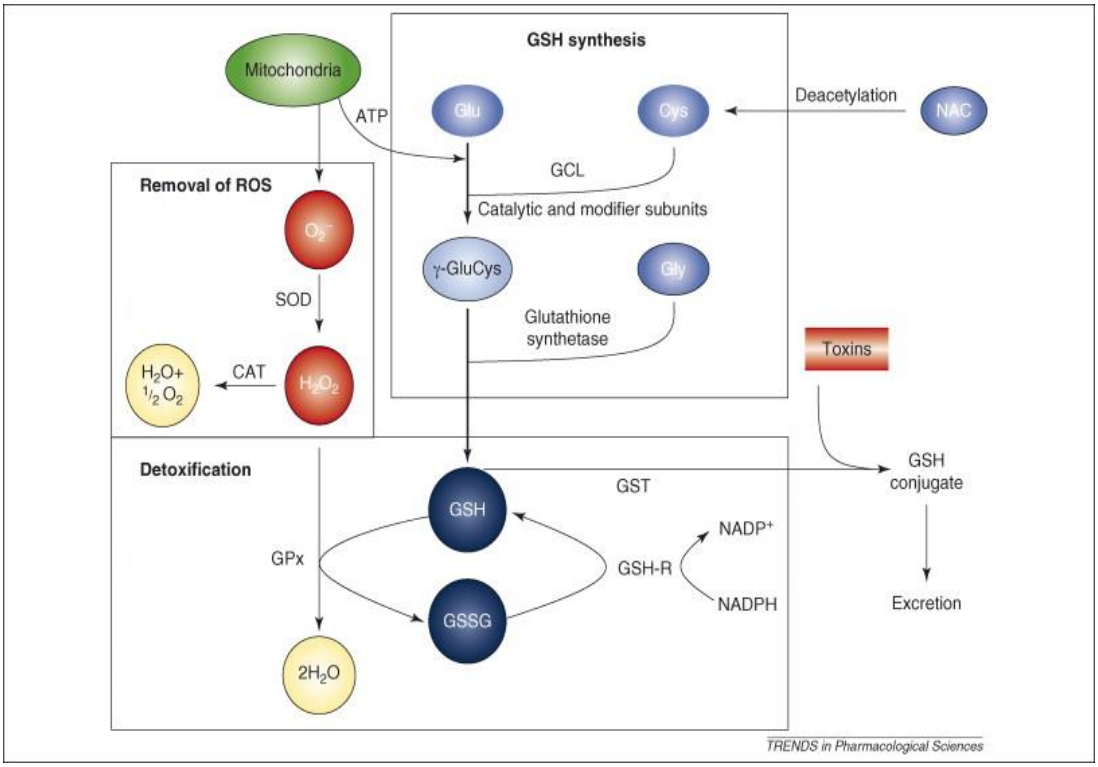


Figure 2.7 Detoxification mechanism by GSTs. (Michael Berk, 2008)

2.10 The Biology of Genetic Polymorphism

Genetic polymorphisms are mainly due to chance or induced mutations or variations among individuals or populations. Such mutations are caused by various factors such as insertions, deletions, recombination, sequence repeats and SNPs [77]. Within a population, the genetic polymorphisms of crucial metabolic enzymes distinguish them into various subgroups that have altered metabolic activity to perform various detoxification or biotransformation reactions. Such polymorphisms generally result in decreased, elevated or complete loss of enzyme activity thus causing an imbalance in the host's inbuilt detoxification mechanism [78].

2.11 Polymorphic expression of *GST*

As shown in table 2.2., among the *GSTs*, a large number of genetic polymorphisms have been reported. This variation in *GST* alleles is frequent in the population and thus makes a significant contribution in an individual's ability to metabolise drugs, his or her susceptibility to any disease and also their response to treatment [79]. Most of the gene deletions have been reported for *GSTT1, GSTM1 and GSTP1* [80].

Table 2.2 The polymorphic expression of GST [81]

Class or super family	**Gene**	**Allele**	**Alteration in gene or nucleotides**	**Protein or amino acid affected**
Mu	*GSTM1*	*GSTM1**A	G519	Lys173
		*GSTM1**B	C519	Asn173
		*GSTM1**0	Gene deletion	No protein
	GSTM3	GSTM3*A	Wildtype	Wildtype protein
		GSTM3*B	3 bp deletion in intron 6	Primary structure
				Unaltered
	GSTM4	GSTM4*A	Wildtype	Wildtype
		GSTM4*B	Changes in introns	Unchanged
Theta	*GSTT1*	*GSTT1**A	Unique gene	Unique protein
		*GSTT1**0	Gene deletion	No protein
	GSTT2	GSTT2*A	A415	Met139
		GSTT2*B	G415	Ile139

2.12 GST Polymorphism And Lung Cancer

GSTM1

The main function of phase II biotransformation enzymes is to catalyze the binding of intermediary metabolites to cofactors and convert them into more hydrophilic products and thus facilitate their elimination [82]. The *GSTM1* gene is polymorphic and is represented by two active alleles and a non-functional null allele which results from the entire *GSTM1* gene deletion mutation. It catalyzes the detoxification of xenobiotics including aromatic hydrocarbons epoxides such as PAHs and DNA hydroperoxides,

which are the products of oxidative stress. Thus it may act as a predisposing factor in different diseases [83].

Glutathione-S-transferase M1 (*GSTM1*) and lung cancer:

Among the different ethnic populations, large variations have been found in the frequencies of *GSTM1* null genotype. As shown in a study by Cotton et al., 2000 and Hayes et al., 2005, the Europeans (42–60%) & Asians (41–63%) exhibit a high frequency of the null genotype for *GSTM1* gene as compared with that of Africans (16–36%). Homozygous deletion of *GSTM1* gene results in *GSTM1**0 allele [84, 85], this causes the loss of enzymatic activity. It has been shown in numerous studies that *GSTM1* polymorphisms affect the detoxification of xenobiotic compounds, which leads to the formation of DNA adducts, ultimately leading to development of cancer [86]. The studies have shown that the polymorphism of *GSTM1* affects the metabolism of xenobiotics compounds and there could be DNA adducts formation which may lead to cancer [87]. In a study conducted by Goto et al., it was reported that *GSTM1* gene, whose catalytic enzyme activity detoxifies the activated metabolites of benzo (a) pyrene, acts as a predisposing factor to lung cancer, mainly associated with smoking. Therefore, it may be associated with overall survival period in patients with NSCLC. The study concluded by stating that the individuals with normal *GSTM1* activity (having *GSTM1* wild genotype) had a larger survival as compared to subjects in which *GSTM1* gene was absent (*GSTM1* null genotype) [88]. In another study, which took breast cancer patients as the subjects, it was reported that, *GSTM1* null genotype was significantly related with the overall survival. Various statistical studies related to *GSTM1* polymorphism have been carried out [89]. Table 2.3 summarises the genotypic frequencies of *GSTM1* among different world populations.

Table 2.3 *GSTM1* genotype frequencies among different world populations.

Authors	**Country/ population**	**Sample no.**	***GSTM1* %**		**p value**
			NULL	**PRESENT**	
Steinhoff C et al., 2000	Central Europe	127	45	55	0.11
Ada et al., 2004	Turkish	133	51.9	48.1	0.01
D'Alo et al., 2004	Italians	273	46.9	53.1	0.06

Naveen et al, 2004	South India	517	30.4	69.6	0.76
Dhruba Mishra et al., 1999	North India	370	33	67	0.1125
Sctiawan et al., 2000	Chinese	477	51	49	0.01
Kiyohara et al., 2000	Japanese	88	55.7	44.3	0.01
Gsur et al., 2001	Caucasians	166	48.8	51.2	0.03
Millikan et al., 2000	USA	392	52	48	0.01
Millikan et al., 2000	African americans	271	28	72	0.54

GSTT1

This polymorphic GST isoform is known to be the most ancient. [90] *GSTT1* is represented by a functional and a non-functional null allele. The null allele results from total or partial deletion of the gene which results in two phenotypes: *GSTT1* null, which is the homozygote of the deleted allele and *GSTT1*-positive, in which at least one copy of the gene is intact [91]. In the human liver, two theta-class *GSTs, GSTT1 and GSTT2,* have been identified [92].

GSTT1 enzymes show some distinguishing features in their catalytic activity as compared to other known GSTs. The major difference is that they have a lower GSH-binding activity. They also have an increased catalytic activity which biotransforms a number of drugs and industrial chemicals such as hydrocarbons, halogenated hydrocarbons and cytostatic drugs. It is also involved in detoxification of ethylene and mono-halomethane. As *GSTM1, T1* also metabolises the by-products of cellular oxidative stress, such as hydroperoxides and oxidised lipids [70, 85].

Glutathione-S-transferase T1 (*GSTT1*) and lung cancer :

According to the epidemiological study reported conducted by Sharma *et al.,* it was suggested that the frequency of *GSTT1* null genotypes is lower in case of Europeans

(13.31%) as compared to high frequency in African (14–57%) and Asian populations (35–48%) [93]. Table 2.4 summarises the genotypic frequencies of *GSTT1* among different world populations.

Table 2.4 *GSTT1* genotype frequencies among different world populations.

Authors	Country/ population	Sample no.	*GSTT1* %		p value
			null	Present	
Steinhoff C et al., 2000	Central Europe	127	13	87	0.43
Ada et al., 2004	Turkish	133	17.3	83.7	1.00
D'Alo et al., 2004	Italians	273	19	81	1.00
Naveen et al., 2004	South India	517	16.8	83.2	1.00
Dhruba Mishra et al., 1999	North India	370	18.4	81.6	0.0064
Sctiawan et al., 2000	Chinese	477	46	54	<0.01
Kiyohara et al., 2000	Japanese	88	44.3	55.7	<0.01
Gsur et al., 2001	Caucasian	166	19.9	80.1	0.86
Millikan et al., 2000	USA	392	16	84	0.85
Millikan et al., 2000	African Americans	271	17	83	1.00

2.13 *GSTs* and cancer

The recent advancements in technology and molecular research has established a strong relationship between the metabolic genes and its interaction with the environment. This has further aided in diagnosis and treatment of several diseases, making it easier to follow a medical algorithm to achieve longer disease free survival. Such an extensively studied disease is cancer, which has caused an epidemic state in past decades. Through numerous epidemiological studies it has been seen that the phase II metabolic genes,

which mainly consists of *GSTs*, play a vital role in detecting an individual's risk, prognosis and treatment outcomes after diagnosis with cancer. Table 2.5 summarises some of the studies done on different types of cancer, which significantly showed an association between *GST* genes and the disease.

Table 2.5. The relation of GST genes with different forms of cancer.

S.No.	References	Study subjects	Results
1.	Xiangzhen Kong *et al*., 2016	Meta-analysis on breast cancer patients	A significant association of *GSTM1* polymorphism with response to chemotherapy. No association was found in case of *GSTT1* genotype.
2.	Djukic *et al.*,	105 patients with muscle invasive bladder cancer	*GSTT1* wild genotype was found to be a prognostic factor, showing a higher death risk in bladder cancer patients.
3.	Abbas M *et al*., 2015	227 cervical cancer (CC) patients	a) Significant association of null *GSTM1* genotype with better survival in CC patients. b) Combined effect of null *GSTT1* and null *GSTM1* genotypes on higher overall survival among females.
4.	Eva Barragan *et al*., 2006	153 patients with acute myeloid leukaemia	The probability of disease free survival was lower in males carrying the null *GSTM1* genotype.
5.	J. Wang *et al*., 2015	262 breast cancer patients	The patients carrying null *GSTM1* genotype had a better response to chemotherapy.

2.14 GSTs and lung cancer

The basis of study in this dissertation is to relate the polymorphic genes, *GSTT1* and *GSTM1* with the disease prognosis and treatment outcomes in lung cancer patients receiving platinum-based chemotherapy. Till date there have been various reports on association of these genes with lung cancer, may be it to determine the hosts' susceptibility, survival or response to treatment. Some studies have postulated and others have significantly proved that the genes encoding phase II metabolic enzymes have a remarkable role in detecting the risk, overall survival and treatment response in patients diagnosed with lung cancer. Though there have been conflicting results, these

genes continue to be of major interest in molecular and diagnostic research. Some of these studies are summarised in table 2.6.

Table 2.6. The relation of GST genes with lung cancer.

S.No.	References	Study subjects	Results
1.	Sweeney *et al*., 2003	274 lung cancer patients	Significant association between males who carried null *GSTM1* genotype with shorter overall survival, independent of histological factors. No association was found with *GSTT1*.
2.	Goto *et al*., 1996	232 NSCLC patients	The worst survival in patients was found associated with null *GSTM1* genotype.
3.	Yang *et al*., 2002 and Przygodzki *et al*., 1998	lung cancer patients	No association was seen between the two GST variants and overall survival in LC patients.
4.	Sreeja *et al*., 2008	422 lung cancer patients	A significant association between null *GSTT1* genotype along with stage was found with shorter overall survival in patients.
5.	Ge H *et al*., 1996	98 NSCLC pateints	The patients carrying null *GSTM1* genotype showed a higher overall survival than the ones carrying *GSTM1* gene.
6.	Sharma *et al*., 2015	270 lung cancer cases and 270 controls	In ADCC patients, the null *GSTM1* genotype was found associated with higher risk of lung cancer. No association was found with *GSTT1* genotype.
7.	W. Jia *et al*., 2016	244 patients with NSCLC	a) A good response to chemotherapy treatment was found in patients carrying the null *GSTM1* genotype. b) The patients carrying the null *GSTM1* genotype showed a better overall survival as compared to the patients carrying the *GSTM1* gene.
8.	Li WeiYing *et al*., 2011	217 NSCLC patients and 198 controls	a) The smokers carrying null *GSTM1* genotype were found to be more susceptible to lung cancer. b) The patients carrying the null *GSTM1* genotype were found to be superior responders to platinum drugs than those carrying the wild *GSTM1* genotype.

9.	Yang and Xian, 2014	Meta analysis of 961 NSCLC patients	The polymorphisms in *GSTM1* gene were significantly related to better treatment response in NSLC patients receiving platinum based chemotherapy.
10.	Ying Gao *et al*., 2017	A meta analysis on lung cancer patients	The combinatorial effect of *GSTT1* and *GSTM1* polymorphisms showed a significant association with increase risk of lung cancer in Indian population.

2.15 GSTs polymorphisms and platinum-based chemotherapy

The knowledge of genetic and environmental interactions can possibly aid in determining the most suitable treatment and the clinical outcome in lung cancer patients [94]. Disease stage and the patients' performance status determine the type of chemotherapy suitable for the patient. Three major types of treatment are given to lung cancer patients: surgery, radiation therapy and chemotherapy [95]. But, for advanced stages, combinations of chemotherapeutic drugs are used. Among them, the most frequently used are platinum compounds (cisplatin or carboplatin) [96].The detoxification or inactivation of platinum drugs is mainly carried out by the glutathione metabolic pathway. Therefore, the genetic polymorphisms corresponding to low metabolic activity by the enzymes may predict useful information about lung cancer prognosis [97].

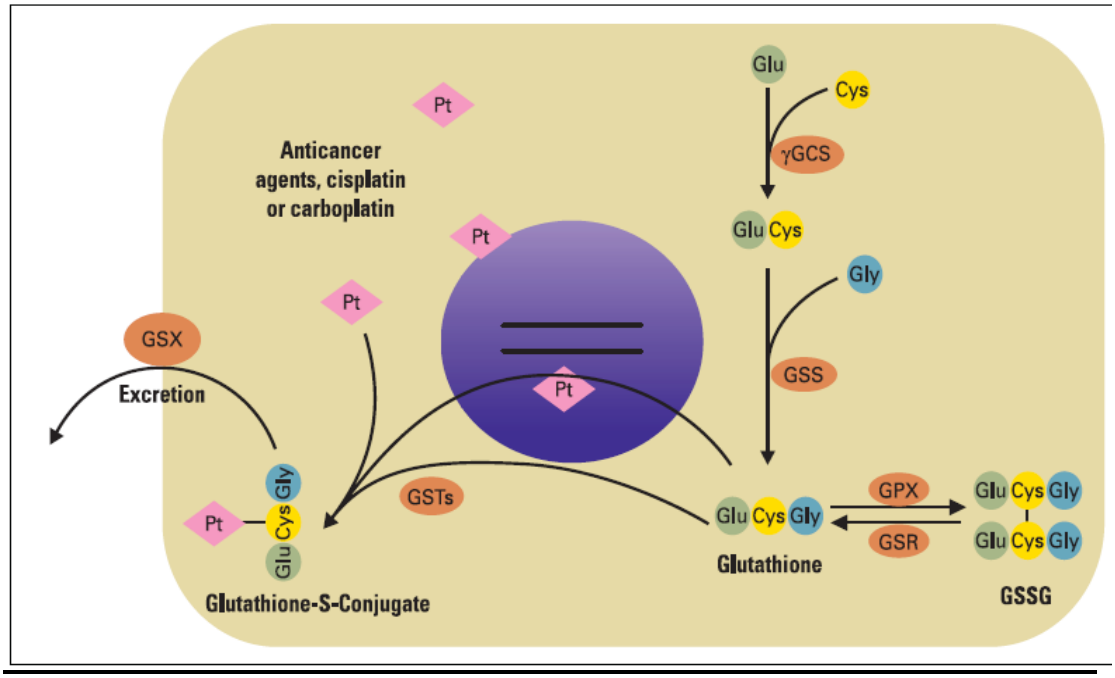
<u>The GSH detoxification system</u>

The major components of this system include:

- glutathione,
- GSH enzymes, and
- The glutathione S-conjugate complex export protein (GS-X pump).

Glutathione is a linear tripeptide of glutamine, cysteine, and glycine. GSTs catalyze the reactions through which GSH conjugates with various xenobiotic compounds or antineoplastic agents such as platinum-drugs. This renders the compound highly

anionic such that it is easily excreted out from the cells by GS-X pump (ATP dependent). Through transhydrogenases, GSH also metabolises free radicals generated by cellular oxidative stress [97].



Glu, glutamate; Cys, cysteine; Pt, platinum-based anticancer drugs; γ GCS, gamma-glutamylcysteine synthetase; GSS, glutathione synthetase; Gly, glycine; GPX, glutathione peroxidase; GSR, glutathione reductase; GSTs, glutathione- S-transferases; GSX, glutathioneconjugation complex export pump; GSSG, glutathione disulfide.)

Figure 2.8.The glutathione pathway and its role in detoxification [97].

The Glutathione System and Anticancer Drug Resistance

Some evidences have proved that through increased GSH-system activity, the tumor cells can become resistant to chemotherapeutic drugs [98]. Figure 2.8 depicts that when platinum compounds (anti-neoplastic) enter the cells; they become conjugated with GSH and are then eliminated. The effect of chemotherapy drugs decreases due to increased GSH level. Elevated GSH level increases the GST enzymatic activity and thus leads to enhanced efflux by the GS-X pump [97].

The Glutathione System and Lung Cancer Prognosis and treatment outcome

It has been postulated in many studies, such as by Andrew M. Gulick et al., 1995, that Glutathione system enzymes could be potential markers in prognosis of lung cancer [99]. This is based on the fact that these enzymes are involved in the detoxification of a wide variety of chemotherapeutic agents. Previous studies by Bai, F et al., 1996 and Oguri, T., 2000 have shown the role of the glutathione system in chemotherapy responses among NSCLC patients. Through these studies it was concluded that the expression of GSTs might decrease a host's response to chemotherapy [100, 101].

The glutathione system and platinum drug toxicities

Two main pathways have been elucidated regarding the molecular mechanism of platinum drugs. These are:

1. The interaction of hydrolyzed platinum drugs with DNA leads to the formation of DNA non-adducts. This leads to inhibition of DNA replication and ultimately apoptosis.
2. The cytoplasmic sulphur-based thiomolecules such as GSH and metallothionein, interact with platinum complexes. This prevents the drugs from binding to DNA, which renders the cell resistant to platinum drugs.

Therefore, it could be concluded that platinum drug resistance is enhanced by decreased drug uptake or enhanced efflux by GS-X pump, as this reduces the desired DNA damage [102, 103].

One of the major limitations of clinical use of platinum drugs is the development of cellular resistance. This could be overcome by maintaining an effective antineoplastic level in the tumor for a specific period. But, this could lead to increased toxicity and ADRs (Adverse Drug Reactions). By regulating the GSH detoxification system, the toxicities could be minimized and the efficacy of chemotherapy drugs could be enhanced [104].

An approach to reduce ADRs is to supplement patients with GSH. This is based on the study by Babu, E et al., 1999, which showed that glutathione inhibits the accumulation of platinum in kidneys, signifying that GSH system has a key role in protecting against

cisplatin-induced toxicity [105]. In another study by Piravano, C et al., 1992, it was found that the patients who received cisplatin along with GSH, had low levels of neurotoxocity [106]. Among the ovarian cancer patients, it was observed that the patients who received a pre-treatment by GSH were more sensitive to chemotherapy and had an improved life quality as compared to those who did not receive GSH pre-treatment [107].

2.16 Gaps in the literature

As mentioned above, numerous studies were carried out worldwide that have aimed to find a significant association of GST genes in determining susceptibility, survival and treatment outcome in cancer patients, mainly those suffering from lung cancer. In this dissertation, for the very first time, North Indian population was taken as study subjects to evaluate the potential role of individual *GSTT1* and *GSTM1* genes in determining the overall survival in lung cancer patients. Also, very limited data is available on the combined effect of these genes on overall survival. Thus, this study takes into account the various genetic combinations of *GSTT1* and *GSTM1* to find a significant association with lung cancer prognosis, with completely new subjects.

Further, the patients were also evaluated based on clinic-pathological parameters such as TNM staging, metastasis, tumor size and lymph node invasiveness. These parameters were used to associate the genetic polymorphisms in *GSTT1* and *GSTM1* with risk of developing lung cancer.

Finally, by studying the role of glutathione metabolic pathway in detoxification of platinum drugs and development of cellular resistance to chemotherapeutic drugs, the patients were evaluated for polymorphisms in their metabolic genes, that is *GSTT1* and *GSTM1*, to determine the individuals' response to platinum-based chemotherapy and study their treatment outcome.

Chapter 3

Aim of study

This dissertation is aimed at studying:

1. The potential role of individual *GSTT1* and *GSTM1* genes in determining the overall survival in lung cancer patients in North Indian population.
2. The effect of various genetic combinations of *GSTT1* and *GSTM1* to find a significant association with lung cancer prognosis.
3. To associate the genetic polymorphisms in *GSTT1* and *GSTM1* with risk of developing lung cancer based on different clinic-pathological parameters.
4. The association of genetic polymorphisms in GSTT1 and GSTM1 in determining the individuals' response to platinum-based chemotherapy and account for their treatment outcome.

Chapter 4

Material and Methods

4.1 Sample Collection

This study accounts for 323 lung cancer patients recruited from the Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh, India. This study has been reviewed and approved by the Institute ethics committee of PGIMER. All the subjects were diagnosed with NSCLC or SCLC and an informed written consent was obtained from them or their representatives. A detailed questionnaire was filled by each patient that contained information on demographic and smoking characteristics such as tobacco habits like smoking of beedi/cigarette etc. The subjects having a regular smoking habit were classified as smokers. The clinic-pathological details such as TNM staging, Tumor size, Lymph node invasiveness, metastasis and clinical response of patients towards chemotherapy were obtained from the hospital records. Gender and other parameters were also taken under consideration. Approximately 3-5ml of venous blood was collected from each participant.

4.2 DNA extraction

From whole blood of lung cancer cases, the genomic DNA was isolated using standard Protein K digestion, phenol/chloroform extraction and ethanol precipitation method (Field *et al* 1999).

Requirements:

- Washing buffer
- Lysis buffer
- Phenol:Chloroform:Isoamylalcohol (25:24:1)
- Chloroform:Isoamylalcohol (24:1)
- Isopropanol
- TE buffer

Preparation of Buffers

Washing buffer, Lysis buffer and TE buffer were prepared as shown in tables below.

Table 4.2.1 Preparation of washing buffer	
Stock concentration	**Working concentration**
1M sucrose	320mM sucrose
100% Triton X-100	1% Triton X-100
100mM Magnesium Chloride	5mM magnesium Chloride
100mM Tris-HCl pH (8.0)	10mM Tris-HCl pH (8.0)

Table 4.2.2 Preparation of lysis buffer	
Stock concentration	**Working concentration**
1M Tris HCl pH (8.00)	400mM Tris HCl pH (8.00)
10% SDS	1% SDS
0.5M EDTA	60mM EDTA
5M NaCl	150mM NaCl
10mg/ml Proteinase-K	100µg/ml proteinase-K

Procedure of DNA Isolation

- Took 5ml of blood and 5ml of Washing Buffer was added and mixed thoroughly. Centrifuged it at 3500rpm for 5 minutes.
- Discarded the supernatant and added 5ml of Washing buffer (1.6ml 1M Sucrose,0.5 ml Triton X-100, 0.25ml MgCl2, 0.5 ml 100mM Tris HCl and

0.26ml of water) to the pellet and re suspended the pellet in the Buffer and centrifuged again (repeat this step thrice).

- Dissolved the pellet in 5ml of Lysis buffer (1 M Tris HCl 2ml,10% SDS 0.5ml ,0.5 M EDTA 0.6ml, 5M NaCl 0.15ml,10mg/ml Proteinase-K 0.05ml and water 1.7ml) and incubated at 44°C overnight.
- Added an equal volume of Phenol: Chloroform: Isoamyl alcohol (PCI) 25:24:1(25ml Phenol, 2.4 ml Chloroform and 0.1ml isoamyl alcohol) and mixed the contents slowly.
- Centrifuged at 8000rpm for 10minutes at 4°C. Took the upper aqueous layer and again add PCI mix and centrifuged.
- Took the aqueous layer and added equal volume of Chloroform: Isoamyl alcohol (24:1).
- Centrifuged it at 6500 rpm for 5 minutes and took the upper layer.
- To the aqueous layer added equal volume of chilled Isopropanol or 2.5 times volume of absolute Ethanol and mixed it gently.
- Freeze it at -20°C for 1-2 hours.
- Centrifuged it at 12,000 rpm for 10 min at 4°C.The supernatant was discarded and the pellet of DNA was washed with chilled 70% Ethanol twice at 10,000 rpm for 5 minutes.
- Decant ethanol and air dry the pellet.
- Dissolved the pellet in 50µl-150µl Tris-EDTA buffer depending on the size of DNA pellet (Bartlett & White, 2003).

4.3 Quantitative and qualitative estimation of DNA template

The Thermo Scientific Nanodrop Spectrophotometer makes use of the fibre optic technology and surface tension which effectively holds 1µl of sample in between two optical surfaces that define the path length in vertical orientation. Removal of fixed containment devices from the system allows the path length to change in real time for a sample. This eliminates the need to perform dilutions and hence is less time-consuming. The quantitative estimation of DNA was done by using UV spectrophotometer and the absorbance was recorded at two wavelengths A260 nm and A280 nm. The ratio of absorbance at 260nm and 280 nm is used to assess the purity of DNA. DNA concentration of the solution was determined by using formula:

{DNA concentration (µg/ml) = O.D at 260nm × 50 × Dilution factor}

Where 50µg/ml of DNA is equal to 1 O.D

Purity of DNA = O.D at 260nm/O.D at 280nm

NOTE: A ratio of 1.8 indicates is accepted for pure DNA; a ratio of 2.0 is generally accepted as pure for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants.

Procedure

- Pipetted 1µl of Deionised water onto the lower optical surface of Nanodrop (Thermo Scientific) to clean it
- Opened the Nanodrop software and select Nucleic acid Module
- Performed a blank measurement by loading 1µl of TE and selecting 'blank' from the screen
- Measured the Nucleic acid sample by loading 1µl of DNA sample and selecting 'measure'

Concentration and purity of DNA samples were calculated automatically.

4.4 Resolution of DNA Fragments on Agarose Gels

Requirements

- Electrophoresis buffer (TAE or TBE)
- Ethidium bromide solution
- Electrophoresis-grade Agarose
- 6X loading dye
- DNA molecular weight markers
- Horizontal gel electrophoresis apparatus
- Gel casting platform
- Gel combs (slot formers)
- DC power supply

Preparing 5X TBE (1000ml)

- Tris base - 54 g
- Boric Acid - 27.5g
- EDTA (0.5M) - 20ml
- Make up final volume with water

Preparing 6X Loading Dye (20ml)

- 0.25% Bromophenol blue - 0.05gm
- 0.25% Xylene Cyanol - 0.05gm
- 40% Sucrose - 8gm
- Make up final volume with TE buffer

Preparation of the Agarose Gel for Electrophoresis

- Prepared an adequate volume of electrophoresis buffer.
- Added the desired amount of Electrophoresis-grade Agarose to a volume of Electrophoresis buffer sufficient for preparing the gel. For example, for genomic DNA 0.7% gel (0.7g agarose in 100ml 0.5X TBE) was prepared while for the PCR products 1.7% gel (1.7g agarose in 100ml 0.5X TBE buffer) was prepared.
- Melted agarose was cooled to 55°C in a water bath before pouring onto the gel platform to prevent warping of the gel apparatus.
- Before pouring, Ethidium bromide solution was added to the melted agarose gel to a final concentration of 0.3µg/ml to facilitate visualization of DNA when seen under UV Transilluminator.
- Poured the melted Agarose onto the gel casting apparatus between 0.5 and 1 cm thick and with the gel comb inserted prior to pouring, making sure that no bubbles are trapped underneath the combs and all bubbles on the surface of the surface of the agarose were removed before the setting of the gel.

Loading and running the gel

- After the gel got solidified, the gel comb was withdrawn with proper care without disrupting the sample wells.
- Placed the gel casting platform containing the set gel in the Electrophoresis tank. Added sufficient Electrophoresis buffer to cover the gel until the tops of the wells are submerged. Made sure no air pockets were trapped within the wells.
- DNA samples were prepared by mixing 5µl DNA with 2µl of 6X loading dye and 2ul water in case of genomic DNA or by mixing 5µl DNA with 2µl of 6X loading dye in case of PCR product.
- Samples were typically loaded into the wells with micropipette. Care was taken to prevent mixing of the samples between wells.
- Appropriate DNA molecular weight marker was also loaded in case of PCR.
- Connected the electrodes to a power pack and allowed the Electrophoresis apparatus to run at 60 V until the marker dyes migrated the desired distance.
- Turned off the electric power, disconnected the leads, and discarded the electrophoresis buffer from the reservoirs
- DNA was visualized by placing the gel on a UV transilluminator and then photographed using Gel Documentation.

4.5 Multiplex Polymerase Chain Reaction (PCR) Amplification of *GSTT1* and *GSTM1*

Making an advanced use of the traditional PCR technique, multiplex PCR potentially amplifies multiple targets by making use of more than one set of specified primers, thus saving considerable time and labour. It makes use of multiple DNA templates and different sets of primers in a single reaction tube. The presence of multiple primer sets may result in mis-priming or cross-hybridization, leading to primer-dimer formation. To avoid this, it becomes necessary that the sets of primers in a multiplex reaction be suitably designed. Ideally, the primers should have similar melting temperatures and each set should be specific for its target template. This technique offers numerous advantages over the single PCR, for example, it is cost effective, provides more information with low quantity of sample, is time saving and has less pipetting errors.

Requirements

- 10X PCR buffer
- BSA
- Forward Primer
- Reverse Primer
- dNTPs
- Taq DNA polymerase
- Water
- DNA sample

The primers used are:

GSTM1 primers:

Forward primer	5’ GAA CTC CCT GAA AAG CTA AAG C 3’
Reverse primer	5’ GTT GGG CTC AAA TAT ACG GTG G 3’
Band size	480bp

GSTT1 primers:

Forward primer	5’-TTC CTT ACT GGT CCT CAC ATC TC 3’
Reverse primer	5’-TCA CCG GAT CAT GGC CAG CA 3’
Band size	215bp

Albumin primers:

Forward primer	5’-GCCCTCTGCT AACAAGTCCT AC-3’
Reverse primer	5’-GCC CTA AAA AGA AAA TCG CCA ATC-3’
Band size	312bp

Table 4.5.1 Representing requirements for Polymerase Chain Reaction (PCR)			
Reagents	**Stock Concentration**	**Working Concentration**	**Volume Used**

Additive 1 BSA	100µg/ml	10µg/ml	16.50µl
PCR Buffer (Mg concentration)	10 X	1.0 X	16.50µl
GSTT1 Primer (Forward)	10µM	0.5µM	8.25µl
GSTT1 Primer (Reverse)	10µM	0.5µM	8.25µl
GSTM1 Primer (Forward)	10µM	0.5µM	8.25µl
GSTM1 Primer (Reverse)	10µM	0.5µM	8.25µl
Albumin Primer (Forward)	10µM	0.3µM	4.95µl
Albumin primer (Reverse)	10µM	0.3µM	4.95µl
Taq Polymerase	5.0U/µl	1.5U	3.30µl
dNTPs	10mM	0.2mM	3.30µl
PCR Grade Water			49.50µl
DNA template	100ng/µl	300ng/µl	3µl

Table 4.5.2 Representing steps of PCR along with their specified temperature.

S. No.	Steps	Temperature	Time
1.	Initial Denaturation	95°C	5 min
2.	Denaturation	94°C	1 min
3.	Annealing	59°C	1 min
4.	Polymerization	72°C	1 min
5.	Final Extension	72°C	5 min

Step 2, 3 and 4 carried out for 30 cycles.

4.6 Statistical Analysis

This study has been carried out by making use of three basic statistical tools available on the Medcalc software version 9.3.6.0. The survival analysis was done by using the Kaplan-Meier estimate and further evaluated by using Cox multivariate hazard proportional analysis. The Hardy–Weinberg equilibrium theory ($p^2+2pq+q^2=1$; where p is the frequency of the wild-type allele and q is the frequency of the variant allele) was used in cases to calculate the genotype frequencies of *GSTT1 and GSTM1* gene polymorphism. To assess the risk for lung cancer and GST polymorphisms adjusted Odds Ratio (ORs) along with 95% Confidence Intervals (CI) was calculated using logistic regression analysis. All p values were two sided, and a p value of <0.05 was considered statistically significant.

Chapter 5

RESULTS

5.1 DNA isolation

5ml blood was used for isolation of genomic DNA. Presence of isolated DNA was affirmed by trans-UV-illuminator using 0.7% agarose gel as shown in Figure.5.1. Concentrated DNA was further diluted with TE to get a concentration of 100 ng/µl and they were used as template for amplification by Polymerase chain reaction.

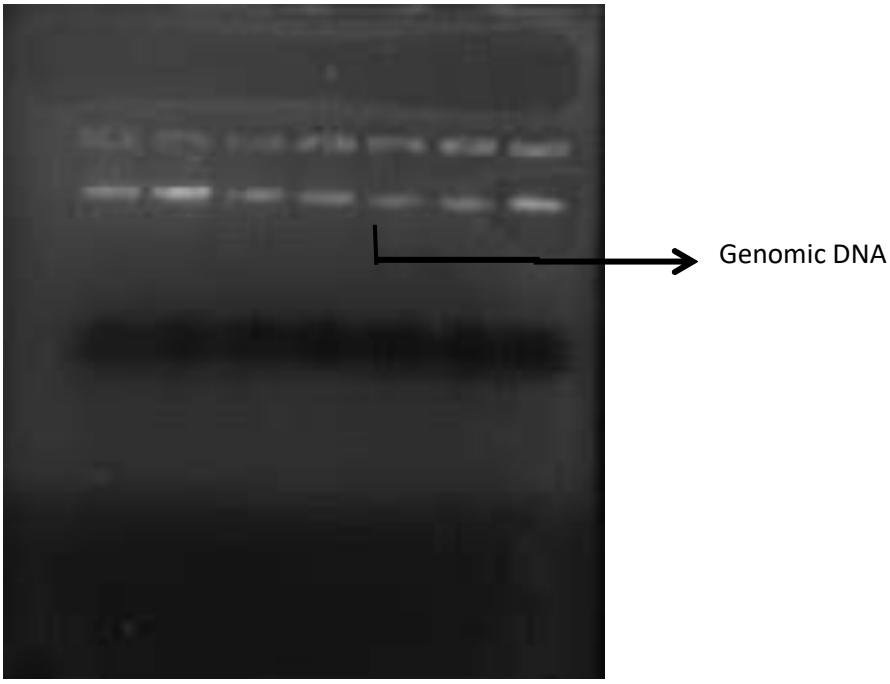


Figure 5.1 Isolated genomic DNA confirmation by gel electrophoresis.

5.2 Multiplex polymerase chain reaction for *GSTT1* and *GSTM1*

Multiplex polymerase chain reaction was performed to simultaneously amplify the *GSTT1* and *GSTM1* genes. The presence of 312bp size amplicon of albumin was taken as a control. The desired size amplicons of 215bp for *GSTT1* and 480bp for *GSTM1* were analyzed using 1.7% agarose by agarose gel electrophoresis as shown in figure 5.2.

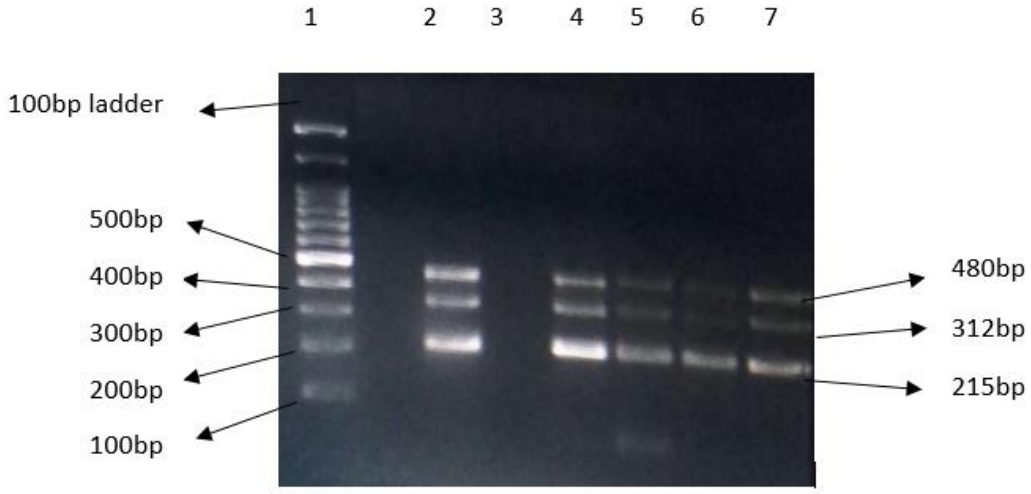


Figure 5.2 PCR amplified DNA products of *GSTM1* (480bp) and *GSTT1* (215bp) gene for LC samples.

5.3 Demographic distribution of lung cancer patients

The demographic characteristics and clinical data of the lung cancer patients are shown in table 5.1. The parameters included age, sex, smoking status, pack years for cases. The clinical and pathological parameters of lung cancer patients were also studied which included histology, tumor stage, tumor size extension, lymph node extension, metastasis along with clinical response parameter (complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD)) for cases. The study recruited total of 323 cases having mean age of 55.1 (9 ± 10.57). It included 281 (86.99%) men and 42 (13%) females. The smokers were over represented as compared to non-smokers (83.59 vs. 16.40 %) which showed that smoking is a significant parameter for occurrence of lung cancer. Pack years was calculated as [((cigarettes or beedis)/20) × No. of years smoked] and was found to be 30.35(±44.70). When the lung cancer cases were stratified on the basis of histology, it was found that, SQCC was the most abundant type of lung cancer (35.60 %) followed by small cell lung cancer (SCLC) and adenocarcinoma (ADCC), with 31.26 % each, while 1.54% had other histological subtype. These were further classified on the basis of Tumor Node Metastatic staging. For this the data was available for 291patients. Among the cases, Stage IV was highly proportionate (50.15 %) followed by Stage III (22.29%). Only 0.92% belonged to stage I and 2.16% of patients were in stage II whereas 8.66% of the

patients were unclassified. Tumor size, lymph node involvement and metastasis data was also included in the study. The tumor size of the patients varied from T1-T4. Maximum of the patients, 162 (50.15%), had T4 type of tumor followed by 72 (22.29%) patients with tumor type T3. 35 (10.83%) had T2 tumor type and 22 (6.81%) had T1 tumor type. Also, for 32 (9.90%) patients, the tumor type was not known. Similarly, out of 323 cases 40.86 % of the lung cancer cases had distant metastasis (M1) and the remaining patients (49.84%) did not show any metastasis. Also, among these cases, 11.76% showed no lymph node involvement, 9.28% showed N1 lymph node involvement, 42.41% of the patients showed the maximum lymph node involvement of type N2 followed by 25.69% showing N3 lymph node involvement and only 0.92% had N4 lymph node involvement. Out of 323 patients, 187 were assessable for clinical response. Among them largest number of patients showed partial response (PR) (30.95 %), 1.54% showed complete response while 19.81% showed stable disease (SD) and 5.57% of patients showed progressive disease after being treated with chemotherapy. Further, on stratifying the patients on the basis of treatment regimen, it was found that maximum of the cases (24.14%) were under regimen 5 (irini cis/ irino carb) and 22.60% were under treatment regimen 1 (doce cis/ doce carb) followed by 21.36% patients under treatment regimen 6 (pem cis/ pem carb). 6.81% cases followed other treatment regimens while for 25.07% cases, the regimen was unknown. While studying the performance status of lung cancer patients, it was found that 39% had KPS between 90 -100, 44.89% had KPS between 70- 80, 13.62% had KPS between 30- 60 and for 2.47% patients the KPS was not known. Similarly, the classification of lung cancer patients on the basis of ECOG showed that 269 (83.28%) patients had ECOG 3 or 4 while 46 (14.24%) had ECOG 0, 1 or 2 and for the remaining 8 (2.47%) of patients, the ECOG was not classified.

Table 5.1. Distribution of demographic characteristics of LC cases.

VARIABLE	**CASES, n(%) N=323**	**VARIABLE**	**CASES, n(%) N=323**
Age(years)		**Lymph node involvement**	
Mean ± SD	55.19 ± 10.57	N0	38 (11.76)
Range	28-86	N1	30 (9.28)
		N2	137 (42.41)
		N3	83 (25.69)
		N4	3 (0.92)
		Unknown	32 (9.90)

Gender		**Metastasis**	
Male	281 (86.99)	M0	161(49.84)
Female	42 (13.00)	M1	132 (40.86)
		Unknown	30 (9.28)
Smoking status		**KPS**	
Smokers	270 (83.59)	30-60	44 (13.62)
Non-smokers	53 (16.40)	70-80	145 (44.89)
		90-100	126 (39.00)
		Unknown	8 (2.47)
Pack years		**ECOG**	
Mean ± SD	30.35 ± 44.70	0	269 (83.28)
		1	46 (14.24)
		Unknown	8 (2.47)
Histologic types		**Regimen**	
SQCC	115 (35.60)	1	73 (22.60)
ADCC	101 (31.26)	5	78 (24.14)
SCLC	101(31.26)	6	69 (21.36)
Others	5 (1.54)	Others	22 (6.81)
Unknown	1 (0.30)	Unknown	81 (25.07)
Tumor size		**TMN staging**	
T1	22 (6.81)	I	3 (0.92)
T2	35 (10.83)	II	7 (2.16)
T3	72 (22.29)	III	151 (46.74)
T4	162 (50.15)	IV	134 (41.48)
Unknown	32 (9.90)	Unknown	28 (8.66)
Objective Response	5 (1.54)		
CR	100 (30.95)		
PR	64 (19.81)		
SD	18 (5.57)		
PD	136 (42.10)		
Unknown			

Abbreviations: SD=Standard Deviation, n=total number of case patients, ADCC= adenocarcinoma, SCLC=small cell lung carcinoma, SQCC= squamous cell carcinoma, CR= complete response, PR= partial response, SD= stable disease, PD= progressive disease, KPS= karnofsky performance scale, ECOG= eastern cooperative oncology groyp, regimen 1= doce cis/ doce carb, regimen 5= irini cis/ irino carb, regimen 6=pem cis/ pem carb

5.4 Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients:

Table 5.2. Genotypic frequencies of *GSTT1* and *GSTM1* among cases and their associated death risk along with overall survival in lung cancer patients							
Genotype	**Overall**						
GSTT1	Dead (273) n%	Alive (50) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	223 (84.15)	42 (15.85)	9.00	-	-	-	-
Null	50 (86.21)	8 (13.79)	6.33	1.06 (0.77-1.45)	0.69	1.06 (0.76-1.49)	0.69
GSTM1	N=273	N=50					
Present	160 (85.56)	27 (14.44)	8.80	-	-	-	-
Null	113 (83.09)	23 (16.91)	8.26	0.96 (0.76-1.22)	0.78	1.04 (0.80-1.34)	0.75

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage. Abbreviation: HR, Hazard ratio; CI, confidence interval.

Overall survival analysis was performed for 323 lung cancer patients and the results are summarized in Table 5.2. Evaluation was made first using univariate Kaplan-meir analysis then adjusted by multivariate Cox hazard proportional analysis for various factors such as age, histology, gender, smoking status, stage and performance status while considering the genotype as an independent factor. On evaluating patients from the day of diagnosis to the last follow-up date, it was found that 15.48% (50) patients were alive while 84.52% (273) were found to be dead due to the disease. By classifying them on the basis of genetic frequencies, it was found that in case of 265 patients having the *GSTT1* gene, 84.15% were dead and 15.85% were alive and in case of 58 subjects carrying the *GSTT1* null genotype, 86.21% were dead and 13.79% were alive. The overall survival period of patients having the *GSTT1* gene was taken as a reference and it was observed that the subjects carrying null *GSTT1* genotype had a lower overall survival period (9 vs. 6.33 months, HR=1.06; log rank p=0.69). On applying the Cox multivariate hazard proportional analysis, no significant association was found between *GSTT1* genotype and the overall survival in lung cancer patients (HR'=1.06; 95%CI=0.76-1.49; p=0.69) as shown in figure 5.3.

Similarly in case of *GSTM1*, the wild genotype was taken as a reference and it was observed that among 187 patients, 160 were dead and 27 were alive, with a MST of 8.80 months. In 136 cases carrying the null *GSTM1* genotype, 83.09% were dead and 16.91 % were alive. It was found that lung cancer patients having the null *GSTM1* genotype had a lower overall survival as compared to that of the cases carrying the *GSTM1* gene (8.26 vs. 8.80 months HR=0.96; log rank p=0.78). On applying the Cox multivariate hazard proportional analysis, no significant association was found between *GSTM1* genotype and the overall survival in lung cancer patients (HR'=1.04; 95%CI=0.80-1.34; p=0.75) as shown in figure 5.4.

Effect of GSTT1 polymorphism on overall survival in lung cancer patients

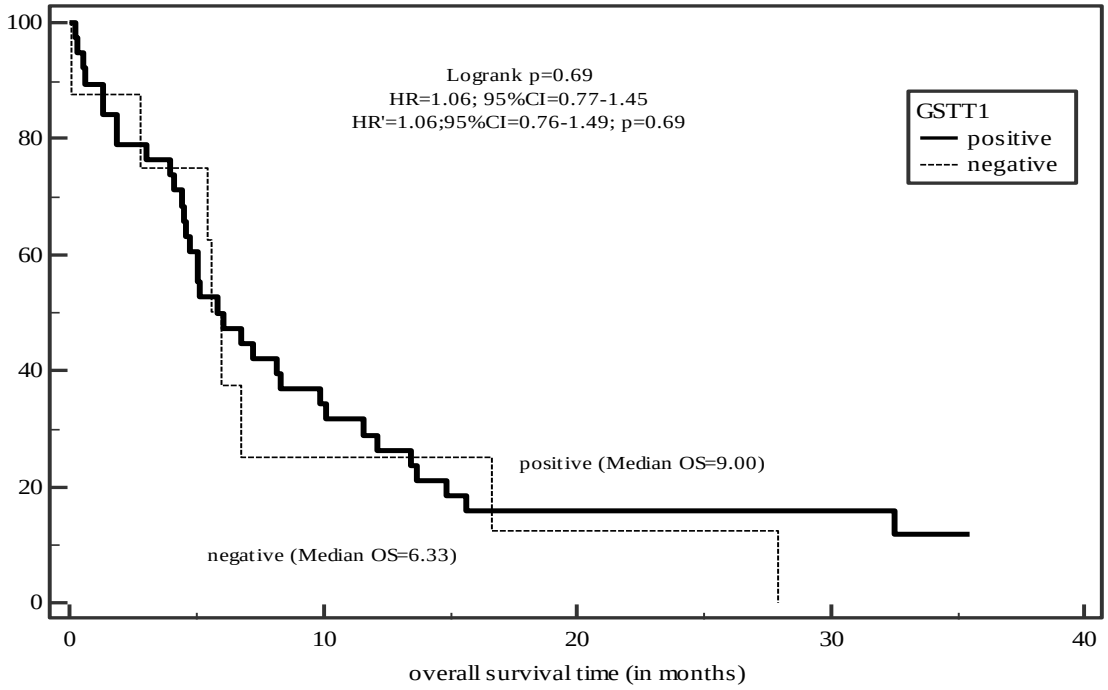


Figure 5.3 Effect of GSTT1 polymorphism on overall survival in lung cancer patients.

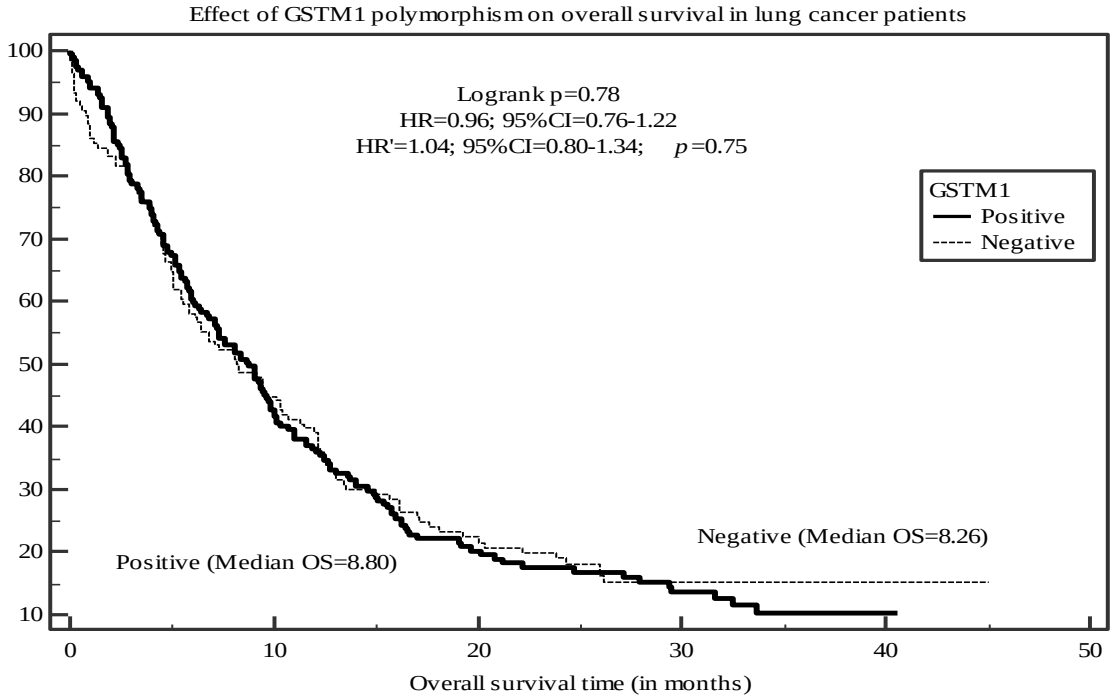


Figure 5.4 Effect of GSTM1 polymorphism on overall survival in lung cancer patients.

5.5 Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients based on histological subtypes:

Table 5.3.1. Genotypic frequencies of *GSTT1* and *GSTM1* among ADCC cases and their association with overall survival in lung cancer patients.

Genotype	**ADCC**						
GSTT1	Dead (83) n%	Alive (18) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	62 (80.52)	15 (19.48)	9.23	-	-	-	-
Null	21 (87.50)	3 (12.50)	6.33	1.24 (0.73-2.09)	0.39	1.60 (0.90-2.81)	0.20
GSTM1	N=83	N=18					
Present	43 (84.32)	8 (15.68)	8.03	-	-	-	-
Null	40 (80)	10 (20)	9.43	0.81 (0.53-1.25)	0.35	0.66 (0.40-1.08)	0.10

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

The results for the association between the genotypic distribution and ADCC histological subtype are summarized in table 5.3.1. It was found that in case of 101 patients with ADCC, 83 (82.18%) were dead and 18 (17.82%) were alive. Based on their classification according to the genotypic distribution, it was found that among 77 patients carrying the *GSTT1* gene, 80.52% were dead and 19.48% were alive. The overall survival period of the individuals carrying the *GSTT1* gene was found to be 9.23 months which is taken as the reference. In this study, it was found that among 24 ADCC patients carrying the null *GSTT1* genotype, 87.50% were dead and 12.50% were alive. In this case it was seen that the MST was much lower as compared to the wild genotype (6.33 vs.9.23 months HR=1.24; log rank p=0.39). After multivariate Cox hazard proportional analysis, the adjusted HR was found to be non-significant (HR'=1.60; 95%CI=0.90-2.81; *p*=0.20) as shown in figure 5.5.

Similarly, it was found that among 51 ADCC patients carrying the *GSTM1* gene, 84.32% were dead while only 15.68% were alive. The overall survival period of these patients was found to be 8.03 months whereas among 50 ADCC patients carrying the *GSTM1* null genotype, 80% were dead and 20% were alive. The overall survival period

was larger as compared to the wild genotype (9.43 vs. 8.03 months HR=0.81, log rank p=0.35). Here also a non-significant death ratio was found after multivariate Cox hazard proportional analysis (HR'=0.66; 95%CI=0.40-1.08; *p*=0.10) as shown in figure 5.6.

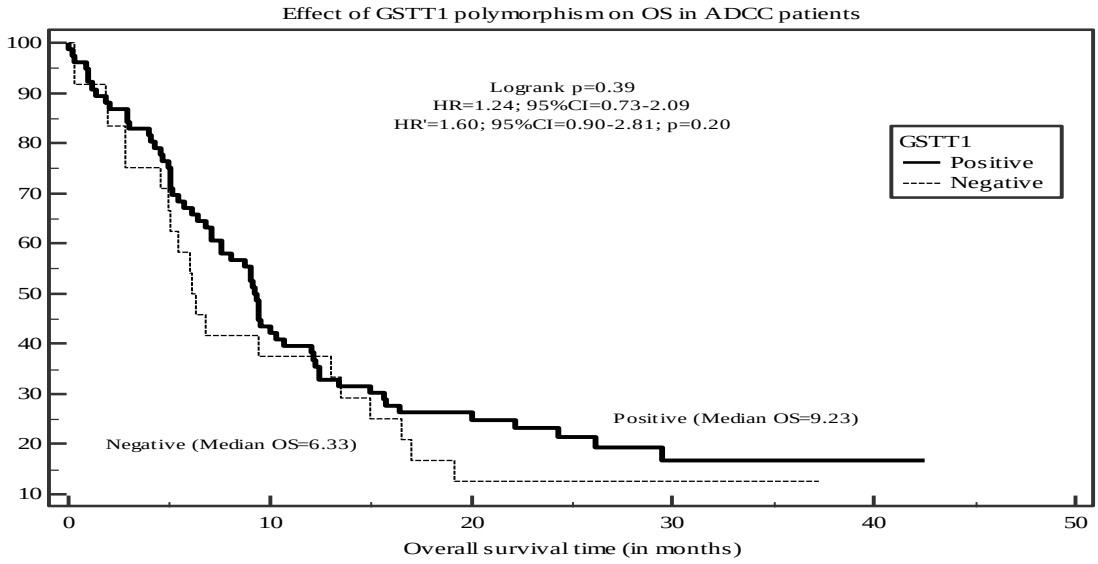


Figure 5.5 Effect of GSTT1 polymorphism on overall survival in ADCC patients.

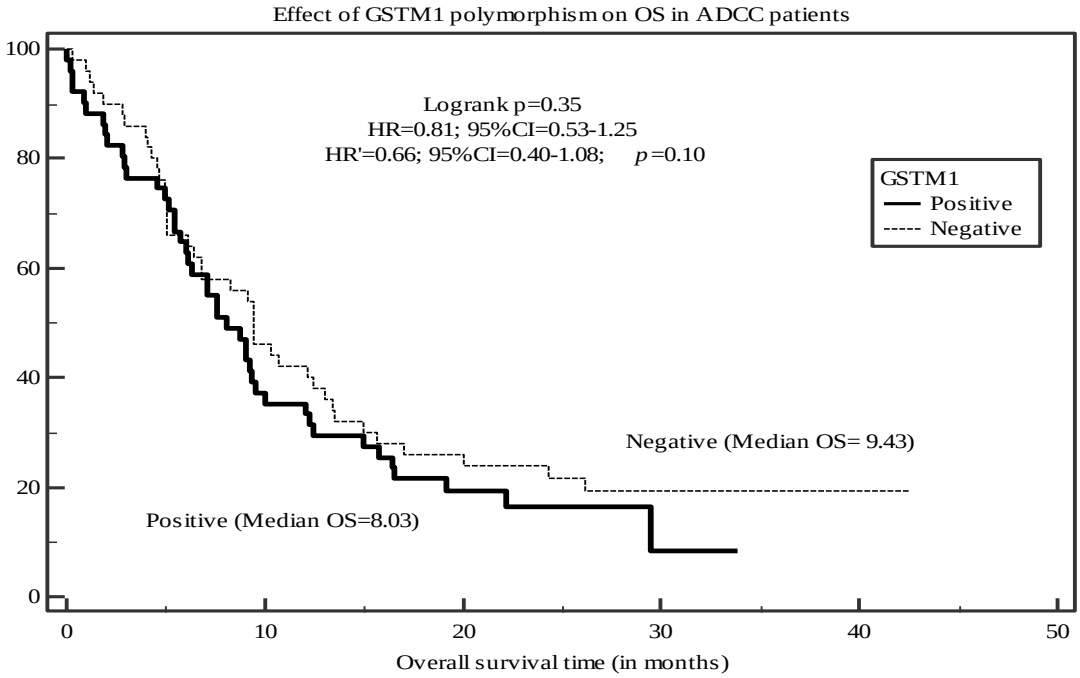


Figure 5.6 Effect of GSTM1 polymorphism on overall survival in ADCC patients.

Table 5.3.2. Genotypic frequencies of *GSTT1* and *GSTM1* among SCLC cases and their association with overall survival in lung cancer patients.							
Genotype	**SCLC**						
GSTT1	Dead (94) n%	Alive (7) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	80 (93.02)	6 (6.98)	8.40	-	-	-	-
Null	14 (93.33)	1 (6.67)	9.00	1.14 (0.63-2.08)	0.63	1.22 (0.62-2.41)	0.55
GSTM1	N=94	N=7					
Present	55 (90.16)	6 (9.84)	10.00	-	-	-	-
Null	39 (97.5)	1 (2.5)	5.00	1.44 (0.93-2.21)	0.07	1.55 (0.98-2.47)	0.08
[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage							

Among 101 SCLC cases, 94 (93.07%) were dead and 7 (6.93%) were alive as shown in table 5.5.2. On further stratifying them on the basis of genotypic distribution, it was found that out of 86 patients carrying the *GSTT1* genotype (MST=8.40 months.), 93.02% were dead and 6.98 % were alive whereas among 15 patients having the null *GSTT1* genotype, 93.33% were dead and only 6.67% were alive. The overall survival in SCLC patients having the null *GSTT1* genotype was found to be larger as compared to the survival in cases carrying the *GSTT1* gene (9.00 vs.8.40 months, HR=1.14; log rank p=0.63). The adjusted HR after multivariate Cox hazard proportional analysis was found to be non-significant (HR'=1.22; 95%CI=0.62-2.41; *p*=0.55) as shown in figure 5.7.

Also, in 61 SCLC patients carrying the *GSTM1* gene, 90.16% were dead and 9.84% were alive and among the 40 SCLC subjects having the null *GSTM1* genotype, 97.5% were dead and 2.5% were alive. In this case, the overall survival period was almost half as compared to the patients carrying the *GSTM1* gene (5 vs.10 months, HR=1.44; log rank p=0.07). After the multivariate Cox hazard proportional analysis, the adjusted HR was found to be non-significant (HR'=1.49; 95%CI=0.95-2.35; *p*=0.08) as shown in figure 5.8.

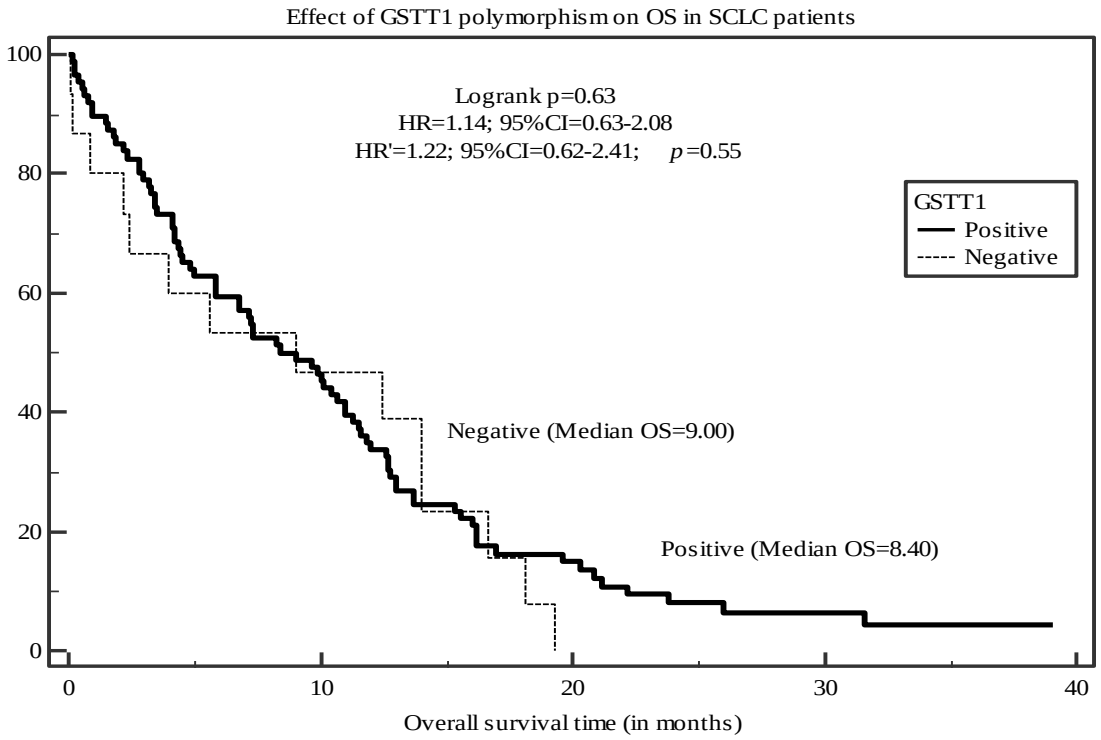


Figure 5.7 Effect of GSTT1 polymorphism on overall survival in SCLC patients.

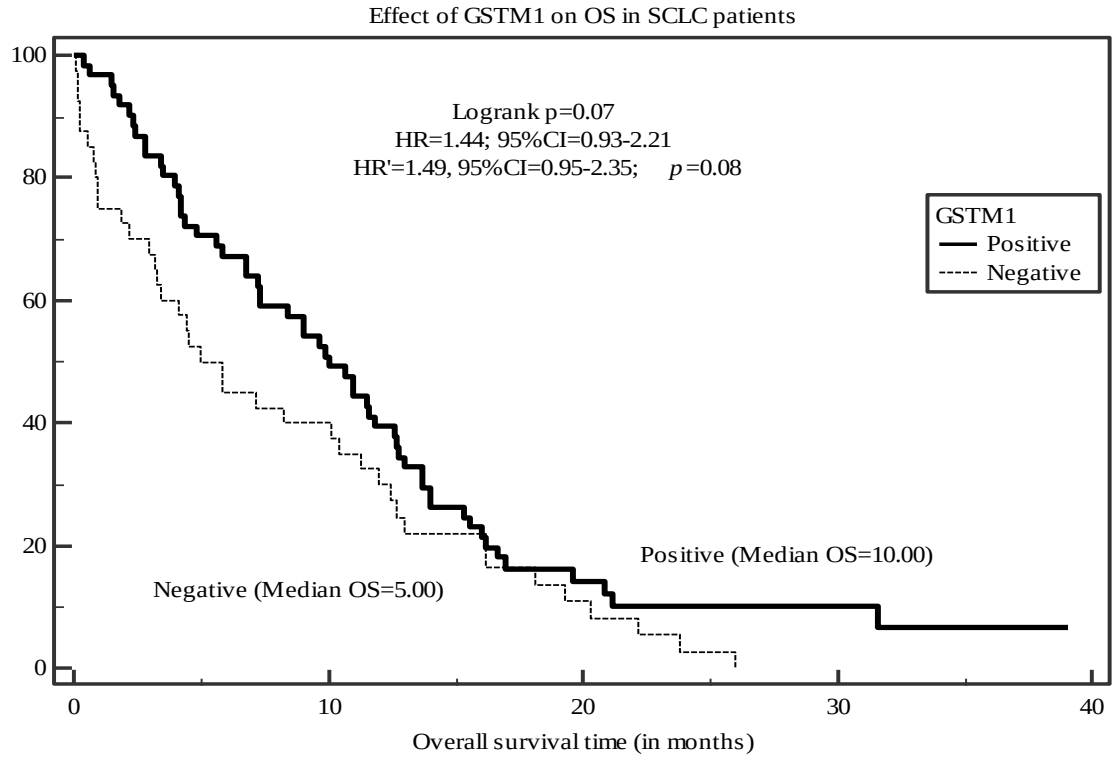


Figure 5.8 Effect of GSTM1 polymorphism on overall survival in SCLC patients.

Table 5.3.3. Genotypic frequencies of *GSTT1* and *GSTM1* among SQCC cases and their association with overall survival in lung cancer patients.							
Genotype	**SQCC**						
GSTT1	Dead (92) n%	Alive (23) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	78 (79.59)	20 (20.41)	7.33	-	-	-	-
Null	14 (82.35)	3 (17.65)	8.06	0.97 (0.55-1.71)	0.93	0.82 (0.51-1.66)	0.79
GSTM1	N=92	N=23					
Present	59 (83.09)	12 (16.91)	7.33	-	-	-	-
Null	33 (75)	11 (25)	8.03	0.86 (0.57-1.31)	0.51	1.11 (0.70-1.74)	0.65
[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage							

Among 115 cases with SQCC, 92 (80%) were dead and 23 (20%) were alive as shown in table 5.3.3. In this study, it was seen that out of 98 SQCC patients that carried the *GSTT1* gene, 79.59% were dead and 20.41% were alive. The MST of subjects with the *GSTT1* gene was found to be 7.33 months whereas in case of 17 SQCC patients that carried the null *GSTT1* genotype, 82.35% were dead and 17.65% were alive and the survival period was found to be larger than the subjects carrying the *GSTT1* gene (8.06 vs. 7.33 months, HR=0.97; log rank p=0.93). In this case, the adjusted HR after applying the multivariate Cox hazard proportional analysis, was found to be non-significant (HR'=0.82; 95%CI=0.43-1.54; *p*=0.54) as shown in figure 5.9.

Similarly in 71 SQCC patients carrying the *GSTM1* gene, 83.09% were dead and 16.91% were alive and in 44 cases having the null *GSTM1* genotype, 75% were dead and 25% were alive. The overall survival period of subjects carrying the null *GSTM1* genotype was found to be larger as compared to the cases having the *GSTM1* gene (8.03 vs. 7.33 months, HR=0.86; log rank p=0.51). After the multivariate Cox hazard proportional analysis the HR was found to be non-significant (HR'=1.11; 95%CI=0.70-1.74; *p*=0.65) as shown in figure 5.10.

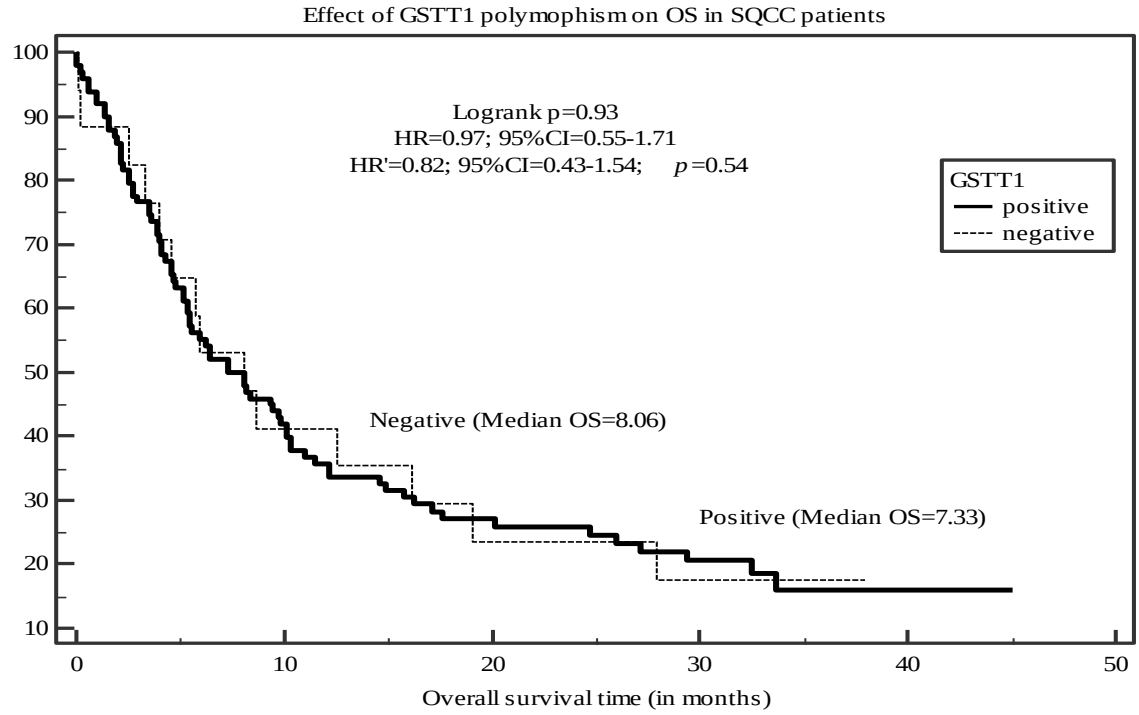


Figure 5.9 Effect of GSTT1 polymorphism on overall survival in SQCC patients.

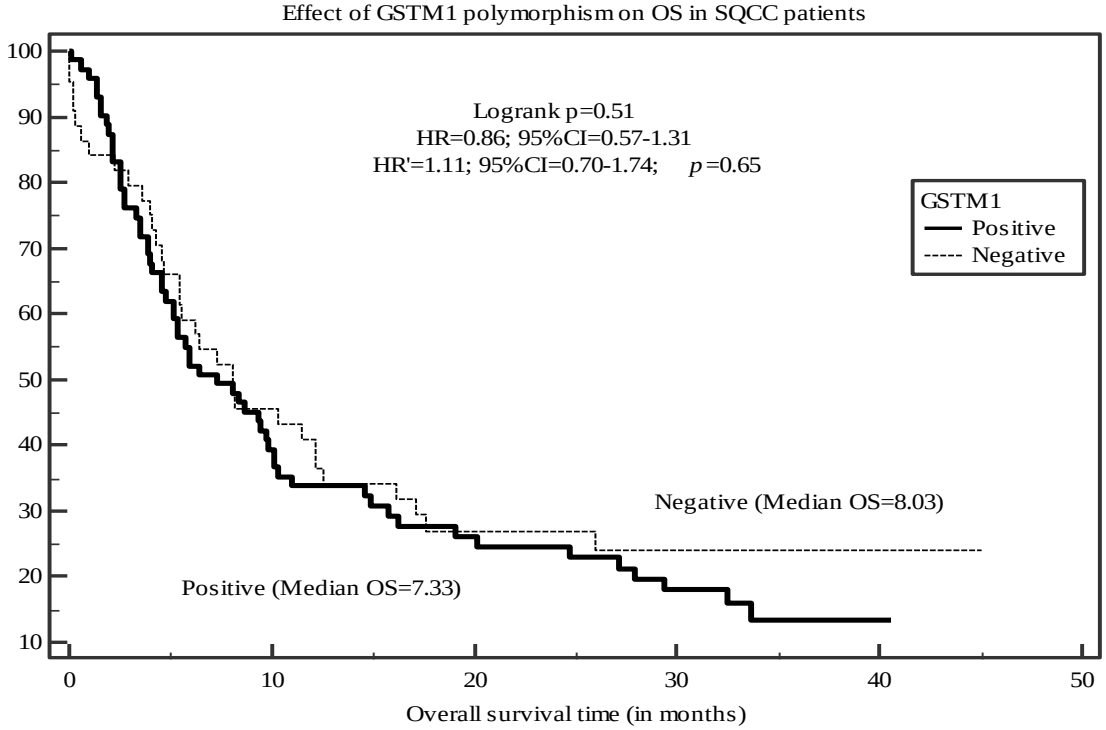


Figure 5.10 Effect of GSTM1 polymorphism on overall survival in SQCC patients.

5.6. Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients on the basis of gender:

Table 5.4.1. Genotypic frequencies of *GSTT1* and *GSTM1* among males and their association with overall survival in lung cancer patients.

Genotype	Males						
GSTT1	Dead (236) n%	Alive (45) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	191 (83.77)	37 (16.23)	9.00	-	-	-	-
Null	45 (84.90)	8 (15.09)	6.76	1.00 (0.72-1.39)	0.95	0.98 (0.69-1.40)	0.94
GSTM1	N=236	N=45					
Present	140 (84.84)	25 (15.16)	9.00	-	-	-	-
Null	96 (82.76)	20 (17.24)	8.20	0.97 (0.75-1.26)	0.85	1.02 (0.77-1.34)	0.88

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

The patients were further classified on the basis of gender and the association with the two genotypes was analyzed. It was found that among 281 males, 83.98% (236) were dead and 16.02% (45) were alive as shown in table 5.4.1. Stratifying on the basis of genotypic distribution it was found that among 228 male patients carrying the *GSTT1* gene, 83.77% were dead and 16.23% were alive and in 53 patients having the *GSTT1* null genotype, 84.90% were dead and 15.09% were alive. On comparing the overall survival period between the two, it was observed that the male patients which carried the *GSTT1* gene, had a larger MST than the patients carrying the null *GSTT1* genotype (9 vs. 6.76 months, HR=1.00; log rank p=0.95). The adjusted HR after the Cox hazard analysis was non-significant (HR'=0.98; 95%CI=0.69-1.40; *p*=0.94).

Further, in 165 male patients carrying the *GSTM1* gene, 84.84% were dead whereas 15.16% were alive and in 116 male patients carrying the null *GSTM1* genotype, 82.76% were dead and 17.24% were alive. The overall survival period was found to be larger in male patients that carried the *GSTM1* gene as compared to the patients carrying the null *GSTM1* genotype (9 vs. 8.20 months, HR=0.97; log rank p=0.85). Here also no

significant value was obtained after applying the Cox hazard analysis (HR'=1.02; 95%CI=0.77-1.34; *p*=0.88).

Table 5.4.2. Genotypic frequencies of *GSTT1* and *GSTM1* among females and their association with overall survival in lung cancer patients.

Genotype	**Females**						
GSTT1	Dead (37) n%	Alive (5) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	32 (86.48)	5 (13.51)	8.03	-	-	-	-
Null	5 (100)	0 (0)	2.86	2.18 (0.60-7.93)	0.09	2.08 (0.69-6.26)	0.19
GSTM1	N=37	N=5					
Present	20 (90.90)	2 (9.09)	6.76	-	-	-	-
Null	17 (85)	3 (15)	6.43	0.90 (0.47-1.72)	0.75	0.87 (0.41-1.85)	0.72

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among the 42 female patients with lung cancer, 88.09% (37) were dead and 11.91% (5) were alive as shown in table 5.4.2. On classifying these patients on the basis of the genotype, it was found that among 37 female patients having the *GSTT1* gene, 86.48% were dead and 13.51% were alive. Also, in 5 female patients carrying the null *GSTT1* genotype, the mortality was 100%. The overall survival period was much higher in female patients carrying the *GSTT1* gene as compared to the patients having the null *GSTT1* genotype (8.03 vs. 2.86 months), however, there was no significant association between *GSTT1* genotype in females and the overall survival after applying multivariate Cox hazard proportional analysis (HR=2.18; 95%CI=0.60-7.93; log rank p=0.09; HR'=2.08; 95%CI=0.69-6.26; *p*=0.19).

Similarly, out of 22 female patients carrying the *GSTM1* gene, 90.90% were dead and 9.09% were alive and among 20 patients having the null *GSTM1* genotype, 85% were dead and 15% were alive. Though the survival period of both, patients carrying the *GSTM1* gene and those having the *GSTM1* null genotype were almost similar (MST=6.76 months and 6.43 months respectively), no significant association was found between *GSTM1* genotype in females and the overall survival after applying the

Cox hazard analysis (HR=0.90; 95%CI=0.47-1.72; log rank p=0.75; HR'=0.87; 95%CI=041-1.85; *p*=0.72).

5.7 Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients on the basis of smoking status:

Table 5.5.1. Genotypic frequencies of *GSTT1* and *GSTM1* among smokers and their association with overall survival in lung cancer patients.

Genotype	**Smokers**						
GSTT1	Dead (230) n%	Alive (40) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	187 (85)	33 (15)	8.03	-	-	-	-
Null	43 (86)	7 (14)	6.33	0.97 (0.70-1.36)	0.89	0.94 (0.65-1.36)	0.76
GSTM1	N=230	N=40					
Present	140 (85.36)	24 (14.64)	8.81	-	-	-	-
Null	90 (84.90)	16 (15.10)	7.13	1.03 (0.79-1.35)	0.77	1.05 (0.80-1.40)	0.68

In this study the smoking status among the lung cancer patients and its association with the overall survival was analysed. It was found that out of 270 patients who smoked, 85.18% (230) were dead and 14.82% (40) were alive as shown in table 5.5.1. On stratifying them on the basis of genotypic distribution of the two genes, it was seen that out of 220 patients who were smokers and carried the *GSTT1* gene, 85% were dead and 15% were alive. The overall survival period of smokers having the *GSTT1* gene was found to be 8.03 months. Similarly among 50 patients having the null *GSTT1* genotype, 86% were dead and 14% were alive. The overall survival period in this case was lower than that of the cases having the wild genotype (6.33 vs. 8.03 months). But, this association was not statistically proved as no significant value was obtained after applying the Cox hazard proportional analysis (HR=0.97; 95%CI=0.70-1.36; log rank p=0.89; HR'=0.94; 95%CI=0.65-1.36; *p*=0.76).

Further, on relating the *GSTM1* genotype with the overall survival in smokers, it was found that out of 164 patients who were smokers and having the *GSTM1* gene, 85.36% were dead and 14.64% were alive and among 106 patients having the null *GSTM1*

genotype, 84.90% were dead and 15.10% were alive. The overall survival in patients carrying the null *GSTM1* genotype was found to be lower than the patients carrying the *GSTM1* gene (7.13 vs. 8.81 months). No relationship was found between *GSTM1* genotype in smokers and the overall survival after applying the multivariate Cox hazard proportional analysis (HR=1.03; 95%CI=0.79-1.35; log rank p=0.77; HR'=1.05; 95%CI=0.80-1.40; *p*=0.68).

Table 5.5.2. Genotypic frequencies of *GSTT1* and *GSTM1* among non- smokers and their association with overall survival in lung cancer patients.

Genotype	**Non-smokers**						
GSTT1	Dead (43) n%	Alive (10) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	36 (80)	9 (20)	9.46	-	-	-	-
Null	7 (87.5)	1 (12.5)	4.96	1.67 (0.62-4.44)	0.20	2.72 (1.09-6.79)	**0.03**
GSTM1	N=43	N=10					
Present	20 (86.95)	3 (13.04)	8.80	-	-	-	-
Null	23 (76.66)	7 (23.33)	9.46	0.77 (0.41-1.42)	0.39	0.89 (0.42-1.92)	0.78

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

On studying the association of genetic polymorphism in non-smokers and the overall survival, it was found that out of 53 patients, 81.13% (43) were dead and 18.87% (10) were alive as shown in table 5.5.2. When classified on the basis of the genotype the patients carried, it was seen that out of 45 non-smoker patients having the *GSTT1* gene, 80% patients were dead and 20% were alive. Further, in 8 patients carrying the null *GSTT1* genotype, 87.5% were dead and 12.5% were alive. The overall survival period of patients having the null *GSTT1* genotype was found to be was almost half as compared to the MST of patients carrying the wild genotype (4.96 vs. 9.46 months, HR=1.67; log rank p=0.20). But when a multivariate Cox hazard proportional analysis was performed, it was seen that a there was statistically significant relationship between the patients carrying the null *GSTT1* genotype and the overall survival period, showing that these patients had a almost 3 fold probability of being at a higher death risk (HR'=2.72; 95%CI=1.09-6.79; ***p*=0.03**) as shown in figure 5.11.

Further, in 23 non-smoker patients having the *GSTM1* gene, 86.95% were dead and 13.04% were alive and also in 30 patients having the null *GSTM1* genotype, 76.66% were dead and 23.33% were alive. The overall survival period in patients carrying the null *GSTM1* genotype was found to be higher than the patients having the *GSTM1* gene (9.46 vs. 8.80 months, HR=0.77; log rank p=0.39). After applying the Cox hazard proportional analysis, the adjusted HR was found to be non-significant (HR'=0.89, 95%CI=0.42-1.92; *p*=0.78) as shown in figure 5.12.

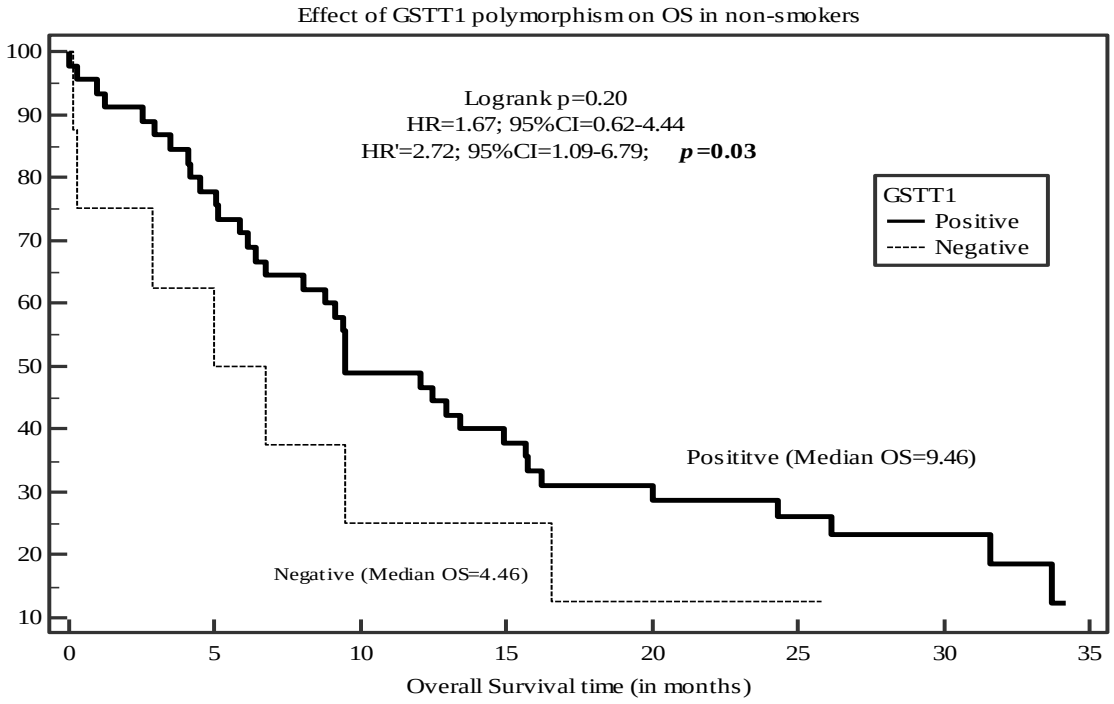


Figure 5.11 Effect of GSTT1 polymorphism on overall survival in non-smokers.

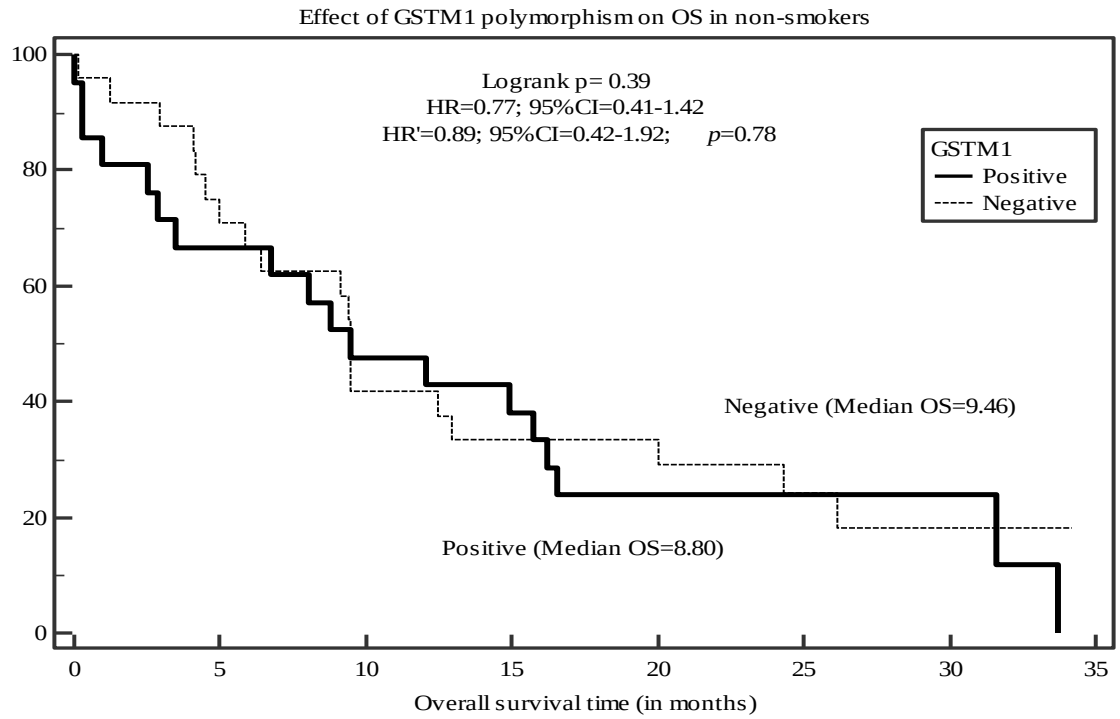


Figure 5.12 Effect of GSTM1 polymorphism on overall survival in non-smokers.

5.8 Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients based on tumor stage:

Table 5.6.1. Genotypic frequencies of *GSTT1* and *GSTM1* among patients in tumor stage III and their association with overall survival in lung cancer patients.

Genotype	**Stage III**						
GSTT1	Dead (128) n%	Alive (23) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	110 (83.33)	22 (16.67)	8.2	-	-	-	-
Null	18 (94.73)	1 (5.27)	5.9	1.41 (0.79-2.48)	0.17	1.59 (0.93-2.71)	0.08
GSTM1	N=128	N=23					
Present	82 (84.53)	15 (15.47)	8.03	-	-	-	-
Null	46 (85.18)	8 (14.82)	6.76	1.59 (0.76-1.59)	0.58	1.15 (0.78-1.67)	0.46

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Further data was stratified on the basis of the tumor III and IV. The analysis showed that out of 151 patients in tumor stage III, 84.77% (128) were dead and 15.23% (23) were alive as shown in table 5.6.1. Based on genotypic distribution of *GSTT1*, it was found that among the 132 patients with stage III tumor and carrying the *GSTT1* gene, 83.33% were dead and 16.67% were alive. The reference survival period was 8.2 months. In 19 cases having the null *GSTT1* genotype, 94.73% were dead and 5.27% were alive. The overall survival period of the patients carrying the null *GSTT1* genotype was found to be lower than the reference. (5.9 vs.8.2 months, HR=1.59; log rank p=0.17). On applying the Cox hazard analysis, no significant association was found between the *GSTT1* genotype and the overall survival of lung cancer patients with stage III tumor (HR'=1.59; 95%CI=0.93-2.71; *p*=0.08).

Among 97 patients carrying the *GSTM1* gene, 84.53% were dead and 15.47% were alive and out of 54 patients having the null *GSTM1* genotype, 85.18% were dead and 14.82% were alive. The overall survival of the patients with stage III tumor carrying the null *GSTM1* was found to be lower as compared to the patients having the *GSTM1* gene (6.76 vs. 8.03 months, HR=1.59; log rank p=0.58). In this case too, no significant association was found between the *GSTM1* genotype and stage III tumor patients (HR'=1.15; 95%CI=0.78-1.67; *p*=0.46).

Table 5.6.2 Genotypic frequencies of *GSTT1* and *GSTM1* among patients with tumor stage IV and their association with overall survival in lung cancer patients.

Genotype	**Stage IV**						
GSTT1	Dead (118) n%	Alive (16) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	89 (88.11)	12 (11.89)	8.03	-	-	-	-
Null	29 (87.87)	4 (12.13)	6.76	0.95 (0.63-1.44)	0.83	1.02 (0.65-1.60)	0.91
GSTM1	N=118	N=16					
Present	66 (88)	9 (12)	8.40	-	-	-	-
Null	52 (88.14)	7 (11.86)	6.76	0.99 (0.69-1.42)	0.96	0.96 (0.65-1.42)	0.85

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

On analysing 134 patients in tumor stage IV, it was found that 88.06% (118) were dead and 11.94% (16) were alive as shown in table 5.6.2. By classifying them on the basis of genotypic distribution of the two genes it was found that out of 101 stage IV tumor patients having the *GSTT1* gene, 88.11% were dead and 11.89% were alive and the MST was 8.03 months whereas out of 33 patients having the null *GSTT1* genotype, 87.87% were dead and 12.13% were alive. The overall survival period in patients carrying the null *GSTT1* genotype was found to be much lower than the reference MST (6.67 vs. 8.03 months, HR=0.95; log rank p=0.83). By analysing the data using Cox hazard analysis, no significant association was found between the *GSTT1* genotype and overall survival in patients with tumor stage IV(HR'=1.02; 95%CI=0.65-1.60; p=0.91).

Similarly, in 75 patients having tumor stage IV and carrying the *GSTM1* gene, 88% were dead and 12% were alive. Also, in 59 patients carrying the null *GSTM1* genotype, 88.14% were dead and 11.86% were alive. The overall survival period of the patients carrying the *GSTM1* gene was larger than the patients carrying the null *GSTM1* genotype (8.40 months vs. 6.76 months, HR=0.99; log rank p=1.42). On applying the multivariate Cox hazard proportional analysis, no significant relationship was found between *GSTM1* and the overall survival of patients with tumor stage IV (HR'=0.96; 95%CI=0.65-1.42; *p*=0.85).

5.9 Association of *GSTT1* and *GSTM1* genotypic distribution with overall survival of lung cancer patients based on their performance status after receiving chemotherapy:

Considering performance status of patients based on Karnofsky performance status scale (KPS) it has been observed that among 126 patients under KPS scale 90-100, 84.92% (107) were dead and 15.08% (19) were alive as shown in table 5.7.1. When further stratified on the basis of genotypic distribution of *GSTT1* and *GSTM1* it was found that out of 103 patients having the *GSTT1* gene, 82.56% were dead and 17.44% were alive whereas among 23 patients having the null *GSTT1* genotype, 73.91% were dead and 26.09% were alive. The overall survival period in the patients carrying the null *GSTT1* genotype was found to be much larger as compared to the survival period of patients with the *GSTT1* gene (14 vs. 9 months, HR=0.64; log rank p=0.09). On

applying the Cox hazard analysis, no significant association was found between *GSTT1* genotype and the overall survival of patients with KPS scale 90-100 (HR'=0.64; 95%CI=0.36-1.12; *p*=0.12) as shown in figure 5.13.

Table 5.7.1. Genotypic frequencies of *GSTT1* and *GSTM1* among patients in KPS scale 90-100 and their association with overall survival in lung cancer patients.

Genotype	**KPS (90-100)**						
GSTT1	Dead (107) n%	Alive (19) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	90 (82.56)	13 (17.44)	9.00	-	-	-	-
Null	17 (73.91)	6 (26.09)	14.00	0.64 (0.41-1.02)	0.09	0.64 (0.36-1.12)	0.12
GSTM1	N=107	N=19					
Present	68 (88.31)	9 (11.69)	9.00	-	-	-	-
Null	39 (79.59)	10 (10.41)	12.00	0.75 (0.51-1.10)	0.15	0.80 (0.52-1.21)	0.30

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Similarly, out of 77 patients carrying the *GSTM1* gene and under the KPS scale of 90-100, 88.31% were dead and 11.69% were alive. Also, in 49 patients having the null *GSTM1* genotype, 79.59% were dead and 10.41% were alive. The overall survival period was larger in patients carrying the null *GSTM1* genotype as compared to the survival period of patients having the *GSTM1* gene (12 vs. 9 months, HR=0.75; log rank p=0.15). In this case also, no significant relationship was found between the *GSTM1* genotype and the overall survival of patients with KPS scale 90-100 after applying the Cox hazard analysis (HR'=0.80; 95%CI=0.52-1.21; *p*=0.30) as shown in figure 5.14.

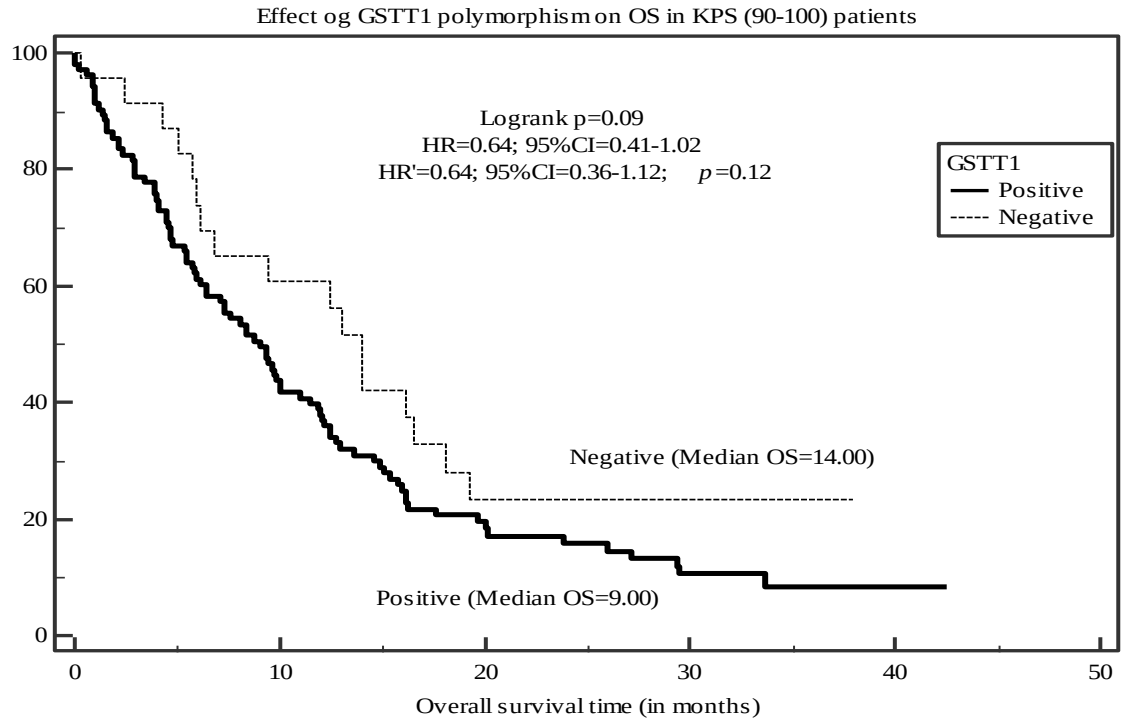


Figure 5.13 Effect of GSTT1 polymorphism on overall survival in patients under KPS scale 90-100.

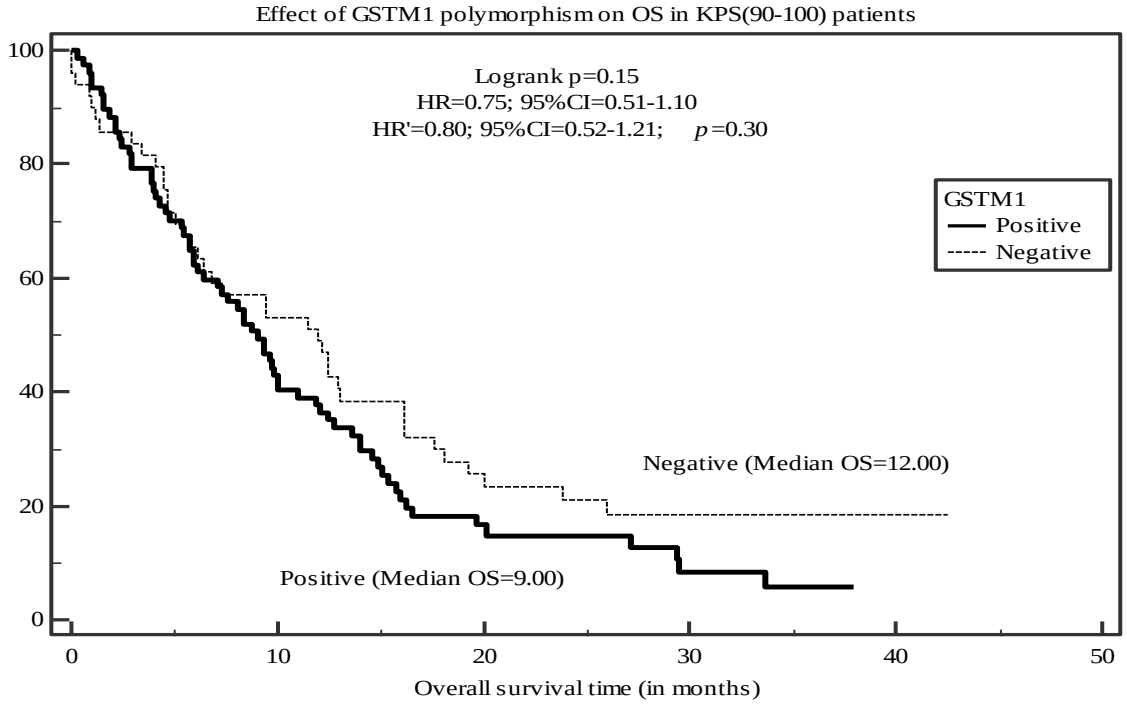


Figure 5.14 Effect of GSTM1 polymorphism on overall survival of patients under KPS range of 90-100.

Table 5.7.2. Genotypic frequencies of *GSTT1* and *GSTM1* among patients in KPS scale 70-80 and their association with overall survival in lung cancer patients.							
Genotype	**KPS (70-80)**						
GSTT1	Dead (125) n%	Alive (20) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	102 (83.60)	20 (16.40)	8.03	-	-	-	-
Null	23 (100)	0 (0)	4.03	2.50 (1.32-4.72)	**<0.0001**	2.34 (1.44-3.82)	**0.0006**
GSTM1	N=125	N=20					
Present	71 (84.52)	13 (15.48)	7.33	-	-	-	-
Null	54 (88.52)	7 (11.48)	5.8	1.18 (0.82-1.69)	0.34	1.22 (0.84-1.77)	0.29
[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage							

On analysing the patients with KPS scale 70-80, it was seen that out of 145 patients, 86.20% (125) were dead and 13.8% (20) were alive as shown in table 5.7.2. Based on genotypic distribution of *GSTT1*, it was found that among the 122 patients under the KPS scale 70-80 and carrying the *GSTT1* gene, 83.60% were dead and 16.40% were alive. The overall survival period was found to be 8.03 months, which is taken as the reference. In 23 patients carrying the *GSTT1* null genotype, the mortality was found to be 100% and the survival period was found to be almost half than the reference (4.03 vs. 8.03 months). In this study, a statistically significant relationship was found between *GSTT1* and overall survival in patients with KPS scale 70-80 (HR=2.50; 95%CI=1.32-4.72; **log rank p=<0.0001;**). After applying multivariate Cox hazard proportional analysis, this relationship was confirmed with a significant adjusted HR value (HR'=2.34; 95%CI=1.44-3.82; ***p*=0.0006**). This showed that the patients with the KPS scale 70-80 and carrying the null *GSTT1* genotype had almost two-fold probability of being at a higher death risk as shown in figure 5.15.

Similarly, among 84 patients having the *GSTM1* gene, 84.52% were dead and 15.48% were alive and among 61 patients having the null *GSTM1* genotype, 88.52% were dead and 11.48% were alive. The MST in patients carrying the null *GSTM1* genotype was

found to be lower than the MST in patients carrying the *GSTM1* gene (5.8 vs. 7.33 months, HR=1.18; log rank p=0.34). No association was observed between *GSTM1* genotype (HR'=1.22; 95%CI=0.84-1.77; *p*=0.29) and overall survival of patients with KPS scale 70-80 as shown in figure 5.16.

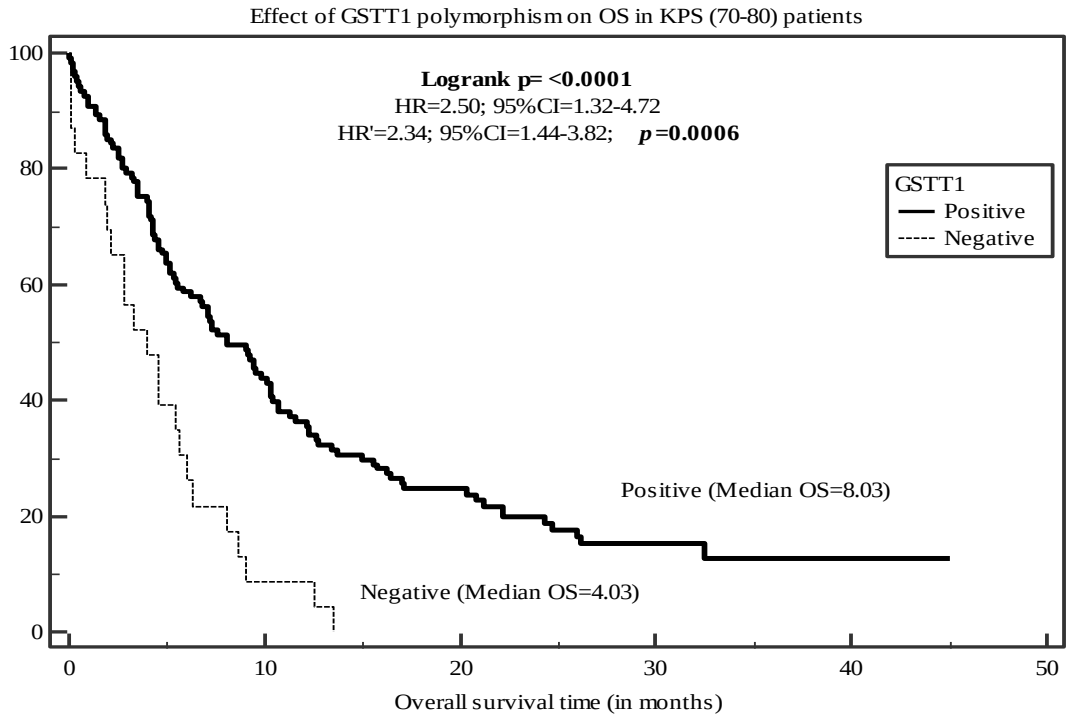


Figure 5.15 Effect of GSTT1 polymorphism on overall survival in patients under KPS range of 70-80

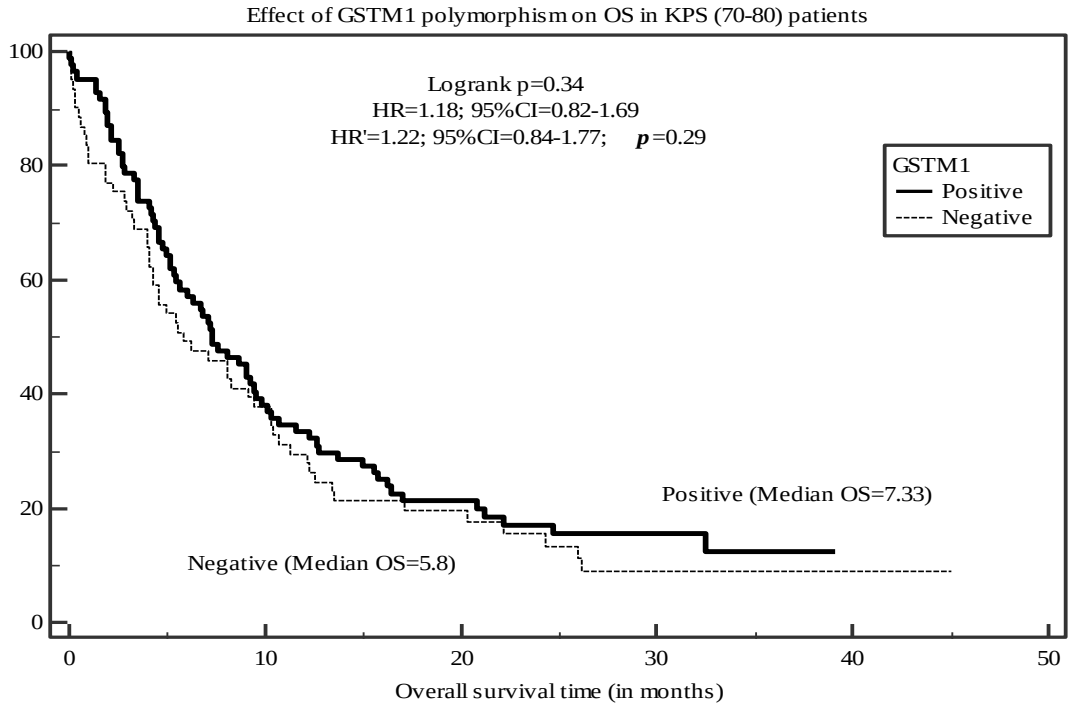


Figure 5.16 Effect of GSTM1 polymorphism on overall survival in patients under KPS range of 70-80

5.10. Association of *GSTT1* and *GSTM1* genetic distribution among cases and their associated death risk based on different treatment regimen.

Table 5.8.1. Genotypic frequencies of *GSTT1* and *GSTM1* among cases treated with doce cis/ doce carb and their association with overall survival in lung cancer patients.							
Genotype	**Doce cis/ doce carb**						
GSTT1	Dead (59) n%	Alive (14) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	51 (82.25)	11 (17.74)	8.11	-	-	-	-
Null	8 (72.72)	3 (27.27)	12.56	0.71 (0.36-1.38)	0.71	1.04 (0.46-2.33)	0.91
GSTM1	N=59	N=14					
Present	36 (87.80)	5 (12.19)	8.63	-	-	-	-
Null	23 (71.87)	9 (28.12)	10.26	0.96	0.89	0.88	0.69

				(0.60-1.55)		(0.49-1.59)	
[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage							

Among 73 patients treated with doce cis/ doce carb, 80.82% (59) patients were dead and 19.17% (14) were alive as shown in table 5.8.1. Based on the genotypic distribution, it was seen that among 62 patients having the *GSTT1* gene, 82.25% were dead and 17.74% were alive whereas in 11 patients carrying the null *GSTT1* genotype, 72.72% were dead and 27.27% were alive. The overall survival period in patients with the null *GSTM1* genotype was found to be larger than in patients with the *GSTM1* gene (12.56 vs. 8.11 months, HR=0.71; log rank p=0.71). However, no association was observed between *GSTT1*genotype on applying the Cox hazard proportional analysis (HR'=1.04; 95%CI=0.46-2.33; *p*=0.91) and the overall survival of patients treated with doce cis/ doce carb.

Similarly in case of 41 patients carrying the *GSTM1* gene, 87.80% were dead and 12.19% were alive whereas in 32 patients carrying the null *GSTM1* genotype, 71.87% were dead and 28.12% were alive. The MST in patients having the null *GSTM1* genotype was found to be larger than the MST in patients carrying the *GSTM1* genotype (10.26 vs. 8.63 months). Here also no association was found between *GSTM1* genotype (HR=0.96; 95%CI=0.60-1.55; log rank p=0.89; HR'=0.88; 95%CI=0.49-1.59; *p*=0.69) and overall survival in patients treated with doce cis/ doce carb.

Table 5.8.2. Genotypic frequencies of *GSTT1* and *GSTM1* among cases treated with Irino cis/ irino carb and their association with overall survival in lung cancer patients.

Genotype	**Irinocis/ irino carb**						
GSTT1	Dead (68) n%	Alive (10) n%	Median OS (months)	HR (95%CI)	Log P	HR' (95%CI)	*P*
Present	60 (86.95)	9 (13.04)	9.86	-	-	-	-
Null	8 (88.88)	1 (11.11)	5.63	1.24 (0.55-2.77)	0.56	1.15 (0.45-2.94)	0.76
GSTM1	N=68	N=10					
Present	39 (84.78)	7 (15.21)	9.86	-	-	-	-
Null	29 (90.62)	3 (9.37)	7.70	1.26 (0.77-2.06)	0.34	1.35 (0.78-2.36)	0.27

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Out of 78 patients treated with irino cis/ irino carb, 87.18% (68) patients were dead and 12.82% (10) were alive as shown in table 5.8.2. Stratified on the basis of genotypic distribution, it was found that in 69 subjects having the *GSTT1* gene, 86.95% were dead and 9.86% were alive whereas in 9 subjects carrying the null *GSTT1* genotype, 88.88% were dead and 11.11% were alive. The overall survival period in patients carrying the null *GSTT1* genotype was found to be smaller than the survival period in patients having the *GSTT1* gene (5.63 vs. 9.86 months, HR=1.24; log rank p=0.56). However, on applying the Cox hazard analysis, no association was observed between *GSTT1* genotype (HR'=1.15; 95%CI=0.45-2.94; *p*=0.76) and the overall survival of patients treated with irino cis/ irino carb.

Similarly in case of 46 patients carrying the *GSTM1* gene, 84.78% were dead and 15.21% were alive whereas in 32 patients carrying the null *GSTM1* genotype, 90.62% were dead and 9.37% were alive. The MST in patients having the null *GSTM1* genotype was found to be smaller than the MST in patients carrying the *GSTM1* gene (7.70 vs. 9.86 months, HR=1.26; log rank p=0.34). Here also no association was found between *GSTM1*genotype and overall survival in patients treated with irino cis/ irino carb on applying the Cox multivariate hazard proportional analysis (HR'=1.35; 95%CI=0.78-2.36; *p*=0.27).

Table 5.8.3 Genotypic frequencies of *GSTT1* and *GSTM1* among cases treated with Pem cis/ pem carb and their association with overall survival in lung cancer patients.

Genotype	**Pem cis/ pem carb**						
GSTT1	Dead (53) n%	Alive (16) n%	Median OS (months)	HR (95% CI)	Log P	HR' (95%CI)	*P*
Present	39 (72.22)	15 (27.77)	10.33	-	-	-	-
Null	14 (93.33)	1 (6.66)	6.76	1.52 (0.76-3.02)	0.17	0.58 (0.77-3.23)	0.20
GSTM1	N=53	N=16					
Present	30 (78.94)	8 (21.05)	9.23	-	-	-	-
Null	23 (74.19)	8 (25.80)	10.66	0.75 (0.44-1.29)	0.30	0.72 (0.38-1.38)	0.33

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Similarly, on analysing the cases treated with pem cis/ pem carb it was observed that out of 69 patients, 76.81% (53) were dead and 23.19% (16) were alive as shown in table 5.8.3. On studying the genotypic effect of *GSTT1*, it was found that in 54 patients having the *GSTT1* gene, 72.22% were dead and 27.77% were alive. In 15 patients carrying the null *GSTT1* genotype, 93.33% were dead and 6.66% were alive. The overall survival period of patients treated with pem cis/ pem carb and carrying the *GSTT1* gene was found to be larger as compared to the patients carrying the null *GSTT1* genotype (10.33 vs. 6.76 months, HR=1.52; lpg rank p=0.17). On applying the Cox analysis, the adjusted hazard ratio was found to be non-significant (HR'=0.58; 95%CI=0.77-3.23; *p*=0.20).

Also, among 38 patients carrying the *GSTM1* gene, 78.94% were dead and 21.05% were alive and in 31 patients carrying the null *GSTM1* genotype, 74.19% were dead and 25.80% were alive. The overall survival period was larger in patients carrying the *GSTM1* null genotype as compared with the patients carrying the *GSTM1* gene (10.66 vs. 9.23 months, HR=0.75; log rank p=0.30). However, no significant association was found between the *GSTM1* genotype and overall survival in lung cancer patients treated with pem cis/ pem carb (HR'=0.72; 95%CI=0.38-1.38; *p*=0.33).

5.11 Association of *GSTT1* and *GSTM1* genotypic distribution with lung cancer susceptibility based on chemotherapy response:

Association of *GSTT1* and *GSTM1* polymorphism with chemotherapy response was summarized in Table 5.9 based on regression analysis. Among 187 patients, the good responders (CR+PR) were found be in higher proportion (56.14%) as compared to the poor responders (SD+PD) which were only 43.85%. It was observed that the patients with complete response (CR) were only 2.67%, the ones with partial response (PR) were 53.47%, 34.22% patients showed stable disease (SD) while 9.62% patients showed progressive disease (PR) after chemotherapeutic treatment. On comparing the two genotypes with the chemotherapeutic response it was observed that among 154 patients carrying the *GSTT1* gene, 56.49% of the patients were good responders and

43.51% were poor responders and in 33 patients carrying the null *GSTT1* genotype, 54.54% were good responders whereas 45.46% were poor responders.

Similarly in 113 patients having the *GSTM1* gene, 54.96% were good responders whereas 45.04% of the patients were poor responders. Also, in 74 patients having the null *GSTM1* genotype, it was seen that 44.59% were good responders and 55.41% were poor responders towards chemotherapy. The relationship between *GSTM1* genotype with lung cancer susceptibility was justified by a significant association stating that the patients carrying the null *GSTM1* genotype proved to be poor responders towards chemotherapy (**AOR=2.00; 95%CI=1.04-3.84; *p*=0.018**). However, no such relationship was found in case of *GSTT1* genotype (AOR=1.00; 95%CI=0.44-2.28; *p*=0.11).

Table 5.9. Relationship of genotypic distribution with clinic-pathological parameter on the basis of response to chemotherapy				
	Response of chemotherapy		**AOR [a] (95% CI)[b]**	***p***
Genotype	**CR+PR (105) n (%)**	**SD+PD (82) n(%)**		
***GSTT1* (positive)**	87 (56.49)	67 (43.51)	-	-
***GSTT1* (negative)**	18 (54.54)	15 (45.46)	1.00 (0.44-2.28)	0.11
	N=105	**N=82**		
***GSTM1*(positive)**	72 (54.96)	41 (45.04)	-	-
***GSTM1*(negative)**	33 (44.59)	41 (55.41)	2.00 (1.04-3.84)	**0.018**
[a] Adjusted Odds ratios, [b] 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender and smoking. Abbreviation: AOR, Adjusted Odds ratio; CI, confidence interval.				

5.12 Association of *GSTT1* and *GSTM1* genotypic distribution with clinic-pathological parameters.

Table 5.10.1. Relationship of different genotypes with the clinical stage and tumor extension.								
Genotype	**Clinical Stage**		**AOR[a] (95% CI) [b]**	***p***	**Primary Tumor Extension**		**AOR[a] (95% CI) [b]**	***p***
	III (151) n (%)	**IV (134) n (%)**			**Tx+T1+T2 (57) n (%)**	**T3+T4 (234) n (%)**		
***GSTT1*$^{+}$**	132 (56.65)	101 (43.35)	-	-	49 (20.58)	189 (79.42)	-	-
***GSTT1*$^{-}$**	19 (36.53)	33 (63.47)	2.64 (1.38-5.04)	**0.003**	8 (15.09)	45 (84.91)	1.38 (0.60-3.17)	0.44
	N=151	**N=134**			**N=57**	**N=234**		
***GSTM1*$^{+}$**	97 (56.39)	75 (43.61)	-	-	33 (19.41)	137 (80.59)	-	-
***GSTM1*$^{-}$**	54 (47.78)	59 (52.22)	1.35 (0.82-2.22)	0.23	24 (19.83)	97 (80.17)	1.08 (0.58-2.01)	0.78
[a] Adjusted Odds ratios, [b] 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender and smoking. Abbreviation: AOR, Adjusted Odds ratio; CI, confidence interval.								

Table 5.10.1 summarized the data for regression analysis showing association of clinic-pathological parameters such as clinical stage and primary tumor extension and risk of lung cancer. While considering histological grade of 291 patients, 19.58% (57) had moderately differentiated tumors (Tx+T1+T2) while 80.41% (234) had fully differentiated tumors (T3+T4). Further stratified on the basis of genotypic distribution of *GSTT1* and *GSTM1*, it was found that among patients having the *GSTT1* gene, 20.58% had moderately differentiated tumors and 79.42% had well differentiated tumors. In 53 patients with the null *GSTT1* genotype, only 15.09% showed moderately differentiated tumors while well differentiated tumors were dominant in 84.91% of the cases.

Similarly in 170 patients carrying the *GSTM1* gene, 19.41% had moderately differentiated tumors while majority of the patients, 80.59% had well differentiated tumors whereas in 121 patients carrying the null *GSTM1* genotype, well differentiated tumours were predominant, occurring in 80.17% of the patients while only 19.83% had moderately differentiated tumors. However, no significant relationship was found between the primary tumor extension and the *GSTT1*genotype (AOR=1.38; 95%CI=0.60-3.17; *p*=0.44) and *GSTM1* genotype (AOR=1.08; 95%CI=0.58-2.01; *p*=0.78).

Based on TNM stage criteria, among 285 lung cancer patients, 52.98% (151) were categorized in stage III and 47.01% (134) in stage IV as shown in table 4.10.1. Based on the genotypic distribution of the two genes, it was seen that among 233 patients having the *GSTT1* gene, 56.65% of the patients were in tumor stage III and 43.35% were in tumor stage IV and out of 52 patients carrying the null *GSTT1* genotype, 36.53% were in tumor stage III and stage IV was predominant in 63.47% patients. From the regression analysis it was found that the cases having the null *GSTT1* genotype had a two-fold increased susceptibility of being in stage IV (**AOR=2.64; 95%CI=1.38-5.04; *p*=0.003**).

Similarly in 172 patients having the *GSTM1* gene, stage III was predominant in 56.39% while 43.61% of patients were in tumor stage IV. Also, in 113 patients having the null *GSTM1* genotype, 52.22% were in tumor stage IV while 47.78% of the subjects were

in stage III. But, no association was found between *GSTM1* genotype and tumor stage (AOR=1.35; 95%CI=0.82-2.22; *p*=0.23).

Table 5.10.2. Relationship of different genotypes with the lymph node invasion and metastasis.

Genotype	Lymph node Invasion		AOR[a] (95% CI) [b]	*p*	Metastasis		AOR[a] (95% CI) [b]	*p*
	N0 (38) n (%)	**N1+N2+N3+N4 (253) n(%)**			**No (161) n (%)**	**Yes (132) n (%)**		
***GSTT1*⁺**	33 (13.86)	205 (86.14)	-	-	138 (57.5)	102 (42.5)	-	-
***GSTT1*⁻**	5 (9.43)	48 (90.57)	1.45 (0.53-3.96)	0.46	23 (43.40)	30 (56.6)	2.12 (1.13-3.98)	**0.019**
	N=38	**N=253**			**N=161**	**N=132**		
***GSTM1*⁺**	15 (8.82)	155 (91.18)	-	-	97 (56.39)	75 (43.61)	-	-
***GSTM1*⁻**	23 (19)	98 (81)	0.40 (0.19-0.84)	**0.014**	64 (52.89)	57 (47.11)	1.05 (0.64-1.73)	0.82

[a] Adjusted Odds ratios, [b] 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender and smoking. Abbreviation: AOR, Adjusted Odds ratio; CI, confidence interval.

Among 293 patients studied for lung cancer metastasis, 54.94% (161) were characterized with absence of metastasis whereas 45.05% (132) showed distant metastasis as shown in table 5.10.2. On the basis of genotypic distribution, it was found that among 240 patients having the *GSTT1* gene, 57.5% of the patients had no metastasis whereas 42.5% showed distant metastasis and in 53 patients having the null *GSTT1* genotype, 43.40% patients showed no metastasis whereas 56.6% showed distant metastasis. This relationship was supported by regression analysis showing that the patients carrying the null *GSTT1* genotype had a two-fold increased susceptibility of having distant metastasis (**AOR=2.12; 95%CI=1.13-3.98; *p*=0.019**).

Similarly in 172 patients carrying the *GSTM1* gene, it was found that 56.39% showed no metastasis while 43.61% showed distant metastasis and in 121 patients having the null *GSTM1* genotype, 52.89% patients had no metastasis while 47.11% showed metastasis. But, no relationship was found between the *GSTM1* genotype and susceptibility to metastasis (AOR=1.05; 95%CI=0.64-1.73; *p*=0.82).

Further, on characterising 291 patients on the basis of lymph node invasion it was found that 13.06% (38) were characterized with the absence of lymph node invasion (N0) while 86.94% (253) showed lymph node invasion (N1+N2+N3+N4). When classified on the basis of genotypic distribution, it was found that among 238 patients having the *GSTT1* gene, 13.86% showed no invasion of the lymph nodes whereas 86.14% showed lymph node invasion. Also, in 53 patients having the null *GSTT1* genotype, 90.57% showed lymph node invasion while only 9.43showed absence of lymph node invasion. However, this relationship could not be justified by regression analysis (AOR=1.45; 95%CI=0.53-3.96; *p*=0.46).

Similarly, among 170 patients carrying the *GSTM1* gene, 91.18% showed invasion of the lymph nodes and only 8.82% had absence of lymph node invasion. Also, in 121 patients carrying the null *GSTM1* genotype, 81% patients showed invasion of the lymph nodes and 19% had no lymph node invasion. This relationship was proved by regression analysis showing that the patients carrying the null *GSTM1* genotype showed a protective effect towards the lymph node invasion in cancer patients. **(AOR=0.40; 95%CI=0.19-0.84; *p*=0.014).**

5.13. Association between combinations of *GSTT1* and *GSTM1* genotypes and the overall survival in lung cancer patients.

Among 323 patients observed for the combined effect of genotypes, 84.52% (273) were dead and 15.47% (50) were alive as shown in table 5.11. Out of 153 patients having the reference genotype, 85.62% were dead and 14.37% were alive. The overall survival period of the subjects carrying both the genes was found to be 9.23 months which was taken as the reference. The MST of patients with *GSTT1* gene and null for *GSTM1* genotype was found to be lower than that of the reference genotype (8.2 vs. 9.23 months, HR=0.95; log rank p=0.73). On applying the Cox hazard analysis, no

significant association was found between this genetic combination and the overall survival in lung cancer patients (HR'=1.02; 95%CI=0.77-1.36; *p*=0.84).

Among 34 patients having the *GSTM1* gene and null for *GSTT1*, 85.29% were dead and 14.70% were alive. The overall survival period of these patients was the least (6 months) among all the genotypic combinations, HR=1.02; log rank p=0.90. When Cox multivariate hazard proportional analysis was applied, no significant association was seen between the above genotypic combination and the overall survival in lung cancer patients (HR'=0.96; 95%CI=0.62-1.47; *p*=0.86).

Table 5.11. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in lung cancer patients.

GENOTYPE	**Overall**						
	DEAD (273) N%	ALIVE (50) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	131 (85.62)	22 (14.37)	9.23	-	-	-	-
***GSTT1*+/*GSTM1*-**	92 (82.14)	20 (17.85)	8.2	0.95 (0.73-1.24)	0.73	1.02 (0.77-1.36)	0.84
***GSTT1*-/*GSTM1*+**	29 (85.29)	5 (14.70)	6.00	1.02 (0.68-1.53)	0.90	0.96 (0.62-1.47)	0.86
***GSTT1*-/*GSTM1*-**	21 (87.5)	3 (12.5)	12.46	1.05 (0.66-1.69)	0.80	1.18 (0.70-1.97)	0.52

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Similarly, out of 24 subjects having both the null genotypes of *GSTT1* and *GSTM1*, 87.5% were dead and 12.5% were alive. The MST of this genotypic combination was found to be the largest as compared to other combinations (12.46 months), however, this combination too had no association with the overall survival in lung cancer patients when studied using Cox multivariate hazard proportional analysis (HR=1.05; 95%CI=0.66-1.69; log rank p=0.80; HR'=1.18; 95%CI=0.70-1.97; *p*=0.52).

5.14 Association between overall survival in lung cancer patients and combination of *GSTT1* and *GSTM1* genotypes based on histological subtypes.

Table 5.12.1. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in ADCC patients.

GENOTYPE	ADCC						
	DEAD (83) N%	ALIVE (18) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
GSTT1+/GSTM1+	33 (84.61)	6 (15.38)	9.00	-	-	-	-
GSTT1+/GSTM1-	29 (76.31)	9 (23.68)	9.46	0.76 (0.46-1.25)	0.27	0.64 (0.36-1.13)	0.13
GSTT1-/GSTM1+	10 (83.33)	2 (16.66)	5.43	1.08 (0.52-2.24)	0.81	1.03 (0.45-2.35)	0.94
GSTT1-/GSTM1-	11 (91.66)	1 (8.33)	6.76	1.06 (0.53-2.13)	0.85	0.85 (0.36-2.01)	0.72

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among 101 patients with adenocarcinoma (ADCC), 82.17% (83) were dead and 17.82% (18) were alive as shown in table 5.12.1. In this study, it was found that in 39 patients having both *GSTT1* and *GSTM1* genes, the overall survival period was found to be 9 months which was taken as the reference. Among 38 patients having the *GSTT1* gene and null *GSTM1* genotype, 76.31% were dead and 23.68% were alive. The overall survival period of the patients with this genetic combination was found to be the largest among all other genetic combinations; that was 9.46 months, HR=0.76; log rank p=0.27. However, on applying Cox hazard analysis, this combination showed no significant association with overall survival in ADCC patients. (HR'=0.64; 95%CI=0.36-1.13; *p*=0.13).

Among 12 patients carrying the *GSTM1* gene and null for *GSTT1* genotype, the overall survival period was found to be the least (5.43 months) among all the genotypic combinations, HR=1.08; log rank p=0.81. On applying the Cox multivariate hazard proportional analysis, it was seen that this combination of *GSTT1* null and *GSTM1* wild

genotype had no significant association with overall survival in ADCC patients (HR'=1.03; 95%CI=0.45-2.35; *p*=0.94).

Similarly, among 12 patients having both the null genotypes of *GSTT1* and *GSTM1*, the overall survival period was found to be 6.76 months, HR=1.06; log rank p=0.85. However, this combination too had no association with the overall survival in ADCC patients, as found by Cox multivariate hazard proportional analysis (HR'=0.85; 95%CI=0.36-2.01; *p*=0.72).

Table 5.12.2. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in SCLC patients.

GENOTYPE	**SCLC**						
	DEAD (94) N%	ALIVE (7) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	47 (88.67)	6 (11.32)	10.66	-	-	-	-
***GSTT1*+/*GSTM1*-**	33 (100)	0 (0)	5.00	1.60 (0.99-2.58)	**0.034**	1.71 (1.03-2.84)	**0.035**
***GSTT1*-/*GSTM1*+**	8 (100)	0 (0)	7.31	1.45 (0.61-3.46)	0.31	1.53 (0.60-3.88)	0.37
***GSTT1*-/*GSTM1*-**	6 (85.71)	1 (14.28)	12.46	1.15 (0.46-2.83)	0.73	1.51 (0.53-4.30)	0.43

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among 101 patients with SCLC, 93.06% (94) were dead and 6.93% (7) were alive as shown in table 5.12.2. Based on the distribution of genotypic combinations, it was seen that among 53 patients carrying both the genes, 88.67 % were dead and 11.32% were alive. As compared to the overall survival period in individuals with presence of both the genes (10.66 months), the patients carrying the null genotypes of *GSTT1* and *GSTM1* showed a larger survival period of 12.46 months followed by the patients carrying only the *GSTM1* gene (MST=7.31 months) and the patients carrying only *GSTT1* genotype (MST=5.00 months).

Among 33 subjects having the *GSTT1*gene and null for *GSTM1* genotype, the mortality rate was 100% and among 8 subjects having *GSTM1* gene and null for *GSTT1*, the

mortality rate was again 100%, HR=1.45; log rank p=0.31. No significant association was found on applying the Cox multivariate hazard proportional analysis (HR'=1.53; 95%CI=0.60-3.88; *p*=0.37) as shown in figure 5.15. In 7 patients having both the null genotypes, 85.71% were dead and 14.28% were alive, HR=1.15; log rank p=0.73. Here also no significant association was found between the SCLC patients and this genetic combination (HR'=1.51; 95%CI=0.53-4.30; *p*=0.43) as shown in figure 5.16.

A significant association was observed in patients having the *GSTT1* gene but null for *GSTM1*. It was found that the individuals carrying such genotypic combination were at the highest death risk as compared to patients with other genotypic combinations, **HR=1.60; 95%CI=0.99-2.58; log rank p=0.034).** This association was further justified by Cox multivariate hazard proportional analysis, which showed a significant adjusted hazard ratio. (**HR'=1.71; 95%CI=1.03-2.84; *p*=0.035)** as shown in figure 5.17.

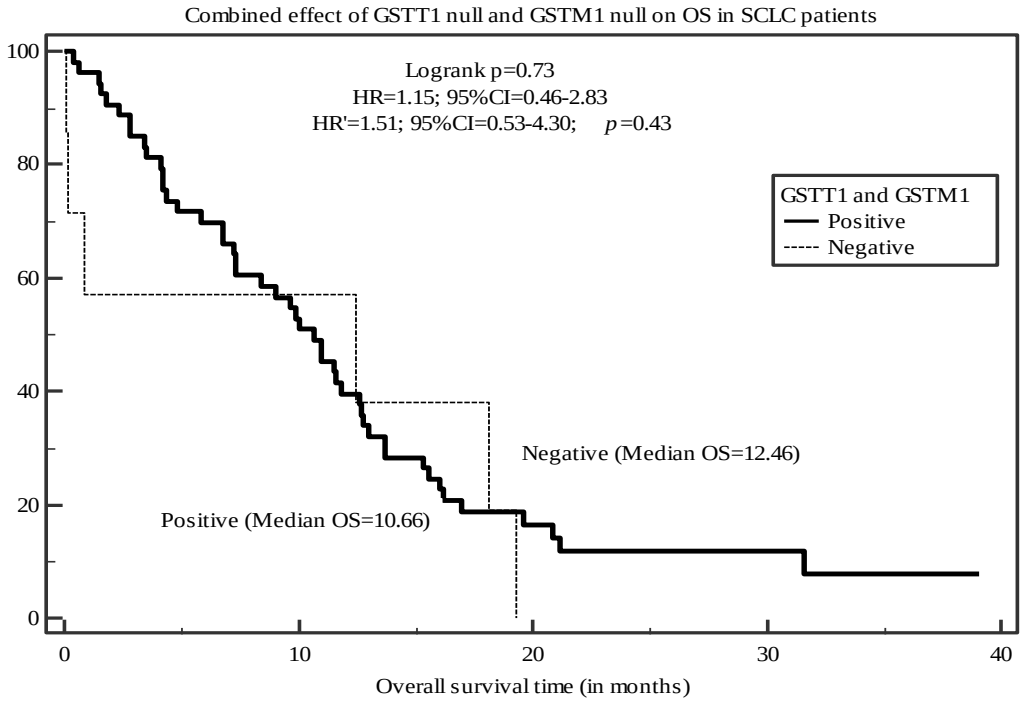


Figure 5.17 Combined effect of GSTT1 null and GSTM1 null genotype on overall survival in SCLC patients

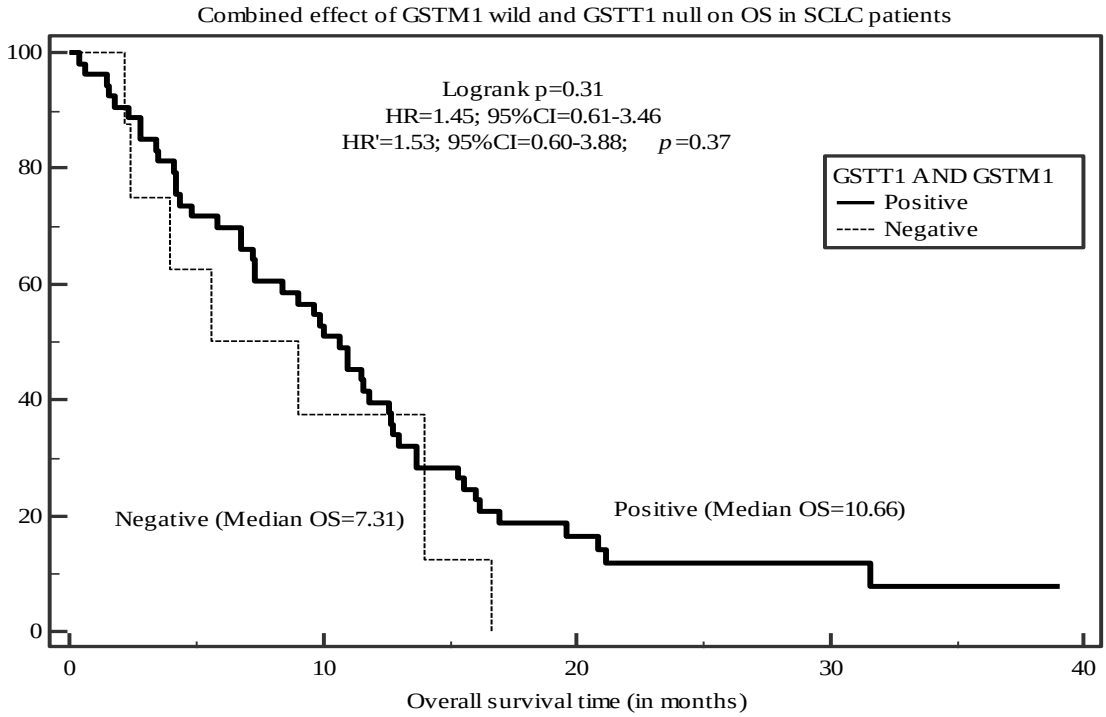


Figure 5.18 Combined effect of GSTM1 wild and GSTT1 null genotype on overall survival in SCLC patients

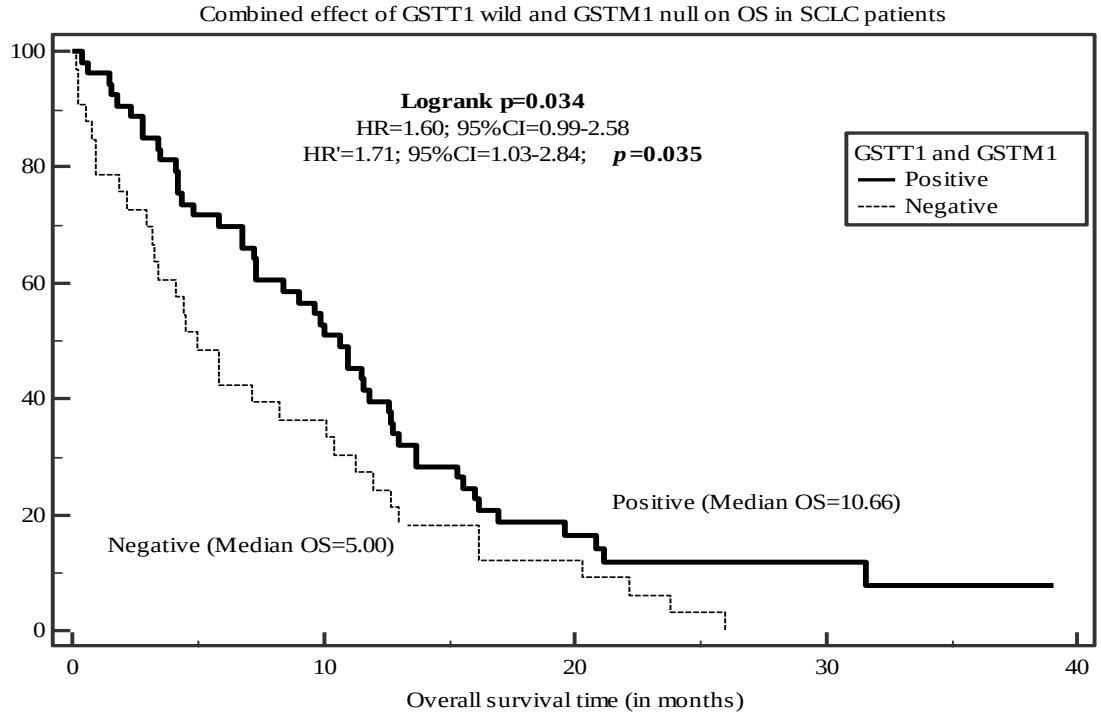


Figure 5.19 Combined effect of GSTT1 wild and GSTM1 null genotype on overall survival in SCLC patients

Of 115 patients with SQCC, 80% (92) were dead and 20% (23) were alive as shown in table 5.12.3. On stratifying them on the basis of genetic combination the patients carry, it was seen that among 58 patients having both the genes, the overall survival period was found 6.4 months, which was the least among all other genetic combinations. Among 40 patients having the *GSTT1* gene and null for *GSTM1* genotype, 72.5% were dead and 27.5% were alive. The overall survival period of such individuals was 8.03 months, HR=0.78; log rank p=0.29. On applying the Cox hazard proportional analysis, the adjusted HR was found to be non-significant (HR'=0.94; 95%CI=0.58-1.53; *p*=82).

Table 5.12.3. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in SQCC patients.

GENOTYPE	**SQCC**						
	DEAD (92) N%	ALIVE (23) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	49 (84.48)	9 (15.51)	6.4	-	-	-	-
***GSTT1*+/*GSTM1*-**	29 (72.5)	11 (27.5)	8.03	0.78 (0.50-1.27)	0.29	0.94 (0.58-1.53)	0.82
***GSTT1*-/*GSTM1*+**	10 (76.92)	3 (23.07)	8.06	0.77 (0.41-1.44)	0.45	0.82 (0.40-1.69)	0.60
***GSTT1*-/*GSTM1*-**	4 (100)	0 (0)	8.3	1.40 (0.43-4.57)	0.50	2.08 (0.66-6.51)	0.20

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among 13 patients having the *GSTM1* gene and null for *GSTT1* genotype, 76.92% were dead and 23.07% were alive. The MST in this case was 8.06 months, HR=0.77; log rank p=0.45 and showed no significant association with overall survival in SQCC patients when analysed by using Cox hazard analysis (HR'=0.82; 95%CI=0.40-1.69; *p*=0.60).

Similarly, out of 4 subjects having both the null genotypes of *GSTT1* and *GSTM1*, the mortality rate was 100%. The overall survival period of such individuals was found to be to be 8.3 months, HR=1.40; log rank p=0.50. This genetic combination also did not show any significant association with the SQCC patients when studied by Cox hazard analysis (HR'=2.08; 95%CI=0.66-6.51; *p*=0.20).

5.15 Association between overall survival in lung cancer patients and combinations of *GSTT1* and *GSTM1* genotypes according to gender.

Among 281 male patients examined for combined effects of *GSTT1* and *GSTM1* genotypes, 83.98% (236) were dead and 16.01% (45) were alive. When stratified on the basis of combinations of the genes, it was found that among 134 male patients carrying both the genes, 85.07% were dead and 14.92% were alive as shown in table 5.13.1. The overall survival period of these patients was found to be 9.33 months and was taken as a reference to compare the MST of patients with other genotypic combinations. Among 94 patients having the *GSTT1*gene and null *GSTM1* genotype, the MST was 8.03 months, HR=0.96; log rank p=0.83. On applying Cox multivariate hazard proportional analysis, no significant association was seen between this genotypic combination and the overall survival in male patients (HR'=1.03; 95%CI=0.75-1.41; *p*=0.82).

Out of 31 patients having the *GSTM1* gene and null *GSTT1* genotype, 83.87% were dead and 16.12% were alive. Through this study, it was observed that the overall survival in male patients carrying the *GSTM1* gene and null *GSTT1* genotype was the least (6.00 months) as compared to the other genotypic combinations, HR=0.98; log rank p=0.94. When analysed on the basis of Cox multivariate hazard proportional analysis, this genotypic combination showed no significant association with overall survival in male patients (HR'=0.96; 95%CI=0.61-1.51; *p*=0.88).

Similarly, out of 22 patients carrying both the null genotypes, 86.36% were dead and 13.63% were alive. The MST of male patients carrying this genotypic combination was found to be the largest (12.46 months) among all other combinations studies, HR=1.00; log rank p=0.97. On applying the Cox hazard proportional analysis, it was seen that this combination too had no association with the overall survival in male patients (HR'=1.06; 95%CI=0.61-1.82; *p*=0.82).

Table 5.13.1. Distribution of genetic combinations of *GSTT1* and *GSTM1* among males and their association with overall survival in lung cancer patients.

GENOTYPE	**Males**						
	DEAD (236) N%	ALIVE (45) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
GSTT1+/GSTM1+	114 (85.07)	20 (14.92)	9.33	-	-	-	-
GSTT1+/GSTM1-	77 (81.91)	17 (18.08)	8.03	0.96 (0.72-1.29)	0.83	1.03 (0.75-1.41)	0.82
GSTT1-/GSTM1+	26 (83.87)	5 (16.12)	6.00	0.98 (0.64-1.50)	0.94	0.96 (0.61-1.51)	0.88
GSTT1-/GSTM1-	19 (86.36)	3 (13.63)	12.46	1.00 (0.61-1.63)	0.97	1.06 (0.61-1.82)	0.82

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among the 42 female patients examined for this study, 88.09% (37) were dead and 11.91% (5) were alive as shown in table 5.13.2. On the basis of genotypic distribution, it was found that in 19 subjects having both the genes, the overall survival of female patients having both the genes was found to be 8.03 months and it was taken as a reference to compare the other MST of female patients carrying other genotypic combinations.

Among 18 patients having the *GSTT1* gene and null *GSTM1* genotype, the overall survival of such patients was 6.43 months, HR=0.92; log rank p=0.81. No significant association was found between this genotypic combination and the overall survival in female patients, when studied by using the Cox multivariate hazard proportional analysis (HR'=0.79; 95%CI=0.35-1.80; *p*=0.58).

Among 3 patients having the *GSTM1* gene and null wild *GSTT1* genotype, the mortality rate was found to be 100%. The overall survival period of such individuals was found

to be the least (2.86 months) as compared to the MST of patients carrying other genotypic combinations, HR=1.83; log rank p=0.31. On applying the Cox hazard analysis, no significant value was obtained (HR'=1.86; 95%CI=0.39-8.75; *p*=0.43).

Similarly, out of 2 patients having both the null genotypes, the mortality rate was 100%. The MST of patients carrying both null genotypes was found to be 4.76 months, HR=2.17; log rank p=0.27. On applying Cox multivariate hazard proportional analysis, the adjusted hazard ratio was found to be non-significant (HR'=3.91; 95%CI=0.57-26.51; *p*=0.16).

Table 5.13.2. Distribution of genetic combinations of *GSTT1* and *GSTM1* among females and their association with overall survival in lung cancer patients.

GENOTYPE	**Females**						
	DEAD (37) N%	ALIVE (5) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	17 (89.47)	2 (10.52)	8.03	-	-	-	-
***GSTT1*+/*GSTM1*-**	15 (83.33)	3 (16.66)	6.43	0.92 (0.46-1.84)	0.81	0.79 (0.35-1.80)	0.58
***GSTT1*-/*GSTM1*+**	3 (100)	0 (0)	2.86	1.83 (0.38-8.62)	0.31	1.86 (0.39-8.75)	0.43
***GSTT1*-/*GSTM1*-**	2 (100)	0 (0)	4.76	2.17 (0.28-16.68)	0.27	3.91 (0.57-26.51)	0.16

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

5.16 Association between overall survival in lung cancer patients and combinations of *GSTT1* and *GSTM1* genotypes according to smoking status.

Among 270 smokers, 85.15% (230) were dead and 14.81% (40) were alive as shown in table 5.14.1. Classifying on the basis of combined effect of *GSTT1* and *GSTM1* genotypes, it was observed that the patients carrying both the null genotypes had the largest survival period of 12.56 months as compared to the patients having both the genes (MST=9.23 months). Also, the patients with presence of only *GSTT1* gene had an overall survival period of 6.76 months and those having only *GSTM1* gene showed the lowest survival period of 6 months.

Also, among 133 patients having both the genes, 85.60% were dead and14.39% were alive and among 87 patients having the *GSTT1* gene and null *GSTM1* genotype, 83.90% were dead and 16.09% were alive, HR=1.03; log rank p=0.80. On applying the Cox multivariate hazard proportional analysis, it was seen that the patients with this genotypic combination had no significant association with the overall survival in smokers (HR'=1.07; 95%CI=0.78-1.46; *p*=0.66).

Table 5.14.1. Distribution of genetic combinations of *GSTT1* and *GSTM1* among smokers and their association with overall survival in lung cancer patients.

GENOTYPE	**Smokers**						
	DEAD (230) N%	ALIVE (40) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
GSTT1+/GSTM1+	114 (85.60)	19 (14.39)	9.23	-	-	-	-
GSTT1+/GSTM1-	73 (83.90)	14 (16.09)	6.76	1.03 (0.77-1.39)	0.80	1.07 (0.78-1.46)	0.66
GSTT1-/GSTM1+	26 (83.87)	5 (16.12)	6.00	0.96 (0.63-1.47)	0.87	0.91 (0.58-1.42)	0.68
GSTT1-/GSTM1-	17 (89.47)	2 (10.52)	12.56	1.03 (0.61-1.73)	0.89	1.08 (0.61-1.92)	0.77

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

In 31 patients with *GSTM1* gene and null *GSTT1* genotype, 83.87% were dead and 16.12% were alive, HR=0.96; log rank p=0.87. In this case too, the Cox hazard

proportional analysis showed a non-significant adjusted HR value (HR'=0.91; 95%CI=0.58-1.42; *p*=0.68). Similarly among 19 patients having both the null genotypes, HR=1.03; log rank p=0.89. On applying the Cox hazard analysis, no statistically significant association was found between this genetic combination and the overall survival in smokers (HR'=1.08; 95%CI=0.61-1.92; *p*=0.77).

Table 5.14.2. Distribution of genetic combinations of *GSTT1* and *GSTM1* among non-smokers and their association with overall survival in lung cancer patients.

GENOTYPE	**Non-smokers**						
	DEAD (43) N%	ALIVE (10) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
GSTT1+/GSTM1+	17 (85)	3 (25)	8.8	-	-	-	-
GSTT1+/GSTM1-	19 (76)	6 (24)	12.46	0.78 (0.40-1.51)	0.45	0.84 (0.35-1.99)	0.70
GSTT1-/GSTM1+	3 (100)	0 (0)	2.86	1.69 (0.37-7.62)	0.38	0.90 (0.16-4.92)	0.90
GSTT1-/GSTM1-	4 (80)	1 (20)	6.67	1.29 (0.36-4.2)	0.63	2.94 (0.63-13.06)	0.16

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

In case of 53 patients who were non-smokers, 81.13% (43) were dead and 18.86% (10) were alive as shown in table 5.14.2. As compared to the overall survival in patients having both the genes (MST=8.8 months), the largest survival period (MST=12.46 months) was found associated with patients having *GSTT1* gene and null *GSTM1* genotype while the shortest was found in patients having the *GSTM1* gene and null *GSTT1* genotype (8.8 vs. 12.46 vs. 2.86 months).

In 20 non-smoker patients carrying both the genes, 85% were dead and 25% were alive. Among 25 subjects having *GSTT1* gene and null *GSTM1* genotype, 76% were dead and 24% were alive, HR=0.78; log rank p=0.45. On applying the Cox multivariate hazard proportional analysis, the adjusted HR value was found to be non-significant (HR'=0.84; 95%CI=0.35-1.99; p=0.70).

In 3 patients having the *GSTM1* gene and null *GSTT1* genotype, the mortality rate was 100%, HR=1.69; log rank p=0.38. No significant association was seen between this genotypic combination and the overall survival in non-smokers (HR'=0.90; 95%CI=0.16-4.92; *p*=0.90). Among 5 subjects carrying both the null genotypes, 80%

were dead and 20% was alive, HR=1.29; log rank p=0.63. On applying Cox multivariate hazard proportional analysis, the adjusted HR value was non-significant showing that no statistically significant association was found between this genotypic combination and overall survival in non-smokers (HR'=2.94; 95%CI=0.63-13.06; *p*=0.16).

5.17. Association between overall survival in lung cancer patients and combinations of *GSTT1* and *GSTM1* genotypes based on tumor stage.

Among 151 patients in stage III of lung cancer, 84.77% (128) were dead and 15.23% (23) were alive as shown in table 5.15.1. On stratifying them the basis of combined effects of both the genotypes, it was found that among 84 patients having both the genes, 83.33% were dead and 16.66% were alive. Among 48 patients carrying the *GSTT1* gene and *GSTM1* null genotype, 83.33% were dead and 16.66% were alive. The overall survival period of stage III patients carrying *GSTT1* gene and null *GSTM1* was found to be 5.83 months, HR=1.14; log rank p=0.48. On applying Cox multivariate hazard proportional analysis, no significant association was found between this genotypic combination and the overall survival in Stage III patients (HR'=1.15; 95% CI=0.76-1.72; *p*=0.49).

Among 13 patients having the *GSTM1* gene and null *GSTT1* genotype, 92.30% were dead and 7.69% were alive. The MST in patients with this genotypic combination was found to be the lowest (5.43 months) as compared to the MST of patients with other studied genotypic combinations, HR=1.60; log rank p=0.12. This association was not statistically significant was studied by applying the Cox hazard proportional analysis (HR'=1.58; 95% CI=0.82-3.05; *p*=0.16).

In 6 patients carrying both the null genotypes of *GSTT1* and *GSTM1*, it was observed that the mortality rate was 100% and the overall survival period was largest (MST= 11.01 months) as compared to the MST 9.36 months of patients carrying both the genes (11.01 vs. 9.36 months), HR=1.35; log rank p=0.47. Also, on applying the Cox multivariate hazard proportional analysis, a statistically significant relationship was found stating that the patients carrying both the null genotypes of *GSTT1* and *GSTM1* had almost three-fold increased probability of death risk as compared to patients

carrying other genetic combinations of these two genes (**HR'=3.67; 95% CI=1.20-11.18; *p*=0.022**).

Table 5.15.1. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in stage III patients.

GENOTYPE	Stage III						
	DEAD (128) N%	ALIVE (23) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	70 (83.33)	14 (16.66)	9.36	-	-	-	-
***GSTT1*+/*GSTM1*-**	40 (83.33)	8 (16.66)	5.83	1.14 (0.77-1.70)	0.48	1.15 (0.76-1.72)	0.49
***GSTT1*-/*GSTM1*+**	12 (92.30)	1 (7.69)	5.43	1.60 (0.77-3.34)	0.12	1.58 (0.82-3.05)	0.16
***GSTT1*-/*GSTM1*-**	6 (100)	0 (0)	11.01	1.35 (0.52-3.50)	0.47	3.67 (1.20-11.18)	**0.022**

[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage

Among 134 patients in stage IV of lung cancer, 88.05% (118) were dead and 11.94% (16) were alive as shown in table 5.15.2. On the basis of the genotypic distribution, it was seen that the patients having both the genes had an overall survival period of 8.4 months, which was taken as the reference for comparing the overall survival of patients with other genotypic combinations of *GSTT1* and *GSTM1*. Further, the overall survival period followed a descending order of 8.06, 7.26 and 6.76 months among the patients having *GSTT1* null, *GSTM1* null and both the null genotypes respectively. On applying the Cox multivariate hazard proportional analysis, the adjusted HR value was found to be non-significant (HR'=0.92; 95% CI=0.58-1.44; *p*=0.71).

Also, in 19 subjects having the *GSTM1* gene and null *GSTT1* genotype, 84.21% were dead and 15.78% were alive, HR=0.82; log rank p=0.50. On applying the Cox hazard analysis, a non-significant value was found for the adjusted death ratio (HR'=0.80; 95% CI=0.43-1.48; *p*=0.48). Further, In 14 patients carrying both the null genotypes, 92.85% were dead and 7.14% were alive, HR=1.02; log rank p=0.93. On applying the Cox multivariate hazard proportional analysis, the adjusted HR was found to be non-significant (HR'=0.84; 95% CI=0.41-1.69; *p*=0.63).

Table 5.15.2. Distribution of genetic combinations of *GSTT1* and *GSTM1* among cases and their association with overall survival in stage IV patients.							
GENOTYPE	**Stage IV**						
	DEAD (118) N%	ALIVE (16) N%	Median OS (months)	HR (95% CI)	LOGP	HR' (95% CI)	P
***GSTT1*+/*GSTM1*+**	50 (89.28)	6 (10.71)	8.4	-	-	-	-
***GSTT1*+/*GSTM1*-**	39 (86.66)	6 (13.33)	7.26	0.92 (0.61-1.41)	0.73	0.92 (0.58-1.44)	0.71
***GSTT1*-/*GSTM1*+**	16 (84.21)	3 (15.78)	8.06	0.82 (0.48-1.41)	0.50	0.80 (0.43-1.48)	0.48
***GSTT1*-/*GSTM1*-**	13 (92.85)	1 (7.14)	6.76	1.02 (0.55-1.89)	0.93	0.84 (0.41-1.69)	0.63
[1] Adjusted Hazard ratios, 95% confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, KPS and Tumor stage							

Chapter 6

Discussion

A new approach for assessment of risk factors contributing to human cancer is by characterising the genes involved in carcinogen metabolism [108]. The genes are also studied for their involvement with the prognosis and treatment outcome of a given therapy against a particular type of cancer [109]. In this study, the focus has been on such metabolic genes which are involved in the detection of lung cancer and also play a vital role in determining the patients' response to chemotherapy, which is the most favourable treatment for severe cases of lung cancer. This study has been carried out in the North Indian population to find the effect of *GSTT1* and *GSTM1* gene polymorphisms, individually and in combinations, on the overall survival of lung cancer patients based on their histological subtypes, smoking status, gender, clinic-pathological parameters and also the patients; response to platinum-based chemotherapy.

Some epidemiological studies have shown the role of genetic variation in GST enzymes in determining the survival in lung cancer patients after its diagnosis [110]. Two hypotheses have been postulated to justify the effect of GST polymorphisms on survival in lung cancer patients [111].

1. Many chemotherapeutic agents and also the by-products of reactive oxygen damage act as potential substrates for glutathione conjugation, catalyzed by GST enzymes. Thus, depending on the individual's GST activity, he or she may differ in their response towards chemotherapy.
2. In tumor tissue, some GST genotypes are found to be related with certain patterns of somatic changes. The patients with low GST activity are therefore considered more susceptible to somatic changes; which might represent more aggressive tumors. Hence, it could be considered that GSTs are associated with individual survival differences. [112]

Considering these factors, this study aimed at analysing the association of overall survival in lung cancer subjects with the two genetic variants of *GST* gene, firstly on the basis of their individual effect and also by various combinations and hence

evaluating their role according to patient's smoking status, gender, histological subtype, performance status and also an individual's response to chemotherapeutic treatment.

Very limited evidences are present stating the role of individuals' genetic differences in GST enzymes in determining their survival after lung cancer diagnosis [112]. But, some plausible mechanisms are given through which it can be inferred that a patient's inherent genetic makeup might affect his or her overall survival [113]. In a study conducted by Goto *et al*., it was reported that *GSTM1* gene, whose catalytic enzyme activity detoxifies the activated metabolites of benzo (a) pyrene, acts as a predisposing factor to lung cancer, mainly associated with smoking. Therefore, it may be associated with overall survival period in patients with NSCLC. The study concluded by stating that the individuals with normal *GSTM1* activity (having *GSTM1* wild genotype) had a larger survival as compared to subjects in which *GSTM1* gene was absent (*GSTM1* null genotype) [114]. In another study, which took breast cancer patients as the subjects, it was reported that, *GSTM1* null genotype was significantly related with the overall survival; however, it did not take into account the other factors, such as stage of the disease, performance status and treatment modalities [115].

Also, some other studies have shown contradictory results. Based on a study by Yang *et al*., it was found that there was no overall difference in survival rates of lung cancer patients based on *GSTM1* genotype [116].Similar results were found in the study conducted by Przygodzki *et al*., which stated that there was no role of *GSTM1* genotype in determining the overall survival in lung cancer patients [117]. The results of this study were in support of these later findings, that is, no significant association was found between the *GSTM1* genotype and the overall survival.

Similarly for *GSTT1* genotype, no significant association was found relating the gene and the overall survival in lung cancer patients. This result is in sync with the findings by Yang *et al*., which showed no overall relationship between the survival rate in lung cancer patients and *GSTT1* genotype [116] and also by Sweeney *et al*., who's study on genetic variants of GST and their association with lung cancer survival, showed that *GSTT1* genotype was unrelated to overall survival [112]. Similar results were found in a study conducted on metastatic colorectal cancer which showed that the deletions in *GSTT1* and *GSTM1* were not related to that survival in cancer patients [118].

It has been evident by numerous scientific findings that tobacco smoke is the leading cause of lung cancer owing to the various types of carcinogens present in it [119]. The inbuilt detoxification system in the body, comprising of phase I and phase II enzymes catalyze the metabolic reactions which detoxify the carcinogens and other harmful chemicals [120]. *GSTT1* and *GSTM1* genes are characterized by well defined polymorphisms. Thus, it might be possible that the metabolic activity of these enzymes is related to the detoxification of smoking-related carcinogens and in turn affect the survival of lung cancer developing due to smoking status of the patients [121]. In a study by Yang *et al*., several Cox models for glutathione-related makers were evaluated in non-smokers, including *GSTT1* and *GSTM1*, but no significant association was found between the two genotypic polymorphisms and the overall survival in lung cancer patients [116]. In the present study, no significant relationship was seen between *GSTM1* genotype and survival rates in smokers as well as non-smokers. But, on applying the Cox multivariate hazard proportional analysis, a statistically significant association was observed between the *GSTT1* null genotype and overall survival in case of non-smokers (HR'=2.72; 95%CI=1.09-6.79**; p=0.03**).

GSTT1 is involved in the metabolism of PAHs, which is mainly found in tobacco smoke and also of pesticides, industrial solvents and other chemicals. In a study conducted by Raimondi *et al*., the role of *GSTT1* was evaluated in lung cancer among never-smokers and no relationship was seen between the *GSTT1* null genotype and lung cancer risk [121]. Also, in a meta and pooled analysis of *GSTT1* and lung cancer, it was stated that there was no significant interaction between smoking and *GSTT1* gene on lung cancer but, *GSTT1* was found to modulate occupation-related lung cancer [122].

In another meta-analytical analysis of 37 studies by Hackshaw *et al*., the role of passive exposure to smoke was confirmed in development of lung cancer. The author estimated that the non-smokers, whose spouses smoked, were more prone to lung carcinogenesis [123]. According to such findings, it could be concluded that the non-smokers in this study carrying the *GSTT1* null genotype, might be exposed to certain occupational hazards or to ETS (Environmental tobacco smoke). Also, it has been illustrated that upon low-dose exposure to carcinogens, the genetic characteristics of the host play a key role in development of lung cancer [124]. This is in support of the hypothesis that the low activity of *GSTT1* to metabolise tobacco carcinogens contributes to lung cancer susceptibility [125]. However, no such association was reported with the survival rates.

In this study, it was found that the overall survival in lung cancer patients, who were non-smokers and carrying the *GSTT1* null genotype, was very low as compared to the patients who were carrying the gene. It was statistically proved by Cox multivariate hazard analysis, in which it was seen that subjects with null *GSTT1* genotype had a 2.7 folds elevated death risk as compared to the subjects having the wild *GSTT1* genotype (HR'=2.72; 95%CI=1.09-6.79; **p=0.03**).

When similar association was analyzed between the effects of genetic polymorphism of *GSTT1* and *GSTM1* with Karnofsky Performance Status (KPS), it was found that *GSTT1* null genotype was associated with the performance status of patients under the scale of 70-80, showing about 2.3 folds increased death risk as compared to the patients with wild *GSTT1* genotype (HR'=2.34; 95%CI=1.44-3.82; **p=0.0006)** but no such statistically significant association was found with *GSTM1*. But, in some previous studies, by Goto *et al*., based on the performance status, it was reported that *GSTM1* polymorphism was significantly associated with the KPS [114].

In this study, various clinic-pathological parameters, such as clinical stage, tumor extension, lymph node invasion and metastasis, were analyzed in association with the two genotypes and it was found that *GSTT1* null genotype was associated with the clinical stage and also with the metastasis of lung cancer. The study showed that the patients in stage IV, with null *GSTT1* genotype had a 2.6 folds higher susceptibility to lung cancer as compared to the patients in clinical stage III (AOR=2.64; 95%CI=1.38-5.04**; p=0.003)**. However, the analysis showed no such association with *GSTM1* genotype.

Similarly, it was seen that the patients with null *GSTT1* genotype showed a 2-fold increased metastasis of lung cancer (AOR=2.12; 95%CI=1.13-3.98; **p=0.019)**. Thus, it was concluded that *GSTT1* genotype is significantly associated with the clinical stage and metastasis in lung cancer patients. While analysing the relationship between the genetic variants of GSTs with the lymph node invasion in lung cancer patients, it was found that *GSTM1* was significantly associated with the lymph node invasion. The present study showed that patients having the *GSTM1* null genotype were less susceptible to lymph node invasion as compared to those carrying the wild *GSTM1* genotype **(AOR=0.40; 95%CI=0.19-0.84; p=0.014)**. This was in accordance with a

study conducted by Goto *et al*., which showed that *GSTM1* polymorphism was associated with lymph node invasion [114].

Although no statistically significant associations were found between the separate *GSTM1* and *GSTT1* polymorphisms and overall SCLC risk, but the combination of the *GSTM1* null genotype and *GSTT1* wild genotype was significantly related to SCLC with the death risk being elevated 1.71 times as compared to the combined *GSTT1* and *GSTM1* wild genotype.

In a study conducted by Sobti, R.C *et al*., 2008, the genetic combinations of *GSTT1* and *GSTM1* were evaluated to associate them with lung cancer, and it was found that the different genotypic combinations were not much related to the development of lung cancer in North Indian population [126]. However, in this study, different combinations of *GSTT1* and *GSTM1* were examined by taking the reference group as the patients carrying both *GSTT1* and *GSTM1* genes. The results showed that the patients with the genotypic combination of wild *GSTT1* and null *GSTM1* genotype had a higher death risk due to lung cancer as compared to the reference group (HR'=1.71; 95%CI=1.03-2.84; **p=0.035)**. This result was found in patients with small cell lung cancer (SCLC).

Also in cases with both null genotypes of *GSTT1* and *GSTM1*, it was found that the death risk increased to 3.67 folds in patients in stage III of lung cancer as compared to the reference group. This result was obtained by adjusting the hazards ratio based on various factors like age, gender, histology, smoking status and performance status (HR=1.35; 95%CI=0.52-3.50; HR'=3.67; 95%CI=1.20-11.18; **p=0.022)**.

The choice of lung cancer chemotherapy depends on the disease stage and is largely influenced by patients' performance status [127]. Platinum drugs, mainly cisplatin or carboplatin, are considered to be the first-line chemotherapy for most metastatic cancers including lung cancer. These drugs interact with the DNA to form DNA adducts which leads to inhibition of nuclear functions and hence apoptosis. Secondly, the platinum complexes also interact with glutathione in cytoplasm, thus preventing the drugs from binding to DNA. This renders the cell resistant. An imbalance in the host's detoxification system results in ineffective or rapid excretion of platinum drugs [128].

Several epidemiological studies have signalled the role of specific variations or mutations in an individual's response to chemotherapy [129]. Such variations may

include altered drug metabolism, changes in the metabolic activity due to genetic polymorphisms, different levels of exposure to carcinogens and also diversity in population [126]. As GST enzyme plays an important role in cellular defence, sufficient activity of GST is crucial at the time of chemotherapy because the water solubility of the drugs increases during the enzyme catalysed conjugation reaction with GSH, and the drugs can be eliminated from the body. [130].The present study, evaluates the patients' overall survival after treatment with platinum-based chemotherapy in relation to genetic polymorphisms in *GSTT1* and *GSTM1*.

In this study, the association of *GSTM1* and *GSTT1* polymorphisms and the outcomes of chemotherapy in patients with lung cancer were examined. The results suggested that *GSTM1* polymorphism plays an important role in influencing the chemotherapy response in patients with lung cancer but no results were found associated to *GSTT1*. Thus it could be stated that *GSTM1* polymorphism might substantially contribute to the future design of individualized cancer treatment for patients with lung cancer [131].

An increasing number of studies have investigated the role of *GSTM1* and *GSTT1* polymorphisms in the susceptibility to chemotherapeutic agent toxicity and have shown that individuals carrying GST variant genotypes are less able to detoxify the metabolites of drugs and carcinogens used for treating colorectal cancer, bladder cancer, osteosarcoma, and breast cancer, among others (Zhang *et al*., 2012; Djukic *et al*., 2013; Duggan *et al*., 2013; Vreuls *et al*., 2013; Kap *et al*., 2014). Kap *et al*. (2014) conducted a study involving 755 patients with colorectal cancer and found that *GSTM1* might be a predictive marker for the success of oxaliplatin therapy. Djukic *et al*. (2013) conducted a study with 105 patients with muscle invasive bladder cancer and reported that the *GSTT1* active genotypes might have a prognostic role for treatments. Many previous studies have reported that GST polymorphisms play an important role in the prognosis of breast cancer (Oliveira *et al*., 2010; Mishra *et al*., 2011; Bai *et al*., 2012; Duggan *et al*., 2013; Tulsyan *et al*., 2013). Tulsyan *et al*. (2013) reported that *GSTM1* and *GSTP1* polymorphisms influence the chemotherapy response and toxicity in patients with breast cancer.

Li *et al*. (2008) reported that the null *GSTM1* was associated with a better response to chemotherapy than the non-null *GSTM1* type in NSCLC patients who received platinum-based chemotherapy [132]. This is in accordance with the study done by Liu

et al. (2016) in which the regression analysis showed that individuals carrying null *GSTM1* were associated with a better response to chemotherapy as compared to present *GSTM1* (OR=1.88; 95%CI= 1.01-3.47**; p=0.03**) [133]. Yang and Xian (2014) performed a meta-analysis of 9 studies, and they found that the *GSTM1* and also a variant of *GSTP1* may influence the treatment response of platinum-based chemotherapy in East-Asian population [134]. Also, in the study conducted by Li, W e*t al*. (2012), a significant difference in *GSTM1* polymorphism was observed between the response and non-response groups [135].

A meta-analysis study by Li Xian *et al*. (2016), showed the association of *GSTM1* polymorphism with response to chemotherapy in breast cancer patients. The cumulative analysis indicated that the breast cancer patients of *GSTM1* null genotype had a poor effect on responsiveness to chemotherapy, especially in Caucasians (OR=0.61; 95%CI=0.45-0.82; p=0.001) [136]. This study also presents similar association between *GSTM1* null genotype and poor response in lung cancer patients treated with platinum-based chemotherapy (AOR=2.00; 95%CI=1.04-3.84; **p=0.018).** This result is supported by another study conducted on acute myeloid leukaemia which showed that GST deletion(s) are an independent prognostic risk factor for response to treatment and overall survival, concluding that the individuals with *GSTM1* or *GSTT1* deletions (or deletions of both) may have an enhanced resistance to chemotherapy and a shorter survival [137].

Chapter 7

CONCLUSION

The present case/control study pertains to patients visiting the Post Graduate Institute of Medical Education and Research, which is a referral center for patients from states like Haryana, Himachal Pradesh, Punjab, Uttar Pradesh, Jammu & Kashmir and Chandigarh. The following points are evident from the present study:

- *GSTT1* and *GSTM1* genotypes were not found to be associated with overall survival in lung cancer patients
- *GSTT1* null genotype was found to be significantly associated with overall survival in non-smokers
- *GSTT1* null genotype was found associated with patient's performance status
- The cases with null *GSTT1* genotype were found to be associated with higher susceptibility for stage III and metastatic lung cancer
- The combined genotypic effect of wild *GSTT1* and null *GSTM1* was found associated with higher death risk in SCLC patients
- *GSTM1* null genotype was associated with poor response in lung cancer patients treated with platinum-based chemotherapy.

In conclusion, this study demonstrated that the polymorphisms in two of the drug metabolizing genes, *GSTT1* and *GSTM1*, contribute majorly in determining the various factors which affect the prognosis and treatment outcome of lung cancer patients in North Indian population. These genetic polymorphisms can become effective biomarkers for studying and framing future diagnostic and therapeutic tools.

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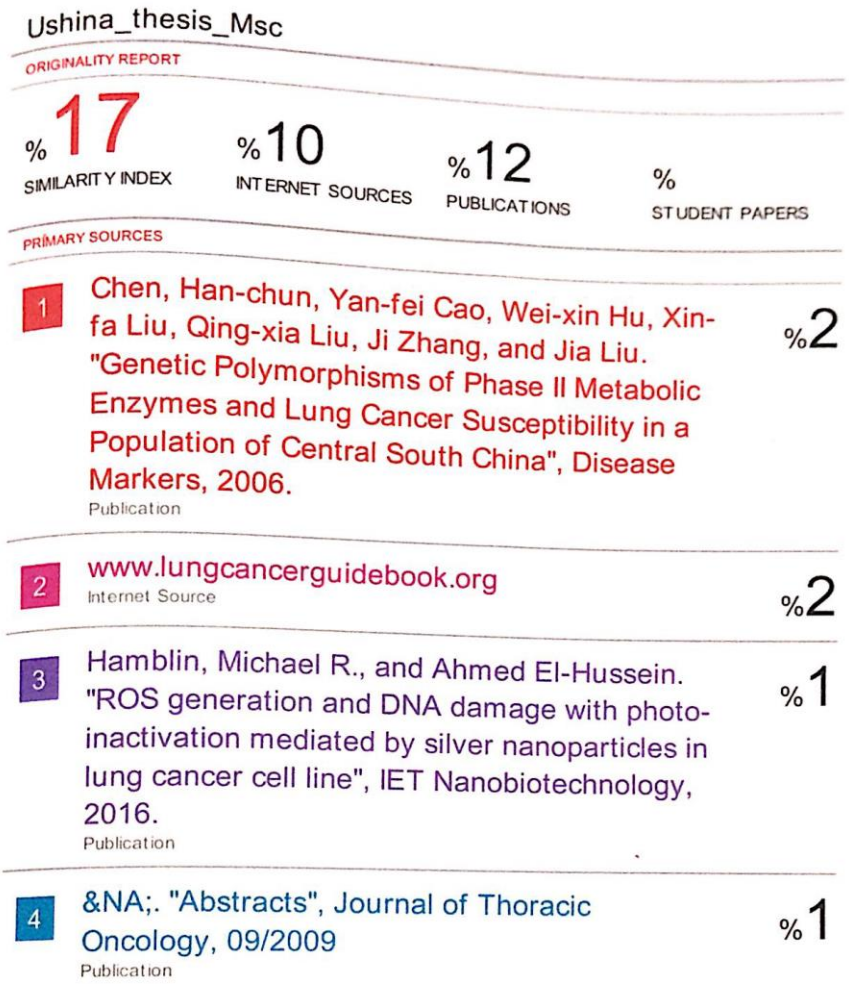
PLAGIARISM REPORT



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ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Chen, Han-chun, Yan-fei Cao, Wei-xin Hu, Xin-fa Liu, Qing-xia Liu, Ji Zhang, and Jia Liu. "Genetic Polymorphisms of Phase II Metabolic Enzymes and Lung Cancer Susceptibility in a Population of Central South China", Disease Markers, 2006. Publication	%2
2	www.lungcancerguidebook.org Internet Source	%2
3	Hamblin, Michael R., and Ahmed El-Hussein. "ROS generation and DNA damage with photo-inactivation mediated by silver nanoparticles in lung cancer cell line", IET Nanobiotechnology, 2016. Publication	%1
4	&NA;. "Abstracts", Journal of Thoracic Oncology, 09/2009 Publication	%1