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Frequency of CYP2C19 Genotypes among Palestinian Population and Detection of its effect on cure rates of GERD by Omeprazole

By
Bayan Khaldi

Supervisor
Prof. Hisham Darwish

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Thesis Approval

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Bayan Emad Issa Khaldi

This thesis was defended successfully on 8/3/2025and approved by:

Committee members	Signature
1. Prof. Hisham M. Darwish: Supervisor	
2. Dr. Thaer Abdelghani: Internal Examiner	
3. Dr. Saad Allahham: External Examiner	

Decleration

I, Bayan Khaldi (student no. 202113090), hereby declare that I am the author of this thesis and that it has not been submitted in any way to any other university or institution for the award of any degree. This thesis does not include any previously published content except in cases where proper acknowledgment has been provided.

The Name of the Student: Bayan Emad Issa Khaldi

ID: 202113090

Signature: Bayan Khaldi

Date: 07/07/2025

Dedication

I dedicate this work to my parents

Emad Khaldi

Kefah Harb

To my sisters and brother

And to my Professor Dr Hisham Darwish for his support in this research subject

Bayan Emad Issa Khaldi

Acknowledgment

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Abstract

Background and Objectives:. Gastroesophageal Reflux Disease (GERD)is a chronic disease caused by the flow of stomach contents in opposite direction upward the esophagus. GERD affects 35% of the adult population in the western world[12]. It is considered a major indication for Proton Pump inhibitors (PPI's) treatment. PPI's including the major drug Omeprazole are first line drugs used as acid inhibitors for many diseases including GERD. However, several patients don't respond or respond poorly to the usual dose of one of the common PPI's. Accordingly, factors that affect treatment success by these drugs should be determined to ensure maximum benefit to patients. For GERD patients who take Omeprazole as treatment, clinical response varies among them according to CYP2C19 polymorphisms. The aim of our study was to assess the frequency of CYP2C19 gene haplotypes among Palestinian GERD patients treated with Omeprazole including CYP2C19/2*, CYP2C19/3*, and CYP2C19/17*.

Methods: The study included 73 Palestinian patients with GERD, 24 were non-responders to Omeprazole and 36 were responders to Omeprazole. DNA samples were obtained and genotyped for CYP2C19/2*, CYP2C19/17*, and CYP2C19/3*by ARMS-PCR. SNPStat software were used to analyze the generated data.

Results: Our data revealed that there was a statically significant difference between responders and non- responders who have G/A and G/G genotypes (P= 0.0001) in CYP2C19/2* that has an association with the clinical response of Omeprazole in GERD patients. However, our data show no statistical significance between responders and non-responders regardless CYP2C19/3* of A/A and G/A genotypes (P=0.72) and CYP2C19/17* of C/C and C/T genotypes (P= 0.2). Our data demonstrate the importance of CYP2C19 polymorphisms screening to assess the suitable dose of Omeprazole for

patients to treat GERD. This helps in minimizing the adverse drug reactions and maximize the effect of Omeprazole as much as possible in treating GERD.

Conclusion: The data revealed significant correlation between CYP2C19/2* genotypes and clinical response of GERD patients to Omeprazole with no significant correlation with other SNP's including CYP2C19/17* and CYP2C19/3*. Further investigations on a larger sample for wider representation of the population and including additional CYP2C19 SNP's that may affect the expression of the gene will provide better correlation between clinical response and the indicated gene variants.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADR: Adverese Drug Reaction

FDA: Food and Drug Administration

G6PD: Glucose-6- Phosphate Dehydrogenase

NAT-2: N- Acetyl Transferase 2

DNA: Deoxyribonucleic acid

MTX: Methotrexate

MTHFR: Methyl Tetra Hydrofolate Reductase

CYP2D6: Cytochrom P2D6

CYP2C19: Cytochrome P2C19

CYP3A4: Cytochrome 3A4

CYP450: Cutochrome P450

CETP: Cholestrol Ester Transfer Protein

GERD: Gastro Esophageal Reflux Disease

ACG: American college of Gastro enterology

NSAIDs: Non Steroidal Anti Inflammatory Drugs

PPI's: Proton Pump Inhibitors

GI: Gastro Intestine

NDMA: Nitroso dimethyl Amine

AHRQ: Agency for Healthcare Research and Quality

D: Dose

F: Bioavailability

AUC: Area Under the Curve

HCL: Hepatic clearance

H.Pylori: Helicobacter Pylori

Hrs: Hours

ARMS: Amplification Refractory Mutation System

CI: Confidence Interval

EQ-5HD: Quality of life score

OR: Odds Ratio

PCR: Polymarase Chain Reaction

SNPs: Single Nucleotide Polymorphisms

BP: Base Pair

Chapter One

Introduction

1.1 Background

1.1.1 Pharmacogenomics Overview

Patients vary in their response to drugs where doses effective in some patients will be ineffective in others. Moreover, adverse drug reactions (ADR) have been implicated as an important cause of hospital admissions. That's why an emerging role for the relation of genetic profile and patients' response to drugs has been studied. Pharmacogenomics is one of the emerging approaches to evaluate medicine, tailoring drug selection and dosing to the patient's genetic features.[3] Pharmacogenetics is the science studying the inter-individual variability in drug response resulting from genetic factors. In this context, drug response concerns therapeutic response and side effects. Together with information on the genetic constitution of individual patients, these guidelines could be a useful tool for individualizing drug choices and pharmacogenetic-based dose adjustment. The ideal drug would be the one effectively treating or preventing disease without any adverse effects in every patient. Unfortunately, no such drug has been developed. It turns out that in daily clinical practice there exists considerable inter-individual variability in drug response: for one patient the drug can prove to be effective without adverse effects whereas in the next patient, the drug is effective but not well tolerated because of adverse effects.[3]

Since different patients respond differently to a drug. it is assessed that genetics can account for 20 to 95 percent of the variability in drug choice and outcomes. While several various non-genetic aspects inspiration the outcomes of drugs, including age, organ function, related therapy, drug connections, and the nature of the illness, there are now abundant instances of items into which inter-individual alterations in

drug reaction are due to sequence variants in genes encoding drug-metabolizing enzymes, drug carriers, or drug targets . Pharmacogenomics describe how human genetic variants influence individual's reaction to drugs, with emphasis on drug metabolism, absorption, distribution and minimize drastic effect. Lately, the Food and Drug Administration (FDA) has developed a strong pharmacogenomic topics in an attempt to prepare medications safer and further valuable so as to adjust the property of now promoted medications, the FDA has appraised clear drug labels to contain Pharmacogenomics data. Presently, over one hundred FDA-approved medications take Pharmacogenomics data on their labels that define genes responsible for medication display, clinical reaction variability, and the possibility of adverse events [3] The most important determinants are physiological characteristics of patients (such as gender, age, weight and fat percentage), pathophysiological characteristics (liver or renal failure, or other concomitant morbidities), hereditary influences, environmental factors (e.g. drug interaction smoking, nutrition), the pharmaceutical quality of the drug and the way the patient uses the drug.

The study of pharmacogenetics originated in the mid-20th century. In those days, primaquine-induced haemolysis was associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In this deficiency, the pentose phosphate cascade in erythrocytes is blocked, resulting in a reduction in the synthesis of reduced glutathione. Reduced glutathione protects erythrocytes against several drug-induced oxidation reactions, thereby preventing haemolysis. A decrease in the availability of reduced gluthation increases the risk of haemolysis, especially in the presence of certain drugs such as primaquine. Primaquine-induced haemolysis was particularly prominent among African

Americans and people originating from the Mediterranean area with G6PD deficiency and diagnosed by means of enzymatic assays.

With the introduction of the polymerase chain reaction (PCR), isolation of individual genetic variations became possible. One of the first examples was the discovery of different subtypes of the enzyme N-acetyl transferase-2 (NAT-2); this is a phase-II enzyme that is relevant in the metabolic pathway of the antituberculosis drug, isoniazid .[3] In some patients, known as "slow acetylators", sustained high plasma levels of isoniazid with a normal dosage causes peripheral neuropathy and liver toxicity. The difference in isoniazid-metabolizing capacity between normal acetylators and slow acetylators was found to be the result of differences in base sequence within the DNA segment encoding for the synthesis of NAT-2.[4]

Pharmacogenetics focuses in particular on :

1. Proteins affecting pharmacokinetic parameters (drug metabolizing enzymes or transporter proteins)
2. Proteins affecting pharmacodynamic parameters (receptors or ion channels)
3. Proteins affecting the pathogenesis of disease. [4]

Pharmacokinetic Polymorphisms

Pharmacokinetics is the science that studies drug handling by the body: absorption, distribution and metabolism of drugs. Until now, most studies in the field of pharmacogenetics have focused on pharmacokinetic polymorphisms. The result of more activity, altered activity or no activity of drug-metabolizing enzymes as a consequence of polymorphisms, in genes encoding for these enzymes, largely influences inter-individual differences in exposure to these drugs. Patients suffering from rheumatoid arthritis are

known to respond differently to methotrexate (MTX). MTX efficacy is thought to be the result of inhibition of the folate cycle, including among other factors, inhibition of methylenetetrahydrofolate reductase (MTHFR). Carriers of MTHFR 1298AA and MTHFR 677CC (wild types for two different alleles) have been found to have an increased chance of a good response to MTX with a relative risk of 2.3 (95% CI: 1.18-4.41) compared with carriers of mutant alleles.[3] Concomitant use of two drugs both being substrates for the same enzyme, or concomitant use of two drugs of which one is an inhibitor or an inducer for the enzyme involved in the metabolism of the other, possibly results in a potentially clinically relevant drug-drug interaction. [4,5]

In carriers of polymorphisms of enzymes involved in drug metabolism, the risk of such clinically relevant drug-drug interactions could be different depending on the type of polymorphism. For example, the antimycotic agent terbinafine is known to be able to influence the plasma level of nortriptyline by inhibition of CYP2D6 activity resulting in a decrease in nortriptyline drug metabolism .[3] The effect of a drug-drug interaction could be more pronounced in carriers of a polymorphism of the gene encoding for the CYP2D6 isoenzyme which decreases CYP2D6 activity. On the other hand, in carriers of gene duplications for active CYP2D6 isoenzymes, an increased activity of CYP2D6 can result in a less relevant drug-drug interaction. The exposure to different drugs at a “standard” dose in carriers of polymorphic CYP P-450 iso-enzymes can display a large variability compared with non-carriers of such polymorphisms resulting in alterations to efficacy or the risk of adverse events .[4]

Pharmacodynamic Polymorphisms

The efficacy or adverse effects induced by a drug are often the result of binding of this drug to a target protein being either a receptor, an enzyme or an ion-channel. Polymorphisms in genes encoding for these target proteins can lead to either up or down-regulation, or a change, such as proteins folding. As a consequence, when patients are given the "standard" dosage, differences in the magnitude of a drug effect between carriers and non-carriers of different polymorphisms are observed. A well-known example of a dynamic polymorphism influencing drug efficacy is the cholesterol ester transfer protein (CETP). CETP has an important role in the transportation of cholesterol to the liver. Research has demonstrated that in carriers of a specific polymorphism in the CETP gene, the use of pravastatin showed no efficacy in preventing the risk for the primary end-point progression of coronary artery disease compared with those not on this drug therapy . It is possible that in the near future, polymorphisms could be taken into account in the decision whether or not to treat individuals with a statin . Blockage of the serotonin-2c receptor is associated with weight gain in patients using antipsychotic drugs , and increases the risk for the development of cardiovascular morbidity and mortality . Different polymorphisms in the gene encoding for the serotonin-2c receptor are known, and these polymorphisms are associated with the development of weight gain in users of antipsychotic drugs . Weight gain is an important reason for discontinuation of treatment with antipsychotic drugs.[3]

1.2 Gastro Esophageal Reflux Disease (GERD)

1.2.1 Gastroesophageal Reflux Disease (GERD) is a chronic disease that is caused by the flow of stomach contents in opposite direction upward the esophagus. This results in annoying symptoms like heartburn and regurgitation. In addition, it might cause more complications including burning sensation in the chest.[6,7]

1.2.2 The American College of Gastroenterology (ACG) guidelines define GERD as "symptoms or complications occurring from the gastric reflux into the esophagus or beyond, into the oral cavity (including larynx) or lung".[6–12].

1.2.3 GERD is usually diagnosed clinically with classic symptoms and response to acid suppression. Heartburn with or without regurgitation is typically sufficient to suspect GERD, especially when these symptoms are worse postprandially.[10] The prevalence GERD range between 8.7-33.1% in the Middle East.[12]. GERD was observed to be more common in those who used non-steroidal anti-inflammatory drugs (NSAIDs) and those who were obese, smokers, and physically inactive[11]. Some food and drinks, like fast food, tea, coffee, carbonated drinks, and greasy food, are correlated to elevated prevalence of GERD.[10]

GERD affects 35% of the adult population in the western world[12]. It is considered a major indication for Proton Pump Inhibitors(PPI's) treatment. However, several patients don't respond or respond poorly to the usual dose of one of the common PPI's. Accordingly, factors that affect the treatment success by these drugs should be determined to ensure maximum benefit to patients [13–15]. PPI's like the major drug Omeprazole are the first line drugs used as acid inhibitors for many diseases including GERD and peptic ulcer. After ingestion, they are absorbed by the small intestine and transported by circulation to the

gastric parietal cells where they bind to and inhibit the proton pumps thus reducing acid secretion.[16–18]

1.3 GERD Diagnosis and Treatment

Diagnosis and treatment guidelines of GERD were published in 1995 and updated in 2005, and lastly reviewed in 2013by the American College of Gastroenterology (ACG)[6,11,12]. GERD management can be managed by various manipulations including food control, lifestyle modifications, medications, and operations. Primary management is usually done by a proton-pump inhibitor like omeprazole[6,7,11]. Some guidelines suggest managing GERD with H2 antagonist before using proton-pump inhibitor because of cost and safety concerns. Diagnosis of GERD depending on latest guidelines involves upper GI endoscopy, esophageal PH monitoring, and mostly clinical diagnosis since other diagnostic tools are invasive with higher cost.[19] GERD treatment requires a stepwise procedure. The purposes is to manage symptoms, to heal esophagitis, and to limit repetitive esophagitis or other complications. The treatment is based on non-pharmacologic treatment, pharmacologic treatment, and surgical treatment[6,7]. Non-pharmacologic treatment such as lifestyle modification including lose weight in overweight and obese subjects, stop smoking, reduce alcohol consumption, raise the head of the bed, and avoid large food intake at least 2h before going to bed at night, especially if the subject has nocturnal symptoms. There is no evidence for a general recommendation to eliminate foods that can trigger reflux symptoms, such as spicy food, citrus fruit, foods with high-fat content, products with caffeine, and carbonated beverages. If the patient finds that any of these foods are associated with their symptoms, eliminating them from the diet can be beneficial[10,11]. Pharmacologic therapy includes antacids. Antacids

approved in the 1970s and are still active in managing mild symptoms of GERD [12]. H2 receptor antagonists and H2 blocker are the first-line factors for cases with moderate symptoms and grades I-II esophagitis. Choices involve cimetidine, famotidine, and nizatidine. The US FDA stated optional recalls of ranitidine from the pharmacies between December 2019 and February 2020 after growing principal attention in September 2019 about medication contamination with the carcinogenic molecule's N-nitrosodimethylamine (NDMA)[7,20,21]. However, Proton pump inhibitors PPIs are the most productive prescriptions available for managing GERD. They have few side effects. While data revealed that PPIs can conflict with calcium homeostasis and increase cardiac conduction deficits. Long-term use of these drugs has also been correlated with bone breaks in postmenopausal females, chronic and acute kidney disorders, community acquired pneumonia, and Clostridium difficile intestinal infection.[6,7,21]Usable PPIs involve omeprazole, lansoprazole, rabeprazole, and esomeprazole. In November 2013, the FDA recommended the first generic versions of rabeprazole sodium delayed-release tablets to manage GERD in adults and teenagers ages 12 and up. In clinical trials, the most usually described opposing reactions to rabeprazole were sore throat, infection, flatulence, and constipation in adults, and diarrhea, headache, and abdominal pain in adolescents. The Agency for Healthcare Research and Quality (AHRQ) concluded that PPIs were superior to H2 receptor antagonists for resolving GERD symptoms at four weeks and healing esophagitis at eight weeks[10–12]. The AHRQ observed no opposition between individual PPIs to reduce symptoms at eight weeks. For symptom relief at four weeks, esomeprazole 20 mg was equivalent, but esomeprazole 40 mg were superior to omeprazole 20 mg[10].

1.4 Omeprazole Metabolism

1.3.1 In general, proton pump inhibitors are administered orally in different doses (D) ranging from 10 to 80 mg/day. Their bioavailability (F) is quite variable ranging from about 40%(omeprazole) to approximately 80% (lansoprazole, pantoprazole) and they show a large interindividual variability in drug disposition[7,21]. All proton pump inhibitors are eliminated by the hepatic route and the polymorphically expressed Cytochrome enzyme calledCYP2C19is primarily responsible for their rate of elimination[11,21].

1.4.2 A variable part of dose is also metabolized by cytochrome enzyme calledCYP3A4and non-enzymatically degraded drugs in the case of rabeprazole[21].

Thus, systemic drug exposure as expressed by the pharmacokinetic term AUC (area under the curve) depends not only on F but also on the systemic hepatic clearance(CL) of the proton pump inhibitors . Since CYP2C19 is involved in the metabolism of all proton pump inhibitors, it can be anticipated that the largest part of the inter-individual variability in the pharmacokinetics of this group of drugs is due to theCYP2C19genotype of the treated patients. The PPI's are then metabolized mainly the liver for excretion by the cytochrome P450 enzymes (CYP)[12].

1.3.3 The main metabolic pathway for PPIs is through the CYP2C19 isoforms as shown in figure 1 below which exhibits differences in their metabolic activity based on their genotypes status where individuals are classified into three classes; rapid, intermediate, and slow metabolizers. As a result, the pharmacokinetics and pharmacodynamics of PPI's

are directly affected.[16,17,22,23] The polymorphisms of CYP2C19 were first discovered in studies conducted on mephenytoin[18].

CYP2C19 encodes a cytochrome P450 enzyme that metabolizes several commonly prescribed medications, including antidepressants, proton pump inhibitors, and clopidogrel (an anti platelet drug).[24]

The CYP2C19 was initially classified into two main categories as rapid and poor metabolizers[5]. However, rapid metabolizers were found to have two subtypes; homozygous mutated alleles classified as rapid metabolizer and heterozygous mutated alleles classified to have slower rate of metabolism and this category was then classified as intermediate metabolizers[13,15,18]

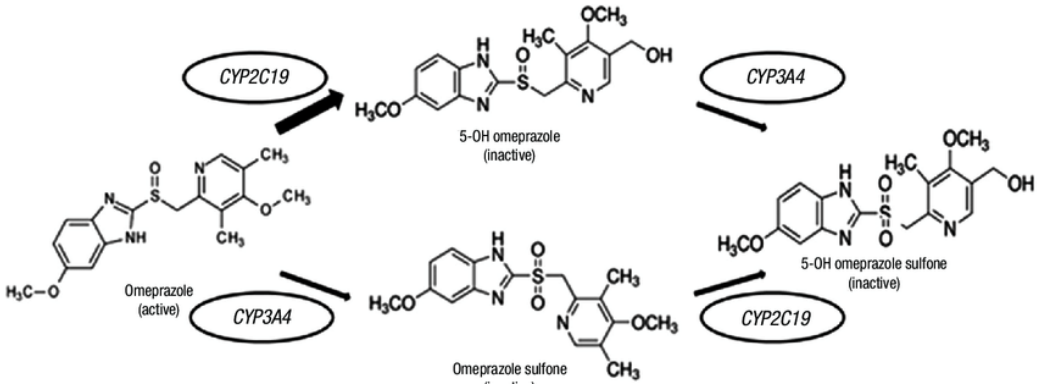


Figure 1.1: Metabolism of Omeprazole by CYP Enzymes 2C19 and 3A4[5]

There are more than 28 variant alleles of CYP2C19 that have been discovered as shown in figure 2. Many of them have relatively low frequencies and are only limited to specific populations or ethnic groups. However, single nucleotide polymorphisms which defines CYP2C19*2 and CYP2C19*3 alleles are the most common and also the most studied alleles as shown in figure 3. These alleles result in null function of the enzyme due to defective splicing mutation (*2) and insertion of a premature stop codon (*3)[25]. Another variant, CYP2C19*17, was also identified during a study of human hepatic proteins [14]Patients who have the CYP2C19*17 allele have increased gene expression and thus

more enzyme is produced. Individuals possessing this polymorphism are described to be ultra-rapid metabolizers[16,25]. Several studies carried out to determine the frequencies of poor metabolizers among many ethnic groups. These studies revealed the frequency among white Americans was 2.5% [17] whereas white Europeans have 3.5%, and the frequency among the Chinese population was around 19.8% [13,17].

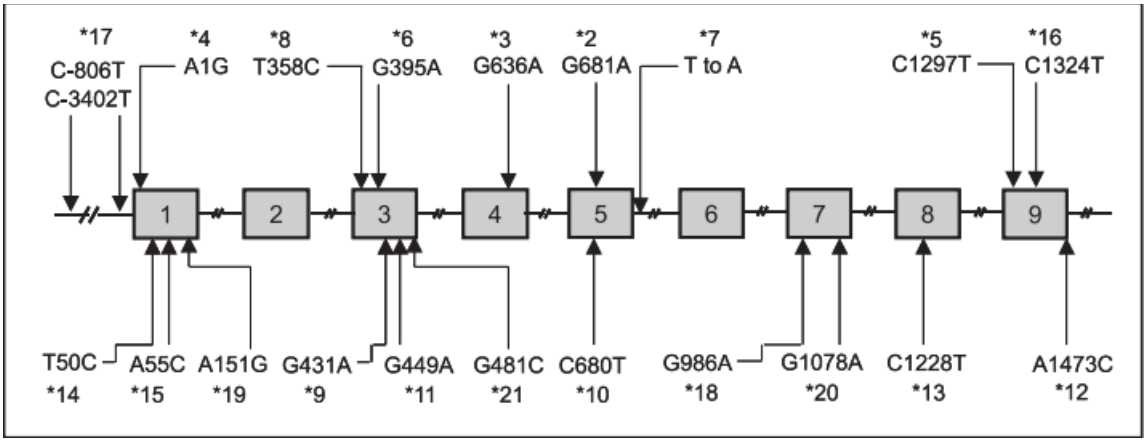


Figure 1.2: CYP2C19 Alleles each with its Location on the CYP2C19 Gene[26]

Allele	SNP	CYP2C19 Function
1*	Normal Allele	Normal function2
2*	681G>A	Loss of Function
3*	636G>A	Loss of Function
17*	-806C>T	Gain of Function

Figure 1.3: Most frequent CYP2C19 Alleles that Affect the Enzymes' Function

Accordingly, when 20 mg of Omeprazole is given once daily, the plasma concentration differs among the three CYP2C19 genotypes as shown in table 2 below. For the poor metabolizers, plasma concentrations were constant for long time after dosing. However, plasma levels of the drug for rapid metabolizers was the lowest after 24 hrs dosing of 20 mg Omeprazole once daily which indicated the dose was considered clinically and

therapeutically insufficient while the drug level of poor metabolizers was found to be 13 times as high as that in the rapid metabolizers group[16–1814]. Other studies on equivalent therapeutic doses of PPI's including 20 mg Omeprazole, 30 mg of Lansoprazole, and 20 mg of Rabeprazole reported same dependency of dug plasma levels on the various CYP2C19 polymorphisms and the difference in acid inhibitory effect of the various PPI's among the three genotypes matched the differences in their plasma concentrations levels[16,18,27]. Eventually, an increase in PPI's dose is recommended for the rapid metabolizers GERD patients who are refractory to the usual dose and a modified dose is needed for poor metabolizer patients[19,24,28–30]. On the basis of their ability to metabolize the proton pump inhibitors, individuals can be classified as extensive metabolizers (homozygous for the wild type allele W/W), heterozygous extensive metabolizers (carrier of only one mutant allele W/I,W/P) or poor metabolizers (two mutant alleles P/P). So far, eight variant alleles(CYP2C19* 2 to *8)have been identified which predict homozygous mutant or compound heterozygous poor metabolizer phenotype. These variants(CYP2C19 *2, *3, *4, *5, *6, *7and*8) are so-called null alleles with no CYP2C19 enzyme activity as a consequence of splice defect(*2), a premature stop codon(*3)or alteration of structure and/or stability of CYP2C19 [4,5,31]. Recently, several newCYP2C19 alleles have been identified (CYP2C19*9 to*15) in individuals from different racial groups with varied ethnic background[21]. At present, however, it is unclear whether these mutations are associated with significant alteration of in vivo enzyme activity .The distribution of poor metabolizers on the basis of phenotype and genotyping tests shows wide inter-ethnic differences. In Caucasian-Europeans, poor metabolizers average 2.8% (range 1.2–3.8%) whereas in Asian/Oceanian populations up to 23% poor metabolizers have been identified[21]

Table 2: *CYP2C19* diplotype-phenotype relationship

Phenotypes	Frequency of patients in different ethnic groups (%)	Genotype	Examples of diplotypes/alleles	Enzyme activity
Normal metabolizers (NMs)	8.6[O]-47.4[A]	Combination of normal function alleles	*1/*1	Full - normal
Intermediate metabolizers (IMs)	24.1[AF]-47[EA]	Combination of normal, decreased, and/or no function alleles	*1/*2, *1/*3	Intermediate (activity between normal and poor metabolizer)
Poor metabolizers (PMs)	2[A]-14.5[EA]	Combination of no function alleles and/or decreased function alleles	*2/*2, *2/*3, *3/*3	Low or absent
Rapid metabolizers (RMs)	1.5[O]-27[C]	One copy of a normal function allele and one copy of an increased function allele	*1/*17	↑ compared to normal metabolizers
Ultrarapid metabolizers (UMs)	0[EA]-4.6[C]	Two increased function alleles	*17/*17	↑ compared to normal metabolizers

O-Oceanian, A-American, AF-African, EA-East Asian, C-Caucasian

1.5 Pharmacokinetics and Pharmacodynamics of Omeprazole: [26]

1.5.1 When AUC values of the proton pump inhibitors – which define drug exposure – are compared between the different phenotypes, it is obvious that poor metabolizers exhibit a 3to 13-times higher drug exposure than extensive metabolizers. On an average, hetero extensive metabolizers AUC-values of the proton pump inhibitors are about 2 to 4-timesh igher than in extensive metabolizers. It is obvious that the largest differences between the phenotypes are seen with omeprazole[11,21]

1.5.2 Regarding the CYP2C19 genotypes and pharmacodynamic action of proton pump inhibitors. With the racemic omeprazole and its S-enantiomer, it can be demonstrated that this response is dependent on AUC (pharmacokinetics)[4,5].Since poor metabolizers of proton pump inhibitors have larger AUC-values than extensive metabolizers, this difference in pharmacokinetics will translate directly into a more pronounced pharmacodynamic response compared to extensive metabolizers.

Evidently, in poor metabolizers, the 24 hour median intra-gastric pH is much higher than in extensive metabolizers and het extensive metabolizers are somewhere in between. All these results suggest a gene-dose effect. In addition, the proton pump inhibitor-induced inhibition of acid secretions more pronounced following multiple

dosing (at day 8) than receiving a single oral dose[3,32]. For all proton pump inhibitors, the dose-concentration (AUC) effect paradigm is clear and a phenotype-dependent drug action has been demonstrated. It was postulated that clinical efficacy of these drugs might be also affected by polymorphisms ofCYP2C19.[3,10,11,21,26,30,32,33]

1.6 Aim of Study

This study aims to determine the Varian's genotypes of CYP2C19 enzyme among Palestinian GERD patients treated with Omeprazole. The frequency of various genotypes will be assessed in this groups of patients and the genotype will be correlated with their response to Omeprazole treatment. The generated data will help to improve the cure rates of GERD in patients by adjusting the correct dose that matches their CYP2C19 genotype. Limited studies were conducted on CYP2C19 genotypes in Palestine[34]. However, many countries have established the frequency of CYP2C19 genotypes among their population that helped improving the cure rates of GERD by selecting the suitable drug and dose according to the genotype of the CYP2C19 gene.

1.7 Hypothesis

Our hypothesis is as follows: Various polymorphisms and mutations in the CYP2C19 gene are present in the Palestinian population and among patients who are diagnosed with GERD and treated with Omeprazole. The variation in response of the patients to the indicated treatment is expected to be mainly due to variation in these polymorphisms and mutations similar but may be distinct from other populations with variations in ethnicity.

Chapter Two

Methodology

2.1 Study Subjects

Sventy three (73) patients, aged 35-68 years old were included in the study who were diagnosed with GERD and treated with daily dose of Omperazole (20 mg-40 mg) as described in Table 1 and subdivided into responders and non-responders to the treatment as summarized in Table 2. The patients were recruited from major medical centers under the supervision of their treating medical staff. All subjects were asked to sign a special consent form indicating their agreement to participate in the study.

Table 3.1: Study Sample Characteristics in Responders and Non- Responders

Category	Median Age	BMI
Responders	45.0	25.4
Non- responders	44.5	25.8
P value	0.9545	0.6234

Table 3.2: Total Number of Responder and Non- Responders Among Study Subjects

Response	Total number
Non Responders	25
Responders	48

2.2 DNA Extraction, Qualification, and Quantification

Venous whole blood samples (3-5 ml) were collected in EDTA tubes, and transferred on ice to the molecular genetics' laboratory at the Arab American University. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit ™(Promega) according to the manufacturer protocol as described below:

1) Five ml of whole blood were drawn into 5 ml K EDTA-containing tube and centrifuged at room temperature for 15 minutes at 1500 X g. The buffy coat (white blood cells) was withdrawn in 300 µl volume and transferred into 1.5 ml Eppendorf tubes .

2) 900 µl of Lysis buffer (1) (10mMTris-HCl, 400mM NACL and 2mM NA2EDTA, pH 8.2) was added to the buffy coat, mixed gently by inverting the tube 6-8 times, the bottom of the tube was flicked to suspend any remaining material .

3) Tubes were incubated for 5 minutes at room temperature; inverted 6-8 time for good mixing and then incubated for an additional 5 minutes at room temperature after flicking the bottom. The tubes were inverted 6-8 times.

4) Tubes were centrifuged for 25 seconds at 10,000 x g.

5) The supernatant (lysed RBCs) was discarded leaving about 25 ul with the pellet, the pellet was vortexed for re-suspension of the white blood cells.

6) 100µl of Lysis buffer (2) (10% SDS, protease K solution,1mg protease K in 1 % SDS and mM Na2EDTA] were added to the tubes, the contents were mixed up and down 5-7 times for complete suspension and lysis of the white blood cells.

7) 100 ul of Precipitation Solution was added to the mixture, mixed vigorously by vortex, and centrifuged for 10 minutes at 10,000 x g.

8) The supernatants were transferred into new clean microfuge tubes.

9) 700 ul of ice-cold isopropanol was added to precipitate the DNA after gentle mixing

10) Tubes were centrifuged at 4° C for 10 minutes at 10,000 x g to pellet the DNA.

11) Supernatants were carefully discarded without dislodging the pellets, washed by 80% ethanol and air dried.

12) DNA was re-suspended in 100 µl rehydration solution.

13) Purified suspended DNA was stored at -20° C until further use for genomic analysis.

Extracted DNA was evaluated for quality by gel electrophoresis using 1% agarose gel, prepared by dissolving 1 gm agarose in 100 ml TAE buffer. DNA purity and concentration were tested using Nanodrop (Nanodrop 2000 C) and all samples had an A260/A280 ratio above 1.7.

2.3 CYP2C19 Gene SNPs Selection

The indicated studied SNPs were selected based on literature resources that documented their location on in the CYP2C19 gene (CYP2C19/2*, CYP2C19/17*, CYP2C19/3*) as part of previous investigations on their association with the pharmacokinetics and pharmacodynamics of Omeprazole in different ethnic groups.[5,35,36]

2.3.1 Genotyping

PCR-ARMS and Primers Design

The genotyping was performed using polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) method. The amplification-refractory mutation system (ARMS) is a convenient method for detecting any mutation involving single base changes or small deletions. ARMS is based on the use of sequence-specific PCR primers that allow amplification of test DNA. Four primers were designed for each SNP; Forward and reverse primer pairs to identify each allele for the three SNPs as shown below in figures 1,2 and 3. All SNPs details and their exact identity are shown in table 3. Genotyping was performed using DNA sequencing or polymerase chain reaction-Amplification Restriction Method System (PCR-ARMS) methods. Polymerase chain reaction (PCR) was carried out to amplify the targeted area DNA fragment of the CYP2C19 gene with SNPstat which specify the three indicated SNPSs CYP2C19/2*, CYP2C19/17*, and

CYP2C19/3* as described in Table 3. Each reaction tube contained 12.5 µl ready mix (lyophilized mixture of Taq polymerase, Mgcl2, dNTP and buffer), 9.5 µl distilled water, 1 µl template genomic DNA (100-200 ng), 1 µl Reverse primer, and 1 µl Forward primer resulting in 25 µl reaction volume.

Figure 2.1 : Primer Pairs (F1/R2 and F2/R1) that Specify the Two Alleles of the -806C>T Loci in CYP2C19 Gene. F1/R2 Pair Identifies the C Allele and F2/R1 Pair Identifies the T Allele. The Brown Colored Region Represents Exon 1 of the Gene.

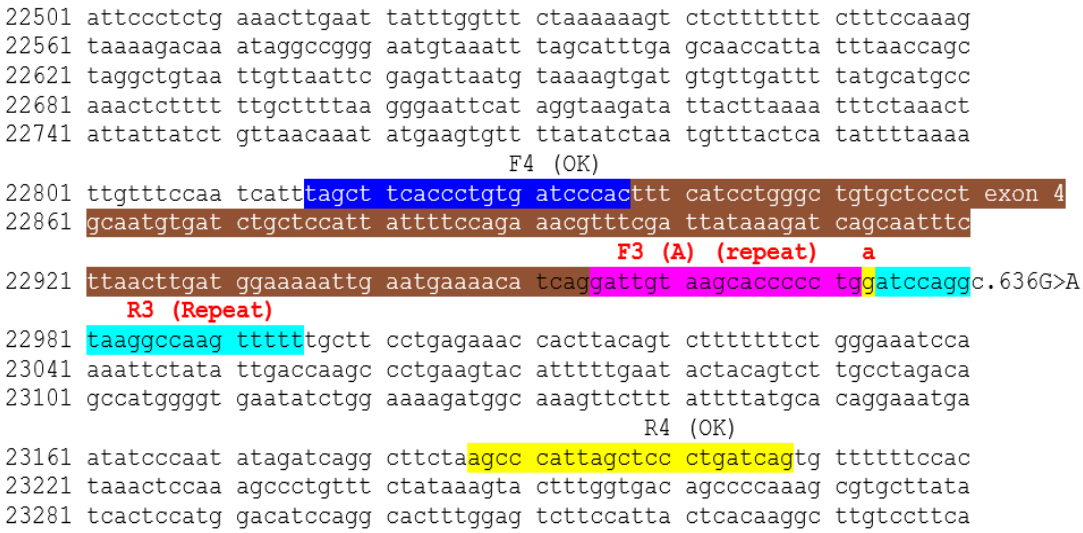


Figure 2.2 : Primer Pairs (F4/R3 and F3/R4) that Specify the Two Alleles of the 636G>A Loci Loci in CYP2C19 Gene. The F4/R3 Pair Identifies the A Allele and F3/R4 Pair Identifies the G Allele.

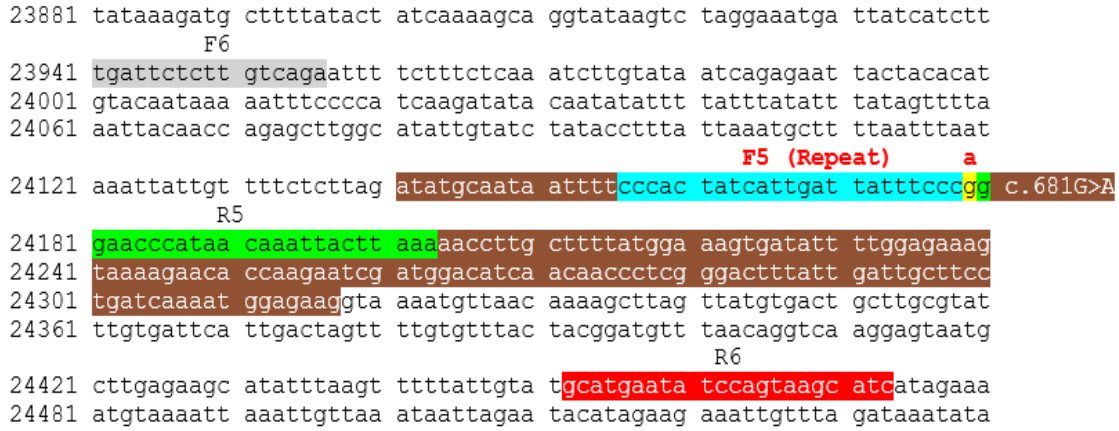


Figure 2.3: Primer pairs (F5/R6 and F6/R5) that specify the two alleles of the 681G>A loci in CYP2C19 gene. The F5/R6 pair identifies the G allele and F6/R5 pair identifies the A allele.

Table 2.1: CYP2C19/2*, CYP2C19/17*, and CYP2C19/3* Detection Details Using the Indicated Forward and Reverse Primers and the Sizes of the Respected Amplified DNA Fragments.

Primers combination	Forward and Reverse sequence	Product size	Mutation	SNP's region	Reference
F1R2	TTGTGTCTTCTGTTCTCAACGT TGAAGGCAGGAATTGTTATTTT	For C allele : 221	C>T	17* Promoter	rs12248560
F2R1	TTTTTTCTCATGAGCATCTCTG GCATTATCTCTTACATCAGAGCTG	For T allele : 185	C>T	17* promoter	rs12248560
F3R4	GATTGTAAGCACCCCCGGA CTGATCAGGGAGCTAATGGGCT	For A allele: 255	G>A	3* Exon 4	rs4986893
F4R3	TAGCTTCACCCTGTGATCCCAC AAAAACTTGGCCTTACCTGGCTC	For G allele: 180	G>A	3* Exon 4	rs4986893
F5R6	CCCACTATCATTGATTATTTCACA GATGCTTACTGGATATTCATGC	For G allele: 270	G>A	2* Exon5	rs4244285
F6R5	TCATCTTTGATTCTCTTGTCAGA TTTAAGTAATTTGTTATGGGTTACC	For A allele: 319	G>A	2* Exon 5	rs4244285

PCR was run on FlexCycler2 thermocycler (Analytik Jena, Germany). The PCR reactions were run on 2.5% agarose gel , electrophoresis was run at 100 V for 40 minutes in Tris-Acetate-EDTA buffer (TAE). The PCR protocols for each SNP are described in Table 4.

Table 2.2: PCR Protocols for the Three Indicated SNP Alleles.

SNP	**Initial denaturation °C(5 min)**	**Denaturation °C** (30 sec)	**Annealing °C (30 sec)**	**Elongation °C(45 sec)**	**Final extension °C** (5 min)
rs4244285	95	95	58	72	72
r4244285	95	95	58	72	72
rs12248560	95	95	58	72	72
rs4986893	95	95	63	72	72
rss4986893	95	95	63	72	72
rs1224856095	95	95	63	72	72

Table 2.3: DNA Fragments Size for Each CYP2C19 SNP's Alleles; the CYP2C19/2*(G and A Allele**),** CYP2C19/3* (G and A Alleles), and CYP2C19/17*(C and T Alleles).

Enzyme Variant	Allele	Product size
CYP2C19/2*	G	270
CYP2C19/2*	A	319
CYP2C19/3*	G	180
CYP2C19/3*	A	255
CYP2C19/17*	C	221
CYP2C19/17*	T	185

2.4 Clinical Criteria for Examining PPI's Response.

Initially, patient history of GERD was assessed. Secondly, the frequency and severity of symptoms was also assessed by frequency based on severity of GERD symptoms. Eventually an additional scale was assessed for the quality of life improvement of each patient defined as EQ-5D score.[7,20] To asses response of GERD patients to treatment, clinical exam was performed with or without an endoscopic examination (Depends on

individual patients' case).[19] Endoscopically proven response to Omeprazole was assessed based on gastric cells (Muscularis, Sub Mucosa, and Mucosa), as Omeprazole acts by irreversibly binding to and inhibiting the hydrogen-potassium ATPase pump on the luminal surface of the parietal cell membrane. So patients with good response will have well characterized, not irritated, and not swollen cell lining. [19] There are three scenarios for response: responder, partial responders, and non-responders. The drug is given to the patient for a 4 weeks trial period before being assessed for the clinical scales. Then, treatment will be continued for another 4 weeks. followed by clinical response evaluation. If the patient is responder the treatment continues as it is. If the patient is non-responder or is partial responder, treatment will be maintained at a higher dose or switched for other types of PPIs like Esomeprazole, Lansoprazole, and Pantoprazol.[20] Responders are patients who have their symbtoms disappeared with score of >3 in EQ-5D quality of life scale and especially GERD-Q score [41]. EQ-5D is an instrument which evaluates the generic quality of life [37]. The descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Partial responders are patients who have symbtoms with score of EQ from 2-3. Whereas non responders are patients who still have symbtoms with the drug given to its maintainance periond (8 weeks) with ascore of EQ < 2 as shown in the table below.

Table 1. The GerdQ questionnaire respondents enter the frequency scores after reflecting on their symptoms over the previous week

Question	Frequency score (points) for symptom			
	0 day	1 day	2–3 days	4–7 days
1. How often did you have a burning feeling behind your breastbone (heartburn)?	0	1	2	3
2. How often did you have stomach contents (liquid or food) moving upwards to your throat or mouth (regurgitation)?	0	1	2	3
3. How often did you have a pain in the centre of the upper stomach?	3	2	1	0
4. How often did you have nausea?	3	2	1	0
5. How often did you have difficulty getting a good night's sleep because of your heartburn and/or regurgitation?	0	1	2	3
6. How often did you take additional medication for your heartburn and/or regurgitation, other than what the physician told you to take? (such as Tums, Rolaids, Maalox?)	0	1	2	3

2.5 Statistical Analysis. 41

Data are presented as mean ± SD, frequencies (%), odds ratio (OR), and 95% confidence interval (CI) when appropriate. The allele frequencies and genotype distributions of CYP2C19 SNPs, the association between SNP genotypes and haplotypes with RPL under different genetic models, as well as linkage disequilibrium tests for these SNPs, were analyzed using the web tool SNPStat (https://www.snpstats.net/start.htm). P-values less than 0.05 were accepted as statistically significant.

2.5.1 Ethical Approval

All methods and procedures performed in this study including study participants were in accordance with the Arab American University's guidelines and regulations. Moreover, ethical approval was obtained from AAUP's graduate studies.

2.5.2 Consent Form

All participants signed an informed consent form before enrolling in this study.

Chapter Three

Results

3.1 Detection of CYP2C19 Gene Variants.

Genotyping of the indicated SNPs in the CYP2C19 gene (CYP2C19/2*, CYP2C19/3* and, CYP2C19/17*) were determined using Polymerase chain reaction (PCR)- Amplified Refractory Mutation System (PCR-ARMS). Detection of 180 bp DNA fragment indicates the G allele for CYP2C19/3* while detection of a 255 bp DNA fragment indicates the A allele. For the CYP2C19/2*, detection 270 bp DNA fragment indicates G allele while detection of 319 bp DNA fragment indicates the A allele. However, for the CYP2C19/17*, detection of a 221 bp DNA fragment indicates the C allele while detection of 185 bp DNA fragment indicates the T allele.

3.1 Genotyping and statistical analysis of the CYP2C19/3* variant.

Genotyping for the CYP2C19/3* variant was performed for responders and non-responders subjects. Figure 3.1 Show a representative gel with the results of 5 responders' subjects (total responders 44). The results show a single product of 180 bp which indicates the G allele.

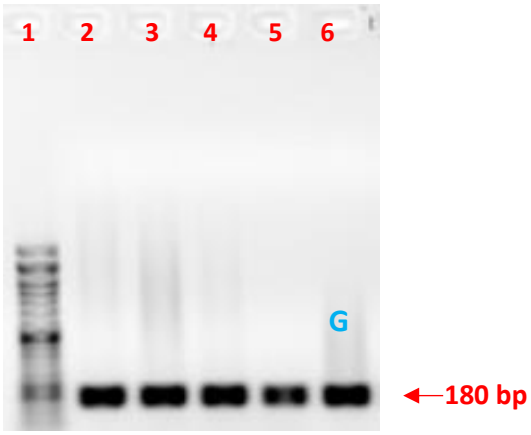


Figure 3.1: Representative Agarose Gel Showing an Amplified 180 bp DNA Fragment Indicating CYP2C19/3*G Allele for 5 Responders' Samples (Lanes 2-6). Lane 1 Shows 50-bp DNA Ladder.

Similarly, genotyping of the CYP2C19/3* A allele was done for both groups (responders and non- responders). A representative gel in figure 3.2 shows the corresponding 255 bp amplified DNA fragment.

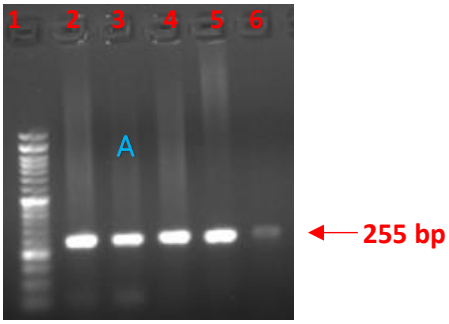


Figure 3.2: Representative Agarose Gel Showing 255 bp Amplified DNA Fragment Indicating the CYP2C19/3* A Allele for 4 Non Responders' Subjects. Lane 1 Shows 50 bp DNA Ladder.

Table 3.1: Upper Section. CYP2C19/3* Allele Frequency Among All Responders and Non- Responders' Subjects. Lower Section Shows the Genotype Frequency of the Same Variant Among all Subjects. Whereas 1*/1* is G/G Genotype (Full Activity), 1*/3* is G/A Genotype(Intermediate Metabolisers), 3*/3* is A/A genotype(Poor Metabolisers.

CYP 2 C19 / 3* Allele Frequency						
Allele	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
A	77	0.53	50	0.52	27	0.54
G	69	0.47	46	0.48	23	0.46

CYP 2 C19 / 3* Genotype Frequency						
Genotype	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
1*/1*	4	0.053	2	0.04	2	0.08
1*/3*	69	0.95	46	0.96	23	0.92

The results presented in table 3.1 show the distribution of the CYP2C19/3* alleles in all study subjects. A proportion of 0.53 (77/146) of all subjects have the A allele and 0.47 (69/146) with the G allele. Among the responders group , the data shows a frequency of 0.52 (50/96) with the G allele and 0.48 (46/96) with the A allele while among the non-responders , the data shows a frequency of 0.54 (27/50) with the G allele and 0.46 (23/50) with the A allele. In addition, Table 3.1 shows a proportion of 0.95 (69/73) frequency with A/G heterozygous genotype among all subjects and a proportion of 0.05 (4/73) with the homozygous G/G genotype. The homozygous A/A genotype could not be detected among all subjects. The data also shows a frequency of 0.96 (46/48) with the heterozygous G/A genotype among the responder group and 0.04 (2/48) with the homozygous G/G genotype

among the responders subjects in comparison with a frequency of 0.92 (23/25) with the G/A genotype and 0.08 (2/25) with A/A genotype among non- responders subjects.

Table 3.2: Association of the CYP2C19/3* variant genotypes between responders and non-responders subjects.

Genotype	Responders	Non-Responders	OR (95% CI)	P – Value
1*/1*	2 (5.3%)	2 (8%)1	1.00	0.72
1*/3*	46(94.7%)	23 (92%)	0.68	0.72

Table 3.2 shows association of the CYP2C19/3*variant genotypes between responders and non- responders. The data shows the 1*/1*genotype represents 5.3% among the responders subjects compared to 8% among non-responders (P value =0.72) while the 1*/3* genotype represents 94.7% among the responders compared to 92% among non-responders (P value = 0.72). These results demonstrate no significant difference between the two indicated genotypes among both groups. Therefore, even though most subjects (responders and non-responders) carry one copy of the weak allele (A), the rs4986893 SNP does not seem play a role in response to Omeprazole among these patients.

3.2 Genotyping and Statistical of the CYP2C19/17* Variant:

Genotyping was done for the CYP2C19/17*variant among all subjects. Figure 3.3 represents 12 subjects of responders (total responders =48). The results show a product size of 221 bp which indicates the C allele and a product size of 185 bp which indicates T allele in a 22 non- responder subjects as shown in figure 3.4 No other bands were observed, indicating the reaction's specificity. Whereas, 1*/1* indicates C/C genotype, 1*/17* indicates C/T genotype, ans 17*/17* indicates T/T genotype.

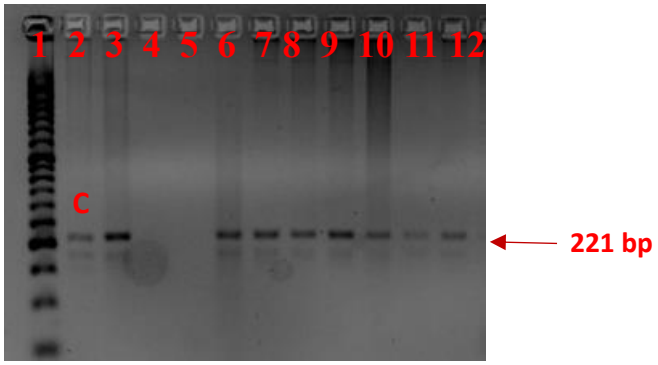


Figure 3.3: Agarose Gel Showing the Indicated Amplified 221 bp DNA Fragment Indicating CYP2C19*17C Allele of Two Responders' Samples. DNA Ladder of 50bp is Shown in (lane 1).

Similarly, genotyping for both groups (responders and non- responders) was done for CYP2C19/17* T allele. A representative gel shows the genotype for responders (as shown in figure 3.4 below.

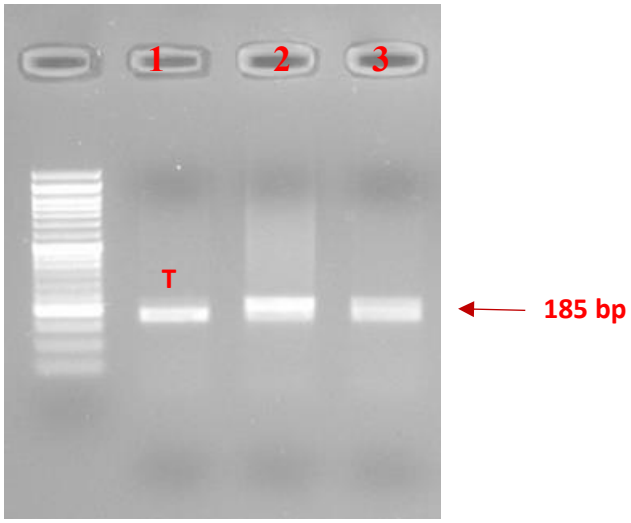


Figure 3.4: Agarose Gel Showing the Indicated an Amplified 185 bp DNA Fragment Indicating CYP2C19*17 T Allele of 3 Responders' Samples (Lanes 2-4). A 50bp Ladder is Shown in Lane 1.

Table3.3: CYP2C19*17 Genotype Frequency Among All Subjects and Among Responders and Non-Responders Subjects.

CYP 2 C19 / 17* Allele Frequency						
Allele	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
C	104	0.71	74	0.77	30	0.6
T	42	0.29	22	0.23	20	0.4

CYP 2 C19 / 17* Genotype Frequency						
Genotype	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
1*/1*	32	0.44	26	0.54	6	0.24
1*/17*	40	0.55	22	0.46	18	0.72
17*/17*	1	0.01	0	0	1	0.04

Table 3.3 (upper section) shows the CYP2C19/17* allele frequency among all subjects. The results show a proportion of 0.71 (104/146) of all subjects with the C allele and 0.29 (42/146) with the T allele. Among the responders group, the data shows a frequency of 0.77 (74/96) with the C allele and 0.31 (22/96) with the T allele while among the non-responders, the data shows a frequency of 0.6 (30/50) with the C allele and 0.4 20(/50) with the T allele. Table 3 (lower section) shows CYP2C19/17* genotype frequencies among all subjects and among responders and non-responder subjects. The data shows a proportion of 0.44(32/73) frequency with 1*/1*genotype in all subjects with a proportion of 0.55 (40/73) with the 1*/17*genotype. The data also shows a frequency of 0.01 (1/73) with the 17*/17* genotype and 0.54 (26/48) with the 1*/1* genotype, 0.46 (22/48) with the 1*/17* among the responders subjects and a frequency of 0.24 (6/25) with 1*/1*

genotype and 0.72 (18/25) with 1*/17* genotype among non- responders subjects. Therefore, even though most subjects (responders and non-responders) carry one copy of the allele (T), the rs12248560 SNP does not seem play a role in response to Omeprazole among these patients.

Table 3.4: The Association of (CYP2C19/17*) Between Responders and non - Responders

Genotype	Responders	Non-Responders	OR (95% CI)	P- Value
1*/1*	26 (54%)	6 (24%)	1.00	0.2
1*/17*	22 (46%)	18 (72%)	1.90 (0.59-6.15)	0.2
17*/17*	0 (0%)	1 (4%)	NA	0.2

Table 3.4 shows the association of CYP2C19/17* between responders' group and non-responders. It shows that 1*/1* genotype has a 37.1% and 1*/17* has 62.9% of responders (P value 0.2).

3.3 Genotyping and Statistical Analysis of the CYP2C19/2* Variant

Genotyping was done for CYP2C19/2*variant among all subjects. Figure 3.5 represents 12 subjects of responders (total responders =48). The results show a product size of 270 bp which indicates the G allele and a product size of 319 bp which indicates A allele in a 9 responder subjects as shown in figure 3.6. No other bands were observed, indicating the reaction's specificity.

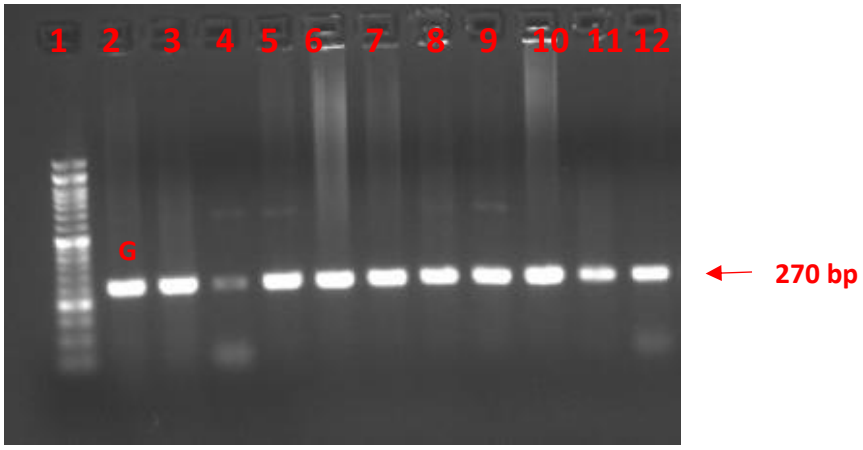


Figure 3.5: Agarose Gel Showing the Indicated an Amplified 270 bp DNA Fragment Indicating CYP2C19/2* G Allele of 12 Responders' Samples (Lanes 2-4). A 50bp ladder is Shown in Lane 1.

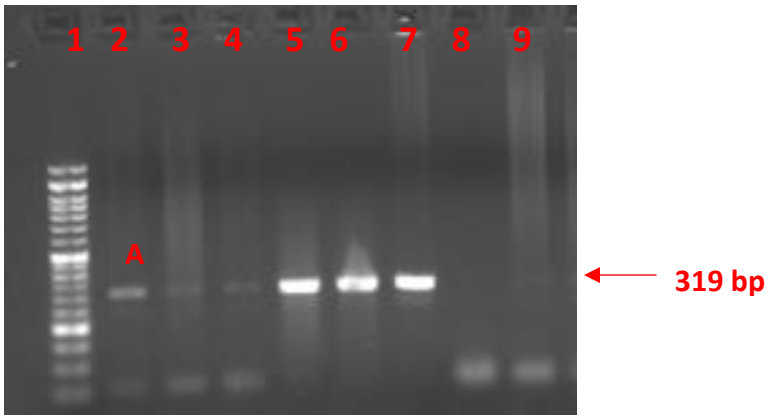


Figure 3.6: Agarose Gel Showing the Indicated an Amplified 319 bp DNA Fragment Indicating CYP2C19/2* A Allele of 9 Responders' Samples (Lane 2-4). A 50bp DNA Ladder is Shown in Lane 1.

Table3.5: CYP2C19*2 Genotype Frequency Among All Subjects, Responders and Non - Responders Subjects.

CYP 2 C19 / 2* Allele Frequency						
Allele	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
G	102	0.7	73	0.76	29	0.58
A	44	0.3	23	0.24	21	0.42

CYP 2 C19 / 2* Genotype Frequency						
Genotype	All subjects		Responders		Non-Responders	
	Count	Proportion	Count	Proportion	Count	Proportion
1*/1*	44	0.6	23	0.48	21	0.84
1*/2*	29	0.4	25	0.52	4	0.16

Table 3.5 (upper section) shows the CYP2C19/2* allele frequency among all subjects. The results show a proportion of 0.69 (102/146) of all subjects with the G allele and 0.3 (44/146) with the A allele. Among the responders' group, the data shows a frequency of 0.76 (73/96) with the G allele and 0.24 (23/96) with the A allele while among the non-responders, the data shows a frequency of 0.58 (29/50) with the G allele and 0.42 (21/50) with the A allele. Table 1(lower section) shows CYP2C19/2*genotype frequencies among all subjects, responders and non-responder subjects. The data shows a proportion of 0.6 (44/73) frequency with 1*/1* genotype in all subjects with a proportion of 0.4 (29/73) with 1*/2* genotype. The data also shows a frequency of 0.48 (23/48) with the 1*/1*genotype and 0.52 (25/48) with the 1*/2* genotype among the responders subjects and a frequency of 0.84 (21/25) with 1*/1* genotype and 0.16 (4/25) with 1*/2* genotype among non- responder's subjects. Therefore, most subjects (responders and non-responders) carry one copy of the allele (A), the rs4244285 SNP seems to play a role in response to Omeprazole among these patients.

Table 3.6: Association of CYP2C19*2 Genotype with Responders Compared Non-Responder Subjects.

Genotype	Responders	Non-Responders	OR (95% CI)	P Value
1*/2*	25 (52%)	4 (16%)	1.00	<0.0001
1*/1*	23 (48%)	21(84%)	15.93	<0.0001

Table 3.6 shows the association of CYP2C19/2* genotypes between responders and non –responders groups . The results show the 1*/2* genotype has significantly higher ratio (52%) among responders compared to non-responders with 16% while for the 1*/1*genotype, the data showed 84% among the non- responders compared to 16% among responders (P value <0.0001). (note as above)

3.4 Haplotype Frequency

The genotype data obtained for the three indicated CYP2C19 SNP's were used for constructing haplotype blocks and their corresponding frequencies. The cumulative frequency of haplotype among all study subjects was 0.4918 with CGA haplotype as shown in table 3.7. The least haplotype frequency in all subjects was CAA (0.1555). No statistically significant correlation between haplotype frequency and clinical response was evident (P=1).

Table 3.7 : Haplotype Frequency of CYP2C19 SNP's (CYP2C19/2*, CYP2C19/17*, and CYP2C19/3*)

Haplotype analysis

Haplotype frequencies estimation (n=73)							
	SNP1	SNP2	SNP3	Total	group.Ca	group.Co	Cumulative frequency
1	C	G	A	0.4918	0.4977	0	0.4918
2	C	A	G	0.191	0.2295	NA	0.6829
3	T	G	G	0.1613	0.1959	0.0317	0.8441
4	T	A	G	0.0908	0.0101	NA	0.935
5	C	G	G	0.0295	0.0437	0.4283	0.9644
6	T	A	A	0.0195	NA	0.2483	0.984
7	T	G	A	0.016	0.0232	0.12	1
8	C	A	A	0	NA	0.1717	1

3.5 Allele Frequencies s of CYP2C19gene Among Responders and Non-Responders Patients.

The frequencies of the various CYP2C19 gene alleles among responders and non-responder GERD patients is described in table 3.8. The frequencies of **CYP2C19/3* alleles (636 G>A) with low enzyme activity** were 0%. 94.2%, and 5.7% for 1*/1*(G/G), 1*/3*(G/A), and 3*/3* (A/A) in the responders group) respectively compared to 0%, 92% and 8% among the non-responders group. In comparison, the frequencies for the CYP2C19/2* alleles (681G>A) with loe in the responders group were 0%, 28.57%, and 71.42% for 1*/1* (G/G), 1*/2* (G/A), and 2*/2* (A/A) respectively compared to 0%, 84% and 16% among the non-responders group. Finally, the frequencies of **CYP2C19/17*** (-806 C/T) with high enzyme activity were 37.14%, 62.85%, and 0% for 1*/1*(C/C), 1*/17* (C/T), and 17*/17* (T/T) respectively for the responders group compared to 24%, 72%

Table 3.8: Observed frequency according to genotype in responders and non- responders

Genotype	Responders(n)	Frequency %	Non-Responders (n)	Frequency %
3*:				
1*/1*	2	5.7%	2	8%
1*/3*	33	94.2%	23	92%
3*/3*	0	0%	0	0%
17*:				
1*/1*	13	37.14%	6	24%
1*/17*	22	62.85%	18	72%
17*/17*	0	0%	1	4%
2*:				
1*/1*	10	28.57%	21	84%
1*/2*	25	71.42%	4	16%
2*/2*	0	0%	0	0%

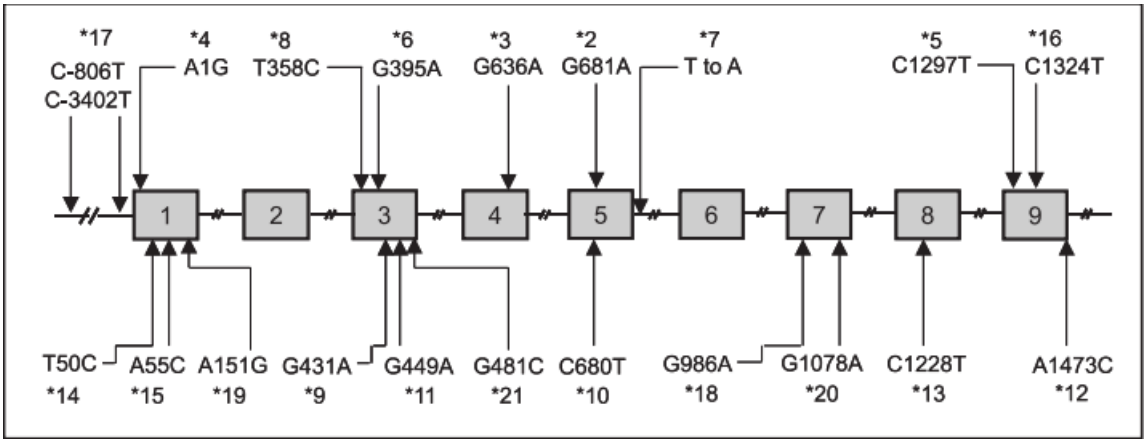
Chapter Four

Discussion

The Genetic variability of *CYP2C19 gene* affects safety or efficacy of many clinically important medications as outlined in the clinical pharmacogenetics implementation consortium (CPIC) dosing guidelines.[35,38] This genetic viability will eventually reflect on the efficacy and excretion rate of the indicated drugs. The present study was designed to investigate the various genotypes of the CYP2C19 enzyme gene and their correlation with treatment response among Palestinian GERD patients treated with Omeprazole. The generated data will provide essential information to improve the cure rates of GERD patients through adjusting the correct dose that matches the CYP2C19 genotype. Previously, a study was conducted to identify the frequency of the various genotypes of this enzyme among healthy individuals [34]. Several countries have established the frequency of CYP2C19 genotypes among their population for improving the cure rates of GERD through selecting the suitable drug and dose [34]. Evidently, all proton pump inhibitors are metabolized by the hepatic route and the polymorphically expressed CYP2C19 enzyme is primarily responsible for their metabolism and eventually elimination[11,21]. In addition, the complete metabolism of proton pump inhibitors like omeprazole is mediated with another cytochrome enzyme , CYP3A4. Since CYP2C19 is the main enzyme involved in the metabolism of all proton pump inhibitors, it can be anticipated that the largest part of the inter-individual variability in the pharmacokinetics of this group of drugs is due to CYP2C19 genotypes in treated patients [12].

Since the main metabolic pathway for PPIs is through the CYP2C19 isoforms, individuals are classified into three classes based on their rate of metabolism by these various isoforms ; rapid, intermediate, and slow metabolizers. As a result, the pharmacokinetics

and pharmacodynamics of PPI's are directly affected.[16,17,22,23] There are more than 28 variant alleles of CYP2C19 that have been identified as described bove in figure 2 and depected below. Several of the identified alleles have relatively low frequencies and are only limited to specific populations or ethnic groups. However, single nucleotide polymorphisms which defines CYP2C19*2 and CYP2C19*3 and CYP2C19*17 alleles are the most common and also the most studied alleles.



The CYP2C19*3 and CYP2C19*17 alleles result in null function of the enzyme due to defective splicing mutation (*2) or insertion of a premature stop codon (*3)[25]. The CYP2C19*17, identified in a study on human hepatic proteins patients who have the CYP2C19*17 allele [14], have increased gene expression of the enzyme and thus higher activity and therefore individuals possessing this polymorphism are described to be ultra-rapid metabolizers[16,25]. Several studies were carried out to determine the frequencies of poor metabolizers among many ethnic groups. These studies revealed the frequency among white Americans was 2.5% [17] whereas among white Europeans was 3.5%, and the frequency among the Chinese population was 19.8% [13,17]. Evidently, when 20 mg of Omeprazole is given once daily, the plasma concentration differs among the three CYP2C19 genotypes. For poor metabolizers, plasma concentrations were constant for long time after dosing (24 hrs). In comparison, plasma levels of the drug for rapid

metabolizers was the lowest after 24 hrs dosing of 20 mg Omeprazole once daily which indicated the dose was considered clinically and therapeutically insufficient while the drug level of poor metabolizers was found to be 13 times higher in the rapid metabolizers group[16–1814]. Other studies on equivalent therapeutic doses of PPI's including 20 mg Omeprazole, 30 mg of Lansoprazole, and 20 mg of Rabeprazole reported the same dependency of dug plasma levels based on the various CYP2C19 polymorphisms and the difference in acid inhibitory effect of the various PPI's among the three genotypes matched the differences in their plasma concentrations levels[16,18,27]. Eventually, an increase in PPI's dose is recommended for the rapid metabolizers GERD patients who are refractory to the usual dose and a reduced modified dose is needed for poor metabolizer patients[19,24,28–30].

Recently, several new CYP2C19 alleles have been identified (CYP2C19*9 to*15) in individuals from different racial groups with varied ethnic background[21]. At present, however, it is unclear whether these mutations are associated with significant alteration of in vivo enzyme activity. The distribution of poor metabolizers on the basis of phenotype and genotyping tests shows wide inter-ethnic differences. In Caucasian-Europeans, poor metabolizers average 2.8% (range 1.2–3.8%) whereas among Asian/Oceanian populations, up to 23% poor metabolizers have been identified.[21,40]

Previous studies investigated the frequency of CYP2C19 polymorphisms with correlation to response for Omeprazole treatment by measuring gastric pH and/or measuring the plasma concentration of Omeprazole[5]. These studies used healthy controls as their study subjects which allows flexibility in management. However, In our study, it was not convenient to obtain frequent gastric samples from GERD patients for frequent gastric

pH monitoring In addition, monitoring plasma concentration of the drug and the various metabolites could not be performed for correlation with CYP2C19 gene alleles variation among the patients subjects due to the lack of the indicated needed facilities. Alternatively, the evaluation for our subjects response to treatment was done by direct clinical evaluation for each patient using endoscopy results and quality of life score (5HD score) which was performed by members of the treatment clinical team. This direct clinical evaluation provided convenient tool for monitoring patients response which is medically recognized for the evaluation of patients response to Omeprazole treatment among GERD patients.[37,40,41] Several previous studies generated their data from healthy volunteers and divided them in groups with the indicated various CYP2C19 polymorphisms for evaluation of their response to their response to treatment using direct pH measurements and plasma metabolites levels following different drug doses . Alternatively, we performed the indicated correlation between the indicated enzyme polymorphisms and standard drug treatment was evaluated in GERD patients subjects who are on continuous treatment with the dug and not healthy subjects.

Our data revealed a statically significant correlation between responders and non-responders concerning the CYP2C19/2* allele (52% vs 16%, P <0.0001). Our results showed 18 out of 24 (patients having the 17* allele were non responders. However, the P value was not significant between genotype and response to Omeprazole. Evidently, 25 out of 29 were responders for the allele 2*. And , 46/48 were responders for the allele 3*. However, P values were not significant between genotype and response to Omeprazole in 3* allele.

In a study in Poland on a large scale worldwide to determine the frequency of these polymorphisms among different ethnic groups. It was been found that CYP2C19 SNPs;

2*,3*, and 17* have frequency of 15%,0.3%, and 20.3% respectively. Whereas the common diplotypes were 1*/17* and 1*/2* with frequency of 26% and 19% respectively. However, least common ones were 2*/17*, 17*/17*, 2*/2* with percentage of 6%,4%, and 2.5% respectively.[38,39,40] The frequency of SNP 17* matches the world population frequency by having 67% of study population had the CYP2C19/17* genotype. Moreover, for the CYP2C19/2*, results also match the world population trend by having about 93%. In addition to that allele 3* was also matching the general frequency trend by 52%.[35]

So far, there are no current or previous studies that done to determine the relationship between genotype a study sample have with his/her response to the PPI's. Our study have done that by discussing the correlation between genotype a GERD patient has and his/her clinical response to Omeprazole.

In general, 68% of the patients in our study have variation in the function of CYP2C19 gene that also matches the general population frequency worldwide around 58%-67%.[36,40] Our data supports the need for investigating the genotype of the most common CYP2C19 polymorphisms in GERD patient for adjusting the dose of Omeprazole. This in turn will lead to the most effective treatment for the symptoms of GERD and improve the life style for the patients by reducing the adverse events that may be caused by the ineffective dosing of PPI's as Omeprazole.

These drug-oriented guidelines have played a critical role in facilitating the clinical implementation of Pharmacogenomics and enable the development of clinical decision support tools for clinicians to understand and more efficiently use Pharmacogenetic testing results in determining drug dosing based on each patients' genetic profiles.[36]

Limitations of our study:

Plasma concentration of Omeprazole is not available in our country where it gives a qualitative description for the plasma levels of Omeprazole in the body. However, we used a qualitative approach where a clinical view was done to classify the patients to responders and not responders. Also, in our study we have used a fixed dose of Omeprazole, where as multidosing approach was not suitable for our patients as this approach was done by many other studies. In addiotion, small sample size may limit the generalizability of the study findings. Finally, some GERD phenotypes may be refractory to treatment and some of our samples may be non-responding to drug just because they have this GERD phenotype.

In Conclusion, the present study focused on investigating potential correlation between specific selected variants in the CYP2C19 gene with their direct impact on the clinical response of Omeprazole in GERD patients among Palestinian subjects. The data revealed significant correlation between the variant CYP2C19/2* and clinical response of Omeprazole among study subjects. However, no significant correlation was found between the clinical response of Omeprazole and CYP2C19/17*(describe) and CYP2C19/3*(describe) alleles. Further investigations should be done with larger sample size that may reveal some new data or correlations.

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الملخص

الخلفية والأهداف: يدرس علم الصيدلة الجيني كيف يختلف المرضى في استجابتهم للأدوية حيث تكون الجرعات الفعالة في بعض المرضى غير فعالة في البعض الآخر. من الناحية العملية والدراسات، وجد أن مرضى الارتجاع المعدي المريئي (GERD) يختلفون في استجابتهم للأوميبرازول. (GERD) هو مرض مزمن يحدث بسبب تدفق محتويات المعدة في الاتجاه المعاكس . يؤثر ارتجاع المريء على 35% من السكان البالغين في العالم الغربي. ويعتبر مؤشرا رئيسيا لعلاج مثبطات مضخة البروتون (PPI)

تعتبر مثبطات مضخة البروتون (PPI) مثل الدواء الرئيسي أوميبرازول (Omeprazole) من أدوية الخط الأول المستخدمة كمثبطات حمض للعديد من الأمراض بما في ذلك مرض الارتجاع المعدي المريئي (GERD) ومع ذلك، فإن العديد من المرضى لا يستجيبون أو يستجيبون بشكل سيئ للجرعة المعتادة لأحد مثبطات مضخة البروتون الشائعة. وعليه يجب تحديد العوامل التي تؤثر على نجاح العلاج بهذه الأدوية لضمان أقصى استفادة للمرضى. بالنسبة لمرضى ارتجاع المريء الذين يتناولون أوميبرازول كعلاج، تختلف الاستجابة السريرية بينهم وفقًا لتعدد أشكال CYP2C19

كان الهدف من دراستنا هو تقييم تواتر الطرز الوراثية لإنزيم CYP2C19 لدى مرضى الارتجاع المعدي المريئي الفلسطينيين المعالجين بالأوميبرازول. على وجه التحديد*CYP2C19/2 ، *CYP2C19/3، و *CYP2C19/17

الطريقة: شملت الدراسة 73 مريضا فلسطينيا يعانون من ارتجاع المريء، 24 من غير المستجيبين للأوميبرازول و 36 من المستجيبين للأوميبرازول. تم الحصول على عينات من الحمض النووي وتنميطها وراثيًا لـ *CYP2C19/2 و *CYP2C19/17و *CYP2C19/3 بواسطة ARMS-PCR تم استخدام برنامج SNPStat لتحليل البيانات الناتجة.

النتائج: كشفت بياناتنا أن هناك فرق ذو دلالة إحصائية بين المستجيبين وغير المستجيبين الذين لديهم أنماط وراثيةG/A وG/G في *CYP2C19/2 والذي له علاقة بالاستجابة السريرية للأوميبرازول في مرض الارتجاع المعدي المريئي. ومع ذلك، لا تظهر بياناتنا أي دلالة إحصائية بين المستجيبين وغير المستجيبين بغض النظر عن *CYP2C19/3 للأنماط الجينية A/A و G/A

و *CYP2C19/17للأنماط الجينية C/C و C/T.توضـــح بياناتنا أهمية فحص تعدد الأشـــكال CYP2C19لتقييم الجرعة المناسبة من أوميبرازول للمرضـى لعلاج ارتجاع المريء. يسـاعد هذا في تقليل التفاعلات الدوائية الضـــارة وتعظيم تأثير أوميبرازول قدر الإمكان في علاج ارتجاع المريء.

الاستنتاج: كشفت البيانات عن وجود ارتباط كبير بين الأنماط الجينية *CYP2C19/2 والاستجابة السريرية لمرضى ارتجاع المريء للأوميبرازول مع عدم وجود ارتباط كبير مع SNPs الأخرى . *CYP2C19/17و .*CYP2C19/3قد تجد المزيد من التحقيقات على عينة أكبر وجود علاقة بين الاستجابة السريرية و .CYP2C19 SNP