بسم الله الرحمن الرحيم

∞∞∞

تم رفع هذه الرسالة بواسطة / هناء محمد علي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: ....

Association of Glutathione S-Transferase P1 (GSTP1) Gene Polymorphism with the Response to Platinum Based Chemotherapy in Patients with Non-Small Cell Lung Cancer

Thesis

Submitted for Partial Fulfillment of Master Degree in Clinical Pathology

By

Martina Nabil Georges Makar

M.B., B.Ch. Ain Shams University

Under Supervision of

Prof. Abeer Ibrahim Abd El-Mageed

Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University

Assist. Prof. Noha Refaat Mohamed

Assistant Professor of Clinical Pathology
Faculty of Medicine - Ain shams University

Assist. Prof. Walaa Ahmed Yousry

Assistant Professor of Clinical Pathology
Faculty of Medicine - Ain shams University

Dr. Ahmed Ali Nagy

Lecturer of Clinical Oncology and Nuclear Medicine
Faculty of Medicine - Ain shams University

Faculty of Medicine, Ain Shams University

2022

Acknowledgments

First and foremost, I feel always indebted to ***Allah*** *the Most Beneficent and Merciful.*

It has been a great honor to proceed into this work under the supervision of ***Prof. Abeer Ibrahim Abd el-Mageed****. Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University. I would like to express my endless gratitude and deepest appreciation for her continuous guidance, valuable suggestion, encouragement and keen supervision throughout the work.*

Indeed, words do fail to express my special thanks and appreciation to ***Dr. Noha Refaat Mohamed****, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University. I will never forget her unlimited help, scientific criticism and wise guidance. To her, words of praise are not sufficient.*

Special thanks are due to ***Dr. Walaa Ahmed Yousry****, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, who offered much of her time and advice for reading and supervising this work and also for her patience, encouragement, continuous support, creative suggestion and useful criticism.*

A lot of appreciation for ***Dr. Ahmed Ali Nagy****, Lecturer of Clinical Oncology and nuclear medicine Faculty of Medicine, Ain Shams University, for his sincere effort and fruitful knowledge regarding the cases.*

Finally, all thanks and gratitude goes to ***my husband and my family****, for pushing me forward in every single step and for always believing in me.*

Martina N. G. Makar

List of Contents

List of Abbreviations

Abb.	Full term
AJCC	American Joint Committee on Cancer
ALK	Anaplastic lymphoma kinase
ALT	Alanine aminotransferase
AP-1	Activator protein-1
ASK1	Apoptosis signal-regulating kinase
AST	Aspartate aminotransferase
BER	Base-excision repair
C. pneumoniae	Chlamydia pneumoniae
CA 125	Cancer antigen 125
CBC	Complete blood count
CEA	Carcinoembryonic antigen
COPD	Chronic obstructive pulmonary disease
CR	Complete response
CT	Computed Tomography
CYP	P-oxidases cytochrome
CYP1A1	Cytochrome P450
DNA	Deoxyribonicleic acid
dNTP	Deoxyribonucleotide triphosphate
E2F	E2 factor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EIA	Enzyme immunoassay
ELSA	Enzyme-linked immunosorbent
ERBB2	Erb-B2 receptor tyrosine kinase 2
FISH	Fluorescence in situ hybridization
GLDH	Glutamate dehydrogenase
GLOBOCAN	Global cancer observatory
GSH	Glutathione
GSR	Glutathione reductase
GSSG	Oxidized glutathione
GSTP1	Glutathione S-transferase p1
GSTs	Glutathione S-transferases
HER2	Human epidermal growth factor receptor 2

List of Abbreviations cont...

Abb.	Full term
HS	Highly significant
IFCC	International Federation for Clinical Chemistry
IHC	Immunohistochemistry
JNK	C-Jun N-terminal kinase
k3 EDTA	Tri-potassium ethylene diamine tetra acetate
KRAS	Kirsten rat sarcoma
LD	Lactate dehydrogenase
Let-7	Lethal-7
MALDI	Matrix-assisted laser desorption ionization
MAPEG	Membrane-associated proteins in eicosanoid and glutathione
MAPK	Mitogen-activated protein kinase
MBD2	Methyl-CpG-binding domain
MDH	Malate dehydrogenase
MET	Mesenchymal–epithelial transition
MGB	Minor groove binder
MiR	OncomiRs
MiRNAs	MicroRNA
MMR	Mismatch repair
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MWW	Mann– Whitney–Wilcoxon
N	Number
NAD	Nicotatinamide adenine dinucleotide
NAT	N-acetyl-transferase
NER	Nucleotide-excision repair
NFQ	Non-fluorescent quencher
NGS	Next-generation sequencing
NS	Non significant
NSCLC	Non-small cell lung cancer
NSE	Neuron-specific enolase
P5P	Pyridoxal-5'-phosphate

List of Abbreviations cont...

Abb.	Full term
PAHs	Polycyclic aromatic hydrocarbons
PAK1	Serine/threonine-protein kinase1
PCR	Polymerase chain reaction
PD	Progressive disease
PD-L	Programmed death ligand 1
PET-CT	Positron-emission tomography-computed tomography
PI3K	Phosphatidylinositol 3-Kinase
PKC	Protein kinase C
PPi	Pyrophosphate
PR	Partial response
Prdx6	Peroxiredoxin VI
ProGRP	Progastrin-releasing peptide
PTEN	Phosphatase and tensin homolog
P-value	Probability value
RECIST	Response Evaluation Criteria In Solid Tumours
RET	Rearranged during transfection
RFLP	Restriction Fragment Length Polymorphism
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ROS1	C-ROS oncogene 1
RS	Thiol radical
RTK	Receptor tyrosine kinases
RT-PCR	Real-time polymerase chain reaction
S	Significant
SBL	Sequencing by ligation
SBS	Sequencing by synthesis
SCC	Squamous cell lung cancer
SCCA	Squamous cell carcinoma antigen
SCLC	Small cell lung cancer

List of Abbreviations cont...

Abb.	Full term
SD	Stable disease
SD	Standard deviation
SNP	Single nucleotide polymorphisms
SPSS	Statistical package of social science
SULT	Sulfotransferases
TB	Tuberculosis
TGFBRII	TGF-β-receptor II
TGF-β	Transforming growth factor-β
Tis	Carcinoma insitu
Tm	Melting temperature
TNFα	Tumor necrosis factor α
TNM	Tumor, Node, Metastasis
TP53	Tumour protein 53
TRAF2	Tumor necrosis factor receptor-associated factor 2
TSP-1	Thrombospondin-1
UGT	UDP-glucuronosyltransferases
UICC	Union for International Cancer Control
UV	Ultraviolet
Val	Valine
VEGF-A	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WB	Wash Buffer
WHO	World Health Organization
X2	Chi square test

List of Tables

List of Tables cont...

List of Figures

INTRODUCTION

Worldwide, lung cancer has a high prevalence and is associated with a high mortality rate. In the last century, the incidence of lung cancer has been rising rapidly and about 2.09 million deaths of lung cancer were estimated in 2018 according to World Health Organization (WHO) ***(Wang et al., 2018).***

The main two types of lung cancer are small cell lung cancer and non-small cell lung cancer. Non-small cell lung cancer (NSCLC) is diagnosed in up to 85% of all cases. It is classified into three subtypes: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. In accordance with the American Joint Committee on Cancer (AJCC), the majority of the patients are cataloged as advanced stage (IIIB–IV) at the time of diagnosis ***(Ramírez et al., 2019).***

Despite multiple advances in therapeutic options over years, platinum based chemotherapy remains the mainstay of adjuvant or first line chemotherapy in NSCLC treatment. Cisplatin-based chemotherapy is slightly superior in terms of response rate and in prolonging the survival without being associated with an increase in severe toxic effects ***(Lin et al., 2018).***

The principal mechanism of action of platinum compounds is the formation of DNA-platinum adducts and subsequently creations of intrastrand or interstrand cross links with DNA which

may cause alteration in the structure of DNA. These phenomena generally lead to apoptosis of cancer cells ***(Mlak et al., 2013).***

Glutathione S-transferases (GSTs) are phase II detoxifying enzymes involved in the maintenance of cell integrity, oxidative stress and protection against DNA damage by catalyzing the conjugation of glutathione to a wide variety of electrophilic substrates ***(Sun et al., 2010).***

The 17 human cytosolic GST subunits are classified as seven gene families according to their biochemical characteristics and amino acid sequence similarities: (GSTA), (GSTM), (GSTT), (GSTP), (GSTO), (GSTZ), and (GSTS). Glutathione S transferase p1 enzyme is the most abundant subunit in lung and brain. It is widely expressed in different human epithelial tissue and is directly involved in the detoxification of cisplatin via the formation of cisplatin-glutathione adducts, which indicates that GSTP1 may play a role in the acquisition of resistance to platinum compound**.** Glutathione S transferase p1 enzyme is encoded by GSTP1 gene which is located on 11q13.2 ***(Li et al., 2019).***

Patients' response to treatment is determined after 2-3 cycles according to the Response Evaluation Criteria In Solid Tumours (RECIST). In order to analyze the response to chemotherapy, patients are classified into responders and non responders.

The responders include: patients with complete response (CR): (disappearance of all target lesions) and patients with

partial response (PR): (more than or equal 30% decrease of all target lesions).

The non-responders include: patients with progressive disease (PD): (more than or equal 20% increase from smallest sum of diameters recorded and 5mm absolute increase over lowest sum) and patients with stable disease (SD): neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD ***(Zhou et al., 2011; Chen et al., 2016).***

AIM OF THE WORK

The aim of the present study was to investigate the association of GSTP1 gene polymorphism with the response to platinum based chemotherapy in patients with NSCLC in order to prevent the non-necessary exposure to the toxic effect of chemotherapy.