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Research Article

DIAGNOSIS OF MYOCARDIAL ISCHEMIA USING PLASMA FREE FATTY ACID AS A BIOMARKER

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ABSTRACT

Objective: Diagnosis of myocardial ischemia (MI) in patients attending emergency intensive care unit (ICU) with symptoms of an acute coronary disease is often difficult. Biochemical markers such as cardiac troponin (cTn) and creatinine kinase MB (CK-MB) may not rise during reversible MI. Previous studies suggest unbound free fatty acid (FFA) increased significantly in ischemic related events. Thus, plasma FFA has shown to be an early biochemical marker. To diagnose MI using plasma FFA as a biomarker.

Methods: Blood samples were collected from 30 ischemic heart disease (IHD) patients admitted to ICU and 30 healthy volunteers for plasma FFA. Patients were diagnosed as IHD based on the clinical presentation, electrocardiogram (ECG), and coronary angiography findings, cTn, CK-MB. Plasma FFA was measured enzymatically with (acyl-CoA synthetase- acyl-CoA oxidase) non-esterified fatty acid kit (Randox Laboratories Ltd., Co.Antrium, United Kingdom) on Bayer RA 50 analyzer in both normals and IHD patients.

Results: Around 93.3% of the patients presented with the chest pain as a major symptom and 6.7% of the patient presented with dyspnea. All the patients showed a positive ECG change and angiographic findings suggestive of IHD. Plasma FFA (1.134±0.21) in IHD was significantly higher ($p<0.0001$) than the control (0.5233±0.13). With respect to lipid profile triglycerides, low-density lipoprotein (LDL), very LDL was significantly higher in MI when compared to normal with $p<0.001$, whereas HDL was significantly higher in normals than the study group with $p<0.05$. There was no statistical difference in total cholesterol and hemoglobin value between the study group and the normals. Further standard biomarker like cTn was elevated in 60% (18) and CK-MB in 63% (19) of the patients when compared to FFA, which was elevated in 86% (26) of the patients.

Conclusion: Thus, plasma FFA can be used as a simple, quick, and early marker of MI. However, should FFAs be measured routinely as a standard diagnostic marker of ischemia still warrants further studies?

Keywords: Myocardial ischemia, plasma FFA, cardiac troponin, creatinine kinase MB

INTRODUCTION

According to WHO, diagnosis of myocardial ischemia/infarction (MI) requires the presence of at least two of the three diagnostic criteria, an appropriate clinical presentation, typical electrocardiogram (ECG) changes and raised cardiac enzymes [1]. In September 2000, the Joint European Society of Cardiology (ESC) and American College of Cardiology (ACC) Committee published its consensus recommendation for a new definition of acute MI [2]. In particular, the ESC/ACC definition of acute MI requires the rise and fall of biochemical markers together with other criteria such as ischemic symptoms and ECG changes [2]. Thus, according to WHO an acute MI could be diagnosed without biochemical markers while the ESC/ACC criteria stipulate that the biomarkers be elevated and, subsequently, be shown to fall in the appropriate clinical context. Accordingly, studies reveal that patients with no ST-segment elevation showed an elevation of biochemical markers and could benefit from aggressive medical therapy [3,4]. The most common and wide determined biochemical markers are CK-MB, troponin I [5,6]. However, these markers may not rise during reversible MI [7]. Further previous studies suggest that unbound free fatty acid (FFA) increases significantly in ischemic related events. Thus, plasma FFA, a better early biochemical marker was evaluated in this study along with routinely used biomarker.

METHODS

In our study, 30 healthy controls and 30 ischemic heart disease (IHD) patients were evaluated for plasma FFA. Symptoms of MI were evaluated using a questionnaire. Furthermore, routine markers like CK-MB and troponin I was measured in these patients. The healthy controls and IHD population were recruited from the outpatient and inpatient cardiology clinic at the Division of Cardiology M.S Ramaiah Memorial Hospital, Bengaluru. The study was undertaken from May 2007 to December 2007. Informed written consent was obtained from each of the participants. The subject's age was between 35 and 75 years. MI was diagnosed by means of ECG and coronary angiogram. Clinically, relevant coronary artery disease (CAD) was defined as the occurrence of at least one stenosis 20% or more in at least one of 15 coronary segments.

Laboratory analysis

5 ml of fasting venous blood sample was collected in ethylenediaminetetraacetic acid vacutainer in supine position. The sample was then centrifuged for 10 minutes at the rate of 2500 g and later the plasma was stored at −20°0C until assayed. Free (non-esterified) fatty acid was measured enzymatically (acyl-CoA synthetase [ACS]-acyl-CoA oxidase) with non-esterified fatty acid kit (Randox Laboratories Ltd., Co., Antrium, United Kingdom) on Bayer RA 50 analyzer. CK-MB and troponin I was assessed using colorimetric method. Furthermore, their lipid profile and hemoglobin (Hb) levels were estimated.

Statistical methods

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean ±± standard deviation (SD) (Min-Max), and results on categorical measurements are presented in number (%). The significance is assessed at 5% level of significance. Student's t-test (two-tailed, independent) and Chi-square test has been used to find the homogeneity of age and gender between two groups. Student's t-test (two-tailed, independent) has been used to find the significance of lipid parameters, FFA (mmol/L) between

cases and controls. Effect size has been used to find the effect of disease on study parameters. Pearson correlation has been used to find the significance of the relationship between FFA and study parameters in controls and cases.

RESULTS

The present study consists of 30 MI (cases) and 30 healthy populations (controls). The mean age of MI patients (cases) and healthy (controls) population were 55.17±13.69 and 53.07±9.92, respectively. Among each group of MI patients and healthy populations 4 (13.3%) were females and 26 (86.7%) were males. The body mass index (BMI) of MI population and controls were 23.05±3.35, 22.19±1.38, respectively (Table 1). The age, sex, and BMI are statistically comparable (p>0.05).

Table 1: Basic characteristics of the study

Basic characteristics	MI	Normal	p value
Number of subjects	30	30	-
Age in years	55.17±13.69	53.07±9.92	0.499
BMI kg/m^2	23.05±3.35	22.19±1.38	0.200
Sex	Male: 26 (86.7%)	Male: 26 (86.7%)	1.000
	Female: 4 (13.3%)	Female: 4 (13.3%)	

BMI: Body mass index, MI: Myocardial ischemia/infarction

Table 2: Comparison between study group and normal group

Group statistics					
Groups	**N**	**Mean**	**SD**	**Independent t test**	**p value**
FFA					
Study group	30	1.1340	0.20652	13.848	0.000***
Normal	30	0.5233	0.12524		
TC					
Study group	30	177.000	36.5485	1.901	0.062 NS
Normal	30	162.067	22.6973		
TG					
Study group	30	164.933	66.7476	4.808	0.000***
Normal	30	100.527	30.4829		
HDL					
Study group	30	39.347	8.4653	2.716	0.009**
Normal	30	45.217	8.2748		
LDL					
Study group	30	114.800	25.2906	4.606	0.000***
Normal	30	89.580	16.1211		
VLDL					
Study group	30	32.9167	13.34947	4.897	0.000***
Normal	30	19.8927	5.82942		
Hb					
Study group	30	14.103	1.4112	0.280	0.781 NS
Normal	30	14.207	1.4522		

*p<0.05 is significant. SD: Standard deviation, FFA: Free fatty acid, TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, Hb: Hemoglobin

Table 3: Correlation of FFA with study parameter

Pair	Cases		Controls		p value
	r value	p value	r value	p value	
FFA versus age	0.029	0.879	0.051	0.788	0.935
FFA versus BMI	0.078	0.682	−0.271	0.147	0.197
FFA versus TC	0.009	0.962	0.097	0.611	0.745
FFA versus TG	0.121	0.526	−0.201	0.287	0.232
FFA versus HDL	0.039	0.840	−0.152	0.422	0.481
FFA versus LDL	−0.074	0.697	0.118	0.534	0.479
FFA versus VLDL	0.118	0.535	−0.216	0.252	0.214

TC: Total cholesterol, FFA: Free fatty acid, TC: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

Plasma FFA levels were significantly higher (p<0.001) in MI patients (1.13±±0.21 mmol/L) when compared to the normal population (0.52±±0.1 mmol/L) (Table 2). FFA also showed a positive correlation with age in both MI and normal, but it was not statically significant (p>0.05). That is, as the age increases the plasma FFA levels also increased. The r value was 0.029 and 0.051 in MI patients and normal individuals, respectively (Table 3). The study showed a positive correlation between plasma FFA and BMI in case of MI but showed a negative correlation in the case of normal population. However, both of them were not statistically significant (p>0.05). The r value was 0.078 and −0.271 in MI patients and normal individuals, respectively.

In our study, in MI patients only the triglycerides (TG) and very low-density lipoprotein (VLDL) levels showed trivial positive correlation with plasma FFA. The r value for TG was 0.121 and VLDL was 0.118. In the control group, only the LDL level showed a trivial positive correlation with plasma FFA.

With respect to lipid profile TG, LDL, VLDL was significantly higher in the study group when compared to normal with p<0.001 whereas HDL was significantly higher in normals than the study group with p<0.05. There was no statistical difference in total cholesterol (TC) and Hb value between the study group and the normals.

Standard biomarker like cardiac troponin (cTn) was elevated in 60% (18) and CK-MB in 63% (19) of the patients when compared to FFA which was elevated in 86% (26) of the patients.

DISCUSSION

The prevalence and mortality rates of MI have been known to be higher in the Indian than the Western population [8]. Many risk factors both modifiable (diabetes mellitus [DM], hypertension, hyperlipidemias, sedentary lifestyle, and smoking) and unmodifiable risk factor (age, male sex, and genetic influence) have been identified. According to the recent ESC/ACC guidelines, diagnosis of MI requires the rise and fall of biochemical markers together with other criteria like ischemic symptoms and ECG changes. The standard biomarkers of MI are CK-MB and cTn [3,9]. However, studies reveal that these markers may not rise during reversible MI [7]. Further, recent studies have suggested that plasma FFA levels are increased in MI; thereby it is known to play an important role in the clinical diagnosis [10]. Despite the success of cTn and CK-MB, there is still a need for the development of early markers that can reliably rule out ACS from the emergency room at presentation and also detect MI in the absence of irreversible myocardial injury. The aim of this study is to identify a new biochemical marker of MI that is elevated even during reversible MI. Further, several prospective and cross-sectional studies have revealed that plasma FFA has strong predictive value for CAD [11]. However, there are very few Indian studies revealing the same. This study was designed to assess the value of plasma FFA as a predictive marker in CAD.

Our study showed that the patients with CAD had significantly higher levels of plasma FFA when compared to age-sex, and BMI-matched control subjects. The result remained statistically significant after adjusting for several possible confounders. These findings extend previous observations of plasma FFA levels in angina pectoris and MI patients. The association between plasma FFA and cardiovascular risk does not establish a cause-effect relation because plasma FFA levels are related to several major lifestyle, and physical characteristics known to be associated with increased risk of CAD.

Further standard biomarker like cTn was elevated in 60% (18) and CK-MB in 63% (19) of the patients when compared to FFA, which was elevated in 86% (26) of the patients. However, biomarkers like cTn and creatinine kinase MB appear in the serum only after 4-10 hrs after the symptom onset and reach a peak at 12-48 hrs [5]. Thereafter, they remain abnormal in the serum for several days, making it difficult to diagnose a reinfarction [6]. Further, many non-ischemic pathophysiologic conditions cause myocardial necrosis thereby causing

the elevation of cTn and CKMB [12-15]. Thus, these markers act as a valuable diagnostic tool only when used together with other clinical information. Furthermore, studies reveal these markers may not rise during reversible MI [7].

Recent studies have explored the rationale for diagnosing MI in advance or in the absence of occurrence of irreversible damage [16,17]. With regard to this, markers like unbound FFA, ischemia – modified albumin precedes necrosis and permits the prevention of its consequences [17]. These markers are also valuable in distinguishing acute MI from non-ischemic causes of myocardial necrosis. Thus in our study, we evaluated the unbound FFA along with the other routine markers.

Recent studies have shown that there is an increase in FFA in acute MI, which can be used for early identification of cardiac injury [18]. In our study also the FFA is significantly elevated in CAD when compared to the normal group. Further, two group of investigator, have proved that this is a sensitive marker that appears in the serum well before the other, more traditional, markers of cardiac necrosis with the sensitivity being more than 90% at the time of admission.

A previous follow-up study demonstrates that high levels of FFAs predict total and cardiovascular mortality; independent of established and emerging cardiovascular risk factors [19]. Moreover, another follow-up study has demonstrated that elevated plasma FFAs predicts sudden cardiac death [19]. This indicates that FFA levels provide additional information on mortality risk beyond established risk factors, supporting the idea of a direct involvement in pathophysiological processes. The increase of FFAs could be due to a surge of catecholamine activity in these patients [20]. The result obtained in our study goes in accordance with the previous study by Pilz *et al*. [19]. The mean value of plasma FFA level in their study was 0.67 but in our study it is 1.13±±0.21. However, in their study there was no significant difference between CAD group and normals.

FFAs is known to increase the activity of protein phosphatase Type 2C, which causes apoptosis of endothelial cells [21], a process that occurs preferentially in the coronary arteries as it serves as the main energy source for the myocardium. Thus, it is possible that the FFAs might play a crucial risk factor causing MI in man by destabilizing the endothelial layer of the coronary vessels [22]. This postulation would perfectly fit within our finding that FFAs are elevated in subjects with unstable CAD and exhibit significant difference between controls and subjects with angiographically proven CAD. The increase of FFAs in the unstable CAD group might be due to a surge of catecholamine activity in these patients [22,23]. Furthermore, a study by Davda *et al*. [24] suggests that increased FFA reduces the activity of the endothelial nitric oxide synthase by inhibiting prostacyclin synthesis thereby increasing the ischemic damage. Furthermore, it induces inflammatory processes, oxidative stress, and lipotoxic [25,26].

Another study by Stepniakowski *et al*. has shown that FFAs may add to the cardiovascular risk by its effect to increase systolic arterial blood pressure [27]. However, in our study such an association was not seen. The probable reason could be because these people were already on antihypertensive drugs. However, there was a slight increase in plasma FFA in patients with IHD and DM when compared to those without diabetes (though not significant). Thus, our study did not show any significant association with any of the traditional risk factors like hypertension, diabetes, smoking, alcohol, family history and also no significant association with age and BMI was noted. However, a previous study by Pilz *et al*. showed significant association with these parameters. However trivial correlation with respect to TG and VLDL in case of IHD was noted. Thus, the lack of correlation with traditional risk factors may be due to the independent role played by FFA in the pathogenesis of IHD. Most of the IHD patients had single-vessel disease 66.6% when compared to double vessel disease and triple vessel disease which was 16.7%, respectively. With respect to lipid parameters it was seen that patient with IHD had significant elevation of TG (176.99±±36.57±, p<0.001), LDL (89.51±±16.02±, p<0.001), and VLDL (19.89±±5.83±, p<0.001) but significant reduction in HDL (45.22±±8.27±, p<0.01) when compared to normals. The TC was high in patient with IHD, but it was not statistically significant. With respect to Cohen's effect size on lipid parameters in IHD patients, the TG had very large effect, LDL, and VLDL had large effect, HDL and TC had moderate effect. Our study goes in accordance with a previous study conducted by Haddad *et al*. [28].

This study has the limitation of small number of cases (n=30) and controls (n=30). However, the study showed a significant elevation of plasma FFA in IHD when compared to normal. This emphasizes the role of FFA as a biomarker for IHD. Should FFAs be measured routinely as a diagnostic cardiovascular marker? Currently, this appears to be hampered by the relatively high day-to-day variability in FFA levels [29]. However, considering our robust and promising results, diagnostic strategies might be devised in the future that overcome this limitation. Apart from this, studies promoting therapeutic strategies to influence FFA metabolism in cardiovascular diseases should be undertaken. These therapies should aim to reduce fatty acid and increase glucose oxidation in the myocardium. A possible diagnostic use of FFA still warrants further studies, but our results may suggest that therapeutic approaches influencing FFA metabolism might improve the prognosis of individuals with or at high risk of cardiovascular disease. Further studies are required to study the role of drugs in reducing the levels of FFA and if such a reduction can also reduce the risk for cardiovascular disease.

Currently, several determinants of FFA levels in health and disease are known. Most of these lifestyle determinants are amenable to change. Thus, the need for adequate lifestyle modification is further stressed by recognition of FFA as a risk factor. Further cardiovascular research revealing FFA as a major diagnostic marker should be considered.

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