

**DETERMINATION OF SERUM FATTY ACIDS PROFILE IN
PATIENTS UNDERGOING MYOCARD INFARCT IN ERBIL**

**Saman Tawfeeq ISMAEL
Master Thesis**

Chemistry Department

**Supervisor: Prof. Dr. Mehmet ÇİFTCI
2017**

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**REPUBLIC OF TURKEY
BİNGÖL UNIVERSITY
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LIST OF ABBRIVATIONS

ALA	: Alpha linolenic acid
AMI	: Acute myocardial infarction
AST	: Aspartate transaminase
BMI	: Body mass index
CHD	: Coronary heart disease
CK	: Creatine kinase
CRP	: C-Reactive protein
Da	: Dalton
DHA	: Docosahexaenoic acid
DBP	: Diastolic blood pressure
ECG	: Electrocardiogram
EC	: Enzyme commission
EDTA	: Ethylene diamine tetra acetic acid
EFA	: Essential fatty acid
EPA	: Eicosapentaenoic acid
kDa	: Kilo Dalton
GC	: Gas chromatography
FFA	: Free fatty acid
HDL	: High density lipoprotein
IHD	: Ischemic heart disease
LDH	: Lactate dehydrogenase
LCFA	: Long chain fatty acid
LDL	: Low density lipoprotein
MS	: Mass spectrophotometry
MCFA	: Medium chain fatty acid
MetS	: Metabolic syndrome

MI	: Myocardial infarction
mmol	: millimols
mg	: milligrams
MUFA	: Monounsaturated fatty acid
P	: Probability
PAP	: Para amino antipyrine
PL	: Phospholipid
PUFA	: Polyunsaturated fatty acid
SFA	: Saturated fatty acid
S	: Serum
SCFA	: Short chain fatty acid
SD	: Standard deviation
SE	: Standard of error
SBP	: Systolic blood pressure
TC	: Total cholesterol
TG	: Total triglycerides
VLCFA	: Very long chain fatty acid
VLDL	: Very low density lipoprotein
WHO	: World health organization

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ERBİL'DE KALP KRİZİ GEÇİREN HASTALARDA SERUM YAĞ ASIDI PROFİLİNİN BELİRLENMESİ

ÖZET

Bu yüksek lisans tez çalışmasında Irak'ın Erbil şehrinde miyokard enfarktüsü geçiren hastalarda yağ asidi profili, lipid profili ve kardiyak biyokimyasal değerler çalışıldı ve sağlıklı bireylerle karşılaştırıldı. Önce kardiyak biyokimyasal veriler normal ve MI gruplarında S. troponin ve CK-MB düzeylerini göstermektedir. Elde edilen sonuçlar, MI grubunda ortalama S. troponin ve CK-MB seviyesinin kontrol grubuna göre anlamlı derecede yüksek olduğunu göstermektedir ($p < 0,0001$).

Daha sonra, yağ asidi bileşimi, kontrol ve MI gruplarındaki toplam doymuş, tekli doymamış ve poli doymamış yağ asitlerinin ortalama \pm SD'sini sağlar; elde edilen sonuçlar, MI grubundaki toplam S. doymuş yağ asitlerinin ortalamasının, MI grubundaki ortalama doymamış yağ asitlerinin ortalamasının, kontrol grubuna göre S. toplam mono-doymamış yağ asitleri, kontrol grubuna göre anlamlı derecede düşük ($P < 0,05$) ve MI grubunda S. çoklu doymamış yağ asitleri kontrol grubuna göre anlamlı olarak düşük ($P < 0,001$) bulundu.

Son olarak, her bir lipid profilinin ortalama \pm SD, MI'daki S. trigliseridlerin ve S. total kolesterolün, MI grubundaki kontrollerden daha yüksekti ($P < 0,001$), S.LDL'nin MI grubunda anlamlı olarak daha yüksek olduğunu gösterdi ($P < 0,0001$), MI grubundaki S.HDL ise kontrol grubuna göre anlamlı olarak daha düşüktü ($P < 0,001$).

Anahtar Kelimeler: Serum, yağ asidi, miyokard, Erbil.

DETERMINATION OF SERUM FATTY ACIDS PROFILE IN PATIENTS UNDERGOING MYOCARD INFARCT IN ERBIL

ABSTRACT

In this master's thesis study, fatty acids profile, lipid profile, and cardiac biochemical's were studied in myocardial infarction and compared with healthy individuals at Erbil province in Iraq. First about cardiac biochemical's shows the mean S. troponin and CK-MB levels in the normal and MI groups. The results obtained indicate that the mean level of S. troponin and CK-MB in MI group were significantly very higher than that of control group ($p < 0.0001$).

Then about fatty acid composition provides the Mean \pm SD, of each of the total saturated, monounsaturated and poly unsaturated fatty acids in control and MI groups, the results obtained that the mean of S. total saturated fatty acids in MI group was non-significantly higher than that of control group, S. total monounsaturated fatty acids in MI significantly ($P < 0.05$) lower than that of control group, and S. total polyunsaturated fatty acids in MI group significantly ($P < 0.001$) lower than that of control group.

Finally, the Mean \pm SD, of each of the lipid profile, indicated that each of S. triglycerides and S. total cholesterol in MI were significantly higher than that of controls ($P < 0.001$), S.LDL in MI group was significantly higher than that of controls ($P < 0.0001$), while S.HDL in MI group was significantly lower than that of control group ($P < 0.001$).

Keywords: Serum, fatty acid, myocard, Erbil.

1. INTRODUCTION

1.1. Introduction to myocardial infarction

Myocardial infarction (MI) means that part of the heart muscle suddenly loses its blood supply (the word infarction means the death of some tissue due to a blocked artery which stops blood from getting past). Without prompt treatment, this can cause damage to the affected part of the heart which leads latterly to a heart attack. An MI is sometimes called a heart attack or a coronary thrombosis (Tim and Beverley 2010; Alan and Goby 2010).

A heart attack occurs when one or more of the coronary arteries that supply blood to the heart are completely blocked and blood to the heart muscle is cut off. The blockage is usually caused by atherosclerosis, the buildup of plaque in the artery walls, and/or by a blood clot in a coronary artery (Glew and Rosenthal, 2007).

1.2. Fatty acids

Fatty acids, measured as components of cholesterol esters or phospholipids present in plasma or serum, reflect intake of dietary fat over the last few weeks (Katan et al. 1997). As such, they are good markers of intake and may be preferred to self-reported intake assessed with a questionnaire, which is prone to underreporting intake (Samaras, Kelly and Campbell 1999). In addition, plasma or serum fatty acid profiles reflect endogenous conversion of ingested fatty acids by desaturation, elongation or both (Faas et al. 1988).

Fatty acids are biologically active molecules with a wide array of effects. For decades, fatty acids have been a focus of dietary recommendations for heart health. Historically, saturated fatty acids (SFAs) have been a target for reduction. More recently, this dietary restriction has been extended to trans-fatty acids. In contrast, unsaturated fatty acids have been considered to be heart healthy. Thus, in recent years, dietary recommendations have

been made to decrease saturated and trans-fatty acids and to emphasize unsaturated fatty acids, since the early 1970s and thereafter, long-chain omega-3 polyunsaturated fatty acids, notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been associated with cardiovascular health benefits (Russo GL, 2009).

1.3. Biochemical Markers of Myocardial Enzymes

Various cardiac enzymes have been used [creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate transaminase (AST)], however at present, cardiac troponins are used in the diagnosis of MI. Troponin is a complex contractile protein comprising of three subunits: C, T, and I. Troponin T and I are cardio specific, used in MI diagnosis (Michael et al. 2005).

Troponin is a complex of globular muscle protein of I band that inhibits contraction by blocking the interaction of actin and myosin; when combined with Ca^{+2} . It so modifies the position of the tropomyosin molecules that interaction takes place.

Troponins rise within a few hours on onset of symptoms and remain elevated for 1-2 weeks. This property enables early as well as late diagnosis. The diagnostic sensitivity of troponin reaches 100% 12 hours after onset of symptoms (Alan et al. 2013).

Myoglobin is the oxygen transporting pigment of muscle type of protein resembling a single subunit of hemoglobin, composed of one globin polypeptide chain and one heme group (containing one iron atom). It combines with O_2 released by erythrocytes stores it and transports it to the mitochondria of muscle cells, where it generates energy by combustion of glucose to CO_2 and water.

Creatine kinase (EC. 2, 7, 3, 2), an Mg^{+2} activated enzyme of the transferase class that catalyzes the phosphorylation of the reaction by ATP to form phosphocreatine. There are three isoenzymes of CK, each having two components composed of M (Muscle), and B (Brain) subunits, CK1 BB is found primarily in brain, CK2 MB is found primarily in cardiac muscle, and CK3 MM is found primarily in muscle skeleton. Differential

determination of isoenzymes is useful for clinical diagnosis; the isoenzyme CK2 MB is specific for diagnosis of MI.

1.4. The Aims of the Study

- 1- To measure the levels of various types of serum fatty acids in normal individuals and MI patient in Erbil
- 2- Determination of serum enzyme creatine kinase (CK), and its isoenzymes in normal individuals and MI patients.
- 3- Estimation of serum troponins in normal individuals and MI patients.
- 4- Evaluation of lipid profile (serum total cholesterol (STC), serum total triglycerides (STG), low density lipoprotein (LDL), and high density lipoprotein (HDL), in both groups (MI patients and normal individuals).

2. LITERATURE REVIEW

2.1. Myocardial Infarction (MI)

Myocardial infarction, commonly known as a heart attack occurs when blood flow stops to a part of the heart causing damage to the heart muscle. An MI may cause heart failure, an irregular heartbeat, or cardiac arrest. The mechanism of an MI often involves the rupture of an atherosclerotic plaque, leading to complete blockage of a coronary artery, unstable collection of lipids (like cholesterol), and white blood cells (especially macrophages) in the wall of an artery. The resulting ischemia and oxygen shortage, if left untreated for a sufficient period of time, can cause damage and/or death (infarction) of heart muscle tissue (Janet, et al. 2008). A number of tests are useful to help with diagnosis, including electrocardiograms (ECGs), blood tests, and coronary angiography (O'Connor et al. 2010). An ECG may confirm an ST elevation MI if ST elevation is present. Commonly used blood tests include troponin and less often creatine kinase (Valensi. et al. 2011).

2.2. Signs and Symptoms

The onset of symptoms in myocardial infarction is usually gradual, over several minutes, and rarely instantaneous. Chest pain is the most common symptom of acute MI and is often described as a sensation of tightness, pressure, or squeezing. Chest pain due to ischemia (a lack of blood and hence oxygen supply) of the heart muscle is termed angina pectoris. Pain radiates most often to the left arm, but may also radiate to the lower jaw, neck, right arm, back, and upper abdomen (Van de Werf et al. 2008).

Shortness of breath (dyspnea) occurs when the damage to the heart limits the output of the left ventricle, causing left ventricular failure and consequent pulmonary edema. Other symptoms include diaphoresis (an excessive form of sweating) (Mallinson 2010)

weakness, light-headedness, nausea, vomiting, and palpitations. These symptoms are likely induced by a massive surge of catecholamine's from the sympathetic nervous system (Little et al. 1986), which occurs in response to pain and the blood flow abnormalities that result from dysfunction of the heart muscle. Loss of consciousness (due to inadequate blood flow to the brain and cardiogenic shock) and sudden death (frequently due to the development of ventricular fibrillation) can occur in MIs (Van de Werf et al. 2008).

2.3. Causes of Myocardial Infarction

Many of the risk factors for myocardial infarction are modifiable and thus many cases may be preventable.

Smoking appears to be the cause of about 36% and obesity the cause of 20% of coronary artery disease. Lack of exercise has been linked to 7–12% of cases. (Lee et al. 2012). Less common causes include stress-related causes such as job stress, which accounts for about 3% of cases, and chronic high stress levels. Tobacco smoking (including second hand smoke) (Steptoe and Kivimäki 2012) and short-term exposure to air pollution such as carbon monoxide, nitrogen dioxide, and sulfur dioxide have been associated with MI. Other factors that increase the risk of MI and are associated with worse outcomes after an MI include lack of physical activity (Mustafic et al. 2012).

There is little evidence that reducing dietary saturated fat or increasing polyunsaturated fat intake affects heart attack risk. Dietary cholesterol also does not appear to have a significant effect on blood cholesterol and thus recommendations about its consumption may not be needed. Trans fatty acids do appear to increase risk. At any given age, men are more at risk than women, particularly before menopause, Family history of ischemic heart disease or MI, particularly if one has a first-degree relative who suffered a 'premature' myocardial infarction (defined as occurring at or younger than age 55 years (men) or 65 (women) (Chowdhury et al. 2014).

Women who use combined oral contraceptive pills have a modestly increased risk of myocardial infarction, especially in the presence of other risk factors, such as smoking

(Khader et al. 2003). Heart attacks appear to occur more commonly in the morning hours (Culić 2007). Evidence suggests that heart attacks are at least three times more likely to occur in the morning than in the late evening (Shaw and Tofler 2009). Old age increases risk of a heart attack (Chowdhury et al. 2014).

2.4. Pathophysiology

The A myocardial infarction occurs when an atherosclerotic plaque slowly builds up in the inner lining of a coronary artery and then suddenly ruptures, causing catastrophic thrombus formation, totally occluding the artery and preventing blood flow downstream. Drawing of the heart showing anterior left ventricle wall infarction

Acute myocardial infarction refers to two subtypes of acute coronary syndrome, namely non-ST-elevated and ST-elevated MIs, which are most frequently (but not always) a manifestation of coronary artery disease (Moe and Wong 2010). The most common triggering event is the disruption of an atherosclerotic plaque in an epicardial coronary artery, which leads to a clotting cascade, sometimes resulting in total occlusion of the artery. Atherosclerosis is the gradual buildup of cholesterol and fibrous tissue in plaques in the wall of arteries (in this case, the coronary arteries), typically over decades. Bloodstream column irregularities visible on angiography reflect artery lumen narrowing as a result of decades of advancing atherosclerosis (Spaan et al. 2008). Plaques can become unstable, rupture, and additionally promote the formation of a blood clot that occludes the artery; this can occur in minutes. When a severe enough plaque rupture occurs in the coronary arteries, it leads to MI (necrosis of downstream myocardium). It is estimated that one billion cardiac cells are lost in a typical MI. (Laflamme and Murry 2005).

If impaired blood flow to the heart lasts long enough, it triggers a process called the ischemic cascade; the heart cells in the territory of the occluded coronary artery die (chiefly through necrosis) and do not grow back. A collagen scar forms in their place. Recent studies indicate that another form of cell death, apoptosis, also plays a role in the process of tissue damage following an MI (Wilson AM 2006). As a result, the person's heart will be permanently damaged. This myocardial scarring also puts the person at risk

for potentially life-threatening abnormal heart rhythms (arrhythmias), and may result in the formation of a ventricular aneurysm that can rupture with catastrophic consequences (Krijnen et al. 2002).

Injured heart tissue conducts electrical impulses more slowly than normal heart tissue. The difference in conduction velocity between injured and uninjured tissue can trigger re-entry or a feedback loop that is believed to be the cause of many lethal arrhythmias. The most serious of these arrhythmias is ventricular fibrillation (V-Fib/VF), an extremely fast and chaotic heart rhythm that is the leading cause of sudden cardiac death. Another life-threatening arrhythmia is ventricular tachycardia (V-tach/VT), which can cause sudden cardiac death. However, VT usually results in rapid heart rates that prevent the heart from pumping blood effectively. Cardiac output and blood pressure may fall to dangerous levels, which can lead to further coronary ischemia and extension of the infarct (Pearson et al. 2003).

Myocardial infarction in the setting of plaque results from underlying atherosclerosis. Inflammation is known to be an important step in the process of atherosclerotic plaque formation (Wilson et al. 2006). C-reactive protein (CRP) is a sensitive but nonspecific marker for inflammation. Elevated CRP blood levels, especially measured with high-sensitivity assays, can predict the risk of MI, as well as stroke and development of diabetes (Wilson et al. 2006). Moreover, some drugs for MI might also reduce CRP levels (Wilson et al. 2006). The use of high-sensitivity CRP assays as a means of screening the general population is advised against, but it may be used optionally at the physician's discretion in those who already present with other risk factors or known coronary artery disease. (Pearson et al. 2003). Whether CRP plays a direct role in atherosclerosis remains uncertain (Wilson et al. 2006). Calcium deposition is another part of atherosclerotic plaque formation. Calcium deposits in the coronary arteries can be detected with CT scans. Several studies have shown that coronary calcium can provide predictive information beyond that of classical risk factors (Detrano et al. 2008). Hyperhomocysteinemia is associated with premature atherosclerosis (Clarke et al. 2011), whether elevated homocysteine in the normal range is causal is controversial (Clarke et al. 2011).

2.5. Diagnosis

Same inferior and right ventricular STEMI as seen on 15 lead ECG. There are additionally some premature ventricular contractions.

A cardiac troponin rise accompanied by typical symptoms, pathological Q waves, ST elevation or depression, or coronary intervention is diagnostic of MI (Alpert et al. 2000). WHO criteria (Anonymous 1979) formulated in 1979 has classically been used to diagnose MI, a patient is diagnosed with MI if two (probable) or three (definite) of the following criteria are satisfied:

Clinical history of ischemic type chest pain lasting for more than 20 minutes.

Changes in serial ECG tracings.

Rise and fall of serum cardiac biomarkers, especially troponins, and reliance on older tests (such as CK-MB) or myoglobin is discouraged. (Lansky et al. 2010). Copeptin may be useful to rule out MI rapidly when used along with troponin. (Lipinski et al. 2014).

2.6. Prevention by Lifestyle Changes

Myocardial infarction and other related cardiovascular diseases can be prevented to a large extent by a number of lifestyle changes.

Recommendations include increasing the intake of wholegrain starch, reducing sugar intake (particularly of refined sugar), consuming five portions of fruit and vegetables daily, consuming two or more portions of fish per week, and consuming 4–5 portions of unsalted nuts, seeds, or legumes per week (Stradling et al. 2014). There is some controversy surrounding the effect of dietary fat on the development of cardiovascular disease. People are often advised to keep a diet where less than 30% of the energy intake derives from fat, a diet that contains less than 7% of the energy intake in the form of saturated fat, and a diet that contains less than 300 mg/day of cholesterol. Replacing saturated with mono- polyunsaturated fat is also recommended, as the consumption of

polyunsaturated fat instead of saturated fat may decrease coronary heart disease (Mozaffarian et al. 2010) Olive oil, rape seed oil and related products are to be used instead of saturated fat. Physical activity can reduce the risk of cardiovascular disease, and people at risk are advised to engage in 150 minutes of moderate or 75 minutes of vigorous intensity aerobic exercise a week. Keeping a healthy weight, drinking alcohol within the recommended limits, and quitting smoking are measures that also appear to reduce the risk of cardiovascular disease. On a population level, public health measures may be used to reduce unhealthy diets (excessive salt, saturated fatty acids and trans fatty acids), and stimulating physical activity. This may be part of regional cardiovascular disease prevention programmes, or through the health impact assessment of regional and local plans and policies (McPherson et al. 2010).

2.7. Epidemiology

Myocardial infarction is a common presentation of coronary artery disease. The World Health Organization estimated in 2004, that 12.2% of worldwide deaths were from ischemic heart disease, with it being the leading cause of death in high- or middle-income countries and second only to lower respiratory infections in lower-income countries. Worldwide, more than 3 million people have STEMIs and 4 million have NSTEMIs a year. STEMIs occur about twice as often in men as women (White et al. 2008).

Rates of death from ischemic heart disease (IHD) have slowed or declined in most high-income countries, although cardiovascular disease still accounted for one in three of all deaths in the USA in 2008. For example, rates of death from cardiovascular disease have decreased almost a third between 2001 and 2011 in the United States (Mozaffarian et al. 2015).

In contrast, IHD is becoming a more common cause of death in the developing world. For example, in India, IHD had become the leading cause of death by 2004, accounting for 1.46 million deaths (14% of total deaths) and deaths due to IHD were expected to double during 1985–2015. Globally, disability adjusted life years (DALYs) lost to ischemic heart disease are predicted to account for 5.5% of total DALYs in 2030, making it the second-

most-important cause of disability (after unipolar depressive disorder), as well as the leading cause of death by this date (Mozaffarian et al. 2015).

2.8. Cardiac Biomarkers

Cardiac enzyme studies measure the levels of enzymes that are linked with injury of the heart muscle. Low levels of these enzymes and proteins are normally found in your blood, but if your heart muscle is injured, such as from a heart attack, the enzymes and proteins leak out of damaged heart muscle cells, and their levels in the bloodstream rise.

The current WHO criteria for the diagnosis of AMI include the presence of two of the following criteria,

1. Clinical symptoms compatible with acute ischemia.
2. ECG abnormalities.
3. A pattern of enzyme release typically of myocardial Injury (Mehta et al. 2000; White 2013).

2.9. Creatine Kinase (CK)

The initial CK-MB rise occurs 4 to 6 hours after the onset of chest pain, peaks at 24 hours, and returns to baseline at 48 to 72 hours. One advantage of CK-MB over other markers is that it remains elevated for longer periods. (Ghormade et al. 2014).

2.9.1. Troponin

Troponin is the gold standard biomarker for myocardial injury and is used commonly in conjunction with creatine kinase-MB (CK-MB) and myoglobin to enable a more rapid diagnosis of acute coronary syndrome ACS. Serum levels can remain elevated for up to 4–7 days for troponin I, and 10–14 days for troponin T (Daubert and Jeremias 2010; Ahmad and Sharma 2012).

Cardiac troponin is more specific than other markers for myocardial injury. Following AMI, cTnI becomes elevated at the same rate as CK-MB, but it remains elevated for 7 to 10 days. As troponin is nearly absolutely specific to myocardial tissue and exhibits high clinical sensitivity, it is the preferred biomarker for myocardial necrosis. Nowadays when all reperfusion strategies have to be instituted within minutes of patients arrival in emergency with ST elevation the role of biomarkers is reducing since they begin to rise after 3-6hrs and the lab report can also take time (Thygesen et al. 2007; Pandey 2011 and Sathyamurthy et al. 2015) Figure 2.1.

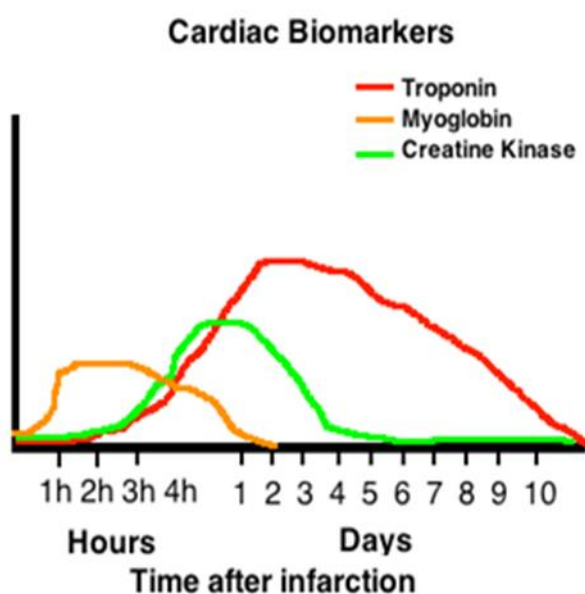


Figure 2.1. Elevation of cardiac biomarker with time after infarction

2.10. Myoglobin

Myoglobin is released into circulation with any damage to muscle tissue, including myocardial necrosis. The markers obvious weakness is the low specificity due to the presence of high levels of myoglobin in skeletal muscle. Myoglobin should be used in conjunction with other serum markers, because its level peaks and falls rapidly in patients with ischemia. Therefore, myoglobin is suggested not to be used on its own but only in the context of other markers, ECG and clinical evaluation (Rosenblat et al. 2012). Myoglobin is released soon after chest pain begins, and increased levels can sometimes be found one to two hours after the AMI has begun. Myoglobin reaches its highest levels

in plasma from 6 to 12 hours after an AMI and disappears from the bloodstream 12-24 hours after the onset owing to its rapid clearance by the kidneys (Bel et al. 2003).

2.11. Fatty Acids

Fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides or phospholipids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP (Marin et al. 2012). Fatty acid chains differ by length, often categorized as short to very long. Short-chain fatty acids (SCFAs) are fatty acids with aliphatic tails of fewer than six carbons (e.g. butyric acid). Medium-chain fatty acids (MCFAs) are fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain triglycerides. Long-chain fatty acids (LCFA) are fatty acids with aliphatic tails 13 to 21 carbons. (Roth 2013). Very long chain fatty acids (VLCFA) are fatty acids with aliphatic tails longer than 22 carbons.

2.11.1. Unsaturated fatty acids

Unsaturated fatty acids have one or more double bonds between carbon atoms. Essential fatty acids (EFAs) are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. Only two fatty acids are known to be essential for humans: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid). In the body, essential fatty acids serve multiple functions. In each of these, the balance between dietary ω -3 and ω -6 strongly affects function. Essential fatty acids play a part in many metabolic processes, and there is evidence to suggest that low levels of essential fatty acids, or the wrong balance of types among the essential fatty acids, may be a factor in a number of illnesses, including osteoporosis (Whitney and Rolfes 2008). Essential fatty acid deficiency results in dermatitis similar to that seen in zinc or biotin deficiency. Omega-9 fatty acids (ω -9 fatty acids or n-9 fatty acids) are a family of unsaturated fatty acids which have in common a final carbon-carbon double bond in the omega-9 position; that is, the ninth bond from the methyl ends of the fatty acid. Some omega-9 fatty acids are common components of

animal fat and vegetable oil. The most omega-9 fatty acid important is oleic acid (18:1, n-9), which is a main component of olive oil.

Omega-6 fatty acids (also referred to as ω -6 fatty acids or n-6 fatty acids) are a family of pro-inflammatory and anti-inflammatory polyunsaturated fatty acids that have in common a final carbon-carbon double bond in the n-6 position, that is, the sixth bond, counting from the methyl end (Nowak 2010). The biological effects of the omega-6 fatty acids are largely produced during & after physical activity for the purpose of promoting growth and during the inflammatory cascade to halt cell damage and promote cell repair by their conversion to omega-6 eicosanoids that bind to diverse receptors found in every tissue of the body.

Linoleic acid (18:2, n-6), the shortest-chained omega-6 fatty acid, is one of many essential fatty acids and is categorized as an essential fatty acid because the human body cannot synthesize it. Mammalian cells lack the enzyme omega-3 desaturase and therefore cannot convert omega-6 fatty acids to omega-3 fatty acids. Closely related omega-3 and omega-6 fatty acids act as competing substrates for the same enzymes. This outlines the importance of the proportion of omega-3 to omega-6 fatty acids in a diet (Bibus and Lands 2015). The conversion of cell membrane arachidonic acid (20:4n-6) to omega-6 prostaglandin and omega-6 leukotriene eicosanoids during the inflammatory cascade provides many targets for pharmaceutical drugs to impede the inflammatory process in atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes, and tumors.

Omega-3 fatty acids also called ω -3 fatty acids or n-3 fatty acids are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the end of the carbon chain (Simopoulos 2002).

Three types of omega-3 fatty acids involved in human physiology are: α -linolenic acid (ALA) (found in plant oils), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (both commonly found in marine oils). Marine algae and phytoplankton are primary sources of omega-3 fatty acids. Common sources of plant oils containing the omega-3 ALA fatty acid include walnut, edible seeds, clay sage seed oil, algal oil,

flaxseed oil, and hemp oil, while sources of animal omega-3 EPA and DHA fatty acids include fish oils, and egg oil (MacLean et al. 2006).

Omega-3 fatty acids are important for normal metabolism. Mammals are unable to synthesize omega-3 fatty acids, but can obtain the shorter-chain omega-3 fatty acid ALA (18 carbons and 3 double bonds) through diet and use it to form the more important long-chain omega-3 fatty acids, EPA (20 carbons and 5 double bonds) and then from EPA, the most crucial, DHA (22 carbons and 6 double bonds) (MacLean et al. 2006).

Evidence does not support a beneficial role for omega-3 fatty acid supplementation in preventing cardiovascular disease (including myocardial infarction and sudden cardiac death) or stroke (Kwak et al. 2012). However, omega-3 fatty acid supplementation greater than one gram daily for at least a year may be protective against cardiac death, sudden death, and myocardial infarction in people who have a history of cardiovascular disease (Casul et al. 2013). No protective effect against the development of stroke or all-cause mortality was seen in this population. Eating a diet high in fish that contain long chain omega-3 fatty acids does appear to decrease the risk of stroke (Delgado et al. 2012).

Evidence suggests that omega-3 fatty acids modestly lower blood pressure (systolic and diastolic) in people with hypertension and in people with normal blood pressure (Miller et al. 2014). Some evidence suggests that people with certain circulatory problems, such as varicose veins, may benefit from the consumption of EPA and DHA, which may stimulate blood circulation and increase the breakdown of fibrin (Wang et al. 2006).

Studies indicate that increased total concentrations of serum free fatty acid or impaired suppression are associated with metabolic risk markers such as metabolic syndrome (Yinghua et al. 2011), abdominal obesity, insulin resistance (Zoratti et al. 2000) and increased familial risk of cardiovascular disease (Carlsson et al. 2000). The composition of FFA has been less studied in this context, however. In particular the relation between fatty acid profile in plasma FFA and myocardial MI has been studied only to a limited extent. According to (Kondo et al. 1986) a low ratio of eicosapentaenoic acid (EPA) to arachidonic acid can be a risk factor for coronary heart disease (CHD), and this is a result of low EPA concentrations. Another study has found the percentage content of arachi-

donic acid in serum FFA lower in patients with fetal MI than in normal subjects (Skuladottir et al. 1988).

Long term intake of fatty acids not formed by the body is reflected in the content of these fatty acids in adipose tissue (Leaf et al. 1995). Coronary risk has been associated with the content of some of these fatty acids in adipose tissue (Seidelin et al. 1992) most often explained as effects of fatty acid intake on plasma lipoproteins, because plasma FFA to a large extent is mobilized into the FA composition of serum phospholipids can be used to track dietary intake of FA for a period of a few weeks and also reflects endogenous FA metabolism (Skeaff et al. 2006), regulated by different enzymes, such as desaturases and elongases. The FA composition in serum can be used as an indicator for the risks of some metabolic and cardiovascular diseases (Vessby et al. 2002). High proportions of palmitic acid (C:16) and low concentrations of linoleic acid (C18:2n-6) in serum cholesteryl esters are characteristic of these populations in Western countries (Warensjo et al. 2006).

In this case-control study we have related the fatty acid pattern of plasma FFA to the risk of having a first MI. The aim was to explore whether the proportions of particular FAs in the serum FFA fraction may be independent markers of MI risk, or whether the findings are parallel to those with adipose tissue or diet. This work is a study investigating association between first MI and FA composition in adipose tissue, diet and serum lipid fractions (Pedersen et al. 2000).

3. MATERIALS AND METHOD

3.1. Design of the Study

The present study is a case-control study.

3.2. Study Populations

The subjects of our study were grouped into two categories:

Healthy controls (group I): Thirty-three (33) randomly selected subjects served as control, all were healthy volunteers and had no evidence for any blood diseases.

Myocardial infarction patients (group II): Forty-one (41) patients with myocardial infarction at hospital of Erbil city were eligible for the study.

3.3. Collection of Blood Samples

Fasting blood samples were collected from these subjects, by using disposable syringes, 5 ml of early morning venous blood samples were drawn aseptically from each subject, 2.0 ml of this volume was collected with ethylene diamine tetra acetic acid (EDTA) tube for hematological evaluation cardiac biomarkers (creatin kinase-MB, troponin I), and the remaining volume (3.0 ml) of blood was collected in a gel tube for 15 minute at room temperature. Serum was separated by centrifugation at 4000 rpm for five minutes, and then each subject serum was stored and frozen at -20 C. The clear serum samples were employed for the estimation of total cholesterol, Triglycerides, LDL-cholesterol, HDL-cholesterol, and fatty acids (saturated, monounsaturated and poly unsaturated fatty acids).

3.4. Time of the Study

The present study including data collection, examination of patients and performance of all the laboratory investigation analysis was carried out from 2 January to 2 June 2016, by collaboration between surgical specialty hospital- cardiac centre and Bingol University of Science and Technology Institute.

3.5. Research Ethics

1. We kept our data in a safe place so that no one can review except our self.
2. We saved the data in our personal computer so that no one could see it.
3. Surely, causes no harm to other human participant in the future.

3.6. Materials

3.6.1. Chemicals

The laboratory chemicals, kits and reagents used in this research are listed below with their companies and their countries of origin:

Table 3.1. Chemicals, reagents and suppliers

No.	Chemicals/Reagents	Company	Country of origin
1	Creatine kinase-MB	Roche, Cobas kit	USA
2	Troponin I	Roche, Cobas kit	Germany
3	S.HDL-cholesterol	Roche, Cobas c kit	Germany
4	S.LDL-cholesterol	Roche, Cobas c kit	Germany
5	S. Cholesterol kit	Roche, Cobas kit	Germany
6	S. Triglyceride kit	Roche, Cobas kit	Germany

3.6.2. Apparatus

The tools have been used in this study are listed in the table below with their companies and their countries of origin:

Table 3.2. List of tools that used in the study

No.	Items	Type/ Company	Country of origin
1	Syringe (5ml,10ml)	Set Medikal	Turkey
2	Gloves		China
3	Eppendrof tube (3ml,5ml)	Vacu test	Italy
4	Water bath	Mammert	Germany
5	Micropipette 100µl-1000µl + tips	Boeco	Germany
6	Chemical tube (Gel and clot act. tube)	Vacu test	Italy
7	Hematology tube (K ₂ EDTA)	Vacu test	Italy
8	Timer	Digital	China

3.6.3. Equipment

The instruments that were used in this study are listed in the table below with their company and origin:

Table 3.3. List of instruments used in this study

No.	Instruments model	Company	Origin
1	Fully automated biochemistry analyzer Cobas C 311	Roche	Germany
2	Fully automated hematological analyzer	Sysmex xt-2000i5 th generation	Japan
3	Centrifuge	Hettlich (D-78532)	Germany
4	Refrigerator	BEKO	Turkey
5	Spectrophotometer	Cecil 7200	Germany

3.7. Experimental Protocols

3.7.1. Determination of Cardiac Biomarkers (Troponin, CK-MB)

The alter triage cardiac panel is a single use fluorescence immunoassay device designed to determine the concentration of CK-MB, and troponin I in EDTA anticoagulated plasma specimens.

The test procedure involves the addition of several drops of an EDTA anticoagulated plasma specimen to the sample port on the test device. After addition of the specimen, the specimen reacts with fluorescent antibody conjugates and flows through the test device by capillary action. Complexes of each fluorescent antibody conjugate are captured on discrete zones specific for each analysis.

The test device is inserted into the Alere triage meter. The meter is programmed to perform the analysis after the specimen has reacted with the reagents within the Test Device. The analysis is based on the amount of fluorescence, the meter detects within a measurement zone on the Test Device. The concentration of the analytes in the specimen is directly proportional to the fluorescence detected. The meter measures the target analyte automatically. The results are displayed on the meter screen in approximately 20 minutes from the addition of specimen. All results are stored in the meter memory to display or print when needed.

3.7.2. Determination of Serum Fatty Acids

In the present study, we used gas chromatography with mass spectrometry (GC-MS) technology to study profiles of free fatty acid (FFA) in MI. This allowed the identification of key FFAs that could distinguish between MI patients and controls; these are likely to provide new biomarkers for the diagnosis of subclinical MI and thereby permit early intervention designed to prevent disease progression.

3.7.2.1. Solvents and Standards

Fatty acid standards: myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1 n-7), stearic acid (C18:0), oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), linolenic acid (C18:3 n-3), and arachidonic acid (C20:4 n-6) at $\geq 99\%$ purity were purchased from Sigma (St. Louis, MO, USA). The 10% H₂SO₄ /CH₃OH solution was freshly prepared by diluting H₂SO₄ (purity $\geq 98.0\%$) with chromatographic grade methanol, n-hexane (chromatographic grade), ethyl acetate (analytical reagent), and NaCl (analytical grade) were purchased from Tianjin Guangfu Chemical Reagent, Co. (Tianjin).

3.7.2.2. Preparation of Standard Solutions

Stock solutions of the 8 fatty acids and an internal standard (heptadecanoic acid) were prepared at 1000 ug/mL in methanol. Working solutions were made up with methanol at concentrations of 3.0–900 ug/mL. All standard solutions were stored at -20°C until required.

3.7.2.3. Sample Preparation

Fasting blood samples were immediately centrifuged at 3000 g for 10 minutes at room temperature and then stored at -80°C before analysis. Samples were randomly selected for the extraction of free fatty acids and GC-MS acquisition. Aliquots (200 μ L) of serum were spiked with internal standard (IS) working solution (200 μ L heptadecanoic acid C17: 0, 200 g/mL); 1 mL 0.05% H₂SO₄ was added to precipitate proteins and fatty acids were extracted with 3mL of ethyl acetate using a vortex mixer for 60 seconds and centrifugation at 4000 rpm for 10 minutes at room temperature. The ethyl acetate phase was evaporated to dryness under N₂. Following the addition of 2 mL of 10% H₂SO₄-CH₃OH to the residue and incubation at 62 °C for 2 hours, 2 mL of saturated NaCl and 2 mL of hexane were sequentially added and mixed for 60 seconds to obtain the fatty acid methyl esters. Organic phases were evaporated to dryness under N₂ gas and samples were taken up into 100 L hexane prior to analysis.

3.7.2.4. Gas Chromatography-Mass Spectrometry (GC-MS)

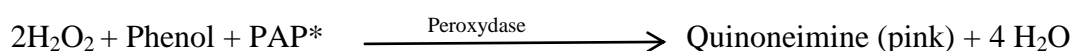
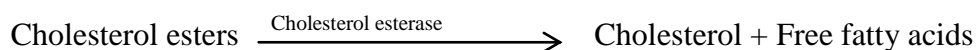
Gas chromatography-mass spectrometry (GC-MS) analysis was performed using gas chromatography coupled to an ion-trap mass spectrometer (Agilent brand and 6975 model, USA). Separation was performed on a capillary column (HP 88, 60 m 0.25 mm inner diameters). Helium was used as the carrier gas with a flow rate of 1.0 mL/minute. The temperature of the injector was 230°C; 1.0 uL aliquots were injected and the split ratio of the injector was 1:10. Free fatty acid methyl esters were separated at constant flow with the following temperature program: (1) 50°C for 2minutes; (2) increase to 200°C at 10°C/min; (3) 200 °C for 10 minutes; (4) increase to 220°C at 10°C/min; and (5) 220°C for 15 minutes. The transfer line was maintained at 230°C. The ion-trap mass spectrometer was operated in electron impact (EI) mode and full scan monitoring mode (m/z 30–450). Source temperature was set at 230°C and electron energy was set at 70 eV.

3.7.3. Determination of Serum Lipid Profiles

3.7.3.1. Serum Total Cholesterol (TC)

The TC was estimated by using Cobas diagnostic kit (Roche/COBAS 311 INTEGRA), with fully automated chemical analyzer.

Principle: An enzymatic method was used for determination of serum total cholesterol the reaction scheme as follows:



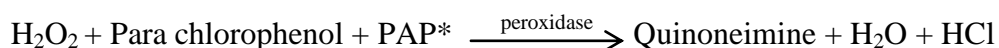
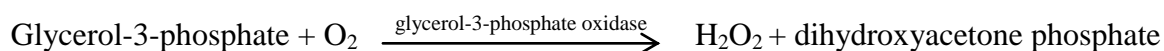
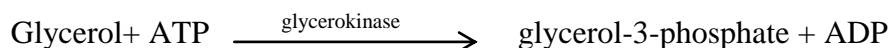
PAP* (4-amino antipyrine) (Allain et al. 1974).

3.7.3.2. Serum Triglycerides (TG)

The TG was estimated by using Cobas diagnostic kit (Roche/COBAS 311 INTEGRA), with fully automated chemical analyzer.

Principle:

Serum Triglyceride was determined automatically by an enzymatic colorimetric method, Fossati and Prencipe method are associated with Trinder reaction. The reaction scheme is as following. (Fossati and Prencipe, 1982):



PAP* 4-amino antipyrine

The absorbance of the colored complex (quinoneimine), which is proportional to the amount of triglyceride in the specimen, is measured at 500 nm.

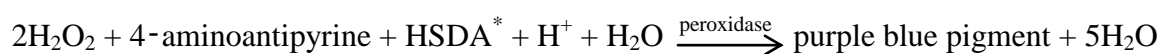
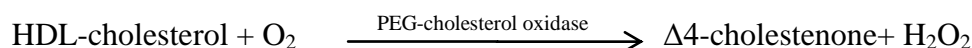
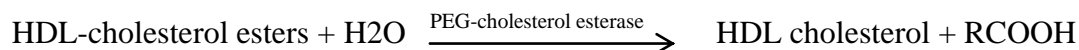
3.7.3.3. Serum High Density Lipoprotein Cholesterol (HDL-C)

The HDL-C was estimated by using COBAS INTEGRA HDL-Cholesterol plus 3rd generation diagnostic kit (Roche/COBAS 311 INTEGRA), with fully automated chemical analyzer.

Principle-Homogeneous enzymatic colorimetric assay:

In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to

the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4 -cholestenone and hydrogen peroxide. (Sugiuchi et al. 1995; Matsuzaki et al. 1996)



HSDA* Sodium N- (2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline
(PEG) polyethylene glycol

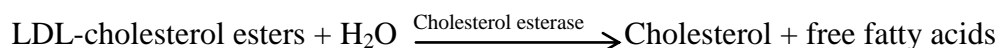
The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

3.7.3.4. Serum Low Density Lipoprotein Cholesterol (LDL-C)

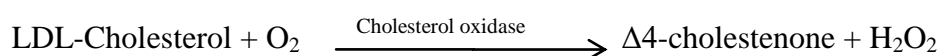
The LDL-C was estimated by using LDL-Cholesterol plus 2nd generation diagnostic kit (Roche/Hitachi Cobas c 311), with fully automated chemical analyzer.

Principle:

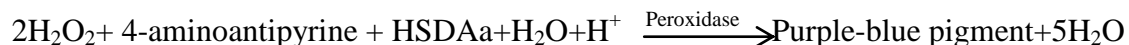
Homogeneous enzymatic colorimetric assay, Reaction scheme principle is as the followings:



Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4 -cholestenone and hydrogen peroxide.



(Abs. max = 585 nm)

HSDAa = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline.

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically (Rifai et al. 1992).

3.8. Statistical Analysis

Data were analyzed with SPSS (statistical package for social science) (Version 20) for Windows 7.0 and Microsoft Excel work sheet 2010. All descriptive data are expressed as mean \pm standard error of the mean (SEM) and standard deviation (SD) for selected variable. Differences between smokers and nonsmokers were assessed using independent sample t tests; Statistical significance was inferred at a two-tailed p value < 0.05 .

4. RESULTS

4.1. Demographics of the MI Patients and Healthy Controls

Table 4.1 provides the number, (Mean \pm SE), of ages, BMI, SBP and DBP in control and MI patients. The results obtained reveal that the mean age and BMI was match between control and MI groups, and also systolic blood pressure (SBP) and diastolic blood pressure (DBP), in control and pregnant groups (mean \pm SE), there is no any significant difference of SBP between control and MI groups, but mean DBP of MI was higher than that of control group, $p < 0.05$.

Table 4.1. Demographics of the MI patients and healthy controls

Parameter	Healthy controls (n=33)	MI patients (n= 41)	P value
Age (years)	53.83 \pm 13.93	55.88 \pm 14.93	0.287 (NS)
BMI (Kg/m ²)	29.55 \pm 5.61	28.95 \pm 6.48	0.386 (NS)
SBP (mm Hg)	125.9 \pm 17.17	130.6 \pm 25.38	0.208 (NS)
DBP (mm Hg)	72.51 \pm 9.33	78.9 \pm 12.81	0.0099 ($p < 0.05$)

4.2. Cardiac Biomarkers

Table (4.2), shows the mean S. troponin and CK-MB levels in the normal and MI groups. The results obtained indicate that the mean level of S. troponin and CK-MB in MI group were significantly very higher than that of control group ($p < 0.0001$).

Table 4.2. Mean \pm SD of biochemical markers of control and MI groups

Parameter	MI patients (n= 41)	Healthy controls (n=33)	P value
Troponin	1.6 \pm 0.36	0.00714 \pm 0.001	$p < 0.0001$
CK-MB	75.88 \pm 15.6	2.232 \pm 0.17	$p < 0.0001$

4.3. Fatty Acids Profiles

Table 4.3, and Figures 4.1- 4.8, shows the Mean \pm SD, of fatty acids myristic, palmitic, stearic, palmitoleic, oleic, linoleic, linolenic and arachidonic acids in control and MI groups, the mean levels of each S. myritic, palmitolic, linolenic and arachidonic acids in MI patients were highly significantly ($P < 0.0001$) lower than that of control group, while S.palmitic acid in MI group non significantly higher than that of control group, and each of the S. steraric , oleic , and linoleic acids in MI were significantly lower than that of control group, ($P = 0.0495, 0.0011, \text{ and } 0.02$) respectively.

Table 4.3. Mean \pm SD of fatty acids composition of control and MI groups

Fatty acids	MI group Numbe (41)	Control group Number (33)	P value
Myristic acid (C 14:0)	0.2837 \pm 0.8197	0.3042 \pm 0.599	p< 0.0001
Palmitic acid (C 16:0)	39.51 \pm 20.22	35.80 \pm 7.045	0.0159
Stearic acid (C18:0)	24.49 \pm 18.58	26.82 \pm 14.96	0.0495
Palmitoleic acid (C 16:1)	0.3166 \pm 0.7654	0.667 \pm 0.8476	p< 0.0001
Oleic acid (C18:1)	9.563 \pm 7.106	11.00 \pm 4.741	p< 0.0011
Linoleic acid (C18:2)	20.24 \pm 13.99	22.73 \pm 8.397	0.02
Linolenic acid (C18:3)	0.0200 \pm 0.1281	0.02485 \pm 0.1427	p< 0.0001
Arachidonic acid (C20:4)	0.5773 \pm 1.261	2.655 \pm 1.739	p< 0.0001

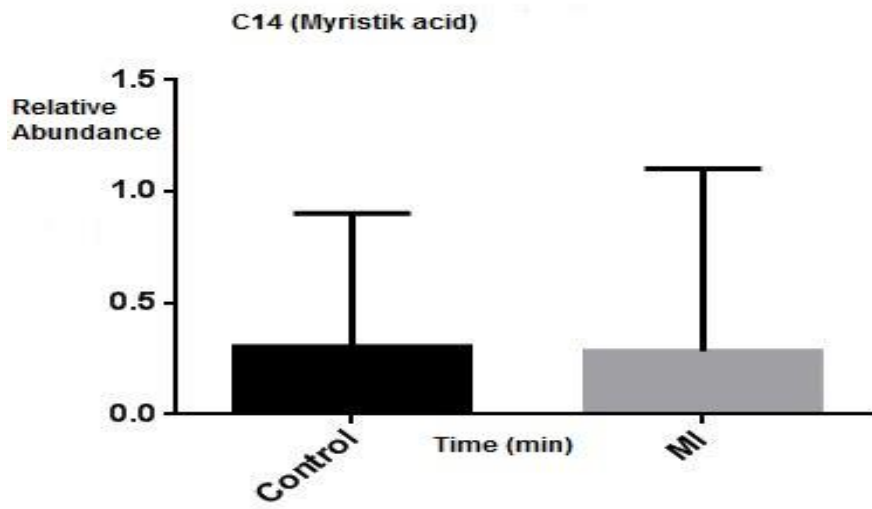


Figure 4.1. Myristic acid in control and MI groups

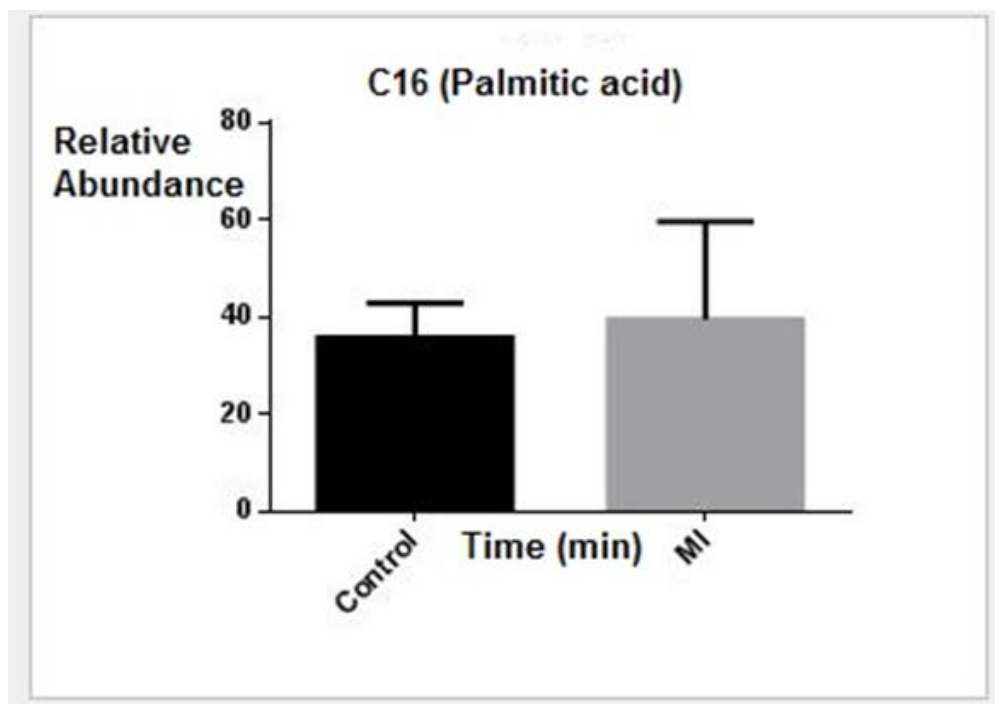


Figure 4.2. Palmitic acid in control and MI groups

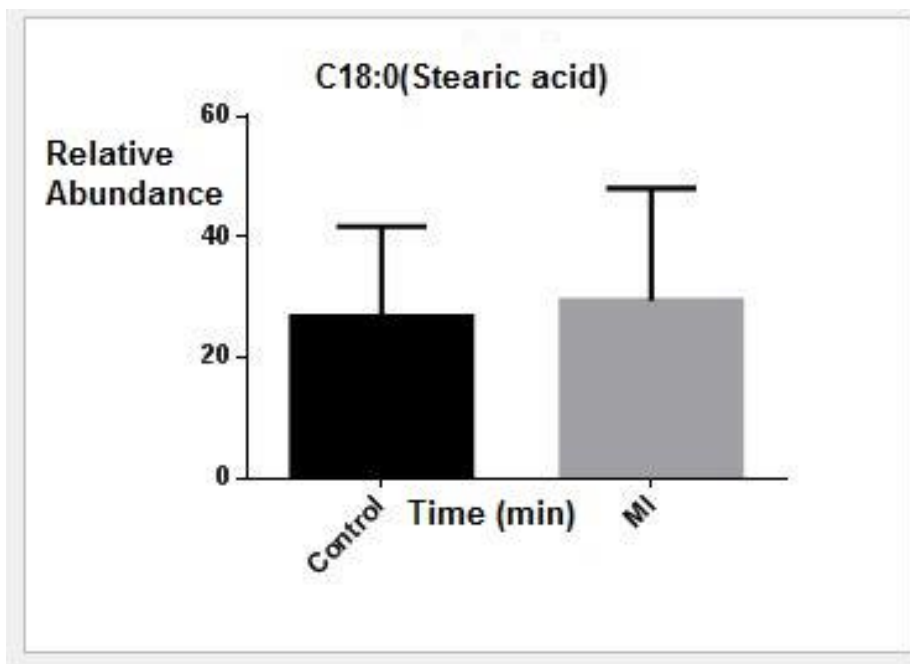


Figure 4.3. Stearic acid in control and MI groups

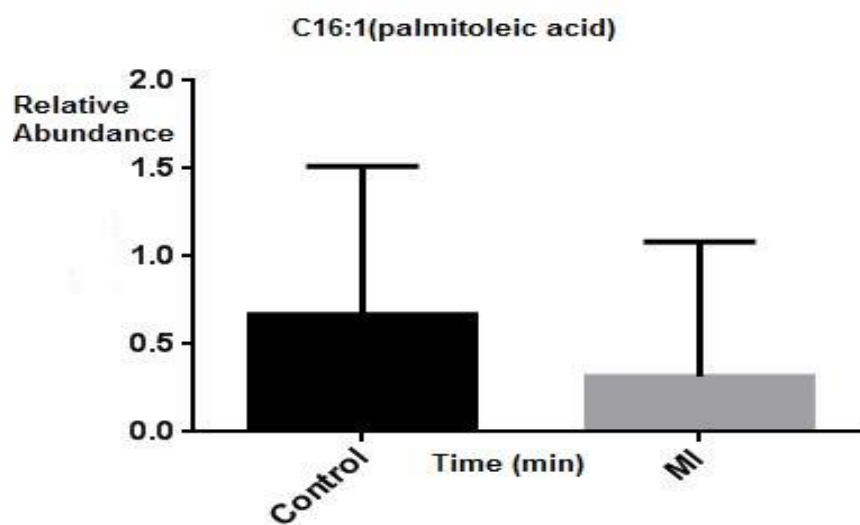


Figure 4.4. Palmitolic acid in control and MI groups

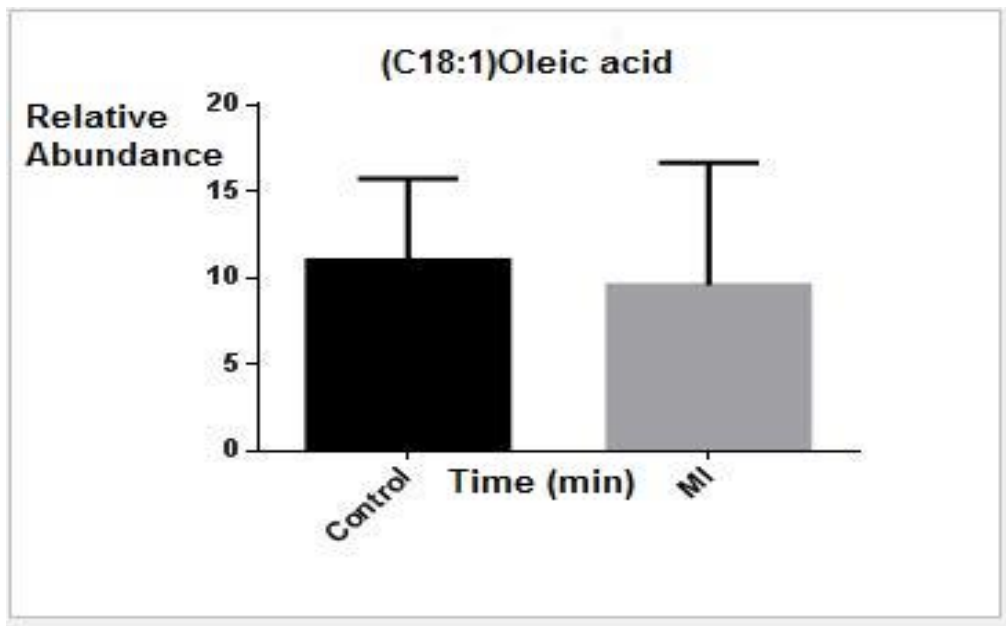


Figure 4.5. Oleic acid in control and MI groups

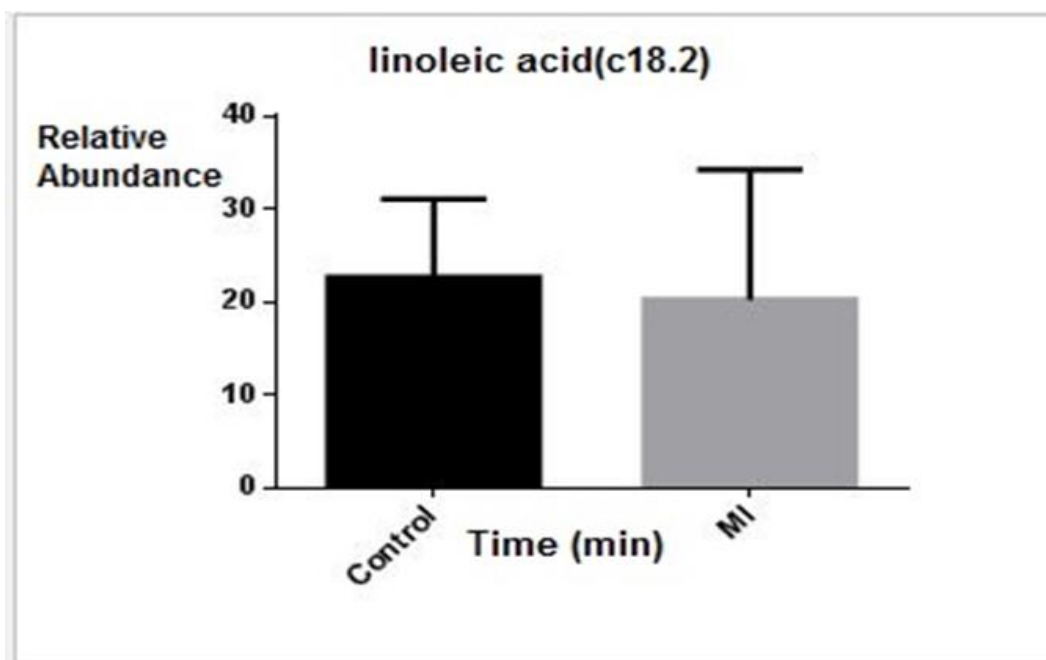


Figure 4.6. Linoleic acid in control and MI groups

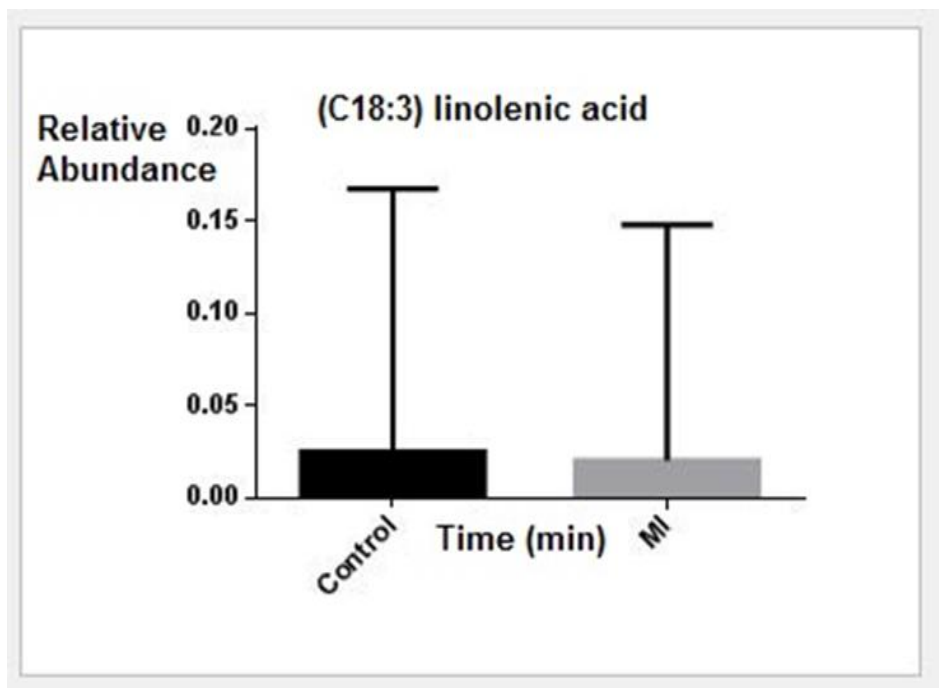


Figure 4.7. Linolenic acid in control and MI groups

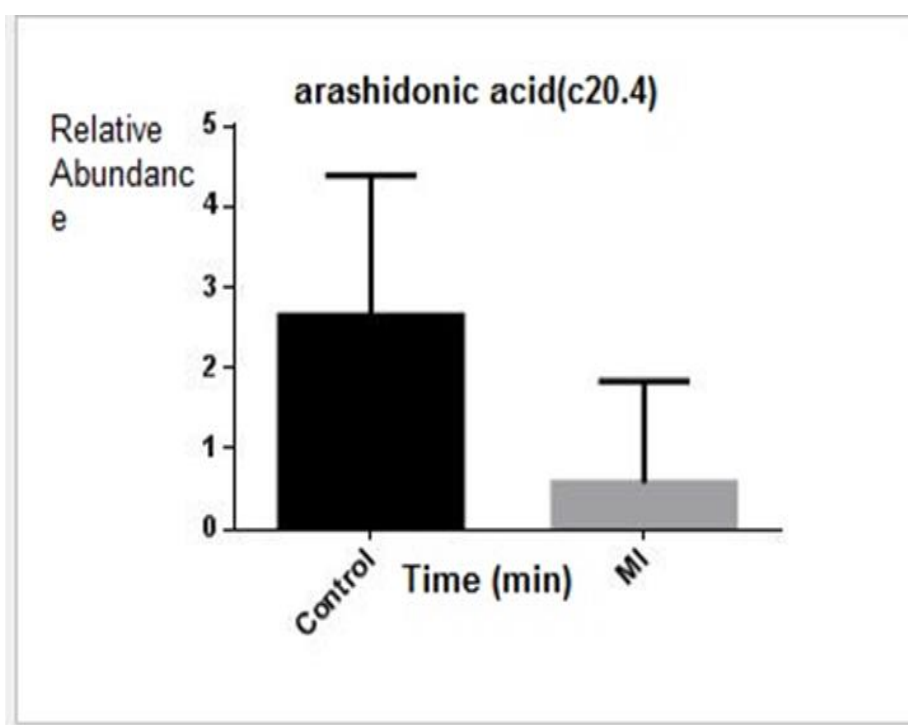


Figure 4.8. Arachidonic acid in control and MI groups

4.4. Profiles of Fatty Acid Types

Table 4.4, provides the Mean \pm SD, of each of the total saturated, monounsaturated and poly unsaturated fatty acids in control and MI groups, the results obtained that the mean of S. total saturated fatty acids in MI group was non-significantly higher than that of control group, S. total monounsaturated fatty acids in MI significantly ($P<0.05$) lower than that of control group, and S. total polyunsaturated fatty acids in MI group significantly ($P<0.001$) lower than that of control group.

Table 4.4. Mean \pm SD of fatty acid type's profiles of control and MI groups

Fatty acids	MI group Number (41)	Control group Number (33)	P value
Total saturated fatty acids (Myristic, Stearic, Palmitic)	64.28 \pm 13.21	62.92 \pm 7.54	NS
Total monounsaturated fatty acids (Oleic, Palmitoleic)	9.88 \pm 3.94	11.67 \pm 2.79	$P<0.05$
Total polyunsaturated fatty acids (Linoleic, Linolenic, Arachidonic)	20.84 \pm 5.13	25.41 \pm 3.43	$P<0.001$

4.5. Lipid Profiles

Table 4.5, Shows the Mean \pm SD, of each of the lipid profile, indicated that each of S. triglycerides and S. total cholesterol in MI were significantly higher than that of controls ($P<0.001$), S.LDL in MI group was significantly higher than that of controls ($P<0.0001$), while S.HDL in MI group was significantly lower than that of control group ($P<0.001$).

Table 4.5. Mean \pm SD of lipid profile levels of control and MI groups

Parameters	MI group Number (41)	Control group Number (33)	P value
S. triglycerides	219 \pm 31.2	138.9 \pm 6.6	$P<0.001$
S. total cholesterol	214.6 \pm 13.4	178.7 \pm 5.23	$P<0.001$
S.LDL	157.22 \pm 6.6	106 \pm 8.9	$P<0.0001$
S.HDL	37.18 \pm 1.6	44.8 \pm 2.4	$P<0.001$

5. DISCUSSION

5.1. Myocardial Infarction

Acute myocardial infarction (AMI) is one of the major causes of morbidity and mortality in the world (Ojha et al. 2008). The most common cause of an AMI is atherosclerosis IHD with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion. Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable (Pasupathi et al. 2009).

5.2. Age distribution

An increase in mortality was observed by AMI in old people. Age is a powerful predictor of short term outcome in AMI. In the present study, the average age of AMI group was 55.88 years, this is identical with the finding reported by each of the (Majid et al. 2007), and (Aziz 2010), who found that the prevalence of AMI was in the mean age of 57 and 59.49 years respectively.

5.3. Cardiac biomarkers

Biochemical markers in (MI) patients and control group:

Biochemical markers play a pivotal role in the diagnosis and management of patients with acute coronary syndrome (ACS). The most common cardiac biomarkers used in the evaluation of acute coronary syndrome are troponin T and I, and CK-MB. Despite the success of the current cardiac markers, there is always a need to discover and develop new biomarkers for diagnosis and management of (AMI).

5.3.1. Troponin

Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that is integral to muscle contraction in skeletal and cardiac muscle. Out of these troponins T and I are the markers of choice for detecting the heart damage (Pasupathi et al. 2009). In our study, we investigate troponin I, because cardiac troponin I is not expressed in skeletal muscle, so is the most sensitive for MI. Serum cardiac troponin I > 0.4 ng/ml suggests myocardial damage.

In the current study, there was highly significant increases of serum cardiac troponin I (cTn I) levels ($P < 0.0001$) in MI patients when compared to the control group. These findings are in accordance with previous studies of (Kasap et al. 2007; Costa et al. 2008 and Khan et al. 2013). This significant increment related strongly to the extent of MI injury. (Chia et al. 2008) found that a strong correlation of troponin I with the extent of myocardial cTn I is a cardiac-specific protein, which rapidly increases after MI by a release of a loosely bound pool, due to degradation of myofilaments in the area of infarction, so more infarct tissue result in more cardiac troponin release.

5.3.2. Creatin Kinase-MB (CK-MB)

Because of superior sensitivity and specificity, the serum troponin have replaced (CK-MB) as the gold standard for the biochemical diagnosis of myocardial injury (Joarder et al. 2013). Higher CK-MB may point more directly to heart damage if you have a heart attack. This result suggested that MI induces a higher level of (CK-MB) secretion or support the hypothesis that injured heart muscle cells release or elevate (CK-MB) in your blood.

The statistical analysis in our study showed that serum (CK-MB) level was significantly increased ($P < 0.0001$) in MI patients compared to the controls. This is in accordance with (Adams et al. 1994; Roqueta et al. 1995; Apple et al. 2009).

Increased (CK-MB) can usually be detected in someone with a heart attack about 3-6 hours after the onset of chest pain. The level of CK-MB peaks in 12-24 hours and then returns to normal within 3-4 days after an (AMI) (Nigam 2007; Basu et al. 2009).

The CK-MB isoenzyme is not considered to be myocardium specific. The combination of CK-MB and cardiac troponin I provide much higher sensitivity and had a much higher negative predictive value for the evaluation of MI than cardiac troponin I or CK-MB alone. In experimental animals, coronary occlusion appears to induce synthesis of CK-MB. This may improve the sensitivity for detection of MI in these groups (Adams et al. 1994; Singh et al. 2011).

5.4. Fatty Acids Profiles

The results of this study shows the Mean \pm SD, of fatty acids myristic, palmitic, stearic, palmitolic, oleic, linoleic, linolenic and arachidonic acids in control and MI groups, the mean levels of each S. myristic, palmitolic, linolenic and arachidonic acids in MI patients were highly significantly ($P < 0.0001$) lower than that of control group, while S. palmitic acid in MI group non significantly higher than that of control group, and each of the S. stearic, oleic, and linoleic acids in MI were significantly lower than that of control group, ($P < 0.0495$, 0.0011 , and 0.02) respectively. Similar results obtained by (Pedersen et al. 2000; Edmond et al. 2008; Elen et al. 2013).

(Olever et al. 2008) concluded that measurement of serum-F.F.A. is a new and valuable early predictive index of the vulnerability of patients with acute myocardial infarction to serious arrhythmias. This relationship between serum-F.F.A. levels and arrhythmias could result from increased catecholamine activity particularly that of noradrenalin, or it could be due directly to an increase in myocardial oxygen consumption caused by the utilization of F.F.A. as the major energy substrate. Both mechanisms would intensify myocardial hypoxia in an already ischemic heart.

Intake of very long-chain n-3 fatty acids as reflected in adipose tissue content is inversely associated with risk of myocardial infarction. Trans fatty acids, linoleic and alpha-linolenic acid were interring correlated and associated with increased risk. It is suggested that the increased risk may be connected to trans- fatty acids or to some other factor associated with margarine consumption (Pedersen et al. 2000). Polyunsaturated fats are inversely associated with the metabolic syndrome, whereas saturated fatty acids are positively associated with the metabolic syndrome, probably through their effect on

lipids, adiposity, insulin, and blood pressure (Edmond et al. 2008). Another study conducted by (Yinghua et al. 2011), they obtained that Serum levels of polyunsaturated fatty acids are low in Chinese men with metabolic syndrome, whereas serum levels of saturated fatty acids, zinc, and magnesium are high.

5.5. Fatty Acid Types Composition

The results obtained that the mean of S. total saturated fatty acids in MI group was non-significantly higher than that of control group, S. total monounsaturated fatty acids in MI significantly ($P < 0.05$) lower than that of control group, and S. total polyunsaturated fatty acids in MI group significantly ($P < 0.001$) lower than that of control group. The same results obtained by (Garemo et al. 2007; Kabagambe et al. 2008; Micallef et al. 2009).

Our study showed that total SFA in the serum PL was higher in the Chinese male population with MetS, whereas total n-3 and n-6 PUFA were lower, especially in 22:6n-3 and 20:4n-6 compared with subjects without MetS. The estimated delta-5 desaturase (D5D) activity decreased in subjects with MetS and was negatively associated with the components of MetS, including BMI, fasting blood glucose, and diastolic blood pressure. High levels of serum SFA in MetS positively contributed to MetS components, such as BMI, serum glucose, blood pressure, and TGs, in Chinese men. Our findings are consistent with previous investigations in the Western population (Kabagambe et al. 2008), in which SFA in serum lipid and erythrocytes were positively associated with MetS. Our results suggest that the increased SFA in serum PL is also common in Chinese men with MetS. Fatty acid composition of serum, platelet, and erythrocyte PLs reflects an individual's type of dietary fat intake (Li et al. 2007). Therefore, compositions of serum PL FAs can be used as a surrogate marker of dietary intake of FAs. The increased serum PL, SFA in the MetS group may represent an increased dietary intake of SFA.

The present study found that n-3 PUFA, especially the proportion 22:6n-3 was decreased and negatively contributed to the risk of MetS in Chinese men. We also found a negative correlation between total n-3 PUFA and 22:6n-3 in serum PL and BMI. Furthermore, total n-3 PUFA was negatively correlated with waist circumference. These findings suggest that n-3 PUFA of serum PL is negatively correlated with obesity, especially

central obesity. Our data are consistent with findings in an Australian population, where obese individuals had significantly lower plasma lipid n-3 PUFA (Micallef et al. 2009). Furthermore, the concentration of n-3 PUFA in serum PL was significantly lowered in obese versus lean, age matched, and females. These findings including ours may indicate that the intake of n-3 PUFA is lowered in individuals with MetS, especially with obesity. Previous studies indicate that n-3 PUFA intake plays an important role in preventing weight gain and improving weight loss (Couet et al. 1997; Garemo et al. 2007).

When acute coronary artery occlusion leads to symptoms, and this is not always the case, there is stimulation of postganglionic sympathetic nerve endings with release of norepinephrine, and of the adrenal medulla with release of epinephrine. Both catecholamines are present in high concentrations in plasma and urine during the first 24-48 hours after the onset of symptoms. The concentrations of these catecholamines in plasma reach high levels within the first few hours after the onset of symptoms and later appear to be related to the severity of the infarct. Norepinephrine acts through, β -adrenergic receptors to activate the adenylyl cyclase system in adipose tissue causing conversion of ATP to cyclic AMP, and cyclic AMP activates a lipolytic system leading to hydrolysis of stored triglycerides to diglycerides, FFA, and also glycerol. While some re-esterification of FFA occurs, the net effect is release of FFA and glycerol into the circulation.") In acute myocardial infarction, plasma FFA concentrations are elevated within 4 hours of the onset of symptoms. The highest values are found on the first day, and by the sixth day normal values are usually reached. Glycerol levels are also elevated. There is a close relationship between blood catecholamine and FFA values in myocardial infarction (Oliver 1972).

5.6. Lipid Profiles

It has long been known that lipid abnormalities are a major risk factor in the occurrence of heart disease, and bearing in mind that the heart belongs to circulatory organs suspicion inevitably arises that hyperlipidemia plays an important role in the onset of MI (Shivananda et al. 2010), in an attempt to shed light on the most important pathological effect of hyperlipidemia on MI, we assayed a series of serum samples of clinically defined stroke and health control.

Patient (MI) group had significantly higher level of uric acid ($P < 0.01$), total cholesterol ($P < 0.01$), triglyceride ($P < 0.01$), low density lipoprotein ($P < 0.01$), very low density lipoprotein ($P < 0.01$) and significantly lower level of high density lipoprotein ($P < 0.01$) than control group. The results of this study also showed a significant difference ($P < 0.01$) between males and females regarding uric acid, total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein and high density lipoprotein in both study groups (Moaed et al. 2014).

The serum lipids and lipoproteins were including S. TCh, TGs, LDL, and HDL. In this study, each of S. triglycerides and S. total cholesterol in MI were significantly higher than that of controls ($P < 0.001$), S.LDL in MI group was significantly higher than that of controls ($P < 0.0001$), while S.HDL in MI group was significantly lower than that of control group ($P < 0.001$). These results are in agreement with results obtained by other investigations (Iqbal et al. 2004), but disagreement with (Kumar et al. 1976), who observed that there is no significant hypercholesterolemia in patients of myocardial infarction in the Chandigarh area (Northern India).

Recent studies revealed that abnormal elevation of any or all lipids and/ or lipoproteins which known as hyperlipidemia is considered as modifiable risk factors that play a crucial role in the pathogenesis of atherosclerosis, MI, and biological etiology of the ischemic stroke (Harikumar et al. 2013). Hyperlipidemia usually classified as either familial caused by specific genetic abnormalities, or acquired when resulting from endocrine, renal or hepatic diseases (Hamad 2009).

The plasma lipids and lipoproteins are including (T. Cholesterol, TGs, Chylomicrons, VLDL, LDL and HDL). The LDL is the major cholesterol carrier of the bloodstream, while chylomicron and VLDL are transports TGs in the blood (Nilsson 2010).

Deposition of cholesterol and cholesterol ester from the plasma lipoproteins into the artery wall, elevates the level of chylomicron, VLDL, and LDL are often causes premature or more severe atherosclerosis, also it may leads to several diseases such as ischemic stroke, heart diseases, diabetes mellitus, hypothyroidism and renal dysfunction,

while HDL has ability in reverse cholesterol transport and having as inverse correlation with the risk of coronary heart disease and ischemic stroke (Murray et al. 2003).

The attenuation of the TG effect after control for HDL, cholesterol may be due to true confounding or more likely is the result of metabolic interactions. Recent data suggest that there are complex metabolic interrelationships between the TG and cholesterol ester-rich lipoproteins. TG levels are elevated in the setting of decreased lipoprotein lipase activity. This leads to higher chylomicron remnant and VLDL levels (both of which may be atherogenic) and lower HDL levels (which clearly promote atherogenesis). Thus, the ratio of TG/HDL may be a valuable marker for abnormal TG metabolism. In addition, lower lipoprotein lipase activity could prolong circulation time of VLDL and may result in increased density of VLDL particles. The subclass patterns of LDL may be dependent in part on VLDL density. A predominance of small dense LDL particles (LDL subclass pattern B), which appear to be more atherogenic, is strongly associated with elevated TG levels and lower HDL levels. Smaller LDL diameter may be the result of smaller more dense VLDL precursors resulting from abnormal TG metabolism (Hodes et al. 1994).

CONCLUSION

In this study, firstly cardiac biomarkers determined in myocardial infarction as a diagnosis of the disease, after that eight fatty acids determined by gas chromatography-mass spectrophotometers (GC-MS), generally saturated fatty acids in MI higher than that of control group, finally serum lipid profile (S.TC, S.TG, LDL, and HDL), each of S.TC, S.TG, and LDL in MI group were significantly higher than that of control group, while S.HDL in MI group was significantly lower than that of control group. The results of this study are summarized in Table 5.1.

Table 5.1 Mean \pm SD of cardiac biomarkers (troponin, CK-MB), fatty acid composition, and lipid profile levels of control and MI groups

Cardiac biomarkers			
Parameter	Healthy controls(n=33)	MI patients (n= 41)	P value
Troponin	0.00714 \pm 0.001	1.6 \pm 0.36	P<0.0001
CK-MB	2.232 \pm 0.17	75.88 \pm 15.6	P<0.0001
Fatty acids composition			
Fatty acids	Control group Number (33)	MI group Number (41)	P value
Myristic acid (C 14:0)	0.3042 \pm 0.599	0.2837 \pm 0.8197	P< 0.0001
Palmitic acid (C 16:0)	35.80 \pm 7.045	39.51 \pm 20.22	0.0159
Stearic acid (C18:0)	26.82 \pm 14.96	24.49 \pm 18.58	0.0495
Palmitolic acid (C 16:1)	0.667 \pm 0.848	0.3166 \pm 0.7654	P< 0.0001
Oleic acid (C18:1)	11.00 \pm 4.741	9.563 \pm 7.106	0.0011
Linoleic acid (C18:2)	22.73 \pm 8.397	20.24 \pm 13.99	0.02
Linolenic acid (C18:3)	0.0249 \pm 0.143	0.0200 \pm 0.128	P< 0.0001
Arachidonic acid (C20:4)	2.655 \pm 1.739	0.5773 \pm 1.261	P< 0.0001
Lipid profile			
Parameters	Control group Number (33)	MI group Number (41)	P value
S. triglycerides	138.9 \pm 6.6	219 \pm 31.2	P<0.001
S. total cholesterol	178.7 \pm 5.23	214.6 \pm 13.4	P<0.001
S.LDL	106 \pm 8.9	157.22 \pm 6.6	P<0.0001
S.HDL	44.8 \pm 2.4	37.18 \pm 1.6	P<0.001

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