PTE/9591-1/2022

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Evaluation of pathogenicity-related oxidative stress biomarkers as well as clinical characteristics, management, and outcomes in acute coronary syndrome

Ph.D. Dissertation

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Pécs, 2021

Dedication

To my family

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Abbreviations

ACS: Acute coronary syndromes

STEMI: ST-segment elevation myocardial infarction

NSTEMI: Non-ST-segment elevation myocardial infarction

UA: Unstable Angina

ST- ACS: ST-segment elevation acute coronary syndrome

AMI: Acute myocardial infarction

CS: Cardiogenic shock

ECG: Electrocardiogram

CVD: Cardiovascular disease

GBD: Global burden of disease

CHD: Coronary heart disease

CAD: Coronary artery disease

CABG: Coronary artery bypass grafting

LVEF: Left ventricular ejection fraction

IHD: Ischemic heart disease

LDL: Low-density lipoproteins

hs-cTn :High-sensitivity cardiac troponin

ROS: Reactive oxygen species

$^1O_2$: Singlet oxygen

H_2O_2: Hydrogen peroxide

O_2•_: Superoxide

·OH: Hydroxyl

SOD: Superoxide dismutase

CAT: Catalase

AA: Ascorbic acid

GSH: Reduced glutathione

ECM: Extracellular matrix

ROS: Reactive oxygen species

NADPH: Nicotinamide adenine dinucleotide phosphate

NOS: Uncoupled nitric oxide synthase

Phe: Phenylalanine

Tyr: tyrosine

m-Tyr: meta-tyrosine

o-Tyr: ortho-tyrosine

p-Tyr: para-tyrosine

ESC: European Society of Cardiology

ACC: American College of Cardiology

AHA: American Heart Association

HPLC: High performance liquid chromatography

ROC: Receiver operating characteristic curve

AUC: Area under curve

PAH: Phenylalanine (4)-hydroxylase

BH4: Tetrahydrobiopterin

ED: Endothelial dysfunction

WHO: World Health Organization

NHLBI: National Heart, Lung, and Blood Institute classified the

BMI: body mass index

WC: waist circumference

HF: Heart failure

TTE: Transthoracic echocardiogram

ED: Emergency department

FMC: First medical contact

Chapter 1: Introduction

Definition of acute coronary syndrome

Acute coronary syndrome (ACS) is a general term used to describe a range of clinical presentations. For instance, ACS is noted to lead to: 1) cardiac arrest; 2) hemodynamic instability with cardiogenic shock (CS) caused by persistent ischemia or mechanical complications; and finally, pain felt at the time of presentation (Collet, 2021; Roffi et al., 2016). Based on the electrocardiogram (ECG), ACS patients can be stratified into two primary groups: those with ST-segment elevation ACS (ST-ACS) and those with non-ST-segment elevation ACS (NSTE-ACS); both of which are explained below:

I) ST-ACS is an abbreviation used for patients with acute chest pain and persistent ST-segment elevation (for at least 20 minutes); which usually reflects a total or partial occlusion of the coronary arteries. Most patients with ST-ACS eventually develop an ST-segment elevation myocardial infarction (STEMI). The primary treatment of such patients is an immediate re-perfusion through primary percutaneous coronary intervention (PCI). Alternatively, such patients might be treated through fibrinolytic therapy (Collet, 2021; Roffi et al., 2016).

II) NSTE- ACS is an abbreviation used for patients with acute chest discomfort, but with no persistent ST-segment elevation. ECG fluctuations for these patients may include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T waves or pseudo-normalization of T waves, finally, the ECG may be normal. However, the pathological correlation at the myocardial level includes myocardial injury (non-ST-segment elevation myocardial infarction; NSTEMI), and less frequently, it includes myocardial ischemia without cell damage (unstable angina; UA) (Collet, 2021; Roffi et al., 2016).

Universal definition of myocardial infarction

Acute myocardial infarction (AMI) is defined as the presence of acute myocardial injury, which can be detected through abnormal cardiac biomarkers. The criteria for MI include an increase and/or decrease in cardiac troponin (preferably high-sensitivity cardiac troponin (hs-cTn) T or I), with ≥1 value higher than the 99th percentile of the upper reference limit. In addition, the criteria include at least one of the following: symptoms of acute myocardial ischemia, new ischemic ECG changes, development of pathological Q waves, ischemic

etiology determined by imaging evidence , and finally, intracoronary thrombus detected on angiography or autopsy (Collet, 2021; Roffi et al., 2016).

Clinical classification of myocardial infarction:

Patient categories with STEMI, NSTEMI, or UA are usually included within the ACS concept. In addition to these categories, MI is divided into different types based on pathological, clinical and prognostic differences, as well as on different treatment strategies (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019). All of these categories are explained below.

Type1 Myocardial infarction

Type1 MI is characterized by atherosclerotic plaque disruption (rupture or erosion) accompanied by intraluminal thrombus in one or more coronary arteries, both of which lead to decreased myocardial blood flow and/or distal embolization and subsequent myocardial necrosis. Plaque rupture can not only be complicated by intraluminal thrombosis (ILT), but also by hemorrhage in the plaque through the disturbed surface (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019)(Figure 1).

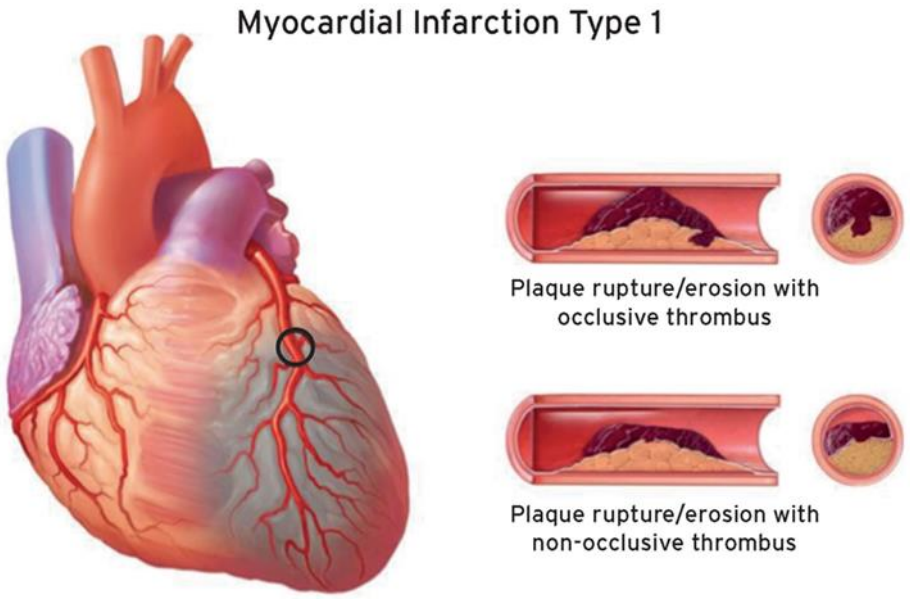


Figure 1: Type 1 myocardial ifarction (Ibanez et al., 2018)

Type 2 Myocardial infarction

Type 2 MI is an imbalance between myocardial oxygen supply and demand, causing ischemic myocardial injury. However, this type is not caused by plaque rupture, and is generally caused by a condition other than coronary artery disease (CAD) (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019) (Figure 2).

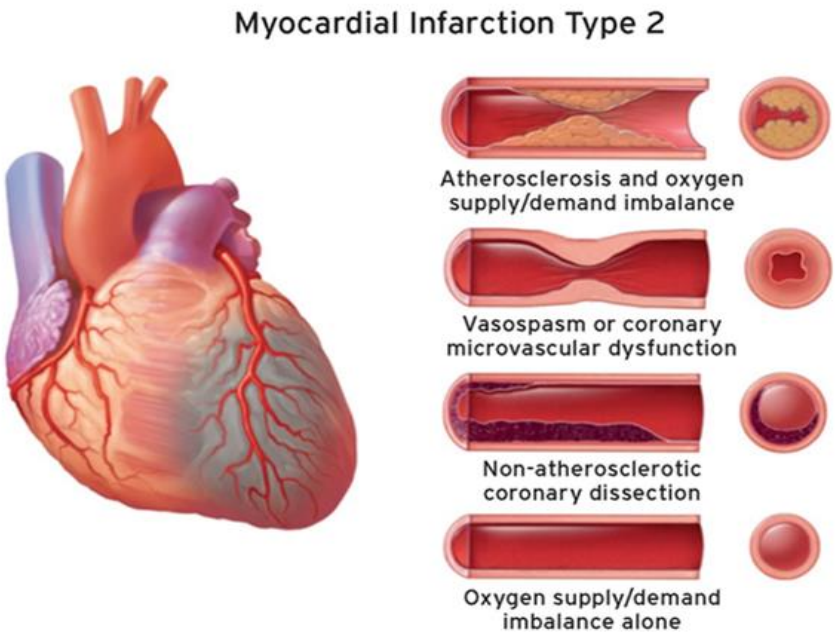


Figure 2: Type 2 myocardial ifarction (Ibanez et al., 2018)

Type 3-5 Myocardial infarction

The universal definition of MI also includes type 3 MI (MI resulting in death when biomarkers are not available), and includes types 4 and 5 which are related to PCI and coronary artery bypass grafting (CABG) respectively (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019).

Epidemiology of acute coronary syndrome

Ischemic heart disease (IHD) is the most increasingly common cause of death around the world. However, during the last three decades in Europe, there has been a general trend aiming to reduce IHD mortality (Ibanez et al., 2018). Today, IHD represents nearly 1.8 million deaths every year; in other words, IHD represents 20% of all deaths in Europe, although there are wide variations between countries (Hartley et al., 2016; Ibanez et al., 2018;

Townsend et al., 2016).

Several studies have demonstrated that STEMI has been relatively decreasing, while NSTEMI has been relatively increasing (Ibanez et al., 2018; McManus et al., 2011). In addition, it has been reported that probably the most comprehensive European registry for STEMI had been found in Sweden with a percentage of 58% (out of a total of 100,000 people) in 2015. In other European countries, the average percentage was less than 50% (ranging from 43 to 144 per 100,000 people in a year) (Ibanez et al., 2018).

Similarly, the reported adjusted incidence rates in the USA decreased from 133 per 100 000 in 1999 to 50 per 100 000 in 2008, whereas the incidence of NSTEMI remained constant or increased slightly (Ibanez et al., 2018). Data from several studies indicated that there had been a consistent pattern for STEMI existence, which is relatively more common among young people than it is among the elderly, and more common among men than it is among women (Ibanez et al., 2018). Thus far, a number of studies have reported that mortality among STEMI patients is influenced by many factors including age, Killip class, duration before treatment, presence of emergency medical system, treatment strategy, history of MI, diabetes mellitus (DM), renal failure, number of diseased coronary arteries, and left ventricular ejection fraction (LVEF) (Ibanez et al., 2018).

Recently, several studies have demonstrated an acute and long-term decrease in STEMI mortality rates due to more use of re-perfusion therapy including PCI, modern antithrombotic therapy, and secondary prevention (Emet et al., 2019; Ibanez et al., 2018). However, mortality rates are still found; particularly, the in-hospital mortality of STEMI patients varied between 4% and 12% (as per the national registries of the European Society of Cardiology (ESC) countries). On the other hand, the mortality rate among STEMI patients was reported to be approximately 10% in one year, as per angiography registries (Emet et al., 2019; Ibanez et al., 2018).

Although IHD develops on average 7 to 10 years later among women compared to men, IHD remains a major cause of mortality among women. Furthermore, ACS occurs 3 to 4 times more often among men than it does among women aging below 60 years; however, after the age of 75, women represent the majority of patients. in the same vein, several studies found that women tend to show more atypical symptoms, up to 30% in some registries, and tend to present later than men (Emet et al., 2019; Ibanez et al., 2018).

Pathophysiology of acute coronary syndrome

The main cause of ACS is atherosclerosis in the coronary arteries. Atherosclerosis is a form of arteriosclerosis where the walls of the arteries are hard, thick and narrow as a result of low-density lipoproteins (LDL) and other lipoproteins accumulation in the sub-endothelial space within the arterial walls (Peate, 2021). LDL are deposited on the tunica intima of the damaged blood vessel where oxidation of LDL takes place. The oxidized LDL then enters the tunica intima of the arterial wall, where they are ingested by macrophages (Moore et al., 2013; Peate, 2021). The lipid-filled macrophages then turn into foam cells. Once the foam cells accumulate in vital numbers, they form a lesion referred to as a fatty streak. Over time, the so-called fatty streak causes a bulge within the lumen of the vessel; which, in turn, restricts blood flow (Moore et al., 2013; Peate, 2021). Affected blood vessels become hard, lose their elasticity, restrict blood flow and eventually occlude the artery. STEMI typically represents a complete occlusion of the coronary artery, whereas in NSTE-ACS (NSTEMI and UA), there is a critical reduction in flow (Bentzon et al., 2014; Hammer & McPhee, 2014). The reason for this is that culprit lesions causing STEMI have greater plaque burden and smaller lumen areas compared to NSTE-ACS (NSTEMI/UA) (Dong et al., 2015; Toutouzas et al., 2011). Morphological characteristics of the atheromatous plaque have been associated with the development of plaque rupture and the pathogenesis of ACS (Toutouzas et al., 2011). Besides the size of plaque rupture, a variable degree of thrombosis associated with the inflammatory process is considered to be the main initiating mechanism of ACS (AL-Ali & Farhan, 2016; Srikanth & Ambrose, 2012).

Oxidative stress

Oxidative stress refers to conditions caused by an imbalance between reactive oxygen species (ROS) and antioxidant systems. This condition of imbalance causes either excessive amounts of free radicals, or a steady decrease in antioxidant capacity. Such conditions can result in the oxidation of proteins, lipids, carbohydrates, and DNA (Lee & Song, 2009; Leiris et al., 2006; Misra et al., 2009; Nita & Grzybowski, 2016; Ozcan & Ogun, 2015; Patlevič et al., 2016).

It is worth mentioning that ROS or free radicals can be any chemical species (atom, ion, or molecule) which contains a single unpaired electron in its outer orbit conferring very high reactivity; examples include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radical ($O^{\bullet -}_2$), and hydroxyl radical ($^{\bullet}OH$) (Lee & Song, 2009; Leiris et al., 2006; Misra et al., 2009; Nita & Grzybowski, 2016; Ozcan & Ogun, 2015; Panth et al., 2016; Patlevič et al., 2016; Vichova & Motovska, 2013).

Since free radicals are highly unstable and highly reactive, they can attack almost all

cellular components, therefore behaving as oxidants or reductants (Lobo et al., 2010; Ray et al., 2012). Thus, free radicals react with the membranes of cells and damage biologically relevant molecules such as lipids, proteins, and DNA. In addition, such free radicals trigger a number of human diseases including cancer, atherosclerosis, Alzheimer's, Parkinson's and many others (Lobo et al., 2010; Ray et al., 2012).

Oxidative stress and atherosclerosis

The pathological processes underlying atherosclerosis disease continue to be incompletely understood. However, there is growing evidence that oxidative stress and inflammation are positively related with the instability of atherosclerotic plaque and the incidence of ACS (Leiris et al., 2006; Victor et al., 2009). Thus far, previous studies have demonstrated that intracellular ROS lead to enhanced oxidative stress in vascular cells and are key mediators of signaling pathways which underlie vascular inflammation in atherogenesis, starting from fatty streak development, through lesion progression, to ultimate plaque rupture (Bradley & Floras, 2016; Vichova & Motovska, 2013).

Furthermore, it was noted that ROS-induced initiation of inflammatory cascades and LDL oxidation lead to: 1) the formation of macrophage-derived foam cells, 2) differentiation and proliferation of vascular smooth muscle cells, 3) the activation of vascular matrix metalloproteinases, and 4) the impairment of the extracellular matrix (ECM) of the affected site. This, in turn, may culminate in atherosclerotic plaque rupture (Vichova & Motovska, 2013) (Figure 3). All in all, previous research has demonstrated increased plasma levels of oxidized LDL in cases of ACS (Ehara Shoichi et al., 2001).

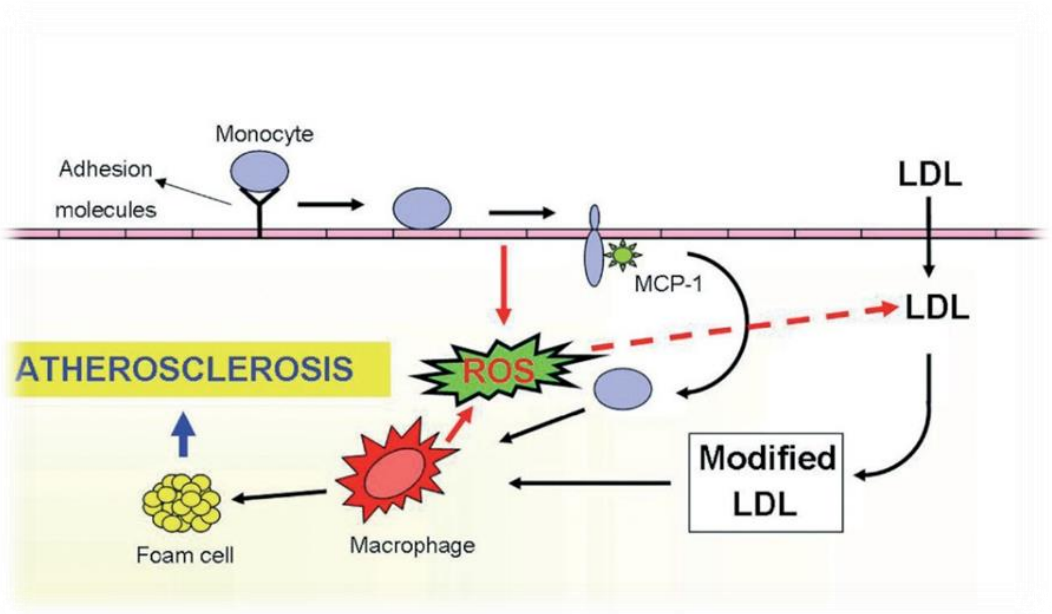


Figure 3: Atherosclerosis and reactive oxygen spesices

Note: This figure was produced by Bonomini et al., (2008), and it is summarized the relationship between atherosclerosis and reactive oxygen spesices in coronary artery diseases.

Oxidative stress and Inflammation

ROS are the contributors of oxidative stress which lead to various diseases and disorders such as cardiovascular disease, cancer, aging, and various neurodegenerative diseases (Twilley & Lall, 2014). There are many sources of ROS including enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH), xanthine oxidase, cyclooxygenases, lipoxygenases, myeloperoxidases, cytochrome P450 monooxygenase, peroxidases, and uncoupled nitric oxide synthase. ROS may be generated intracellularly, extracellularly, or in specific intracellular compartments. They can also be generated by polymorphonuclear lymphocytes via NADPH oxidase apart from vascular cells (Mittal et al., 2014; Molnár et al., 2016; Paravicini & Touyz, 2008).

Many reports have shown that polymorphonuclear leukocytes are major sources of ROS in macro inflammatory processes (Mittal et al., 2014; Molnár et al., 2016; Paravicini & Touyz, 2008). Moreover, one of the major sources of ROS in the inflammatory processes is NADPH which was originally recognized to play an important role in cellular defense in phagocytic cells (Mittal et al., 2014; Molnár et al., 2016; Paravicini & Touyz, 2008). Furthermore, Xantin oxidase may also be a major source of superoxide radical in inflammatory processes (Feoli et al., 2014).

The excessively produced ROS can oxidize biomolecules. Additionally, it can structurally modify proteins and genes so as to trigger signaling cascades, which, in turn, can lead to the onset and progression of inflammatory diseases. ROS-induced activation of transcription factors and pro-inflammatory genes lead to the onset of inflammation (Chatterjee, 2016). Inflammation causes immune cells to secrete various cytokines and chemokines in order to recruit various other immune cells to the site of oxidative stress/infection. Reflexively, an enhanced ROS generation by immune cells causes oxidative stress and tissue injury in the site of inflammation (Chatterjee, 2016).

Oxidative stress and inflammation are closely interrelated, one of which can be easily induced by the other (Biswas, 2016; Chatterjee, 2016). Both oxidative stress and inflammation cause injury to cells including endothelium. Endothelial dysfunction (ED), in turn, promotes a pro-inflammatory environment as evidenced by increased endothelial expression of adhesion molecules, and it is also evidenced by the imbalance of arachidonic acid metabolites and chemoattractant molecules (Biswas, 2016; Chatterjee, 2016; Chrissobolis et al., 2011). Forming a positive feedback loop, vascular inflammation leads to ED. In the same vein, ROS is considered as signaling molecules which contribute to ED in experimental and clinical atherosclerosis (Panth et al., 2016).

Oxidative stress markers

Oxidative stress can be measured by direct and/or indirect methods, using diverse methods (Arauz et al., 2016; Molnár et al., 2016). The most feasible method is the direct detection of ROS and free radical accumulation. Unfortunately, direct detection of ROS and other free radicals is not an easy task. Particularly, these molecules are highly reactive due to their unpaired electron, and therefore have a very short half-life. Thus, persistent oxidative damage is generally analyzed by measuring byproducts including amino acid derivatives, nucleic acids and lipid peroxidation. Rather than measuring radicals directly, research tends to focus on measuring stable oxidation products derived from free radicals such as phenylalanine (Phe) derivatives. (Arauz et al., 2016; Molnár et al., 2016).

Phe is an essential aromatic amino acid in humans. Specifically, it plays a key role in the biosynthesis of other amino acids, and it is important in the structure and function of many proteins and enzymes. Phe develops into tyrosine which is used in the biosynthesis of dopamine and norepinephrine neurotransmitters (Liu et al., 2018; Molnár et al., 2016). It is worth noting that Phe develops into three different structural isomers of tyrosine (Tyr) , namely, para-, meta- and ortho-tyrosine (*p*-Tyr, *m*-Tyr and *o*-Tyr, respectively) (Liu et al.,

2018; Molnár et al., 2016). The formation of these Tyr isomers (by oxidizing •OH of Phe) includes a two-step process in which •OH first attacks the phenyl ring as an additive reaction to produce a highly reactive hydroxyl phenylalanine radical substance. Then, this intermediate product rapidly undergoes a secondary reaction to generate a stable tyrosine isomer through one of three mechanisms: (1) abstraction; (2) oxygenation; or (3) disproportionation (Ipson & Fisher, 2016).

As mentioned in the literature review, *m*- and *o*-Tyr are produced exclusively by •OH in the human body. Detection of •OH is technically difficult because of its low concentration and extremely short duration. However, stable amino acid species derived from •OH can be relatively simply detected. Thus, *m*- and *o*-Tyr are reliable markers of •OH (Ipson & Fisher, 2016). As a result, when measured as a biomarker for •OH, elevations in the concentrations of both *m*- and *o*-Tyr have been observed in many diseases in which oxidative stress is thought to play a pivotal pathological role (Ipson & Fisher, 2016). For example, one study found higher levels of *m*-Tyr and *o*-Tyr in burn patients compared to controls between days two and five (Kovacs et al., 2020). Another study found higher levels of *m*-Tyr and *o*-Tyr in human cataract lenses (Fu et al., 1998). All in all, *m*-Tyr serum levels among the experimental group's sepsis patients were found to be higher than among the control group on the second day (Szélig et al., 2016).

Among amino acid-derived oxidative stress markers, Phe-derivatives offer a specific advantage in regard to detection. Particularly, the aromatic ring bears an autofluorescence; thus, both phenylalanine, as well as the tyrosine isoforms exert a specific fluorescence; i.e., Phe at wavelengths of 258 nm (excitation) and 288 nm (emission), while *p*-, *m*- and *o*-Tyr at 275 nm (excitation) and 305 nm (emission). Therefore, they can be detected using fluorescent methods, such as high-performance liquid chromatography (HPLC) with a fluorescent detector, without derivatization, not only using derivatization or a mass-spectrometric method (Ishimitsu et al., 1989; Molnár, Nemes, et al., 2005; Molnár, Wagner, et al., 2005).

Oxidative stress markers and acute coronary syndrome

Oxidative stress plays a pivotal role in the pathogenesis of several cardiovascular diseases including atherogenesis, ischemic-reperfusion injury, and cardiac remodelling (Vichova & Motovska, 2013). Under conditions of oxidative stress in which the levels of free radicals are elevated, •OH can oxidize the benzyl ring of Phe, and produce various Tyr isomers (i.e., *m*-, *o*-, and *p*-Tyr). These Tyr isomers vary according to the location of the hydroxyl group on the benzyl ring (Figure 4) (Ipson et al., 2019; Ipson & Fisher, 2016).

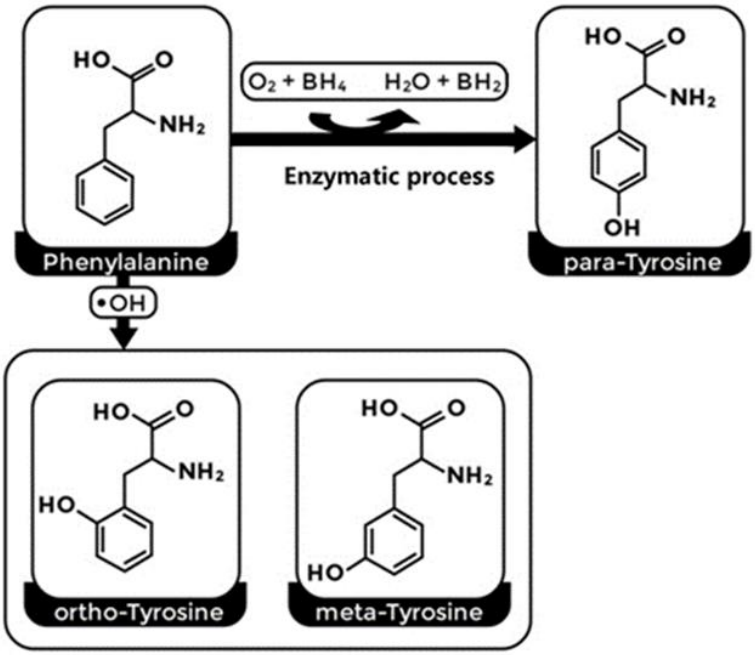


Figure 4: Oxidation of phenylalanine into different tyrosine isoforms due to the activity of the phenylalanine. Hydroxylase enzyme or under conditions of oxidative stress (mainly via hydroxyl free radicals).

Normal physiological enzymatic processes also produce *p*-Tyr from Phe. This happens predominantly in the kidney due to activating the phenylalanine-4-hydroxylase (PAH) enzyme (Ipson & Fisher, 2016). The enzymatically produced *p*-Tyr is more plentiful than the free radically derived *p*-Tyr. Therefore, *p*-Tyr is viewed as the physiological isoform (Ipson et al., 2019; Ipson & Fisher, 2016). Recent evidence suggests that oxidative stress induced by immune activation and inflammation may destroy the cofactor tetrahydrobiopterin (BH4) enzyme, and impair the activity of PAH. As a result, the conversion of Phe to Tyr by PAH may be diminished (Murr et al., 2014).

In the human body, *m*- and *o*-Tyr amino acids cannot be formed enzymatically; instead, they are produced as a result of the reaction between the hydroxyl free radical and the benzyl ring of Phe. Further, the accumulation of *m*-Tyr has been reported to adversely affect cells; this suggests a direct role of *m*-Tyr in oxidative stress damage (Ipson et al., 2019). As a result, increases in *m*-Tyr levels are commonly used as a biomarker of oxidative stress (Ipson et al., 2019). Specifically, *m*-Tyr and *o*-Tyr are viewed as free radical markers (Ipson et al., 2019; Ipson & Fisher, 2016; Molnár et al., 2016), which may play a role in chronic inflammation during the initiation and progression of ACS (Cziráki et al., 2012; Molnár, 2015; Szélig et al., 2016). However, no single study has investigated Tyr isomers in ACS

patients. Therefore, the present study is designed to compare these parameters between ACS patients (experimental group) and healthy people (control group) through blood samples taken from both groups. Furthermore, the present study is designed to make a comparison between patients with STEMI and NSTEMI. Finally, the study is also designed to make a comparison between the serum levels of Phe and Tyr isomers at the aortic root and distal to the culprit lesion in two groups of patient.

Risk factors and comorbidities

Non-modifiable factors: Age and Gender

Advanced age is considered as one of the major risk factors for CAD. It is also dealt with as an independent predictor of poor outcomes following ACS. It is associated with an increase in susceptibility to endothelial dysfunction and reduced endothelial repair, both of which lead to arterial calcification with the progressively increased vessel rigidity and stiffness. Consequently, plaque ruptures take place in the absence of symptoms and usually lead to coronary arterial narrowing. It is worth noting that ACS occurrence is evidently associated with coronary atherosclerotic plaque burden, metabolic activity, and conditions that promote vascular thrombosis (Arbab-Zadeh et al., 2012; Odden et al., 2011).

Advanced age is independently associated with the occurrence of both STEMI and NSTEMI, and also associated with increased possibility of mortality (Libungan et al., 2014). In the same vein, a review paper of the epidemiology, clinical features, and prognosis of AMI showed that over 60% of AMI cases occurred to patients aged > 65 years, and approximately one-third occurred to those aged >75 years (Docherty, 2010). This is consistent with a study done by Dai et al., (2016) which stated that the highest incidence rate of ACS among older adult patients was among the age group of 65 - 75 years old. In addition, Dai et al. found that about 60% of hospital admissions were from this age group which also represented the highest rate of death (approximately 85%) due to ACS.

Regarding the mortality rate when associated with an age group, it is reported that an increase of 10 years of age was associated with a doubled rate of mortality in 24 months (33% vs. 17%, $P = 0.001$). These findings came in line with results obtained from another study which noted that the mortality rate among patients over the age of 85 is at least three times higher than in the younger age group of 65 years (Dai et al., 2016).

In terms of gender, men have the double of cardiovascular diseases (CVD) when compared to women. Whenever ACS is present among men, it has a tendency to become

more severe than it does among women (Roth et al., 2018). This is consistent with a study conducted to compare clinical data and prognostics of patients with STEMI in 2010 and 2011; the database contained 3,038 men out of 4,981 patients (Piros et al., 2017). It was noticed that women are often older when they are afflicted with ACS at an average age of 71.8 years compared with 65 years for men. The onset of CAD among women at an older age compared with men is thought to be due to the protective role of circulating estrogens on the vascular endothelium (Dai et al., 2016). The data revealed that the women were significantly older than men (67.7±13.5 vs. 60.5±12.5 years; $p<0.001$). Further, elderly women hospitalized with NSTEMI have worse clinical outcomes compared with men. These outcomes are consistent with those obtained from Dai et al., (2016), which indicated that within the first year of AMI, 26% of women die while only 19% of men do. In addition, within 5 years, 47% of women die while 36% of men do.; both suffering from heart failures (HF) or strokes (Dai et al., 2016). Another study showed that overall CVD among Europeans caused 51% of deaths among women and 42% among men (Dai et al., 2016).

Non-modifiable factors: Family history

ACS is strongly influenced by the family factor. Specifically, first-degree relatives of patients with CAD have a higher risk of developing ACS when compared to other people (Roth et al., 2017). Additionally, an increased risk of heart disease has been found among individuals with a family history of premature CAD (in male first-degree relatives < 55 years and in female first-degree relatives < 65 years) (Osadnik et al., 2018).

It has also been noted that the genetics and shared environmental exposures play a major role in the development of CAD (McManus et al., 2011). Furthermore, it was noticed that individuals with genetic disorders such as genetic polymorphisms are more susceptible to atherogenic abnormalities when exposed to certain environmental stimuli compared to those who do not have this genetic disorder (Kovacic & Bakran, 2012).

Modifiable factors: Hypertension

Hypertension is one of the primary modifiable risk factors for CVD and its prevalence and severity both increase with age (Egan et al., 2013; Matsuzawa & Lerman, 2014). According to the US National Health and Nutrition Examination Survey (NHANES), 70% of adults ≥65 years have hypertension (Mozaffarian et al., 2015). In 2010, 1.4 billion people globally had hypertension which contributes to 18 million CVD deaths annually (Egan et al., 2019). According to the registries and survey, the hypertensive risk factor for patients with ACS is more likely to be associated with older age females who also have a higher prevalence of

comorbidities (Dorobantu et al., 2014; Picariello et al., 2011).

It is worth noting that the term hypertension has been used to refer to situations in which a systolic BP ≥140 and/or diastolic BP ≥90 mmHg (Egan et al., 2013). According to guidelines on HTN which are set by the Eighth Joint National Committee (JNC-8), the hypertension control for treated and untreated patients was defined as BP <140/<90 (Abel et al., 2015; Armstrong, 2014).

Modifiable factors: Diabetes Mellitus

Diabetes Mellitus (DM) is considered as one of the highest independent risk factors of ACS. Pathogenesis of diabetes has involved several general mechanisms that lead to accelerating the atherosclerotic process. Additionally, the most morbidity and mortality rates among patients with DM are induced as a result of atherosclerosis complications. Furthermore, DM patients afflicted with ACS have worse cardiovascular outcomes compared to patients who do not have diabetes (Balasubramaniam et al., 2012).

For example, an end result has shown that the 7-year incidence of recurrent MI was 45% in diabetic sufferers versus 19% in nondiabetic patients. Further, the mortality rate during that period was 42.0% and 15.4% in DM patients with and without history of acute MI respectively; this demonstrates poorer outcomes amongst patients with diabetes following ACS (Balasubramaniam et al., 2012). Today, about 15% to 35% of people admitted to hospitals with ACS are aware about diabetes; on the other hand, more than 15% have undiagnosed diabetes (Tardif et al., 2013, 2018).

Finally, it should be noted that, according to the American Diabetes Association (ADA), patients are considered to have diabetes only in specific circumstances. These circumstances include fasting plasma glucose values of ≥ 7.0 mmol/L (126 mg/dl), 2-h post-load plasma glucose values of ≥ 11.1 mmol/L (200 mg/dl), HbA1c values of ≥ 6.5% (48 mmol/mol); or finally, a random blood glucose value of ≥ 11.1 mmol/L (200 mg/dl) in the presence of signs and symptoms (Association, 2018).

Modifiable factors: Obesity

Obesity is a significant risk factor contributing to the development of atherosclerosis and consequently ACS. It is also associated with an increased risk of morbidity and mortality as well as reduced life expectancy. Obesity is a complex, multifactorial, and largely preventable chronic disease resulting from both genetic and environmental factors (Hruby & Hu, 2015; Wang & Nakayama, 2010). Research indicates that obesity causes insulin resistance and is

strongly associated with health problems such as type 2 diabetes, high blood pressure, dyslipidemia, and impaired glucose metabolism, all of which exacerbate atherosclerosis. It was recently suggested that some forms of obesity are associated with chronic low-grade inflammation that leads to accelerating atherosclerosis (Shimano, 2009; Slattery et al., 2010; Wang & Nakayama, 2010). According to the global burden of disease (GBD), more than 4 million people dying each year as a result of being overweight or obese in 2017 (H. Dai et al., 2020).

Overweight and obesity are defined as abnormal or excessive fat accumulation that leads to a health risk. According to the World Health Organization (WHO), a body mass index (BMI) over 25 is considered overweight, and a BMI over 30 is obese (Hruby & Hu, 2015; Singh et al., 2011; Slattery et al., 2010).

Modifiable factors: Dyslipidemia

Dyslipidemia is an important risk factor for ACS and is an important feature of metabolic syndrome. It involves high abnormal serum total cholesterol (TC), low-density lipoprotein (LDL-C), triglycerides (TG), or low levels of high-density lipoprotein cholesterol (HDL-C) (Bandeali & Farmer, 2012; Kajikawa et al., 2015). When LDL cholesterol levels rise, they accumulate in the arterial wall and are oxidized and taken up by foam cells, exacerbating the process of atherosclerosis and causing it to develop. On the other hand, elevated HDL plays a vital role in the reduced risk for atherosclerosis. Specifically, elevated HDL removes cholesterol from the foam cells and inhibits the oxidation of LDL. It also limits the inflammatory processes that underlie atherosclerosis (Bandeali & Farmer, 2012; Kajikawa et al., 2015). It should be mentioned that new evidence has recently shown that elevated TG is associated with endothelial dysfunction, leads to the progression of CAD, and leads to the formation of new lesions (Kajikawa et al., 2015).

It should be noted that the American Heart Association (AHA) has various classifications regarding TC, LDL-C, TG, and HDL-C; all of which are detailed below.

I) If TC level is below 200 mg/dl (5.2 mmol/L), it is considered desirable and at a low risk for heart diseases. If the TC is between 200 and 239 mg/dl (5.2-6.2 mmol/L), it is considered as a borderline-high risk for heart diseases. Finally, if the TC is 240 mg/dl (6.2 mmol/L) or above, it is considered as a high risk for heart diseases (Jellinger et al., 2017).

II) Second, the AHA classified the LDL-C as follows: first, LDL-C below 70 mg/ dL (1.8 mmol/L) is best for people who have heart disease or diabetes. Second, LDL-C below

100 mg/dL (2.6 mmol/L) is optimal for people at risk of heart disease. Third, LDL-C between100 and 129 mg/dL (2.6-3.3 mmol/L) is considered near-optimal if there is no heart disease and high if there is a heart disease. Fourth, LDL-C between 130 and 159 mg/dL (3.4-4.1 mmol/L) is considered as a borderline high risk if there is no heart disease. Fifth, LDL-C between160 and 189 mg/dL (4.1-4.9 mmol/L) is considered as a high risk if there is no heart disease and very high if there is heart disease. Finally, when the LDL-C is 190 mg/dL (4.9 mmol/L) or above, it is considered as a very high risk (van Deventer et al., 2011).

III) Third, HDL-C level < 50 mg/dl (1.3 mmol/L) is considered too low or poor for females, and < 40 mg/dl (1 mmol/L) is considered too low or poor for males. Moreover, 40-49 mg/dL (1-1.3 mmol/L) is considered acceptable for males, and 50-59 mg/dL (1.3-1.5 mmol/L) is acceptable for females. Finally, when HDL-C level is > 60 mg/dl (1.6 mmol/L), it is considered high or best for both of gender (HDL Cholesterol - The 'Good' Cholesterol Explained, 2018).

IV) In the fourth group of criteria, when the TG level is below 150 mg/ dl (1.7 mmol/L), it is considered as desirable. When it is from 150to 199 mg/dl (1.7-2.2 mmol/L), it is considered as borderline–high TGs. Additionally, when the TG level is 200–499 mg/dl (2.3-5.6 mmol/L), it is considered as high. Finally, when the TG is 500 mg/ dl (5.6 mmol/L) or more, it is considered a very high TG level (Welty, 2013).

Modifiable factors: Smoking

Smoking is a major preventable risk factor for ACS induced via initiation and progression of atherosclerosis. The pathophysiological mechanisms that involve the association between cigarette smoking and cardiovascular dysfunction are still largely vague (Siasos et al., 2014). However, a variety of studies have proved that cigarette smoking induces various risks. These risks include oxidative stress of LDL, vascular inflammation, platelet coagulation, and vascular dysfunction. Moreover, cigarette smoking was found to impair serum lipid profile in current smokers, chronic smokers, active smokers, and passive smokers. Finally, cigarette smoking was noted to result in detrimental effects on the cardiovascular system (Siasos et al., 2014).

In this study, Current smokers, ex-smokers, and never-smokers are defined according to the WHO criteria as: I) A current smoker is any person who smokes any tobacco product either daily or occasionally at the time of the survey. II) An ex-smoker (previous) is a person who was formerly a daily smoker but has not smoked in the last 6 months. III) A never-smoker is a person who either has never smoked at all or has never been a daily smoker and

has smoked <100 cigarettes (or the equivalent amount of tobacco) in his/her lifetime (*WHO | WHO Report on the Global Tobacco Epidemic 2011*, 2018.).

Diagnosis

The present study deployed clinical history, physical examination, ECG, and cardiac troponins to help identify patients with ACS at increased risk of adverse outcome(s). All of the afore-mentioned criteria are detailed in the sections below.

Clinical presentation

The discomfort associated with unrecognized angina (UA) is deemed more severe than stable angina. Occurs at rest and it is usually described as the typical presentation chest pain of ACS. It is characterized by clear pain and it is often described as a retrosternal sensation of pressure, heaviness or discomfort (angina) aching, tightness or burning radiating to the left arm, neck or jaw. Some patients may experience additional symptoms including an abdominal pain, sweating, nausea, dizziness, lightheadedness, unexplained anxiety, weakness or fatigue, palpitation or paleness (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019).

Atypical presentation includes the epigastric pain, stimulating indigestion and dyspnea, which are more common among women, elderly people and in patients with diabetes, chronic renal disease or dementia. In few cases, syncope may be the clear symptom of ACS. On the other hand a strong pain is not usually ischemic when it is stabbing or pleuritic, reproducible with palpation or with movement, or when it is able to be localized at the tip of one finger (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019).

It is reported that the relief of chest pain by administration of sublingual nitroglycerin in the emergency department (ED) setting does not always indicate the existent of ACS. On the other hand, the occurrence of pain is usually accompanied by the five most important history-related factors that help identify ischemia due to CAD., These factors (mentioned descendingly according to their importance) are the nature of the anginal symptoms, history of CAD, gender, advanced age, and the number of traditional risk factors present (eg, HT, hypercholesterolemia, cigarette smoking, DM, family history of premature CAD, and renal insufficiency). Traditional risk factors present have actually been found to be weak predictors of the likelihood of acute ischemia, although their presence relates to poor outcomes for patients with established ACS (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019).

In addition, the initial clinical presentation is highly predictive of early prognosis. Particularly, chest pain at rest carries a worse prognosis than symptoms stimulated during

physical exertion. In patients with intermittent symptoms, an increasing number of episodes preceding the index event also adversely affect prognosis. In addition, tachycardia, hypotension, HF and new mitral regurgitation at presentation predict poor prognosis and call for rapid diagnosis and management (Collet, 2021; Ibanez et al., 2018; Roffi et al., 2016; Thygesen et al., 2019).

Physical examination

Physical examination is the most frequent conventional diagnosis done for patients with suspected ACS, but it is usually unremarkable in patients with suspected NSTE-ACS. Physical examination incorporates chest examination, auscultation, and measurement of heart rate and blood pressure. It is usually conducted in order to exclude non-cardiac causes of chest pain and non-ischemic cardiac disorders through providing signs that can help in determining the differential diagnosis. For examples, unequal pulses or a murmur of aortic regurgitation indicate possible aortic dissection, whereas a pericardial friction rub suggests acute pericarditis (Amsterdam et al., 2014; Collet, 2021; Ibanez et al., 2018; Roffi et al., 2016; Thygesen et al., 2019).

Moreover, abdominal disorders may reflect upper gastrointestinal diseases such as esophageal spasm, esophagitis, gastric ulcer, cholecystitis, and pancreatitis. In addition, pallor, sweating or tremor may indicate precipitating conditions such as anemia and thyrotoxicosis. On the other hand, there exist other presentations which are suggestive of alternative diagnoses; these presentations include differences in blood pressure between the upper and lower limbs or between the arms, irregular pulse, jugular vein distension, and pain reproduced by chest or abdominal palpation (Amsterdam et al., 2014; Collet, 2021; Ibanez et al., 2018; Roffi et al., 2016; Thygesen et al., 2019).

The physical examination is also carried out to identify any precipitating reasons for myocardial ischemia, and also to assess the hemodynamic outcomes of the acute ischemic event. The physical examination of a patient with chest pain may reveal the signs associated with the pain itself that point towards a large area of ischemia and high risk including diaphoresis, cold and sweaty skin, sinus tachycardia, a third or fourth heart sound, bibasilar crackles, and hypotension (Amsterdam et al., 2014; Collet, 2021; Ibanez et al., 2018; Roffi et al., 2016; Thygesen et al., 2019).

Electrocardiogram (ECG)

ECG is the first-line diagnostic tool conducted for patients with suspected ACS. It provides

important information about the presence, extent, and severity of myocardial ischemia through checking the electrical activity of the heart from different views. European Society of Cardiology (ESC) guidelines state that a qualified physician should conduct and interpret the results of the 12-lead ECG for patients with chest pain or other symptoms suggestive of ACS. Such procedures should be conducted within 10 minutes after the patient's arrival at the emergency room or, ideally, at first medical contact (FMC) with the emergency medical services in the pre-hospital setting (Collet, 2021; Ibanez et al., 2018).

According to ESC guidelines, the ECG in the setting of NSTE-ACS may be normal in more than 30% of patients, while characteristic abnormalities for this condition include ST-segment depression, transient ST-segment elevation, and T-wave changes (Collet, 2021; Roffi et al., 2016; Thygesen et al., 2019). Furthermore, if standard leads are inconclusive and the patient has signs or symptoms indicating persistent myocardial ischemia, it is recommended that additional leads should be recorded. For instance, left circumflex artery occlusion or right ventricular MI may be detected only in V7–V9, V3R and V4R, respectively. Besides, in patients with suggestive signs and symptoms of ACS, detection of persistent ST elevation indicates STEMI, which requires immediate reperfusion (Collet, 2021; Ibanez et al., 2018; Roffi et al., 2016; Thygesen et al., 2019).

Moreover, it is recommended that additional 12-lead ECGs should be re-conducted in the case of uncertain diagnosis especially with persistent or recurrent symptoms. Particularly, the ECGs should be re-conducted because uncertain diagnosis doesn't necessarily mean that there is no possibility of ACS; rather, ACS often occurs to 1%-6% of such patients. However, in patients with bundle branch block (BBB) or pacing, ECG is not helpful in diagnosing NSTE-ACS (Collet, 2021; Ibanez et al., 2018).

Cardiac biomarkers: High-sensitivity cardiac troponin

Cardiac biomarkers are usually measured in order to complete both of clinical assessment and ECG for diagnosis and risk stratification of all patients with chest pain and suspected ACS. Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are the most specific and sensitive biomarkers in comparison with Creatine Kinase–MB (CK-MB iso-enzyme) and myoglobin regarding reflects of cardiomyocyte injury. It is worth noting that the level of troponin begins to rise within a few hours (3-6 h) after the onset of myocardial injury and remains elevated for 10–14 days. On the other hand, the peak up level of troponin usually occurs after 12–48 h (Xu et al., 2013).

Therefore, troponin is helpful in detecting myocardial damage in a patient who wants to check

several days after the onset of symptoms. In the context of acute chest pain with a negative result obtained within a period of 3-6 h, patients should repeat troponin assay 6-12 h after the symptoms onset. Furthermore, because troponin levels remain elevated for a prolonged period after myocardial necrosis, their usefulness is in detecting recurrent myocardial damage and in comparing CK-MB markers. Those which had a shorter half-life and isoenzyme are useful in diagnosing infarct extension (re-infarction) and per procedural MI. However, in the context of myocardial ischemia (chest pain, ECG changes, or new wall motion abnormalities), troponin elevation indicates MI (Xu et al., 2013).

Since 5th-generation high-sensitivity cardiac troponin T (hs-cTn) assays have been developed, ESC guidelines prefer to use hs-cTn T in all patients with suspected ACS. Hs-cTn T can detect troponin at concentrations 10 to 100-fold (i.e. usually within 1 h from symptom onset if using high-sensitivity assays). Additionally, hs-cTn T can detect troponin after symptom onset and remains elevated for a variable period of time (usually several days), which is lower than conventional cTn T assays. Therefore, MI can now be detected more frequently and earlier in patients suffering from chest pain (Collet et al., 2021; Ibanez et al., 2018).

Using the hs-cTn T assay improves the overall diagnostic accuracy in patients with suspected AMI, while a negative result also has a high negative predictive value. The gain in sensitivity may be particularly important in patients with a short duration from symptom onset to admission. Measurement of cardiac troponin T with the hs-cTn T assay may provide strong prognostic information in patients with ACS, stable CAD, and heart failure and even in the general population; however, increased sensitivity comes at the cost of decreased specificity. Serial testing, clinical context and co-existing diseases, are likely to become increasingly important for the interpretation of hs-cTn T assay results (Collet et al., 2021; Ibanez et al., 2018).

Non-invasive imaging: Functional evaluation

A transthoracic echocardiogram (TTE) is the most common type of echocardiogram, which is a still or moving image of the internal parts of the heart using ultrasound. TTE should be routinely available in ED and chest pain units and performed by qualified physicians for all patients during hospitalization for ACS (Collet et al., 2021; Ibanez et al., 2018).

This imaging modality is useful in identifying abnormalities suggestive of myocardial ischemia or MI. It should be noted that the diagnostic and prognostic value of conventional echocardiography might improve due to various factors such as: impaired myocardial

perfusion detected by contrast echocardiography, or reduced regional function using strain and strain rate imaging in the absence of significant wall motion abnormalities (Collet et al., 2021; Ibanez et al., 2018). Furthermore, TTE can help in detecting alternative pathologies associated with chest pain. Such pathologies include acute aortic dissection, pericardial effusion, aortic valve stenosis, hypertrophic cardiomyopathy, mitral valve prolapse, or right ventricular dilatation suggestive of acute pulmonary embolism (Collet et al., 2021; Ibanez et al., 2018).

Similarly, echocardiography is the diagnostic tool of choice for patients with hemodynamic instability of suspected cardiac origin. Evaluation of left ventricular (LV) systolic function, latest by the time of hospital discharge, is important to estimate prognosis. Further, echocardiography (as well as other imaging modalities) can provide this information (Collet et al., 2021; Ibanez et al., 2018). Finally, based on ESC guidelines, stress imaging can be performed (during hospitalization or soon after discharge) for patients who are free from chest pain for several hours,without ischemic changes on ECGs, and with normal hs-cTn (Collet et al., 2021; Ibanez et al., 2018).

Non-invasive imaging: Anatomical evaluation

Coronary computed tomography angiography (CCTA) allows visualization of the coronary arteries, and a normal scan excludes CAD. CCTA has a high net present value (NPV) to exclude ACS (by excluding CAD). It also has an excellent outcome in patients presenting to the ED with low-to-intermediate pre-test probability for ACS and a normal CCTA. Importantly, computed tomography (CT) imaging can effectively exclude other causes of acute chest pain that, if untreated, are associated with high mortality rates. These causes are the pulmonary embolism and aortic dissection (Collet et al., 2021; Ibanez et al., 2018).

Aims

The overall aim was to evaluation of pathogenicity-related oxidative stress biomarkers as well as clinical characteristics, management, and outcomes in acute coronary syndrome.

Specific Aims

1. Serum concentrations of phenylalanine and tyrosine isomers in patients with acute coronary syndrome (I.)

- We aimed to examine the association of Phe and Tyr isomers (*m*-, *o*-, and *p*-Tyr) with oxidative stress following myocardial injury.

2. Assessment of serum phenylalanine and tyrosine isomers in patients with ST-segment elevation versus non-ST-segment elevation myocardial infarction (II.)

- We aimed to compare patients with ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI) and the serum levels of Phe and Tyr isomers at the aortic root and distal to the culprit lesion in both groups.

Sub- Aims

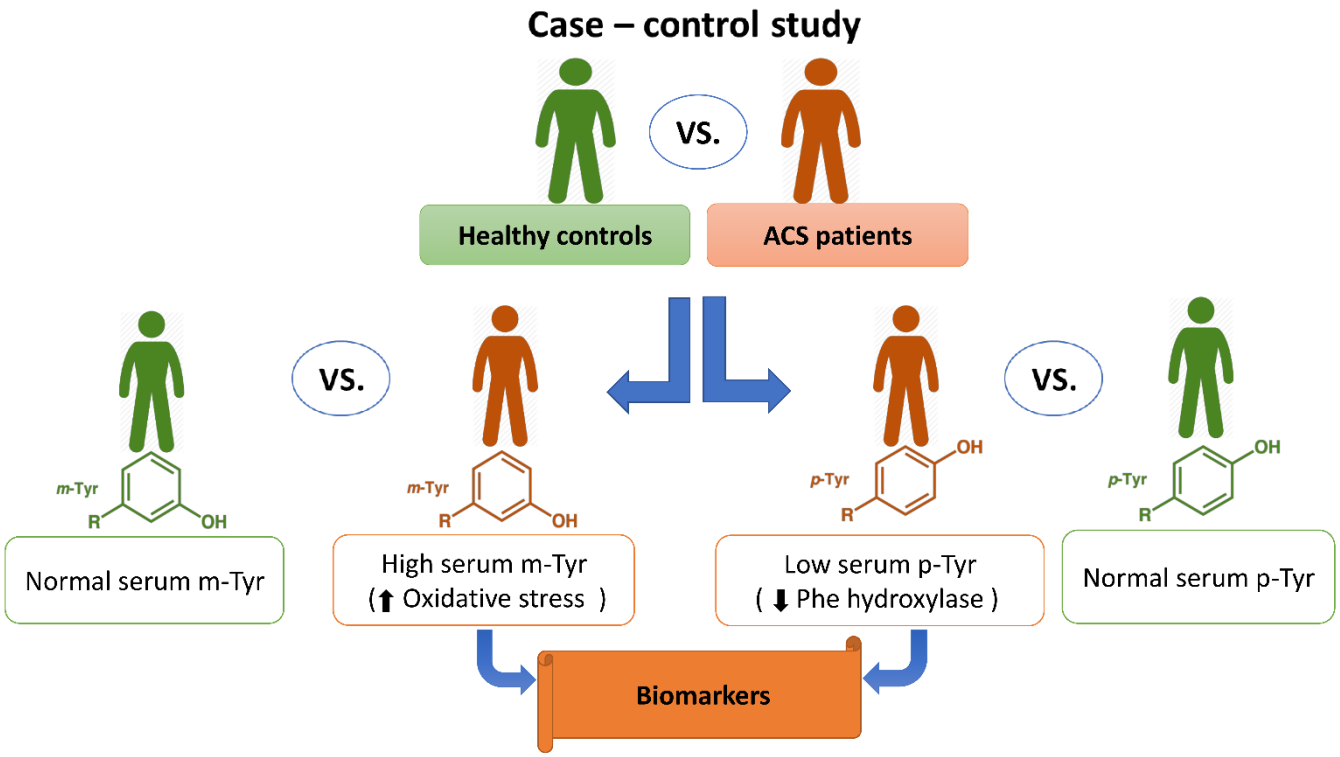
1. Comparison of baseline characteristics, clinical management and outcomes for patients with acute coronary syndrome

- We aimed to describe current characteristics of patients admitted for ACS in Hungary compared to Iraq and to analyses whether in-hospital and 30 days post discharge outcomes variations are explained by differences in patients' baseline characteristics and clinical management.

Chapter 2: Main study

Serum concentrations of phenylalanine and tyrosine isomers in patients with acute coronary syndrome

Graphical abstract



Patients and Methods

Patients

A cohort of 44 patients (11 men, 33 women) admitted to the Department of Interventional Cardiology of the Heart Institute of Pécs University Clinical Center (Pécs, Hungary) were part of this case-control study of ACS patients. The diagnosis of ACS was defined according to the ST segment deviation: STEMI and NSTEMI. Patients aged 30 years and older with confirmed diagnosis of STEMI or NSTEMI were included. The exclusion criteria comprised the lack of serum samples of adequate volume or an uncertain diagnosis of ACS. None of the ACS patients had inflammatory disease or cancer that could impact the tyrosine isomer concentration.

Clinical and angiographic evaluation

General medical history was collected and physical examinations, standard laboratory tests, and 12-lead electrocardiograms were performed in all patients upon admission. The type of ACS was assigned based on American Heart Association and American College of Cardiology guidelines (Amsterdam et al., 2014). The extent of coronary artery disease (CAD) was ascertained by coronary angiography and was categorized according to the number of coronary arteries with obstructive CAD (defined as angiographic stenosis of ≥50%) into 0-, 1-, 2- or 3-vessel disease.

Control group

Control serum samples were obtained by collecting blood from 26 healthy volunteers who were healthcare workers at the same Heart Institute mentioned above, with a gender distribution of 11 men and 15 women. A biobank similar to that of ACS patients was obtained by selecting volunteers aged 25-72 years. None of the volunteers had cardiovascular disease, risk factors of CAD, or used immunosuppressive drugs.

Ethics

This study was approved by the Regional and Institutional Research Ethics Committee (4511/2016) of the University of Pécs and was conducted in accordance with the ethical guidelines of the 2003 Declaration of Helsinki. All participants gave their informed consent.

Blood collection and laboratory analysis

Blood samples were drawn from the aortic root and the radial artery for ACS patients, while for the control group, they were drawn by venipuncture. Serum was obtained through

centrifugation (3000 rpm, 10 min) and was stored at −80°C until further examination. Afterwards, 125 μL of trichloroacetic acid (Reanal Private Ltd., Budapest, Hungary) was added to 500 μL of serum and the samples were incubated on ice for 30 minutes. The precipitate was subsequently separated by centrifugation. The supernatant was filtered by a syringe filter (0.2 μm; Millipore, Billerica, MA, USA) before analysis.

Serum *m*-Tyr, *o*-Tyr, *p*-Tyr, and Phe levels were determined using reversed-phase-high performance liquid chromatography (rp-HPLC), using a C18 silica column (250 × 4 mm) with isocratic sodium acetate/acetic acid as the mobile phase, on a Shimadzu LC-20 system (Shimadzu USA Manufacturing Inc., Canby, OR, USA) with fluorescence detection (Shimadzu, RF-10Axl; λ_{ex} = 275 nm/λ_{em} = 305 nm for Tyr, λ_{ex} = 258 nm/ λ_{em} = 288 nm for Phe), as described in more detail previously (Molnár, Wagner, et al., 2005). Concentrations of the compounds were calculated using an external standard, and in some cases, ratios of the individual amino acids were also used. A representative HPLC chromatogram is depicted in Figure 5.

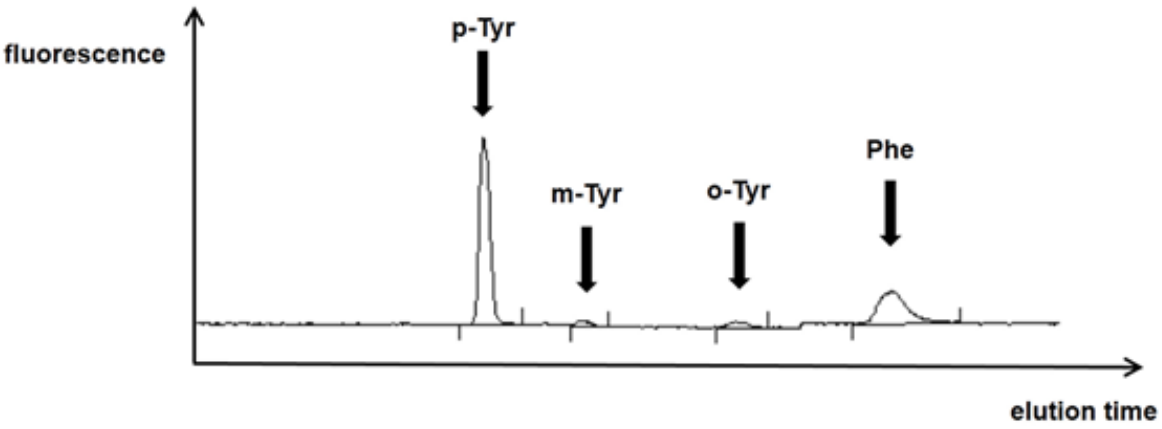


Figure 5: Original registrate showing HPLC separation of *p*-, *m*-, *o*-Tyr, and Phe

Statistical analysis

SPSS software, version 22.0 (IBM Corporation, Armonk, New York, United States) was used for statistical analysis. Continuous variables were expressed as mean (SD) or median and interquartile range. Categorical variables were expressed as percentages or frequencies. Normal distribution was assessed with the Shapiro‐Wilk test. Comparisons between the ACS patient and healthy controls were performed using the $\chi 2$ test for categorical variables, the *t* test for normally distributed continuous variables, and the Mann‐Whitney test for skewed continuous variables. To assess the correlation between the amino acid parameters and baseline characteristics of patients with ACS, we used Spearman rank correlation. *P* values of less than 0.05 were considered statistically significant.

Results

A comparison of baseline characteristics and amino acid parameters for ACS patients showed no significant differences between the STEMI and NSTEMI subgroups; therefore, ACS patients were treated as a single group. Similar results have been published previously (Al-Sadoon et al., 2021). Gender distribution did not differ significantly between ACS patients and healthy controls (men /women, 11/33 vs 11/15, respectively; $P = 0.13$), but the mean age was significantly higher in the ACS group (mean [SD], 68.1 [9.4] years vs 47.5 [12.7] years, respectively; $P = 0.02$). Demographic and clinical data of the study population are summarized in Table 1.

Table 1: Baseline characteristics of study population.

Variables	ACS patients (n=44)	Healthy controls (n=26)	*p*-value
Age, y, mean (SD)	68.1 (9.4)	47.5 (12.7)	0.02
Male, n (%)	11 (25.0%)	11 (42.3%)	0.13
Female, n (%)	33 (75.0%)	15 (58.0%)	
Smoking, n (%)	17 (38.6%)	6 (23.1%)	0.14
Hypertension, n (%)	35 (79.5%)	7 (26.9%)	<0.001
Diabetes mellitus, n (%)	16 (36.4%)	0 (0.0%)	<0.001
Serum creatinine (μmol/L), mean (SD)	75.4 (25.3)	84.00 (18.0)	0.15
eGFR, median (IQR 25–75)	93.0 (75.7- 99.7)	97.0 (48.7- 110.7)	0.58
Diagnosis of ACS			
STEMI, n (%)	23 (52.3%)		NA
NSTEMI, n (%)	21 (47.7%)		NA
Extent of CAD			
Single vessel disease, n (%)	37 (84.1%)		NA
Double vessel disease, n (%)	6 (13.6%)		NA
Triple vessel disease, n (%)	1 (2.3%)		NA

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; eGFR, estimated glomerular filtration.

Our data showed that while serum phenylalanine concentrations did not differ between ACS patients and controls (Figure 6A), serum *p*-Tyr levels were significantly lower in the ACS group than in controls (median, 34.9 vs 54.1 μmol/l; $P < 0.001$) (Figure 6B). Serum *m*-tyrosine concentrations were significantly higher in ACS patients than in controls (median, 14.6 vs 6.1 nmol/l; $P < 0.001$) (Figure 6C), whereas serum *o*-tyrosine concentrations did not differ between the two groups (Figure 6D). Moreover, the serum *p*-tyrosine/ phenylalanine ratio was lower in ACS patients compared with controls (median, 0.9 vs 1.4 μmol/μmol; P <0.001) (Figure 6E). In contrast, the serum *m*-tyrosine/ phenylalanine ratio was higher in ACS patients compared with controls (median, 0.3 vs 0.1 nmol/μmol; P <0.001) (Figure 6F).

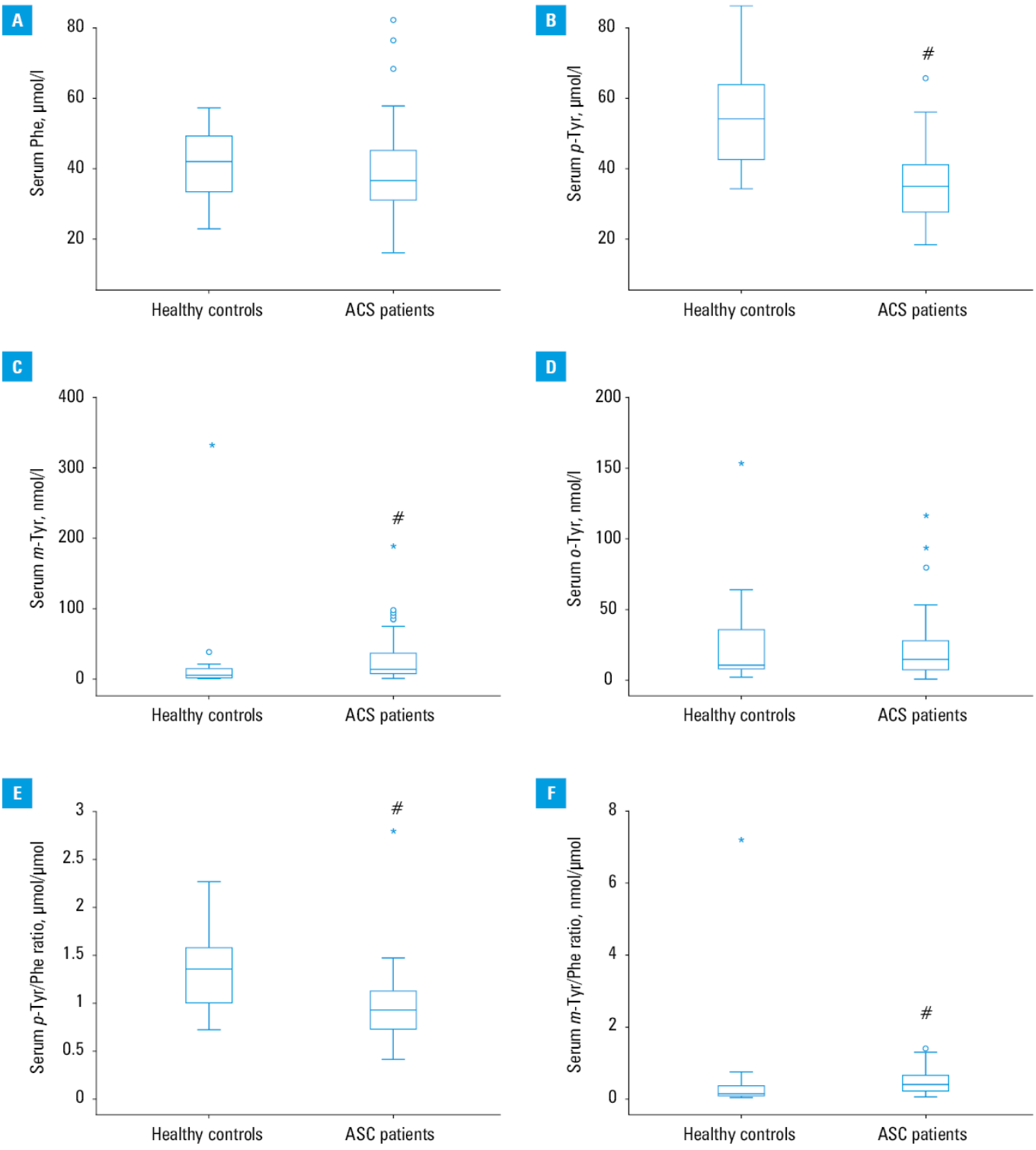


Figure 6 : Box plots of phenylalanine (Phe) (**A**), *para*-tyrosine (*p*-Tyr) (**B**), *meta*-tyrosine (*m*-Tyr) (**C**), *ortho*-tyrosine (*o*-Tyr) (**D**), *para*-tyrosine / phenylalanine ratio (**E**), and *meta*-tyrosine / phenylalanine ratio (**F**) in ACS patients (n = 44) and healthy controls (n = 26). Boxes denote medians and interquartile ranges, whiskers, minimum and maximum values, circles represent outliers, and asterisks, extreme outliers. Hashtags indicate *P* values of less than 0.001.

When comparing serum *p*-tyrosine/ phenylalanine ratios from our recalculated data on different illnesses, we found that this ratio was significantly lower in all patients compared with controls (Figure 7).

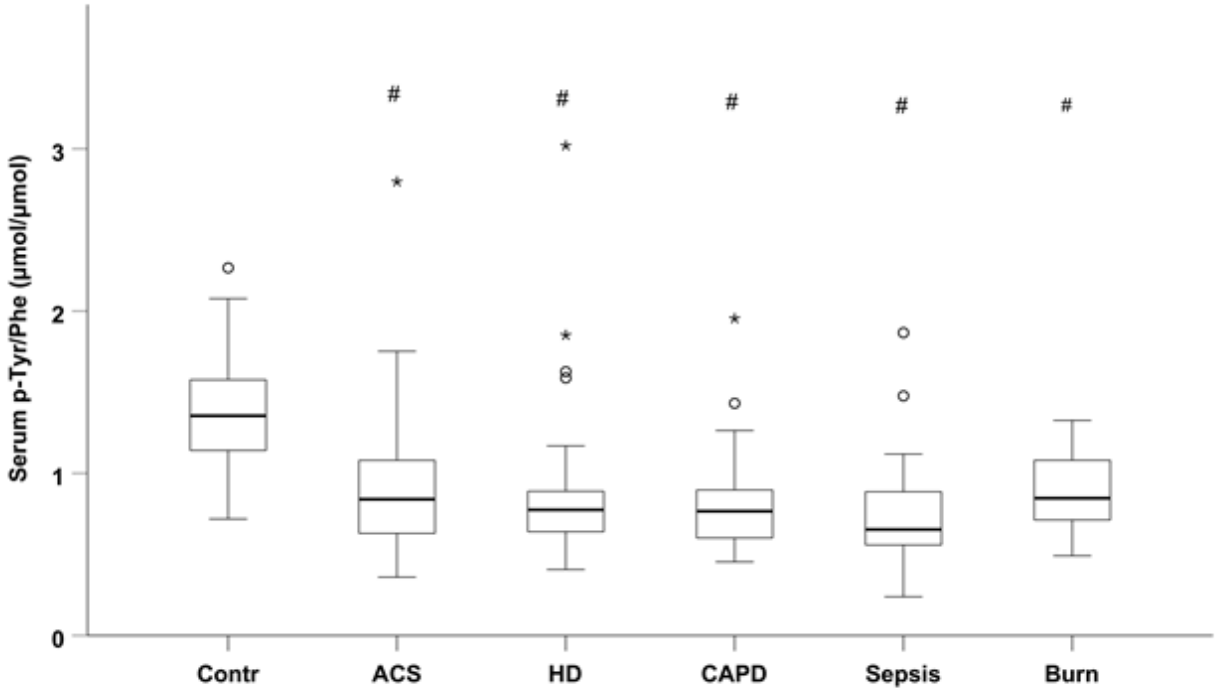


Figure 7: Serum *p*-Tyr/Phe ratios in different illnesses and in healthy controls

Note. The data for HD and CAPD patients are from Kun et al., Redox Rep, pp. 190-198, Sep, 2014 *(Kun et al., 2014)*. The data for septic patients are from L. Szélig et al., Redox Rep, pp. 180–189, Jul. 2016 *(Szélig et al., 2016)*. The data for burned patients are from P. Kovacs et al., Immunobiology, p. 151917, May 2020 *(Kovacs et al., 2020)*. # $P<0.001$ vs. Contr.

Abbreviations: Contr= control; ACS= acute coronary syndrome; HD= hemodialysis; CAPD= continuous ambulatory peritoneal dialysis.

In both ACS patients and controls, no gender differences were found for phenylalanine or *p*-, *m*-, or *o*-tyrosine. None of these amino acid parameters were correlated with age in either group. Moreover, creatinine levels and estimated glomerular filtration rates were not significantly correlated with either phenylalanine or *p*-, *m*-, or *o*-tyrosine in ACS patients. Serum *m*-tyrosine levels did show a positive correlation with *p*-tyrosine/ phenylalanine ratios in different vessel segments (Figure 8).

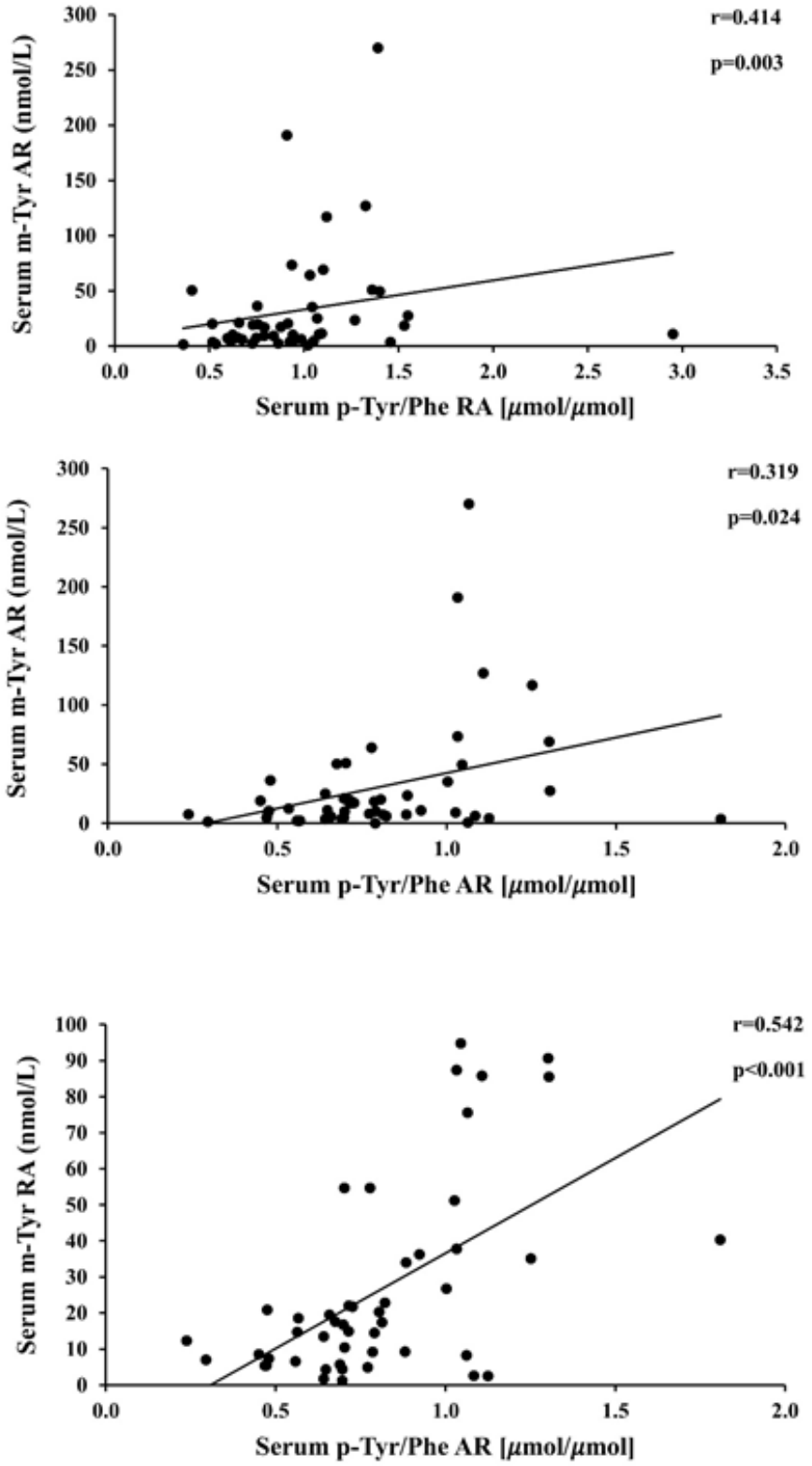


Figure 8: Correlation of *p*-Tyr/Phe ratios with m-Tyr in different vessel segments
Abbreviations: AR= aortic root; RA= radial artery. R= Spearman's rho test, *P*= 0.05.

Discussion

In the present study, we found that the serum *p*-tyrosine concentration and the *p*-tyrosine/ phenylalanine ratio were both lower in ACS patients compared with controls. Similar results were described in patients with diseases associated with inflammation and immune activation, such as sepsis, diabetes, and renal failure as well as in burn patients (Kovacs et al., 2020; Kun et al., 2014; Molnár et al., 2005; Szélig et al., 2016). It is worth noting that there are several possible explanations for the existence of lower serum *p*-Tyr levels in patients. One possible explanation is that the levels of the precursor phenylalanine were low. In the current study, we found that serum phenylalanine levels in our patients were not lower than those of the controls (Figure 6A). This finding helped exclude the possibility that lower levels of phenylalanine precursors were the primary cause for the lower serum levels of *p*-Tyr. Another possible explanation has to do with impaired renal synthesis of serum *p*-Tyr (Szélig et al., 2016). In previous studies, it has been reported that low serum *p*-Tyr levels caused by low PAH activity are mainly recognised in patients with severe impairment of glomerular function (Szélig et al., 2016). In our study, no correlation could be found between serum creatinine levels and serum *p*-Tyr levels (data not shown). This finding suggests that at this phase of renal damage, lower *p*-Tyr levels could occur without severe impairment of glomerular function. A third possible explanation is that lower serum p-Tyr levels-caused by reduced PAH activity-could be a result of a deficiency of the enzyme cofactor BH4 which is used in the enzymatic reaction (Szélig et al., 2016). The current data are in line with the results obtained from our previous studies that we recalculated (the data presented in Figure 7 are based partly on literature data) (Kovacs et al., 2020; Kun et al., 2014; Murr et al., 2014; Szélig et al., 2016). These results further support the idea that diminished conversion of phenylalanine to tyrosine by PAH may be due to increased production of reactive oxygen species, which can cause a decrease in the enzyme cofactor BH4 that takes part in the enzymatic reaction (Kovacs et al., 2020; Molnár, Wagner, et al., 2005; Murr et al., 2014; Szélig et al., 2016). Therefore, the observed decrease in the serum *p*-tyrosine concentration and the *p*-tyrosine/ phenylalanine ratio could be attributed to reduced enzymatic production of cofactor BH4.

Another important finding was that the serum *m*-tyrosine concentration and the *m*-tyrosine/ phenylalanine ratio were both higher in ACS patients compared with controls. Similar results were detected in patients with diseases in which oxidative stress is thought to play a pivotal pathological role, such as sepsis, lens cataracts, as well as in burn patients and those after trauma and acute ischemic stroke (Ipson & Fisher, 2016; Kovacs et al., 2020;

Molnár, 2015; Molnár et al., 2016). These results indicate that increased serum concentration of *m*-tyrosine and the *m*-tyrosine/ phenylalanine ratio in ACS patients may reflect oxidative stress induced by inflammation (Ipson & Fisher, 2016; Molnár, 2015; Molnár et al., 2016; Szélig et al., 2016).

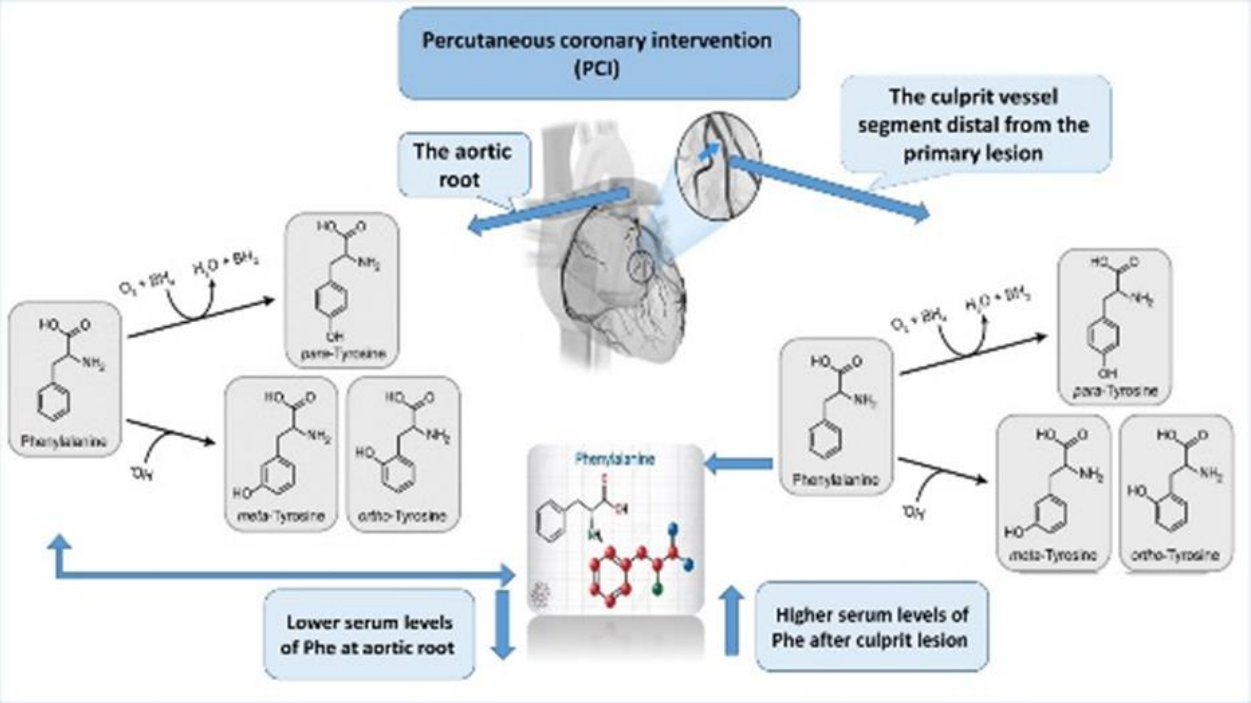
Conclusion

The results of this study showed that increased serum *m*-tyrosine levels can reflect oxidative stress induced by inflammation after myocardial injury, similarly to the observed decrease in *p*-tyrosine levels.

Chapter 3: Main study

Assessment of serum phenylalanine and tyrosine isomers in patients with ST-segment elevation versus non-ST-segment elevation myocardial infarction

Graphical abstract



Materials and Methods

Study population

This prospective study was performed according to regulations issued by the local ethics committee (4511/2016) of the Medical Faculty and Doctoral School of the Health Sciences of the University of Pécs and compiled in accordance with the ethical guidelines of the 2003 Declaration of Helsinki. Written consent was obtained from all patients or their nearest relatives after they were informed clearly about the details of the study.

The study was conducted on 44 patients diagnosed with ACS who were admitted to the cardiac catheterization laboratory [Department of Interventional Cardiology, University of Pécs Clinical Centre (Pécsi Tudományegyetem/Heart Institute)] between January 1, 2017, and March 3, 2017. Patients with ACS were divided into two groups according to ST-segment deviation: STEMI and NSTEMI, based on European Society of Cardiology and American College of Cardiology/American Heart Association (ACC/AHA) guidelines (Amsterdam et al., 2014; European Society of Gynecology (ESG) et al., 2011; Roffi et al., 2016).

The extent of CAD was ascertained by coronary angiography. The extent of CAD was determined according to the number of coronary arteries with obstructive CAD (defined as ≥50% angiographic stenosis): 0-, 1-, 2- or 3-vessel disease. On angiography, of course an occlusive lesion easily recognizable as infarct-related artery (IRA). Occasionally, in cases of NSTEMI, to define the culprit lesion is not so easy in patient with multivessel coronary artery disease. Thus, the identification of the culprit lesion is usually achieved by a combination of factors, including angiographies characteristics and information from non-invasive examinations (ECG, echocardiography). In case of non-occlusive MI the ESC/AHA definition was used the determine existing of CAD.

Clinical and biochemical parameters

Personal and medical histories of all study patients were recorded. Arterial blood samples were taken from the aortic root using a guiding catheter and from the culprit vessel segment distal from the primary lesion using an aspiration catheter, during the percutaneous coronary intervention. The database has been published previously (Al-Sadoon, 2020). Serum were obtained by centrifugation (1008g, 10 minutes) and stored at −80°C until further examination. Serum *m*-Tyr, *o*-Tyr, *p*-Tyr, and Phe levels were determined by reverse-phase high performance liquid chromatography (Shimadzu USA Manufacturing INC, Canby, OR, USA) using a C18 silica column (250 × 4 mm) with fluorescence detection [Tyr (λem = 275 nm/λem

= 305 nm) and Phe (λex = 258 nm/λex = 288 nm)], as described previously (Molnár, Wagner, et al., 2005).

Statistical analyses

SPSS software, version 22.0 (IBM Corporation, USA), was used for statistical analyses. Continuous variables are expressed as the mean ± standard deviation or median and interquartile range (25–75%). Categorical variables are expressed as percentages and frequencies. Differences between the STEMI and NSTEMI groups were assessed using the chi-square test for categorical variables, the Student's t test for normally distributed continuous variables, and the Mann-Whitney U test for skewed continuous variables. For pairwise comparisons of each group, the Wilcoxon test was used, depending on the normal distribution. To assess the correlation between the amino acid parameters and baseline characteristics of patients with ACS, we used the Spearman's rho test. *P* values less than 0.05 were considered statistically significant.

Results

Baseline characteristics of patients with ACS

Demographics and patient data are summarized in Table 2. Forty-four patients were included in the study: 23 with STEMI and 21 with NSTEMI. The mean age of the participants was 68.1 ± 9.4 years, and most were female (75.0%). A previous history of hypertension, smoking, and diabetes mellitus was found for 79.5%, 38.6%, and 36.4% of the patients, respectively. Moreover, most patients (84.1%) had one-vessel disease. There was no significant difference in smoking, diabetes mellitus, and extent of CAD, with the exception of hypertension, between patients with STEMI and NSTEMI.

Table 2: Baseline characteristics of the study patients.

Characteristics	STEMI (n= 23)	NSTEMI (n= 21)	Total (n= 44)	*P* value
Age, y (mean ± SD)	66.87± 8.745	69.57 ± 10.201	68.16 ± 9.455	0.35
Male/ Female, n (%)	6/17 (26.1/ 73.9)	5/16 (23.8/ 76.2)	11/33 (25.0/ 75.0)	0.57
Smoking, n (%)	6 (26.1)	11 (52.4)	17 (38.6)	0.06
Hypertension, n (%)	15 (65.2)	20 (95.2)	35 (79.5)	0.01
Diabetes mellitus, n (%)	7 (30.4)	9 (42.9)	16 (36.4)	0.29
Culprit lesions				
LM, n (%)	1 (4.3)	0 (0.0)	1 (2.3)	0.33
LAD, n (%)	12 (52.1)	1 (4.8)	13 (29.5)	0.29
Cx, n (%)	5 (21.7)	16 (76.1)	21 (47.7)	0.25
RCA, n (%)	9 (39.1)	0 (0.0)	9 (20.5)	0.77
Extent of CAD				
One vessel disease, n (%)	21 (91.3)	16 (76.2)	37 (84.1)	0.32
Two vessel disease, n (%)	2 (8.7)	4 (8.7)	6 (13.6)	
Three vessel disease, n (%)	0 (0.0)	1 (4.8)	1 (2.3)	

Note: Abbreviations: CAD= coronary artery disease; STEMI= ST-segment elevation myocardial infarction; NSTEMI= non ST-segment elevation myocardial infarction; LM: left main; LAD: left anterior descending; Cx: circumflex artery; RCA: right coronary artery. Data are expressed as mean ± SD for the continuous variable and percentages (%) and frequencies (n).

Comparison of the amino acid parameters for patients with ACS

Serum Phe levels were significantly higher distal to the culprit lesion than at the aortic root (44.7 vs. 35.5 µmol/L, $P = 0.002$) in patients with STEMI. Serum *p*-Tyr/Phe and *m*-Tyr/Phe ratios were significantly lower distal to the culprit lesion than at the aortic root (0.7 vs. 0.9 µmol/µmol, $P = 0.024$; 0.1 vs. 0.4 nmol/µmol, $P = 0.018$, respectively) in patients with STEMI (Table 3).

Table 3: Serum levels of phenylalanine and tyrosine isomers in patients with STEMI.

Parameters	Aortic root	The culprit lesion	*P* value
Serum Phe [μmol/L]	35.5 (26.7- 44.9)	44.7 (39.0- 58.6)	<0.00
Serum *p*-Tyr [μmol/L]	31.2 (26.7- 41.6)	32.0 (30.2- 37.0)	0.31
Serum *m*-Tyr [nmol/L]	17.6 (10.1- 36.2)	10.39 (7.1- 37.1)	0.24
Serum *o*-Tyr [nmol/L]	16.6 (6.6- 32.6)	11.9 (6.9- 36.7)	0.92
Serum *p*-Tyr/Phe [μmol/μmol]	0.9 (0.7- 1.1)	0.7 (0.6- 0.8)	0.02
Serum *m*-Tyr/Phe [nmol/μmol]	0.4 (0.2- 0.6)	0.1 (0.1- 0.4)	0.01

Note: Abbreviations: STEMI= ST-segment elevation myocardial infarction; Phe = phenylalanine; *p*-Tyr = para-tyrosine; *m*-Tyr = meta-tyrosine; *o*-Tyr = ortho-tyrosine. All data are expressed as median (IQR: 25–75%).

There were no statistically significant differences with respect to changes in serum levels of Phe and Tyr isomers distal to the culprit lesion compared to the aortic root in patients with NSTEMI (Table 4).

Table 4: Serum levels of phenylalanine and tyrosine isomers in patients with NSTEMI.

Parameters	Aortic root	The culprit lesion	*P* value
Serum Phe [μmol/L]	37.4 (34.0 - 46.9)	40.2 (33.6 - 47.8)	0.76
Serum *p*-Tyr [μmol/L]	35.6 (30.5 - 40.6)	32.8 (26.5 - 40.2)	0.20
Serum *m*-Tyr [nmol/L]	11.6 (6.7 - 57.2)	18.4 (6.1 - 38.8)	0.49
Serum *o*-Tyr [nmol/L]	13.1 (7.9 - 23.2)	11.9 (5.2 - 21.6)	0.54
Serum *p*-Tyr/Phe [μmol/μmol]	0.8 (0.7 - 1.1)	0.8 (0.6 - 0.9)	0.13
Serum *m*-Tyr/Phe [nmol/μmol]	0.2 (0.1 - 0.6)	0.3 (0.1 - 0.6)	0.43

Note: Abbreviations: NSTEMI= non-ST-segment elevation myocardial infarction; Phe= phenylalanine; *p*-Tyr= para-tyrosine; *m*-Tyr= meta-tyrosine; *o*-Tyr= ortho-tyrosine. All data are expressed as median (IQR: 25–75%).

As shown in Table 5, there were no significant differences between patients with STEMI and NSTEMI with regard to serum levels of Phe and Tyr isomers, whether distal to the culprit lesion or at the aortic root.

Table 5 : Comparison of the amino acid parameters for patients with ACS.

Parameters	Location	STEMI	NSTEMI	*P* value
Serum Phe [μmol/L]	Aortic root	35.5 (26.7- 44.9)	37.4 (34.0 - 46.9)	0.14
	The culprit lesion	44.7 (39.0 - 58.6)	40.2 (33.6 - 47.8)	0.28
Serum *p*-Tyr [μmol/L]	Aortic root	31.2 (26.7 - 41.6)	35.6 (30.5 - 40.6)	0.35
	The culprit lesion	32.0 (30.2 - 37.0)	32.8 (26.5 - 40.2)	0.63
Serum *m*-Tyr [nmol/L]	Aortic root	17.6 (10.1 - 36.2)	11.6 (6.7 - 57.2)	0.22
	The culprit lesion	10.39 (7.1 - 37.1)	18.4 (6.1 - 38.8)	0.37
Serum *o*-Tyr [nmol/L]	Aortic root	16.6 (6.6 - 32.6)	13.1 (7.9 - 23.2)	0.59
	The culprit lesion	11.9 (6.9 - 36.7)	11.9 (5.2 - 21.6)	0.48
Serum *p*-Tyr/Phe [μmol/μmol]	Aortic root	0.9 (0.7 - 1.1)	0.8 (0.7 - 1.1)	0.63
	The culprit lesion	0.7 (0.6 - 0.8)	0.8 (0.6 - 0.9)	0.54
Serum *m*-Tyr/Phe [nmol/μmol]	Aortic root	0.4 (0.2 -0.6)	0.2 (0.1 - 0.6)	0.20
	The culprit lesion	0.1 (0.1 -0.4)	0.3 (0.1 - 0.6)	0.36

Note: Abbreviations: ACS=acute coronary syndrome; STEMI= ST-segment elevation myocardial infarction; STEMI= ST-segment elevation myocardial infarction; Phe= phenylalanine; *p*-Tyr= para-tyrosine; *m*-Tyr= meta-tyrosine; *o*-Tyr= ortho-tyrosine. All data are expressed as median (IQR: 25–75%).

Correlation between serum amino acid parameters and baseline patient characteristics

We examined the associations of the amino acid parameters with demographics and clinical data for patients, according to their diagnoses. Subject age, gender, smoking status, hypertension, and diabetes mellitus all failed to show any significant correlation with amino acid parameters at the aortic root or distal to the culprit lesion in patients with STEMI and NSTEMI (data not shown). Serum *m*-Tyr levels at the aortic root showed a negative correlation with the extent of CAD in patients with NSTEMI (rho = –0.446, r2 = 0.096; *P* = 0.043), whereas there was no significant correlation in patients with STEMI (rho = –0.236-, r2 = 0.050; *P* = 0.129) (Figure 9).

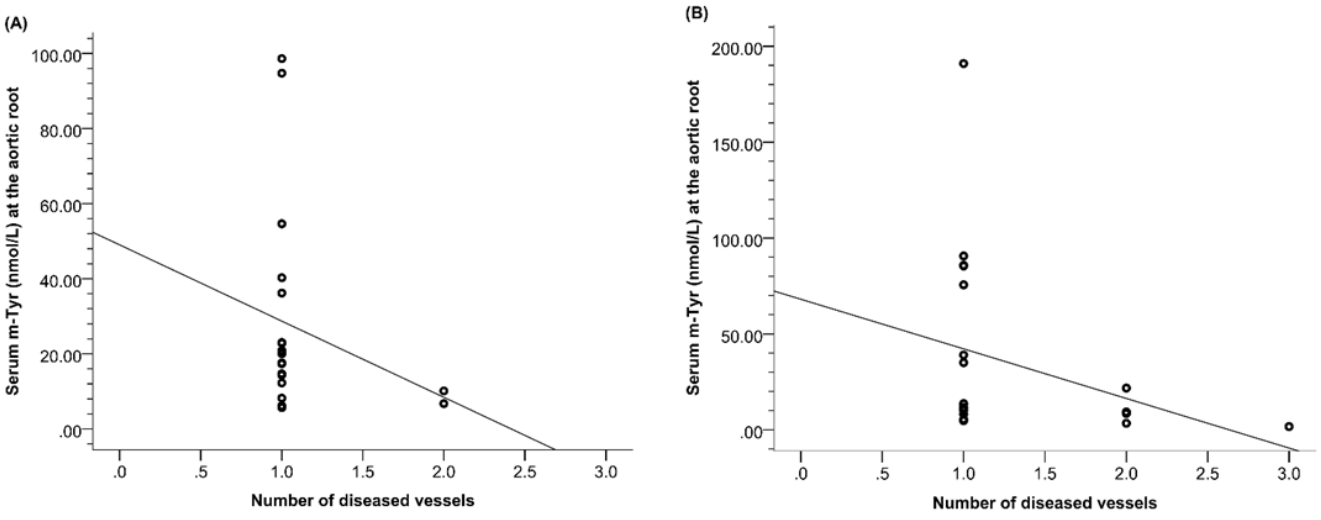


Figure 9 : Scatter plot of serum *m*-Tyr levels at the aortic root versus the extent of CAD (represented as number of diseased vessels) in patients with (A) STEMI and (B) NSTEMI.

Discussion

As mentioned in the literature, the pathological processes underlying vascular diseases are not fully understood; however, there is increasing evidence that oxidative stress and inflammation are positively associated with the rupture of atherosclerotic plaques and the incidence of ACS (Vichova & Motovska, 2013). The findings from this study suggest that certain oxidative stress markers may be associated with the extent of myocardial damage located proximally to the aortic root or distally from the culprit lesion in patients with STEMI and in those with

NSTEMI.

In the current study, there were significantly higher levels of serum Phe distal from the culprit lesion compared to the aortic root in patients with STEMI; while there were slightly higher levels in patients with NSTEMI, this difference was not significant. Similar results have been described in patients suffering from diseases associated with inflammation and immune activation, such as ovarian carcinoma, HIV-1 infection, and sepsis, as well as in patients after trauma and acute ischemic stroke (Ormstad et al., 2016; Ploder et al., 2008; Ribas et al., 2011; Zangerle et al., 2010). These findings may be explained by the fact that increased serum Phe levels can be caused by the diminished conversion of Phe into Tyr by phenylalanine hydroxylase (Murr et al., 2014; Ormstad et al., 2016; Ploder et al., 2008; Ribas et al., 2011; Zangerle et al., 2010). The observed increase in serum Phe levels in STEMI patients could be attributed to the number of damaged cells and disruptions in tissue function.

In the present study, there was no evidence of a statistically significant difference between serum levels of *p*-Tyr, *m*-Tyr, and *o*-Tyr distal to the culprit lesion compared to the aortic root in patients with STEMI and NSTEMI. Furthermore, serum concentrations of Phe and Tyr isomers did not show any significant differences between patients with STEMI and NSTEMI, whether distal to the culprit lesion or at the aortic root. A possible explanation for this finding may be the brief period of ischemia, as there is a short time period between the clinical data obtained and the duration of ischemia. Several reports have shown that elevations in some tyrosine isomers correspond to the duration of ischemia, indicating that hydroxyl radical production is associated with prolonged periods of ischemia (Ipson & Fisher, 2016; O'Neill et al., 1996; Szélig et al., 2016). Another possible explanation is that only a small number of patients were studied, which may bias the results obtained during the study.

However, the results of the current study revealed that serum *p*-Tyr levels were slightly higher, while serum *m*-Tyr and *o*-Tyr levels were slightly lower, in the distal region of the culprit vessel compared to the aortic root in patients with STEMI. In contrast, serum *p*-Tyr and *o*-Tyr levels were slightly lower, while serum *m*-Tyr levels were slightly higher, in the distal region of the culprit vessel compared to the aortic root in patients with NSTEMI. These findings were unexpected and suggest that serum *p*-Tyr levels clearly differ from those of *m*-Tyr and *o*-Tyr in patients with STEMI and NSTEMI. A possible cause of this difference may be the two pathways of tyrosine isomer synthesis: *p*-Tyr is primarily produced enzymatically under physiological conditions, mainly in the kidneys, and is synthesized to a much lower extent under conditions of oxidative stress, whereas *m*-Tyr and *o*-Tyr are only formed non-

enzymatically under conditions of oxidative stress (Szélig et al., 2016).

The results of this study showed no significant association between serum amino acid parameters and baseline patient characteristics except for serum *m*-Tyr levels; they are negatively correlated with the extent of CAD at the aortic root in patients with NSTEMI. These results suggest that serum amino acid changes may be caused by the effects of oxidative stress and inflammation during myocardial infarction.

The clinical significance of this study is to discover that changes in the Phe and Tyr isomers (*m*-, *o*-, and *p*-Tyr) are associated with oxidative stress after myocardial injury, which may play a role in chronic inflammation during initiation and progression of ACS. The limitation of this study is that only a small number of patients have been studied and a time period of ischemia is not specified. This research has thrown up many questions in need of further investigation.

Conclusions

Our data suggest that changes in serum levels of different Tyr isomers can mediate the effects of oxidative stress during myocardial infarction. The contribution of this study is to confirm the association of changes in the Phe and Tyr isomers with oxidative stress following myocardial injury.

Chapter 4: Sub-study

Comparison of baseline characteristics, clinical management and outcomes for patients with acute coronary syndrome

Introduction

ACS has become the most important cause of premature death and disability, spreading to cover a vast population amongst men and women globally. This is certainly related to the high prevalence of common risk factors of CAD, such as older age, male gender, family history of CAD, DM, hyperlipidemia, HT and smoking (Arnett et al., 2019; Hajar, 2017; Radovanovic et al., 2010; Rashed et al., 2018). Which are likely resulted from the vast significant changes in the lifestyle of people.

However, clinical outcomes of ACS depend on these risk factors as well as other relative factors such as type of ACS, previous medical history and time from onset symptoms to presentation. Whereas, a study showed that there was a correlation between ACS type in presentation and incidence of cardiovascular death in long-term follow-up (Fanaroff et al., 2016). Another study showed that ACS outcomes are time-dependent and inversely related to delays in appropriate treatment (Frisch et al., 2017). Furthermore, many large national registries have demonstrated variations in ACS outcomes due to differences in disease severity, therapeutic management, and socioeconomic characteristics in these countries (André et al., 2014).

To lessen these outcomes variations, AHA/ACC and ESC have published guidelines provide recommendations on ACS management strategies by revascularization (such as; PCI, thrombolysis, and CABG),combine with medical therapy that includes anti-ischemic, antiplatelet, anticoagulant, and lipid-lowering drugs (André et al., 2014; Kassaian et al., 2015; McNamara et al., 2014).

Recently, many of the national studies have started to register the prevalence of ACS and it has shown the "gaps" between evidence and practice as well as the implementation of the ACS guidelines between countries (André et al., 2014; Andrikopoulos et al., 2016). Due to the reasons illustrated above, international experiences emphasized the necessity of ACS registries, and this demand has been affirmed by publications of recent years (Pedoe, 1978).

However, the first Hungarian Myocardial Infarction Registry was launched on 1 January 2010 with the voluntary participation of 12 centres and the number of centres contributing data was growing steadily; on 1 September 2013, there were 59 healthcare centres providing data (92% of the hospitals caring ≥50 myocardial infarction cases per year). This situation is different in Iraq, where there is no clear national registered survey demonstrating ACS; although, this country shares the burden of ACS with the other countries worldwide.

We began a restrained study to investigate the prevalence, management and prognosis of ACS. In addition, to assess the application of guideline recommendations in Hungary compared to Iraq. The aim of this study was to describe current characteristics of patients admitted for ACS in Hungary compared to Iraq and to analyse whether in-hospital and 30 days post discharge outcomes variations are explained by differences in patients' baseline characteristics and clinical management.

Methods

Study design and population

A prospective cohort study was conducted at two cardiac centers between May 2018 to May 2019. The study included 164 ACS patients; 64 patients from the Pécs Heart Institute in Hungary and 100 patients from Al-Nasiriyah Heart Center in Iraq. The study complies in accordance with the ethical guidelines of the Declaration of Helsinki 2003, and was approved by the local ethical committee of the Doctoral School of Health Sciences of the University of Pécs and Dhi-Qar health director/Al-Nasiriyah Heart Center. Written consent was obtained from each patient after they were informed clearly about the details of the study. The diagnosis of ACS was defined as follows: STEMI and NSTEMI/UA. ACS diagnosis was based on ESC and AHA/ACC guidelines (Amsterdam et al., 2014; Arnett et al., 2019; Collet, Thiele, Barbato, Barthélémy, Siontis, et al., 2021; Roffi et al., 2016). Specifically, present chest pain, changes in the ECG and levels of cardiac biomarkers.

Patient characteristics, management and outcomes

Baseline variables of study interest include: demographic data (age and gender), risk factors (smoking, DM, HT, dyslipidemia and family history of CAD, previous medical history (prior UA, prior MI, prior renal failure, prior PCI and prior CABG. Key hospital presentation variables included symptom onset, heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) on arrival, creatinine levels, troponin levels, and ejection fraction (EF); in-hospital clinical management variables included angiography, PCI and CABG; in-hospital and 30 days post discharge outcomes.

Control subjects Statistical analysis

SPSS version 22.0 (IBM Corporation, USA) was used for the statistical analysis. Continuous variables are expressed as the mean ± standard or median and interquartile range (IQR). Categorical variables are expressed as percentages and frequencies. Differences between the two countries were evaluated using the chi-square for the categorical variables and the

Student T-test or the Mann–Whitney U test of continuous variables, based on normal distribution. Univariate multivariate logistic regression was performed to assess for predictors of in-hospital and 30 days post discharge major adverse cardiovascular events (MACE, defined as death, re-infarction and stroke). We included covariates from the Global Registry of Acute Coronary Events (GRACE) Risk Model to serve as the basis of our investigation into potential in- and post-hospital targets for intervention. Values of $P < 0.05$ were considered as significant.

Results

Patient demographic and clinical characteristics

The overall demographic and clinical characteristics of patients in the two countries showed some similarities, but there were many notable differences (Table 6). The patients were younger in Iraq (61 vs. 68 years, *P*=0.001) and often had a family history of CAD (9.4% vs 24.0%, *P*=0.018) than those in Hungary. Conversely, Hungarian patients more often had hypertension (89.1% vs. 68.0%, *P*=0.002), dyslipidemia (64.1% vs. 42.0%, *P*=0.006), prior MI (98.4% vs. 24.0%, *P*=0.000), prior PCI (92.2% vs. 23.0%, *P*=0.000) and prior CABG (21.9% vs.1.0%, *P*=0.000) than Iraqi patients. As regards clinical characteristics at admission, Iraqi patients were more often presented with typical chest pain (96.0% vs. 82.8%, P=0. 004), higher creatinine levels (85.0 vs. 75.0 μmol/L, *P*=0.005) than Hungarian patients. By contrast, Hungarian patients were more often presented with higher diastolic blood pressure (90 vs. 80 mm Hg, *P*=0.020) than Iraq patients.

Table 6 : Baseline characteristics of the study patients

Variables	Hungary n=64	Iraq n= 100	*P* value
Demographics			
Age, years	68 (62 -74)	61(50 - 69)	*< 0.001
Male gender	39 (60.9%)	72 (72.0%)	0.140
Risk factors			
Current smoking	15 (23.4%)	33 (33.0%)	0.189
Hypertension	57 (89.1%)	68 (68.0%)	*0.002
Diabetes mellitus	25 (39.1%)	46 (46.0%)	0.382
Dyslipidemia	41 (64.1%)	42 (42.0%)	*0.006
Family history of CAD	6 (9.4%)	24 (24.0%)	*0.018
Prior MI	63 (98.4%)	24 (24.0 %)	*< 0.001
Prior PCI	59 (92.2%)	23 (23.0 %)	*< 0.001
Prior CABG	14 (21.9%)	1 (1.0%)	*< 0.001
Clinical features on presentation			
Symptom onset to presentation >6 hours	29 (45.3%)	57 (57.0%)	0.144
Typical chest pain	53 (82.8 %)	96 (96.0%)	*0.004
Heart rate (beats/min)	85.6 ±17.6	82.3 ±17.6	0.242
Systolic blood pressure (mm Hg)	135 (130-150)	135 (116-148)	0.116
Diastolic blood pressure (mm Hg)	90 (80-90)	80 (70-90)	*0.020
Creatinine (μmol/L)	75.0 (60.0-89.0)	85.0 (66.5-105.0)	*0.005
Ejection Fraction $\leq 30\%$	3 (4.7%)	4 (4.0%)	0.832

Note: :Data are expressed as mean ± SD or median (IQR) for the continuous variables. Data are expressed as n (%) percentages and frequencies for the categorical variables. Values of * =$P < 0.05$ were considered as significant. Abbreviations: CAD=coronary artery disease; UA =unstable angina; MI= myocardial infarction; PCI=percutaneous coronary intervention; CABG=coronary artery bypass graft.

In-hospital procedure and managments

Overall, Iraqi patients were more often diagnosed with STEMI (64.0% vs. 26.6%). In contrast, Hungarian patients were more often diagnosed with NSTEMI (73.4% vs. 36.0%). Concerning management strategies overall, PCI was performed more often in Hungary (92.2% vs.70.0%, *P*=0.001) than in Iraq. Likewise, CABG was performed in Hungary (10.9% vs. 0.0 %, *P*=0.001) while not performed in Iraq (Table 7). With regard reperfusion therapy for patients diagnosed with STEMI, primary PCI has also been performed more frequently in Hungary (94.1% vs. 71.9%, *P* = 0.048) than in Iraq (Table 7).

Table 7 : In-hospital management strategies of the study patients

Variables	Hungary n=64	Iraq n= 100	*P* value
Key investigations			
Positive cardiac enzyme (TnI; CK; CK-MB)	57 (89.1%)	84 (84.0%)	0.362
Diagnosis at discharge			
STEMI	17/64 (26.6%)	64/100 (64.0%)	*0.000
NSTEMI/UA	47/64(73.4%)	36 /100(36.0%)	
In-hospital therapy			
PCI	59/64 (92.2%)	70/100 (70.0%)	*0.001
CABG	7/64(10.9%)	0/100 (0.0%)	*0.001
Reperfusion therapy for patients diagnosed with STEMI			
Primary PCI	16/17 (94.1%)	46 /64(71.9%)	*0.046

Note: Data are expressed as n (%) percentages and frequencies. Values of * =$P < 0.05$ were considered as significant Abbreviations: TnI= troponin; CK = Creatine kinase; CK-MB= Creatine kinase-MB; STEMI=ST-elevation myocardial infarction; LBBB: left bundle branch block; NSTEACS =non-ST elevation myocardial infarction; UA =unstable angina; PCI=percutaneous coronary intervention; CABG=coronary artery bypass graft.

In-hospital and 30 days post-discharge outcomes and predictors

During hospital admission, there were no statistically significant differences between Hungary and Iraq in relation to mortality rate (6.3% vs. 3.0%; respectively), cardiogenic shock (7.8% vs. 5.0%, respectively), stroke (0.0% vs. 2.0%, respectively) and MACE (14.1% vs. 11.0%, respectively). However, Hungarian patients had a higher rate of in-hospital re-infraction (14.1% vs. 4.0%, *P*=0. 020) than Iraqi patients (Table 8). There were no statistically significant differences when comparing out-hospital events between Hungary and Iraq with regard mortality rate (1.7% vs. 3.1%, respectively), re-infraction (8.3% vs. 13.4%, respectively), cardiogenic shock (0.0% vs. 3.1%, respectively), stroke (6.3 % vs. 2.0 %, respectively), and MACE (15.0% vs. 13.5%, respectively) (Table 8).

Table 8: In-hospital and 30 days post-discharge outcomes of the study patients

In Hospital outcomes	Hungary n=64	Iraq n= 100	*P* value
Death	4 (6.3%)	3 (3.0%)	0.315
Re-infraction	9 (14.1%)	4 (4.0%)	*0.020
Cardiogenic shock	5 (7.8%)	5 (5.0%)	0.463
Stroke	0 (0.0%)	2 (2.0%)	0.255
MACE (death, re-infraction and stroke)	9 (14.1%)	9 (9.0%)	0.312
30 Days post-discharge outcomes	Hungary n=64	Iraq n= 100	*P* value
Death	5 (7.8%)	6 (6.0%)	0.651
Re-infraction	10 (15.6%)	17 (17.0%)	0.817
Cardiogenic shock	5 (7.8%)	7 (7.0%)	0.845
Stroke	4 (6.3%)	2 (2.0%)	0.161
MACE (death, re-infraction and stroke)	15 (23.4%)	24 (24.0%)	0.934

Note: Data are expressed as n (%) percentages and frequencies. Values of * =$P < 0.05$ were considered as significant. Abbreviations: MACE= major adverse cardiovascular events

Table 9 and 10 demonstrates the predictors of the major adverse cardiovascular events within in-hospital and 30 days post discharge for study patients. Patients presenting with STEMI had a higher risk of in-hospital MACE [odds ratio (OR), 95% confidence interval = 0.087, 95% CI 0.020 to 0.376, *P*= 0.001] and 30 days post discharge MACE (OR = 0.308, 95% CI 0.101 to 0.937, *P*= 0.038) than patients presenting with NSTEMI/UA. Furthermore, symptom onset to presentation >6 hr was associated with both in-hospital MACE (OR = 1.858, 95% CI 0.556 to 6.215, *P*= 0.021) and 30 days post discharge MACE (OR = 1.143, 95% CI 0.395 to 3.309, *P*= 0.018).

Table 9 : Independent predictors of the major adverse cardiovascular events within in-hospital for study patients

Variaable	In- hospital MACE	*P* value
	OR (95% CI)	
Age, years	0.973 (0.926, 1.022)	0.278
Heart rate (beats/min)	0.975 (0.940, 1.011)	0.176
Systolic blood pressure (mm Hg)	1.011 (0.987, 1.036)	0.377
Creatinine (μmol/L)	1.017 (0.994, 1.040)	0.142
Ejection Fraction $\leq$ 30%	15.042 (1.516, 149.264)	*0.021
STEMI vr. NSTEMI/ UA	0.087 (0.020, 0.376)	*0.001
Symptom onset to presentation >6 hr	1.858 (0.556, 6.215)	0.314

Note: Multivariate logistic regression model to evaluate predictors of in-hospital major adverse cardiovascular events (MACE; death, re-infarction and stroke). Adjusted for GRACE risk score variables [age, heart rate, systolic blood pressure, serum creatinine, cardiac enzyme (positive vs. negative), and ST segment deviation using STEMI vr. NSTEMI /UA as reference], and symptom onset to presentation (>6 vr.<6 hr). Values of * =$P < 0.05$ were considered as significant

Table 10 : Independent predictors of the major adverse cardiovascular events within 30 days post discharge for study patients

Variaable	30 days post discharge MACE	*P* value
	OR (95% CI)	
Age, years	1.032 (0.987, 1.078)	0.167
Heart rate (beats/min)	0.976 (0.944, 1.010)	0.166
Systolic blood pressure (mm Hg)	1.007 (0.985, 1.031)	0.527
Creatinine (μmol/L)	1.015 (0.995, 1.034)	0.138
Ejection Fraction $\leq 30\%$	10.759 (1.505, 76.929)	*0.018
STEMI vr. NSTEMI/ UA	0.308 (0.101, 0.937)	*0.038
Symptom onset to presentation >6 hr	1.143 (0.395, 3.309)	0.805

Multivariate logistic regression model to evaluate independent predictors of 30 days post discharge major adverse cardiovascular events (mace; death, re-infarction and stroke). Adjusted for GRACE risk score variables [age, heart rate, systolic blood pressure, serum creatinine, cardiac enzyme (positive vs. negative), and ST segment deviation using STEMI vr. NSTEMI /UA as reference], and symptom onset to presentation (>6 vr.<6 hr). Values of * =$P < 0.05$ were considered as significant

Discussion

In this comparative study, we found that clinical management in terms of PCI and CABG procedures for overall patients was more often in Hungary than in Iraq. Furthermore, our data showed there were a higher rate of in-hospital complication in Hungary than Iraqi patients.

Comparison of baseline characteristics in both countries

Our findings showed that there were many notable differences upon demographic and clinical characteristics in this comparisons. The younger age of the patients in Iraq may reflect the unphysical activity, a higher prevalence of diabetes, stress and the genetic factor of family history of CAD. These findings were consistent with several records proving that the prevalence of patients with ACS in the Arab Middle East are about a decade younger than in developed countries and have a higher prevalence of diabetes (Almahmeed et al., 2012; Suwaidi et al., 2010; Zubaid et al., 2017). However, both of countries patients were older

adult. The results are directly in line with previous findings indicating a high incidence of ACS among elderly patients in the 65-75 age group, and approximately 60% of hospital admissions. This age group represents the highest death rate by ACS 85% (Dai et al., 2016).

The higher prevalence of hypertension, dyslipidemia, previous MI and previous PCI in Hungary may indicate unhealthy lifestyles. According to the latest finding of a population survey in the Hungarian countryside, HT affect approximately 2.4 million people out of a total population of 10 million in Hungary. Additionally, the prevalence of HT in the age group of around 60 years is comparable to the values observed in other parts of the world (Farsang et al., 2004). Furthermore, another research based on information stored in Hungarian Myocardial Infarction Registry (HUMIR) found that the occurrence of DM, HT, peripheral vascular disease, and prior of myocardial infarction and stroke were significantly more frequent in Hungarian patients (Piros et al., 2017). The increased prevalence of HT among young and middle-aged Hungarians compared with Canadians could represent an essential contributor to the high CV mortality and stroke rates in Hungary (Steiner et al., 2012).

In accordance with the present results, previous studies have demonstrated that a higher proportion of diabetic patients was above the target values in Hungary than the means of the European surveys. There was a higher proportion of smokers in the Hungarian samples, while the proportion of obese and overweight patients was similar to the European sample (Jancsó et al., 2020). Previous study evaluating the epidemiology of smoking in Hungary noted that 36.1% of adult population smoke cigarettes (29.9% on a daily basis). 40.6% of males while 31.7% of females smoke regularly (rates of daily smokers are 34.6% and 25.3%, respectively) (Tombor et al., 2010). Several reports showed that the prevalence of overweight in Hungary was 40.4% among men and 31.3% among women, while for obesity 32.0% and 31.5%, respectively. Abdominal obesity was 37.1% in males and 60.9% in females (Rurik et al., 2014).

The clinical characteristics (symptom onset to presentation, heart rate, and systolic blood pressure) from both country suggest a reasonably similar infarction severity. The higher rate of typical chest pain and creatinine levels in Iraq patients may indicate the younger age and ACS type because most patients have STEMI (Almahmeed et al., 2012; Suwaidi et al., 2010; Zubaid et al., 2017).

Comparison of clinical management in both countries

The results indicate that Iraqi patients were more often diagnosed with STEMI. The higher

prevalence of STEMI among Iraqi patients can be explained by the younger age and one another possible reason is that the study was conducted in CCU where all patients with STEMI were admitted while NSTEMI and UA included those patients with serious condition only, according to the number of patients and the hospital's ability to receive cases. Interestingly, the use of angiography was higher in Iraq than in Hungary, but PCI and CABG rates were significantly lower. With regard reperfusion therapy for patients diagnosed with STEMI, primary PCI was treated more frequently in Hungary than in Iraq. This finding was consistent with data recorded form Hungarian Myocardial Infarction Registry, where primary percutaneous coronary intervention (PPCI) was performed in 91.1% of STEMI patients. However, this major issue lies in the impact of financial and administrative factors on the medical decision-making process and the quality of the services provided. Where, there is a shortage of hospitals with cath laboratory facilities and equipment in Iraq, despite the large numbers of patients. The present study confirmed the results of previous clinical trials that showed a wide variation in practical performance between countries in managing ACS patients (McNamara et al., 2014). However, these basic findings are consistent with research showing that patterns of management within a clinical trial do not necessarily reflect management in routine clinical practice (McNamara et al., 2014).

Comparison of outcomes in both countries

Data indicate a similarity in mortality in both countries during hospital admission and after 30-day follow-up, while the complications were higher in Hungary during hospital admission. This might due to the higher prevalence of risk factors such as older age, hypertension, dyslipidemia, previous MI and previous PCI in Hungary than Iraq. Concerning outcomes predictors of overall study patients, our data showed the patients presenting with STEMI and had a higher risk of major adverse cardiovascular events (MACE) in-hospital and 30 days post discharge than NSTEMI/UA. Furthermore, symptom onset to presentation >6 hr was associated with both in-hospital and 30 days post discharge major adverse cardiovascular events. Several studies indicate that the type of ACS in presentation is associated with differences in relative long-term mortality, where the hospital mortality rate is higher in patients with STEMI compared to NSTE-ACS (Fanaroff et al., 2016). Another studies showed that ACS outcomes are time-dependent and inversely related to delays in appropriate treatment (Frisch et al., 2017; Hicks Karen A. et al., 2015; Zègre-Hemsey et al., 2018).

Conclusion

Our results showed that variations in ACS outcomes are due to differences in socio-economic characteristics, disease severity, and therapeutic management in both countries.Moreover, there is a obvious impact of financial and administrative factors on medical decision-making and quality of services provided.

Future Perspective:

Several studies over the past two decades have demonstrated the importance of oxidative stress in the development of atherosclerosis and myocardial injury. Elevated concentrations of a variety of oxidative stress markers were linked with a more frequent occurrence of cardiac events. However, assessment of oxidative stress markers could modify risk stratification, diagnosis and prevention of patients with suspected ACS. The accumulation of m-tyrosine and *o*-tyrosine has been reported to adversely affect cells, indicating a direct role for *m*-Tyr in the effects of oxidative stress. As a result, increases of *m*-Tyr and *o*-Tyr levels are commonly used as a biomarker of oxidative stress. Therefore, previous studies have justified the association of *m*-Tyr and *o*-Tyr levels with other oxidative stress markers. However, no single study exists which investigated of Tyr isomers in ACS patients.

Therefore, this thesis was designed to examine the association of Phe and Tyr isomers (*m*-, *o*-, and *p*-Tyr) with oxidative stress following myocardial injury. Furthermore, to compare patients with STEMI and NSTEMI, and the serum levels of Phe and Tyr isomers at the aortic root and distal to the culprit lesion in both patient groups.

In this thesis, one of the more significant findings to emerge is that increased serum *m*-Tyr levels can reflect oxidative stress induced by inflammation after myocardial injury, similar to the observed decrease in *p*-Tyr levels. The second major finding was that changes in serum levels of different Tyr isomers in STEMI and NSTEMI patients can mediate the effects of oxidative stress during myocardial infarction. The limitation of this thesis is that only a small number of patients have been studied and a time period of ischemia is not specified.

Further research needs to examine more closely the links between myocardial infarction and Tyrosine isomers as well as the Phe/Tyr ratio. Moreover, examine the association of Phe and Tyr isomers with immune activation and inflammation in patients with ACS.

Summary of Findings :

The findings of this thesis were summarized according to the specific aims stated in Chapter one.

We examined the association of phenylalanine (Phe) and Tyr isomers with oxidative stress following myocardial injury.

Based on that we found the following:

- Serum *p*-Tyr levels and *p*-Tyr/Phe ratio were significantly lower in the ACS patients than in controls.
- Serum *m*-Tyr level and *m*-Tyr/Phe ratio were significantly higher in the ACS patients than in controls.
- Serum *p*-Tyr/Phe ratio was significantly lower in all patients compared to controls.
- Serum *m*-Tyr levels did show a positive correlation with *p*-Tyr/Phe ratios in different vessel segments.

The significance of our new findings is that increased serum *m*-Tyr levels can reflect oxidative stress induced by inflammation after myocardial injury, similar to the observed decrease in *p*-Tyr levels.

We compared patients with STEMI and NSTEMI and the serum levels of Phe and Tyr isomers at the aortic root and distal to the culprit lesion in both groups.

We found the following:

- Serum levels of Phe were significantly higher distal to the culprit lesion compared to the aortic root in patients with STEMI.
- Serum *p*-Tyr/Phe and *m*-Tyr/Phe concentration ratios were both lower distal to the culprit lesion than at the aortic root in patients with STEMI.
- There were no statistically significant differences with respect to changes in serum Phe and Tyr isomers distal to the culprit lesion compared to the aortic root in patients with NSTEMI.

The new findings demonstrate that changes in serum levels of different Tyr isomers in STEMI and NSTEMI patients can mediate the effects of oxidative stress during myocardial infarction.

We aimed to describe current characteristics of patients admitted for ACS in Hungary compared to Iraq and to analyses whether in-hospital and 30 days post discharge outcomes variations are explained by differences in patients' baseline characteristics and clinical management.

We found the following:

- Iraqi patients were more often diagnosed with STEMI.
- Hungarian patients were more often diagnosed with NSTEMI.
- The patients were younger in Iraq and often had a family history of CAD than those in Hungary.
- Hungarian patients more often had hypertension, dyslipidemia, prior MI, prior PCI and prior CABG than Iraqi patients.
- PCI and CABG were performed more in Hungary than in Iraq.
- In-hospital mortality and 30 days post discharge were low in both countries without any major differences.
- Hungarian patients recorded a higher events rate during hospitalization and after 30 days post discharge from Iraqi patients.

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Acknowledgements

Before all, greatest thanks to "Allah"

First of all, I am greatly indebted to my supervisor, Dr Verzár Zsófia for her invaluable help and advice during my doctoral research.

I am grateful to the Doctoral School of Health Sciences, first of all to Prof. Dr. József Bódis, the Head of the Doctoral School, to Prof. Dr. Endre Sulyok, Secretary of the Doctoral School of Health Sciences, and to Dr. Viktória Prémusz, coordinate of the Doctoral School.

I am also grateful to the leaders of the Faculty of Health Sciences, especially to Dr habil András Oláh and Prof Dr Pongrác Ács for providing the opportunity for performing my scientific work.

I would like to express my gratitude for all who supported me during my work especially the professional team from Prof. Dr. István Wittmann, Chair of the 2nd Department of Medicine and Nephrological Center, Faculty of Medicine, to Dr. Gergő Attila Molnár, and Dr.Szilárd Kun who provided the insight and experience that greatly assisted the research.

I would like to express my deepest thanks to my friend, Dr. Qasim Ali to help and encourage me during my study period.

Finally, my heartfelt thanks to my family, my wife, and my children, Barra, Saif Al-Din and Bahaa Al-Din for their support, patience and tolerance over the past years.

List of Publications

In extenso publications

Articles related to the dissertation

1. Al-Sadoon, I., Wittmann, I., Kun, S., Ahmann, M., Konyi, A., & Verzár, Z. (2021). Assessment of serum phenylalanine and tyrosine isomers in patients with ST-segment elevation vs non-ST-segment elevation myocardial infarction. Journal of Clinical Laboratory Analysis, 35(2), e23613. https://doi.org/10.1002/jcla.23613. Q 2, IF 2.352.
2. Al-Sadoon I, Wittmann I, Molnár GA, et al. Serum concentrations of phenylalanine and tyrosine isomers in patients with acute coronary syndrome. Pol Arch Intern Med. 2021; 131; 16107. doi:10.20452/pamw.16107. Q3, IF 3.277.
3. Al-Sadoon, I. (2020). Phenylalanine, para-tyrosine, ortho-tyrosine and meta-tyrosine for ST-segment elevation VS. Non-ST-segment elevation acute coronary syndrome. 1. [Data set]. Mendeley. https://doi.org/10.17632/tgr3dd7wj4.1

Articles in the field of the dissertation

1. Qasim Ali Khasal, Ied AlSadoon, Fatima J Shinjar. (2020). STRESSFUL LIFE EVENTS OF PATIENT WITH ISCHEMIC HEART DISEASE AT AL-NASIRIYA HEART CENTER – International Journal of Psychosocial Rehabilitation,24(6), 7414-7423. https://www.psychosocial.com/article/PR260746/18854/

Articles in other topics

1. Nahla Saleh Hasan, Murtadha Kadhim Yasir, Qasim Ali Khasal, Mishaal Zoori Jabbar, Abdulrahman Abbas Jasim, Ied Al_Sadoon (2020). The Use of Complementary and Alternative Medicine among Diabetic Patients in Nasiriya City, Medico Legal Update, 20 (1): Medico Legal Update.
2. Khasal, Q. A., Dabis, H. A., Al_Sadoon, I., & Sachit, A. A. (2019). Assessment of Metered-dose Inhalers Technique among Patients with Chronic Respiratory Disorders at Al-Hussein Teaching Hospital in Al-Nasiriyah City. Indian Journal of Forensic Medicine & Toxicology, 13(3), 243. https://doi.org/10.5958/0973-9130.2019.00203.2

Abstracts and oral presentations

1. Ied Al Sadoon , István Wittmann Prof. Dr , Szilárd Kun MD PhD , Mercedes Ahman , Attila Konyi MD PhD , Zs.Verzár MD PhD, Assessment of Oxidative Stress Markers in Patients with Acute Coronary Syndrome: Potential to Modify Risk Stratification and Treatment, In: Csiszár, Beáta; Bódog, Ferenc (ed.) Medical Conference for PhD Students and Experts of Clinical Sciences: Book of abstractsPécs, Hungary: University of Pécs Doctoral Student Self-Government , (2019) p. 9.
2. Ied Al-Sadoon, István Wittmann, GA Molnár, Szilard Kun, Mercédesz Ahmann, Attila Konyi, Zsófia Verzár (2020). Investigation of Serum Phenylalanine and Tyrosine Isomers in Acute Coronary Syndrome Patients. 9th INTERDISCIPLINARY DOCTORAL CONFERENCE 2020 BOOK OF ABSTRACTS. Pécs, Hungary : Doctoral Student Association of the University of Pécs (2020) 384 p. pp. 55-55. , 1 p.
3. Ied, A. S., Wittmann, I., Kun, S., Ahmann, M., Pakai, A., Oláh, A., Boncz, I., & Verzár, Z. (2020). PCV95 SERUM Phenylalanine and Tyrosine Isomers in ACUTE Coronary Syndrome Patients. Value in Health, 23, S503–S504. https://doi.org/10.1016/j.jval.2020.08.586
4. Ied, Al-Sadoon; Mercédesz, Ahmanna; Hussein, Al-Kenzawib; Zsófia, Verzár. Comparison of baseline characteristics, clinical management and outcomes for patients with acute coronary syndrome. Medical Conference for PhD Students and Experts of Clinical Sciences 2021: Book of Abstracts. Pécs, Hungary: Doctoral Student Association of the University of Pécs (2021) 128 p. pp. 6-6. , 1 p.

Appendix

List of Tables

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Ethical approval (1)

PÉCSI TUDOMÁNYEGYETEM
Klinikai Központ
Regionális és Intézményi Kutatás-Etikai Bizottsága

dr. Kónyi Attila és
dr. Szokodi István
egyetemi docensek
PTE KK
Szívgyógyászati Klinika
Társvizsgálatvezetők

Pécs, 2012.06.16.

Tisztelt Tanár Úrak!

A PTE-KK Regionális és Intézményi Kutatás – Etikai Bizottsága **2012.06.15.**-ei ülésén megtárgyalta az Önök által benyújtott dokumentumokat:

Cím: Gyulladásos faktorok, citokinek és kemokinek **szintjének vizsgálata** intrakoronáris plakkban, trombusban valamint plazmában stabil angina, valamint AICS miatt perkután koronária intervención, illetve koszorúér bypass műtétre kerülő betegek esetén

Mellékletek:
(1.) a vizsgálat tudományos háttere;
(2.) vizsgálati protokoll;
(3.) Betegtájékoztató és
(4.) beleegyező nyilatkozat;
(5.) a résztvevő klinika és intézet igazgatóinak támogató nyilatkozata;

Döntés: a PTE KK RIKEB engedélyezi a klinikai vizsgálatok protokoll szerinti kivitelezését

Ügyiratszám: 4511.

Szivélyes üdvözlettel

Dr. Kosztolányi György
egyetemi tanár
a bizottság elnöke

dr. Kocsis Béla
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E-mail: foigazgatoi.hivatal@kk.pte.hu

Ethical approval (2)

UNIVERSITY OF PÉCS
CLINICAL CENTER
Regional and Local Research Ethics Committee

Pécs, 07. April 2018.

Certificate

To Whom It May Concern,

Principal investigator: Ied Ali Omar Al-Sadoon PhD student
Cardiovascular Health Sciences Programme
Doctoral School of Health Sciences at University of Pécs, Hungary

Leader of Programme: Zsófia Verzár MD PhD Med. habil. assoc. professor
Department of Emergency Medicine Clinical Center University of Pécs Hungary

Title: Clinical Characteristics, Management and Outcome of patients with acute coronary syndrome in two different cultures

Documents:
(1.) Scientific background and references;
(2.) Research Protocol: socio-demographics, medical history, clinical presentation, risk factors: Questionnaire (1) and Questionnaire (SF-36);
(3.) diagnostic and therapeutic modalities;
(4.) quality of life measurement;
(5.) data collection;
(6.) Infermed consent to participate in the research study;
(7.) 11. supplement Statistical and Scientific Data Collection;
(8.) supporting letters from the Leader of the Programme and the Secretary of the Doctoral School of Health Sciences at University of Pécs, Hungary

Decision: The Ethics Committee as the Institutional Review Board **discussed** and **accepted** this application on its meeting held on 6th of April 2018. **The Board approves and supervises of the data management** of anonymous clinical data of case reports necessary for thesis work. All parts of the thesis work should keep the act LXIII of 1992 on the Protection of Personal Data and Publicity of Information of Public Interest and its updates.

Record number: **7143 – PTE 2018.**

PTE Centrum Kutatás-Etikai Bizottság

Yours sincerely

György Kosztolányi
Professor of Human Genetics
Chairman of the Regional Research Ethics Committee of the Medical Center, Pécs
Institutional Review Board
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Béla Kocsis
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Institutional Review Board
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fax 00-36-72-536253

Data Availability Statement

The dataset, the questionnaires and the inform consent form are available from the author upon reasonable request.

Submission of the doctoral dissertation and declaration of the originality of the dissertation

The undersigned,

Name: Al-Sadoon
Maiden name: Ied Ali Omar

Mother's maiden name: Lilwa Washeel

Place and time of birth: Thi-Qar, Iraq, 01/01/1984

on this day submitted my doctoral dissertation entitled

EVALUATION OF PATHOGENICITY-RELATED OXIDATIVE STRESS BIOMARKERS AS WELL AS CLINICAL CHARACTERISTICS, MANAGEMENT, AND OUTCOMES IN ACUTE CORONARY SYNDROME

to the

PR-2. Cardiovascular Health Science

of the Doctoral School of Health Sciences, Faculty of Health Sciences, University of Pécs.

Names of the consultant(s): Dr. Verzár Zsófia

At the same time, I declare that

- I have not submitted my doctoral dissertation to any other Doctoral School (neither in this country nor abroad),
- my application for degree earning has not been rejected in the past two years,
- in the past two years I have not had unsuccessful doctoral procedures,
- my doctoral degree has not been withdrawn in the past five years,
- my dissertation is independent work, I have not presented others' intellectual work as mine, the references are definite and full, on preparation of the dissertation I have not used false or falsified data.

Dated: 21/12/2021

Ied Al-Sadoon
Candidate

Dr. Verzár Zsófia
Supervisor