Genetic polymorphisms of GSTP1,XRCC1, XPC and ERCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer patients of Bangladesh

A thesis submitted by
Most.UmmeBushra
for the degree of
Doctor of Philosophy
in
Clinical Pharmacy and Pharmacology

Department of Clinical Pharmacy and Pharmacology
Faculty of Pharmacy
University of Dhaka
Dhaka1000,
Bangladesh
November, 2016

DECLARATION

Not any portion of this work referred to in this thesis paper has been submitted for another degree or qualification of the University of Dhaka or any other University or any other institute of learning.

DEDICATION

Dedicated to my parents and teachers who always inspire me in every steps of my life

Acknowledgement

First of all, I praise Almighty Allah for giving me the opportunity and strength to carry on and complete this research. I express my deepest regards and gratitude to my respected teacher and my supervisor Professor Dr. AbulHasnat, Department of Clinical Pharmacy

and Pharmacology, University of Dhaka, Bangladesh for his excellent guidance, patience, and support in this research. I am very grateful for his continuous encouragement and confidence in me to apply novel methods and ideas. I will forever cherish my experience working in his lab. I feel greatly honored in expressing my gratefulness to current Dean of Faculty of Pharmacy- Professor Dr. Md. Saiful Islam and current Chairman of Department of Clinical Pharmacy and Pharmacology- Professor Bilkis Begum. I am grateful to late Professor Dr. HabiburRahman, Professor Dr. Md. Abdur Rashid and Profesor Dr. NazmulQais, Professor Md. AbdurRahman.

I would like to thank all of my colleagues in the lab, past and present, for their technical assistance, theoretical guidance, moral support and above all for maintaining amicable environment in the lab. It was a great benefit to join a group with such intelligent, humble, and caring people. My sincere thanks and gratitude goes to Dr. Mohammad Safiqul Islam, an older brother of the department and a senior colleague of the lab, for his continuous support and encouragement.

I also state my thanks to Dr. Siddiqul Islam, Abdullah Al Mamun, MaizbhaUddin Ahmed, Muhammad Shahdaat Bin, NazmaParvin, SalmaParvin, Md. Robiul Islam, Nahid, Opu, Raju and others. I would like to express my deep appreciation and respect to my father Md. MoqbulHossain and mother NurjahanAkter. All are their endless love, heartfelt affection, overall moral support and a lot of sacrifices that bring me up to this level. Finally, I am deeply indebted to my family and friends for their continued support, especially my husband Dr. Md. ArifurRahman and two wonderful kids- Nausheen and Maryam who were my constant source of inspiration for the work.

Genetic polymorphisms of GSTP1, XRCC1, XPC and ERCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer patients of Bangladesh

Abstract:

Background: Lung cancer is the second leading cause of cancer related death worldwide as well as in Bangladesh. Platinum-based chemotherapy is considered as the first-line treatment for lung cancer. But non-small cell lung cancer (NSCLC) is relatively insensitive to chemotherapy compared to other type of lung cancer. Chemotherapy resistance and platinum-induced toxicities are major obstacle for successful chemotherapy in NSCLC patients. The goal of this study is to evaluate the role of genetic polymorphism of GSTP1 (rs1695), XRCC1 (rs25487), XPC (rs2228001) and ERCC1 (rs11615) genes to the response and toxicities produced by platinum based chemotherapy used in the treatment of non-small cell lung cancer.

Methods: Two hundred and eighty five patients with non-small cell lung cancer were recruited from different public and private hospitals of Bangladesh who received platinum based chemotherapy. Each volunteer signed an informed consent document before entering the study. A measure of 3ml of venous blood was collected into a tube with EDTA-Na_2 and stored at -80^0C. After extraction of genomic DNA, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to analyze the genetic polymorphisms of GSTP1, XRCC1, XPC and ERCC1 genes. Then amplified DNA fragments were digested with restriction enzymes and gel electrophoresis was carried out to identify the targeted alleles. The assessment of chemotherapy induced toxicities was done with the help of common terminology criteria for adverse events (CTCAEv4.0).CT scan was performed for one hundred and fifty patients before and after chemotherapy for the evaluation of response to treatment. The American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system (sixth edition) and Response Evaluation Criteria In Solid Tumors (RECIST) were used to evaluate the pathological response of primary tumor and axillary lymph nodes.

Results: In our study, Patients carrying AG and AG+GG genotypes of GSTP1 polymorphism showed a significantly good response to platinum based chemotherapy than did those carrying AA genotype ($p = 0.034$ and $p = 0.037$ respectively). AG genotype and at least one variant A allele (AA + AG) of XRCC1 (rs25487) showed an elevated response to chemotherapy than did those carrying the GG genotype ($p = 0.027$,

p = 0.002). No significant association was found in case of XPC and ERCC1 polymorphisms with response to chemotherapy in our study. In the case of toxicity evaluation, patients carrying GG genotype of GSTP1 had higher risk for the development of neutropenia (p = 0.043) compared to those with AA genotype. The patients carrying AG genotype and at least one variant A (AA+AG) genotype of XRCC1 had an elevated risk for the development of anemia, neutropenia, thrombocytopenia and gastrointestinal toxicity ($p<0.05$) compared to those with GG genotype. Patients carrying AC genotype of XPC had significantly higher risk of neutropenia than did those carrying AA genotype (p = 0.016). No significant relationship of ERCC1 polymorphism with any platinum induced toxicity and response was found in our study ($p > 0.05$).The response to the treatment as well as toxicity was not significantly associated with different demographic and clinicophathological characteristics in our study.

Discussion: Our result indicates that GSTP1 polymorphism was strongly associated with the response of chemotherapy due to the reduced detoxification ability of patients carrying G allele. Furthermore, patients carrying GG genotype hadhigher risk for the development of neutropenia compared to those with AA genotype. This might be due to differential capacities of normal and malignant cells in dealing with drug cytotoxicity, which could be further attributed to somatic changes incurred during tumorigenesis in cancer cells. An elevated response was also found in case of XRCC1 due to increased DNA damage and chemotherapy sensitivity by G allele. The patients carrying AG genotype and at least one variant A genotype of XRCC1 had an elevated risk for the development of different type of toxicities. This might be due to increased DNA damage, reduced repairing capacity and chemotherapy sensitivity by G allele. Patients carrying AC genotype of XPC were found to be associated with neutropenia compared to those with AA genotype. This might be due to the potentially influence of the acute hematological toxicity in platinum based chemotherapy, owing to the decreased repair capacity of DNA damaged by platinum based drug in myelocytes.

Conclusion: Although we have some limitations in this study and major one is that we selected only GSTP1 XRCC1, XPC and ERCC1, which account for the phase II metabolism, base excision repair and nuclear excision repair pathway, our findings, open some windows in further wide range of researches in the field of personalized medicine. Our study suggests that GSTP1, XRCC1 and XPC might affect the clinical outcome of

patients with advanced NSCLC receiving platinum-based chemotherapy. This observation could be used in personalized chemotherapy strategies to increase the response rate and prolonged survival time. On the basis of these findings, it may be possible to use these polymorphisms in the future as a biomarker to predict the outcomes of personalized platinum chemotherapy.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

A	Adenine (where referring to a nucleotide)
ADR	Adverse Drug Reaction
Arg (R) Arginine	
BMI	Body Mass Index
bp	Base Pair
C	Cytosine (where referring to a nucleotide)
ºC	Degrees Celsius
cDNA	Complimentary DNA
CH_3CH_2OH	Ethanol
CH_3COONa	Sodium Acetate
$C_4H_{11}NO_3$	Tris(hydroxymethyl)aminomethane
$C_{10}H_{16}N_2O_8$	Ethylenediaminetetraacetic Acid
$C_{12}H_{25}OSO_3Na$	Sodium Dodecyl Sulphate
C_{max}	Maximum Serum Concentration
CMBI	Centre for Molecular and Biomolecular

		Informatics
	CNS	Central Nervous System
	cSNP	Coding Single Nucleotide Polymorphism
	Cys (C)	Cysteine
	dbSNP	Single Nucleotide Polymorphism Database
	dH_2O	Distilled Water
	DME	Drug-Metabolizing Enzyme
	DNA	Deoxyribonucleic Acid
	dNTP	Deoxynucleotide Triphosphate
	EDTA	Ethylenediaminetetraacetic Acid ($C_{10}H_{16}N_2O_8$)
	EM	Extensive Metaboliser
	EtBr	Ethidium Bromide
	EtOh	Ethanol (CH_3CH_2OH)
	F	Forward Primer
G	Guanine	
	G6PD	Glucose-6-Phosphate Dehydrogenase
	Gln (Q)	Glutamine
	HGP	Human Genome Project
	His (H)	Histidine
	http	Hypertext Transfer Protocol
	Ile (I)	Isoleucine
	IM	Intermediate Metaboliser
	Inc.	Incorporated
	indel	Insertion/Deletion
	ins	Insertion

iSNP	Intergenic Single Nucleotide Polymorphism
kb	Kilobase
KCl	Potassium Chloride
kDa	Kilodalton
kg	Kilogram
K_m	Michaelis-Menten constant
L	Litre
Leu (L)	Leucine
log	Logarithm
Lys (K)	Lysine
M	Molar (moles per litre)
Met (M)	Methionine
MDR1	Multidrug Resistance 1
mg	Milligram
$MgCl_2$	Magnesium Chloride
ml	Millilitre
min	Minimum
max	Maximum
mM	Millimolar
mm^3	Cubic Millimetre
mRNA	Messenger Ribonucleic Acid
n	Sample Size
N	Any Nucleotide (where referring to a nucleotide)
NaCl	Sodium Chloride

NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium Hydroxide
NAT2	N-acetyltransferase 2
NCBI	National Centre for Biotechnological Information
NEB	New England Biolabs
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics
PGP	Phosphoglycoprotein
p^H	Potential Hydrogen
Phe (F)	Phenylalanine
PK	Pharmacokinetics
PM	Poor Metaboliser
Pre-mRNA	Preliminary Messenger Ribonucleic Acid
Pro (P)	Proline
pSNP	Perigenic Single Nucleotide Polymorphism
R	Reverse Primer
®	Registered Trademark
REase	Restriction Enzyme/Restriction Endonuclease
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RNAseRibonuclease	
rpm	Revolutions per Minute
rSNP	Random Single Nucleotide Polymorphism

SD	Standard deviation
SDS	Sodium Dodecyl Sulphate ($C_{12}H_{25}OSO_3Na$)
SSCP	Single Strand Conformation Polymorphism
T	Thymine (where referring to a nucleotide)
TBE	Tris Borate EDTA
Thr (T)	Threonine
TM	Trademark
Tris	Tris(hydroxymethyl)aminomethane
U	Unit (enzyme quantity)
UK	United Kingdom
UM	Ultra-rapid Metaboliser
UN	United Nations
URTI	Upper Respiratory Tract Infection
USA	United States of America
UTR	Untranslated Region
UV	Ultraviolet
V	Volts
V_{max}	Maximum Reaction Velocity
vs	Versus
v/v	Volume per Volume
WHO	World Health Organisation
www	World Wide Web
w/v	Weight per Volume
$	Dollar

>	Greater Than (except where referring to a nucleotide change)
<	Less Than
µ	Mu (Micro)
µg/mcg	Microgram
mgMiligram	
gm	Gram
µl	Microlitre
L	litre

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER THREE: RESULTS AND DISCUSSION

1. Introduction

1.1 Lung Cancer

Lung cancer is one of the most frequently occurring cancers in the world in terms of mortality and incidence (Jemal *et al.,* 2011). It is also the most common type of cancer in Bangladesh (24.9% of incidence and 28.6% of mortality) (Ferlay *et al.,* 2010). It comprises 13% of total new cancer cases and 18% of all death caused by cancer (Ferlay *et al.,* 2010). It is the most commonly diagnosed cancer and leading cause of death in males (Jemal *et al.,* 2011). Various factors are responsible for lung cancer. Besides cigarette smoking, environmental tobacco smoking, occupational exposure, racial, gender and ethnic differences are thought to be responsible for the development of lung cancer (Weitberg, 2002). Carcinogenesis is influenced by cigarette smoking as well as genetic predisposition. Tissue injury (e.g. from tobacco smoke, reflected in the discolored smoking related lungs) initially occurs in the form of genetic and epigenetic changes (e.g. mutations, loss of heterozygosity and promoter methylation) and global transcriptomechanges (e.g. inflammation and apoptosis pathways). These changes can persist longterm and eventually lead to aberrant pathway activation and cellular function (e.g. dysregulated proliferation and apoptosis) to produce premalignant changes, including dysplasia and clonal patches. Additional changes can result in angiogenesis, invasion and early-stage cancer and advanced cancer and metastasis (Sato *et al.,* 2007). Radical treatment depends primarily on early detection, since patients with advanced and metastatic disease have 5 year survival rate less than 5% (Hirsch *et al*., 2001).

In Bangladesh, the present scenario is that, the incidence of lung cancer is increasing in an alarming rate. The number of lung cancer patients in Bangladesh was a staggering amount of almost 196000 among those aged 30 years or more (Haque, 2011). The report of IARC (2010) demonstrates that in 2008 new lung cancer cases in Bangladesh were 14951 and it is estimated to rise to 43048 by the year 2030.

1.1.1 Epidemiology

Worldwide, lung cancer is the most common cancer among men in terms of both incidence and mortality, and among women has the third highest incidence, and is second after breast cancer in mortality. In 2012, there were 1.82 million new cases globally and 1.56 million deaths due to lung cancer, representing 19.4% of all deaths from cancer (WHO, 2014). The highest rates are in North America, Europe and East Asia, with over a third of new cases in 2012 in China, rates in Africa and South Asia are much lower (Jemal *et al*., 2004).

The population segment most likely to develop lung cancer is people aged over 50 who have a history of smoking. In contrast to the mortality rate in men, which began declining more than 20 years ago, womens' lung cancer mortality rates have been rising over the last decades, and are just recently beginning to stabilize. For every 3–4 million cigarettes smoked, one lung cancer death occurs. The influence of "Big Tobacco" plays a significant role in the smoking culture. Young nonsmokers who see tobacco advertisements are more likely to take up smoking (NCI, 2010) The role of passive smoking is increasingly being recognized as a risk factor for lung cancer, leading to policy interventions to decrease undesired exposure of nonsmokers to others' tobacco smoke. Emissions from automobiles, factories and power plants also pose potential risks. Lung cancer rates are currently lower in developing countries. With increased smoking in developing countries, the rates are expected to increase in the next few years, notably in China and India. Lung cancer is the second most common cancer in the UK (around 43,500 people were diagnosed with the disease in 2011), and it is the most common cause of cancer death (around 35,400 people died in 2012).

1.1.2 Pathology of lung Cancer

Similar to many other cancers, lung cancer is initiated by activation of oncogenes or inactivation of tumor suppressor genes. Oncogenes are believed to make people more susceptible to cancer. Proto-oncogenes are believed to turn into oncogenes when exposed to particular carcinogens. Mutations in the *K-ras* proto-oncogene are responsible for 10–30% of lung adenocarcinomas (Herbst, 2008). The epidermal growth factor receptor (EGFR) regulates cell proliferation, apoptosis, angiogenesis, and tumor invasion. Mutations and amplification of EGFR are common

in non-small-cell lung cancer and provide the basis for treatment with EGFR-inhibitors. Her2/neu is affected less frequently. Chromosomal damage can lead to loss of heterozygosity. This can cause inactivation of tumor suppressor genes. Damage to chromosomes 3p, 5q, 13q, and 17p are particularly common in small-cell lung carcinoma. The p53 tumor suppressor gene, located on chromosome 17p, is affected in 60-75% of cases. Other genes that are often mutated or amplified are c-MET, NKX2-1, LKB1, PIK3CA, and BRAF (Aviel-Ronen *et al*., 2006).

1.1.3 Risk Factors of Lung cancer

The etiologies of lung cancer can be divided into modifiable and unmodifiable risk factors. The unmodifiable risks include gender, African American race and genetic predisposition. The modifiable factors include exposure to tobacco smoke, environmental tobacco smoke, occupational lung carcinogens, air pollution, and diet (Brownson *et al*., 1997; Risch *et al*., 1993). Some underlying lung diseases also increase the risk of lung cancer.

1.1.3.1 Unmodifiable Risk Factors

- **Gender**

The male predominance of lung cancer is a result of the substantial smoking habits of males compared to female (Beckett, 1993). When differences in smoking initiation, duration, and intensity are adjusted, male and female lung-cancer rates are more comparable (Beckett, 1993). There is some evidence, however, that female smokers and nonsmokers may have a higher susceptibility to lung cancer than males (McDuffie *et al*., 1987; Zang and Wynder, 1996). Lifetime nonsmoking females have between two and seven times the risk of developing lung cancer than male nonsmokers (Brownson *et al*., 1997; Risch *et al*., 1993). The increased susceptibility is also apparent in smokers. Female smokers have a sharper increase in the risk of lung cancer; with increasing cigarette consumption compared to male smokers (Osnan *et al*., 1993; Risch *et al*., 1993). Zang and Wynder (1996) have proposed some possible explanations for this increased susceptibility, including lower metabolism of tobacco constituents in females, variations in the cytochrome P-450 enzymes, and possibly estrogen effects on tumor development.

- **Race**

Although cigarette smoking is usually the cause of lung cancer, the racial disparity in lung-cancer incidence and mortality is not entirely related to differences in smoke exposure (Dela-Cruz *et al.*, 2011).The fraction of deaths attributable to smoking at ages above 50 is greater for black males than for white males. Among men, current smoking status explains about 20 % of the black excess relative risk in all-cause mortality at ages above 50 without adjustment for socioeconomic characteristics (Ho and Elo, 2013).Therefore, factors other than smoke exposure must influence the incidence and mortality of lung cancer in the African American population. Genetic differences in lung-cancer susceptibility have been the focus of extensive study. CYP1A1 is a gene that codes for some of the enzymes involved in the metabolism of polynuclear aromatic hydrocarbons (PAHs) (Gelboin, 1980; Guengerich and Shima, 1991). PAHs are carcinogens that are abundant in cigarette smoke, coke, coal gasification, and diesel exhaust (Hecht, 2002).

- **Genetic Predisposition**

A genetic predisposition to lung cancer is suggested by the observation that between 10% and 15% of smokers develops lung cancer. Therefore, considerable investigative efforts have explored genetic predisposing risk factors. Familial clustering of bronchogenic carcinoma occurs. Shaw and colleagues retrospectively compared patients with lung cancer to age-matched community controls, and found an odds ratio for lung cancer of 1.8 (95% CI: 1.3–2.5) for those with at least one first-degree relative with lung cancer (Shaw *et al*., 1991). The increased risk was directly proportional to the number of affected first-degree relatives. The risk associated with spousal cancer was significant for small-cell carcinoma, a finding that may reflect shared smoking habits. Finally, McDuffie found that 30% of patients with lung cancer had more than two relatives with cancer compared to 23% of controls (McDuffie *et al*., 1991). A genetic prevalence of lung cancer also applies to lifetime nonsmoking patients. After controlling for confounding factors like second-hand smoke, several studies have concluded that the risk of lung cancer in nonsmoking patients increases with the number of first-degree relatives with cancer (Wu *et al*., 1996 ; Poole *et al*., 1999). The elevated risk varies from 30% and 70%, depending

upon the number of relatives affected, the level of smoke exposure, and whether the first-degree relative is a parent, sibling, or child (Schwartz *et al*., 1996).

1.1.3.2 Modifiable Risk Factors

- **Smoking**

Tobacco smoking is the major risk factor of lung cancer. It is responsible for 90% of lung cancer (IARC, 2004). Moreover it accounts for 85% of lung cancer in males (Higgins *et al*., 1988). In case of smoker males there is higher risk of developing lung cancer than smoker females. The average smoker has a 10-fold increased risk of developing lung cancer compared with nonsmokers (US PHS, 1980); the risk for heavy smokers is greater than or equal to 15–25 times that of a nonsmoker for developing lung cancer (US PHS, 1980). The risk decreases with increasing years of smoking cessation. The risk of lung cancer in pipe and cigar smokers is less than in cigarette smokers, but greater than for nonsmokers. There is conflicting evidence in the medical literature regarding the comparative lung-cancer risks of cigar and pipe smoking. Some studies suggest that pipe smokers have a greater relative risk for lung cancer than cigar smokers (IARC 2004). Others note that differences in inhalation may explain a higher relative risk in cigar smokers . The increase of lung cancer risk in case of smoking cigar or pipe, low tar, light cigarette is as much as regular cigarette smoking whereas smoking tendency is increased by menthol cigarettes (Tindle *et al*., 2006; Gandhi *et al*., 2009).

- **Passive smoking**

When a person is not a smoker but inhales smoke from another person's smoking then he is called a passive smoker. According to several reports it is a cause of lung cancer in case of nonsmokers (IARC, 2004; USDHHS, 2010). Nonsmokers who reside with a smoker have a 24% more chance of developing lung cancer. More than 21,000 deaths occur worldwide because of passive smoking and in USA approximately 3400 people die each year (NCI, 2010) due to passive smoking.

- **Occupational Exposure**

Although cigarette smoking causes the majority of bronchogenic carcinomas, occupational exposures account for between 3% and 17% of lung cancers (Nemery, 1990; Unia *et al.*, 1994). Table 1.1 lists the current occupational human carcinogens as categorized by the International Agency for Research on Cancer (IARC), as well as examples of occupations at high risk for carcinogen exposure. In general, the mechanisms by which these agents cause lung cancer are not established, but are partly caused by their effects on the DNA or by promoting the growth of initiated cells.

Table 1.1: Occupational Human Lung Carcinogens

Evidence	**Agent**	**IARC**	**High-risk occupations**	**Relative risk**
Sufficient	Arsenic	1980(192), 1987(a) 139	Cooper smelting, manufacturing arsenical pesticides, veterinary medications, electronic devices, semiconductor devices	3.69*
	Asbestos	1977(193), 1987(a) 139	Maintenance and construction workers, brake repair and maintenance, shipbuilding and repair, mining of fibrous materials	2.0*
	BCME, CCME,	1987(a) 139, 1993 (92)	Production of chemicals in textiles, paints and home insulation	5.0-11.0 (89,90)
	Cadmium	1993 (92)	Electroplating, welding, smelting, refining, laser cutting diamond cutting, enameling, electrodes in batteries, and alloys	1.49*
	Chromium VI	1980 (192),	Chromate and chromate pigment	2.78*

		1990 (122)	production, welding, chrome plating	
	Coke production and coal gasification	1984 (113)	Coal gasification workers, industrial work involving the destructive distillation of coal	N/A
	Nickel sulfides & nickel oxides	1990 (122)	Production of stainless steel, nonferrous alloys, electro- plating and manufacture of batteries, nickel miners, leaching, sintering, calcining, roasting	1.56*

Evidence	**Agent**	**IARC**	**High-risk occupations**	**Relative risk**
	Radon progeny	1988 (189)	Underground mining for Uranium and other metals, and in processing ores and radioactive materials	1.5-15 (125,126)
	Soot	1985 (190), 1987(a) (139)	Chimney-sweeps	N/A
	Mustard gas	1975 (191)	Used in WWI and manufactured up to WWII for warfare production; no longer manufactured	N/A
	Vinyl chloride	1979 (127)	Production of polyvinyl chloride, vinyl chloride production and polymerization	N/A
Limited	Acrylonitrile	1979 (127), 1987(a) (139)	Acrylic fibers in textile and home furnishing manufacturing, pipes and fittings	N/A
	Diesel exhaust	1989 (145)	Railroad workers, truck drivers, mechanics	1.31*
	Crystalline silica	1987(b) (146)	Masonry, stone work, concrete and gypsum, pottery, mining and quarrying of coal and other minerals, sand blasting, polishing, grinding	1.33*

Possible	Art glass, glass containers, pressed ware	1993 (92)	Leaded crystal, art glass workers, glass blowing	N/A
	Manmade fibers (glass wool, rock wool)	1988 (192)	Thermal and acoustical insulation, production and manufacturing of manmade fibers	N/A

N/A = not assessed.

*Adapted from Steenland: Relative Risks from selected studies of occupational lung carcinogens

- **Arsenic**

Arsenic is a ubiquitous element that occurs in organic and inorganic forms. Humans are exposed to it through the environment, occupations, and medications (Chiou *et al*., 1995). The general population may be exposed through contaminated water, seafood, and wine. Workers involved in smelting and refining copper, gold, and lead ores, as well as the production of pesticides and various pharmaceutical substances, are at risk for arsenic-induced cancers. Although arsenic is not mutagenic in animal species, it is carcinogenic in humans (Landrigan, 1981). There is substantial evidence that arsenic causes cancers of the skin, lung, liver, bladder, kidney, and prostate (Chen *et al*., 1985; Chen *et al*., 1986; Chen *et al*., 1992). Studies from Taiwan, Chile, and Argentina show an increased risk of lung cancer in human populations that consume water with high arsenic concentrations (Ferreccio *et al*., 1998; Hopenhayn-Rich *et al*., 1998, Smith *et al*., 1998).

- **Radon**

Epidemiological data suggests that radon can cause lung cancer. It is a naturally occurring radioactive gas which occurs due to the radioactive decay of uranium and in the uranium using countries it is considered as the second major cause of lung cancer (Sethi *et al*., 2012).

- **Nickel**

Inhalation of nickel causes lung and nasal sinus cancers**.** People doing metalworking, nickel mining and smelting, sand blasting, stainless steel manufacturing, paint and varnish manufacturing, and welding are at increased risk of high nickel exposure.

- **Asbestos**

A rare form of lung cancer known as mesothelioma is caused by high exposure to asbestos. Tobacco smoking and asbestos give synergistic effects.

- **Polycyclic Aromatic Hydrocarbons (PAHs)**

Incomplete burning of coal, oil, gas, garbage or other organic substances like tobacco causes the release of polycyclic aromatic hydrocarbons. But diesel fuel exhausts are the main source of PAHs. PAHs are very potent carcinogens developing lung cancer by producing DNA adducts (Hecht, 2002).

- **Viruses**

Human Papillomavirus causes lung cancer (Cheng *et al*., 2001). John Cunningham virus (Zheng *et al*., 2007) and Cytomegalovirus also causes lung cancer (Giuliani *et al*., 2007).

- **Diet**

The effect of diet is not yet established. The supplement of beta carotene increases the risk of lung cancer in smokers.

1.1.4 Classification of lung cancer

The current World Health Organization (WHO) classification for lung carcinomas is presented in Table 1.2. There is a simple classification for treatment purposes i.e. small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC) (Travis *et al*., 2015). In contrast to this the histopathologic classification of lung carcinoma continues to evolve. Although the four major categories traditionally recognized—squamous-cell carcinoma (SCC), adenocarcinoma, large-cell carcinoma (LCC), and small-cell carcinoma—remain in the present classification, the

histologic features of each and its variants continue to be refined. This is particularly true of the NSCLC, whereas some variants of SCLC recognized in previous classifications have been eliminated in recognition of the morphologic spectrum of small-cell carcinoma and the lack of clinical significance of such subtypes as the oat cell and intermediate cell variants. Despite considerable progress in establishing a reproducible, unified, and conceptually gratifying classification scheme for neuroendocrine lung tumors, including typical and atypical carcinoids, small-cell carcinoma, and large-cell neuroendocrine carcinoma, based on morphologic criteria, the current classification does not place these tumors under one category. Large-cell neuroendocrine carcinoma, previously classified with small-cell carcinoma, is included with other variants of large-cell carcinoma in the current WHO classification, at least until clinical patterns suggest that it should be categorized with these other neuroendocrine tumors. Moreover, further evidence beyond morphologic similarity is needed to link small-cell carcinoma to the carcinoid tumors. Ideally, a classification scheme should have practical value in terms of affecting therapeutic decisions, as well as promoting an appreciation of the morphologic relationships among the SCLC and NSCLC. Adenocarcinomas were sub typed by Noguchi into pathological sub-types A-F. These sub-types, A to F, are associated with progressively poorer prognoses in terms of long term survival:

A. Localized bronchioalveolar carcinoma (LBAC)
B. LBAC with foci of collapsed alveolar structures
C. LBAC with foci of active fibroblastic proliferation
D. Poorly differentiated adenocarcinoma
E. Tubular adenocarcinoma
F. Papillary adenocarcinoma with compressive and destructive growth

Table 1.2: Histological Classification of Lung Carcinomas (Travis *et al*., 2015)

Histologic type	**Variant**
SCC	Papillary
	Clear-cell
	Small-cell

	Basaloid
Adenocarcinoma	Bronchioloalveolar, mucinous, nonmucinous, or intermediate
	Acinar
	Papillary
	Solid adenocarcinoma with mucin
	Rare variants
Adenosquamous carcinoma	Large-cell neuroendocrine carcinoma
	Basaloid carcinoma
	Lymphoepithelioma-like carcinoma
	Clear-cell carcinoma
	LCC with rhabdoid features
LCC	Combined small-cell carcinoma
Small-cell lung carcinoma	Carcinomas with spindle and/or giant cells
Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements	Pleomorphic carcinoma
	Spindle-cell carcinoma
	Giant-cell carcinoma
	Carcinosarcoma
	Pulmonary blastoma
	Others
Carcioid tumor	Typical carcinoid
	Atypical carcinoid
Carcinomas of salivary-gland type	Mucoepidermoid carcinoma
	Adenoid cystic carcinoma

Others
Unclassified carcinoma

1.1.5 Stages of Lung Cancer

Accurate lung-cancer staging is essential for designing treatment programs and for determining a prognosis. Most importantly, a consistent, reproducible staging system is imperative for the execution of meaningful clinical trials of old and new therapeutic modalities, and for the extrapolation of the outcomes of trials to the general population. The International System for Staging Lung Cancer is widely accepted as an accurate and reproducible staging system for non small-cell lung cancer (NSCLC) (Naruke *et al*., 1997). The staging system for small-cell lung cancer (SCLC) presently divides this disease into limited and extensive stage. However, there continues to be numerous proposals for a more meaningful staging system for SCLC (Abrams *et al*., 1998).

Table 1.3: Primary tumor (T)

TX	Positive malignant cytology finding with no observable lesion
Tis	Carcinoma in situ
T1	Diameter of 3 cm or smaller, is surrounded by lung or visceral pleura, and is without invasion more proximal than the lobar bronchus
T2	Diameter greater than 3 cm and/or has extension to the visceral pleura, atelectasis, obstructive pneumonitis that extends to the hilar region but does not involve the whole lung or tumor of a main bronchus more than 2 cm distal from the carina
T3	A tumor of any size that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium; or, associated atelectasis or obstructive pneumonitis of the entire lung or, tumor in the main bronchus less than 2 cm distal to the carina
T4	A tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; or, separate tumor nodules in the same lobe; or, tumor with a malignant pleural effusion

Table 1.4: Regional lymph nodes (N)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to ipsilateral peribronchial and/or ipsilateral hilar lymph nodes, and intrapulmonary nodes including involvement by direct extension of the primary tumor
N2	Metastasis to ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis to contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

Table 1.5: Metastasis (M)

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis present

Table1.6: TNM Stage

Stage	**TNM**	**Survival rate**
Stage IB	T2N0M0	5 year survival rate of 50 – 60%
Stage IIA	T1N1M0	5 year survival rate of 40 – 50%
Stage IIB	T2N1M0 or T3N0M0	5 year survival rate of 25 -40%
Stage IIIA	T3N1M0 or T(1-3)N2M0	5 year survival rate of 10 -35%
Stage IIIB	T4N(0-3)M0 or T(1-4)N3M0	5 year survival rate of 5%
Stage IV	T(1-4)N(0-3)M1	5 year survival rate less than 5%

A simple two stage classification is used for NSCLC instead of the detailed TNM staging because of the aggressive nature of the small cell lung carcinoma. For small tumors that are confined to the chest (mediastinum and supraclavicular node) limited stage SCLC is used. This is correlated with a 2 year survival rate of 20%. For distance metastases extensive stage SCLC is

used. It is also used for extensive tumors that cannot be incorporated into the limited stage. The prognosis for extensive stage SCLC is a 2 year survival rate of 5% (Mountain, 1997; Dollinger *et al*., 2002).

1.1.6 Detection and diagnosis of lung cancer

There are different tests that are used to examine for detection, diagnosis and finding out the stage of non-small cell lung cancer.

- **Chest x-ray**: **Imaging tests.**

  An X-ray image of lungs can reveal an abnormal mass or nodule but small lesions in lungs cannot be detected on an X-ray. It is a type of energy beam that can go through the body and onto film, making a picture of areas inside the body. X-rays pass through the patient onto film (Radiological Society of North America, 2016).

- **CT scan**

It is a procedure that makes a series of detailed pictures of areas inside the body, such as the chest, taken from different angles. The pictures are made by a computer linked to an x-ray machine. A dye may be injected into a vein or swallowed to help the organs or tissues show up more clearly. This procedure is also called computed tomography, computerized tomography, or computerized axial tomography (American Thoracic Society, 2015).

- **Sputum cytology**

It is a procedure in which a pathologist views a sample of sputum (mucus coughed up from the lungs) under a microscope which can sometimes reveal the presence of lung cancer cells (American Cancer Society, 2016).

- **Fine-needle aspiration (FNA) biopsy of the lung**

The tissue or fluid from the lung using a thin needle is removed. A CT scan, ultrasound, or other imaging procedure is used to locate the abnormal tissue or fluid in the lung. A small incision may be made in the skin where the biopsy needle is inserted into the abnormal tissue or fluid. A sample is removed with the needle and sent to the laboratory. A pathologist then views the sample under a microscope to look for cancer cells. A chest x-ray is done after the procedure to make sure no air is leaking from the lung into the chest (American Cancer Society, 2016).

- **Bronchoscopy**

It is a procedure to look inside the trachea and large airways in the lung for abnormal areas. A bronchoscope is inserted through the nose or mouth into the trachea and lungs. A bronchoscope is a thin, tube-like instrument with a light and a lens for viewing. It may also have a tool to remove tissue samples, which are checked under a microscope for signs of cancer (American Cancer Society, 2016).

- **Thoracoscopy**

It is a surgical procedure to look at the organs inside the chest to check for abnormal areas. An incision is made between two ribs, and a thoracoscope is inserted into the chest. A thoracoscope is a thin, tube-like instrument with a light and a lens for viewing. It may also have a tool to remove tissue or lymph node samples, which are checked under a microscope for signs of cancer. In some cases, this procedure is used to remove part of the esophagus or lung. If certain tissues, organs, or lymph nodes can't be reached, a thoracotomy may be done. In this procedure, a larger incision is made between the ribs and the chest is opened (American Cancer Society, 2016).

- **Thoracentesis**

The fluid from the space between the lining of the chest and the lung, using a needle is removed by this technique. A pathologist views the fluid under a microscope to look for cancer cells (American Cancer Society, 2016).

- **Immunohistochemistry study**

A laboratory test in which a substance such as an antibody, dye, or radioisotope is added to a sample of cancer tissue to test for certain antigens. This type of study is used to tell the difference between different types of cancer (American Cancer Society, 2016).

1.1.7 Treatment of lung cancer

There are different types of treatment for patients with non-small cell lung cancer. Different types of treatments are available for patients with non-small cell lung cancer (American Cancer Society, 2016).

Some treatments are standard and some are being tested in clinical trials. A treatment clinical trial is a research study meant to help improve current treatments or obtain information on new treatments for patients with cancer. When clinical trials show that a new treatment is better than the standard treatment, the new treatment may become the standard treatment. Nine types of standard treatment are used:

- **Surgery**

Four types of surgery are used to treat lung cancer (American Cancer Society, 2016). They are-

- **Wedge resection:**

Wedge resection is a surgery to remove a tumor and some of the normal tissue around it. When a slightly larger amount of tissue is taken, it is called a segmental resection (American Cancer Society, 2016).

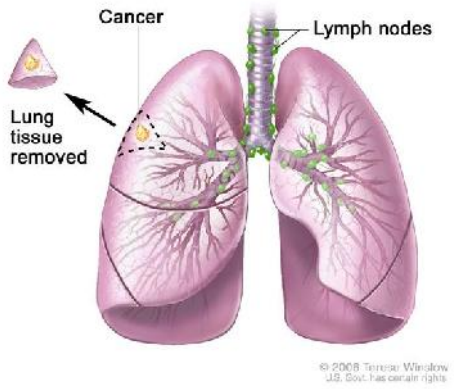


Figure 1.1: Wedge resection of the lung

- **Lobectomy**

Lobectomy is a surgery to remove a whole lobe (section) of the lung is lobectomy (American Cancer Society, 2016).

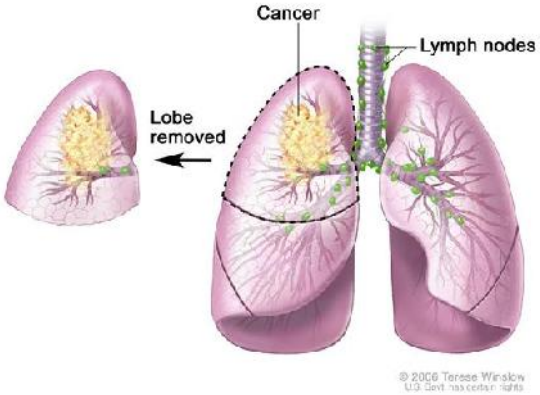


Figure 1.2: Lobectomy

- **Pneumonectomy**

Surgery to remove one whole lung is pneumonectomy (American Cancer Society, 2016).

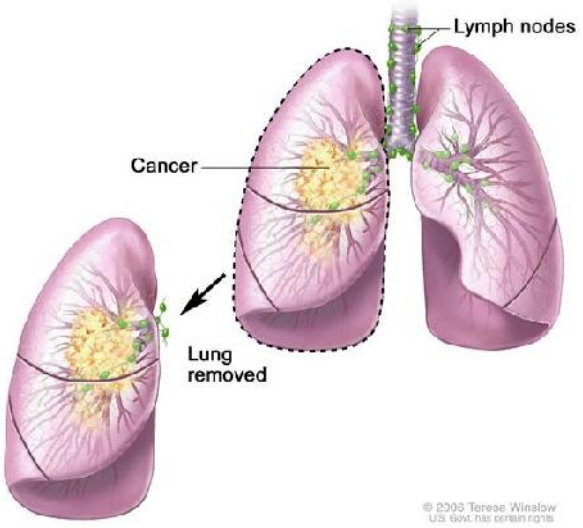


Figure 1.3: Pneumonectomy of the lung

- **Sleeve resection**

Surgery to remove part of the bronchus is sleeve resection (American Cancer Society, 2016).

- **Radiation therapy**

Radiation therapy is a cancer treatment that uses high-energy x-rays or other types of radiation to kill cancer cells or keep them from growing. There are two types of radiation therapy. External radiation therapy uses a machine outside the body to send radiation toward the cancer. Internal radiation therapy uses a radioactive substance sealed in needles, seeds, wires, or catheters that are placed directly into or near the cancer (American Cancer Society, 2016).

- **Radiosurgery**

Radiosurgery is a method of delivering radiation directly to the tumor with little damage to healthy tissue. It does not involve surgery and may be used to treat certain tumors in patients who cannot have surgery. The way the radiation therapy is given depends on the type and stage of the cancer being treated. It also depends on where the cancer is found. For tumors in the airways, radiation is given directly to the tumor through an endoscope (American Cancer Society, 2016).

- **Chemotherapy**

Chemotherapy is a cancer treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle, the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the cerebrospinal fluid, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy). The way the chemotherapy is given depends on the type and stage of the cancer being treated (American Cancer Society, 2016).

- **Targeted therapy**

Targeted therapy is a type of treatment that uses drugs or other substances to identify and attack specific cancer cells without harming normal cells (American Cancer Society, 2016). Monoclonal antibodies and tyrosine kinase inhibitors are two types of targeted therapy being used in the treatment of non-small cell lung cancer. Tyrosine kinase inhibitors are targeted therapy drugs that block signals needed for tumors to grow. Tyrosine kinase inhibitors may be used with other anticancer drugs as adjuvant therapy (American Cancer Society, 2016).

Tyrosine kinase inhibitors used to treat non-small cell lung cancer include erlotinib and gefitinib. They are types of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Crizotinib

is a type of tyrosine kinase inhibitor that is used to treat non-small cell lung cancer with certain gene changes.

- **Monoclonal antibody therapy**

Monoclonal antibody therapy is a cancer treatment that uses antibodies made in the laboratory from a single type of immune system cell. These antibodies can identify substances on cancer cells or normal substances that may help cancer cells grow. The antibodies attach to the substances and kill the cancer cells, block their growth, or keep them from spreading. Monoclonal antibodies are given by infusion. They may be used alone or to carry drugs, toxins, or radioactive material directly to cancer cells. Monoclonal antibodies used to treat non-small cell lung cancer include bevacizumab and cetuximab. Bevacizumab binds to vascular endothelial growth factor (VEGF) and may prevent the growth of new blood vessels that tumors need to grow. Cetuximab binds to epidermal growth factor receptor (EGFR) and works to stop cancer cells from growing and dividing (Scott *et al*., 2012).

- **Laser therapy**

Laser therapy is a cancer treatment that uses a laser beam (a narrow beam of intense light) to kill cancer cells (Lee *et al*., 2002).

- **Photodynamic therapy (PDT)**

Photodynamic therapy (PDT) is a cancer treatment that uses a drug and a certain type of laser light to kill cancer cells. A drug that is not active until it is exposed to light is injected into a vein. The drug collects more in cancer cells than in normal cells. Fiberoptic tubes are then used to carry the laser light to the cancer cells, where the drug becomes active and kills the cells. Photodynamic therapy causes little damage to healthy tissue. It is used mainly to treat tumors on or just under the skin or in the lining of internal organs. When the tumor is in the airways, PDT is given directly to the tumor through an endoscope (Kato, 1998).

- **Electrocautery**

Electrocautery is a treatment that uses a probe or needle heated by an electric current to destroy abnormal tissue. For tumors in the airways, electrocautery is done through an endoscope (Lee *et al*., 2002).

1.1.8 Treatment Options by Stage

Treatment of non-small cell lung cancer depends on the stage of the disease (American Cancer Society, 2016).

- **Stage 0 (Carcinoma in Situ)**

Treatment of stage 0 may include the following:

- Surgery (wedge resection or segmental resection).
- Photodynamic therapy using an endoscope.
- Electrocautery, cryosurgery, or laser surgery using an endoscope.

- **Stage I Non-Small Cell Lung Cancer**

Treatment of stage I non-small cell lung cancer may include the following:

- Surgery (wedge resection, segmental resection, sleeve resection, or lobectomy).
- External radiation therapy (for patients who cannot have surgery or choose not to have surgery).
- A clinical trial of chemotherapy or radiation therapy following surgery.
- A clinical trial of surgery followed by chemoprevention.
- A clinical trial of treatment given through an endoscope, such as photodynamic therapy (PDT).

- **Stage II Non-Small Cell Lung Cancer**

Treatment of stage II non-small cell lung cancer may include the following:

- Surgery (wedge resection, segmental resection, sleeve resection, lobectomy, or pneumonectomy).

- Chemotherapy followed by surgery.
- Surgery followed by chemotherapy.
- External radiation therapy (for patients who cannot have surgery or choose not to have surgery).
- A clinical trial of radiation therapy following surgery.

- **Stage IIIA Non-Small Cell Lung Cancer**

Treatment of stage IIIA non-small cell lung cancer that can be removed with surgery may include the following:

- Surgery followed by chemotherapy.
- Chemotherapy followed by surgery.
- Surgery followed by chemotherapy combined with radiation therapy.
- Surgery followed by radiation therapy.
- A clinical trial of new combinations of treatments.

Treatment of stage IIIA non-small cell lung cancer that cannot be removed with surgery may include the following:

- Chemotherapy and radiation therapy given as separate treatments over the same period of time.
- External radiation therapy alone (for patients who cannot be treated with combined therapy, as palliative treatment to relieve symptoms and improve the quality of life).
- Internal radiation therapy or laser surgery, as palliative treatment to relieve symptoms and improve the quality of life.
- A clinical trial of new combinations of treatments.

Non-small cell lung cancer of the superior sulcus, often called Pancoast tumor, begins in the upper part of the lung and spreads to nearby tissues such as the ribs and vertebrae. Treatment of Pancoast tumors may include the following:

- Radiation therapy alone.
- Radiation therapy followed by surgery.
- Chemotherapy and radiation therapy given as separate treatments over the same period of time, followed by surgery.

- Surgery alone.
- A clinical trial of new combinations of treatments.

Some stage IIIA non-small cell lung tumors that have grown into the chest wall may be completely removed. Treatment of chest wall tumors may include the following:

- Surgery.
- Surgery and radiation therapy.
- Radiation therapy alone.
- Chemotherapy combined with radiation therapy and/or surgery.
- A clinical trial of new combinations of treatments.

- **Stage IIIB Non-Small Cell Lung Cancer**

Treatment of stage IIIB non-small cell lung cancer may include the following:

- Chemotherapy followed by external radiation therapy.
- Chemotherapy and radiation therapy given as separate treatments over the same period of time.
- Chemotherapy followed by surgery.
- External radiation therapy alone for patients who cannot be treated with chemotherapy.
- External or internal radiation therapy as palliative therapy, to relieve pain and other symptoms and improve the quality of life.
- Clinical trials of new radiation therapy schedules and new combinations of treatments.

- **Stage IV Non-Small Cell Lung Cancer**

Treatment of stage IV non-small cell lung cancer may include the following:

- Combination chemotherapy.
- Maintenance therapy with an anticancer drug to help keep cancer from progressing, after combination chemotherapy.
- Combination chemotherapy and targeted therapy with a monoclonal antibody.
- Targeted therapy with a tyrosine kinase inhibitor.

- External radiation therapy as palliative therapy, to relieve pain and other symptoms and improve the quality of life.
- Laser therapy and/or internal radiation therapy.
- A clinical trial of new drugs and combinations of treatments.

- **Treatment option for recurrent Non-Small Cell Lung Cancer**

Recurrent non-small cell lung cancer is cancer that has recurred after it has been treated. The cancer may come back in the brain, lung, or other parts of the body.Treatment of recurrent non-small cell lung cancer may include the following:

- External radiation therapy as palliative therapy, to relieve pain and other symptoms and improve the quality of life.
- Chemotherapy.
- Targeted therapy with a tyrosine kinase inhibitor.
- Laser therapy or internal radiation therapy using an endoscope.
- Radiosurgery (for certain patients who cannot have surgery).
- Surgery to remove a very small amount of cancer that has spread to the brain (American Cancer Society, 2016).

1.1.9 Worldwide Prevalence of lung cancer

By considering incidence and mortality lung cancer is the most prevalent cancer in world (Jemal *et al*., 2011) It comprises 13% of the total new cancer cases (1.6 million) and 18% of the total cancer deaths (Ferlay *et al*., 2010) (Table 1.8). It is the most commonly diagnosed cancer (17% of the total new cancer cases) and leading cause of death in males (23% of the total cancer deaths) (Jemal *et al*., 2011) (Table 1.6) whereas it is the fourth most commonly diagnosed cancer (8.5%) and the second leading cause of cancer death (12.8%) in females (Ferlay *et al*., 2010; Jemal *et al*., 2011) (Table 1.7).

Table 1.7: Worldwide Incidence, Mortality, 5 years prevalence of top 5 Cancers in both sexes [Incidence and mortality data for all ages, 5-year prevalence for adult population only, ASR (W) and proportions per 100,000] (Ferlay *et al*., 2010**).**

Cancer Type	**Incidence**			**Mortality**			**5 year prevalence**		
	Number	**(%)**	**ASR (W)**	**Number**	**(%)**	**ASR (W)**	**Number**	**(%)**	**Prop**
Lung	1608055	12.7	22.9	1376579	18.2	19.3	1677416	5.8	34.1
Breast	1384155	10.9	38.9	458503	6.1	12.4	5189028	18	210.7
Colorectm	1235108	9.8	17.2	609051	8.1	8.2	3260890	11.3	66.3
Stomach	988602	7.8	14	737419	9.7	10.3	1598440	5.5	32.5
Prostate	899102	7.1	27.9	258133	3.4	7.4	3200372	11.1	130.4
Lung	1608055	12.7	22.9	1376579	18.2	19.3	1677416	5.8	34.1

Table 1.8: Worldwide Incidence, Mortality, 5 years prevalence of different types of Cancers in Males [Incidence and mortality data for all ages, 5-year prevalence for adult population only, ASR (W) and proportions per 100,000] (Fearly *et al*., 2010)

Cancer type	**Incidence**	**Mortality**	**5-year prevalence**

	Number	**(%)**	**ASR (W)**	**Number**	**(%)**	**ASR (W)**	**Number**	**(%)**	**Prop.**
Lung	1092056	16.5	33.8	948933	22.5	29.2	1121619	8.3	45.7
Prostate	899102	13.6	27.9	258133	6.1	7.4	3200372	23.7	130.4
Colorectal	663904	10	20.3	320397	7.6	9.6	1765422	13.1	71.9
Stomach	640031	9.7	19.7	463930	11	14.2	1050306	7.8	42.8
Liver	523432	7.9	16	478134	11.3	14.5	433207	3.2	17.6
Esophagus	326245	4.9	10.1	276007	6.5	8.5	335707	2.5	13.7
Bladder	294345	4.4	8.9	112308	2.7	3.3	904169	6.7	36.8
Non-Hodgkin lymphoma	199736	3	6	1094484	2.6	3.3	427038	3.2	17.4
Leukemia	195456	3	5.8	143555	3.4	4.3	278754	2.1	11.4
Lip, oral cavity	170496	2.6	5.2	83109	2	2.6	401075	3	16.3
Kidney	169155	2.6	5.2	72019	1.7	2.2	466631	3.5	19
Pancreas	144859	2.2	4.4	138377	3.3	4.2	91997	0.7	3.8
Larynx	129651	2	4.1	70336	1.7	2.2	366566	2.7	14.9
Brain, nervous system	126815	1.9	3.8	97251	2.3	2.9	171827	1.3	7
Other pharynx	108588	1.6	3.4	76458	1.8	2.4	229030	1.7	9.3
Melanoma of skin	101807	1.5	3.1	25860	0.6	0.8	385064	2.8	15.7
Gallbladder	58375	0.9	1.8	42949	1	1.3	69252	0.5	2.8
Nasopharynx	57852	0.9	1.7	35984	0.9	1.1	153736	1.1	6.3
Multiple myeloma	54923	0.8	1.7	37795	0.9	1.1	112421	0.8	4.6
Testis	52322	0.8	1.5	9874	0.2	0.3	201562	1.5	8.2
Thyroid	49211	0.7	1.5	11206	0.3	0.3	195097	1.4	7.9
Hodgkin lymphoma	40265	0.6	1.2	18256	0.4	0.5	114537	0.8	4.7

All cancers excl. non-melanoma skin cancer	6617844	100	202.8	4219626	100	127.9	13514868	100	550.6

Table 1.9: Worldwide Incidence, Mortality, 5 years prevalence of different types of Cancers in Females [Incidence and mortality data for all ages, 5-year prevalence for adult population only, ASR (W) and proportions per 100,000] (Fearly *et al*., 2010).

Cancer type	**Incidence**			**Mortality**			**5-year prevalence**		
	Number	**(%)**	**ASR (W)**	**Number**	**(%)**	**ASR (W)**	**Number**	**(%)**	**Prop.**
Breast	1384155	22.9	38.9	458503	13.7	12.4	5189028	33.9	210.7
Colorectal	571204	9.4	14.6	288654	8.6	7	1495468	9.8	60.7
Cervix uteri	530232	8.8	15.2	275008	8.2	7.8	1555341	10.2	63.2
Lung	515999	8.5	13.5	427586	12.8	10.9	555797	3.6	22.6
Stomach	348571	5.8	9.1	273489	8.2	6.9	548134	3.6	22.3
Corpus uteri	288387	4.8	8.2	73854	2.2	1.9	1097620	7.2	44.6
Liver	226312	3.7	6	217592	6.5	5.7	180006	1.2	7.3
Ovary	224747	3.7	6.3	140163	4.2	3.8	549850	3.6	22.3
Thyroid	163968	2.7	4.7	24177	0.7	0.6	667377	4.4	27.1
Non-Hodgkin Lymphoma	156695	2.6	4.2	82115	2.5	2.1	344983	2.3	14
Esophagus	155400	2.6	4.2	130526	3.9	3.4	146438	1	5.9
Leukemia	154978	2.6	4.3	113606	3.4	3.1	221120	1.4	9
Pancreas	133825	2.2	3.3	128292	3.8	3.1	80495	0.5	3.3
Brain, nervous system	111098	1.8	3.1	77629	2.3	2.2	145212	0.9	5.9
Kidney	104363	1.7	2.8	44349	1.3	1.1	277559	1.8	11.3
Melanoma of skin	97820	1.6	2.7	20512	0.6	0.5	370814	2.4	15.1
Lip, oral cavity	92524	1.5	2.5	44545	1.3	1.2	209581	1.4	8.5
Bladder	88315	1.5	2.2	37974	1.1	0.9	268292	1.8	10.9
Gallbladder	86828	1.4	2.2	66638	2	1.7	99206	0.6	4
Multiple myeloma	47903	0.8	1.2	34658	1	0.9	98276	0.6	4
Other pharynx	28034	0.5	0.8	19092	0.6	0.5	59008	0.4	2.4

Hodgkin lymphoma	27654	0.5	0.8	11646	0.3	0.3	81808	0.5	3.3
Nasopharynx	26589	0.4	0.8	15625	0.5	0.4	68975	0.5	2.8
Larynx	21026	0.3	0.6	11556	0.3	0.3	59131	0.4	2.4
All cancer expt non-melanoma skin cancer	6044710	100	164.4	3345176	100	87.2	15288300	100	620.8

1.2 Pharmacogenetics

Pharmacogenetics is the study of the role of inheritance in interindividual and interpopulation variations in drug response (Vogel, 1959; Meyer, 2004; Robert *et al.*, 2005). The rationale and ultimate aim of pharmacogenetics is the possibility that knowledge of an individual's genetic make-up could be used to enhance drug therapy by maximising drug efficacy while minimising drug toxicity (Linder *et al.*, 1997; Meyer, 2000; Lesko and Woodcock, 2004; Weinshilboum and Wang, 2004). The ultimate goal of pharmacogenetics, therefore, is targeted pharmacological treatment of patients based on their genetic determinants of drug efficacy and toxicity, so that they are more likely to respond favourably with fewer or no unfavourable side effects (Evans and Relling, 1999; Evans and Johnson, 2001).

Genetic diversity contributes to both disease susceptibility and variability in response to drug therapy. Pharmacogenomics is a discipline focused on examining the genetic basis for individual variations in response to therapeutics (Firth *et al*., 1986; Butler and Rizza, 1991; Zimmet, 1992; Dinneen *et al*., 1992). Although the task of developing individualized medicines tailored to patient's genotypes poses a major scientific challenge, pharmacogenomics is already starting to influence how physicians or scientists design clinical trials and its impact on the practice of medicine is forthcoming (Kaprio *et al*., 1992; Weyer *et al*., 1999). Recent evidence suggests that most prescribed medications are effective in no more than 60% of the individuals in whom they are used, and a significant number of patients also develop major adverse effects. Better understanding of the genetic factors that regulate patient's responsiveness to drugs is therefore needed to elucidate the molecular mechanisms involved and allow for development of new therapeutic strategies that match each patient and the most suitable drug (Committee on diabetic twins, 1988; Kumar *et al*., 1993; Medici *et al*., 1999). Pharmacogenetics and Pharmacogenomics

constitute an especial young field of research in the domain of pharmacology. Both work on genetic variations which occur in individuals resulting reduced drug efficacy and more adverse drug reactions.

Pharmacogenetics, emphasizes the diversity of patients and their genetic background, set their response to a given drug therapy, making understood the biological variability whereas pharmacogenomic considers the effects they cause in an individual (patient) different medications. The differences are studied on gene expression induction and repression of genes.

Various factors have in the past been held accountable for the observed differences in drug response and include age, concomitant diseases, gender, interactions with other drugs, misdoing, renal and hepatic function, as well as lifestyle variables such as smoking and alcohol consumption (Evans and Relling, 1999; Meisel *et al.*, 2000; Bachmann, 2002; Oscarson, 2003; Schwartz, 2003).

There is, however, an ever-increasing body of evidence that suggests that genetic differences between individuals and even entire populations can be an important and at the same time predominant factor influencing drug response variability (Evans and Relling, 1999; Meyer, 2000; Lesko and Woodcock, 2004).

In fact, it is estimated that genetics is responsible for 15% to 95% (depending on the drug or class of drug) of the observed interindividual variability in drug disposition and effects. This increasing awareness of the significant role that genetic polymorphisms play in drug response variability, together with rapid developments in genomic technologies and the completion of the HGP, has given rise to the field of pharmacogenetics.

As the influence of pharmacogenetics on drug discovery and development and drug treatment regimens increases, there will undoubtedly be a move away from the current approach of standardised treatments towards more individualised, ‘tailor-made’ therapies (Roses, 2000; Liggett, 2004). Despite the fact that this concept of individualised drug treatment seems very helpful and

is largely the product of recent advances in human molecular biology, the scientific foundation on which it is based has a relatively long history.

1.2.1 Benefits of Pharmacogenetics

- **More Powerful Medicines**

The differential gene expressions and proteomics are screening technologies that detect different levels and patterns of gene and protein expression in tissues (Blackstock and Weir, 1999; Kozian and Kirschbaum, 1999). The identification of disease susceptible genes and study of the function of the susceptibility gene variants can be used to identify targets that will be related to the disease in patients and will therefore be validated (Monica and Gorey, 2007). Drugs may be developed targeting specific cells that will maximize therapeutic effects but decrease damage to nearby healthy cells. Pharmaceutical companies will be able to create drugs based on the proteins, enzymes, and RNA molecules associated with genes and diseases. This will facilitate drug discovery and allow drug makers to produce a therapy more targeted to specific diseases (Reid *et al*., 2003).

- **Enhancing efficacy**

Many medicines are not effective for everyone with a particular disease. Some common treatments for diabetes, depression and asthma are only effective in around 60% of patients. Pharmacogenetics could allow doctors to prescribe medicine only for those patients most likely to respond. Alternatively, new medicines could be designed on the basis of genetic information about the cause of disease (McLeod and Evan, 2001).

- **Advanced Screening for Disease**

Technologies such as differential gene expression, transgenic animal models, proteomics, in-situ hybridization and immuno-histo chemistry are used to imply relationships between a gene and a disease process (Roses, 2000). Knowing one's genetic code will allow a person to make adequate lifestyle and environmental changes at an early age so as to avoid or lessen the severity of a genetic disease. Likewise, advance knowledge of particular disease susceptibility will allow

careful monitoring, and treatments can be introduced at the most appropriate stage to maximize their therapy.

- **Improvements in the Drug Discovery and Approval Process**

To discover a new drug sometimes many targets are chosen on the basis of scientific hypotheses and do not lead to effective medicines because the initial hypotheses are subsequently disproved (Curran, 1998; Marton *et al.*, 1998). However, using pharmacogenetics a limited number of molecular target families have been identified, including the receptors and enzymes, for which high through put screening is now possible. A good target is one against which many compounds can be screened rapidly to identify active molecules (hits). These hits can be developed into optimized molecules (leads), which have properties of well-tolerated and effective medicines (Monica and Gorey, 2007).

The drug approval process should be facilitated as trials are targeted for specific genetic population groups --providing greater degrees of success. The cost and risk of clinical trials will be reduced by targeting only those persons capable of responding to a drug.

- **Decrease in the Overall Cost of Health Care**

Decreases in the number of adverse drug reactions, the number of failed drug trials, the time it takes to get a drug approved, the length of time patients are on medication, the number of medications patients must take to find an effective therapy, the effects of a disease on the body (through early detection), and an increase in the range of possible drug targets will promote a net decrease in the cost of health care.

- **Better Vaccines**

Vaccines made of genetic material, either DNA or RNA; promise all the benefits of existing vaccines without all the risks. They will activate the immune system but will be unable to cause infections. They will be inexpensive, stable, easy to store, and capable of being engineered to carry several strains of a pathogen at once.

- **Safer Drugs**

Instead of the standard trial-and-error method of matching patients with the right drugs, doctors will be able to analyze a patient's genetic profile and prescribe the best available drug therapy from the beginning. Not only will this take the guesswork out of finding the right drug, it will speed recovery time and increase safety as the likelihood of adverse reactions is eliminated. Pharmacogenetics has the potential to dramatically reduce the estimated 100,000 deaths and 2 million hospitalizations that occur each year in the United States as the result of adverse drug response.

- **More Accurate Methods of Determining Appropriate Drug Dosages**

The other anticipated benefits of pharmacogenetics include more accurate methods of determining appropriate drug dosages. The ability of a person's body to process a drug that is his genetic profile will form the basis for dosage calculations instead of the weight and age of the patient (Monica and Gorey, 2007).

1.2.2 Genetic Polymorphism of human

A genetic polymorphism is defined as a deoxyribonucleic acid (DNA) sequence variant which is stable within a population and occurs with a frequency equal to or greater than 1% (Bachmann, 2002; Lash *et al.*, 2003; Nebert and Vessell, 2004). There is a considerable level of variability between individuals at the genetic level, as manifested by the polymorphisms present within their genome (Sachidanandam *et al.*, 2001; Oscarson, 2003). Over 90% of these polymorphisms are believed to be accounted for by changes in a single nucleotide, namely Single nucleotide polymorphisms (SNPs), with the remainder of the variation caused by insertions and deletions (indels), variable number tandem repeats (VNTRs) and microsatellites (Quirk *et al.*, 2004; Marsh and McLeod, 2006). However, unlike many other previously characterized polymorphisms, such as VNTRs and microsatellites, SNPs are often found within the coding and regulatory regions of genes and thus can have functional consequences for gene expression and gene product functionality (Campbell *et al.*, 2000; Gray *et al.*, 2000).

Genetic polymorphisms are natural variations in the genomic DNA sequence present in at least or more than 1% of the population. For example, a place in the genome where 93 percent of

people have a T and the remaining 7 percent have an A is a polymorphism. Genetic polymorphisms in therapeutic targets and metabolic pathways can significantly impact both drug efficacy and toxicity.

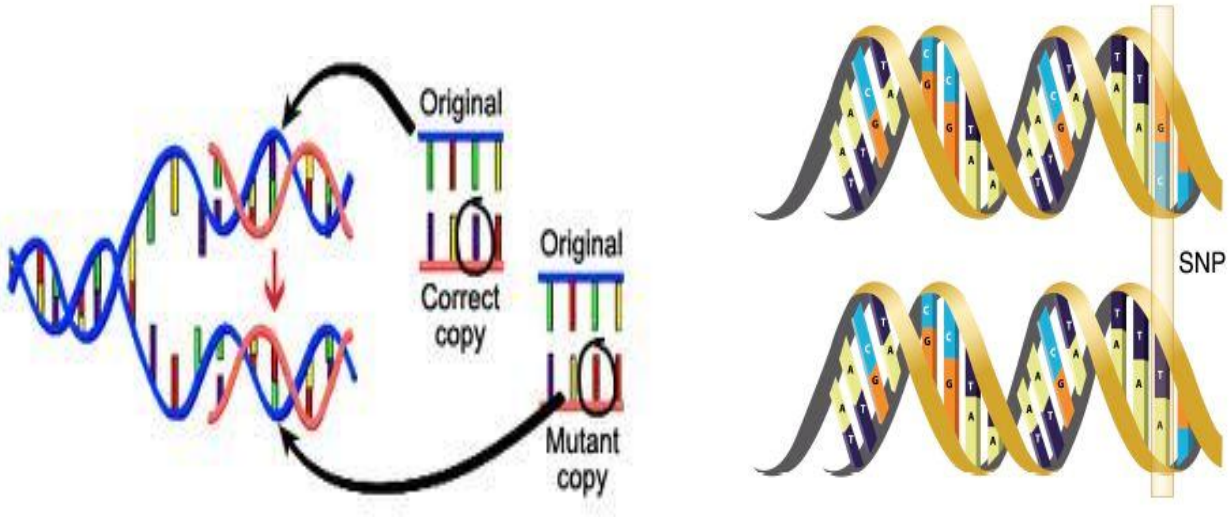


Figure 1.4: Mutation, SNP & Polymorphism (Broad Institute of Harvard & MIT)

If one of the possible sequences is present in less than 1 percent of people (99.9%) of people have a G and 0.1 percent have a C), then the variation is called a mutation. Informally, the term mutation is often used to refer to a harmful genome variation that is associated with a specific human disease, while the word polymorphism implies a variation that is neither harmful nor beneficial. Many pharmacogenetic polymorphisms have been identified, but the most frequent genetic changes being single nucleotide polymorphisms (SNPs), almost 90% (Rongcun and Richard, 2003; Rob *et al.*, 2003).

Other types of genetic polymorphism include variation caused by insertions and deletions (indels), variable number tandem repeats (VNTRs) and microsatellites (Quirk *et al.*, 2004; Marsh and McLeod, 2006).

These polymorphisms may be present in coding and noncoding regions, potentially altering protein function or abundance of key regulatory molecules, and thereby influencing the efficacy and toxicity of therapy or resulting in predispostion to diseases (Rongcun and Richard, 2003).

Genetic Mutation is a change in the nucleotide sequence of a DNA molecule. Genetic mutations are a kind of genetic polymorphism. The term "mutation," as opposed to "polymorphism," is generally used to refer to changes in DNA sequence which are not present in most individuals of a species and either have been associated with disease (or risk of disease) or have resulted from damage indicted by external agents (such as viruses or radiation).

Recent studies have indeed suggested that the presence of sequence variants, such as pSNPs, within intronic regions could affect basic preliminary-mRNA (pre-mRNA) splicing mechanisms and thereby cause altered levels of normal transcripts (Pagani *et al.*, 2003). A pSNP within the 3′-untranslated region (UTR) following the coding sequence may affect the intracellular stability of the mRNA gene transcript (Quirk *et al.*, 2004).

1.2.3 Single Nucleotide Polymorphism

A single nucleotide polymorphism is a source variance in a genome. A SNP (snip) is a single base mutation in DNA. SNPs are the most simple form and most common source of genetic polymorphism in the human genome (90% of all human DNA polymorphism) (Lippert *et al.*, 2002).

There are two types of nucleotide base substitutions resulting in SNPs:

- **A transition substitution** occurs between purines (A, G) or between pyrimidines (C, T). This type of substitution constitutes two third of all SNPs. (Schwartz *et al.*, 1996*).*
- **A transversion substitution** occurs between a purine and a pyrimidine.

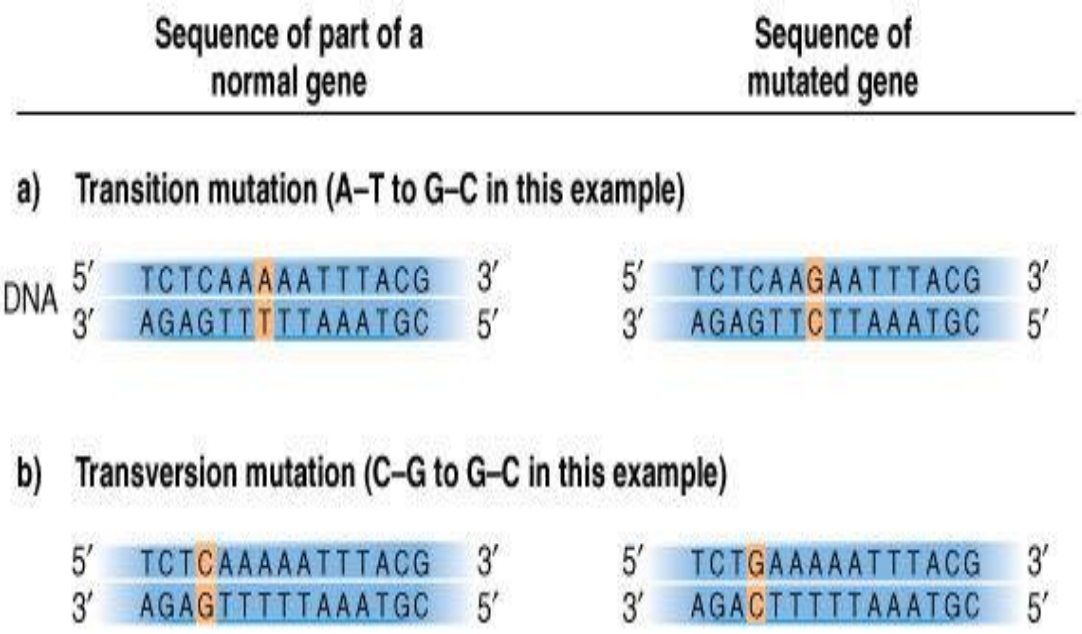


Figure 1.5: Schematic of SNP

1.2.4 Position of SNP

SNPs are found throughout the genome, e.g. in exons, introns, intergenic regions, in promoters or enhancers, etc. Hence, they are more likely to yield, upon collection, a functional or physiologically relevant allele than other sorts of polymorphism. What is of extreme interest in this regard is the nature of the effect that a simple base pair substitution can have on a trait or disease. Thus, a SNP in coding region may directly impact a relevant protein, an intronic SNP can influence splicing, (Krawezak *et al*., 1992) a SNP in a promoter can influence gene expression etc (Drazen *et al*., 1999). The degree to which each kind of SNP influences phenotypic expression is likely to receive a great deal of attention as more and more SNPs are identified and studied.

1.2.5 Distribution of SNP

SNPs are not uniformly distributed over the entire human genome, neither over all chromosomes and neither within a single chromosome (Guo and Jamison, 2005). There are one third as many SNPs within coding regions as non-coding region SNPs. It has also been shown that sequence

variation is much lower for the sex chromosomes. Within a single chromosome, SNPs can be concentrated about a specific region, usually implying a region of medical or research interest. For instance, the sequence that encodes proteins that present antigens to the immune system in chromosome 6 displays very high nucleotide diversity compared to the other areas of that chromosome.

1.2.6 Sequence Variation

Sequence variation caused by SNPs can be measured in terms of nucleotide diversity, the ration of the number of base differences between two genomes over the number of bases compared. This is approximately 1/1000 (1/1350) base pairs between two equivalent chromosomes (Lancia *et al*., 2001).

1.2.7 SNP discovery and SNP genotyping

SNP analysis techniques fall into two distinct classes:

- SNP discovery
- SNP genotyping (SNP scoring).

SNP discovery is the detection of novel polymorphisms, while SNP genotyping is the identification of specific alleles in a known polymorphism (Weaver, 2000).

Classical SNP discovery methods discriminate between DNA strands carrying alternative alleles without necessarily identifying either the exact position of the polymorphism or the allele present. For example, single-strand conformational polymorphism (SSCP) analysis exploits the ability of single DNA strands to fold into complex tertiary structures through intramolecular base pairing.

The tertiary structures of strands carrying alternative bases at a given position will differ and this will affect their electrophoretic mobility in a non-denaturing gel. The exact position of the polymorphism is then confirmed by DNA sequencing which may be carried out in the traditional fashion, by chain termination, or using oligonucleotide chips (sequencing by hybridization, resequencing).

Also many SNPs were initially detected by comparing the sequences of genomic clones or expressed sequence tags (ESTs) deposited in public and proprietary databases.

1.2.8 Coding region of SNP

A SNP in a coding region may have two different effects on the resulting protein:

- **Synonymous** the substitution caused no amino acid change to the protein it produces. This is also called a silent mutation.
- **Non-Synonymous** the substitution results in an alteration of the encoded amino acid (Nei and Kumar , 2000). A missense mutation changes the protein by causing a change of codon. A nonsense mutation results in a misplaced termination codon. One half of all coding sequence SNPs results in non-synonymous codon changes.

SNPs may occur in regulatory regions of genes. These SNPs are capable of changing the amount of timing of a proteins production. Such SNPs are much more difficult of find and understand and gene regulation itself is not yet clearly understood.

1.2.9 STRs, VNTRs

Other types of genetic polymorphism result from the insertion or deletion of a section of DNA. The most common type of such 'insertion/deletion' polymorphism is the existence of variable numbers of repeated base or nucleotide patterns in a genetic region. Repeated base patterns range in size from several hundreds of base pairs, known as 'variable number of tandem repeats' (VNTRs or 'minisatellites'), to the more common 'microsatellites' consisting of two, three or four nucleotides repeated some variable number of times (Quirk *et al.,* 2004; Marsh and McLeod, 2006). Microsatellites are often referred to as 'simple tandem repeats' (STRs). Repeat polymorphisms often result in many alleles or variants (e.g. several different repeat sizes) within the population and are thus considered 'highly polymorphic'.

1.2.10 Restriction Fragment Length Polymorphisms (RFLPS)

One of the earliest and most widely used genotyping methods, restriction fragment length polymorphism (RFLP) analysis, works on the principle of allele-specific enzymatic cleavage. An RFLP is generated when an SNP occurs at a restriction endonuclease recognition sequence, and one allele preserves the sequence while the other destroys it. If we consider any DNA fragment with three adjacent restriction sites, with the middle one containing an SNP, then digestion of amplified genomic DNA with the appropriate restriction endonuclease will produce either a single large fragment (if the central restriction site is absent) or two smaller fragments (if the central restriction site is present and cleavage occurs). The fragments are then separated by gel electrophoresis can be compared to others to detect the differences. These differences are called restriction fragment length polymorphisms (RFLPs) (Saiki *et al*., 1985, Osborn *et al*., 2000).

1.2.11 Importance of Genetic Polymorphism in Clinical & Pathological Investigation

The study of polymorphism has many uses in medicine, biological research, and law enforcement. Genetic diseases may be caused by a specific polymorphism. Scientists can look for these polymorphisms to determine if a person will develop the disease, or risks passing it on to his or her children. Besides being useful in identifying people at risk for a genetically based disease, knowledge of polymorphisms that cause disease can provide valuable insight into how the disease develops (Smith *et al*., 1998). Polymorphisms located near a disease gene can be used to find the gene itself, through mapping. In this process, researchers look for polymorphisms that are co inherited with the disease. By finding linked polymorphisms on smaller and smaller regions of the chromosome, the chromosome region implicated in the disease can be progressively narrowed, and the responsible gene ultimately can be located (Harris, 1980)

A related use of polymorphism is widely employed in agriculture. If a polymorphism can be identified that is associated with a desirable characteristic in an agriculturally important plant or animal, then this polymorphism can be used as a genetic flag to identify individuals that have the desirable characteristic (Clegg, 1986). Using this technique, known as marker-assisted selection, breeding programs aimed at improving agriculturally important plants and animals can be made

more efficient, since individuals that have the desired trait can be identified before the trait becomes apparent.

Polymorphisms can be used to illuminate fundamental biological patterns and processes. By studying polymorphisms in a group of wild animals, the familial relationships (brother, sister, mother, father, etc.) between them can be determined. The amount of interbreeding between different groups of the same species (gene flow) can be estimated by studying the polymorphisms they contain. This information can be used to identify unique populations that may be important for survival of the species. Sometimes it is not immediately obvious if two different groups of organisms should be classified as different species.

1.3 Pharmacogenomics of drug metabolizing, BER and NER enzyme

The personalized medicine integrates the uniqueness of an individual with respect to the pharmacokinetics and pharmacodynamics of a drug. It holds promise as a means to provide greater safety and efficacy in drug design and development which is particularly important in oncology whereby most clinically used anticancer drugs have a narrow therapeutic window and exhibit a large interindividual pharmacokinetic and pharmacodynamic variability. This variability can lead to therapeutic failure or severe toxicity. Understanding of how genetic variations influence drug disposition and action could help in tailoring cancer therapy based on individual's genetic makeup. Pharmacogenomics is the study of how variations in the human genome affect the response to medications (Marsh and McLeod, 2006). Each drug, after it enters the body, interacts with numerous proteins, such as carrier proteins, transporters, metabolizing enzymes, and multiple types of receptors. These protein interactions determine drug pharmacokinetics (i.e., drug absorption, distribution, metabolism, and excretion) and pharmacodynamics (i.e., target site of action, pharmacological and toxicological effects).Drugs trigger downstream secondary events may impact additional gene or protein expression responses and can also vary among patients. As a result, the overall response to a drug is determined by the interplay of multiple genes that are involved in the pharmacokinetic and pharmacodynamic pathways of a drug. In general, important genetic variation in drug effect can be envisioned at the level of drug metabolizing enzymes, drug transporters and drug targets (Evans and Relling, 1999).

1.3.1 Drug metabolizing-enzymes

Drug metabolizing enzymes are proteins which catalyze the biochemical modifications of xenobiotics (eg, drugs) and endogenous chemicals (e.g., hormones, neurotransmitters). They are divided into two categories: Phase I (functionalizing) enzymes that introduce or remove functional groups in a substrate through oxidation, reduction, or hydrolysis; and Phase II (conjugating) enzymes that transfer moieties from a cofactor to a substrate. Essentially all of the major human metabolizing enzymes exhibit genetic polymorphisms at the genomic level. Many of these enzymes have clinically relevant genetic polymorphisms (Evans and Relling, 1999). A gene is considered to be polymorphic when the frequency of a variant allele in a specific population is at least 1%.

- **Phase I enzymes**

Phase I metabolizing enzymes include those involved in:

- Oxidation – cytochrome P450, alcohol dehydrogenase, aldehyde dehydrogenase, dihydropyrimidine dehydrogenase, monoamine oxidase, and flavin-containing monooxygenase;
- Reduction – nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase and reduced cytochrome P450;
- Hydrolysis – epoxide hydrolase, esterases, and amidases.

- **Phase II enzymes**

The most important Phase II enzymes that exhibit functional and clinical relevant genetic polymorphisms are uridine diphosphate glucuronosyltransferase (UGT), sulfotransferase (SULT), glutathione S-transferases (GST), N-acetyltransferase (NAT), and thiopurine methyltransferase (TPMT).

- **Glutathione S-transferases (GST)**

The super family of human GST catalyzes the conjugation of glutathione (GSH) to a wide range of endogenous metabolites and xenobiotics. The common metabolites are alkylating and free radical generating anticancer drugs (Lo and Ali-Osman, 2007). Glutathion S Transferases are categorized into three main families: cytosolic/nuclear, mitochondrial, and microsomal. The cytosolic GSTs are further divided into seven classes: alpha, mu, omega, pi, sigma, theta, and zeta. Besides their enzymatic function, GSTs also possess nonenzymatic functions, in which they act as regulators of cell signaling and posttranslational modification pathway in response to stress, growth factors, and DNA damage, and in cell proliferation, cell death, and other processes that ultimately lead to tumor growth and drug resistance. In determinants of cancer susceptibility, therapeutic response, and prognosis, GSTs have greater importance (Lo and Ali-Osman, 2007).

1.3.2 Base Excision repair enzyme

Base excision repair (BER) is a cellular mechanism that repairs damaged DNA throughout the cell cycle which is responsible primarily for removing small, non-helix-distorting base lesions from the genome (Seeberg *et al*., 1995). It is important for removing damaged bases that could otherwise cause mutations by mispairing or lead to breaks in DNA during replication. Such damage typically results from deamination, oxidation, or methylation. Much of the damage is the result of spontaneous decay of DNA, although similar damage may also be caused by environmental chemicals, radiation, or treatment with cytostatic drugs. BER is initiated by DNA glycosylases, which recognize and remove specific damaged or inappropriate bases, forming AP sites. These are then cleaved by an AP endonuclease. The resulting single-strand break can then be processed by either short-patch or long-patch BER.

1.3.3 Nucleotide Excision repair enzyme

Nucleotide excision repair is a DNA repair mechanism. DNA damage occurs constantly because of chemicals such as intercalating agents, radiation and other mutagens. Three excision repair pathways exist to repair single stranded DNA damage: Nucleotide excision repair (NER), base excision repair (BER), and DNA mismatch repair (MMR) (Wood *et al*., 2001). While the BER

pathway can recognize specific non-bulky lesions in DNA, it can correct only damaged bases that are removed by specific glycosylases.

It is a particularly important excision mechanism that removes DNA damage induced by ultraviolet light which results in bulky DNA adducts - these adducts are mostlythymine dimers and 6,4-photoproducts. Recognition of the damage leads to removal of a short single-stranded DNA segment that contains the lesion. The undamaged single-stranded DNA remains and DNA polymerase uses which is a template to synthesize a short complementary sequence. Final ligation to complete NER and form a double stranded DNA is carried out by DNA ligase. It can be divided into two subpathways: global genomic NER (GG-NER or GGR) and transcription coupled NER (TC-NER or TCR). The two sub pathways differ in how they recognize DNA damage but they share the same process for lesion incision, repair, and ligation. The importance of NER is evidenced by the severe human diseases that result from in-born genetic mutations of NER proteins.

It has been suggested to be the main cellular defense mechanism against platinum-induced intra-strand crosslinks in DNA repair pathway (Kalikaki *et al*., 2009). Genes involve in NER pathway, including its up-stream genes, are considered as candidate genes for prediction of different response to platinum-based chemotherapy in NSCLC patients (Behera *et al*., 2012). Some SNPs have been acted as important factors contributing to the activity of NER pathways. Thus it would influence platinum-based outcome in NSCLC patients.

1.4 The drugs: Platinum based drugs

Platinum is cytotoxic drug. It disrupts double-stranded DNA in cells by forming platinum-DNA adducts. These adducts cause interstrand crosslinking, induce bulky distortion of DNA, and inhibit DNA replication (Rudd *et al.*, 1995; Poklar *et al.*, 1996). These DNA lesions can be mended by DNA repair mechanisms; therefore, the activities of repair molecules can play a role in clinical outcomes, response to chemotherapy, and survival of patients treated with platinum-based chemotherapy (Johnson, 2001)

The platinum based drugs are cisplatin ,cicisplatinum, platamin, neoplatin, cismaplat or *cis*-diamminedichloridoplatinum (II) (CDDP). Cisplatin was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum

complexes react in the body, binding to DNA and causing the DNA strands to crosslink, which ultimately triggers cells to die in a programmed way.

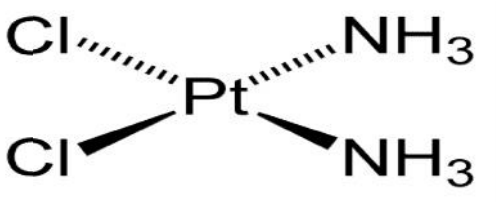


Figure 1.6: Structure of Cisplatin

It is one of the most potent antitumor agents for a wide variety of solid tumors. Its cytotoxic mode of action is mediated by its interaction with DNA to form DNA adducts, primarily intrastrand crosslink adducts, which activate several signal transduction pathways, including those involving ATR, p53, p73, and MAPK, and culminate in the activation of apoptosis. DNA damage-mediated apoptotic signals, however, can be attenuated, and the resistance that ensues is a major limitation of cisplatin-based chemotherapy. The mechanisms responsible for cisplatin resistance are several, and contribute to the multifactorial nature of the problem. Resistance mechanisms that limit the extent of DNA damage include reduced drug uptake, increased drug inactivation, and increased DNA adduct repair. Origins of these pharmacologic-based mechanisms, however, are at the molecular level. Mechanisms that inhibit propagation of the DNA damage signal to the apoptotic machinery include loss of damage recognition, over expression of HER-2/neu, activation of the PI3-K/Akt (also known as PI3-K/PKB) pathway, loss of p53 function, overexpression of antiapoptotic bcl-2, and interference in caspase activation. The molecular signature defining the resistant phenotype varies between tumors, and the number of resistance mechanisms activated in response to selection pressures dictates the overall extent of cisplatin resistance.

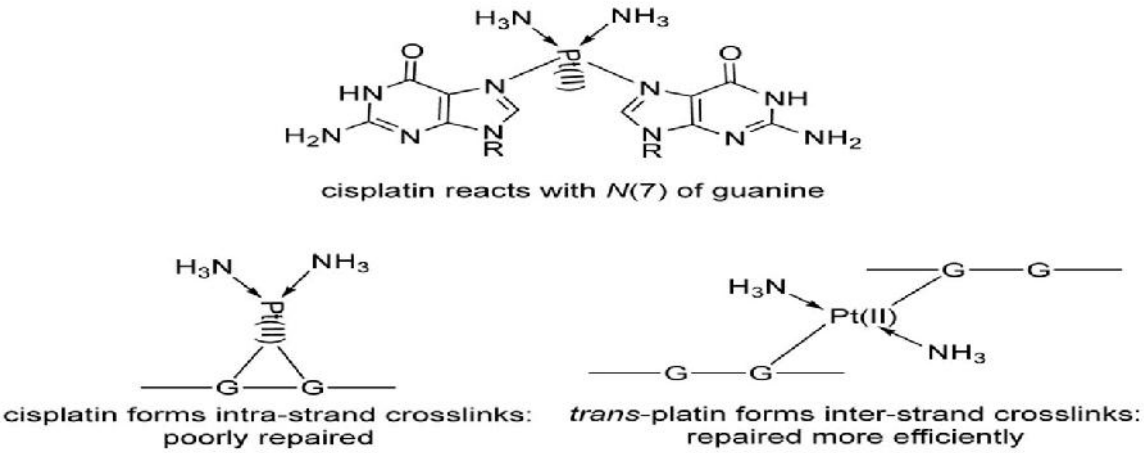


Figure 1.7: Cisplatin causes DNA lesions by forming intra-strand and inter-strand cross-links that result in DNA distortion and inhibition of DNA replication.

Carboplatin,or cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) is a drug used against some forms of cancer mainly ovarian carcinoma, lung, head and neck cancers as well as endometrial, esophageal, bladder, breast and cervical; central nervous system or germ cell tumors; osteogenic sarcoma, and as preparation for a stem cell or bone marrow transplant (Knox *et al*., 1986; Natarajan *et al*., 1999). It was introduced in the late 1980s and has since gained popularity in clinical treatment due to its vastly reduced side effects compared to its parent compound cisplatin.

Figure 1.8: structure of carboplatin

Relative to cisplatin, the greatest benefit of carboplatin is its reduced side effects, particularly the elimination of nephrotoxic effects.Nausea and vomiting are less severe and more easily controlled. The main drawback of carboplatin is its myelosuppressive effect. This causes

the blood cell and platelet output of bone marrow in the body to decrease quite dramatically, sometimes as low as 10% of its usual production levels. The nadir of this myelosuppression usually occurs 21–28 days after the first treatment, after which the blood cell and platelet levels in the blood begin to stabilize, often coming close to its pre-carboplatin levels. This decrease in white blood cells (neutropenia) can cause complications, and is sometimes treated with drugs like filgrastim. The most notable complication of neutropenia is increased probability of infection by opportunistic organisms, which necessitates hospital readmission and treatment with antibiotics. It is less potent than cisplatin; depending on the strain of cancer, carboplatin may only be 1/8 to 1/45 as effective. The clinical standard of dosage of this drug is usually a 4:1 ratio compared to cisplatin; that is, for a dose that usually requires a particular dose of cisplatin, four times as much carboplatin is needed to achieve the same effectiveness. The stable property of carboplatin is a mixed blessing: once uptake of the drug occurs, its retention half-life is considerably longer than cisplatin, but it is also this inertness that causes carboplatin to go right through the human body, and up to 90% of the carboplatin given can be recovered in urine.

1.5 The gene: GSTP1, XRCC1, XPC and ERCC1

1.5.1 The gene: GSTP1

Glutathion S transferase is Phase II metabolising enzyme. This type of enzyme has a advantage of carrying electrophilic groups intrinsically in a structure, or obtained from phase I metabolism, for making conjugation of xenobiotics with donor molecules like glutathione (GSH), UDP glucuronic acid, or 3_-phosphoadenosine-5_- phosphosulfate (PAPS). This enzyme possesses catalytic properties for detoxifying endogenous reactions with GSH to protect cellular macromolecules from damage resulted from the toxic effect of a wide range of endogenous and exogenous molecules such as cytotoxic, mutagens, carcinogens, and chemotherapeutic agents. The resulting glutathione adducts get increased property of the solubility and polarity leading to either excretion or further metabolism. The human cytosolic GST subunits are divided into seven gene families in accordance with their biochemical characteristics as well as amino acid sequence similarities: (GSTA), (GSTM), (GSTT), (GSTP), (GSTO), (GSTZ), and (GSTS) (Board *et al*., 1997). GSTP1 is the most abundant subunit which is found ubiquitously in different human epithelial tissue. It gets conjugation to provide protection particularly against the

cytotoxic effects of different chemotherapeutic agents, such as anthracyclines, alkylating agents, and their metabolites. It pushes forth the hypothesis that allelic variation in the gene associated with less effective detoxification of potential anticancer agents may result in an enhanced sensitivity to chemotherapy. Glutathione S-transferase P1 [GSTP1, A single nucleotide substitution (A→G) at position 313, which results in replacement of isoleucine (Ile) with valine (Val) at codon 105] is linked to the sensitivity of cancer cells to platinums (Watson *et al*., 1998; Peklak-Scott *et al*., 2008).

1.5.2 The gene: XRCC1

X-ray repair cross-complementing group 1 (XRCC1) protein plays a key role in base excision repair pathway which serves as a scaffold protein in both single-strand break repair and base excision repair activities (Lindahl and Wood, 1999). The transcription in XRCC1 has shown a significant correlation with platinum resistance among NSCLC cell lines (Weaver *et al*., 2005). XRCC1 protein could bind to platinum-containing DNA duplexes (Zhu and Lippard, 2009). In erythrocytes, human placental aflatoxin B1 (AFB1-DNA) adducts have been shown to respond to environmental insults and somatic glycophorin A (GPA) variants have been shown to respond to tobacco smoking. It has been reported that the 399Gln allele is significantly associated with higher levels of this type of genotoxic damage which suggest that the Arg399Gln amino acid variant may alter the phenotype of the XRCC1 protein, causing deficient DNA repair (Lunn *et al*., 1999).

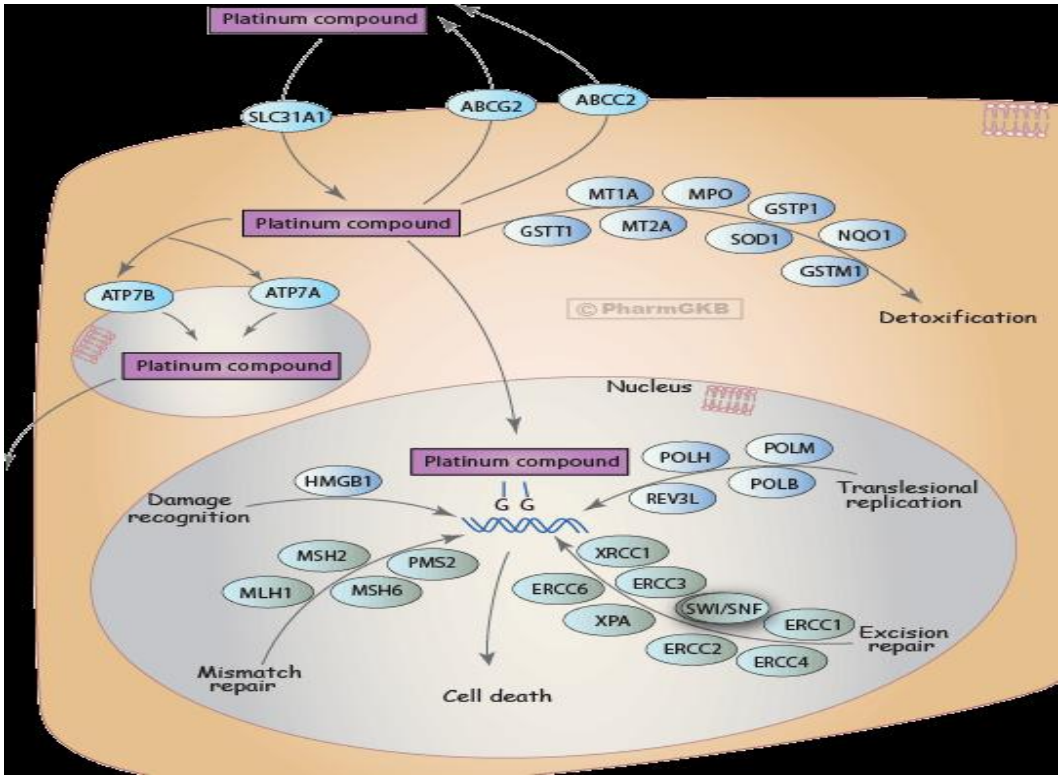


Figure 1.9: Role of GSTP1, XRCC1 and ERCC1 gene in metabolism of platinum based drug

1.5.3 The gene: XPC

Xeroderma pigmentosum group C (XPC) is a nuclear excision repair gene which is mainly involved in the recognition of DNA damage. It plays an important role in the repair of bulky lesions, such as pyrimidine dimmers, photoproducts, larger chemical adducts and cross-links, and in the maintenance of genomic stability (Sugasawa *et al*., 1998; Min and Pavletich, 2007). It gene spans 33 kb and includes 16 exons (82–882 bp) and 15 introns (0.08–5.40 kb) on chromosome 3p25 and encodes a protein containing 940 amino acids which is linked to the sensitivity of cancer cells to platinums. The most common polymorphisms, Lys939Gln (rs2228001) in exon 15 and a poly (AT) insertion/deletion polymorphism in intron 9 which has been associated with an increased risk of many human malignancies (Francisco *et al*., 2008). Individuals homozygous for the *XPC* PAT polymorphism have an increased risk of developing lung cancer (Marin *et al*., 2004) where as PAT polymorphism in the *XPC* gene has been associated with an increased risk of developing different types of cancer, including smoking-

related cancers (Shen *et al*., 2001; Casson *et al*., 2005; Kietthubthew *et al*., 2006) or melanoma (Blankenburg *et al*., 2005).

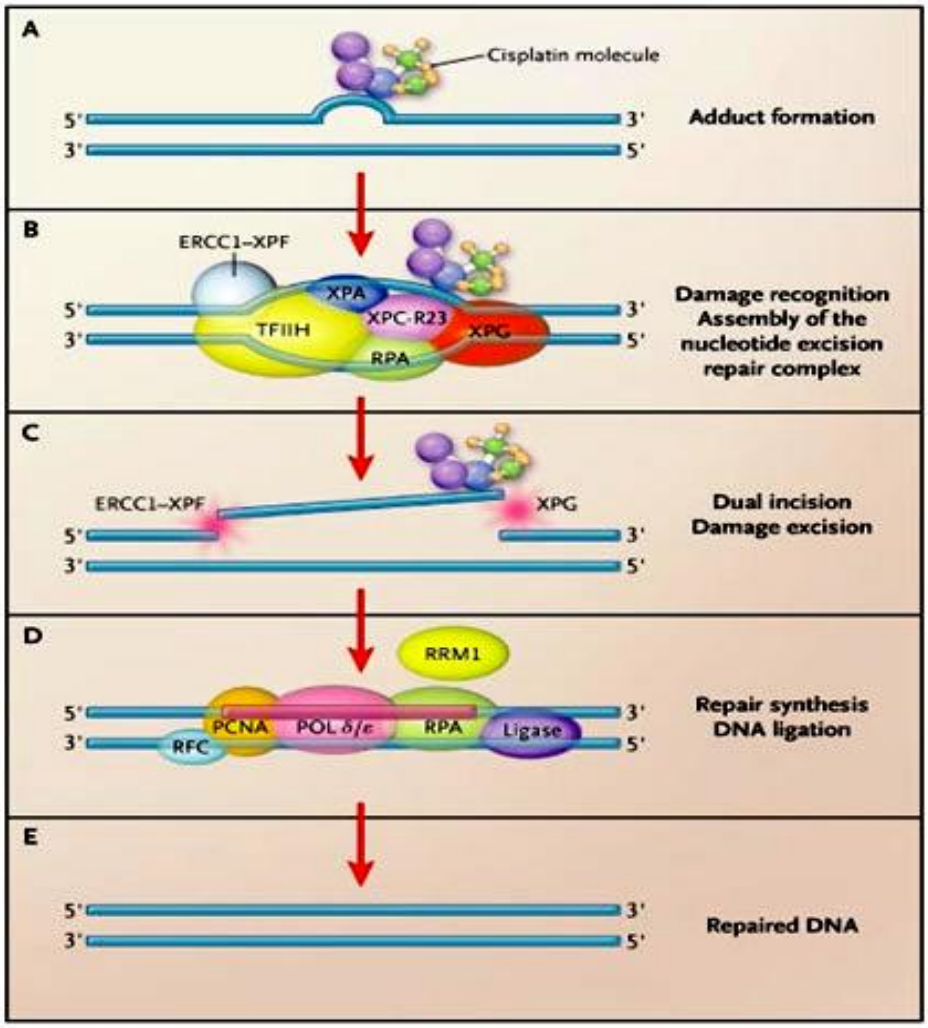


Figure 1.10: Role of XPC gene in metabolism of platinum based drug

1.5.4 The gene: ERCC1

Excision repair cross-complementing group 1 (ERCC1) protein plays important role in nucleotide excision repair pathway by repairing DNA damage and adducts. Single nucleotide polymorphisms of DNA repair genes are suspected to influence the risk of lung cancer. This

gene is located in chromosome 19q13.2. SNPs in exons of DNA repair genes may influence their protein activity, resulting in differences of individual NER and DNA repair capacity (DRC) that may affect the susceptibility of lung cancer. The common polymorphism of ERCC1 gene is at codon 118 (C > T substitution at exon 4, without amino acid change--Asn/Asn, rs11615).

Loss of heterozygosity of NER genes has been observed in lung carcinomas which reduce mRNA expression. NER genes have been observed in peripheral blood lymphocytes (PBLs) from lung cancer patients. The expression of NER genes in PBLs may be used as a surrogate for estimating expression levels of these genes in proliferating tissues (Cheng *et al*., 2000). In a host-cell reactivation assay, ERCC1 mRNA levels in PBLs correlated with DNA repair capacity (DRC). Reduced DRC is related to lung oncogenesis (Wei *et al*., 1996), while elevated DRC has been related to chemoresistance in NSCLC (Zeng-Rong *et al*., 1995; Bosken *et al*., 2002). Low levels of ERCC1 expression also correlated with significantly longer survival in gemcitabine–cisplatin-treated advanced NSCLC patients (Lord *et al*., 2002).

1.6 Justification of the study

Platinum-based chemotherapy is considered as the first-line treatment for non small cell lung cancer, but it is relatively insensitive to chemotherapy compare to SCLC (Jemal *et al*., 2011). So more attention should take to improve the chemotherapy efficacy in NSCLC patients (Harrington and Smith, 2008). Cisplatin, carboplatin and oxaliplatin are the first-, second- and third-generation of platinum-containing anti-cancer chemotherapy drugs, respectively combined with other chemotherapeutic agents like gemcitabine, vinorelbine, etoposide, paclitaxel, docetaxel and pemet (Program, 2011). However, both of the chemotherapy resistance and platinum-induced toxicities such as anemia, leucopenia, neutropenia, thrombocytopenia and gastrointestinal symptoms such as vomiting, anorexia and nausea are common. So it is the major obstacle for successful chemotherapy in NSCLC patients (Monneret, 2011).

Personalized medicine tailors intervention to individual variation in risk and treatment response. It has been considered as one of the potential medicine models that will greatly improve the clinical efficacy of platinum-based chemotherapy. The use of genetic information has been played a major role in the design of personalized medicine (Guo, 2010). Personalized medicines

have been widely researched in several complex diseases, such as diabetes, seizures, and so on. As the SNP detection technologies and integrative network-based computational methods are rapidly developed, more researches have investigated the potential ability of using certain SNPs to predict the treatment response and chemo toxicities in cancer patients.

Polymorphism of GSTP1, XRCC1, XPC and ERCC1 genes might cause a large inter individual difference in the plasma concentration of drugs. In addition, anticancer therapies are notoriously reported due to having a narrow therapeutic range. Higher concentration in the patient's body causes toxicity and a lower concentration reduces the efficacy of the drugs. So the role of pharmacogenomics, which is expected to provide a predictive way for severe drug toxicity, comes to the front line.

GSTP1 gene is crucial for responsible to the cell defence system. These phase II detoxification enzymes are involved in the detoxification of a variety of chemotherapeutics including platinum. The role of GSTP1 in the detoxification of platinum based drugs indicates the possible implication of those polymorphisms to the efficacy of those chemotherapeutic agents. A313G in exon 5 is one of the common single nucleotide polymorphisms in GSTP1that lead to amino acid substitutions. In addition, studies suggest that the variant genotype results in diminished enzymatic activity. Thus, individuals with variant GSTP1 genotypes may possess increased susceptibility to cancer but good responses to chemotherapy due to decreased detoxification of carcinogens and chemotherapeutic agents.

XRCC1 is a key component of the BER pathway (Hoeijmakers, 2001; Whitehouse *et al*., 2001). It is involved in the repair of other types of DNA damage caused by platinum, including DNA double-strand breaks (Audebert *et al*., 2004). This protein is critical for repairing DNA damage induced by the platinum based anticancer drugs cisplatin (DDP) and carboplatin (CBP) (Mohrenweiser *et al*., 2002) which may markedly impact the efficacy of platinum-based therapy against NSCLC. Additionally, the XRCC1 transcript abundance level has been demonstrated to correlate with cisplatin chemoresistance in NSCLC cell lines (Weaver *et al*., 2005). Therefore, it may have some relationship with the efficacy of platinum chemotherapy. Theoretically, genetic polymorphisms in this gene should modulate DNA repair capacity and hence influence the

anticancer effect of platinum drugs and reveal platinum sensitivity in NSCLC patients. Several recent studies have found the relationship between XRCC1 polymorphisms and clinical outcome of platinum-containing drugs in non small cell lung cancer patients (Gao *et al*., 2006; Jin *et al*., 2006; Hong *et al*., 2009; Sun *et al*., 2009; Ding *et al*., 2010; Qian *et al*., 2010).

XPC and ERCC1 are nucleotide excision repair genes. NER pathway is a major form of DNA repair pathway in which the main cellular defense mechanism is occured against platinum-induced intra-strand crosslinks in DNA repair pathway (Kalikaki *et al*., 2009). Genes involve in NER pathway are considered as candidate genes for prediction of different response to platinum-based chemotherapy in NSCLC patients (Behera *et al*., 2012). Polymorphisms of the XPC gene may contribute to variations in DNA repair capacity and in the genetic susceptibility to different cancers. Many studies have found that polymorphisms in XPC are associated with the risk of esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma, squamous cell carcinoma of the head and neck, breast cancer, renal cell carcinoma, bladder cancer, advanced colorectal adenoma, oral squamous cell carcinoma, lung cancer, and pancreatic adenocarcinoma (Vogel *et al*., 2005; Hirata *et al*., 2006; Huang *et al*., 2006; Kietthubthew *et al*., 2006; Kristensen *et al*., 2006; Zhou *et al*., 2006; An *et al*., 2007; Figueroa *et al*., 2007). But the relationship between XPC polymorphism and the outcome of advanced NSCLC patients to platinum-based chemotherapy has rarely been studied (Yuan *et al*., 2005).

ERCC1 is responsible for recognition of DNA damage and removal of the damaged nucleotides in NER. SNPs in exons of DNA repair genes may influence their protein activity, resulting in differences of individual NER and DNA repair capacity (DRC) that may affect the susceptibility of lung cancer. SNP of this gene may modulate repair capacity and contribute to individual variations in chemotherapy outcome. In vitro analysis showed that the *C/T* SNP at codon 118 of the *ERCC1* gene affected mRNA and protein levels which leads to differential platinum sensitivity (Yu *et al*., 2000). Furthermore, clinical data suggested a possible correlation of this SNP with clinical outcome and tumor response to platinum-based chemotherapy in advanced NSCLC (Isla *et al*., 2004; Ryu *et al*., 2004; Park *et al*., 2011).

Platinum based chemotherapy provides an opportunity for the direct assessment of tumor response. It also helps us to estimate the toxicity to therapy without interference of other treatments. On the basis of these different types of preclinical and clinical data, we made the hypothesis that the genetic polymorphisms in the major drug-metabolizing enzyme, BER and NER enzyme involved in platinum based drug predict inter individual variability in the treatment response as well as toxicity. To test this hypothesis, we examined the role of genetic polymorphisms in GSTP1, XRCC1, XPC and ERCC1 genes on the response and toxicity of the therapy with 285 patients recruited from different public and private hospitals of Bangladesh, received platinum based chemotherapy. Besides no pharmacogenetic study has yet done on Bangladeshi population and the studies on the role of these gene polymorphisms to the response and toxicities.

2.Materials and Methods

2.1 Subject Selection

Two hundred and eighty five non-small cell lung cancer patients were recruited randomly from different private and public hospitals of Bangladesh (Ahsania Mission Cancer and General Hospital, Dhaka Medical College Hospital, Bangabandhu Sheikh Mujib Medical University and Delta Medical College and Hospital) from the mid of 2013 to the end of 2015. They were histologically or cytologically proven staged IIIB or IV NSCLC. They met the following criteria: an Eastern Cooperative Oncology Group performance status ≤1, age >18 years, adequate bone marrow reserve (leukocyte count $>4.0 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L), adequate liver (bilirubin level ≤1.5 mg/dL, alanine aminotransferase/aspartate aminotransferase <3 times the upper limit of normal) and renal function (creatinine level ≤1.5 mg/dL).The exclusion criteria were active infections, concomitant malignancy, or a second primary malignancy, recent myocardial infarction, unstable angina, symptomatic brain metastases, spinal cord compression, uncontrolled massive pleural or pericardial effusion and previous chemotherapy.

The study was conducted in accordance with the International Conference of Harmonization (ICH) for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki and its further amendments (adopted by the 18th WMA general assembly, Helsinki, Finland, June 1964 and last amendment in Edinburgh on October 2000). Ethical permission was taken to approve the protocol and consent form of the clinical investigation from Bangladesh Medical Research Council (BMRC).

We recruited 285 patients age matched for toxicity assessment, but for response evaluation, CT scan reports were available for 150 patients and thus we excluded 135 patients from response evaluation part of this study. Each patient signed an informed consent document before entering the study and was free to withdraw from the study at any time without any obligation. Hematologic and non-hematologic toxicities of all patients were recorded at day 1 and 8 of every treatment course. Toxicities were assessed using National Cancer Institute common terminology criteria (version 4.0).Assessment of tumor response was carried out by computed tomography

scan after every three cycles. Responses were assessed using Response Evaluation Criteria in Solid Tumors (RECIST).

A basic demographic information and medical history was obtained by means of a short questionnaire. Their mean age ± SD was 53.9 ± 9.96 (range 18 to 78) years. Their mean body weight was 67.1 ± 9.07 (range 38 to 88) kg and mean body mass index (BMI) was 25.30 ± 3.21 (18.4 to 39.6) kg/m^2. Among them, 77.54% patients were male and 22.46% patients were females. Male to female ratio was 3.45:1. Out of 285 patients, 65.61% was smokers and 34.38% was non-smokers. Smoker to non-smoker ratio was 1.93:1. Most of the patients (74.39%) came from urban area in this study. In the present study most of the patients were economicallylower-middle class (34.04%) (Table 2.1)

Table 2.1: Demographic data and clinicopathological characteristics of NSCLC patients

Characteristics	Number of patients	Percentage (%)
Age (years)		
<50	113	39.65
>50	172	60.35
Body Weight (Kg)		
<55	107	37.54
>55	178	62.46
Body max index (kg/m^2)		
Underweight (<18.5)	133	46.67
Normal (18.5-24.9)	112	39.30
Overweight (≥25)	29	10.18
Obese (≥ 30)	11	3.86
Gender		
Male	221	77.54

Female	64	22.46
Socioeconomic status		
Poor	31	10.88
Lower-Middle	97	34.04
Upper-Middle	95	33.33
Rich	62	21.75
Family History		
Yes	135	47.37
No	150	52.63
Residence		
Urban	212	74.39
Rural	73	25.61
Tobacco smoking		
Smoker	187	65.61
Non-smoker	98	34.38
TNM stage		
IIIB	214	75.09
IV	71	24.91
Histology		
Adenocarcinoma	121	42.45
Squamous carcinoma	103	36.14
Other	61	21.40

Patient consent form:

A form regarding the approval of the patients is included in the appendix portion**.**

Data collection form:

The form needed to encompass all the information about the patients is jostled down in a data collection form.

2.1.1 Chemotherapy regimen

All participants received one of the following platinum-based combination chemotherapy regimen:

Cisplatin(DDP)/Carboplatin(CBP) plus gemcitabine (GEM),

Cisplatin(DDP)/Carboplatin(CBP) plus vinorelbine (NVP),

Cisplatin(DDP)/Carboplatin(CBP) plus paclitaxel (PT)/ docetaxel (DOC).

The dose of that chemotherapy regimen was :DDP(cisplatin) 30 mg/m^2; CBP (carboplatin) AUC = 4–5 g; PT (paclitaxel) 175 mg/m^2 , DOC (docetaxel) 75 mg/m^2; GEM (gemcitabine) 1 g/m^2; NVB (vinorelbine) 25 mg/m^2.All chemotherapeutic drugs were administered intravenously and it was repeated at three-weekly intervals for up to six cycles unless unacceptable toxicity, disease progression or patients' refusal to continue treatment.

2.1.2 Determination of stages of Lung Cancer

Accurate lung-cancer staging is essential for designing treatment programs and for determining a prognosis. Most importantly, a consistent, reproducible staging system is imperative for the execution of meaningful clinical trials of old and new therapeutic modalities, and for the extrapolation of the outcomes of trials to the general population. The International System for Staging Lung Cancer is widely accepted as an accurate and reproducible staging system for non-small cell lung cancer (NSCLC) (Naruke *et al*., 1997).

Primary tumor (T)

TX	Positive malignant cytology finding with no observable lesion
Tis	Carcinoma in situ
T1	Diameter of 3 cm or smaller, is surrounded by lung or visceral pleura, and is without invasion more proximal than the lobar bronchus
T2	Diameter greater than 3 cm and/or has extension to the visceral pleura, atelectasis, obstructive pneumonitis that extends to the hilar region but does not involve the whole lung or tumor of a main bronchus more than 2 cm distal from the carina
T3	A tumor of any size that directly invades any of the following: chest wall

	(including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium; or, associated atelectasis or obstructive pneumonitis of the entire lung or, tumor in the main bronchus less than 2 cm distal to the carina
T4	A tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; or, separate tumor nodules in the same lobe; or, tumor with a malignant pleural effusion

Regional lymph nodes (N)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to ipsilateralperibronchial and/or ipsilateralhilar lymph nodes, and intrapulmonary nodes including involvement by direct extension of the primary tumor
N2	Metastasis to ipsilateralmediastinal and/or subcarinal lymph node(s)
N3	Metastasis to contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

Metastasis (M)

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis present

TNM Stage

Stage	**TNM**
Stage IB	T2N0M0
Stage IIA	T1N1M0
Stage IIB	T2N1M0 or T3N0M0
Stage IIIA	T3N1M0 or T(1-3)N2M0

Stage IIIB	T4N(0-3)M0 or T(1-4)N3M0
Stage IV	T(1-4)N(0-3)M1

2.2 Response and Toxicity evaluation criteria

Clinical response of tumor was estimated according to the Response Evaluation Criteria in Solid Tumors (RECST) criteria where complete response is defined as disappearance of tumor for at least four weeks; at least a 30% decrease of the longest diameter of tumor for more than 4 weeks is determined as partial response; progressive disease is termed as at least a 20% increase of the longest diameter of tumor; neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease is evaluated as stable disease.

The sixth edition of American Joint Committee on Cancer (AJCC) tumor-node metastasis (TNM) staging system was applied for evaluation of both clinical stages before chemotherapy and pathological response of primary tumor and axillary lymph nodes after the treatment. Chemotherapy induced toxic reaction was assessed according to the Common Terminology Criteria for Adverse Events (CTCAE v4.0)

2.2.1 Response evaluation method

We evaluated response to drug in accordance with Response Evaluation Criteria in Solid Tumor (RECIST). 150 non-small cell lung cancer patients were taken for whom CT scan reports of before and after three cycle of chemotherapy was found. This method is internationally recognized and followed method. We categorized our patients in different level of this method considering patient's different histopathological data and diagnosis test report. We took the help of expert physicians, oncologist, pathologist and laboratory expert of respective hospital to categorize patients. Patients who showed complete response (CR) and partial response (PR) after treatment were considered as responder, and patients with stable disease (SD) and progressive disease (SD) were regarded as non-responder.

The Response Evaluation Criteria in Solid Tumors (RECIST) were published in February 2000 by the European Organization for Research and Treatment of Cancer (EORTC), the National Cancer Institute of the United States, and the National Cancer Institute of Canada Clinical Trials

Group. RECIST criteria are used to evaluate a patient's response to the therapy used to treat their disease.

Baseline documentation of Target lesions:

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total that are representative of all involved organs should be identified as target lesions recorded, and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which to characterize the objective tumor.

Evaluation of target lesions

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started
- Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

We found that patients with younger age (<50 years) had less response rate than the older aged patients (> 50 years) (n= 23, 15.33%). Male patients were found more as responder compared to female patients (n=47, 31.33%). The response rate to chemotherapy in patients with more weight (>55kg) had greater response rate than less weighted patients (<55 kg) (n=47, 31.33%). Male patients were more responder compared to female patients (n =47, 31.33%). Patients of economically lower middle class had elevated response rate than the poor (n= 29, 19.33%) (Table 2.2).

Table 2.2: Comparison of response rate to chemotherapy with demographic and clinicopathological characteristics of patients			
Characteristics	No. of Patients (150)	Responder (65)	Non responder (85)
Age			
<50	54	23 (15.33%)	31 (20.67%)
>50	96	42 (28%)	54 (36%)
Body Weight			
< 55	45	18 (12%)	27 (18%)
>55	105	47 (31.33%)	58 (38.67%)
Body max index (kg/m^2)			
Underweight (<18.5)	78	38 (25.33%)	40 (26.67%)
Normal (18.5-24.9)	65	25 (16.67%)	40 (26.67%)
Overweight (≥25)	5	1 (0.67%)	4 (2.67%)
Obese (≥ 30)	2	1 (0.67%)	1 (0.67%)
Gender			
Male	102	47 (31.33%)	55 (36.67%)
Female	48	18 (12%)	30 (20%)
Socioeconomic status			
Poor	12	4 (2.67%)	8 (5.33%)
Lower-Middle	62	29 (19.33%)	33(22%)
Upper-Middle	58	26 (17.33%)	32(21.33%)
Rich	18	6 (4.0%)	12(8.0%)
Family History			
Yes	85	34 (22.67%)	51 (34%)

No	65	31 (20.67%)	34 (22.67%)
Residence			
Urban	111	50 (33.33%)	61 (40.67%)
Rural	39	15 (10%)	24 (16%)
Smoking Habit			
Smoker	103	44 (29.33%)	64 (42.67%)
Non-smoker	47	21 (14%)	26 (17.33%)
TNM stage			
IIIB	128	50 (33.33%)	73 (48.67%)
IV	22	10 (6.67%)	12 (8.0%)
Histology			
Adenocarcinoma	77	34 (22.67%)	43 (28.67%)
Squamous carcinoma	68	30 (20%)	38 (25.33%)
Other	5	1 (0.67%)	4 (2.67%)

2.2.2 Toxicity evaluation method

We followed Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for adverse event analysis. Different diagnostic test reports and expert doctors' advice were taken for grading of adverse events and staging of cancer. Several criteria and signs and symptoms as stated in the guide line were considered as tools for adverse event identification and grading.

CTCAE Terms

An Adverse Event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or

procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each CTCAE v4.0 term is a MedDRA LLT (Lowest Level Term).

Grades

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).

Grade 3 Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4 Life-threatening consequences: urgent intervention indicated.

Grade 5 Death: related to AE.

The different grade of hematological toxicity and gastrointestinal toxicity were recorder in a prescribed questionnaire.Off the 285 patients, 96 (28.42%) patients were found with severe anemia(grade III + grade IV) and 189 (71.57%) patients were found with mild and moderate anemia(grade $\leq$ II) (Table 2.3). Again, we found that 31.22% patients were with severe neutropenia and 68.22% patients were with mild and moderate neutropenia. The percentage of different grade of others hematological and GI toxicity is showed in Table 2.3.

Table 2.3: Percentage of different grade of toxicities in NSCLC patients

Toxicity	Grade $\leq$ II		Grade III +Grade IV	
	Number	Percentage	Number	Percentage
Anemia	189	71.57	96	28.42
Neutropenia	196	68.77	89	31.22
Leukopenia	214	75.09	71	24.91
Throbocytopenia	238	83.51	47	16.49
Gastrointestinal Toxicity	232	81.80	53	18.20

Anemia

It is usually defined as a decrease in the amount of red blood cells (RBCs) or hemoglobin in the blood. It can also be defined as a lowered ability of the blood to carry oxygen. Anemia was categorized into four grades based on Hb levels: grade 1 (11.9 to 10.0 g/dL); grade 2 (9.9 to 8.0 g/dL); grade 3 (7.9 to 6.5 g/dL); and grade 4 (life-threatening, <6.5 g/dL).Among 285 patients, 96 (28.42%) patients were found with severe (grade III + grade IV) anemia whereasmore patients were found with mild and moderate (grade ≤ II) anemia (189, 71.57%). Patients with older age (>50 years) had increased risk of severe anemia than younger patients (<50 years) (n= 62, 21.73%). Male patients had higher risk of severe anemia compared to female patients (n=78, 27.37%). Smokers were found more with severe anemia compared to non-smokers (n =68, 23.86%) (Table 2.4).Relationship of different grade of anemiawith demographic and clinicopathological characteristic of patients is shown in Table 2.4.

Table 2.4: Relationship of different grade of anemiawith demographic andclinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Anemia Grade III + IV (96)	Mild and moderate Anemia Grade ≤ II (189)
Age			
<50	113	34 (11.93%)	79 (27.72%)
>50	172	62 (21.75%)	110 (38.60%)
Body Weight			
< 55	107	35(12.87%)	72 (25.26%)
>55	178	61 (21.40%)	117 (41.05%)
Body max index (kg/m^2)			
Underweight (<18.5)	133	51 (17.89%)	82 (28.77%)
Normal (18.5-24.9)	112	33(11.58%)	79 (27.72%)
Overweight (≥25)	29	8(2.81%)	21 (7.37%)
Obese (≥ 30)	11	4(1.40%)	7 (2.46%)

Gender			
Male	221	78 (27.37%)	143 (50.18%)
Female	64	18(6.32%)	46 (16.14%)
Socioeconomic status			
Poor	31	11(3.86%)	20 (7.02%)
Lower-Middle	97	35 (12.28%)	62 (21.75%)
Upper-Middle	95	32 (11.23%)	63 (22.11%)
Rich	62	18(6.32%)	44 (15.44%)
Family History			
Yes	150	56 (19.65%)	94 (32.98%)
No	150	40 (14.04%)	95 (33.33%)
Residence			
Urban	212	67 (23.51%)	145 (50.88%)
Rural	73	29 (10.18%)	44 (15.44%)
Smoking Habit			
Smoker	187	68 (23.86%)	117 (41.05%)
Non-smoker	98	28(9.82%)	70 (24.56%)
TNM stage			
IIIB	214	71(24.91%)	143 (50.18%)
IV	71	25(8.77%)	46 (16.14%)
Histology			
Adenocarcinoma	121	46 (16.14%)	75 (26.32%)
Squamous carcinoma	103	33 (11.57%)	70 (24.56%)

Other	61	17(5.96%)	44 (15.44%)

Neutropenia

It is an abnormally low concentration of neutrophils. Neutrophils make up the majority of circulating white blood cells and serve as the primary defense against infections by destroying bacteria, bacterial fragments and immunoglobulin-bound viruses in the blood. Neutropenia was categorized into four grades based on neutrophil levels: grade 1: Neutrophil count decreased <LLN - 1500/mm^3; grade 2: <1500 - 1000/mm^3; grade 3: <1000 - 500/$mm^3$and grade 4: <500/mm^3. Off 285 patients, 89 (31.22%) patients were found with severe neutropenia (grade III + grade IV) and 196 (68.77%) patients were found withmild and moderate neutropenia (grade I and grade II).Male patients had higher risk of severe neutropeniacompared to female patients (n=74, 25.96%). Elevated risk of severe neutropenia was observed with those who had a family history of NSCLC compared to those who had not any family history (n=53, 18.59%). Patients living in urban area were found more with severe neutropenia compared to those living in rural area(n= 63, 22.11%)(Table 2.5).Relationship of different grade of neutropeniawith demographic and clinicopathological characteristic of patients is shown inTable 2.5.

Table 2.5: Relationship of different grade of neutropeniawith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Neutropenia Grade III+IV (89)	Mild and Moderate Neutropenia Grade ≤ II (196)
Age			
<50	113	31(10.87%)	82 (28.77%)
>50	172	58 (20.35%)	114 (40%)
Body Weight			
< 55	107	31 (10.87%)	76 (26.67%)
>55	178	58 (20.35%)	120 (42.11%)
Body max index(kg/m^2)			

Underweight (<18.5)	133	49 (17.19%)	84 (29.47%)
Normal (18.5-24.9)	112	32 (11.23%)	80 (28.07%)
Overweight (≥25)	29	7(2.46%)	22 (7.72%)
Obese (≥ 30)	11	3 (1.05%)	8 (2.81%)
Gender			
Male	221	74 (25.96%)	147 (51.58%)
Female	64	15 (5.26%)	49 (17.19%)
Socioeconomic status			
Poor	31	9 (3.16%)	22 (7.72%)
Lower-Middle	97	34 (11.93%)	63 (22.11%)
Upper-Middle	95	30 (10.53%)	65 (22.81%)
Rich	62	16 (5.61%)	46 (16.14%)
Family History			
Yes	150	53 (18.59%)	97 (34.04%)
No	136	36 (12.63%)	99 (34.74%)
Residence			
Urban	212	63 (22.11%)	149 (52.28%)
Rural	73	26 (9.12%)	47 (16.49%)
Smoking Habit			
Smoker	187	65 (22.81%)	122 (42.81%)
Non-smoker	98	24 (8.42%)	74 (25.96%)
TNM stage			
IIIB	214	68 (23.86%)	146 (51.23%)

IV	71	21 (7.37%)	50 (17.54%)
Histology			
Adenocarcinoma	121	44 (15.44%)	77 (27.02%)
Squamous carcinoma	103	31 (10.88%)	72 (25.26%)
Other	61	14 (4.91%)	47 (16.49%)

Leukopenia

It is a decrease in the number of white blood cells (leukocytes) found in the blood, which places individuals at increased risk of infection. Leukopenia was categorized into four grades based on WBC levels: Grade 1: White blood cell decreased <LLN - 3000/mm^3;Grade 2: <3000 - 2000/mm^3; Grade 3: <2000 - 1000/$mm^3$and Grade 4: <1000/mm^3.Among 285 patients, 24.91% patients were found with severe leukopenia and 75.09% patients were found with mild and moderateleukopenia. We found that older patients (>50 years) had increased risk of severe leukopenia than younger patients (<50 years) (n= 45, 15.79%). Male patients had higher risk of severe leukopeniacompared to female patients (n=60, 21.05%). Patients living in urban area were found more with severe leukopenia compared to those living in rural area(n= 56, 19.65%)(Table 2.6).Relationship of different grade of leukopeniawith demographic and clinicopathological characteristic of patients is shown inTable 2.6.

Table 2.6:Relationship of different grade of leukopeniawith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Leukopenia Grade III + IV (71)	Mild and moderate Leukopenia Grade ≤ II (214)
Age			
<50	113	26 (9.12%)	87 (30.53%)
>50	172	45 (15.79%)	127 (44.56%)
Body Weight			

< 55	107	22 (7.72%)	85 (29.82%)
>55	178	49 (17.19%)	129 (45.26%)
Body max index (kg/m^2)			
Underweight (<18.5)	133	39 (13.68%)	94 (32.98%)
Normal (18.5-24.9)	112	25 (8.77%)	85 (29.82%)
Overweight (≥25)	29	5 (1.75%)	24 (8.42%)
Obese (≥ 30)	11	2 (0.70%)	9 (3.16%)
Gender			
Male	221	60 (21.05%)	161 (56.49%)
Female	64	11 (3.86%)	53 (18.60%)
Socioeconomic status			
Poor	31	6 (2.11%)	25 (8.77%)
Lower-Middle	97	27 (9.47%)	70 (24.56%)
Upper-Middle	95	27 (9.47%)	68 (23.86%)
Rich	62	11 (3.86%)	51 (17.89%)
Family History			
Yes	150	39 (13.68%)	111 (38.95%)
No	135	32 (11.23%)	103 (36.14%)
Residence			
Urban	212	56 (19.65%)	156 (54.74%)
Rural	73	15 (5.26%)	58 (20.35%)
Smoking Habit			
Smoker	187	52 (18.25%)	135 (47.37%)

Non-smoker	98	19 (6.67%)	79 (27.72%)
TNM stage			
IIIB	214	58 (20.35%)	156 (54.74%)
IV	71	13 (4.56%)	58 (20.35%)
Histology			
Adenocarcinoma	121	36 (12.63%)	85 (29.82%)
Squamous carcinoma	103	23 (8.07%)	80 (28.07%)
Other	61	12 (4.21%)	49 (17.19%)

Thrombocytopenia

It is a condition in which blood platelet count is decreased. Platelets (thrombocytes) are colorless blood cells that help blood clot. Platelets stop bleeding by clumping and forming plugs in blood vessel injuries. Thrombocytopenia was categorized into four grades based on platelet levels: Grade 1: Platelet count decreased <LLN - 75,000/mm^3; Grade 2: <75,000 - 50,000/mm^3; Grade 3: <50,000 - 25,000/mm^3 and Grade 4: <25,000/mm^3.Out of 285 patients, 16.49% patients were found with severe thrombocytopenia and 83.51% patients were found with mild and moderate thrombocytopenia. We found that older patients (>50 years) had increased risk of severe thrombocytopenia than younger patients (<50 years) (n= 34, 11.93%). Male patients had higher risk of severe thrombocytopenia compared to female patients (n=40, 14.04%). Patients living in urban area were found more with severe thrombocytopenia compared to those living in rural area(n= 40, 14.04%)(Table 2.7).Relationship of different grade of thrombocytopeniawith demographic and clinicopathological characteristic of patients is shown inTable 2.7.

Table 2.7: Relationship of different grade of thrombocytopeniawith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Thrombocytopenia Grade III + IV (47)	Mild and moderate Thrombocytopenia Grade ≤II (238)
Age			
<50	113	13 (4.56%)	100 (35.09%)
>50	172	34 (11.93%)	138 (48.42%)
Body Weight			
< 55	107	12 (4.21%)	95 (33.33%)
>55	178	35 (12.28%)	143 (50.18%)
Body max index (kg/m^2)			
Underweight (<18.5)	133	29 (10.18%)	104 (36.49%)
Normal (18.5-24.9)	112	15 (5.26%)	97 (34.04%)
Overweight (≥25)	29	2 (0.70%)	27 (9.47%)
Obese (≥ 30)	11	1 (0.35%)	10 (3.51%)
Gender			
Male	221	40 (14.04%)	181 (63.51%)
Female	64	7 (2.46%)	57 (20%)
Socioeconomic status			
Poor	31	4 (1.40%)	27 (9.47%)
Lower-Middle	97	17 (5.96%)	80 (28.07%)
Upper-Middle	95	19 (6.67%)	76 (26.67%)
Rich	62	7 (2.46%)	55 (19.30%)
Family History			

Yes	150	21 (7.37%)	129 (45.26%)
No	135	26 (9.12%)	109 (38.25%)
Residence			
Urban	212	40 (14.04%)	172 (60.35%)
Rural	73	7 (2.46%)	66 (23.16%)
Smoking Habit			
Smoker	187	36 (12.63%)	151 (52.98%)
Non-smoker	98	11 (3.86%)	87 (52.53%)
TNM stage			
IIIB	214	39 (13.68%)	175 (61.40%)
IV	71	8 (2.81%)	63 (22.11%)
Histology			
Adenocarcinoma	121	21 (7.37%)	100 (35.09%)
Squamous carcinoma	103	19 (6.67%)	84 (29.47%)
Other	61	7 (2.46%)	54 (18.95%)

Gastrointestinal disorders

Gastrointestinal disorder is characterized by frequent and watery bowel movements. It was categorized into four grades (Grade 1: <4 stools per day over baseline; mild increase in ostomy output compared to baseline; Grade 2: Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline ;Grade 3: Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL and Grade 4: Life-threatening consequences; urgent intervention indicated).Relationship of different grade of GI toxicitywith demographic and clinicopathological characteristic of patients is shown inTable 2.8.

Table 2.8. Relationship of different grade of GI toxicitywith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe GI Toxicity Grade III + IV(53)	Moderate GI Toxicity Grade ≤ II (232)
Age			
<50	113	16 (5.61%)	97 (34.04%)
>50	172	37 (12.98%)	135 (47.37%)
Body Weight			
< 55	107	14 (4.91%)	93 (32.63%)
>55	178	39 (13.68%)	139 (48.77%)
Body max index (kg/m^2)			
Underweight (<18.5)	133	32 (11.23%)	101 (35.44%)
Normal (18.5-24.9)	112	18 (6.32%)	94 (32.98%)
Overweight (≥25)	29	2 (0.70%)	27 (9.47%)
Obese (≥ 30)	11	1 (0.35%)	10 (3.51%)
Gender			
Male	221	45 (15.79%)	176 (61.75%)
Female	64	8 (2.81%)	56 (19.65%)
Socioeconomic status			
Poor	31	5 (1.75%)	26 (9.12%)
Lower-Middle	97	21 (7.37%)	76 (26.67%)
Upper-Middle	95	19 (6.67%)	76 (26.67%)
Rich	62	8 (2.81%)	54 (18.95%)
Family History			

Yes	150	29 (10.18%)	121 (42.46%)
No	135	24 (8.42%)	111 (38.95%)
Residence			
Urban	212	45 (15.79%)	167 (58.60%)
Rural	73	8 (2.81%)	65 (22.81%)
Smoking Habit			
Smoker	187	39 (13.68%)	148 (51.93%)
Non-smoker	98	14 (4.91%)	84 (29.47%)
TNM stage			
IIIB	214	44 (15.44%)	170 (59.65%)
IV	71	9 (3.16%)	62 (21.75%)
Histology			
Adenocarcinoma	121	24 (8.42%)	97 (34.04%)
Squamous carcinoma	103	21 (7.37%)	82 (28.77%)
Other	61	8 (2.81%)	53 (18.60%)

2.6 Study End Point

Prospective study was done to evaluate the role of the GSTP1 (rs1695), XRCC1 (rs25487), XPC (rs2228001) and ERCC1 (rs11615) polymorphism in the response of intact tumor to platinum based chemotherapy with 150 non-small cell lung cancer patients. Response Evaluation Criteria in Solid Tumors (RECIST) was applied to assess the Tumor response after 3 weeks from the completion of three cycle's chemotherapy. Patients were divided into two groups as responders (complete + partial response) and non-responders (static + progressive disease).

In the second part of the study, the role of the GSTP1 (rs1695), XRCC1 (rs25487), XPC (rs2228001) and ERCC1 (rs11615) polymorphism on platinum based chemotherapy was evaluated on 285 patients. Patient showing drug induced hematological toxicities and gastrointestinal toxicities were assessed according to the Common Terminology Criteria for Adverse Events. The highest grade toxicity occurred during the course of treatment of an individual patient was taken as a tool for the analysis.

2.4 Materials

The list of instruments, consumable materials, chemical and reagents, restriction enzymes and solutions are attached in the appendix part.

2.5 Venous blood collection

After explanation and counseling about the study, approximately 3 ml of venousblood was collected from each patient in a sterile eppendorf tube containingethylenediaminetetra acetic acid disdium (EDTA-Na2). Then samples were stored at -80ºC until DNA extraction.

2.6Preparation of DNA Isolation Reagents

Preparation procedure of cell lysisbuffer, nuclear lysisbuffer, Sodium Perchlorate (5 M), Tris-EDTA (TE) Buffer (1x) andTAE buffer (10x) is attached in the appendix part.

2.7 Genomic DNA Isolation

DNA was isolated from blood by previously published method (Daly et al., 1998). Briefly, 3 ml blood was taken in a 50 ml Falcon centrifuge tube. 20 ml Lysis Buffer was added to it. Then it was mixed gently for 2 minutes by inversion and It was then centrifuged for 10 minutes at 3000 rpm at 4°C by using UNIVERSAL 240V 50-60Hz Refrigerated Bench- Top Centrifuge Machine (Hettich GmbH & Co., Germany). The supernatant was discarded into a bottle containing enough savlon. The pellet was collected. 2 ml Nuclear Lysis Buffer and 0.5 ml of 5 M Sodium perchlorate were added to it. Then the tube was mixed in a rotary mixture at room temperature for about 15 min so that pellet was dissolved completely. The sample tube was then incubated at

65°C for 30 min. (Heidolph Unimax-2010 Incubator, Wolf Laboratories Limited, UK). Then 2.5 ml of chilled Chloroform was added to it. It was then mixed in a rotary mixture for 10 min at room temperature. Then the tube was centrifuged at 1500 rpm for 5 min. (37°C). The DNA containing phase (uppermost phase) was transferred to a fresh autoclaved 15 ml polypropylene tube using a disposable Pasteur pipette. Two volumes of Ethanol (double that of DNA phase) was added to it. It was then mixed immediately by slow gentle inversion until all cloudiness was disappeared. DNA was seen to come out of the solution as a white 'cotton-wool' pellet. The white 'cotton-wool pellet' was collected with a disposable microbiology loop. The loop was air dried. The DNA was dissolved in 200µl TE Buffer contained in a 1.5 ml screw cap tube. Then the tube was kept at 65°C overnight. Then it was taken back and stored in freezer (-40°C).

2.8Quantification of genomic DNA

The quantity and purity of DNA isolated from blood samples were assessed by using a UV Spectrophotometer (UV Prove v2.1) at 260 nm. In order to ensure complete sample homogeneity, which is critical when measuring genomic DNA concentration and purity with this instrument, samples were very gently shaken on a vortex shaker for approximately 30 minutes before measurements were taken. A sample volume of 1.5 to 2 µl was pipetted onto the fibre optic measurement surface. Working solutions of genomic DNA were made up to a standard concentration of 50 ng/µl with Nuclease free water, except in cases where the sample had an initial concentration of less than 50 ng/µl, in which case an undiluted aliquot was taken as a working solution.

For calculation of DNA concentration of samples free of RNA, the following conversion factor is used: 1 OD260 = 50 mg of DNA/ml.DNA concentration in µg/µl was calculated as follows:

$$\text{DNA Concentration } (\mu g/\mu ml) = \frac{\text{OD 260X50 (dilution factor)X 50 } \mu g/ml}{1000}$$

OD260/OD280 should be = 1.7-1.9 (OD=Optical density)

A value out of this range is not acceptable. It may indicate that the DNA sample is not in solution or that there are contaminants (i.e., protein) in the sample that may inhibit subsequent

reactions.All working solutions of genomic DNA were stored at -40ºC until genotype analysis. The purity and integrity of isolated genomic DNA were also assessed by means of agarose gel electrophoresis. A sample volume of 5 µl (50-70 ng/µl) was resolved on a 1% (w/v) agarose gel.

2.9Genotyping of single nucleotide polymorphism (SNPs) of GSTP1 (rs1695), XRCC1 (25487), XPC (rs2228001)& ERCC1 (rs11615)

In order to facilitate the accurate genotyping of the volunteer's DNA samples for the selected SNPs, PCR-RFLP was employed due to its affordability, ease of use and reliability. This method of genotyping entails the restriction enzyme (REase) digestion of polymerase chain reaction (PCR) amplification product. The subsequent digestion or lack of digestion, of PCR amplification product due to the presence or absence of an SNP within the REase recognition site allows for accurate and reliable genotyping and the consequent determination of SNP frequencies within a sample cohort.The classification of an SNP genotype as 'wild-type' or 'variant' was done according to accepted nomenclature and the relevant reference sequences available from the National Centre for Biotechnological Information (NCBI) .

2.9.1 DNA Amplification by PCR (Polymerase Chain Reaction)

The relevant genomic target regions, containing the SNPs of interest, were amplified by means of primer-directed PCR using thermo stable DNA polymerase, as originally described by (Saiki et al., 1985; Saiki et al., 1988). This primer-directed PCR method facilitates the in vitroamplification of single-copy genomic DNA sequences by a factor of more than ten million with extremely high sequence specificity.

2.9.2 Primer Design

There are some guidelines for primer design:

i. PCR primers should be generally 15-30 nucleotides long.

ii. Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.

iii. Should avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.

iv. Should avoid primer self-complementarities or complementarities between the primers to prevent hairpin formation and primer dimerization.

v. Should check for possible sites of non-desirable complementarities between primers and the template DNA.

vi. Differences in melting temperatures (Tm) of the two primers should not exceed 5°C.

By considering all the factors, the primers for the study were designed. The sequences of the primers used and their sizes are presented in Table 2.9.

Table 2.9:Name of the allele, sequence of the designed primer with their size and melting point

No	Allele	Primer Sequence	Size (Bp)
1	GSTP1 FP	5-ACCCCAGGGCTCTATGGGAA-3	022c2 200200
	GSTP1 RP	5-TGAGGGCACAAGAAGCCCCT-3	20
2	XRCC1 FP	5'-TTGTGCTTTCTCTGTGTCCA-3'	20
	XRCC1 RP	5'-TCCTCCAGCCTT TTCTGATA-3'	20

3	XPC FP	5’-GGAGGTGGACTCTCTTCTGATG-3’	22
	XPC RP	5’-TCAAAGGGTGAGTGGGCTTTGG-3’	22
4	ERCC1 FP	5’-AGGACCACAGGACACGCAGA-3’	22
	ERCC1 RP	5’-CATAGAACAGTCCAGAACAC-3’	22

FP=Forward Primer; RP=Reverse Primer

Primers are obtained from Jena Bioscience.

2.10Required conditions for PCR

GoTaq® Flexi DNA Polymerase, reaction buffer, dNTPs and $MgCl_2$were used for the PCR amplification of the relevant genomic target regions, containing the SNPs of interest.A 25 µl reaction volume was used containing 1.0 µl of genomic DNA (50-70 ng/µl), 5.0 µl of 5X Colorless GoTaq® reaction buffer, 4.0 µl of $MgCl_2$ (25 mM), 2.0 µl of dNTPs (2.5 mM), 1.0 µl of each primer (10 µM), 0.1 µl of GoTaq® DNA polymerase (5 U/µl), and 10.9 µl of Nuclease free water.PCR conditions to synthesize GSTP1, XRCC1, XPC and ERCC1 alleles with their respective lengths are given in Table2.10.

Table 2.10: PCR conditions to synthesize GSTP1 (rs1695), XRCC1 (25487), XPC (rs2228001) & ERCC1 (rs11615) alleles and their respective lengths.

Allele	**PCR Conditions (35 Cycles)**	**Size Of PCR Products (bp)**
GSTP1 (rs1695)	94°C 2 min 94°C 30 sec 57°C 30 sec 72°C 1min (35 cycles)	176

XRCC1 (rs25487)	94°C 2 min 94°C 30 sec 59°C 45 sec 72°C 30 sec (35 cycles)	615
XPC (rs2228001)	94°C 2 min 94°C 30 sec 57°C 45 sec 72°C 30 sec (35 cycles)	453
ERCC1 (rs11615)	96°C 5 min 94°C 30 sec 61°C 1 min 72°C 1 min (35 cycles)	525

2.11 Restriction Enzyme Digestion

After PCR amplification, 25µl of the PCR products for GSTP1, XRCC1, ERCC1 and XPC were digested with approximately 2 unit of *Bsm*AI, 2 units of *Hpa*II, *BsrD1* and *PvuII*respectively obtained from Jena Biosciences, India. Incubation conditions are listed in Table 2.11. Electrophoreses was done for the digested products using 2% agarose gel.

Table 2.11: The restriction enzymes, digestion condition and length of the expected fragments on digestion to diagnose GSTP1(rs1695), XRCC1(rs25487), XPC(rs2228001) and ERCC1(rs11615) alleles

Allele	**Res**	**Digestion conditions**	**Expected fragments (bp)**
GSTP1 (rs1695)	*BsmA*I	Incubation at 55° C overnight	NH 176 HE 85, 91, 176 MH 83, 93

XRCC1 (rs25487)	*Hpa*II	Incubation at 37° C overnight	NH 615 HE 375, 240, 615 MH 375,240
XPC (rs2228001)	*Pvu*II	Incubation at 37° C for 6hours	NH 453 HE 453,183, 270 MH 183, 270
ERCC1 (rs11615)	*BsrD1*	Incubation at 65° C for 6hours	NH 525 HE 525, 369, 156 MH 369, 156

NH: Normal Homozygote; HE: Heterozygote; MH: Mutant Homozygote

2.12 Visualization of PCR Products and REase Digestion Fragments

PCR amplification products were visualised by means of agarose gel electrophoresis in order to allow for size estimation and thus confirmation of amplification of the desired genomic target region. REase digestion fragments that were of sufficient size (>100 bp) and size differential between fragments (>30 bp) were also visualized on agarose gel. EZ Load™ Molecular ruler (100 bp)was used for size estimation of PCR amplification products, which served as confirmation that amplification of the desired genomic target region had occurred, as well as for quantification of PCR product prior to REase digestion reactions. EZ Load™ 100 bp DNA ladder was also used for size estimation of all REase digestion fragments, allowing for accurate and reliable genotyping of samples. EZ Load™ 100 bp DNA ladder is thus evident in lane 1 of all agarosegel photos. All agarosegels were visualised under ultraviolet (UV) light and photographed with a Gel Documentation and Analysis System.

2.13Gel Electrophoresis

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose gel electrophoresis of DNA is used to determine the presence and distinguish the type of nucleic acids obtained after extraction and to analyze digestion products. Desired DNA fragments can be physically isolated for various purposes such as sequencing, probe preparation, or for cloning fragments into other vectors. Both agarose and polyacrylamide gels are used for DNA analysis. Agarose gels are usually run to size larger fragments (greater than 200 bp) and polyacrylamide gels are run to size fragments less than 200 bp.Typicallyagarose gels are used for most purposes and polyacrylamide gels are used when small fragments, such as digests of 16S rRNA genes, are being distinguished. Regular agarose gels may range in concentration from 0.6 to 3.0%.

Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatine-like slab. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is forced through the pores of the gel by the electrical current.

Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose in the gel and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA in the gel will be visualized by the use of Ethidium Bromide, added to the gel. Ethidium bromide binds to DNA and illuminates when exposed to ultraviolet light, causing the DNA to ‘glow’.

All PCR products were resolved by electrophoresis in 2% (w/v) agarose gel at 80 volts (V). The REase digestion fragments were also observed in 2% (w/v) agarose gel. All REase digestion fragments were resolved at 80 V, so as to ensure sufficient resolution to allow for accurate genotyping.

2.14Agarose gel electrophoresis procedure

All agarose gels were made with and resolved in 1X tris acetate ethylenediaminetetraacetic acid (TAE) buffer, which was made and stored as a 10X stock solution and diluted to the required working concentration as was needed. In order to facilitate the visualization of DNA within the agarose gel under UV light, 1 µg of ethidium bromide (EtBr) per ml agarose solution was added - i.e. 0.01% (v/v) EtBr stock solution (10 mg/ml). Description of that procedure is included in appendix portion.

2.15 PCR-RFLP OF GSTP1 (rs1695)

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGG
TGAATGACGGCRTGGAGGACCTCCGCTGCAAATACXTCTCCCTCWTCTAYASC
AACTATGWRAGCATCTGCACCAGGGTTGGGCACKGGGRGCTGAACAAAGAAA
GGGGCTTCTTGTGCCCTCA

Yellow……..>Prime rsequence

Green……> *SNP of Interest*

After completing PCR amplification with appropriate reagents a PCR product ofGSTP1 (rs1695) was obtained. The PCR product size was 176 bp and this was visualized in 2%(w/v)

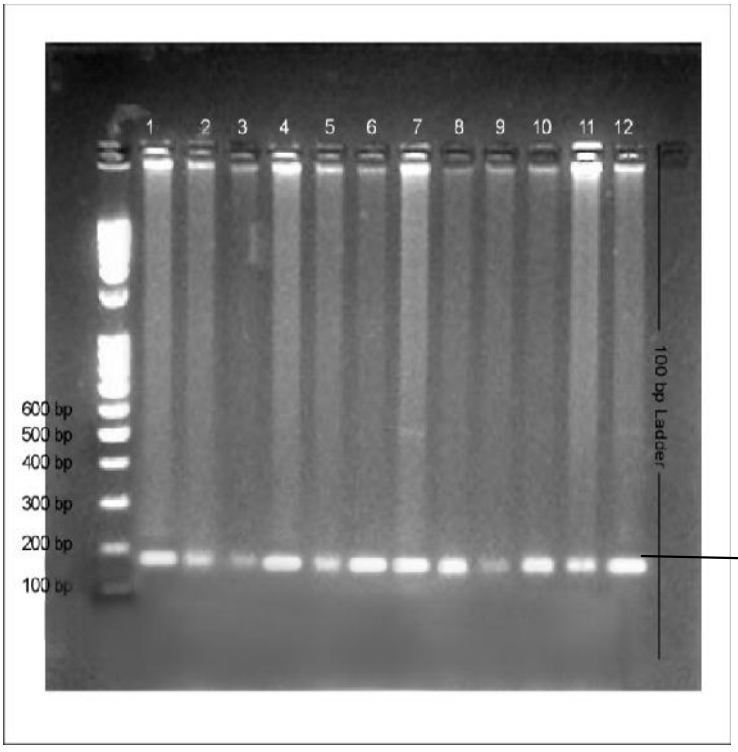


Figure 2.1: PCR product of GSTP1(176 bp)

2.15.1 Fragmentation Pattern of GSTP1 (rs1695)

The fragments were visualized in agarose gel (3%) after digestion of the PCR product with restriction enzyme *BsmAI*.

Table 2.12: Name of the restriction enzyme of GSTP1 (rs1695)with its sites of digestion

Restriction Enzyme	Sites of digestion
BsmAI	**5'…….....GTCTC(N)$_1$……3'** **3'……..CAGAG(N)$_5$……5'**

Table 2.13: Type of nucleotide changes, cutting sites and fragments of the allele in caseof GSTP1 (rs1695)

Change	Fragments (bp)	Type
When X= A in both chromosomes (A/A)	176	Normal Homozygote
When X= G in one chromosome (A/G)	83, 93, 176	Heterozygote
When X=G in both chromosome (G/G)	83, 93	Mutant Homozygote

When X= A in both of the sister chromosomes: (NORMAL HOMOZYGOTE)(A/A)

When X=A in both of the sister chromosomes, there will be no cutting in the bothchromosome and only one fragment with 176bp will be obtained and this isconsidered as normal homozygote. The figure of this fragmentation is included in the appendix portion**.**

When X=G in one of the sister chromosome: (HETEROZYGOTE) (A/G)

When X=G in one of the sister chromosome, there will be one cutting site at 93bp.So, there will be 3 fragments (83, 93 and 176bp) for two sister chromosomes.The figure of this fragmentation is included in the appendix portion**.**

When X=G in both of the sister chromosomes: (Mutant Homozygote) (G/G)

When X=G in both of the sister chromosomes, there will be one cutting between at 93 bp in both of the chromosomes and two fragments with 93 and 83 bp will be obtained.The figure of this fragmentation is included in the appendix portion**.**

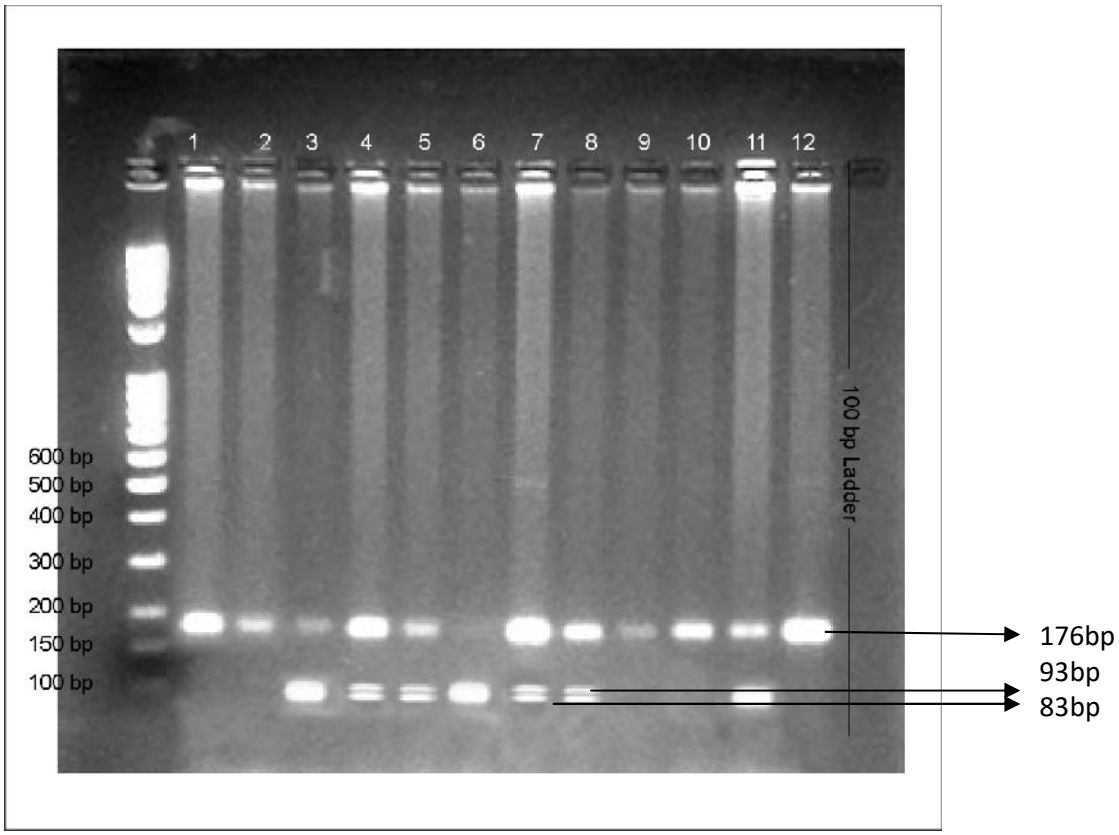


Figure 2.2: Restriction Enzyme (*BsmAI*) digestion fragment of GSTP1 (rs1695) (3% agarose gel)

2.16 PCR-RFLP of XRCC1 (rs25487)

After completing PCR amplification with appropriate reagents a PCR product ofXRCC1 (rs25487)was obtained. The PCR product size was 615bp and this was visualized in2% (w/v) agarose gel

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACT
TGCTTTCTTTCTCACTGCATTCTGTAGCCTTTGTCTTCTCTCTGATTTTT
GCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAATCT
CATGATCTGTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTG
TCTCGTTCCCCTTTGCCCCTCAGATCACACCTAACTGGCATCTTCACTT
CTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCAG
GTCCTAGGCCTGGGAGGCCGCATCGTGCGTAAGGAGTGGGTGCTGGA
CTGTCACCGCATGCGTCGGCGGCTGCCCTCCCRGAGGTAAGGCCTCAC
ACGCCAACCCTGCTCCTTATCCTGTGCTGGGCAATGCCAGGAATCTGG
AGGGGAGTCAGACTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCA
GCCCAGAGCCAGCAGACTACTGAGAGGAGCGGGGCAGGGGCTGGGGT
ACTCCAGATGAGGGAAGGAGGACCTGCCTGGGAGGTCACAGAGGGCT
CTGTGAAGCCTGCTTATCAGAAAAGGCTGGAGGA

Yellow Primer Sequence

Green → SNP of interest

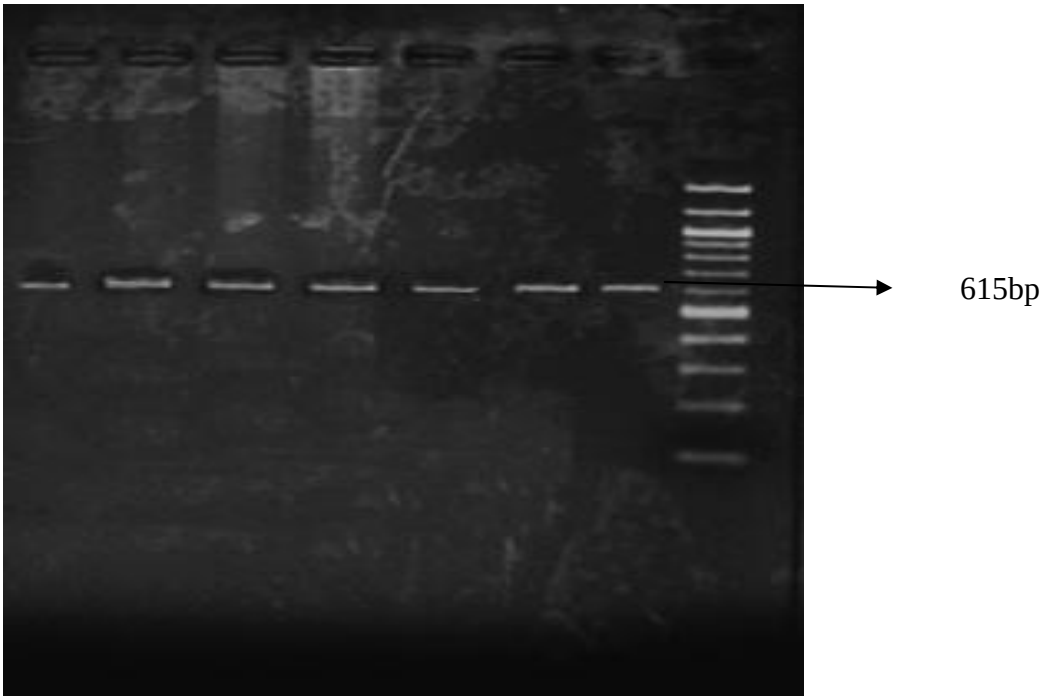


Figure2.3: PCR product of XRCC1(615 bp)

2.16.1 Fragmentation Pattern of XRCC1 (rs25487)

The fragments were visualized in agarose gel (3%) after digestion of the PCR productwith *HpAII*.

Table 2.14: Name of the restriction enzyme of XRCC1 (rs25487) with its sites of digestion

Restriction Enzyme	Sites of digestion
HpAII	5'... C ▼C G G ... 3' 3'... G G C ▲C ... 5'

Table 2.15: Type of nucleotide changes, cutting sites and fragments of the allele in caseof XRCC1 (rs25487)

Change	Fragments (bp)	Type
When X= G in both chromosomes (G/G)	**615**	Normal Homozygote
When X= A in one chromosome (A/G)	**375, 270, 615**	Heterozygote
When X=A in both chromosome (A/A)	**375,270**	Mutant Homozygote

When X= G in both of the sister chromosomes: (NORMAL HOMOZYGOTE) (G/G)

When X=G in both of the sister chromosomes, there will be no cutting in the bothchromosome and only one fragment with 615bp will be obtained and this isconsidered as normal homozygote. The figure of this fragmentation is included in the appendix portion**.**

When X=A in one of the sister chromosome: (HETEROZYGOTE) (A/G)

When X=A in one of the sister chromosome, there will be one cutting site at 315bp. So, there will be 3 fragments (375, 270 and 615bp) for two sister chromosomes. The figure of this fragmentation is included in the appendix portion**.**

When X=G in both of the sister chromosomes: (Mutant Homozygote) (G/G)

When X=G in both of the sister chromosomes, there will be one cutting between at 375bp in both of the chromosomes and two fragments with 375bp and 240bp will be obtained. The figure of this fragmentation is included in the appendix portion**.**

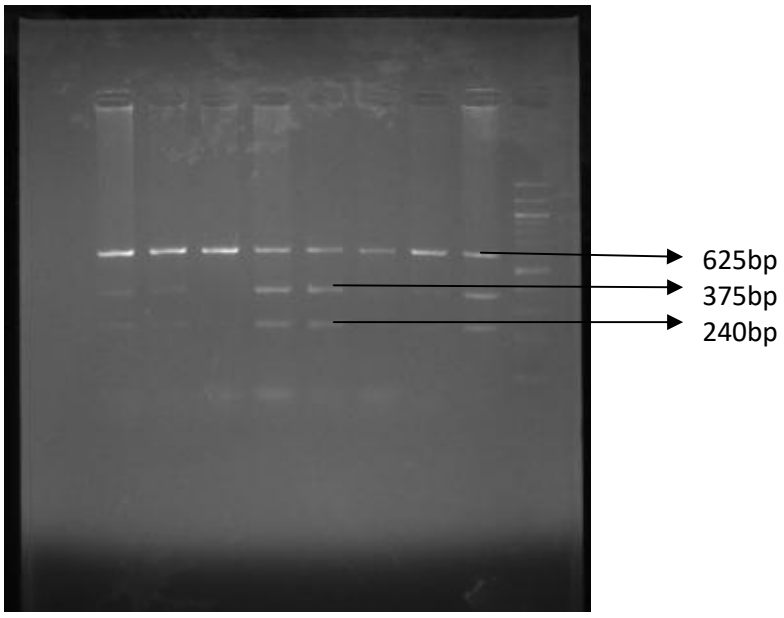


Figure 2.4: Restriction Enzyme (*HpAII*) digestion fragment of XRCC1 (rs25487) (3% agarose gel)

2.17 PCR-RFLP of XPC (rs2228001)

After completing PCR amplification with appropriate reagents a PCR product of XPC was obtained. The PCR product size was 453bp and this was visualized in 2% (w/v) agarose gel

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGC
CAGGATACTGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGC
TGAAGGGTGGGCCCAAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCT
GTTCCCATTTGAGMAGCTGTGAGCTGAGCGCCCACTAGAGGGGCACCCACCAGTTG
CTGCTGCCCCACTACAGGCCCCACACCTGCCCTGGGCATGCCCAGCCCCTGGTGGTG
GGGGCTTCTCTGCTGAGAAGGCAAACTGAGGCAGCATGCACGGAGGCGGGGTCAGG
GGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCCCGTCTGGCTCCAGCCCTTGT
CAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAAAGCCCACTCACCCT
TTGA

Yellow……..>Primersequence

Green……> *SNP of Interest*

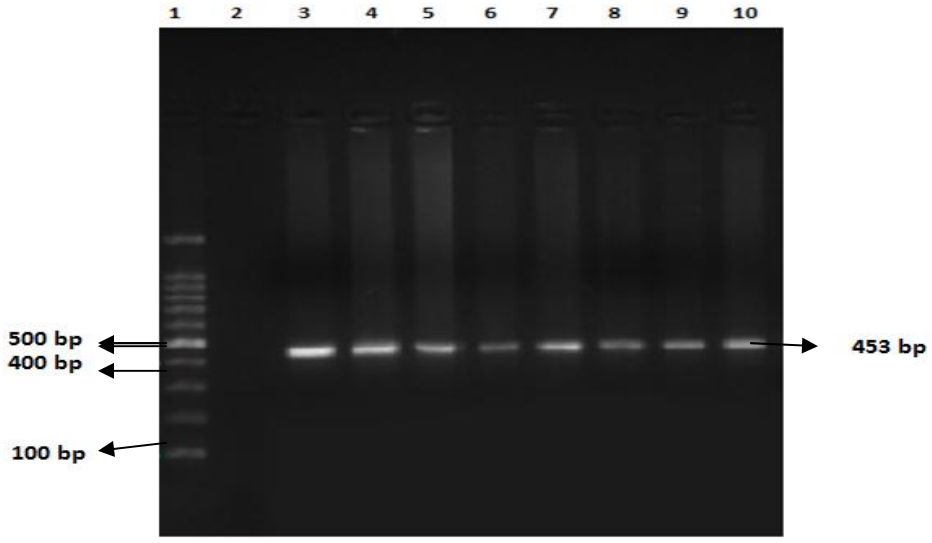


Figure 2.5: PCR product of XPC (rs2228001)

2.17.1 Fragmentation Pattern of XPC (rs2228001)

The fragments were visualized in agarose gel (3%) after digestion of the PCR product with restriction enzyme *pvuII*.

Table 2.16: Name of the restriction enzyme of XPC (rs2228001) with its sites of digestion

Restriction Enzyme	Sites of digestion
PvuII	5'... C A G ▼C T G ... 3' 3'... G T C ▲G A C ... 5'

Table 2.17: Type of nucleotide changes, cutting sites and fragments of the allele in case of XPC (rs2228001)

Change	Fragments (bp)	Type
When X=A in both chromosome(A/A)	453	Normal Homozygote (NH)
When X=C in one Chromosome(A/C)	453, 183, 270	Heterozygote(HE)
When X=C in both Chromosome(C/C)	183, 270	Mutant Homozygote(MH)

When X= A in both of the sister chromosomes: (NORMAL HOMOZYGOTE)(A/A)

When X=A in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 453bp will be obtained and this is considered as normal homozygote. The figure of this fragmentation is included in the appendix portion**.**

When X=C in one of the sister chromosome: (HETEROZYGOTE) (A/C)

When X=C in one of the sister chromosome, there will be one cutting site at 183 bp. So, there will be 3 fragments (183, 270 and 453 bp) for two sister chromosomes. The figure of this fragmentation is included in the appendix portion**.**

When X=C in both of the sister chromosomes: (Mutant Homozygote) (C/C)

When X=G in both of the sister chromosomes, there will be one cutting between at 183bp in both of the chromosomes and two fragments with 183 and 270 bp will be obtained. The figure of this fragmentation is included in the appendix portion**.**

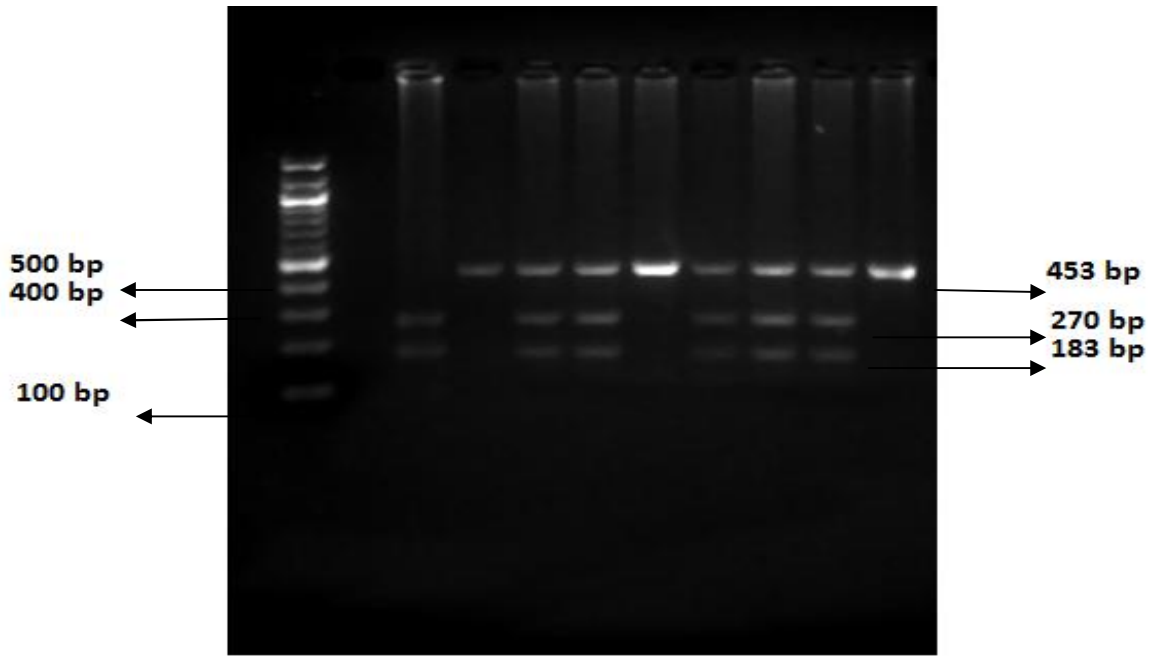


Figure 2.6Restriction Enzyme (*pvuII*) digestion fragment of XPC(rs2228001) (3% agarose gel)

2.18 PCR-RFLP ofERCC1 (rs11615)

After completing PCR amplification with appropriate reagents a PCR product of ERCC1 (rs11615) was obtained. The PCR product size was 525 bp and this was visualized in 2% (w/v) agarose gel.

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAG
ACCCGGGGACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCT
GGATCAGAGGATCAGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGA
CCCTGCCACAGGTGGGAGGAGGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGG
AGGTGTGGGAAGAGGTGGGAGGAGGGAGGAGGTGCAAGAAGAGGTGGAGGAGGG
CCCTGTGGTTATCAAGGGTCATCCCTATTGATGGCTTCTGCCCTTCGTCCCTCCCCAG
AGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTGCCCTGGGAATTTGGCGACGT
AATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTTCCTCAGGTGAGCTCT
GCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGTCATGTC
CCAGTGTTCTGGACTGTTCTATG

Yellow……> Primer sequence

Green……> SNP of interest

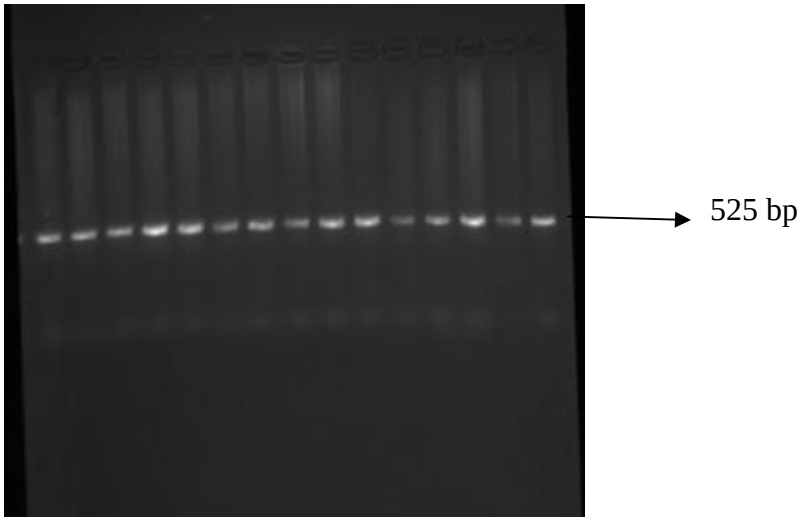


Fig: 2.7 PCR product of ERCC1(rs11615)

2.18.1 Fragmentation Pattern of ERCC1 (rs11615)

The fragments were visualized in agarose gel (3%) after digestion of the PCR product with restriction enzyme *BsrDI*.

Table 2.18: Name of the restriction enzymeof ERCC1 (rs11615) with its sites of digestion

Restriction Enzyme	Sites of digestion
BsrD1	5'... G C A A T G $(N)_2$ ▼ ... 3' 3'... C G T T A C ▲ ... 5'

Table 2.19: Type of nucleotide changes, cutting sites and fragments of the allele in case of ERCC1 (rs11615)

Change	Fragments (bp)	Type
When X= C in both chromosomes (C/C)	**525**	Normal Homozygote (NH)
When X= T in one chromosome (C/T)	**369, 156, 525**	Heterozygote (HE)
When X=T in both chromosome (T/T)	**369, 156**	Mutant Homozygote (MH)

When X= C in both of the sister chromosomes: (NORMAL HOMOZYGOTE)(C/C)

When X=C in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 525bp will be obtained and this is considered as normal homozygote. The figure of this fragmentation is included in the appendix portion**.**

When X=T in one of the sister chromosome: (HETEROZYGOTE) (C/T)

When X=T in one of the sister chromosome, there will be one cutting site at 368bp. So, there will be 3 fragments (156, 369 and 525bp) for two sister chromosomes. The figure of this fragmentation is included in the appendix portion**.**

When X=T in both of the sister chromosomes: (Mutant Homozygote) (T/T)

When X=T in both of the sister chromosomes, there will be one cutting between at 369 bp in both of the chromosomes and two fragments with 156 and 369 bpwill be obtained. The figure of this fragmentation is included in the appendix portion**.**

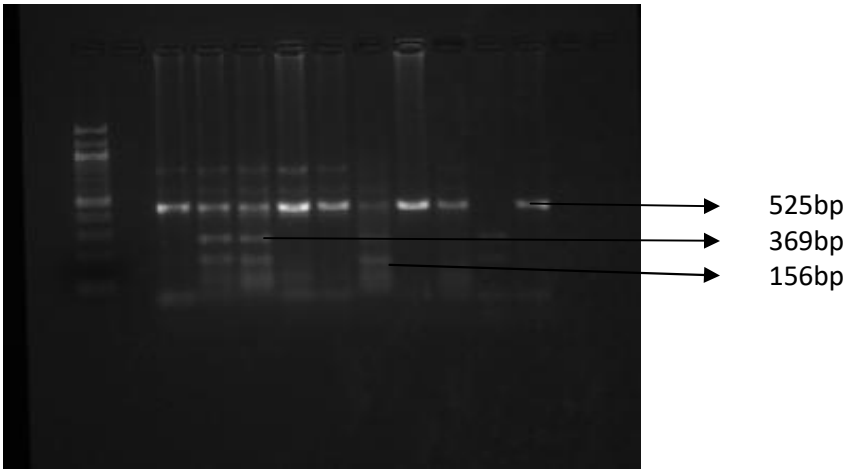


Figure 2.8 Restriction Enzyme (*BsrDI*) digestion fragment of ERCC1 (3% agarose gel)

2.19 Statistical Analysis

The statistical significance of differences in genotype frequencies between patientswith different treatment outcomes and toxicities were determined by the Chi-squaretest. Binary logistic regression was applied for all analysis variables to evaluate risk asodds ratios (AORs) with 95 % confidence intervals (95 % CIs). All statistical analyseswere done applying the SPSS software, version 17.0 (SPSS, Chicago, IL, USA).

3. Results

3.1 Demographic and clinicopathological characteristics of patients

The demographic andclinicopathological characteristics of all the recruited patients receiving platinum based chemotherapy (n= 285) including patient's age, TNM staging, lymph node status, histology, tumor gradewere recorded and a chi square test was done toanalyze these recorded values in terms of response and toxicity.

Table 3.1: Demographic data and clinicaopathological characteristics of NSCLC patients

Characteristics	Number of patients	Percentage (%)
Age (years)		
<50	113	39.65
>50	172	60.35
Body Weight (Kg)		
< 55	107	37.54
>55	178	62.46
Body max index (kg/m^2)		
Underweight (<18.5)	133	46.67
Normal (18.5-24.9)	112	39.30
Overweight (≥25)	29	10.18
Obese (≥ 30)	11	3.86
Gender		
Male	221	77.54
Female	64	22.46
Socioeconomic status		
Poor	31	10.88
Lower-Middle	97	34.04
Upper-Middle	95	33.33

Rich	62	21.75
Family History		
Yes	150	52.63
No	135	47.37
Residence		
Urban	212	74.39
Rural	73	25.61
Tobacco smoking		
Smoker	187	65.61
Non-smoker	98	34.38
TNM stage		
IIIB	214	75.09
IV	71	24.91
Histology		
Adenocarcinoma	121	42.45
Squamous carcinoma	103	36.14
Other	61	21.40

3.2 Response evaluation

The patients were required to complete at least two cycles of chemotherapy to evaluate treatment response according to RECIST criteria. Patients with complete response (CR) and partial response (PR) after treatment were considered as responders, and patients with stable disease (SD) and progressive disease (PD) were regarded as non-responders. In response evaluation study, we prospectively recruited 150 NSCLC patients from different hospitals for whom the CT scan reports of before and after chemotherapy were found.

3.2.1 Association of responseto chemotherapywithclinic-pathological characteristic of patients

We found that patients with younger age (<50 years) had less response rate thanthe older aged patients (> 50 years) (n= 23, 15.33%).The response rate to chemotherapy in patients with more

weight (>55kg) had greater response rate than less weighted patients (<55kg)(n=47, 31.33%).Male patients were more responder compared to female patients (n =47, 31.33%). Patients of economically lower middle class had elevated response rate than the poor (n= 29, 19.33%) (Table 3.2).No significant association ofresponse to chemotherapy was found with demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.2).

Table 3.2:Association of response to chemotherapy with demographic and clinicopathological characteristics of patients

Characteristics	No. of Patients (150)	Responder (65)	Non responder (85)	OR (95% CI)	P Value
Age					
<50	54	23 (15.33%)	31 (20.67%)	Ref.	-
>50	96	42 (28%)	54 (36%)	1.04 (0.53 -2.05)	0.89
Body Weight					
< 55	45	18 (12%)	27 (18%)	Ref.	-
>55	105	47 (31.33%)	58 (38.67%)	1.22 (0.60 - 2.47)	0.59
Body max index (kg/m^2)					
Underweight (<18.5)	78	38 (25.33%)	40 (26.67%)	Ref.	-
Normal (18.5-24.9)	65	25 (16.67%)	40 (26.67%)	0.66 (0.34 -1.28)	0.22
Overweight (≥25)	5	1 (0.67%)	4 (2.67%)	0.26(0.03 -2.47)	0.24
Obese (≥ 30)	2	1 (0.67%)	1 (0.67%)	1.05 (0.06 -1.74)	0.97
Gender					
Male	102	47 (31.33%)	55 (36.67%)	Ref.	-
Female	48	18 (12%)	30 (20%)	1.18 (0.58 – 2.29)	0.66
Socioeconomic status					
Poor	12	4 (2.67%)	8 (5.33%)	Ref.	-
Lower-Middle	62	29 (19.33%)	33 (22%)	1.76 (0.48 -6.45)	0.40

Upper-Middle	58	26 (17.33%)	32 (21.33%)	1.63 (0.44 -6.01)	0.47
Rich	18	6 (4.0%)	12 (8.0%)	1.0 (0.212 -4.7)	1.00
Family History					
Yes	85	34 (22.67%)	51 (34%)	Ref.	-
No	65	31 (20.67%)	34 (22.67%)	1.36 (0.71 -2.62)	0.35
Residence					
Urban	111	50 (33.33%)	61 (40.67%)	Ref.	-
Rural	39	15 (10%)	24 (16%)	0.76 (0.36 -1.61)	0.48
Smoking Habit					
Smoker	103	44 (29.33%)	64 (42.67%)	Ref.	-
Non-smoker	47	21 (14%)	26 (17.33%)	1.17 (0.59 – 2.35)	0.65
TNM stage					
IIIB	128	50 (33.33%)	73 (48.67%)	Ref.	-
IV	22	10 (6.67%)	12 (8.0%)	1.11 (0.45 – 2.75)	0.83
Histology					
Adenocarcinoma	77	34 (22.67%)	43 (28.67%)	Ref.	-
Squamous carcinoma	68	30 (20%)	38 (25.33%)	0.99 (0.52 – 1.93)	1.0
Other	5	1 (0.67%)	4 (2.67%)	0.32 (0.03 – 2.96)	0.31

3.2.2Association of GSTP1 (rs1695) polymorphism with response to chemotherapy

The response rate to chemotherapy in patients withAG, AA and GG allele of GSTP1 was48%,41% and 11% respectively. Patients carrying AG and (AG+GG) genotype of GSTP1 showed a significant response to chemotherapy than did those carrying AA genotype (OR = 2.12, 95% Cl = 1.06 -4.27, p = 0.034 and OR = 2.01, 95% Cl = 1.04-3.87, p = 0.037, respectively) (Table 3.4).

Table3.3: Response variation of patients with GSTP1 (rs1695) polymorphism

GSTP1 (rs1695)						
	Total Responder (65)			Total Non responder (85)		
Genotype	Complete Response (8)	Partial Response (57)	Total responder (65)	Stable Disease (78)	Progressive Disease (7)	Total non responder (85)
AA (77)	5	22	27	46	4	50
AG (58)	2	29	31	25	2	27
GG (15)	1	6	7	7	1	8
AG+GG (73)	3	36	39	32	3	35

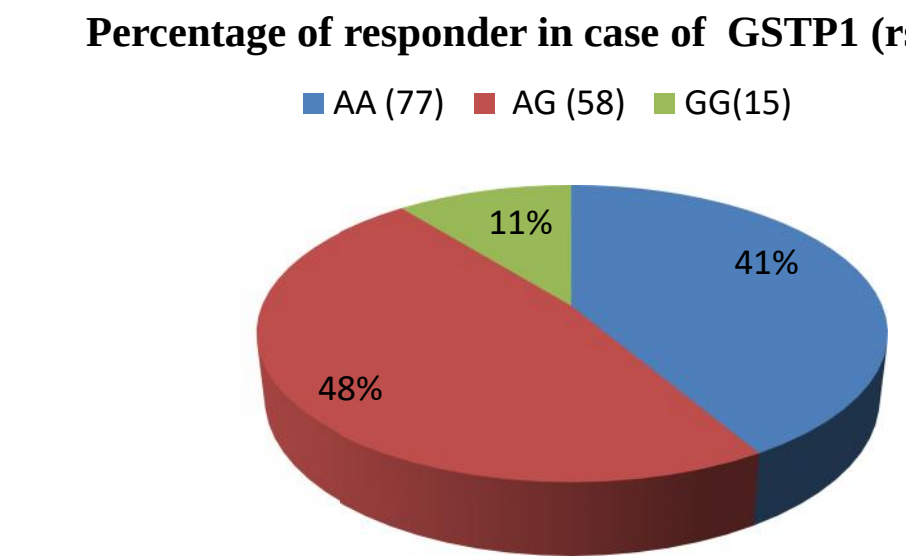


Figure 3.1: Percentage of responder of different genotypes in case of GSTP1 (rs1695) polymorphism

Table 3.4: Association of GSTP1 (rs1695) polymorphism with response to platinum-based chemotherapy

GSTP1 (rs1695)				
Genotype	Responder (CR + PR) (n = 65)	Non responder (SD + PD) (n = 85)	Odds Ratio (95% CI)	P value
AA (77)	27	50	Reference	-
AG (58)	31	27	2.12 (1.06 - 4.27)	0.034
GG (15)	7	8	1.62 (0.53 -4.95)	0.397
AG+GG (73)	38	35	2.01 (1.04 -3.87)	0.037

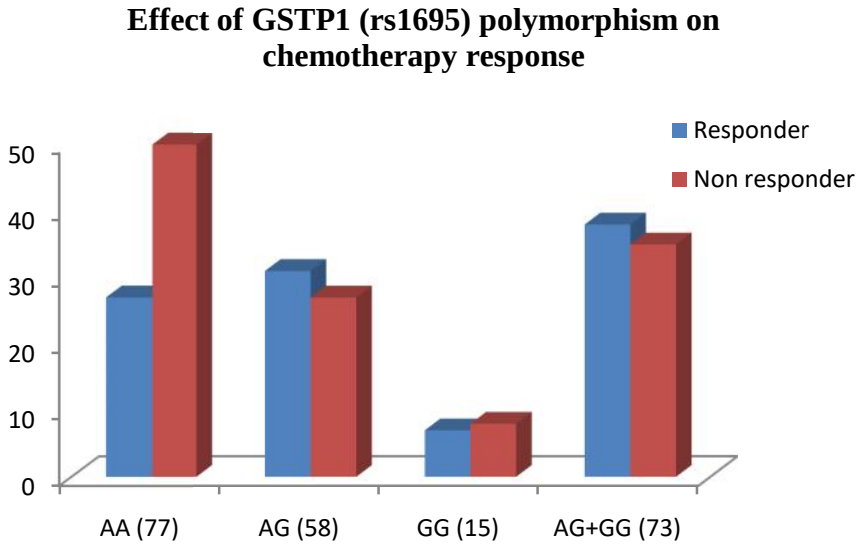


Figure 3.2: Effect of GSTP1 (rs1695) polymorphism on chemotherapy response

3.2.3. Association of XRCC1 (rs25487) polymorphism withresponse to chemotherapy

From the variation of response among the different XRCC1 (rs25487) genotype carriers, it wasobservedthat patients carrying GG genotype showed poorer response rate comparedthat of theAG genotype(49%).Individual carrying AG genotype and carrying at least one A allele (AA + AG) of XRCC1polymorphism had significantly elevated response compared to those withGG genotype (OR = 2.16, 95% Cl = 1.09 -4.28, p = 0.027; OR = 2.18, 95% Cl = 1.13 -4.22, p = 0.002, respectively) (Table 3.6).

Table3.5: Response variation of patients with XRCC1 (rs25487) polymorphism

XRCC1 (rs25487)						
	Total Responder (65)			Total Non responder (85)		
Genotype	Complete Response (8)	Partial Response (57)	Total responder (65)	Stable Disease (78)	Progressive Disease (7)	Total non responder (85)
GG (81)	3	25	28	51	2	53
AG (60)	2	30	32	25	3	28
AA (9)	3	2	5	2	2	4
AA+AG (69)	5	32	37	25	5	32

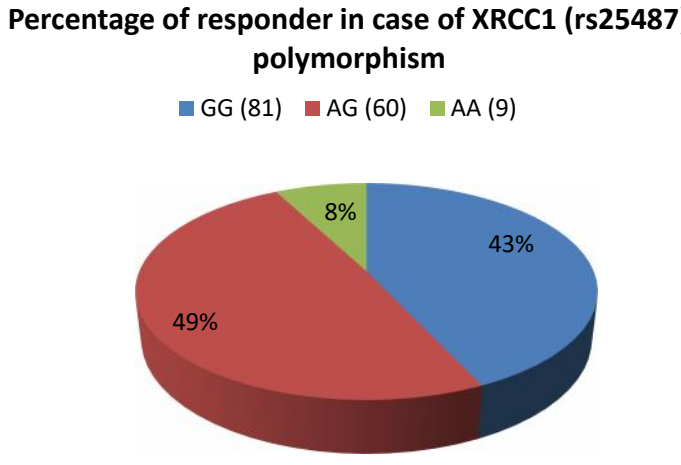


Figure 3.3: Percentage of responder of different genotypes in case of XRCC1 (rs25487) polymorphism

Table3.6: Association of XRCC1 (rs25487) polymorphism with response to platinum-based chemotherapy

XRCC1 (rs25487)				
Genotype	Responder (CR + PR) (n = 65)	Non responder (SD + PD) (n = 85)	Odds Ratio (95% CI)	P value
GG (81)	28	53	Reference	-
AG (60)	32	28	2.16 (1.09 -4.28)	0.027
AA (9)	5	4	2.36 (0.58 -9.52)	0.225
AA+AG (69)	37	32	2.18 (1.13 -4.22)	0.002

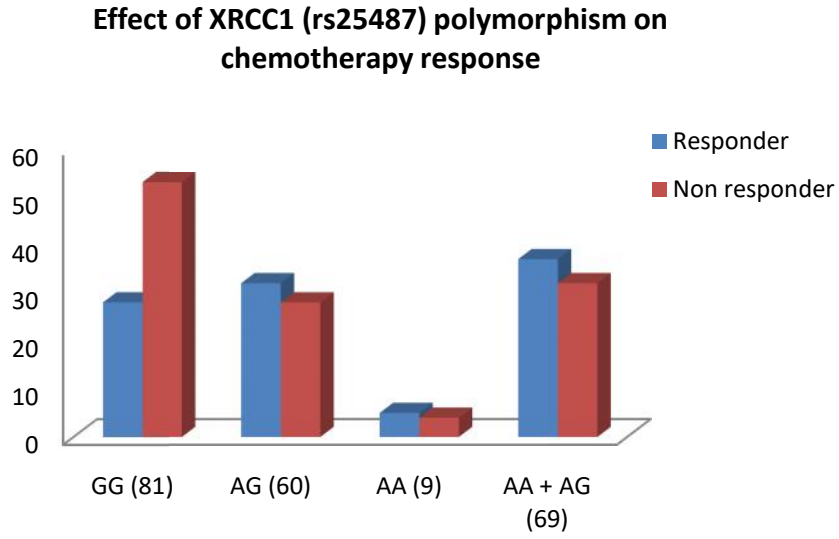


Figure 3.4: Effect of XRCC1 (rs25487) polymorphism on chemotherapy response

3.2.4Association of XPC (rs2228001) polymorphism with response to chemotherapy

From the variation of response among the different XPC (rs2228001) genotype carriers, an increased chance of treatment response was found in patients with AA genotype versus AC genotype (45%, 29responders).There was no significant association of XPC polymorphism in response to chemotherapy in this study (Table 3.8).

Table3.7: Response variation of patients with XPC (rs2228001) polymorphism

XPC (rs2228001)						
	Total Responder (65)			Total Non responder (85)		
Genotype	Complete Response (8)	Partial Response (57)	Total responder (65)	Stable Disease (78)	Progressive Disease (7)	Total non responder (85)
AA (61)	3	26	29	29	3	32
AC (72)	2	23	25	44	3	47
CC (17)	3	8	11	5	1	6
AC+CC (89)	6	31	36	49	4	53

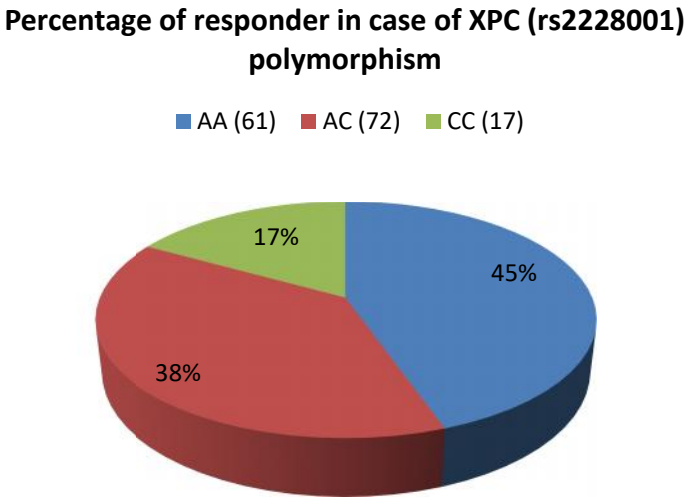


Figure 3.5: Percentage of responder of different genotypes in case ofXPC (rs2228001)

Table3.8: Association of XPC (rs2228001) polymorphism with response to platinum-based chemotherapy

XPC (rs2228001)				
Genotype	Responder (CR + PR) (n = 65)	Non responder (SD + PD) (n = 85)	Odds ratio (95%CI)	P value
AA (61)	29	32	Reference	-
AC (72)	25	47	0.59 (0.29 -1.18)	0.135
CC (17)	11	6	2.02 (0.66 - 6.17)	0.215
AC+CC (89)	36	53	0.75 (0.39 – 1.45)	0.390

3.2.5Association of ERCC1 (rs11615) polymorphism with response to chemotherapy

In our study, patients carrying the CC genotype of ERCC1 showed poorer response to chemotherapy than did those carrying the CT or TT genotype(n=32, 49%).We found no significant association between that polymorphism and response to a platinum based regimen in this study (Table 3.10).

Table3.9: Response variation of patients with ERCC1 (rs11615) polymorphism

ERCC1 (rs11615)						
	Total Responder (65)			Total Non responder (85)		
Genotype	Complete Response (8)	Partial Response (57)	Total responder (65)	Stable Disease (78)	Progressive Disease (7)	Total non responder (85)
CC (83)	2	30	32	49	2	51
CT (52)	3	21	24	25	3	28
TT (15)	3	6	9	4	2	6
CT+TT (67)	6	27	33	29	5	34

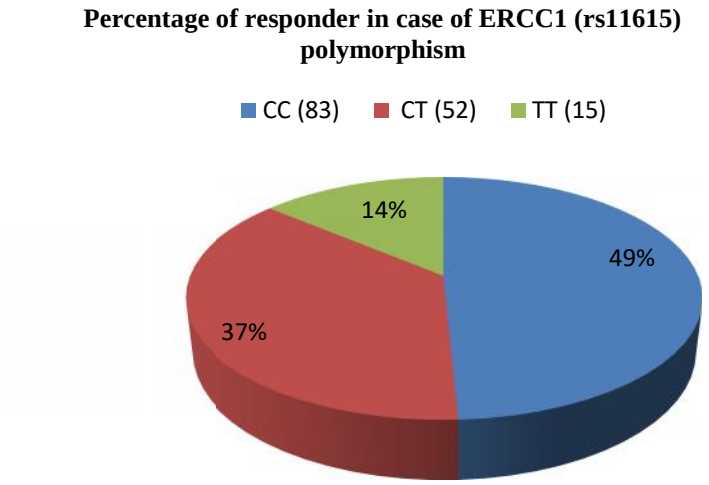


Figure 3.6: Percentage of responder of different genotypes in case ofERCC1 (rs11615) polymorphism

Table3.10: Association of ERCC1 (rs11615) polymorphism with response to platinum-based chemotherapy

ERCC1 (rs11615)				
Genotype	Responder (CR + PR) (n = 65)	Non responder (SD + PD) (n = 85)	Odds Ratio (95% CI)	P value
CC (83)	32	51	Ref	-
CT (52)	24	28	1.37 (0.68 – 2.76)	0.387
TT (15)	9	6	2.39 (0.78 -7.35)	0.128
CT+TT (67)	33	34	1.55 (0.81 -2.97)	0.190

3.3 Toxicity evaluation

Platinum based chemotherapy induced toxic reaction was assessed according to the Common Terminology Criteria for Adverse Events (CTCAE v4.0) (Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 4.0, DCTD, NCI, NIH, DHHS 2003.In the case of toxicity evaluation study, we prospectively recruited 285 NSCLC patients from different hospitals.We divided the patients into two categories: mild plus moderate toxicity (grade ≤ II) and severe toxicity (grade III + grade IV) according to the National Cancer Institute as wellas clinical chemotherapeutic guideline.

3.3.1Association of anemia with demographic and clinicopathologicalcharacteristic of patients

Hematological toxicity such as anemia is the most common types of all adverse events.Off the 285 patients, 96 (28.42%) patientswere found with severe (grade III + grade IV) anemia whereasmore patients were found with mild and moderate(grade ≤ II) anemia(189, 71.57%).We found that older patients (>50 years) had increased risk of severe anemia than younger patients (<50 years) (n= 62, 21.73%). Male patients had higher risk of severe anemia compared to female patients (n=78, 27.37%). Smokers were found more with severe anemia compared to non-smokers (n =68, 23.86%).No significant association of anemia was found between demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.11).

Table 3.11.:Association of different grade of anemiawith demographic andclinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Anemia Grade III + IV (96)	Mild and moderate Anemia Grade ≤ II(189)	OR (95% CI)	P Value
Age					
<50	113	34 (11.93%)	79 (27.72%)	Ref.	-
>50	172	62 (21.75%)	110 (38.60%)	1.31 (0.78 – 2.17)	0.29

Body Weight					
< 55	107	35 (12.87%)	72 (25.26%)	Ref.	-
>55	178	61 (21.40%)	117 (41.05%)	1.07 (0.64 – 1.78)	0.79
Body max index (kg/m^2)					
Underweight (<18.5)	133	51 (17.89%)	82 (28.77%)	Ref.	-
Normal (18.5-24.9)	112	33 (11.58%)	79 (27.72%)	0.67 (0.39 – 1.15)	0.15
Overweight (≥25)	29	8 (2.81%)	21 (7.37%)	0.61 (0.25 -1.49)	0.28
Obese (≥ 30)	11	4 (1.40%)	7 (2.46%)	0.92 (0.26 – 3.30)	0.90
Gender					
Male	221	78 (27.37%)	143 (50.18%)	Ref.	-
Female	64	18 (6.32%)	46 (16.14%)	0.72 (0.39 – 1.32)	0.29
Socioeconomic status					
Poor	31	11 (3.86%)	20 (7.02%)	Ref.	-
Lower-Middle	97	35 (12.28%)	62 (21.75%)	1.03 (0.44 – 2.39)	0.95
Upper-Middle	95	32 (11.23%)	63 (22.11%)	0.92 (0.39 -2.16)	0.85
Rich	62	18 (6.32%)	44 (15.44%)	0.74 (0.30 – 1.86)	0.53
Family History					
Yes	150	56 (19.65%)	94 (32.98%)	Ref.	-
No	135	40 (14.04%)	95 (33.33%)	0.71 (0.43 – 1.16)	0.17
Residence					
Urban	212	67 (23.51%)	145 (50.88%)	Ref.	-
Rural	73	29 (10.18%)	44 (15.44%)	1.43 (0.82 – 2.47)	0.21
Smoking Habit					
Smoker	187	68 (23.86%)	117 (41.05%)	Ref.	-

Non-smoker	98	28(9.82%)	70 (24.56%)	0.70 (0.41 – 1.18)	0.19
TNM stage					
IIIB	214	71(24.91%)	143 (50.18%)	Ref.	-
IV	71	25(8.77%)	46 (16.14%)	1.09 (0.62 – 1.92)	0.75
Histology					
Adenocarcinoma	121	46 (16.14%)	75 (26.32%)	Ref.	-
Squamous carcinoma	103	33 (11.57%)	70 (24.56%)	0.77 (0.44 – 1.34)	0.35
Other	61	17(5.96%)	44 (15.44%)	0.63 (0.32 – 1.23)	0.18

3.3.1.2 Association between GSTP1 (rs1695) polymorphism and platinum induced anemia

The percentage ofgrade ≤ II anemia in patients carrying AA, AG and GG genotype of GSTP1was48%, 39% and 13% respectively. No significant association was found between GSTP1polymorphism and the platinum induced anemia in this study($p>0.05$) (Table 3.13).

Table3.12: Different grades of anemia caused by platinum based chemotherapy in patients carrying GSTP1(rs1695) polymorphism

Anemia				
Allele	Genotype	Grade ≤ II (189)	Grade III (81)	Grade IV (15)
GSTP1 (rs1695)	AA (138)	90	40	8
	AG (117)	74	37	6
	GG (30)	25	4	1

	AG+GG (147)	99	41	7

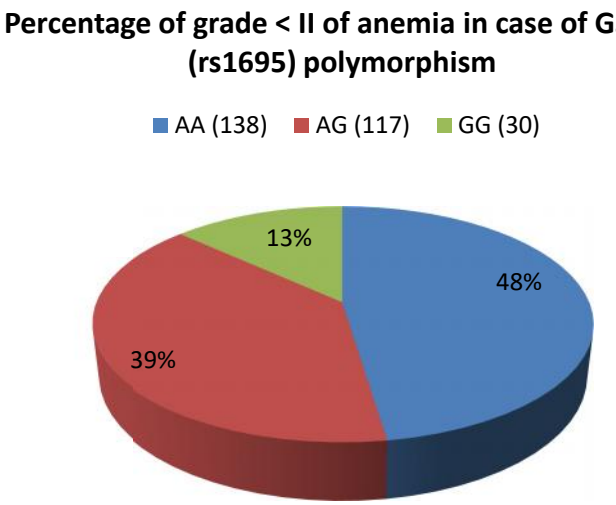


Figure 3.7: Percentage of grade ≤ II of anemia of different genotypes in case of GSTP1 (rs1695)

Table 3.13: Association between GSTP1 (rs1695) and platinum induced anemia

Anemia					
Allele	Genotype	Grade III + Grade IV (96)	Grade ≤II (189)	Odds Ratio (95% CI)	P value
GSTP1 (rs1695)	AA (138)	48	90	Reference	-
	AG (117)	43	74	1.09 (0.65 – 1.82)	0.744
	GG (30)	5	25	0.38 (0.14 – 1.04)	0.060
	AG+GG (147)	48		0.91 (0.56 – 1.49)	0.702

			99		

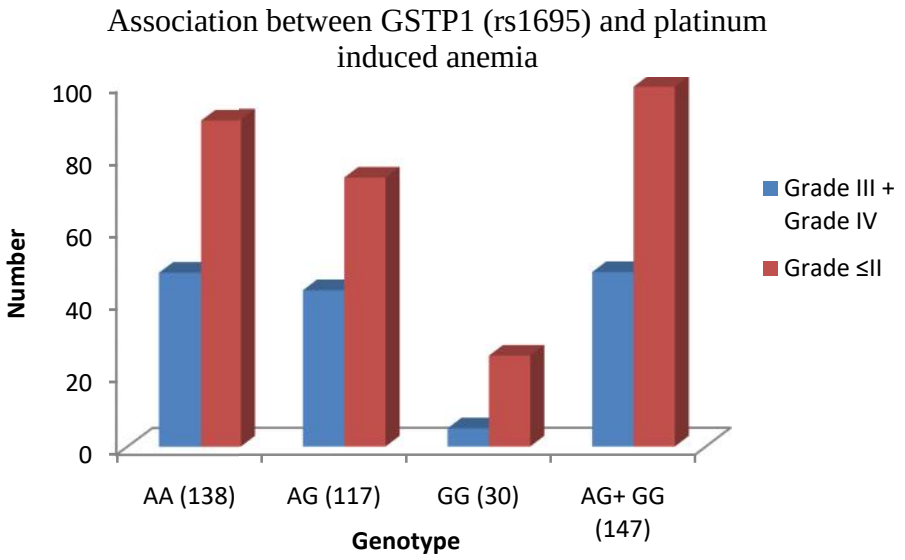


Figure 3.8: Association between GSTP1 (rs1695) and platinum induced anemia

3.3.1.3 Association between XRCC1 (rs25487) polymorphism and platinum induced anemia

In our study, the percentage of grade ≤ II anemiain patients carrying GG, AG and AA genotype of XRCC1 was 63%, 30% and 7% respectively. Patients with AG genotype and (AA+AG) genotypeof XRCC1hadsignificantlyincreased risk of developing anemia compared tothose with GG genotype.(OR = 2.39, 95% Cl=1.42 – 4.02, p = 0.001 and OR = 2.14, 95% Cl = 1.30-3.53, p = 0.003, respectively) (Table 3.15).

Table3.14: Different grades of anemia caused by platinum based chemotherapy in patientscarrying XRCC1 (rs25487) polymorphism

Anemia				
Allele	Genotype	Grade ≤ II (189)	Grade III (81)	Grade IV (15)
XRCC1 (rs25487)	GG (163)	120	36	7
	AG (104)	56	42	6
	AA (18)	13	3	2
	AA+AG (122)	69	45	8

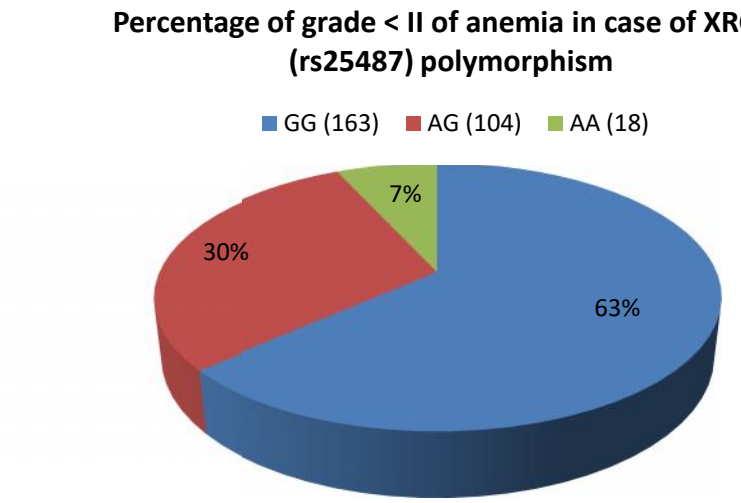


Figure 3.9: Percentage of different grades of anemia caused by platinum based chemotherapy in patients carrying XRCC1 (rs25487) polymorphism

Table 3.15: Association between XRCC1 (rs25487) polymorphism and platinum induced anemia

Anemia					
Allele	Genotype	Grade III + Grade IV (96)	Grade ≤II (189)	Odds Ratio (95% CI)	P value
XRCC1 (rs25487)	GG (163)	43	120	Reference	-
	AG (104)	48	56	2.39 (1.42 – 4.02)	0.001
	AA (18)	5	13	1.07 (0.361 – 3.18)	0.898
	AA+AG (122)	53	69	2.14 (1.30 – 3.53)	0.003

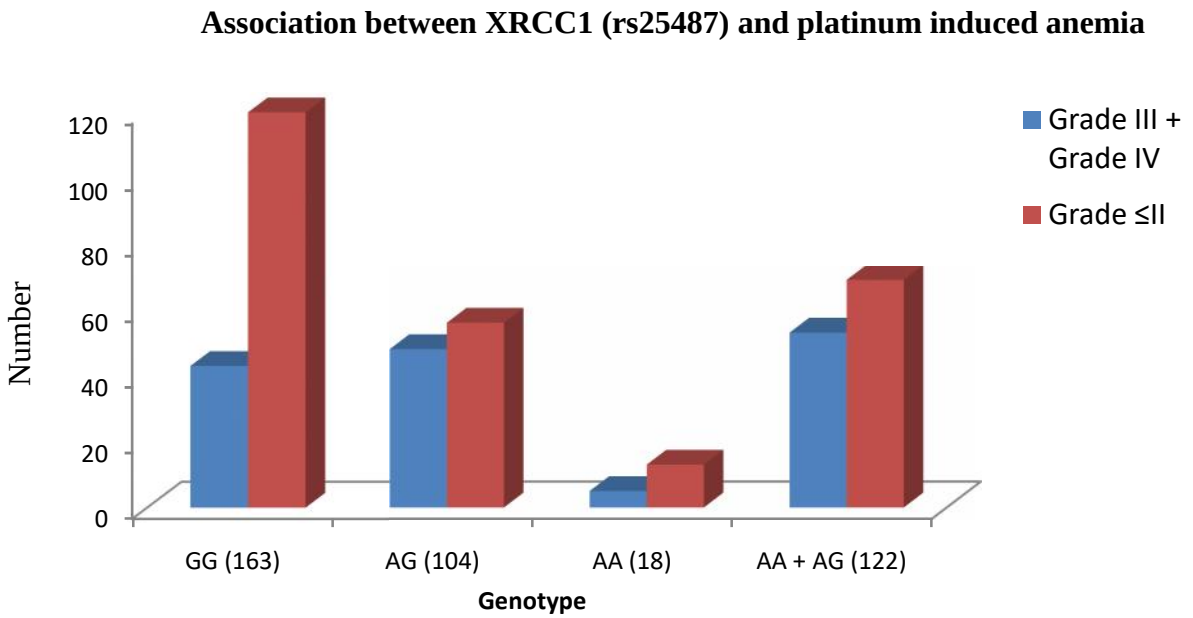


Figure 3.10: Association between XRCC1 (rs25487) polymorphism and platinum induced anemia

3.3.1.4 Association between XPC (rs2228001) polymorphism and platinum induced anemia

In case of XPC polymorphism, grade ≤ II anemia was found in 46% of patients carrying AA genotype, 43% of those AC genotype and 11% of those CC genotype.In this study, there was no statistically significant association between XPC polymorphism and the risk of developinganemia (p=0.054 for the AC genotype and p= 0.051 for the CC genotype, when compared with the AA genotype) (Table 3.17).

Table3.16: Different grades of anemia caused by platinum based chemotherapy in patients carryingXPC (rs2228001)

Anemia				
Allele	Genotype	Grade ≤ II (189)	Grade III (81)	Grade IV (15)
XPC (rs2228001)	AA (124)	87	29	8
	AC (138)	81	50	7
	CC (23)	21	2	0
	AC+CC (161)	102	52	7

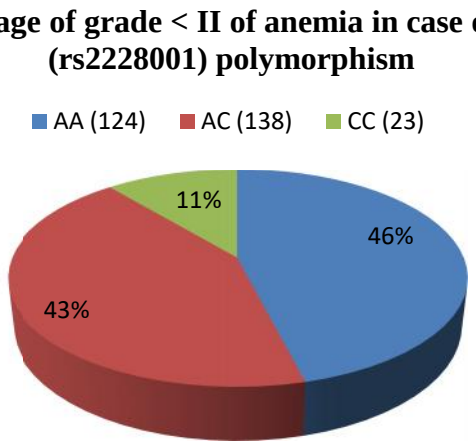


Figure 3.11: Percentage of different grades of anemia caused by platinum based chemotherapy in patients carrying XPC (rs2228001) polymorphism

Table 3.17: Association between XPC (rs2228001) polymorphism and platinum induced anemia

Anemia					
Allele	Genotype	Grade III + Grade IV (96)	Grade ≤II (189)	Odds Ratio (95% CI)	P value
XPC (rs2228001)	AA (124)	37	87	Reference	-
	AC (138)	57	81	1.65 (0.99 – 2.76)	0.054
	CC (23)	2	21	0.22 (0.05 – 1.00)	0.051
	AC+CC (161)	59	102	1.36 (0.82 – 2.24)	0.229

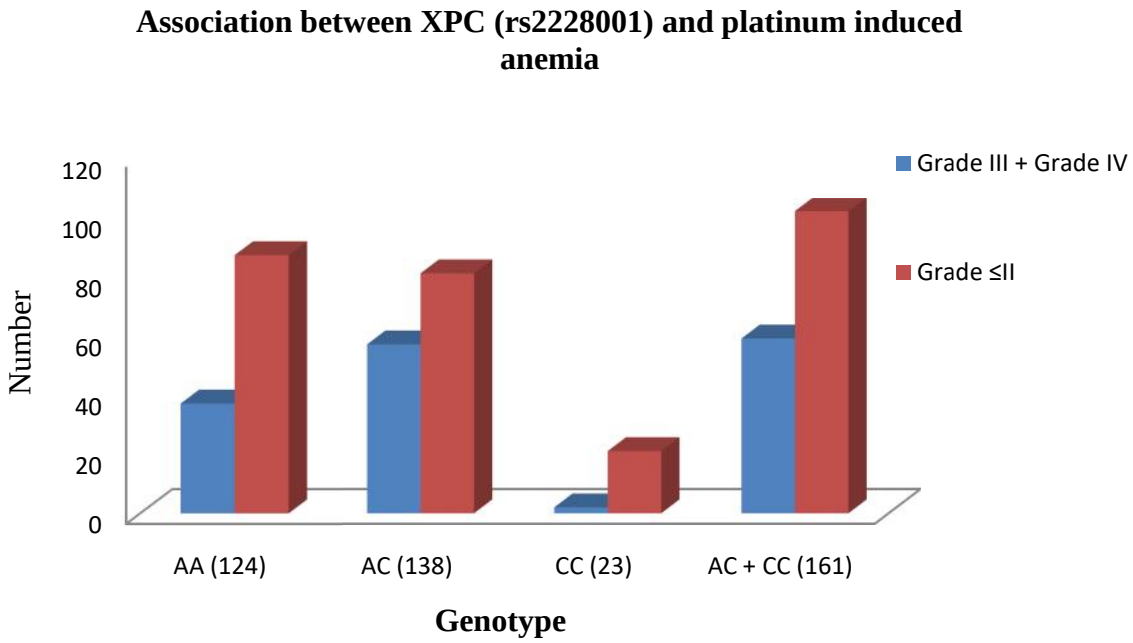


Figure 3.12: Association between XPC (rs2228001) polymorphism and platinum induced anemia

3.3.1.5 Association between ERCC1 (rs11615) polymorphism and platinum induced anemia

In case of ERCC1 polymorphism, the percentage of grade ≤ II anemiaof patients carrying CC, CT and TT genotype was 55%, 33% and 12% respectively. No significant association was found between this polymorphism and platinum induced anemia in this study (Table 3.19).

Table3.18: Different grades of anemia caused by platinum based chemotherapy in patients carryingERCC1 (rs11615)

Anemia				
Allele	Genotype	Grade ≤ II (189)	Grade III (81)	Grade IV (15)
	CC (156)	104	47	5

ERCC1 (rs11615)				
	CT (97)	62	28	7
	TT (32)	23	6	3
	CT+TT (129)	85	34	10

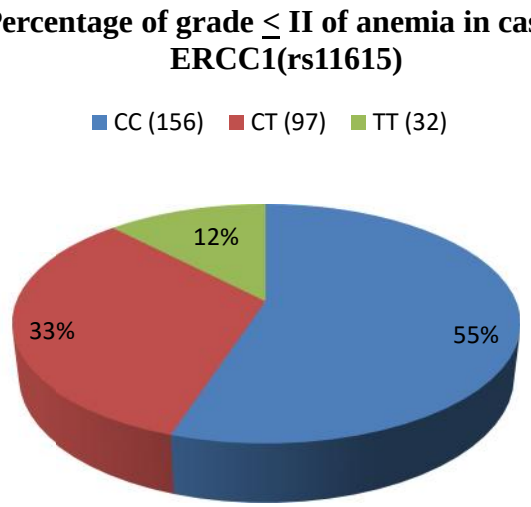


Figure 3.13: Percentage of different grades of anemia caused by platinum based chemotherapy in patients carrying ERCC1 (rs11615)

Table 3.19: Association between ERCC1 (rs11615) and platinum induced anemia

Anemia					
Allele	Genotype	Grade III + Grade IV (96)	Grade ≤II (189)	Odds Ratio (95% CI)	P value
ERCC1 (rs11615)	CC (156)	52	104	Reference	-
	CT (97)	35	62	1.13 (0.66 – 1.92)	0.655
	TT (32)	9	23	0.78 (0.34 – 1.81)	0.567
	CT+TT (129)	44	85	1.03 (0.63 -1.69)	0.890

3.3.2Association of neutropenia with demographic and clinicopathological characteristic of patients

Neutropenia is one of the most common hematological toxicity found inNSCLC patients, when treated with platinum based chemotherapy.Off 285 patients, 89 (31.22%) patients were found with severe neutropenia (grade III + grade IV) and 196 (68.77%) patients were found withmild and moderate neutropenia (grade I and grade II).We found that older patients (>50 years) had increased risk of severe neutropenia than younger patients (<50 years) (n= 58, 20.53%). Male patients had higher risk of severe neutropeniacompared to female patients (n=74, 25.96%). Elevated risk of severe neutropenia was observed with those who had a family history of NSCLC compared to those who had not any family history (n=53, 18.59%). Patients living in urban area were found more with severe neutropenia compared to those living in rural area(n= 63, 22.11%)(Table 3.20).No significant association of neutropenia was found between demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.20).

Table 3.20:Association of different grade of neutropeniawith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Neutropenia Grade III+IV (89)	Moderate Neutropenia Grade ≤ II (196)	OR (95% CI)	P Value
Age					
<50	113	31(10.87%)	82 (28.77%)	Ref.	-
>50	172	58 (20.35%)	114 (40%)	1.34 (0.80 – 2.26)	0.26
Body Weight					
< 55	107	31 (10.87%)	76 (26.67%)	Ref.	-
>55	178	58 (20.35%)	120 (42.11%)	1.18 (0.70 – 1.99)	0.52
Body max index(kg/m^2)					
Underweight (<18.5)	133	49 (17.19%)	84 (29.47%)	Ref.	-
Normal (18.5-24.9)	112	32 (11.23%)	80 (28.07%)	0.69 (0.40 -1.17)	0.17
Overweight (≥25)	29	7(2.46%)	22 (7.72%)	0.55 (0.22 – 1.37)	0.20
Obese (≥ 30)	11	3 (1.05%)	8 (2.81%)	0.64 (0.16 – 2.54)	0.53
Gender					
Male	221	74 (25.96%)	147 (51.58%)	Ref.	-
Female	64	15 (5.26%)	49 (17.19%)	0.61 (0.32 -1.16)	0.13
Socioeconomic status					
Poor	31	9 (3.16%)	22 (7.72%)	Ref.	-
Lower-Middle	97	34 (11.93%)	63 (22.11%)	1.32 (0.55 – 3.18)	0.54
Upper-Middle	95	30 (10.53%)	65 (22.81%)	1.13 (0.46 – 2.74)	0.79
Rich	62	16 (5.61%)	46 (16.14%)	0.85 (0.33 – 2.22)	0.74
Family History					

Yes	150	53 (18.59%)	97 (34.04%)	Ref.	-
No	135	36 (12.63%)	99 (34.74%)	0.67 (0.40 – 1.11)	0.12
Residence					
Urban	212	63 (22.11%)	149 (52.28%)	Ref.	-
Rural	73	26 (9.12%)	47 (16.49%)	1.31 (0.75 – 2.30)	0.35
Smoking Habit					
Smoker	187	65 (22.81%)	122 (42.81%)	Ref.	-
Non-smoker	98	24 (8.42%)	74 (25.96%)	0.61 (0.35 – 1.06)	0.08
TNM stage					
IIIB	214	68 (23.86%)	146 (51.23%)	Ref.	-
IV	71	21 (7.37%)	50 (17.54%)	0.90 (0.50 -1.62)	0.73
Histology					
Adenocarcinoma	121	44 (15.44%)	77 (27.02%)	Ref.	-
Squamous carcinoma	103	31 (10.88%)	72 (25.26%)	0.75 (0.43 – 1.32)	0.32
Other	61	14 (4.91%)	47 (16.49%)	0.52 (0.26 -1.05)	0.07

3.3.2.1 Association between GSTP1 (rs1695) polymorphism and platinum induced neutropenia

The percentage of grade ≤ II neutropeniain patients carrying AA, AG and GG genotype of GSTP1 was 48%, 39% and 13% respectively. Patients with GG genotypeof GSTP1 hadhigher risk for the development of neutropenia compared to those with AA genotype(OR = 0.32, 95% Cl =0.11 -0.97, p = 0.043) (Table 3.22).

Table3.21: Different grades of neutropenia caused by platinum based chemotherapy in patients carryingGSTP1 (rs1695)

Neutropenia				
Allele	Genotype	Grade ≤ II (196)	Grade III (77)	Grade IV (12)
GSTP1 (rs1695)	AA (138)	93	40	5
	AG (117)	77	35	5
	GG (30)	26	2	2
	AG+GG (147)	103	37	7

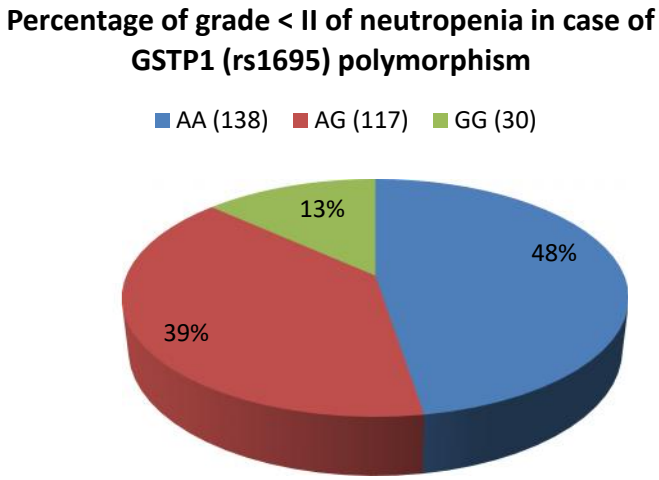


Figure 3.14: Percentage of different grades of neutropenia caused by platinum based chemotherapy in patients carrying GSTP1 (rs1695)

Table 3.22: Association between GSTP1 (rs1695) and platinum induced neutropenia

Neutropenia					
Allele	Genotype	Grade III + Grade IV (89)	Grade ≤II (196)	Odds Ratio (95% CI)	P value
GSTP1 (rs1695)	AA (138)	45	93	Reference	-
	AG (117)	40	77	1.07 (0.64 – 1.80)	0.789
	GG (30)	4	26	0.32 (0.11 – 0.97)	0.043
	AG+GG (147)	44	103	0.883 (0.535 – 1.46)	0.626

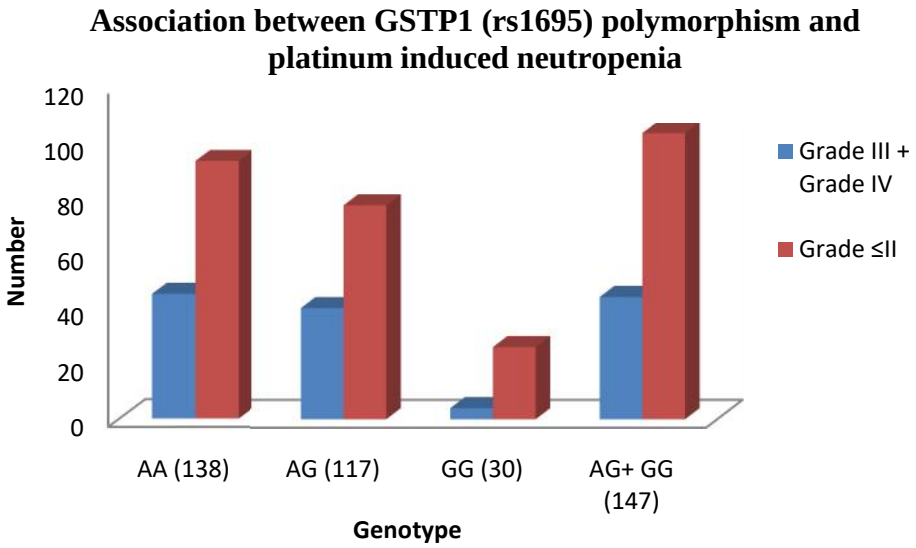


Figure 3.15: Association between GSTP1 (rs1695) polymorphism and platinum induced neutropenia

3.3.2.2 Association between XRCC1 (rs25487) polymorphism and platinum induced neutropenia

The percentage of grade ≤ II neutropenia in patients carrying GG, AG and AA genotype of XRCC1 was 62%, 30% and 8% respectively. Patients with XRCC1 399 variant allele AG and(AA + AG) had higher risk of neutropeniacompared to those with GG genotype(OR = 2.26, 95% Cl =1.34 - 3.84, p = 0.002 and OR = 1.93, 95% Cl = 1.16 - 3.20, p = 0.011 respectively)(Table 3.24).

Table3.23: Different grades of neutropenia caused by platinum based chemotherapy in patients carryingXRCC1 (rs25487) polymorphism

Neutropenia				
Allele	Genotype	Grade ≤ II (196)	Grade III (77)	Grade IV (12)
XRCC1 (rs25487)	GG (163)	122	35	6
	AG (104)	59	40	5
	AA (18)	15	2	1
	AA+AG (122)	74	42	6

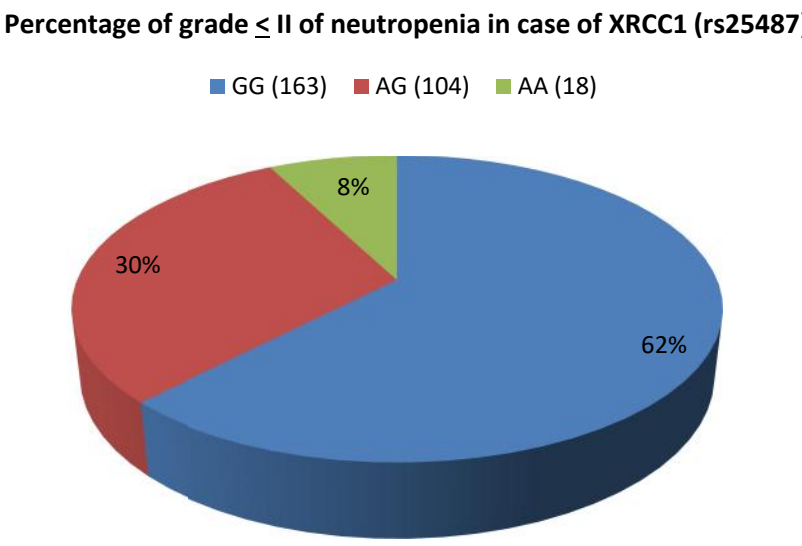


Figure 3.16: Percentage of different grades of neutropenia caused by platinum based chemotherapy in patients carrying XRCC1 (rs25487) polymorphism

Table 3.24: Association between XRCC1 (rs25487) polymorphism and platinum induced neutropenia

Neutropenia					
Allele	Genotype	Grade III + Grade IV (89)	Grade ≤II (196)	Odds Ratio (95% CI)	P value
XRCC1 (rs25487)	GG (163)	41	122	Reference	-
	AG (104)	45	59	2.26 (1.34 – 3.84)	0.002
	AA (18)	3	15	0.59 (0.16 – 2.16)	0.430
	AA+AG (122)	48	74	1.93 (1.16 – 3.20)	0.011

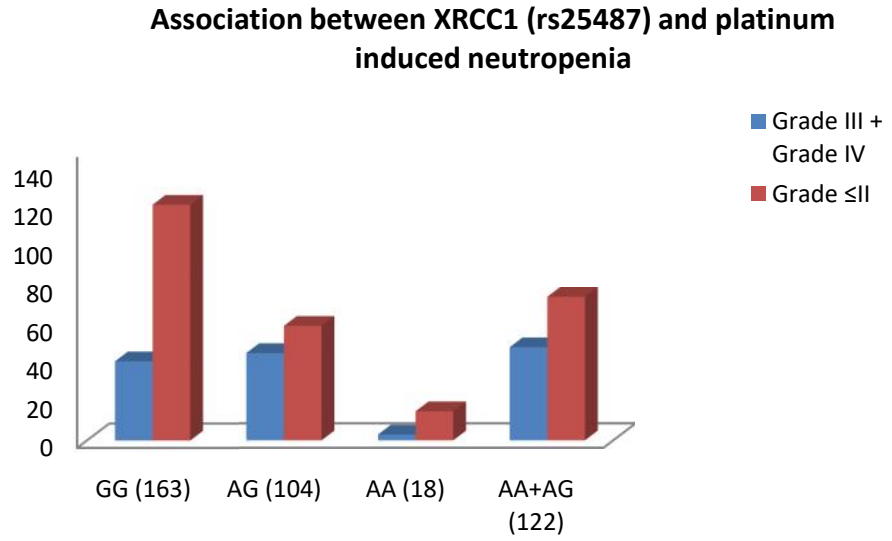


Figure 3.17: Association between XRCC1 (rs25487) polymorphism and platinum induced neutropenia

3.3.2.3 Association between XPC (rs2228001) polymorphism and platinum induced neutropenia

In case of XPC polymorphism, grade ≤ II neutropenia was found in 47% of patients homozygous for the wild-type AA genotype, in contrast to 42% of those heterozygous AC genotype and 11% of those homozygous CC genotype. Patients carrying AC genotype of XPC had significantly higher risk of neutropenia than did those carrying AA genotype (OR = 1.91, 95% Cl =1.13 -3.23, p = 0.016) (Table 3.26).

Table 3.25: Different grades of neutropenia caused by platinum based chemotherapy in patients carryingXPC (rs2228001)

Neutropenia				
Allele	Genotype	Grade ≤ II (196)	Grade III (77)	Grade IV (12)
XPC (rs2228001)	AA (124)	92	25	7
	AC (138)	83	51	4
	CC (23)	21	1	1
	AC+CC (161)	104	52	5

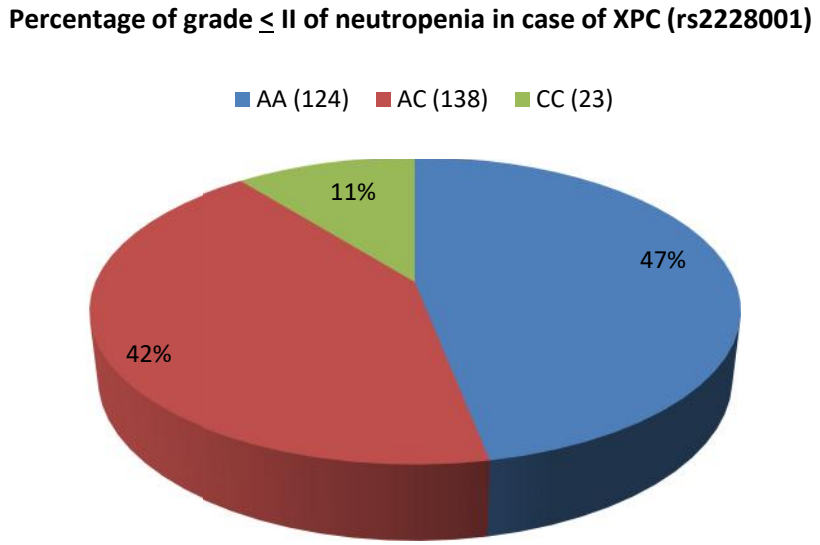


Figure 3.18: Percentage of different grades of neutropenia caused by platinum based chemotherapy in patients carryingXPC (rs2228001)

Table 3.26: Association between XPC (rs2228001) and platinum induced neutropenia

Neutropenia					
Allele	Genotype	Grade III + Grade IV (89)	Grade ≤II (196)	Odds Ratio (95% CI)	P value
XPC (rs2228001)	AA (124)	32	92	Reference	-
	AC (138)	55	83	1.91 (1.13 – 3.23)	0.016
	CC (23)	2	21	0.27 (0.06 – 1.23)	0.092
	AC+CC (161)	57	104	1.58 (0.94 – 2.64)	0.084

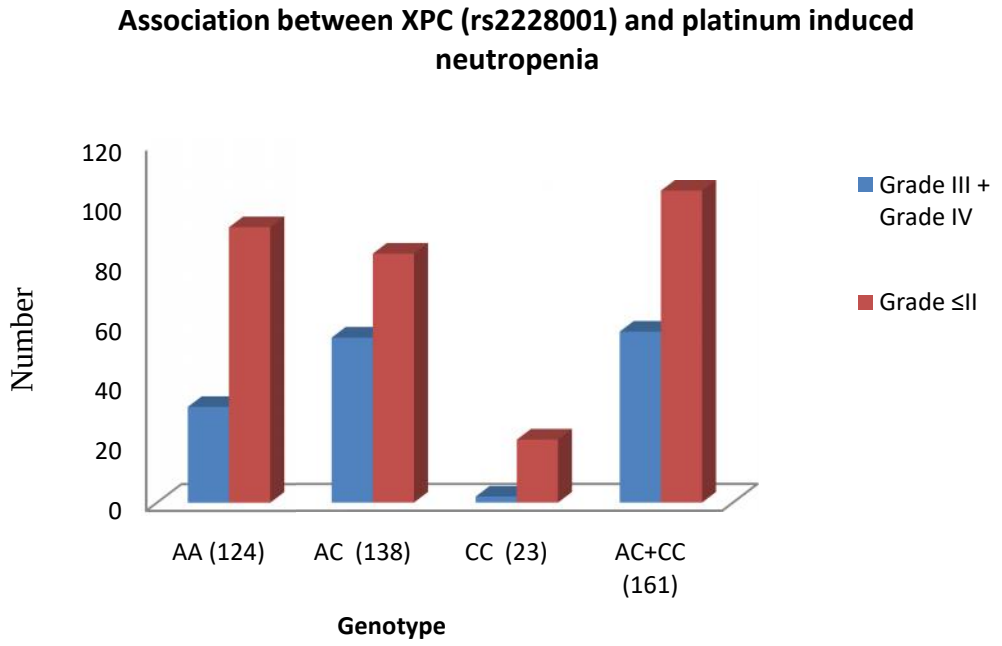


Figure 3.19: Association between XPC (rs2228001) polymorphism and platinum induced neutropenia

3.3.2.4 Association between ERCC1 (rs11615) polymorphism and platinum induced neutropenia

In case of ERCC1 C118T, the percentage of grade ≤ II neutropeniain patients carrying wild-type CC genotype,heterozygous CT genotype and homozygous TT genotype was 55%, 32% and 13% respectively. In this study, there was no statistically significant association between ERCC1 polymorphism and the risk of developing neutropenia for the CT and TT genotype, when compared with the CC genotype (p =0.38 and 0.176 respectively) (Table 3.28).

Table 3.27: Different grades of neutropenia caused by platinum based chemotherapy in patients carryingERCC1 (rs11615)

Neutropenia				
Allele	Genotype	Grade ≤ II (196)	Grade III (77)	Grade IV (12)
ERCC1 (rs11615)	CC (156)	108	41	7
	CT (97)	62	31	4
	TT (32)	26	5	1
	CT+TT (129)	88	36	5

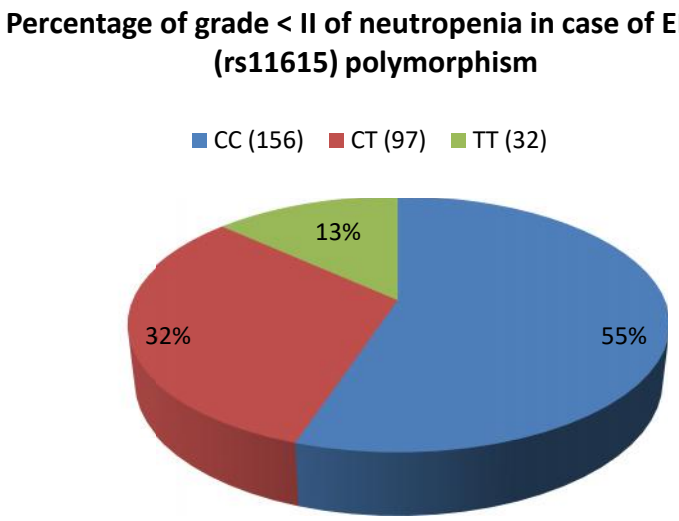


Figure 3.20: Percentage of different grades of neutropenia caused by platinum based chemotherapy in patients carryingERCC1 (rs11615)

Table 3.28: Association between ERCC1 (rs11615) and platinum induced neutropenia

Neutropenia					
Allele	Genotype	Grade III + Grade IV (89)	Grade ≤II (196)	Odds Ratio (95% CI)	P value
ERCC1 (rs11615)	CC (156)	48	108	Reference	-
	CT (97)	35	62	1.27 (0.74 – 2.17)	0.382
	TT (32)	6	26	0.52 (0.20 – 1.34)	0.176
	CT+TT (129)	41	88	1.05 (0.63 – 1.73)	0.854

3.3.3 Association of leukopenia with demographic and clinicopathological characteristic of patients

Leukopenia is another type of hematological toxicity caused by theplatinum based chemotherapy. Out of 285 patients, 24.91% patients were found with severe leukopenia and 75.09% patients were found with mild and moderateleukopenia.We found that older patients (>50 years) had increased risk of severe leukopenia than younger patients (<50 years) (n= 45, 15.79%). Male patients had higher risk of severe leukopeniacompared to female patients (n=60, 21.05%). Elevated risk of severe leukopenia was observed with those who had a family history of NSCLC compared to those who had not any family history(n=39, 13.68%). Patients living in urban area were found more with severe leukopenia compared to those living in rural area(n= 56, 19.65%)(Table 3.29).No significant association of leukopenia was found between demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.29).

Table 3.29: Association of different grade of leukopeniawith demographic and clinicopathological characteristic of patients

Characteristics	No. of Patients	Severe Leukopenia Grade III + IV (71)	Mild and moderate Leukopenia Grade ≤ II (214)	OR (95% CI)	P Value
Age					
<50	113	26 (9.12%)	87 (30.53%)	Ref.	-
>50	172	45 (15.79%)	127 (44.56%)	1.19 (0.68 – 2.06)	0.55
Body Weight					
< 55	107	22 (7.72%)	85 (29.82%)	Ref.	-
>55	178	49 (17.19%)	129 (45.26%)	1.47 (0.83 – 2.60)	0.19
Body max index (kg/m^2)					
Underweight (<18.5)	133	39 (13.68%)	94 (32.98%)	Ref.	-
Normal (18.5-24.9)	112	25 (8.77%)	85 (29.82%)	0.71 (0.40 – 1.27)	0.25
Overweight (≥25)	29	5 (1.75%)	24 (8.42%)	0.50 (0.18 – 1.41)	0.19

Obese (≥ 30)	11	2 (0.70%)	9 (3.16%)	0.54 (0.11 – 2.59)	0.44
Gender					
Male	221	60 (21.05%)	161 (56.49%)	Ref.	-
Female	64	11 (3.86%)	53 (18.60%)	0.56 (0.27 – 1.14)	0.11
Socioeconomic status					
Poor	31	6 (2.11%)	25 (8.77%)	Ref.	-
Lower-Middle	97	27 (9.47%)	70 (24.56%)	1.61 (0.59 – 4.34)	0.35
Upper-Middle	95	27 (9.47%)	68 (23.86%)	1.65 (0.61 -4.48)	0.32
Rich	62	11 (3.86%)	51 (17.89%)	0.94 (0.30 -2.71)	0.85
Family History					
Yes	150	39 (13.68%)	111 (38.95%)	Ref.	-
No	135	32 (11.23%)	103 (36.14%)	0.88 (0.52 – 1.52)	0.65
Residence					
Urban	212	56 (19.65%)	156 (54.74%)	Ref.	-
Rural	73	15 (5.26%)	58 (20.35%)	0.72 (0.38 – 1.37)	0.32
Smoking Habit					
Smoker	187	52 (18.25%)	135 (47.37%)	Ref.	-
Non-smoker	98	19 (6.67%)	79 (27.72%)	0.62 (0.34 -1.13)	0.12
TNM stage					
IIIB	214	58 (20.35%)	156 (54.74%)	Ref.	-
IV	71	13 (4.56%)	58 (20.35%)	0.60 (0.31 – 1.18)	0.14
Histology					
Adenocarcinoma	121	36 (12.63%)	85 (29.82%)	Ref.	-
Squamous carcinoma	103	23 (8.07%)	80 (28.07%)	0.68 (0.37 – 1.24)	0.21

Other	61	12 (4.21%)	49 (17.19%)	0.58 (0.28 -1.21)	0.15

3.3.3.1 Association between GSTP1 (rs1695) polymorphism and platinum induced leukopenia

In case of GSTP1 polymorphism, grade ≤ II leukopenia was found in 49% of patients homozygous for the wild-type AA genotype, in contrast to 39% of those heterozygous AG genotype and 12% of those homozygous GG genotype. No significant association was found between GSTP1 polymorphism and leukopeniafor the patients carrying AG and GG genotype compared to those with AA genotype in this study (p =0.353 and 0.213 respectively)(Table 3.31).

Table 3.30: Different grades of leukopenia caused by platinum based chemotherapy in patients carryingGSTP1(rs1695)

Leukopenia				
Allele	Genotype	Grade ≤ II (214)	Grade III (63)	Grade IV (8)
GSTP1 (rs1695)	AA (138)	105	28	5
	AG (117)	83	32	2
	GG (30)	26	3	1
	AG+GG (147)	109	35	3

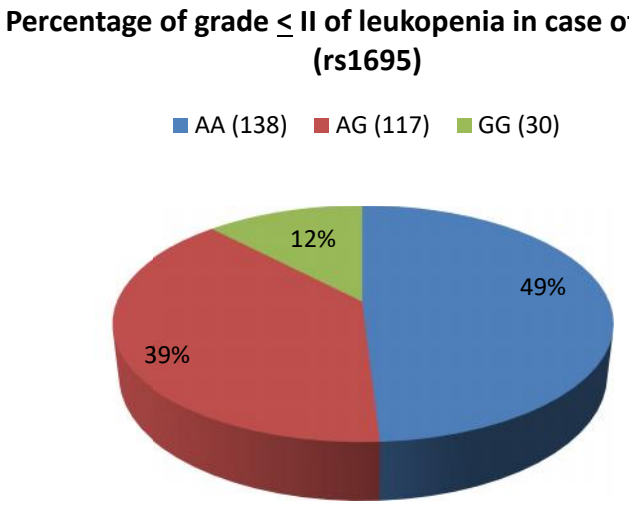


Figure 3.21: Percentage of different grades of leukopenia caused by platinum based chemotherapy in patients carrying GSTP1 (rs1695)

Table 3.31: Association between GSTP1 (rs1695) polymorphismand platinum induced leukopenia

Leukopenia					
Allele	Genotype	Grade III + Grade IV (71)	Grade ≤II (214)	Odds Ratio (95% CI)	P value
GSTP1 (rs1695)	AA (138)	33	105	Reference	-
	AG (117)	34	83	1.30 (0.75 -2.28)	0.353
	GG (30)	4	26	0.49 (0.16 – 1.51)	0.213
	AG+GG (147)	38	109	1.11 (0.65 – 1.89)	0.706

3.3.3.2 Association between XRCC1 (rs25487) polymorphism and platinum induced leukopenia

The percentage of grade ≤ II leukopeniain patients carrying GG, AG and AA genotypeof XRCC1 was 61%, 32% and 7% respectively. In this study, there was no statistically significant association between XRCC1 polymorphism and the risk of developingleukopeniafor the patients with AG and AA genotypecompared to those with GG genotype (p =0.07 and 0.54 respectively) (Table 3.33).

Table 3.32: Different grades of leukopenia caused by platinum based chemotherapy in patients carryingXRCC1 (rs25487)

Leukopenia				
Allele	Genotype	Grade ≤ II (214)	Grade III (63)	Grade IV (8)
XRCC1 (rs25487)	GG (163)	130	29	4
	AG (104)	69	32	3
	AA (18)	15	2	1
	AA+AG (122)	84	34	4

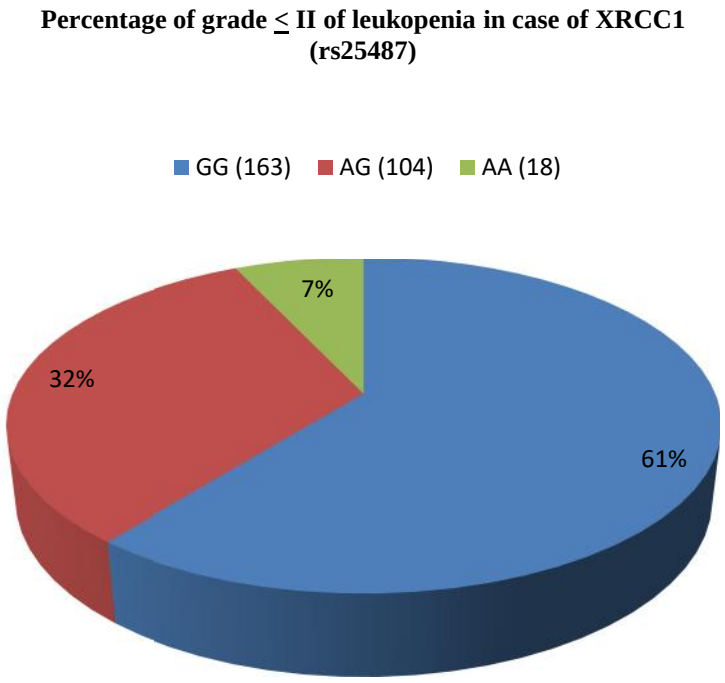


Figure 3.22: Percentage of different grades of leukopenia caused by platinum based chemotherapy in patients carrying XRCC1 (rs25487) polymorphism

Table 3.33: Association between XRCC1 (rs25487) and platinum induced leukopenia

Leukopenia					
Allele	Genotype	Grade III + Grade IV (71)	Grade ≤II (214)	Odds Ratio (95% CI)	P value
XRCC1 (rs25487)	GG (163)	33	130	Reference	-
	AG (104)	35	69	1.69 (0.96 – 2.97)	0.07
	AA (18)	3	15	0.67 (0.18 – 2.44)	0.540
	AA+AG (122)	38	84	1.51 (0.87 – 2.60)	0.140

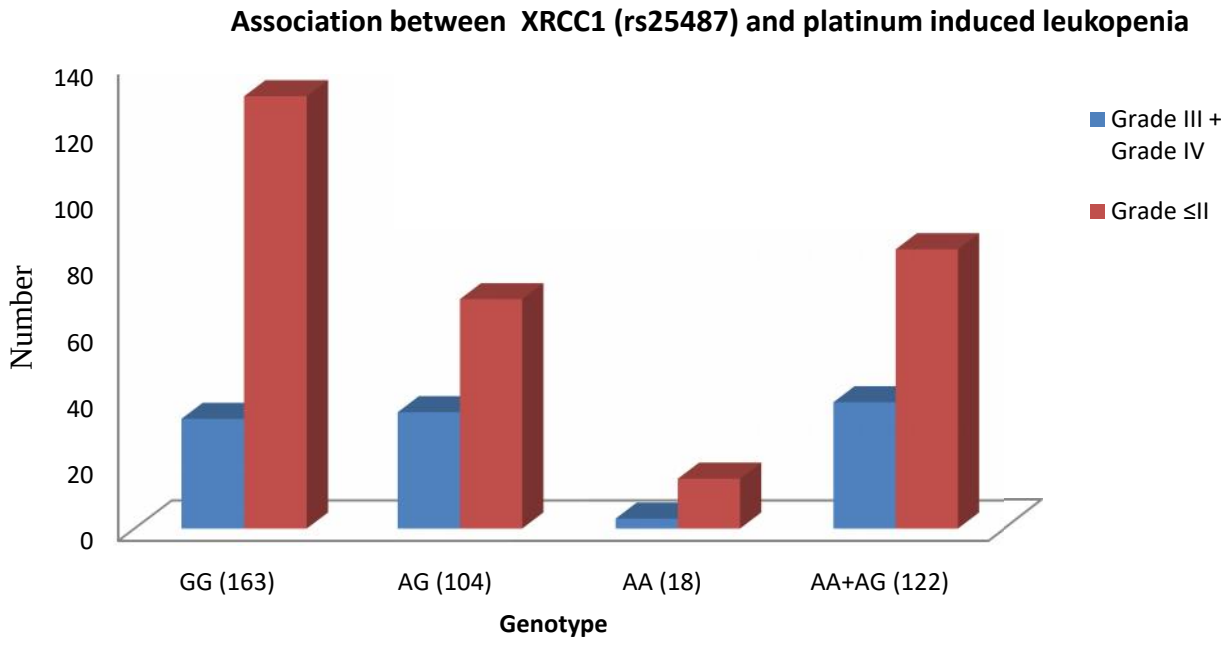


Figure 3.23: Association between XRCC1 (rs25487) and platinum induced leukopenia

3.3.3.3 Association between XPC (rs2228001) polymorphism and platinum induced leukopenia

In case of XPC, grade ≤ II leukopenia was found in 46% of patients homozygous for the wild-type AA genotype, in contrast to 44% of those heterozygous AC genotype and 10% of those homozygous CC genotype. Patients carrying AC genotype of XPC polymorphism had significantlyincreased risk of leukopenia than did those carrying AA genotype (OR = 1.85, 95% Cl =1.05 -3.27, p = 0.033) (Table 3.35)

Table 3.34: Different grades of leukopenia caused by platinum based chemotherapy in patients carrying XPC (2228001)

Leukopenia				
Allele	Genotype	Grade ≤ II (214)	Grade III (63)	Grade IV (8)
XPC (rs2228001)	AA (124)	99	21	4
	AC (138)	94	40	4
	CC (23)	21	2	0
	AC+CC (161)	115	42	4

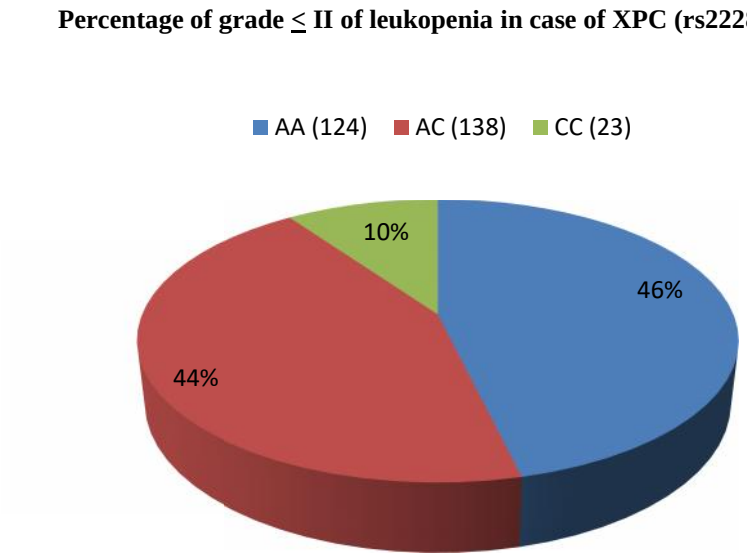


Figure 3.24: Percentage of different grades of leukopenia caused by platinum based chemotherapy in patients carrying XPC (rs2228001)

Table 3.35: Association between XPC (rs2228001) and platinum induced leukopenia

Leukopenia					
Allele	Genotype	Grade III + Grade IV (71)	Grade ≤II (214)	Odds Ratio (95% CI)	P value
XPC (rs2228001)	AA (124)	25	99	Reference	-
	AC (138)	44	94	1.85 (1.05 – 3.27)	0.033
	CC (23)	2	21	0.38 (0.03 -1.72)	0.207
	AC+CC (161)	46	115	1.58 (0.91 – 2.76)	0.105

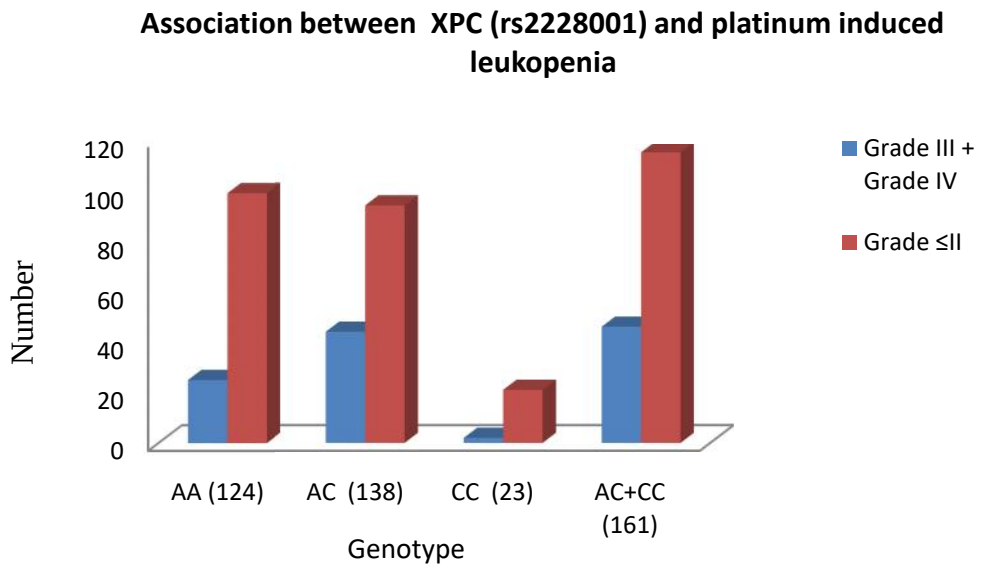


Figure 3.25: Association between XPC (rs2228001) and platinum induced leukopenia

3.3.3.4 Association between ERCC1 (rs11615) polymorphism and platinum induced leukopenia

The percentage of grade ≤ II leukopeniain patients carrying CC, CT and TT genotype of ERCC1 was 53%, 35% and 12% respectively. In this study, there was no statistically significant association between ERCC1 polymorphism and the risk of developingleukopeniafor the patients with CT and TT genotypecompared to those with CC genotype (p =0.57 and 0.34 respectively) (Table 3.37).

Table 3.36: Different grades of leukopenia caused by platinum based chemotherapy in patients carryingERCC1 (rs11615)

Leukopenia				
Allele	Genotype	Grade ≤ II (214)	Grade III (63)	Grade IV (8)
ERCC1 (rs11615)	CC (156)	114	37	5
	CT (97)	74	21	2
	TT (32)	26	5	1
	CT+TT (129)	100	26	3

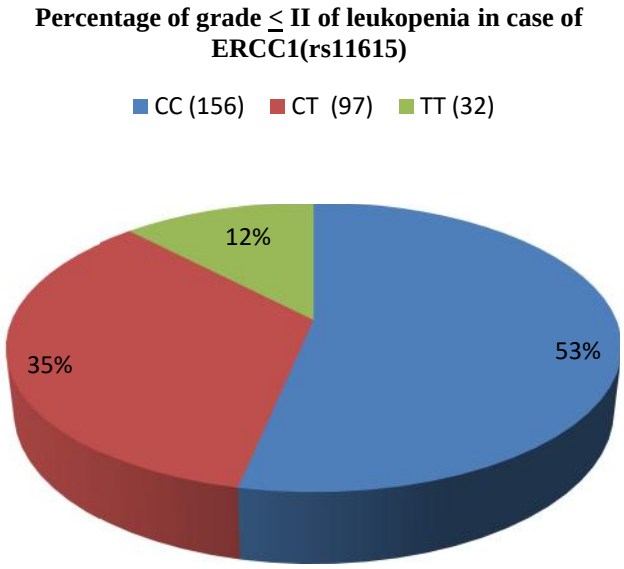


Figure 3.26: Percentage of different grades of leukopenia caused by platinum based chemotherapy in patients carrying ERCC1 (rs11615)

Table 3.37: Association between ERCC1 (rs11615) and platinum induced leukopenia

Leukopenia					
Allele	Genotype	Grade III + Grade IV (71)	Grade ≤II (214)	Odds Ratio (95% CI)	P value
ERCC1 (rs11615)	CC (156)	42	114	Reference	-
	CT (97)	23	74	0.84 (0.47 - 1.52)	0.57
	TT (32)	6	26	0.63 (0.24 – 1.62)	0.34
	CT+TT (129)	29	100	0.79 (0.46 – 1.36)	0.39

3.3.4Association of thrombocytopenia with demographic and clinicopathological characteristics of patients

Thrombocytopenia is another hematological toxicity caused by the platinum based chemotherapy. Out of 285 patients, 16.49% patients were found withsevere thrombocytopenia and 83.51% patients were found with mild and moderate thrombocytopenia. We found that older patients (>50 years) had increased risk of severe thrombocytopenia than younger patients (<50 years) (n= 34, 11.93%). Male patients had higher risk of severe thrombocytopeniacompared to female patients (n=40, 14.04%). Patients living in urban area were found more with severe thrombocytopenia compared to those living in rural area(n= 40, 14.04%)(Table 3.38).No significant association of thrombocytopenia was found between demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.38).

Table 3.38: Association of different grade of thrombocytopeniawith demographic and clinicopathological characteristics of patients

Characteristics	No. of Patients	Severe Thrombocytopenia Grade III + IV (47)	Mild and moderate Thrombocytopenia Grade ≤II (238)	OR (95% CI)	P Value
Age					
<50	113	13 (4.56%)	100 (35.09%)	Ref.	-
>50	172	34 (11.93%)	138 (48.42%)	1.90 (0.95 -3.77)	0.07
Body Weight					
< 55	107	12 (4.21%)	95 (33.33%)	Ref.	-
>55	178	35 (12.28%)	143 (50.18%)	1.94 (0.96 – 3.92)	0.07
Body max index (kg/m^2)					
Underweight (<18.5)	133	29 (10.18%)	104 (36.49%)	Ref.	-
Normal (18.5-24.9)	112	15 (5.26%)	97 (34.04%)	0.55 (0.28 -1.10)	0.09

Overweight (≥25)	29	2 (0.70%)	27 (9.47%)	0.27 (0.06 – 1.18)	0.08
Obese (≥ 30)	11	1 (0.35%)	10 (3.51%)	0.36 (0.04 – 2.92)	0.34
Gender					
Male	221	40 (14.04%)	181 (63.51%)	Ref.	-
Female	64	7 (2.46%)	57 (20%)	0.56 (0.24 -1.31)	0.18
Socioeconomic status					
Poor	31	4 (1.40%)	27 (9.47%)	Ref.	-
Lower-Middle	97	17 (5.96%)	80 (28.07%)	1.43 (0.44 -4.63)	0.55
Upper-Middle	95	19 (6.67%)	76 (26.67%)	1.63 (0.53 – 5.40)	0.38
Rich	62	7 (2.46%)	55 (19.30%)	0.86 (0.23 – 3.19)	0.82
Family History					
Yes	150	21 (7.37%)	129 (45.26%)	Ref.	-
No	135	26 (9.12%)	109 (38.25%)	0.68 (0.36 – 1.28)	0.23
Residence					
Urban	212	40 (14.04%)	172 (60.35%)	Ref.	-
Rural	73	7 (2.46%)	66 (23.16%)	0.46 (0.19 -1.07)	0.07
Smoking Habit					
Smoker	187	36 (12.63%)	151 (52.98%)	Ref.	-
Non-smoker	98	11 (3.86%)	87 (52.53%)	0.53 (0.26 -1.09)	0.09
TNM stage					
IIIB	214	39 (13.68%)	175 (61.40%)	Ref.	-
IV	71	8 (2.81%)	63 (22.11%)	0.57 (0.25 -1.29)	0.18
Histology					
Adenocarcinoma	121	21 (7.37%)	100 (35.09%)	Ref.	-

Squamous carcinoma	103	19 (6.67%)	84 (29.47%)	1.07 (0.54 – 2.14)	0.83
Other	61	7 (2.46%)	54 (18.95%)	0.62 (0.25 – 1.54)	0.30

3.3.4.1 Association between GSTP1 (rs1695) polymorphism and platinum induced thrombocytopenia

In case of GSTP1 polymorphism, grade ≤ IIthrombocytopenia was observed in 50% of patients homozygous forthe wild-type AA genotype, in contrast to 39% ofthose heterozygous AG genotype and 11% of those homozygous GG genotype. We did not find significant association between GSTP1 polymorphism and platinum induced thrombocytopenia (Table 3.40).

Table 3.39: Different grades of thrombocytopenia caused by platinum based chemotherapy in patientscarrying GSTP1 (rs1695)

Thrombocytopenia				
Allele	Genotype	Grade ≤ II (238)	Grade III (42)	Grade IV (5)
GSTP1 (rs1695)	AA (138)	120	16	2
	AG (117)	92	22	3
	GG (30)	26	4	0
	AG+GG (147)	118	26	2

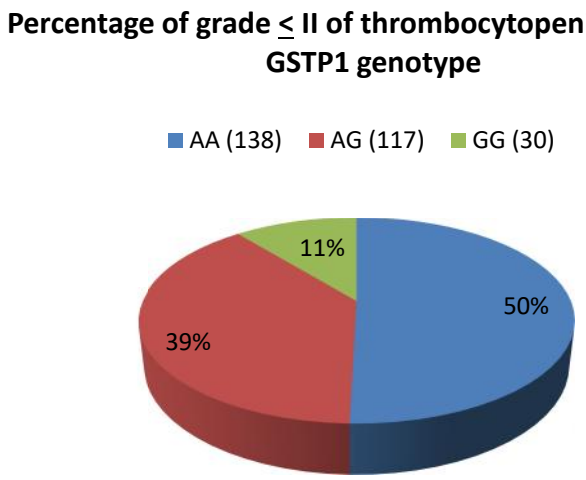


Figure 3.27: Percentage of different grades of thrombocytopenia caused by platinum based chemotherapy in patients carrying GSTP1 (rs1695)

Table 3.40: Association between GSTP1 (rs1695) and platinum induced thrombocytopenia

Thrombocytopenia					
Allele	Genotype	Grade III + Grade IV (47)	Grade ≤II (238)	Odds Ratio (95% CI)	P value
GSTP1 (rs1695)	AA (138)	18	120	Reference	-
	AG (117)	25	92	1.81 (0.93 – 3.52)	0.079
	GG (30)	4	26	1.03 (0.32 – 3.28)	0.966
	AG+GG (147)	29	118	1.64 (0.86 – 3.11)	0.131

3.3.4.2 Association between XRCC1 (rs25487) polymorphism and platinum induced thrombocytopenia

The percentage of grade ≤ II thrombocytopenia in patients carrying GG, AG and AA genotypes of XRCC1 polymorphism was 60%, 34% and 6% respectively. Patients carrying AG genotype and (AA + AG) genotypesof XRCC1 polymorphism had higher risk of developing thrombocytopenia compared with that of the GG genotype (OR = 2.14, 95% Cl =1.12 - 4.12, p = 0.022 and OR = 2.03, 95% Cl = 1.08 - 3.83, p = 0.028 respectively) (Table 3.42).

Table 3.41: Different grades of thrombocytopenia caused by platinum based chemotherapy in patientscarrying XRCC1 (rs25487) polymorphism

Thrombocytopenia				
Allele	Genotype	Grade ≤ II (238)	Grade III (42)	Grade IV (5)
XRCC1 (rs25487)	GG (163)	143	19	1
	AG (104)	80	20	4
	AA (18)	15	3	0
	AA+AG (122)	95	23	4

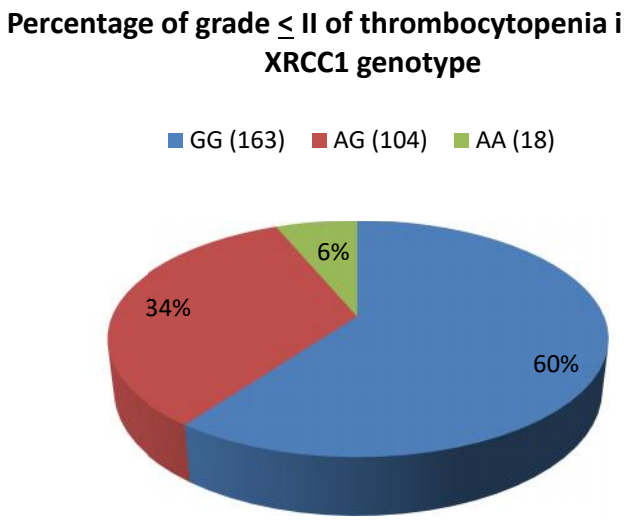


Figure 3.28: Percentage of different grades of thrombocytopenia caused by platinum based chemotherapy in patients carrying XRCC1 (rs25487) polymorphism

Table 3.42: Association between XRCC1 (rs25487) and platinum induced thrombocytopenia

Thrombocytopenia					
Allele	Genotype	Grade III + Grade IV (47)	Grade ≤II (238)	Odds Ratio (95% CI)	P value
XRCC1 (rs25487)	GG (163)	20	143	Reference	-
	AG (104)	24	80	2.14 (1.12 – 4.12)	0.022
	AA (18)	3	15	1.43 (0.38 – 5.38)	0.597
	AA+AG (122)	27	95	2.03 (1.08 – 3.83)	0.028

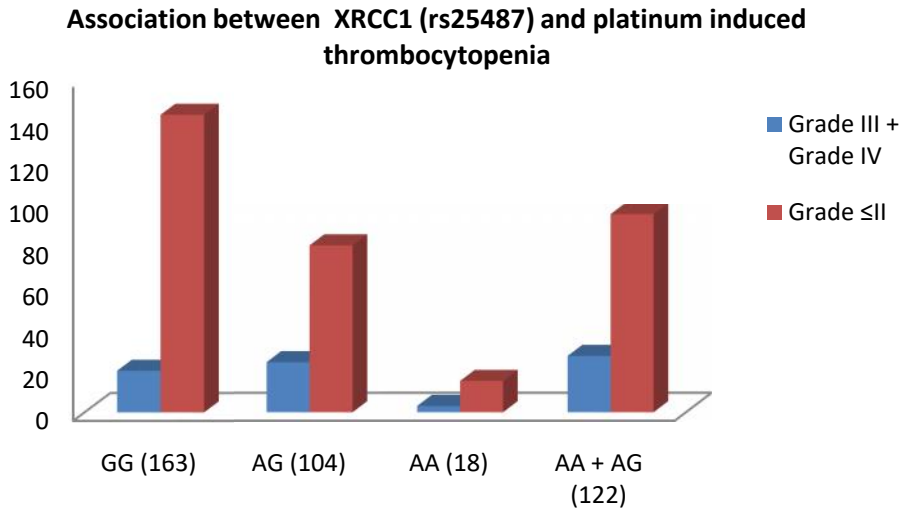


Figure 3.29: Association between XRCC1 (rs25487) and platinum induced thrombocytopenia

3.3.4.3 Association between XPC (rs2228001) polymorphism and platinum induced thrombocytopenia

In case of XPC polymorphism, grade ≤ II thrombocytopenia was found in 46% of patients homozygous for the wild-type AA genotype, in contrast to 45% of those heterozygous AC genotype and 9% of those homozygous CC genotype. In this study, there was no statistically significant association between XPC polymorphism and the risk of developingthrombocytopeniafor the patients with AC and CC genotype compared to those with AA genotype (p =0.16 and 0.51 respectively) (Table 3.44).

Table 3.43: Different grades of thrombocytopenia caused by platinum based chemotherapy in patients carrying XPC (rs2228001)

Thrombocytopenia				
Allele	Genotype	Grade ≤ II (238)	Grade III (42)	Grade IV (5)
XPC (rs2228001)	AA (124)	107	16	1
	AC (138)	110	24	4
	CC (23)	21	2	0
	AC+CC (161)	131	26	4

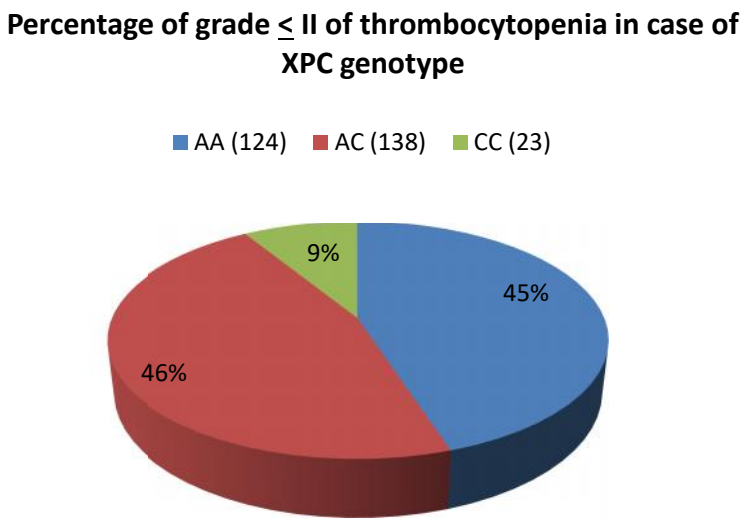


Figure 3.30: Percentage of different grades of thrombocytopenia caused by platinum based chemotherapy in patients carrying XPC (rs2228001)

Table 3.44: Association between XPC (rs2228001) and platinum induced thrombocytopenia

Thrombocytopenia					
Allele	Genotype	Grade III + Grade IV (47)	Grade ≤II (238)	Odds Ratio (95% CI)	P value
XPC (rs2228001)	AA (124)	17	107	Reference	-
	AC (138)	28	110	1.60 (0.83 – 3.10)	0.161
	CC (23)	2	21	0.60 (0.13 – 2.79)	0.514
	AC+CC (161)	30	131	1.44 (0.75 – 2.75)	0.268

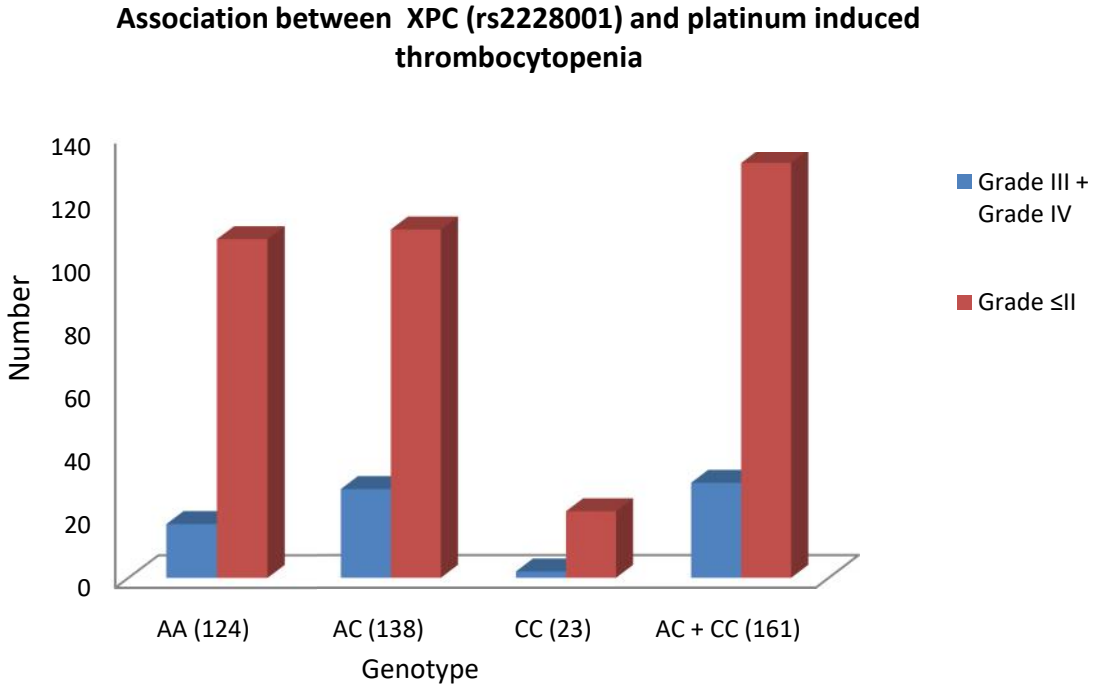


Figure 3.31: Association between XPC (rs2228001) and platinum induced thrombocytopenia

3.3.4.4 Association between ERCC1 (rs11615) polymorphism and platinum induced thrombocytopenia

In case of ERCC1 C118T, grade ≤ II thrombocytopenia was observed in 56% of patients homozygous for the wild-type CC genotype, in contrast to 33% of those heterozygous CT genotype and 11% of those homozygous TT genotype.No statistically significant association between ERCC1 polymorphism and the risk of developingthrombocytopeniawas found for the patients with CT and TT genotypecompared to those with CC genotype (p =0.251 and 0.503 respectively) (Table 3.46).

Table 3.45: Different grades of thrombocytopenia caused by platinum based chemotherapy in patientscarrying ERCC1 (rs11615)

Thrombocytopenia				
Allele	Genotype	Grade ≤ II (238)	Grade III (42)	Grade IV (5)
ERCC1 (rs11615)	CC (156)	134	19	3
	CT (97)	78	17	2
	TT (32)	26	6	0
	CT+TT (129)	104	23	2

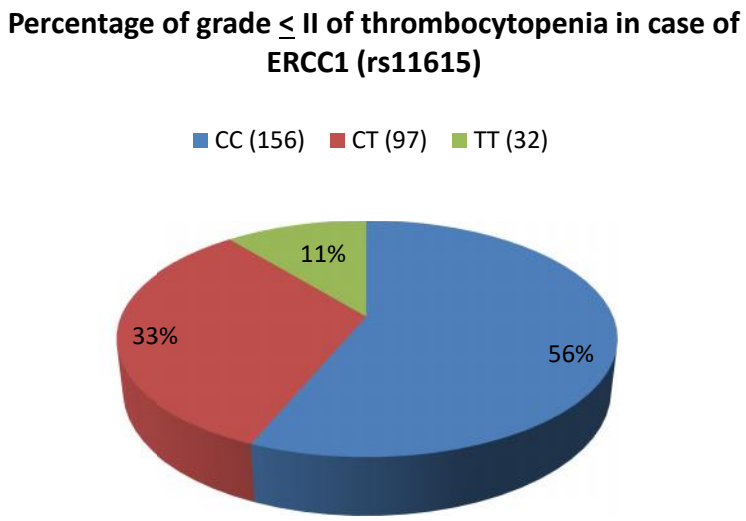


Figure 3.32: Percentage of different grades of thrombocytopenia caused by platinum based chemotherapy in patients carrying ERCC1 (rs11615)

Table 3.46: Association between ERCC1 (rs11615) and platinum induced thrombocytopenia

Thrombocytopenia					
Allele	Genotype	Grade III + Grade IV (47)	Grade ≤II (238)	Odds Ratio (95% CI)	P value
ERCC1 (rs11615)	CC (156)	22	134	Reference	-
	CT (97)	19	78	1.48 (0.76 – 2.91)	0.251
	TT (32)	6	26	1.41 (0.52 – 3.80)	0.503
	CT+TT (129)	25	104	1.46 (0.78 – 2.74)	0.234

3.3.5 Association of different grade of GI toxicity with demographic data and clinicopathological characteristics of patients

Gastrointestinal toxicity is very common in NSCLC patients among platinum-based chemotherapy induced toxicities. Off the 285 patients, 18.20% patients were found with severe GI toxicity (grade III + grade IV) whereasmore patients, 81.80% were found with mild and moderate GI toxicity (grade ≤ II). We found that older patients (>50 years) had increased risk of GI toxicity than younger patients (<50 years) (n= 32, 12.98%). Male patients had higher risk of severe GI toxicitycompared to female patients (n=45, 15.79%). No significant association of GI toxicity was found between demographic and clinicopathological characteristic of patients in our study ($p > 0.05$) (Table 3.47).

Table 3.47: Association of different grade of GI toxicitywith demographic data and clinicopathological characteristics of patients

Characteristics	No. of Patients	Severe GI Toxicity Grade III + IV(53)	Mild & moderate GI Toxicity Grade ≤ II (232)	OR (95% CI)	P Value
Age					
<50	113	16 (5.61%)	97 (34.04%)	Ref.	-
>50	172	37 (12.98%)	135 (47.37%)	1.66 (0.87 – 3.16)	0.12
Body Weight					
< 55	107	14 (4.91%)	93 (32.63%)	Ref.	-
>55	178	39 (13.68%)	139 (48.77%)	1.86 (0.96 -3.62)	0.07
Body max index (kg/m^2)					
Underweight (<18.5)	133	32 (11.23%)	101 (35.44%)	Ref.	-
Normal (18.5-24.9)	112	18 (6.32%)	94 (32.98%)	0.60 (0.32 -1.15)	0.12
Overweight (≥25)	29	2 (0.70%)	27 (9.47%)	0.23 (0.05 -1.04)	0.06
Obese (≥ 30)	11	1 (0.35%)	10 (3.51%)	0.32 (0.04 -2.56)	0.28
Gender					
Male	221	45 (15.79%)	176 (61.75%)	Ref.	-

Female	64	8 (2.81%)	56 (19.65%)	0.56 (0.25 -1.26)	0.16
Socioeconomic status					
Poor	31	5 (1.75%)	26 (9.12%)	Ref.	-
Lower-Middle	97	21 (7.37%)	76 (26.67%)	1.44 (0.49 -4.20)	0.51
Upper-Middle	95	19 (6.67%)	76 (26.67%)	1.30 (0.44 – 3.83)	0.63
Rich	62	8 (2.81%)	54 (18.95%)	0.77 (0.23 – 2.59)	0.67
Family History					
Yes	150	29 (10.18%)	121 (42.46%)	Ref.	-
No	135	24 (8.42%)	111 (38.95%)	0.90 (0.50 – 1.64)	0.74
Residence					
Urban	212	45 (15.79%)	167 (58.60%)	Ref.	-
Rural	73	8 (2.81%)	65 (22.81%)	0.46 (0.20 – 1.02)	0.06
Smoking Habit					
Smoker	187	39 (13.68%)	148 (51.93%)	Ref.	-
Non-smoker	98	14 (4.91%)	84 (29.47%)	0.63 (0.32 – 1.23)	0.18
TNM stage					
IIIB	214	44 (15.44%)	170 (59.65%)	Ref.	-
IV	71	9 (3.16%)	62 (21.75%)	0.56 (0.26 -1.22)	0.14
Histology					
Adenocarcinoma	121	24 (8.42%)	97 (34.04%)	Ref.	-
Squamous carcinoma	103	21 (7.37%)	82 (28.77%)	1.04 (0.54 -1.99)	0.92
Other	61	8 (2.81%)	53 (18.60%)	0.61 (0.26 -1.45)	0.26

3.3.5.1 Association between GSTP1 (rs1695) polymorphism and platinum induced gastrointestinal toxicity

The percentage of grade ≤ II GI toxicity in patients carrying wild-type AA, heterozygous AG and homozygous GG genotype of GSTP1 was 51%, 38% and 11% respectively. There was no statistically significant association between GSTP1 polymorphism and the risk of developingGI toxicityfor the patients with AG and GG genotypecompared to those with AA genotype (p =0.081 and 0.793 respectively) in this study (Table 3.49).

Table 3.48: Different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patientscarrying GSTP1 (rs1695)

Gastrointestinal toxicity				
Allele	Genotype	Grade ≤ II (232)	Grade III (47)	Grade IV (6)
GSTP1 (rs1695)	AA (138)	117	18	3
	AG (117)	89	16	2
	GG (30)	26	3	1
	AG+GG (147)	115	19	3

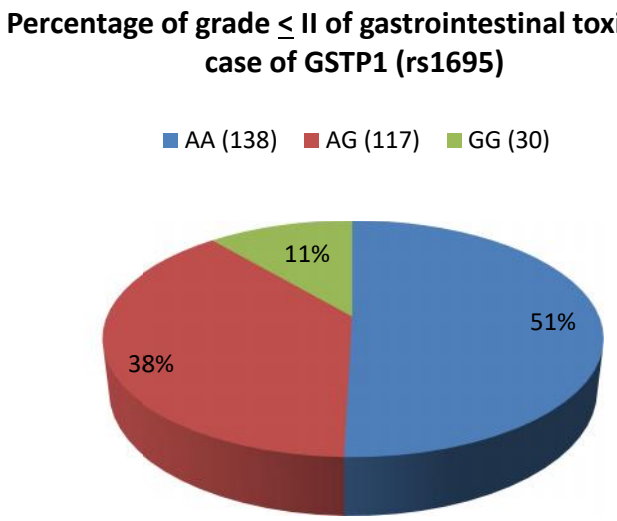


Figure 3.33: Percentage of different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patients carrying GSTP1 (rs1695)

Table 3.49: Association between GSTP1 (rs1695) and platinum induced gastrointestinal toxicity

Gastrointestinal Toxicity					
Allele	Genotype	Grade III + Grade IV (53)	Grade ≤II (232)	Odds Ratio (95% CI)	P value
GSTP1 (rs1695)	AA (138)	21	117	Reference	-
	AG (117)	28	89	1.75 (0.93 – 3.29)	0.081
	GG (30)	4	26	0.857 (0.27 – 2.71)	0.793
	AG+GG (147)	32	115	1.55 (0.84 – 2.85)	0.157

3.3.5.2 Association between XRCC1 (rs25487) polymorphism and platinum induced gastrointestinal toxicity

In case of XRCC1, grade ≤ II GI toxicity was found in 62% of patients homozygous for the wild-type GG genotype, in contrast to 32% of those heterozygous AG genotype and 6% of those homozygous AA genotype. Patients carrying AG genotype of XRCC1 had increasedrisk of gastrointestinal toxicity than did those carrying GG genotype (OR=2.89,95%CI =1.54-5.45, p=0.001). Furthermore, individuals carrying at least one A allele (AA+AG) of XRCC1had statistically increased risk of gastrointestinal toxicity when compared with that of theGG genotype (OR=2.65, 95%CI=1.43 -4.90, p=0.002) (Table 3.51).

Table 3.50: Different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patientscarrying XRCC1 (rs25487) polymorphism

Gastrointestinal toxicity				
Allele	Genotype	Grade ≤ II (232)	Grade III (47)	Grade IV (6)
XRCC1 (rs25487)	GG (163)	143	18	2
	AG (104)	74	26	4
	AA (18)	15	3	0
	AA+AG (122)	89	29	4

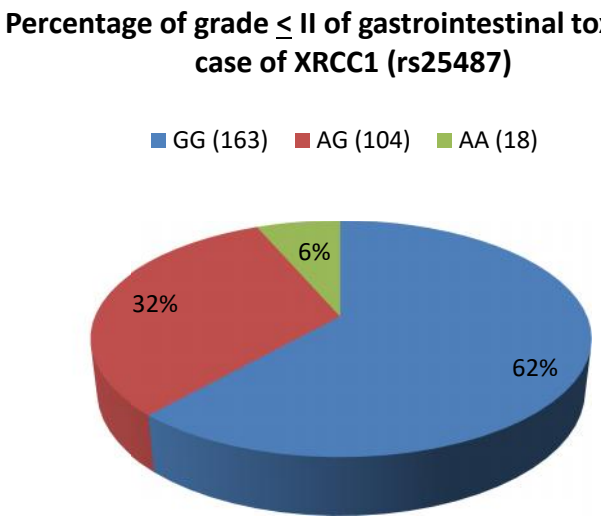


Figure 3.34: Percentage of different grades of neutropenia caused by platinum based chemotherapy in patients carrying XRCC1 (rs25487) polymorphism

Table 3.51: Association between XRCC1 (rs25487) polymorphism and platinum induced gastrointestinal toxicity

Gastrointestinal Toxicity					
Allele	Genotype	Grade III + Grade IV (53)	Grade ≤II (232)	Odds Ratio (95% CI)	P value
XRCC1 (rs25487)	GG (163)	20	143	Reference	-
	AG (104)	30	74	2.89 (1.54 -5.45)	0.001
	AA (18)	3	15	1.43 (0.38 -5.38)	0.597
	AA+AG (122)	33	89	2.65 (1.43 -4.90)	0.002

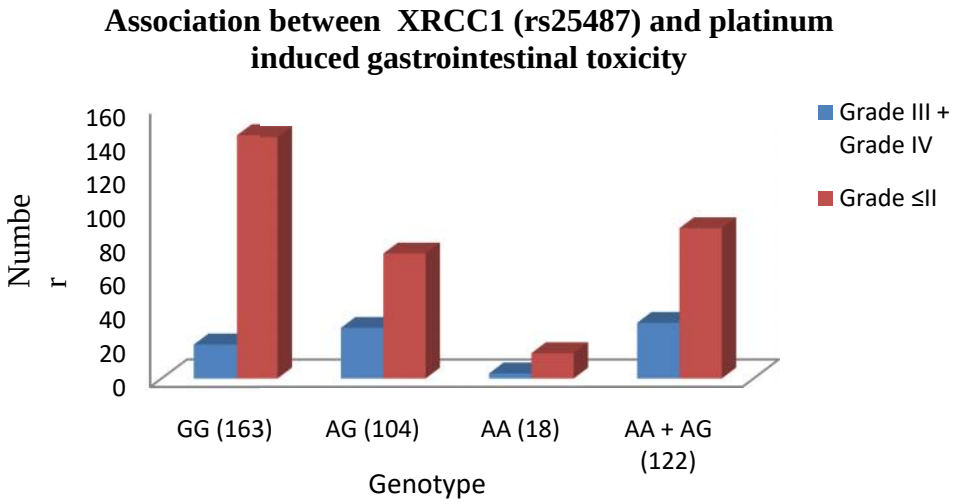


Figure 3.35: Association between XRCC1 (rs25487) polymorphism and platinum induced gastrointestinal toxicity

3.3.5.3 Association between XPC (rs2228001) polymorphism and platinum induced gastrointestinal toxicity

The percentage of grade ≤ II GI toxicity in patients carrying AA, AC and CC genotype of XPC was 47%, 44% and 9% respectively. There was no statistically significant association between XPC polymorphism and the risk of developing GI toxicityfor the patients with AC and CC genotype compared to those with AA genotype in our study (p =0.722 and 0.261 respectively) (Table 3.53).

Table 3.52: Different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patientscarrying XPC (rs2228001)

Gastrointestinal toxicity				
Allele	Genotype	Grade ≤ II (232)	Grade III (47)	Grade IV (6)
XPC (rs2228001)	AA (124)	101	20	3
	AC (138)	110	25	3
	CC (23)	21	2	0
	AC+CC (161)	131	27	3

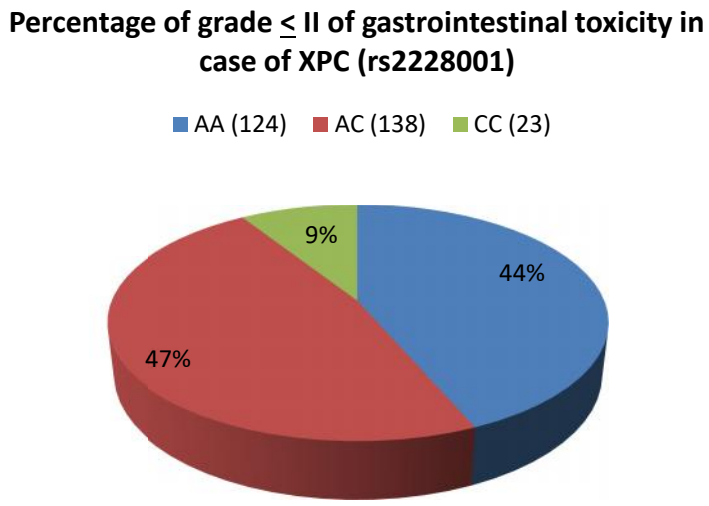


Figure 3.36: Percentage of different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patients carrying XPC (rs2228001)

Table 3.53: Association between XPC (rs2228001) and platinum induced gastrointestinal toxicity

Gastrointestinal Toxicity					
Allele	Genotype	Grade III + Grade IV (53)	Grade ≤II (232)	Odds Ratio (95% CI)	P value
XPC (rs2228001)	AA (124)	23	101	Reference	-
	AC (138)	28	110	1.12 (0.61 – 2.07)	0.722
	CC (23)	2	21	0.42 (0.09 - 1.91)	0.261
	AC+CC (161)	30	131	1.01 (0.55 - 1.84)	0.985

3.3.5.4 Association between ERCC1 (rs11615)polymorphism and platinum induced gastrointestinal toxicity

In case of ERCC1 C118T, grade ≤ II GI toxicity was found in 55% of patients homozygous for the wild-type CC genotype, in contrast to 34% of those heterozygous CT genotype and 11% of those homozygous TT genotype.No statistically significant association between ERCC1 polymorphism and the risk of developingGI toxicity was foundfor the patients with CT and TT genotype compared to those with CC genotype in our study (p =0.745 and 0.915 respectively)(Table 3.55).

Table 3.54: Different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patientscarrying ERCC1 (rs11615)

Gastrointestinal toxicity				
Allele	Genotype	Grade ≤ II (232)	Grade III (47)	Grade IV (6)
ERCC1 (rs11615)	CC (156)	128	25	3
	CT (97)	78	17	2
	TT (32)	26	5	1
	CT+TT (129)	104	22	3

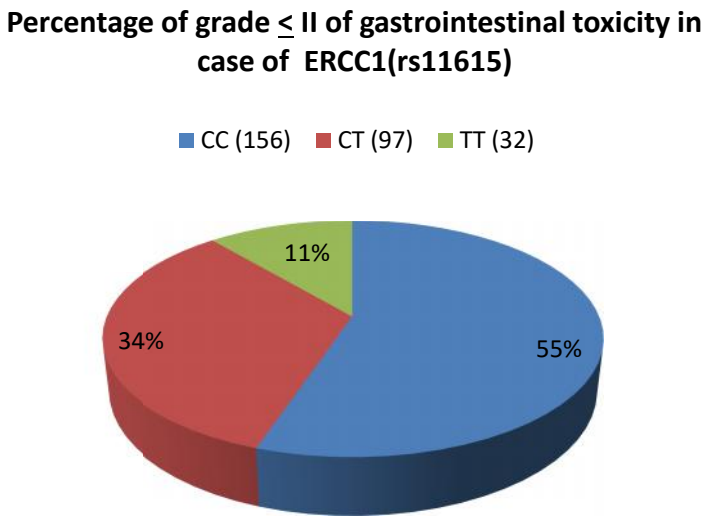


Figure 3.37: Percentage of different grades of gastrointestinal toxicity caused by platinum based chemotherapy in patients carrying ERCC1 (rs11615)

Table 3.55: Association between ERCC1 (rs11615) and platinum induced gastrointestinal toxicity

Gastrointestinal Toxicity					
Allele	Genotype	Grade III + Grade IV (53)	Grade ≤II (232)	Odds Ratio (95% CI)	P value
ERCC1 (rs11615)	CC (156)	28	128	Reference	-
	CT (97)	19	78	1.11 (0.58 – 2.13)	0.745
	TT (32)	6	26	1.05 (0.39 - 2.80)	0.915
	CT+TT (129)	25	104	1.09 (0.60 – 1.99)	0.757

4. Discussion

Treatment response and chemotherapy induced toxicities are important issue in the treatment of cancer patients. Individualized drug dosage can reduce unnecessary toxicity and improve therapeutic efficacy.Oncologists could predict the efficacy and toxicity according to the pharmacological effects and drug dosage regimen, patient's KPS, organ functions, and previous treatments, etc.But the toxicity may be quite different for the same drug dosage. We believe that the treatment efficacy and toxicity could vary for patients with different genotypes. In this study, we examined 285 patients receiving platinum based chemotherapy to establish the relationship between GSTP1 (rs1695), XRCC1 (rs25487), XPC (rs2228001) and ERCC1 (rs11615) gene polymorphisms and toxicity produced by this chemotherapy as well as we assessed the association of chemotherapy response with mentioned genetic polymorphisms of 150 patients for whom the CT scan reports of before and after chemotherapy were available.

The demographic and clinicopathological characteristics of all the recruited patients were recorded and a chi square test was done toanalyze these recorded values in terms of response and toxicity.Out of 285, aged over 50 years of patients was found more (60.35%) than younger (<50 years). This might be due to that ageing is a process associated with chronic respiratory disease like lung cancer. 77.54% patients were male whereas 22.46% patientswere female. The highest incidence in male might be due to higher rates of smoking and to the occupational factors to which males were more often exposed. Sometimes females were reluctant to consult doctors. Perhaps, for this reason, the number of female patients was found less in our study. Economically poor class patients were less available compared to other classes (10.88%). This might be due to the expensive platinum based treatment. It might be one of the reasons of low availability of poor patients in our study. So, this picture might not represent the actual situation.52.63% patients had the family history of lung cancer.This might be due to genetic predisposition.Out of 285 patients, 65.61% was smokers and 34.38% was non-smokers. This might be due to the association of lung cancer with smoking. Most of the patients (78.6%) came from urban area in this study. This might be due to unhealthier lifestyles and the urban air pollution (Table 3.1).

Glutathione S-transferases (GSTs) are phase II metabolic enzymes that are involved in the detoxification of mutagenic and cytotoxic DNA-reactive molecules mediated by glutathione conjugation. Glutathione S-transferase P1 (GSTP1) is a subclass of GSTs, which plays a role in detoxification of platinum compounds. This enzyme is also an important mediator of both intrinsic and acquired resistance to platinum (Peklak-Scott et al., 2008). Previous studies showed a single nucleotide substitution (A to G) at position 313 induces replacement of isoleucine (IIe) with valine (Val) at codon 105, has been found to modify enzyme activity and affinity for electrophilic substrates (Watson et al., 1998), and previous epidemiologic studies have shownthe polymorphism of GSTP1 has a profound impact on chemotherapy for NSCLC, especially for platinum (Zhou et al.,2011).

Several previous studies have reported the association of this polymorphism withplatinum induced toxicity and treatment response of patients with advanced NSCLC (Stoehlmacher et al., 2004; Kalikaki et al., 2009; Ada et al., 2010; Ke et al., 2012). Sun et al. found that the A→G change of GSTP1 A313G polymorphism significantly increased platinum-based chemotherapy response in advanced NSCLC patients (Sun et al.,2010).However, a few studies conducted in Chinese population suggested GSTP1 was associated inversely with response to chemotherapy (Xu et al., 2010; Zhou et al., 2011).

Patients carrying AG and AG+GG genotypes of GSTP1 polymorphism showed a significantly good response to platinum based chemotherapy than did those carrying AA genotype ($p = 0.034$ and $p = 0.037$ respectively) in our study (Table 3.4). Furthermore,patients with GG genotype of GSTP1 had higher risk for the development of neutropenia compared with those carrying AA genotype ($p = 0.043$) (Table 3.22).

The possible explanation as GSTP1, the major type of detoxifying enzyme plays a vital role in activation and detoxification of platinum based drugs and its metabolites. It is found in many normal and malignant tissues and has the significant role in the metabolic pathway of this drug (Fuertes et. al, 2003). Polymorphism of single-nucleotide substitution in the coding sequence of GSTP1 (rs1695 A>G) give rise to Ile105Val and amino acid substitutions present within the substrate-binding site of GSTP1 are reported to have the differences in its catalytic activity

(Zhang et al.,2011).The GSTP1 105Val variant is known to have the relationship with a lower thermalstability and altered catalytic activity to a wide range of substrates in comparison withGSTP1105Ile (Yang and Xian, 2014).Patients with homozygous AA (Ile/Ile) areknown to have the highest level of GSTP1 activity and it reduced in heterozygotes AG (Ile/Val) to some extent and further reduction in activity is observed in those patients who have mutant homozygotes GG and contain two copies of valine (Val/Val**)** (Watson et al.,1998; Sunet. al, 2010)**.** In case of G (Val) carriers, plasma drug level will be increased as drug metabolism becomes slow leading to more response. Again patients carrying GG genotype of GSTP1 polymorphism had higher risk for the development of neutropenia compared to those with AA genotype. This might be due to differential capacities of normal and malignant cells in dealing with drug cytotoxicity, which could be further attributed to somatic changes incurred during tumorigenesis in cancer cells.

The X-ray repair cross complementing group 1 (XRCC1) gene is a major member of the base excision repair (BER) pathway. The XRCC1 gene product plays an important role in the BER pathway by acting as a scaffold for other DNA repair proteins, such as DNA polymerase β and DNA ligase III. It is well known that this gene has a prominent function in the pathway of efficiently repairing DNA damage induced by DNA alkylating agents like platinum based drug. So the capacity of repairing the damaged cancer cell would also be changed by the polymorphisms of XRCC1, and the cytotoxic effects and response to chemotherapy would be altered (Zhou et al., 2011).

Currently, several studies have assessed the association between XRCC1 polymorphism and response to platinum based drug of patients with NSCLC (Xu et al., 2011; Liao et al., 2012; Zhao et al., 2013; Du et al., 2014; Zhang et al., 2014). But chemotherapy toxicity was focused by only a few studies (Giachino et al., 2007; Tibaldi et al., 2008). Wang et al found that patients carrying at least one variant XRCC1 Arg399Gln allele have a 2.5-fold increased risk of grade 3 or 4 gastrointestinal toxicity when treated with first-line cisplatin-based chemotherapy in lung cancer (Wang et al., 2008). Yu et al found that patients carrying the AA genotype had shown a good response to platinum based treatment compare to those with the GG genotype (Yu et al., 2014).

In case of XRCC1, we found that patients carrying AG genotype and at least one variant A allele (AA + AG) showed a significantly good response to chemotherapy than did those carrying the GG genotype (p = 0.027, p = 0.002) (Table 3.6). Patients with AG genotype and patients carrying at least one variant A (AA+AG) genotype of XRCC1 were found to be significantly associated with anemia, neutropenia, thrombocytopenia and gastrointestinal toxicity compared to those with GG genotype(p = 0.001, p = 0.003) (Table 3.15); (p = 0.002 and p = 0.011) (Table 3.24) ; (p = 0.022 and p = 0.028) (Table 3.42) and (p=0.001 and p=0.002) (Table 3.51) respectively.

Patients carrying AG genotype and at least one variant A allele (AA + AG) of XRCC1 polymorphism showed an elevated response compared to those with GG. The possible cause might be that XRCC1 polymorphism was considered of increasing chemotherapy sensitivity which would reduce the function of DNA repair and would lead to the effect of increased DNA damage. It has been demonstrated that cells with a switch from arginine to glutamine, such as the Arg/Gln or Gln/Gln would make a negative effect on the DNA repair activity. Theoretically, cells with substitution of arginine to glutamine would show larger amounts of DNA damage, and the response of platinum-based chemotherapy should be turn better (Lunn et al., 1999).

Patients with AG genotype and patients carrying at least one variant A (AA+AG) genotype were found to be significantly associated with different types of toxicities compared to those with GG genotype. This might be that XRCC1 was involved in DNA single-strand break repair, and also played an important role in the BER pathway; the polymorphisms of this gene might alleviate DNA repair capacity. Thus the capacity of repairing the damaged cancer cell would also be alleviated by the polymorphism of XRCC1 and the cytotoxic effects of chemotherapy would be enhanced.

XPC is one of the genes in the nucleotide excision repair (NER) pathway and is involved in the damage recognition, open complex formation and reparation. This pathway is primarily responsible for eliminating a wide variety of DNA lesions, and thus is an important defense mechanism against structurally unrelated DNA lesions. Functional SNPs in protein coding regions may alter amino acids sequence and even protein function. Polymorphism of XPC occurs in the protein coding regions and cause amino acids substitution in functional domain, thus it is

reasonablethat the functional polymorphisms of XPC will alter DNA repair capacity and susceptibility to lung cancer. In vitro and in vivo studies have demonstrated that XPC in the NER pathway is involved in the pharmacokinetics of platinum-based drugs and platinum resistance of cancer patients (Rosell et al., 2003; Azuma et al., 2007). Thus XPC may modulate DRC and alter outcome of platinum based chemotherapy.

XPC polymorphism was associated with an increased risk for lung cancer in several studies (Shen, 2005; Hu, 2005). Sakanoet al found that XPC was shown to increase the susceptibility to severe toxicities and was significantly associated with grade 3/4 neutropeniainduced by platinum based chemotherapy (Sakanoet al., 2010). Zhu et al demonstrated that in stage III, an A-C transition of the XPC gene was significantly associated with the response to a platinum-based regimen. The heterozygous A/C genotype had a much poorer response than the wild A/A genotype by 0.074 times (Zhu et al., 2010).

In our study, we found no statistically significant association of XPC (rs2228001) gene polymorphism in response to chemotherapy ($p > 0.05$) (Table 3.8).Patients carrying AC genotype of XPC had significantly higher risk of neutropenia than did those carrying AA genotype ($p = 0.016$) (Table 3.26).

No significant association between XPC polymorphism and response to chemotherapy was found in our study. The one possible cause might be due to inadequate sample size or other causes yet to be determined. Patients with AC genotype were found to be associated with neutropenia. This might be due to thatXPC polymorphism might potentially influence the acute hematological toxicity in platinum based chemotherapy, owing to the decreased repair capacity of DNA damaged by platinum based drug in myelocytes.

ERCC1 is a major component of the NER pathway. It encodes a subunit for the NER pathway and is involved in repairing the interstrand and intrastrand cross-links induced by platinum based chemotherapy (Liang et al., 2010).The DNA repair capacity of ERCC1 was considered to be a critical mechanism of resistance to platinum-based drugs (Goode et al., 2002). Therefore,

variants of ERCC1 rs11615 may have an impact on the clinical outcome of non- small cell lung patients receiving platinum-based chemotherapy.

Many studies found no significant association between ERCC1 rs1695 and response to platinum based chemotherapy (Lord et al., 2002; Azuma et al., 2007; Booten et al., 2007; Fujii et al., 2008; Hwang et al., 2008; Azuma et al., 2009; Lee et al., 2009; Ota et al., 2009; Wang et al., 2010; Ozdemir et al., 2013) which was similar to our result. Again some previous studies on ERCC1 found significant relationship with treatment response (Isla et al., 2004; Ryu et al., 2004; Zhou et al., 2004; Kalikaki et al., 2009; Chen et al., 2010; Li et al., 2010; Wei et al., 2010) which was contradictory with our result and many other results.

In the case oftoxicity evaluation, many studies failed to find a significant association between this polymorphism and any platinum-induced toxicity (Ryu et al.,2004; Suk et al., 2005; Tibaldi et al., 2008; Gandara et al., 2009; Kalikaki et al., 2009; Chen et al., 2010; Ludovini et al., 2011; Vinolus et al., 2011).

There was no statistically significant relationship of ERCC1 polymorphism with any platinum induced toxicity and response in our study($p > 0.05$). The lack of getting such association in this study might reflect a true finding, or inadequate sample size, differential mediation of myelosuppression by treatment which was not fully understood or other causes yet to be determined.

Although we have some limitations, our study has contributed significant information on the prognostic and predictive value of these polymorphisms and may prove to be useful tools whilst working towards individualizing NSCLC treatment strategies. So it may help us to identify those patients who will get more benefit from the platinum based therapy and those who are at risk of platinum-induced ADRs leading to a better prognosis and quality of life for patients with non - small cell lung cancer.

5. Conclusion

Lung cancer is the second most leading cause of death in cancer patients and continues to be a serious global problem.In lung cancer, approximately 85% of patients are histologicallydiagnosed with non-small cell lung cancer (NSCLC) in which most of the patients are confirmed during theadvanced stages presenting with stage IIIB or IV due to thedelay in clinical diagnosis. It is an aggressive malignant carcinoma which presents with a high growth rate, widespreadmetastases, poor prognosis and disappointing estimated. The survival rate is 5 year rates of about 15%. Platinum-basedchemotherapy, such as cisplatin or carboplatin in combinationwith gemcitabine, paclitaxel, docetaxel and etoposide,is considered as the standard first line treatment forNSCLC patients. However, platinum resistance is one of the major obstaclesfor successful chemotherapy.

In our study, we found statistically significant association between GSTP1 rs1695 and XRCC1 rs25487 polymorphisms with response to platinum based chemotherapy. Furthermore, in the case of toxicity study, we found significant association between GSTP1 rs1695, XRCC1 rs25487 and XPC rs2228001 polymorphisms with platinum induced toxicities.

Our study suggests that GSTP1 rs1695,XRCC1 rs25487 and XPC rs2228001polymorphisms might affect the clinical outcome of patients with advanced NSCLC receiving platinum-based chemotherapy. This observation could be used in personalized chemotherapy strategies to increase the response rate and prolonged survival time. On the basis of these findings, it may be possible to use these polymorphisms in the further as a biomarker to predict the outcomes of personalized platinum based chemotherapy.

Abrams J, Doyle LA, Aisner J. Staging, prognostic factors, and special considerations in small cell lung cancer. SeminOncol1988; 15: 261–277.

Ada AO, C Kunak S, Hancer F et al. CYP and GST polymorphisms and survival in advanced non-small cell lung cancer patients. Neoplasma 2010; 57, 512-521.

American Cancer Society.(2016) Lung cancer (Non-small cell). [Online] Available from: http:/www.cancer.org. [Accessed on 25 June 2016]

American Thoracic Society.(2015) Decision aid for lung cancer screening with computerized tomography (CT). [Online] Available from: http"/www.thoracic.org. [Accessed on 16 April 2016]

An J, Liu Z, Hu Z, Li G, Wang LE, Sturgis EM et al. Potentially functional single nucleotide polymorphisms in the core nucleotide excision repair genes and risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev 2007; 16: 1633-1638.

Audebert M, Salles B, Calsou P. Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J BiolChem 2004; 279: 55117-55126.

Aviel-Ronen S, Blackhall FH, Shepherd FA, Tsao MS. "K-ras mutations in non-small-cell lung carcinoma: a review". Clinical Lung Cancer (Cancer Information Group) 2006; 8 (1): 30-38.

Azuma K, Komohara Y, Sasada T, Terazaki Y, Ikeda J et al. Excision repair cross-complementation group 1 predicts progression-free and overall survival in non-small cell lung cancer patients treated with platinum-based chemotherapy. Cancer Sci 2007; 98: 1336–1343.

Azuma K, Sasada T, Kawahara A, Hattori S, Kinoshita T et al. Expression of ERCC1 and class III beta-tubulin in non-small cell lung cancer patients treated with a combination of cisplatin/docetaxel and concurrent thoracic irradiation. Cancer ChemotherPharmacol 2009; 64: 565–573.

Bachmann KA. Genotyping andphenotyping the Cytochrome P-450 enzymes. American J Therapeutics 2002; 9: 309-316.

Beckett WS.Epidemiology and etiology of lung cancer.Clin Chest Med 1993;14(1):1–15.

Behera M, Heine JJ, Sica GL, Fowler EE, Tran H, Fu RW, Gal AA, Hermann R, Mayfield W, Khuri FR, Owonikoko TK, Ramalingam SS. Survival Analysis of Patients With Stage I Non-

Small-Cell Lung Cancer Using Clinical and DNA Repair Pathway Expression Variables. Clin Lung Cancer 2012; 14(2): 128-138.

Blackstock WP, Weir MP. Proteomics quantitative and physical mapping of cellular proteins.Trends Biotechnol 1999; 17: 121-127.

Blankenburg S, Konig IR, Moessner R, Laspe P, Thoms KM, Krueger U, Khan SG, Westphal G, Berking C, Volkenandt M, Reich K, Neumann C, Ziegler A, Kraemer KH, Emmert S. Assessment of 3 xerodermapigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case-control study. Carcinogenesis 2005; 26: 1085-1090.

Board PG, Baker RT, Chelvanayagam G, Jermiin LS. Zeta, a novel class of glutathione transferases in a range of species from plants to humans.Biochem J 1997; 328: 929–935.

Booten R, Ward T, Ashcroft L, Morris J, Heighway J et al. ERCC1 mRNA expression is not associated with response and survival after platinumbased chemotherapy regimens in advanced non-small cell lung cancer. J ThoracOncol 2007; 2: 902–906.

Booten R, Ward T, Heighway J, Ashcroft L, Morris J, Tratcher N. Glutaniose-S-transferase P1 isoenzyme polymorphisms, platinum based chemotherapy and non-small cell lung cancer. J ThoracOncol 2006; 1(7): 679-683.

Bosken CH, Wei Q, Amos CI et al. An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer. J Natl Cancer Inst 2002; 94: 1091–1099.

Brownson RC, Alavanja MC, Caporaso N, Berger E, Chang JC. Family history of cancer and risk of lung cancer in lifetime non-smokers and long-term ex-smokers.Int J Epidemiol1997; 26(2): 256–263.

Butler PC, Rizza RA.Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients.Diabetes 1991; 40: 73–81.

Cabelguenne A, Loriot MA, Stucker I, Blons H et al. Glutathione-associated enzymes in head and neck squamous cell carcinoma and response to cisplatin-based neoadjuvant chemotherapy. Int. J. Cancer 2001; 93: 725-730.

Cai Y, Geacintov NE and Broyde S. Nucleotide excision repair efficiencies of bulky carcinogen-DNAadducts are governed by a balance between stabilizing and destabilizing

Campbell DA, Valdes A, Spurr N. Making drug discovery a SN(i)P. Drug Discovery Today 2000; 5: 388-396.

Casse C, Hu YC and Ahrendt SA.The XRCC1 codon 399 Gln allele is associated with adenine to guanine p53 mutations in non-small cell lung cancer. Mutat. Res 2003; 528: 19-27.

Casson AG, Zheng Z, Evans SC, Veugelers PJ, Porter GA, Guernsey DL. Polymorphisms in DNA repair genes in the molecular pathogenesis of esophageal (Barrett) adenocarcinoma. Carcinogenesis 2005; 26: 1536-1541.

Chen CJ, Chin CW, Wu MM, Kuo TT. Cancer potential in liver, lung, bladder, and idney due to ingested arsenic in drinking water.Br J Cancer 1992; 66:888–892.

Chen CJ, Chuang YC, Lin TM, Wu HY. Malignant neoplasms among residents of a blackfoot disease endemic area in Taiwan: high arsenic artesian well water and cancers. Cancer Res 1985; 45: 5895–5899.

Chen CJ, Chuang YC, You SL, Lin TM, Wu HY. A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan. Br J Cancer 1986; 53(3): 399–405.

Chen S, Huo X, Lin Y, Ban H, Lin Y, Li W, Zhang B, Au WW, Xu X. Association of MDR1 and ERCC1 polymorphisms with response and toxicity to cisplatin-based chemotherapy in non-small-cell lung cancer patients. Int J Hyg Environ Health 2010; 213(2): 140-145.

Chen S, Zhang J, Wang R, Luo X, Chen H. The platinum-based treatments for advanced non-small cell lung cancer, is low/negative ERCC1 expression better than high/positive ERCC1 expression? A meta-analysis.Lung Cancer 2010 [Epub ahead of print].

Cheng L, Spitz MR, Hong WK et al. Reduced expression levels of nucleotide excision repair genes in lung cancer: a case-control analysis. Carcinogenesis 2000; 21: 1527–1530.

Cheng YW, Chiou HL, Sheu GT et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res 2001; 61: 2799-2803.

Chiou HY, Hsueh YM, Liaw KF, Horng SF, Chiang MH, Pu YS et al. Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. Cancer Res 1995; 55(6): 1296–1300.

Clegg MT. Genetics of crop improvement. AmerZool 1986. 26: 821-833.

Committee on Diabetic Twins, Japan Diabetes Society. Diabetes mellitus in twins: a cooperative study in Japan. Diabetes Res ClinPract 1988; 5: 271–280.

Curran ME. Potassium ion channels and human disease: Phenotypes to drug targets? CurrOpin Biotech 1998; 9: 565-572.

Daly AK, Monkman SC, Smart J, Steward A, Choletron S. Analysis of Cytochrome P450 polymorphisms. Meth MolBiol 1998; 107; 405-22.

Dela-Cruz CS, Tanoue LT, Matthay RA.Lung Cancer: Epidemiology, Etiology, and Prevention. Clin Chest Med 2011; 32(4). doi:10.1016/j.ccm.2011.09.001.

Ding C, Liu L, Song H. Polymorphism in XRCC1 and sensitivity to platinbased chemotherapy in advanced non-small cell lung cancer. China Pharm 2010; 13: 1399-1401.

Dinneen S, Gerich J, Rizza R. Carbohydrate metabolism in noninsulin-dependent diabetes mellitus. N Engl J Med 1992; 327: 707–713.

Dollinger M, Tempero M, Rosenbaum E, Mulvihill S. Everyone's guide to cancer therapy. 4th ed. Kansas City: Andrews McMeel Publishing; 2002.

Drazen JM, Yandava CN, Dube L et. al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Genet 1999; 22: 168–170.

Du Y, Su T, Zhao L, Tan X, Chang W, Zhang H, Cao G. Associations of polymorphisms in DNA repair genes and MDR1 gene with chemotherapy response and survival of non-small cell lung cancer. PLoS One 2014; 9: e99843.

Evans WE, Johnson JA. Pharmacogenomics: The inherited basis for interindividual differences in drug response. Annual Review of Genomics and Human Genetics 2001; 2: 9-39.

Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999; 286: 487–491.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on cancer; 2010. Available from: http://globocan.iarc.fr; [Accessed on 25 June 2016]

Ferreccio C, Gonzalez PC, Mislosavjlevic SV, Marshall GG, Sancha AM. Lung cancer and arsenic exposure in drinking water: a case-control study in northern Chile. Cadernos de SaudePublica1998; 14(3S): 193–198.

Figueroa JD, Malats N, Real FX, Silverman D, Kogevinas M, Chanock S et al. Genetic variation in the base excision repair pathway and bladder cancer risk. Hum Genet 2007; 121: 233-242.

Firth RG, Bell PM, Marsh HM, Hansen 1, Rizza RA. Postprandial hyperglycemia in patients with non insulin dependent diabetes mellitus: role of hepatic and extrahepatic tissues. J Clin Invest 1986; 77: 1525–1532.

Francisco G, Menezes PR, Eluf-Neto J, Chammas R. XPC polymorphisms play a role in tissue-specific carcinogenesis: a meta-analysis. Eur J Hum Genet 2008; 16(6): 724-734.

Fuertes MA, Castilla J, Alonso C et. al. Cisplatin biochemical mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. Curr Med Chem 2003; 10: 257-266.

Fujii T, Toyooka S, Ichimura K, Fujiwara Y, Hotta K et al. ERCC1 protein expression predicts the response of cisplatin-based neoadjuvant chemotherapy in non-small-cell lung cancer. Lung Cancer 2008; 59: 377–384.

Furuta T, Ueda T, Aune G et al. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. Cancer Res 2002; 62: 4899–4902.

Gandara DR, Kawaguchi T, Crowley J, Moon J, Furuse K, Kawahara M, Teramukai S, Ohe Y, Kubota K, Williamson SK, Gautschi O, Lenz HJ, McLeod HL, Lara PN Jr, Coltman CA Jr, Fukuoka M, Saijo N, Fukushima M, Mack PC. Japanese-US common-arm analysis of paclitaxel plus carboplatin in advanced non-small-cell lung cancer: a model for assessing population-related pharmacogenomics. J ClinOncol 2009; 27(21): 3540-3546.

Gandhi KK, Foulds J, Steinberg MB et al. Lower quit rates among African American and Latino menthol cigarette smokers at a tobacco treatment clinic. Int J ClinPract 2009; 63(3): 360-367.

Gao CM, Shi MQ, Wu JZ, Cao HX, Feng JF, Xu L. Polymorphisms in XRCC1 gene and sensitivity to gemcitabine/cisplat in chemotherapy in non small cell lung cancer. Practical J Cancer 2006; 21: 351-353.

Gelboin HV. Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation

Giachino DF, Ghio P, Regazzoni S et al. Prospective assessment of XPD Lys751Gln and XRCC1 Arg399Gln single nucleotide polymorphisms in lung cancer. Clin Cancer Res 2007; 13: 2876-2881.

Giuliani L, Jaxmar T, Casadio C, Gariglio M, Manna A, D'Antonio D, Syrjanen K, Favalli C, Ciotti M. Detection of oncogenic viruses SV40, BKV, JCV, HCMV, HPV and p53 codon 72 polymorphism in lung carcinoma. Lung Cancer 2007; 57(3): 273-281.

Goode EL, Ulrich CM , Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11: 1513-1530.

Gray IC, Campbell DA, Spurr NK. Single nucleotide polymorphisms as tools in human genetics. Human Molecular Genetics 2000; 9: 2403-2408.

Guengerich FP, ShimaDT: Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol 1991; 4(4): 391-407.

Guo S. NER pathway and platinum toxicity and future research priorities. Med Decis Making 2010; 30(3): 328-340.

Guo Y, Jamison DC. The distribution of SNPs in human gene regulatory regions.BMC genomics 2005; 6: 140.

Gurubhagavatula S, Liu G., Park S, Zhou W, Su L, Wain JC, Lynch TJ, Neuberg DS, Christiani DC. XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J ClinOncol 2004; 22(13): 2594-2601.

Haque AFMR. Prevention and control of noncommunicable diseases: The Bangladesh perspective. Commonwealth Health Ministers' Update 2011; Pro-Brook Publishing Limited, Melton, Woodbridge, Suffolk, 2011; 100-101.

Harrington SE, Smith TJ. The role of chemotherapy at the end of life: "when is enough, enough?".JAMA 2008; 299(22): 2667-2678.

Harris H. Principles of human biochemical genetics.3rd edition. New York: Elsevier/North Holland Biomedical; 1980: 311.

Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002; 3(8): 461-469.

Herbst RS, Heymach JV, Lippman SM. Lung cancer. New England J Med 2008; 359(13): 1367–1380.

Higgins ITT, Mahan CM, Wynder EL. Lung cancer among cigar and pipe smokers.Prev Med 1988; 17: 116–128.

Hirata H, Hinoda Y, Matsuyama H, Tanaka Y, Okayama N, Suehiro Y et al. Polymorphisms of DNA repair genes are associated with renal cell carcinoma. BiochemBiophys Res Commun 2006; 342: 1058-1062.

Hirsch FR, franklin WA, Gazdar AF, Bunn JR. Early detection of lung cancer: clinical perspectives of recent advances in biology & radiology. Clin cancer Res 2001; 7: 5-22.

Ho JY, Elo IT. The contribution of smoking to black-white differences in US mortality. Demography 2013; 50(2): 545-568.

Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer.Nature 2001; 411: 366-374.

Hong CY, Xu Q, Yue Z, Zhang Y, Yuan Y.Correlation of the sensitivity of NP chemotherapy in non-small lung cancer with DNA repair gene XRCC1 polymorphism. Chin J Cancer 2009, 28: 1291-1297.

Hopenhayn-Rich C, Biggs ML, Smith AH. Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. Int J Epidemiol1998; 27(4): 561-569.

Hu Z. DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. Int. J. Cancer 2005; 115: 478–483.

Huang WY, Berndt SI, Kang D, Chatterjee N, Chanock SJ, Yeager M et al. Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: XPC polymorphisms modify smoking-related risk. Cancer Epidemiol Biomarkers Prev 2006; 15: 306-311.

Hwang IG, Ahn MJ, Park BB, Ahn YC, Han J et al. ERCC1 expression as a prognostic marker in N2(+) nonsmall-cell lung cancer patients treated with platinum-based neoadjuvant concurrent chemoradiotherapy. Cancer 2008; 113: 1379–1386.

IARC, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on cancer; 2010. Available from: http://globocan.iarc.fr; [Accessed on 15 June 2016]

International Agency for Research on Cancer.IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.vol. 83. Lyon, FR: IARC; 2004. Tobacco Smoke and Involuntary Smoking; 53–1187.

Isla D, Sarries C, Rosell R et al. Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. Ann Oncol2004; 15: 1194 –1203.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global Cancer Statstics. CA Cancer J Cin 2011; 61(2): 69-90.

Jemal A, Tiwari RC, Murray T et al."Cancer statistics, 2004". CA: a Cancer J for Clinicians 2004; 54(1): 8–29.

Jin YW, Liu J, Wang ZH. Prediction of XPD and XRCC1 gene polymorphisms in advanced non-small cell lung cancer patients receiving platinum-based chemotherapy. Shandong Yi Yao 2006; 46: 42-43.

Johnson DH. Gemcitabine for the treatment of non-small-cell lung cancer. Oncology (Williston Park)2001; 15(3 Suppl. 6): 33-39.

Kalikaki A, Kanaki M, Vassalou H, Souglakos J, Voutsina A, Georgoulias V, Mavroudis D. DNA repair gene polymorphisms predict favorable clinical outcome in advanced non small-cell lung cancer. Clin Lung Cancer 2009; 10(2): 118-123.

Kaprio J, Tumiletho J, Koskenvuo M, Romanov K, Renuanen A, Erikson J, Stengaard J, Kesaaniemi YA. Concordance for type1 (insulin-dependent) and type2 (non-insulindepen- dent) diabetes mellitus in a population based cohort of twins in Finland.Diabetologia 1992; 35: 1060–1067.

Kato H. Photodynamic therapy for lung cancer - A review of 19 years' experience. J PhotochemPhorobio 1998; 42(2): 96-99.

Ke HG, Li J, Shen Y, You QS, Yan Y, Dong HX, Liu JH, Shen ZY.Prognostic significance of GSTP1, XRCC1 and XRCC3 polymorphisms in non-small cell lung cancer patients. Asian Pac J Cancer Prev 2012; 13: 4413-4416.

Khrunin AV, Moisseev A, Gorbunova V and Limborska S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. Pharmacogenomics J 2010; 10: 54-61.

Kietthubthew S, Sriplung H, Au WW, Ishida T. Polymorphism in DNA repair genes and oral squamous cell carcinoma in Thailand.Int J Hyg Environ Health 2006; 209: 21-29.

Knox RJ, Friedlos F, Lydall DA, Roberts JJ. "Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum(II) and cis-diammine-(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA". Cancer Res 1986; 46: 1972–1979.

Kozian DH, Kirschbaum BJ. Comparative gene expression analysis.Trends Biotechnol 1999; 17**:** 73-78.

Krawezak M, Reiss J, Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum Genet 1992; 90: 41–54.

Kristensen VN, Edvardsen H, Tsalenko A, Nordgard SH, Sørlie T, Sharan R et al. Genetic variation in putative regulatory loci controlling gene expression in breast cancer. ProcNatlAcadSci USA 2006; 103: 7735-7740.

Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, Dwyer JH, Roy- Burman P, Bray GA, Mack TM. North-American twins with IDDM: genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. Diabetes 1993; 42: 1351–1363.

Lancia,G, Bafna V, Istrail S, Lippert R Schwartz R. SNPs problems, complexity, and algorithms.In Proceedings of the Ninth Annual European Symposium on Algorithms (ESA'01) 2001; 182–193.

Landrigan PJ. Arsenic—state of the art.Am J Ind Med 1981; 2(1): 5–14.

Lash LH, Hines RN, Gonzalez FJ, Zacharewski TR, Rothstein MA. Genetics and susceptibility to toxic chemicals: do you (or should you) know your genetic profile? The Journal of Pharmacology and Experimental Therapeutics 2003; 305: 403-409.

Lee HW, Choi YW, Han JH, Kim JH, Jung JH et al. Expression of excision repair cross-complementation group 1 protein predicts poor outcome in advanced non-small cell lung cancer patients treated with platinum-based doublet chemotherapy. Lung Cancer 2009; 65: 377–382.

Lee P, Kapeli E, Mehta AC. Therapeutic bronchoscopy in lung cancer: Laser therapy, electrocautery, brachytherapy, stents and photodynamic therapy. ClicHest Med 2002; 23(1): 241-256.

Lesko LJ, Woodcock J. Translation of pharmacogenomics and pharmacogenetics: a regulatory perspective. Nature Reviews Drug Discovery 2004, 3: 763-769.

Li F, Sun X, Sun N, Qin S, Cheng H, Feng J et al. Association Between Polymorphisms of ERCC1 and XPD and Clinical Response to Platinum-Based Chemotherapy in Advanced Non-Small Cell Lung Cancer. Am J ClinOncol 2010 [Epub ahead of print].

Liang J, Li QF, Yao RY, Lu HY et. al. Association between genetic polymorphisms of ERCC1, XRCC1, GSTP1 and survival of advanced gastric cancer patients treated with oxaliplatin/5-Fu-based chemotherapy. ZhonghuaZhong Liu ZaZhi 2010;32: 515-519.

Liao WY, Shih JY, Chang GC, Cheng YK, et al. Genetic polymorphism of XRCC1 Arg399Gln is associated with survival in non-small-cell lung cancer patients treated with gemcitabine/platinum. J. Thorac. Oncol 2012; 7: 973-981.

Liggett SB. Genetically modified mouse models for pharmacogenomic research. Nature Reviews Genetics 2004, 5: 657-663.

Lindahl T, Wood RD. Quality control by DNA repair. Science 1999; 286: 1897–1905.

Linder MW, Prough RA, Valdes R. Pharmacogenetics: a laboratory tool for optimizing therapeutic efficiency. Clinical Chemistry 1997; 43: 254-266.

Lippert R, Schwartz R, Lancia G, Istrail S. Algorithmic strategies for the single nucleotide polymorphism haplotype assembly problem. Brie¯ngs in Bioinformatics 2002; 3(1): 1-9.

Liu D, Wu J, Shi GY, Zhou HF, Yu Y. Role of XRCC1 and ERCC5 polymorphisms on clinical outcomes in advanced non-small cell lung cancer. Genet Mol Res 2014; 13: 3100-3107.

Lo HW, Ali-Osman F. Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. CurrOpinPharmacol 2007; 7: 367–374.

Lord RVN, Brabender J, Gandara D et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small-cell lung cancer. Clin Cancer Res 2002; 8: 2286-2291.

Ludovini V, Floriani I, Pistola L, Minotti V, Meacci M, Chiari R, Garavaglia D, Tofanetti FR, Flacco A, Siggillino A, Baldelli E, Tonato M, Crin L. Association of cytidinedeaminase and xerodermapigmentosum group D polymorphisms with response, toxicity, and survival in cisplatin/gemcitabine-treated advanced non-small cell lung cancer patients. J ThoracOncol 2011; 6(12): 2018-2026.

Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorinA variant frequency. Cancer Res 1999; 59: 2557–2561.

Marin MS, Lopez-Cima MF, Garcia-Castro L, Pascual T, Marron MG, Tardon A. Poly (AT) polymorphism in intron 11 of the XPC DNA repair gene enhances the risk of lung cancer. Cancer Epidemiol Biomarkers Prev 2004; 13: 1788-1793.

Marsh S, McLeod HL. Pharmacogenomics: from bedside to clinicalpractice. Human Molecular Genetics 2006; 15: 89-93.

Marton MJ, DeRisi JL, Bennett HA, Iyer VR, Meyer MR, Roberts CJ et al. Drug target validation and identification of secondary drug target effects using DNA microarrays. Nat Med 1998; 4: 1293-1301.

McDuffie HH, Klaassen DJ, Dosman JA. Female-male differences in patients with primary lung cancer.Cancer 1987; 59: 1825–1830.

McDuffie HH, Klaassen DJ, Dosman JA. Men, women and primary lung cancer—a Saskatchewan personal interview study.J ClinEpidemiol1991; 44: 537–544.

McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. Annu Rev PharmacolToxicol 2001; 41: 101-121.

Medici F, Hawa M, Ianari A, Pyke DA, Leslie RD. Concordance rate for typeIIdiabetesmellitus in monozygotic twins: actual analysis. Diabetologia 1999; 42: 146–150.

Meisel C, Roots I, Cacorbi I, Brinkmann U, Brockmöller J. How to manage individualized drug therapy: application of pharmacogenetic knowledge of drug metabolism and transport. Clinical Chemistry and Laboratory Medicine 2000; 38: 869-876.

Meyer UA. Pharmacogenetics and adverse drug reactions. The Lancet 2000; 356: 1667-1671.

Meyer UA. Pharmacogenetics-five decades of therapeutic lessons from genetic diversity. Nature Reviews Genetics 2004; 5: 669-675.

Min JH, Pavletich NP. Recognition of DNA damage by the Rad4 nucleotide excision repair protein. Nature 2007; 449(7162): 570-575.

Mohrenweiser HW, Xi T, Vazquez-Matias J, Jones IM.Identification of 127 amino acid substitution variants in screening 37 DNA repair genes in humans.Cancer Epidemiol. Biomarkers Prev 2002; 11:1054-1064.

Monica RP, Gorey S. Pharmacogenomics and modern therapy. 2007; 69(2): 167-172.

Monneret C. Platinum anticancer drugs: From serendipity to rational design. Ann Pharm Fr2011; 69(6): 286-295.

Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111(6): 1710-1717.

Naruke T, Tsuchiya R, Kondo H et al. Implications of staging in lung cancer. Chest1997; 112: 242–248.

Natarajan G, Malathi R, Holler E. "Increased DNA-binding activity of cis-1,1-cyclobutanedicarboxylatodiammineplatinum(II) (carboplatin) in the presence of nucleophiles and human breast cancer MCF-7 cell cytoplasmic extracts: activation theory revisited". BiochemPharmacol 1999; 58 (10): 1625–1629.

National Cancer Institute; SEER stat fact sheets: Lung and Bronchus. Surveillance Epidemiology and End Results. 2010

Nebert DW, Vessell ES. Advances in Pharmacogenomics and individualised drug therapy: exciting challenges that lie ahead. European Journal of Pharmacology 2004, 500: 267-280.

Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford University Press; 2000.

Nemery B. Metal toxicity and the respiratory tract.EurRespir J 1990; 3: 202–219.

Oguri T, Fujiwara Y, Katoh O, Daga H, et al. Glutathione S-transferase-pi gene expression and platinum drug exposure in human lung cancer.Cancer Lett 2000; 156: 93-99.

Osann KE, Anton-Culver H, Kurosaki T, Taylor T. Sex differences in lung-cancer risk associated with cigarette smoking. Int J Cancer 1993; 54: 44–48.

Osborn AM, Moore ERB, Timmis NT. An evaluation of terminal-restriction fragment length polymorphism (TRFLP) analysis for the study of microbial community structure and dynamics. Env.Microbiol 2000; 2: 39-50.

Oscarson M. Pharmacogenetics of drug metabolizing enzymes: importance for personalised

Ota S, Ishii G, Goto K, Kubota K, Kim YH et al. Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small cell lung cancer treated with cisplatin-based chemotherapy. Lung Cancer 2009; 64: 98–104.

Ott K, Lordick F, Becker K, Ulm K, Siewert J, Hofler H et al. Glutathione-S-transferase P1, T1 and M1 genetic polymorphisms in neoadjuvant-treated locally advanced gastric cancer: GSTM1-present genotype is associated with better prognosis in completely resected patients. Int J Colorectal Dis 2008; 23(8): 773–782.

Ozdemir O, Ozdemir P, Veral A, Uluer H et al. ERCC1 expression does not predict survival and treatment response in advanced stage non-small cell lung cancer cases treated with platinum based chemotherapy. Asian Pac. J. Cancer Prev 2013; 14: 4679-4683.

Pagani F, Stuani C, Tzetis M, Kanavakis E, Efthymiadou A, Doudounakis S et al. New type of disease causing mutations: the example of the composite exonic regulatory elements of splicing in CFTR exon 12. Human Molecular Genetics 2003; 12: 1111-1120.

Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ. A xerodermapigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. Cancer Res 2001; 61: 8654-8658.

Park DJ, Zhang W, Stoehlmacher J et al. ERCC1 gene polymorphism as a predictor for clinical outcome in advanced colorectal cancer patients treated with platinum-based chemotherapy. ClinAdvHematolOncolPharmacolPhysiol2011; 38(9): 632-637.

Peklak-Scott C, Smitherman PK, Townsend AJ et al. Role of glutathione S-transferase P1-1 in the cellular detoxification of cisplatin. Mol Cancer Ther 2008; 7(10): 3247–3255.

Poklar N, Pilch DS, Lippard SJ, Redding EA, Dunham SU, BreslauerKJ . "Influence of cisplatinintrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex". ProcNatlAcadSci USA 1996; 93(15): 7606–7611.

Poole CA, Byers T, Calle EE, Bondy J, Fain P, Rodriguez C. Influence of a family history of cancer within and across multiple sites on patterns of cancer mortality risk for women.Am J Epidemiol1999; 149(5): 454–462.

Program NT, Cisplatin. Rep Carcinog2011; 12: 110-111.

Qian XP, Qiu LX, Yang Y, Jiang M, Zhang Y, Yu LX, Wang LF, Hu WJ, Liu BR. Predictive value of base-excision repair gene polymorphisms in advanced non-small cell lung cancer patients receiving platinum-based chemotherapy. Mod Oncol 2010; 18: 1303-1304.

Qiu L, Wang ZX, Shi XQ, Wang ZZ. Associations between XPC polymorphisms and risk of cancers: a meta-analysis. Eur J Cancer 2008; 44: 2241-2253.

Quirk E, McLeod H, Powderly W. The pharmacogenetics of antiretroviral therapy: A review of studies to date. HIV/AIDS 2004; 39: 98-106.

Radiological Society of North America. (2016) X-ray (Radiography) - Chest. [Online] Available from: http:/www.radiologyinfo.org. [Accessed on 22 July 2016]

Reed E, Yu JJ, Davies A et al. Clear cell tumors have higher mRNA levels of ERCC1 and XPB than other histological types of epithelial ovarian cancer. Clin Cancer Res 2003; 9: 5299–5305.

Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M, Wijnholds J, Borst P. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidalantiinflammatory drugs. ProcNatlAcadSci USA 2003; 100(16): 9244-9249.

Risch HA, Howe GR, Jain M, Burch DJ, Holowaty EJ, Miller AB. Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type.Am J Epidemiol1993; 138: 281–293.

Robb TB, Beerli P, Nickerson DA, Edwards SV. The utility of single nucleotide polymorphisms in inferences of population history. TRENDS in Ecology and Evolution Vol.18 No.5 May 2003.

Robert J, Le Morvan V, Smith D, Pourquier P, Bonnet J. Predicting drug response and toxicity based on gene polymorphisms. Critical Reviews in Oncology/Haematology 2005; 54: 171-196.

Rongcun Y, Richard BSR. Genetic Polymorphism and Tumor Immunotherapy. Current Pharmacogenomics 2003; 1: 37-57.

Rosell R, Taron M, BarnadasA et. al. Nucleotide excision repair pathways involved in Cisplatin resistance in non-small-cell lung cancer. Cancer Control 2003;10: 297-305.

Roses AD.Pharmacogenetics and the practice of medicine. Nature 2000 ; 405: 857-865.

Rudd GN, Hartley JA, Souhami RL."Persistence of cisplatin-induced DNA interstrand crosslinking in peripheral blood mononuclear cells from elderly and young individuals". Cancer Chemother.Pharmacol 1995; 35(4): 323–326.

Ryu JS, Hong YC, Han HS, Lee JE, Kim S, Park YM, Kim YC, Hwang TS. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. Lung Cancer 2004; 44(3): 311-316.

Sachidanandam R, Weissman D, Schidt SC, Kakol JM, Stein LD, Marth G et al.A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001; 409: 928-933.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT. Primer-directed enzymatic amplification of DNA with a thermo stable DNA polymerase. Science 1988; 239: 487-491.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia.Science 1985; 230: 1350-1354.

Sakano S, Hinoda Y, Sasaki M, Wada T, Matsumoto H et al. Nucleotide excision repair gene polymorphisms may predict acute toxicity in patients treated with chemoradiotherapy for bladder cancer. Pharmacogenomics 2010; 11: 1377-1387.

Sato M, Shames DS, Gazdar AF, Minna JD. A translational view of the molecular pathogenesis of lung cancer. J ThoracOncol 2007; 2(4): 327-343.

Schwartz AG, Yang P, Swanson GM. Familial risk of lung cancer among nonsmokers and their relatives. Am J Epidemiol1996; 144(6): 554–562.

Schwartz JB. The influence of sex on pharmacokinetics. Clinical Pharmacokinetics 2003; 42: 107-121.

Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nature reviews cancer 2012; 12: 278-287.

Seeberg E, Eide L, Bjoras M. The base excision repair pathway. Trends BiochemSci 1995; 20(10): 391-397.

Sethi TK, El-Ghamry MN, Kloecker GH.Radon and lung cancer.ClinAdvHematolOncol 2012; 10(3): 157-164.

Shaw GL, Falk RT, Pickle LW, Mason TJ, Buffler PA. Lung cancer risk associated with cancer in relatives. J ClinEpidemiol1991; 44: 429–437.

Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J 2001; 360: 1-16.

Shellard SA, Fichtinger-Schepman AM, Lazo JS and Hill BT. Evidence of differential cisplatin-DNA adduct formation, removal and tolerance of DNA damage in three human lung carcinoma cell lines. Anticancer Drugs 1993; 4: 491-500.

Shen H, Sturgis EM, Khan SG, Qiao Y, Shahlavi T, Eicher SA, Xu Y, Wang X, Strom SS, Spitz MR, Kraemer KH, Wei Q. An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Res 2001; 61: 3321-3325.

Shen M. Polymorphisms in the DNA nucleotide excision repair genes and lung cancer risk in Xuan Wei, China. Int J Cancer 2005; 116: 768–773.

Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water.Am J Epidemiol1998; 147(7): 660–669.

Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, Yu MC et al. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst 2002; 94(12): 936–942.

Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S et al. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 2004; 91(2): 344–354.

Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP et al. Xerodermapigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol Cell 1998; 2(2): 223-232.

Suk R, Gurubhagavatula S, Park S, Zhou W, Su L, Lynch TJ, Wain JC, Neuberg D, Liu G, Christiani DC. Polymorphisms in ERCC1 and grade 3 or 4 toxicity in non-small cell lung cancer patients. Clin Cancer Res 2005; 11(4): 1534-1538.

Sun N, Sun X, Chen B et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advancednon-small cell lung cancer. Cancer ChemotherPharmacol 2010; 65, 437-446.

Sun X, Li F, Sun N, Shukui Q, Baoan C, Jifeng F, Lu C, Zuhong L, Hongyan C, YuanDong C, Jiazhong J, Yingfeng Z. Polymorphisms in XRCC1 and XPG and response to platinum-based chemotherapy in advanced non-small cell lung cancer patients. Lung Cancer 2009; 65: 230-236.

Takebayashi Y, Nakayama K, KanzakiA et al. Loss of heterozygosity of nucleotide excision repair factors in sporadic ovarian, colon and lung carcinomas: implication for their roles of carcinogenesis in human solid tumours. Cancer Lett 2001; 174: 115-125.

Thompson LH, Brookman KW, Jones NJ, Allen SA, Carrano AV. Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange. Mol Cell Biol 1990; 10(12): 6160-6171.

Tibaldi C, Giovannetti E, Vasile E, Mey V, Laan AC, Nannizzi S, Di Marsico R, Antonuzzo A, Orlandini C, Ricciardi S, Del Tacca M, Peters GJ, Falcone A, Danesi R. Correlation of CDA, ERCC1, and XPD polymorphisms with response and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 2008; 14(6): 1797-1803.

Tindle HA, Rigotti NA, Davis RB, Barbeau EM, Kawachi I, Shiffman S. Cessation amongsmokers of "light" cigarettes: results from the 2000 national health interview survey. Am J Public Health 2006; 96(8): 1498-1504.

Travis WD, Brambilla E, Nicholson AG et. al. The 2015 World Health Organization Classification of Lung Tumors. J Thoracic oncol 2015; 10(9): 1243-1260.

U.S. Department of Health and Human Services. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010.

Unia MM, Gazdar AF, Carbone DP, Minna JD. The Biology of Lung Cancer.Textbook of Respiratory Medicine, 2nd edition.Edited by Murray and Nadel. 1994.

Unsal M, Akpolat I and Kandemir B. Glutathione-S transferase-pi expression in non small cell lung cancer in the assessment of response to chemotherapy. Saudi Med. J 2003; 24: 493-498.

US Public Health Service: The Health Consequences of Smoking for Women. A Report of the Surgeon General.Rockville, MD, US Department of Health and Human Services, Office on Smoking and Health, 1980.

Viñolas N, Provencio M, Reguart N, Cardenal F, Alberola V, Snchez-Torres JM, Barn FJ, Cobo M, Maestu I, Moreno I, Mesa C, Izquierdo A, Felip E, Lpez-Brea M, Mrquez A, Snchez-Ronco M, Tarn M, Santarpia MC, Rosell R. Single nucleotide polymorphisms in MDR1 gen correlates with outcome in advanced non-small-cell lung cancer patients treated with cisplatin plus vinorelbine. Lung Cancer 2011; 71(2): 191-198.

Vogel F. Moderneprobleme der Humangenetik.Ergeb Inn Med Kinderheilkd 1959; 12: 52-125.

Vogel U, Overvad K, Wallin H, Tjønneland A, Nexø BA, Raaschou-Nielsen O. Combinations of polymorphisms in XPD, XPC and XPA in relation to risk of lung cancer. Cancer Lett 2005; 222: 67-74.

Wang X, Zhao J, Yang L, Mao L, An T, et al. (2009) Positive expression of ERCC1 predicts a poorer platinum-based treatment outcome in Chinese patients with advanced non-small-cell lung cancer. Med Oncol 2010; 27(2): 484-490.

Wang Z, Xu B, Lin D et al. XRCC1 polymorphisms and severe toxicity in lung cancer patients treated with cisplatin-based chemotherapy in Chinese population. Lung Cancer 2008; 62: 99-104.

Watson MA, Stewart RK, Smith GB et. al. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998; 19: 275–280.

Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC. ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatinchemoresistance in non-small cell lung cancer cell lines. Mol Cancer 2005; 4: 18-25.

Weaver TA. High-throughput SNP discovery and typing for genome-wide genetic analysis.New Technologies of Life Sciences. A Trends Guide 2000;36-42.

Wei Q, Cheng L, Hong WK et al. Reduced DNA repair capacity in lung cancer patients. Cancer Res 1996; 56: 4103–4107.

Wei SZ, Zhan P, Shi MQ, Shi Y, Qian Q, Yu LK et al. Predictive value of ERCC1 and XPD polymorphism in patients with advanced non-small cell lung cancer receiving platinum-based chemotherapy: a systematic review and meta-analysis. Med Oncol 2010 [Epub ahead of print].

Weinshilboum R, Wang L. Pharmacogenomics: bench to bedside. Nature Reviews Drug Discovery 2004; 3: 739-748.

Weitberg BA. Cancer of the Lung: From Molecular Biology to Treatment Guidelines. 2002.

Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type2 diabetes mellitus. J Clin Invest 1999; 104: 787–794.

Whitehouse CJ, Taylor RM, Thistlethwaite A, Zhang H et al. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 2001; 104: 107-117.

Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. Science 2001;291: 1284-1289.

World Health Organization.2014 World Cancer Report. [Online] Available from: http:/www.searo.who.int/publications/bookstore/documents/9283204298/en/ [Accessed on 22 April,2016]

Wu AH, Fontham ET, Reynolds P, Greenberg RS, Buffler P, Liff J et al. Family history of cancer and risk of lung cancer among lifetime nonsmoking women in the United States. AmJ Epidemiol1996; 143(6): 535–542.

Xu C, Feng D, Li L et al. The expression and prognosticsignificanceofERCC1andGST-piinlungcancer.Zhongguo Fei Ai ZaZhi 2010; 13: 195-200.

Xu C, Wang X, Zhang Y, Li L. Effect of the XRCC1 and XRCC3 genetic polymorphisms on the efficacy of platinum-based chemotherapy in patients with advanced non-small cell lung cancer.ZhongguoFei Ai ZaZhi 2011; 14: 912-917.

Yang Y, Xian L. The association between the GSTP1 A313G and GSTM1 null/present poly-morphisms and the treatment response of the platinum-based chemotherapy in non-small cell lung cancer (NSCLC) patients: a meta-analysis. TumourBiol 2014; 35: 6791-6799.

Yoon SM, Hong YC, Park HJ, Lee JE et al. The polymorphism and haplotypes of XRCC1 and survival of non-small-cell lung cancer after radiotherapy.Int J RadiatOncolBiolPhys 2005; 63: 885-891.

Yu JJ, Lee KB, Mu C et al. Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene.Int J Oncol 2000;16(3);550-560.

Yu Y, Liu D, Wu J, Shi GY, Zhou HF. Role of XRCC1 and ERCC5 polymorphisms on clinical outcomes in advanced non-small cell lung cancer. Genet Mol Res 2014; 13 (2): 3100–3107.

Yuan P, Miao XP, Zhang XM, Wang ZH, Tan W, Sun Y, Xu BH, Lin DX. Polymorphisms in nucleotide excision repair genes XPC and XPD and clinical responses to platinum-based chemotherapy in advanced non-small cell lung cancer.Zhonghuayixuezazhi 2005; 85(14): 972-975.

Yuan P, Miao XP, Zhang XM, Wang ZH, Tan W, Zhang XR et al. Correlation of genetic polymorphisms in nucleotide excision repair system to sensitivity of advanced non-small cell lung cancer patients to platinum-based chemotherapy. Cancer (Chin) 2005; 24: 1510-1513.

Zang EA, Wynder EL. Differences in lung cancer risk between men and women: examination of the evidence. J Natl Cancer Inst1996; 88: 183–193.

Zeng-Rong N, Paterson J, Alpert L et al. Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy. Cancer Res 1995; 55: 4760–4764.

Zhang BL, Sun T, Zhang BN, Zheng S, Lü N, Xu BH, Wang X, Chen GJ, Yu DK, Lin DX. Polymorphisms of GSTP1 is associated with differences of chemotherapy response and toxicity in breast cancer. Chin Med J (Engl) 2011; 124(2):199- 204.

Zhang L, Ma W, Li Y, Wu J, Shi GY. Phar-macogenetics of DNA repair gene polymorphisms in non-small-cell lung carcinoma patients on platinum-based chemotherapy. Genet Mol Res 2014; 13: 228-236.

Zhao W, Hu L, Xu J, Shen H, Hu Z, Ma H, Shu Y, Shao Y, Yin Y. Polymorphisms in the base excision repair pathway modulate prognosis of platinum-based chemotherapy in advanced non-small cell lung cancer. Cancer ChemotherPharmacol 2013; 71: 1287-1295.

Zheng H, Abdel Aziz HO, Nakanishi Y, Masuda S, Saito H, Tsuneyama K, Takano Y. Oncogenic role of JC virus in lung cancer. J Pathol 2007; 212(3): 306-315.

Zhou F, Yu Z, Jiang T, et al. Genetic polymorphisms of GSTP1 and XRCC1: prediction of clinical outcome ofplatinum-based chemotherapy in advanced non-small cell lung cancer (NSCLC) patients. Swiss Med Wkly 2011; 141: w13275.

Zhou RM, Li Y, Wang N, Zhang XJ, Dong XJ, Guo W. Correlation of XPC Ala499Val and Lys939GIn polymorphisms to risks of esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma. Cancer (Chin) 2006; 25: 1113-1119.

ZhouW, Gurubhagavatula S, Liu G et al. Excision repair cross-complementation group 1polymorphism predicts overall survival in advanced non-small cell lung cancer patientstreatedwith platinum-based chemotherapy. Clin Cancer Res 2004;10: 4939-4943.

Zhu G, Lippard SJ. Photoaffinity labeling reveals nuclear proteins that uniquely recognize cisplatin-DNA interstrand cross-links. Biochemistry 2009; 48: 4916–4925.

Zhu X, Sun X, Chen B, Sun N, Cheng H, Li F, Zhang H, Feng J, Qin S, Cheng L, Lu Z. XPC Lys939Gln polymorphism is associated with the decreased response to platinum based chemotherapy in advanced non-small-cell lung cancer. Chinese Medical Journal 2010; 123(23): 3427-3432.

Zimmet PZ. Kelly West Lecture 1991. Challenges in diabetes epidemiology – from Westtotherest.Diabetes Care 1992; 15: 232–252.

Reference

DATA COLLECTION FORM

Questionnaires

Genetic polymorphisms of GSTP1, XRCC1, XPC and ERCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer patients of Bangladesh

1. Identification

1.1 ID. Code:

1.2 Name: **1.3 Age (yrs) :**

1.4 Marital Status: Married/Unmarried **1.5. Sex:** Male/ Female

1.6. Mailing address: **1.7Telephone No.:**

2 Personal History

2.1 Area of residence	Rural	Urban	S-Urban	Others
2.2 Where have you spent at least ¾th or more of your life time?				
2.3 Education level:	Illiterate	SSC or equivalent	HSC or equivalent	Graduate
2.4 Occupation:	Student	Professional	Technical	Other
2.5 Family expense/month:				
2.6 Impression about social class:	Rich	Upper middle	Lower middle	Poor
2.7 Smoking habit :	Never	Ex- smoker	Current smoker	

3. Biophysical Characteristics

3.1 Height (cm):

3.2 Weight (kg):

3.3 Pulses/min:

3.4 BMI

3.4 Temperature (^{0}F):

3.5 BP (Sys/Dias):

3.6 BSA

4. Type of lung cancer

4.1. NSCLC

a)Adenocarcinoma **b) Squamous cell carcinoma** **c) Bronchoalveolarcarcinoma**

d) Large cell carcinoma **e) Adenosquamous**

4.2. Treatment period :

4.3 TNM stage:	
4.4 Tumor position:	
4.5 Initial Tumor volume (cm^3):	
4.6 Tumor volume after 4 weeks of chemotherapy(cm^3)	
4.7 Cycles	

5. Prescribed drugs

	Drugs	Dose (mg)		Drugs	Dose (mg)
a)	Cycplatin/Carboplatin		f)	Pacitaxel	
b)	Docetaxel		**g)**	Topotecan	
c)	Gemcitabine		h)	Irinotecan	
d)	Vinorelbine		i)	Etoposide	
e)	Premetexed				

6. **Toxic effects:**

Toxicity	**Grades**			
	1	2	3	4
Hematological toxicity				
Gastointestinal toxicity				
Diarrhoea				
Constipation				
Allopecia				
Others				

Name of the investigator:

Signature:

PATIENT CONSENT FORM

I, the undersigned, authorize the research student to consider me as a patient for his/her research work. I understand that I can change my mind at any time to withdraw myself as patient during this research work.

Patient's consent to study treatment

Please tick as appropriate

1. Do you have complete idea about the type, ultimate goal and methodology of the research? Yes No

2. Are you aware that you don't have to face any physical, mental and social risk for this? Yes No

3. There will be no chance of injury in any of your organs; are you aware of this? Yes No

4. Have you got any idea about the outcome of this experiment? Yes No

5. Have you decided intentionally to participate in this experiment? Yes No

6. Do you think this experiment violate your human rights? Yes No

7. Are you sure that all the information regarding you will be kept confidentially? Yes No

8. No remuneration will be provided for this experiment, are you aware

of this? Yes No

After reading the above mentioned points, I am expressing my consent to participate in this experiment as a patient.

Patient's signature and Date:_____

Patient's Name:_____

Address:_____

Witness:_____

Please return the signed copy to the research student and keep an extra copy for yourself.

_____

Signature of the researcher

Department of Clinical Pharmacy and Pharmacology

Faculty of Pharmacy

University of Dhaka

Instruments

Instruments	Sources
UNIVERSAL 240V 50i60Hz Refrigerated Bench-Top Centrifuge	Hettich GmbH & Co., Germany
MJ Mini Gradient Thermal Cycler	Bio-Rad Laboratories, USA
Alpha Imager HP (Gel Doc. System)	Alpha Innotech Corporation, USA
Gel Electrophoresis Machine (Elite)	Wealtech, Germany
UV Probe V.2.1 Spectrophotometer	Shimadzu, USA
P^H Meter (Cyber Scan 500)	Eutech ,Singapore
Water Bath	Siemens, USA
Micropipette	Bio-Rad Laboratories, USA
Distillation Plant (Distinction D4000)	BibbySterlin Ltd., UK
Ultrapure Water System (Arium 611)	Sartorius, Germany
Microcentrifuge Machine (Mikro 20)	Hettich GmbH & Co., Germany
Freeze (-40^0 C)	Siemens, USA
Vortex Mixer Machine (Rotamixer-9590)	Hook & Tucker Instruments Ltd., UK
Autoclave Machine	Yongfeng Enterprise Co., UK
Heidolph Unimax-2010 Incubator	Wolf Laboratories Ltd., UK
Freeze (-80^0 C)	DAIREI,Sweden

Consumable materials

Materials	Sources
Reagent Bottle (250, 500, 1000 ml)	Schott GL-45, German
Conical Flasks	Schott GL-45, Germany
Pipettes (Precicolor)	HBG, Germany
Eppendorf Tube (1.5 ml)	Hamburg, Germany
Pipette Tips	ALA, USA
PCR Tubes (0.2/0.5 ml)	Bio-Rad Laboratories, USA
Falcon Tubes (50 ml)	Hamburg, Germany
Polypropylene Tubes (15 ml)	Hamburg, Germany

Chemicals and reagents

Agarose

Type	DNA Size (kbp)	Gel Strength (gm/cm^2)
HS	0.5-30	>2000 (1.5%)
H	1-200	>2800 (1.5%)
X	0.01-1.0	>1000 (3.0%)

1600	0.01-1.0	>1400 (1.5%)

Other Reagents

Reagents	**Sources**
Triton-X 100	Sigma Chemical Company, USA
Sodium Lauryl Sulphate	Sigma Chemical Company, USA
Ethanol	Sigma Chemical Company, USA
Chloroform	Sigma Chemical Company, USA
Sodium Perchlorate	Sigma Chemical Company, USA
Glacial Acetic Acid	Sigma Chemical Company, USA
Sodium Chloride	Sigma Chemical Company, USA
Sucrose	Sigma Chemical Company, USA
Magnesium Chloride	Sigma Chemical Company, USA
Tris-HCl	Sigma Chemical Company, USA
EDTA-Na_2	Sigma Chemical Company, USA
Nuclease Free Water	Promega Corporation, USA
Ethidium Bromide	BDH, UK
Sample Loading Dye, 6X	Promega Corporation, USA
Taq DNA polymerase	NEB, USA
Standard Reaction Buffer	NEB, USA
$MgCl_2$ Solution	NEB, USA
Deoxyribonucleotide Solution Mix (dNTP)	NEB, USA
Quick-Load® 50bp DNA Ladder	NEB, USA
100 bp DNA ladder	NEB, USA

Restriction enzymes

Allele	RE	Recognition sites
GSTP1 (rs1695)	*BsmAI*	5'…….....GTCTC(N)$_1$……3' 3'……CAGAG(N)$_5$……5'
XRCC1 (rs25487)	*HpAII*	5'…..…..C ▼ CGG…..…..3' 3'……GGC ▼ C……5'
XPC (rs2228001)	*PvuII*	5'... C A G ▼ C T G ... 3' 3'... G T C ▲ G A C ... 5'
ERCC1 (rs11615)	*BsrD1*	5'... G C A A T G (N)$_2$ ▼ ... 3' 3'... C G T T A C ▲ ... 5'

⟶ **Cutting site**

Solutions

Name	Composition
TAE Buffer (10x)	0.4 M Tris-Base (Tris (Hydroxymethyl)-amino methane), 11.4 % (v/v) /0.2 M Glacial acetic acid 0.01 M EDTA-Na$_2$,p^H to 7.6 with Glacial acetic acid or Tris.
TBE Buffer(10x)	0.89 M Tris-Base Boric acid, 20 mMEDTA-Na$_2$, p^H to 8.0 with Boric acid or Tris.

TE Buffer(1x)	10 mM (Tris-HCl (Tris (Hydroxymethyl) amino methane hydrochloride), 0.001M EDTA-Na_2, p^H to 8.0 withTris.

Cell Lysis Buffer

To prepare 1 L buffer, 10 mMTris-(hydroxymethyl)-amino methane, 320 mM Sucrose, 5 mM MgCl2 were taken in a 1L buffer container and it was diluted to 850 ml with Milli-Q water. pH was adjusted to 8.0 by adding Glacial acetic acid. After autoclaving 1% Triton X-100 was added to it and the total solution was made up to 1L by Milli-Q water and it was stored at 4˚C.

Nuclear Lysis Buffer

400 mMTris-(hydroxymethyl)-amino methane, 60 mM EDTA-Na_2 , 150 mM Sodium chloride were taken in a 1L buffer container and it was added to 850 ml with Milli-Q water. pH was adjusted to 8.0 by adding Glacial acetic acid. After autoclaving, 1% Sodium lauryl sulphate was added to it and the total solution was made up to 1L by adding Milli-Q water and stored at room temperature.

Sodium Perchlorate (5 M)

61.22 gm of Sodium perchlorate was dissolved in 100 ml Milli-Q water and stored at 4˚C.

Tris-EDTA (TE) Buffer (1x)

10 ml of 1M Tris - (Hydroxymethyl) - amino methane and 2 ml of 500 mM EDTA-Na_2 were mixed in buffer container and then diluted to 1L by adjusting pH to 8.0 and stored at 4˚C. The final concentration of Tris - (Hydroxymethyl) - amino methane and EDTA-Na_2 were 10Mm and 1mM respectively.

TAE buffer (10x)

0.4 M Tris -(Hydroxymethyl)-amino methane, 11.4 %(v/v)/0.2 M Glacial acetic acid and 0.01 M EDTA-Na were taken in a buffer container and diluted to 1L after adjusted pH to 7.6 and stored at room temperature.

Procedure of agarose gel electrophoresis

A. Casting a gel

An appropriate volume of 1X Tris-acetate-EDTA (TAE) buffer with an appropriate amount of agarose (these values are determined based on the gel dimensions and the desired percentage of agarose) was mixed in a conical flask. The flask was swirled to evenly distribute the agarose.

2. The solution was then heated in the microwave oven for 1 minute. Protective gloves were worn and the flask was removed from the microwave oven (before it boiled over), swirled, and reheated while keeping constant watch to be sure it did not boil over. When it started to boil, boiling was stopped and swirled again repeating the process until all of the agarose went into solution.

3. The flask was allowed to cool. The gel was poured when the temperature of the solution was 55-65° C.

4. The gel apparatus was prepared for casting the gel while the agarose was cooling.

5. Prior to pouring the gel, Ethidium bromide was added to the dissolved agarose and swirled to mix.

6. The gel was poured into the casting tray and the comb was adjusted to keep the wells perpendicular. The gel was allowed to cool and was hardened (20-30 minutes) prior to use.

B. Preparing the gel for electrophoresis

8. A few ml of 1X TAE buffer was added to the well area of the gel and the comb was carefully removed by pulling straight up.

9. The electrophoresis tank was filled with buffer solution (1X TAE) and the gel was placed (In the casting tray) on the tank platform.

C. Preparing samples for loading/running the gel

10. An appropriate volume of loading dye (6X) was added to the sample (1 µl of 6X sample dye for every 5 µl of sample).

11. The sample was loaded using a 1-10 µl micropipette. The marker was also loaded at Lane-1.

12. After the gel had been loaded, the cover was gently placed on the apparatus and the Power leads were hooked up. The power was adjusted to 80 volts (constant voltage). The gel was run until the first dye front (bromophenol blue) had migrated about two-thirds the length of the gel and the second dye front (xylene cyanol) had migrated approximatelyone-third of the length of the gel.

13. The power was turned off before removing the gel for photographing.

14. The gel was placed on the UV transilluminator to visualize the DNA.

2.15.1 Fragmentation Pattern of GSTP1 (rs1695)

When X= A in both of the sister chromosomes: (NORMAL HOMOZYGOTE) (A/A)

When X=A in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 176 bp will be obtained and this is considered as normal homozygote.

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

BsmA1 recognition site

GCRTGGAGGACCTCCGCTGCAAATACXTCTCC**CTCWTCTAYASCAACTATGWRAGCATCTG**

CACCAGGGTTGGGCACKGGGRGCTGAACAAAGAAAGGGGCTTCTTGTGCCCTCA

5′……....GTCTC(N)$_1$……..3′

3′……..CAGAG(N)$_5$……..5′

Restriction enzyme BsmA1

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

GCRTGGAGGACCTCCGCTGCAAATACX**TCTCCCTCWTCTAYASCAACTATGWRAGCATCTG**

CACCAGGGTTGGGCACKGGGRGCTGAACAAAGAAAGGGGCTTCTTGTGCCCTCA

(No digestion, one fragment 176bp)

When X=G in one of the sister chromosome: (HETEROZYGOTE) (A/G)

When X=G in one of the sister chromosome, there will be one cutting site at 93 bp. So, there will be 3 fragments (83, 93 and 176 bp) for two sister chromosomes.

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

BsmA1 recognition site

GCRTGGAGGACCTCCGCTGCAAATACXTCTCC**CTCWTCTAYASCAACTATGWRAGCATCTG**

CACCAGGGTTGGGCACKGGGRGCTGAACAAAGAAAGGGGCTTCTTGTGCCCTCA

5′…….. GTCTC(N)$_1$……..3

3′……..CAGAG(N)$_5$……..5′

Restriction enzyme BsmA1

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

GCRTGGAGGACCTCCGCTGCAAATACGTCTCC

(Fragment 1: 93bp)

CTCWTCTAYASCAACTATGWRAGCATCTGCACCAGGGTTGGGCACKGGGRGCTGAACAAAG

AAAGGGGCTTCTTGTGCCCTCA

(Fragment 2: 83 bp)

D

(Fragment 3: 176 bp uncut sister chromosome)

When X=G in both of the sister chromosomes: (Mutant Homozygote) (G/G)

When X=G in both of the sister chromosomes, there will be one cutting between at 93 bp in both of the chromosomes and two fragments with 93 and 83 bp will be obtained.

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

BsmA1 recognition site

GCRTGGAGGACCTCCGCTGCAAATACXTCTCCCTCWTCTAYASCAACTATGWRAGCATCTG

CACCAGGGTTGGGCACKGGGRGCTGAACAAAGAAAGGGGCTTCTTGTGCCCTCA

5′……..GTCTC(N)$_1$……..3′

3′……..CAGAG(N)$_5$……..5′

Restriction enzyme BsmA1

ACCCCAGGGCTCTATGGGAAGGACCAKCAGGAGGCAGCCCYGGTGGACAYGGTGAATGACG

GCRTGGAGGACCTCCGCTGCAAATACGTCTCC

(fragment 1 : 93bp)

CTCWTCTAYASCAACTATGWRAGCATCTGCACCAGGGTTGGGCACKGGGRGCTGAACAAAG

AAAGGGGCTTCTTGTGCCCTCA

DI

(fragment 2 : 83bp)

Figure 2.2: Restriction Enzyme (*BsmAI*) digestion fragment of GSTP1 (rs1695) (3% agarose gel)

2.16.1 Fragmentation Pattern of XRCC1 (rs25487)

When X= G in both of the sister chromosomes: (NORMAL HOMOZYGOTE) (G/G)

When X=G in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 615 bp will be obtained and this is considered as normal homozygote.

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTGTAGCC
TTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAATCTCATGATCT
GTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCAGATCACACCTAACTG
GCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCAGGTCCTAGGCCTGGGAGGCC
GCATCGTGCGTAAGGAGTGGGTGCTGGACTGTCACCGCA

TGCGTCGGCGGCTGCCCTCCCRCAGCTAAG(No HpAII recognition site 'CCTGTGCTGGGCAATGCCAGGA
ATCTGGAGGGGAGTCAGACTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCAGCCCAGAGCCAGCAGACTACTGAGAG
GAGCGGGGCAGGGGCTGGGGTACTCCAGATGAGGGAAGGAGGACCTGCCTGGGAGGTCACAGAGGGCTCTGTGAAGCCT
GCTTATCAGAAAAGGCTGGAGGA

5'... C C G G ... 3'
3'... G G C C ... 5'

Restriction enzyme HpAII

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTGTAGCC
TTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAATCTCATGATCT
GTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCAGATCACACCTAACTG
GCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCAGGTCCTAGGCCTGGGAGGCC
GCATCGTGCGTAAGGAGTGGGTGCTGGACTGTCACCGCATGCGTCGGCGGCTGCCCTCCCRGAGGTAAGGCCTCACACG
CCAACCCTGCTCCTTATCCTGTGCTGGGCAATGCCAGGAATCTGGAGGGGAGTCAGACTGGGGCCTGCCAGCGGAGAAC
ACAGGTGGTCCCAGCCCAGAGCCAGCAGACTACTGAGAGGAGCGGGGCAGGGGCTGGGGTACTCCAGATGAGGGAAGGA
GGACCTGCCTGGGAGGTCACAGAGGGCTCTGTGAAGCCTGCTTATCAGAAAAGGCTGGAGGA

(No digestion, one fragment 615bp)

When X=A in one of the sister chromosome: (HETEROZYGOTE) (A/G)

When X=A in one of the sister chromosome, there will be one cutting site at 315 bp. So, there will be 3 fragments (375, 270 and 615 bp) for two sister chromosomes.

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTGTAG
CCTTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAATCTCATG
ATCTGTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCAGATCACACC
TAACTGGCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCAGGTCCTAGGCCT
GGGAGGCCGCATCGTGCGTAAGGAGTGGGTGCTGGACTGT

HpAII recognition site

CACCGCATGCGTCGGCGGCTGCCCTCCCRGAGGTAAGGCCTCACACGCCAACCCTGCTCCTTATCCTGTGCTGGGCA
ATGCCAGGAATCTGGAGGGGAGTCAGACTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCAGCCCAGAGCCAGCAG
ACTACTGAGAGGAGCGGGGCAGGGGCTGGGGTACTCCAGATGAGGGAAGGAGGACCTGCCTGGGAGGTCACAGAGGG
CTCTGTGAAGCCTGCTTATCAGAAAAGGCTGGAGGA

```
5'... C C G G ... 3'
3'... G G C C ... 5'
```

Restriction enzyme HpAII

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTGTAG
CCTTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAATCTCATG
ATCTGTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCAGATCACACC
TAACTGGCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCAGGTCCTAGGCCT
GGGAGGCCGCATCGTGCGTAAGGAGTGGGTGCTGGACTGTCACCGCATGCGTCGGCGGCTGCCCTCC
(Fragment 1: 375bp)

CRGAGGTAAGGCCTCACACGCCAACCCTGCTCCTTATCCTGTGCTGGGCAATGCCAGGAATCTGGAGGGGAGTCAGA
CTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCAGCCCAGAGCCAGCAGACTACTGAGAGGAGCGGGGCAGGGGCT

When X=G in both of the sister chromosomes: (Mutant Homozygote) (G/G)

When X=G in both of the sister chromosomes, there will be one cutting between at 375 bp in both of the chromosomes and two fragments with 375 bp and 240 bp will be obtained.

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTG
TAGCCTTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAAT
CTCATGATCTGTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCA
GATCACACCTAACTGGCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCA
GGTCCTAGGCCTGGGAGGCCGCATCGTGCGTAAGGAGTGGGTGCTGGACTGT

HpAII recognition site

CACCGCATGCGTCGGCGGCTGCCCTCCCRGAGGTAAGGCCTCACACGCCAACCCTGCTCCTTATCCTGTGCTGG
GCAATGCCAGGAATCTGGAGGGGAGTCAGACTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCAGCCCAGAGC
CAGCAGACTACTGAGAGGAGCGGGGCAGGGGCTGGGGTACTCCAGATGAGGGAAGGAGGACCTGCCTGGGAGGT
CACAGAGGGCTCTGTGAAGCCTGCTTATCAGAAAAGGCTGGAGGA

5'... C C G G ... 3'
3'... G G C C ... 5'

Restriction enzyme HpAII

TTGTGCTTTCTCTGTGTCCACTATGCTGCATGCTTTCTCTCTCTCTCACTTGCTTTCTTTCTCACTGCATTCTG
TAGCCTTTGTCTTCTCTCTGATTTTTGCATCTCTCCCTTGGTCTCCAACCTCTTTTTGTTTCTCCCACCTCAAT
CTCATGATCTGTCTGTCTGTCTGTCTCTCTCTCTCTCTGTCTGTCTCCCCTGTCTCGTTCCCCTTTGCCCCTCA
GATCACACCTAACTGGCATCTTCACTTCTGCCCCCCACCAGCTGTGCCTTTGCCAACACCCCCAAGTACAGCCA
GGTCCTAGGCCTGGGAGGCCGCATCGTGCGTAAGGAGTGGGTGCTGGACTGTCACCGCATGCGTCGGCGGCTGC
CCTCC

(Fragment 1: 375 bp)

CRGAGGTAAGGCCTCACACGCCAACCCTGCTCCTTATCCTGTGCTGGGCAATGCCAGGAATCTGGAGGGGAGTC
AGACTGGGGCCTGCCAGCGGAGAACACAGGTGGTCCCAGCCCAGAGCCAGCAGACTACTGAGAGGAGCGGGGCA
GGGGCTGGGGTACTCCAGATGAGGGAAGGAGGACCTGCCTGGGAGGTCACAGAGGGCTCTGTGAAGCCTGCTTA
TCAGAAAAGGCTGGAGGA

2.17.1 Fragmentation Pattern of XPC (rs2228001)

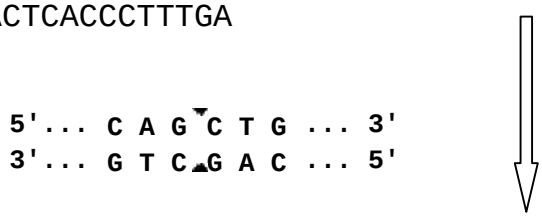
When X= A in both of the sister chromosomes: (NORMAL HOMOZYGOTE) (A/A)

When X=A in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 453 bp will be obtained and this is considered as normal homozygote.

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGAT
ACTGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGC
CC

No PvuII recognition site

AAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAGCTGTG
AGCTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCACACC
TGCCCTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGAGGC
AGCATGCACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCCCG
TCTGGCTCCAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAAA
GCCCACTCACCCTTTGA



Restriction enzyme PvuII

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGAT
ACTGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGC
CCAAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAGCTG
TGAGCTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCACA
CCTGCCCTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGAG
GCAGCATGCACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCC
CGTCTGGCTCCAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCA
AAGCCCACTCACCCTTTGA

When X=C in one of the sister chromosome: (HETEROZYGOTE) (A/C)

When X=C in one of the sister chromosome, there will be one cutting site at 183 bp. So, there will be 3 fragments (183, 270 and 453 bp) for two sister chromosomes.

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGATAC
TGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGCCC

PvuII recognition site

AAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAGCTGTGAG
CTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCACACCTGCC
CTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGAGGCAGCATG
CACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCCCGTCTGGCTC
CAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAAAGCCCACTCAC
CCTTTGA

5'... C A G C T G ... 3'
3'... G T C G A C ... 5'

Restriction enzyme PvuII

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGATAC
TGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGCCCAA
GAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAG

(Fragment 1: 183 bp)

CTGTGAGCTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCAC
ACCTGCCCTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGAGG
CAGCATGCACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCCCGT
CTGGCTCCAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAAAGCC
CACTCACCCTTTGA

When X=C in both of the sister chromosomes: (Mutant Homozygote) (C/C)

When X=G in both of the sister chromosomes, there will be one cutting between at 183 bp in both of the chromosomes and two fragments with 183 and 270 bp will be obtained.

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGATA
CTGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGCCC

PvuII recognition site

AAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAGCTGTGA
GCTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCACACCTG
CCCTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGAGGCAGC
ATGCACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCCCGTCTG
GCTCCAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAAAGCCCA
CTCACCCTTTGA

5'... C A G ▼C T G ... 3'
3'... G T C ▲G A C ... 5'

Restriction enzyme PvuII

GGAGGTGGACTCTCTTCTGATGAAGAGGAGGGGACCAGCTCTCAAGCAGAAGCGGCCAGGATA
CTGGCTGCCTCCTGGCCTCAAAACCGAGAAGATGAAGAAAAGCAGAAGCTGAAGGGTGGGCCC
AAGAAGACCAAAAGGGAAAAGAAAGCAGCAGCTTCCCACCTGTTCCCATTTGAGMAG

(Fragment 1: 183 bp)

CTGTGAGCTGAGCGCCCACTAGAGGGGCACCCACCAGTTGCTGCTGCCCCACTACAGGCCCCA
CACCTGCCCTGGGCATGCCCAGCCCCTGGTGGTGGGGGCTTCTCTGCTGAGAAGGCAAACTGA
GGCAGCATGCACGGAGGCGGGGTCAGGGGAGACGAGGCCAAGCTGAGGAGGTGCTGCAGGTCC
CGTCTGGCTCCAGCCCTTGTCAGATTCACCCAGGGTGAAGCCTTCAAAGCTTTTTGCTACCAA
AGCCCACTCACCCTTTGA

Figure 2.6 Restriction Enzyme (*pvuII*) digestion fragment of XPC (rs2228001) (3% agarose gel)

2.18.1 Fragmentation Pattern of ERCC1 (rs11615)

When X= C in both of the sister chromosomes: (NORMAL HOMOZYGOTE) (C/C)

When X=C in both of the sister chromosomes, there will be no cutting in the both chromosome and only one fragment with 525 bp will be obtained and this is considered as normal homozygote.

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCC

BsrDI recognition site

CTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTGCCCTGGGAAT
TTGGCGACGTAATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTTCCTCAGGTGA
GCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGTCATGTCC
CAGTGTTCTGGACTGTTCTATG

5'... G C A A T G $(N)_2$ ... 3'
3'... C G T T A C ... 5'

Restriction enzyme BsrDI

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCCCTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTGCCC
TGGGAATTTGGCGACGTAATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTTCCT
CAGGTGAGCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGT
CATGTCCCAGTGTTCTGGACTGTTCTATG

When X=T in one of the sister chromosome: (HETEROZYGOTE) (C/T)

When X=T in one of the sister chromosome, there will be one cutting site at 368 bp. So, there will be 3 fragments (156, 369 and 525 bp) for two sister chromosomes.

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCC

BsrDI recognition site

CTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTGCCCTGGGAAT
TTGGCGACGTAATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTTCCTCAGGTGA
GCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGTCATGTCC
CAGTGTTCTGGACTGTTCTATG

Restriction enzyme BsrDI

5'... G C A A T G $(N)_2$ ... 3'
3'... C G T T A C ... 5'

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCCCTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTG

(Fragment 1: 369 bp)

DI

CCCTGGGAATTTGGCGACGTAATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTT
CCTCAGGTGAGCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAG
GGTCATGTCCCAGTGTTCTGGACTGTTCTATG

(Fragment 2: 156bp)

When X=T in both of the sister chromosomes: (Mutant Homozygote) (T/T)

When X=T in both of the sister chromosomes, there will be one cutting between at 369 bp in both of the chromosomes and two fragments with 156 and 369 bp will be obtained.

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCC

BsrDI recognition site

CTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTGCCCTGGGAAT
TTGGCGACGTAATTCCCGACTATGTGCTGGGCCAGAGCACCTGTGCCCTGTTCCTCAGGTGA
GCTCTGCGGCGCCACCCCAGACTTCAGGAAGGGCACCCCACTGGCCTGGGAGGGTCATGTCC
CAGTGTTCTGGACTGTTCTATG

Restriction enzyme BsrDI

5'... G C A A T G $(N)_2$ ... 3'
3'... C G T T A C ... 5'

AGGACCACAGGACACGCAGACTGGGGCTGGAGGGAGAGGGCTGAGCTGGAGACAGACCCGGG
GACCCTTTAGGAAAGGCCTGGTGCCAGTGGGCATGGAGGGGAGGAGCCTGGATCAGAGGATC
AGGGACTGTCCAGGGTTAGGAGGAGAGAGAAGCTGGAAAAGACCCTGCCACAGGTGGGAGGA
GGGAGGAGGTGTGGGAAGAGGTGCGAGGAGGCAGGAGGTGTGGGAAGAGGTGGGAGGAGGGA
GGAGGTGCAAGAAGAGGTGGAGGAGGGCCCTGTGGTTATCAAGGGTCATCCCTATTGATGGC
TTCTGCCCTTCGTCCCTCCCCAGAGGGGCAATCCCGTACTGAAGTTCGTGCGCAAYGTG

(Fragment 1: 369 bp)