Relation of Genetic Polymorphism of ABCG2 Gene and Occurrence of Oxaliplatin- Induced Peripheral Neuropathy in Patients with Gastrointestinal Tract Cancer

A Thesis Submitted for Fulfillment of Master Degree in Pharmaceutical Sciences (Clinical Pharmacy)

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List of Abbreviations

	Full Term
5-FU	F – Fluorouracil
A	Absorbance
ABC	ATP-binding cassette transporters on plasma membranes
ABCG2	ATP-binding cassette, subfamily G, isoform 2 protein
ADL	Activities of daily living
AEs	Adverse events
ALT	Alanine transaminase enzyme
ART	Antiretroviral therapy
AST	Aspartate transaminase enzyme
ATP	Adenosine Triphosphate
BBB	Blood brain barrier
Bp	Base pair
CA 19.9	Carbohydrate tumor-associated antigen
CIPN	Chemotherapy-induced peripheral neuropathy
CI	Confidence interval
CMT	Charcot-Marie-Tooth disease
CNS	Central nervous system
CRC	Colorectal cancer
CTCAE	Common terminology criteria for adverse events
D5W	Dextrose and water
DACH	Diaminocyclohexane
DNA	Deoxyribose nucleic acid
DRG	dorsal root ganglion
DsDNA	Double-strand DNA
EC	Esophageal cancer
ECOG	Eastern Cooperative Oncology Group
FDA	Food and drug administration
FOLOFX	FOL–Folinic acid (leucovorin), F – Fluorouracil (5-FU), and OX– Oxaliplatin.
g DNA	Genomic DNA
GBC	Gallbladder carcinoma
GC	Gastric carcinoma
gDNA	Genomic DNA
GI	Gastrointestinal
GIT	Gastrointestinal tract
GST	Glutathione S-transferases
H2O	Dihydrogen Monoxide (water)
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus disease
HRM	High resolution melting technique
IBS	Irritable bowel syndrome

List of Abbreviations

	Full Term
IENF	Intraepidermal nerve fiber
IGF1	Insulin-like Growth Factor-1
LDI	Laser Doppler imager technique
MDR	Multidrug resistance
NCI	National cancer institute
NCI-CTC	National cancer institute of health-common terminology criteria
NSAIDs	Non-steroidal anti-inflammatory drugs
NTC	No template control
OR	Odds ratio
OIPN	Oxaliplatin induced peripheral neuropathy
OIIT	Oxaliplatin-induced immune thrombocytopenia
PAC	Pancreatic adenocarcinoma
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PN	Peripheral neuropathy
PNS	Peripheral nervous system
PNQ	Patient Neurotoxicity Questionnaire
PV	Pain Vision Device
QOL	Quality of life
QPCR	Quantitative polymerase chain reaction
QST	Quantitative sensory testing
RT	Room temperature
SNPs	Single nucleotide polymorphisms
TBE	Tris/Borate/EDTA
Tm	Melting temperature
TNS	Total neuropathy score
US	United State
UV	Ultraviolet-visible
WHO	World health organization

Abstract

Background: Oxaliplatin use in gastrointestinal malignancies is limited by neurotoxicity. The aim of the current study was to identify single-nucleotide polymorphisms (SNPs) in ABCG2 gene involved in oxaliplatin-induced peripheral neuropathy (OIPN) among Egyptian Colorectal Cancer (CRC) patients treated with oxaliplatin-based chemotherapy (CT) together with its association with other patient characteristics.

Patients and methods: All eligible Colorectal Cancer patients were included in the study if they were between 18-80 years old and excluded if they had a neurologic disease or a disease impairing neurologic function. OIPN was evaluated and graded on day 1 of each CT cycle according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE v.4). The Genomic DNA was extracted from blood to detect SNPs within the ABCG2 gene (at rs2231137and at rs3114018) using High Resolution Melting (**HRM**) technique followed by direct sequencing method for every melting cluster using 3500 genetic analyzer for the samples selected from each cluster.

Results: The occurrence of grade 2-3 OIPN was higher in patients carrying the G/G genotype of ABCG2 (rs2231137) than those with G/A genotype at the same locus (rs2231137) (96.7% versus 82.0%; p =0.05). While, grade 2-3 OIPN occurrence was not significantly different in patients carrying genotypes (C/C, AC/AC and A/A) of ABCG2 at rs3114018 (92.9%, 82.1% and 80.0% respectively; *p* =0.309).

From a total of 47 females and 33 males; 41(87.2%) females and 29 (87.9%) males suffered from different grades of sensory and motor peripheral neuropathy. Regarding sensory oxaliplatin-induced peripheral neuropathy (OIPN), females had higher risk of grade II-III PN than males (92.7% versus 58.6% respectively; p =0.001) For motor OIPN, females were more susceptible to grade II-III PN versus males (84.6% versus 56.5% respectively; p=0.015).

Conclusion: The occurrence of OIPN among Egyptian Colorectal Cancer (CRC) patients was more associated with the G/G allele genotype of ABCG2 (rs2231137). While patients carrying different genotypes (C/C, AC/AC and

A/A) of ABCG2 at rs3114018 were similarly associated with OIPN in this patient population**.** The high grade (grade II-III) Oxaliplatin-induced sensory and motor peripheral neuropathy occurred more frequently in females versus males Egyptian colorectal cancer patients.

Keywords: Oxaliplatin-Induced Peripheral Neuropathy, ABCG2, Colorectal Cancer, High Resolution Melting Technique, 3500 Genetic Analyzer, females, males.

Introduction

Gastrointestinal tract (GIT) cancer, especially colorectal , gastric and pancreatic cancers are a global epidemiological health concern (**Lambert R and Hainaut P 2007)**. There were estimated 1,500,000 new cases of GIT cancer worldwide in 2005 and the number is expected to rise to 2,110,000 in 2025 **(Jemal A, Bray F et al., 2011).** Gastric and colon cancers were the second and third most common causes of cancer-related mortality worldwide in 2008, respectively, accounting for over 1 million deaths **(Ferlay J, Shin HR et al., 2010)**.

Generally, GIT cancer is known to be a multifactorial disease induced by complex interactions between environmental and genetic factors **(Pharoah PD, Dunning AM et al., 2004**). Previous studies suggest that lifestyle, dietary, genetic factors and other environmental exposures, might have played a role in causing GIT cancer **(McCormack VA and Boffetta P 2011**). However, the majority of genetic variants that influence susceptibility to GIT cancer are not well-known **(Lichtenstein P, Holm NV et al., 2000).** Genetic factors may be important contributors to the risk of GIT cancer, efficacy and adverse events (AEs) severity of chemotherapy **(Lochhead P, Frank B et al., 2011; Ye F, Liu Z et al., 2013; Custodio A, Moreno-Rubio J et al., 2014).**

The World Cancer Research Fund estimated that half of the cases may be prevented by dietary and other lifestyle changes **(http://www.wcrf.org/)**. Early detection leading to early treatment will save lives **(http://www.ueg.eu/press/crceurope/)** Therefore, strategies for prevention, early detection and treatment modalities are highly ensured. The various treatments plan of GIT cancer include surgery, chemotherapy and/or radiation therapy vary depending on the size, location, extent of tumor and the patient's overall health **(Earle CC, Maroun J et al., (2002)**.

As mentioned, Chemotherapy is routinely used alone or in combination with other therapies to treat patients following cancer diagnosis. However, chemotherapy-induced peripheral neuropathy (CIPN) is a type of neuropathic pain that is a major disabling side effect of several commonly used chemotherapeutic agents. Its

development may require chemotherapy dose reduction or cessation, which may increase cancer-related morbidity and mortality (**Gutiérrez-Gutiérrez G**, **Sereno M**, **et al., 2010**).

Chemotherapy-induced peripheral neuropathy is a predominantly sensory neuropathy but it may also occur as a motor dysfunction and occasionally it can be accompanied by dysfunction of the autonomic nervous system. **(Boland BA, Sherry V et al., 2010)**

Incidence rates of CIPN available in the literature are highly variable, ranging from 10 to 100%. The influencing factors include: type of antineoplastic agent, treatment schedule being administered (dose, number of cycles, …), combinations of different neurotoxic agents, patient characteristics (age, pre-existing causes of peripheral neuropathy as diabetes mellitus, Renal or hepatic dysfunction, vitamin B12 deficiency), and the diagnostic scale of assessment of the CIPN **(Balayssac D, Ferrier J, et al., 2011).**

Chemotherapy induced peripheral neuropathy CIPN is typically seen in about 40% **(Windebank AJ and Grisold W 2008; Paice JA 2011; Vincenzi B, Frezza AM et al., 2013)** of patients treated with platinum based treatments, including Oxaliplatin. It is assumed that platinum compounds, which bind irreversibly to the DNA, induce apoptosis of sensory neurons (**Von Schlippe M, Fowler CJ et al., 2001).** The persistent cumulative injury caused by antineoplastic agents mostly affect sensory nerve cell bodies in the Dorsal root ganglia and/or the afferent and efferent axons of the peripheral nervous system **(Quasthoff and Hartung, 2002;Windebank AJ and Grisold W 2008).** These typically lead to severe neurotoxicity which results in sensory and motor neurodegeneration, and is classified as pain associated with touch, 'pins and needles' and numbness as well as loss of motor coordination **(Paice JA 2011).** These symmetrical sensory complications are routinely identified in the extremities e.g. fingers and toes, with symptoms persisting from weeks/months to many years. Moreover, the symptoms of CIPN may remain after discontinuation of treatment with platinum compounds **(van den Bent MJ 2005; Pietrangeli A, Leandri M et al., 2006; Windebank AJ and W Grisold 2008; Vincenzi B, Frezza AM et al., 2013)**. Clinical studies suggest that genomic findings can translate into

improvements in clinical practice (**Evans WE and McLeod HL 2003; Rieder MJ, Reiner AP et al., 2005)**.

A previous study suggested an association between genetic variants of ABCG2 gene and severe peripheral neuropathy adverse events end points of oxaliplatin chemotherapy **(Custodio A, Moreno-Rubio J et al., 2014)**.

The diagnosis of CIPN is mainly clinical, detailed history and physical examination are the most important methods for early detection. Several toxicity grading scales have been developed to assess the severity of peripheral neurotoxicity. The grading is based on symptoms and functional capacity reported by the patient and findings in physical examination. The most commonly used scale is NCI-CTCAE (Common Terminology Criteria for Adverse Events). Other grading scales used in clinical practice are the World health organization (WHO), Eastern Cooperative Oncology Group (ECOG) scales and the oxaliplatin grading scale of Levi, Patient Neurotoxicity Questionnaire (PNQ) and The Total Neuropathy Score (TNS) (**Griffith KA**, **Zhu S et al., 2017)**.

As anticancer therapy is often associated with severe adverse events (AEs), there is an increasing demand for effective supportive care strategies preventing or ameliorating drug-induced toxicity. The addition of a pharmacist to the health care team may ensure appropriate medication use, maximize adherence, minimizing treatment-related toxicity; therefore, focus on optimizing supportive care strategies **(Liekweg A, Westfeld M et al. 2012).** The concordance and communication between patients and pharmacists may improve patients' understanding of pharmacotherapy. Pharmacist intervention has been shown to result in improved patient care. Pharmacist counseling cannot change the physical AEs of adjuvant systemic therapy but may be able to prevent deterioration of Quality of life (QOL) by emotionally preparing the patient to tolerate the AEs **(Kawaguchi T, Iwase S et al., 2012)**.

1. Gastrointestinal Cancers

1.1. Epidemiology and Types:

Cancers of the digestive system can be mainly divided into esophageal cancer (EC), gastric carcinoma (GC), colorectal cancer (CRC), a gallbladder carcinoma (GBC), hepatocellular carcinoma (HCC) and pancreatic adenocarcinoma (PAC). According to the global cancer statistics in 2012, HCC was identified as the second most frequently diagnosed cancers among men in less developed countries. Esophageal cancer EC, had highest rates in East Asia, and caused 400,200 deaths worldwide in 2012. While there were around 1.4 million cases of CRC patients and 693,900 deaths that occurred due to CRC **(Torre LA, Bray F et al., 2015).**

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and a major cause of cancer related mortality. CRC is a multistep process in which a combination of genetic and epigenetic factors leads to alterations in normal colonic mucosa to develop into invasive cancer **(Vatandoo N, Ghanbari J et al., 2016)**. The major etiological and risk factors for CRC have been identified. Genetic and environmental alterations interact in a complex way **(Bahrami A, Hassanian SM et al., 2017; Bahrami A, Hesari A, et al., 2017)**. The overall hereditability of CRC has been estimated to be 20–30%, and while important for familial CRC, the genetic factors have not been clarified **(Vatandoost N, Ghanbari J et al., 2016).**

1.2. Causes/Risk Factors of GIT Cancer:

The exact cause of GIT cancer is unknown; however, researchers have learned that there are certain risk factors associated with the development of GIT cancer. These factors differ according to the type of GIT cancers and include:

1.2.1 Lifestyle Factors:

The Incidence of GIT cancer correlates greatly with certain lifestyle factors, including: Diet, Alcohol consumption and smoking.

- **Diet** an increase of fiber, raw fruit and vegetable consumption is associated with a significant decrease of GIT cancer including; gastric, Pancreatic and colorectal cancer risk. Consumption of nitrates, refined carbohydrates (white