RESEARCH PAPER | Chemistry | Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

Correlation of serum Malonialdehyde Acetylaldehyde Adduct to serum Malondialdehyde as oxidative stress markers in Acute Coronary Syndrome patients

KEYWORDS: MDA, MAA, ACS

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ABSTRACT *Background: Advances in our understanding of the pathophysiology of acute coronary syndrome (ACS) have led to the marked increase in development of new biomarkers for its diagnosis. One of these markers is a molecule, known as malondialdehyde–acetaldehyde or MAA also appeared to indicate the presence of coronary artery disease.*

Aim: to investigate the relationship between MAA-adducted proteins and parameters of oxidative stress on the one side and different types of acute coronary syndromes on the other.Subject and methods: the present study is a case-control study conducted during the period from April, 2004 till the end of December 2005, which includes measurement of serum malondialdehyde by chemical method and malondialdehyde-acetyl aldehyde by Enzyme Linked ImmunoSorbant method on 138 patients with Acute Coronary Syndrome attending the Cardiac Care Unit at Al-Kadhimiya Teaching Hospital with a matching group of 40 apparently volunteer subjects for age and sex which were considered as a control. Results: the serum (malondialdehyde & malondialdehyde-acetyl aldehyde) are significantly elevated in Acute Coronary Syndrome patients (Acute Myocardail Infarction and Unstable Angina) (P<0.001) as compared with controls; however, these parameters are not altered significantly (P>0.001) when patients groups are compared with each other. Both malondialdehyde & malondialdehyde-acetyl aldehyde are significantly correlated (r=0.9, P<0.001 in all study groups whether Acute Coronary Syndrome or control groups.In conclusion: all patients with coronary diseases have oxidative stress. This is supported by the finding of high serum (malondialdehyde & malondialdehyde-acetyl aldehyde) which are markers of oxidative stress. Measurement of malondialdehyde-acetyl aldehyde protein adduct by many kits that are commercially available; as this adducts may be playing a role in the development and/or progression of vascular disease such as atherosclerosis, this biomarker is still under intense investigation and lack specificity.

Introduction

Advances in our understanding of the pathophysiology of acute coronary syndrome (ACS) have led to the marked increase in development of biomarkers for diagnosis, risk stratification, therapeutic decision-making, and assessment of clinical outcomes. Patients with ACS are subdivided into the following 2 major categories based on the 12-lead electrocardiogram (ECG): those with new ST-elevation on the ECG that is diagnostic of acute ST-elevation myocardial infarction (STEMI) and those who present with ST-segment depression, T-wave changes, or no ECG abnormalities [non-ST elevation ACS (NSTEACS)]. The latter encompasses both unstable angina (UA) and non-ST-elevation myocardial infarction (NSTEMI).[1,2] This group comprises a growing number of patients with ACS and is emerging as a major public health problem worldwide, especially in Western countries, Asia and other developing countries.[3-5]

There are some new groups of biomarkers that may become useful for the diagnosis of ACS. Most of these biomarkers are still under intense investigation. Most of the prospective studies have demonstrated that these markers were of prognostic value for ACS. However, the diagnostic significance of these markers remains to be elucidated; Elevated levels of malondialdehyde , a product of lipid peroxidation, and oxidised low-density lipoprotein are reported to have a significant association with the risk of death or MI in ACS patients. [6] , Since almost all acute coronary syndromes (ACSs) result from thrombus formation in pre-existing atherosclerosis [1],

Findings obtained from recent studies have demonstrated that malondialdehyde and acetaldehyde can react together with proteins in a synergistic manner and form hybrid protein conjugates, which have been designated as malondialdehyde-acetaldehyde (MAA)-protein adducts. [7] These adducts are immunogenic because significant increases in circulating antibody titers against MAA-adducted proteins have been more recently in human suffering from atherosclerotic lesions. However, the biomarkers of cardiovascular risk are multiple and show the evolution of the atherosclerotic lesion and its ability to rupture. [8]The oxidative stress is a risk factor significant that their testing is necessary in complications of atherosclerosis [8].

Emerging data suggest that acute presentations of coronary artery disease may involve a complex interplay between the vessel wall, inflaMAAtory cells, and the coagulation cascade. Considering the role of oxidative stress in the development of endothelial dysfunction and atherosclerotic disease [9], the aim of our study was to investigate the relationship between MAA-adducted proteins and parameters of oxidative stress on the one side and different types of acute coronary syndromes on the other.

Subjects & Methods:

Patients:

The study was a case-control study conducted on One hundred thirty eight patients admitted to coronary care unit (CCU) in AL- Al-Kadhimiya teaching hospital during the period from April 2004 to December 2005 were included in this study. Nine patients died and fourty six were ruled out from the study because twenty-two of them had previous history of IHD and twenty-four had other diseases that may affect the parameters to be diagnosed. These diseases included infections, trauma, alcohol-induced liver cirrhosis, malignancies, rheumatoid arthritis, nephrotic syndrome, chronic active hepatitis, biliary cirrhosis.

The remaining one hundred thirty eight patients were aged (35-75year). Patients with AMI meet at least two of the WHO criteria of the study:

a history of chest pain, evolutionary changes on the ECG, and elevation of cardiac enzymes. The patients were diagnosed depending on the result of the following examination:

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1-Clinical examination.
2- ECG.
3-Chest X-ray.
4-Lipid profile.
5-Cardiac Enzymes.
6-Cardiographic findings.

The studied subjects were divided into three groups:
Group 1:
Eighty-four patients with AMI (46 male and 38 female). These patients were subdivided into two groups:

A- Sixty patients with QMI (32 male and 28 female).
B- Twenty-four patients with NQMI (14 male and 10 female).

Group 2:
Fifty-four patients suffered from unstable angina (29 male and 26 female).

Group 3:
The control subjects were ninty volunteers (52 male, 38 female) who had no evidence of CHD.

Blood Samples:
Ten milliliters of venous blood samples were drawn from each patient in supine position, without application of tourniquet. utilizing disposable plastic syringes during the first day from admission to CCU. Samples were transferred into clean new plane tube, centrifuged at 4°C for 15 minutes at 4000×g. and the separated plasma were transferred into a new plane tube where fibrin mesh was removed by a wood stick leaving a free serum which was stored in Eppendrof tube and was used for measurement of MDA and MAA-protein addict. Samples were processed within 5h after blood collection and kept frozen until analysis which was done within 1 month after collection of blood according to ELISA kit manual. Haemolysed samples were discarded.

C-Methods
Separation and detection of MAA protein adduct was accomplished by ELISA kit (OxiSelect™ MDA Adduct ELISA Kit-cell biolabs, inc).

Measurement of serum MDA was done by the method of Draper and Hadley [10] based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532nm.

D. Statistical analysis:
Statistical analysis was done using Excel system version 2007 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significancy of mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results:
As expected for patients with ACS (AMI & UA), serum MAA and serum MDA, were significantly elevated as compared with controls (P-value >0.001 for both in groups) as seen in Table 1. However; serum MAA and serum MDA were not significantly altered in AMI (G1) when compared to UA (G2) (P-value >0.001) as seen in Table 1.

A significant positive correlation was found between serum MAA and serum MDA in AMI patients (r = 0.94, P-value < 0.001) and UA patients (r = 0.95, P-value < 0.001), also this correlation was found in healthy controls (r = 0.96, P-value < 0.001) as seen in Figures 1, 2 and 3.

Discussion:
A simple blood test may reveal that patients with heart ailments are actually suffering from the more dangerous coronary artery disease. Coronary artery disease results when plaque grows in the arteries until blood flow to the heart is constricted and if enough plaque builds up, a piece could break off and block blood flow to the heart, which results in a heart attack or stroke. clues to help understand inflaMAAtory conditions such as arthritis and alcoholic liver disease, focused on a molecule that is a strong indicator of inflaMAAtion. The molecule, known as malondialdehyde–acetaldehyde or MAA also appeared to indicate the presence of coronary artery disease [11].

ACS is the main cause of mortality and a major cause of morbidity and disability worldwide [12]. Atherosclerosis, the most common pathologic process underlying cardiovascular disease, represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall [13]. Oxidation of low density lipoproteins is considered a key initial step in atherosclerosis and Coronary Heart Disease (CHD) development and progression [14]. Various risk factors have been attributed in the genesis of ACS such as heredity, dyslipidemia, obesity, hypertension and lifestyle stress.

In our study we have clearly shown that levels of MDA and MAA are raised in patients affected by ACS with a strong positive correlation between them as oxidative stress markers, especially in AMI; these results are in agreement with other studies in which oxidative stress play a central role as liver disease[15],leukemia [16], lung diseases [17] and atherosclerosis [18].

These results are not surprising, given the outstanding role of MDA in the formation of protein adducts and hybrid adducts with acetaldehyde, which perpetuates tissue damage and promotes fibrogenesis [19]. MDA adducts trigger a potent immune response [20]. Activated T cells secrete cytokines, which contribute to myocardial damage. Therefore, MDA adducts are responsible, at least in part, for T-cell activation and cytokine production. However, variable immune responses to antigens formed as a result of MDA protein modification may explain in part the inter-individual variability of the severity of coronary heart disease.

To the best of our knowledge, this is the first study of the association between ACS and oxidative stress. By its very nature, oxidative stress is a difficult parameter to measure effectively in vivo. Oxidative damage to biomolecules is an accepted surrogate measure of oxygen-centred free radical generation and a range of plasma markers of lipid and protein peroxidation have been identified [21]. However, there is considerable debate as to the benefits of one measure over another and, in studies where more than one marker has been measured; there are not necessarily consistent effects across different markers of oxidative stress in response to the same stimulus, suggesting that different markers might reflect disturbances in different processes that contribute to oxidative stress. With this in mind, our approach was to measure a traditional marker with respect to a new marker to provide as broad a picture as possible, whilst at the same time potentially contributing additional information as to the mechanisms involved in the process. MDA is an end-product of lipid peroxidation that has been associated with a range of risk factors for cardiovascular disease [22]. MAA is a key mediator of atherosclerosis and plasma concentrations have been found to be elevated in a range of hepatic, pulmonary, malignant and cardiovascular diseases [1, 22]. Plasma MAA and MDA concentrations may also be associated with plaque instability [23];

Given the relevance of these two markers in our patient group, it was considered sensible to measure them all were sufficient sample was available.

Modifications of proteins or lipoproteins have many deleterious effects in a number of diseases including atherosclerosis. Plasma soluble protein modifications result in their binding to scavenger receptors, stimulating the release of

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pro-inflaMAAtory cytokines and become immunogenic, elicit autoantibody formation and the generation of T cells responses [20]. The covalent binding of acetaldehyde and malondialdehyde to proteins to form the MAA adduct has been demonstrated to be a major player in both coronary heart disease and more recently atherosclerosis [23]. The formation of these modified self-proteins and their biological consequences provide a potential mechanism by which atherosclerotic lesions form. [24].

One question that arises is how these two chemicals (MAA and MDA) are present at the same time in the body to make these modified self-proteins. MDA from lipid peroxidation could be attributed to the high fat diet people consume every day, especially as increased fatty liver (alcoholic or non-alcoholic) and incidences of atherosclerosis are on the rise. MAA could come from the breakdown of MDA to MAA, or the oxidation of alcohol from drinking [15], fermentation of food in the gut [25] metabolism of threonine by threonine aldolase in rodents only [26] and smoking [27]. In fact, cigarette smoke extract has been shown to react with MDA and proteins to make MAA modified proteins [27]. Given the highly oxidative state of smoking, it has been shown that oxidative stress increases the amount of lipid peroxidation in the lungs which increases the plasma and tissue levels of MDA [17]. Importantly, this could be one potential mechanism of how cigarette smoking may be a co-factor and increases the risk of cardiovascular disease; Therefore, the immune response to MDA modified proteins is most likely to the dihydropyridine structure (predominant epitope in MAA), and suggests that MAA adducts may be playing a role in the development and/or progression of vascular disease such as atherosclerosis [18].

Finally, there are some new groups of biomarkers that may become useful for the diagnosis of ACS as MAA protein adduct for which many kits are commercially available. Although MAA adducts may be playing a role in the development and/or progression of vascular disease such as atherosclerosis, this biomarker is still under intense investigation.

The author declare that they have no conflict of interest.

Table (1): The mean serum (MDA and MAA) in ACS and control groups (presented as mean + SD).

Variable	G1 (n=84)	G2 (n=54)	G3 (n90)
s. MDA (nmol/mL)	11.66 ± 3.89*	6.08 ± 2.99**§	1.31 ± 0.74
s.MAA (pmol/mg)	4.7 ± 1.7*	3.8 ± 1.8**§	0.5 ± 0.3

(G1): Acute Myocardial Infarction (AMI).
(G2): Unstable Angina (UA).
(G3): Control subjects.

* t-test: G1 versus G3, p < 0.001

** t-test: G2 versus G3, p<0.001

§ t-test: G2 versus G1, p>0.05

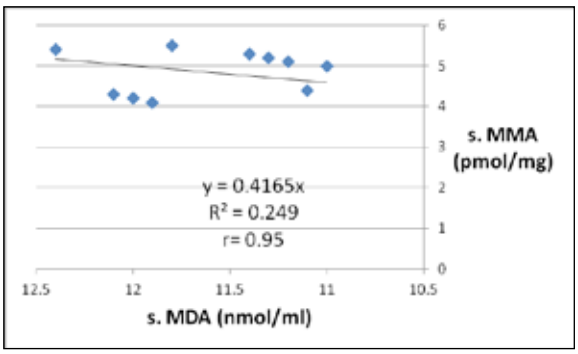

Figure (1): Correlation between serum (MDA & MAA) in G1: ACS patients with AMI. (n=84; r = 0.94; P< 0.001).

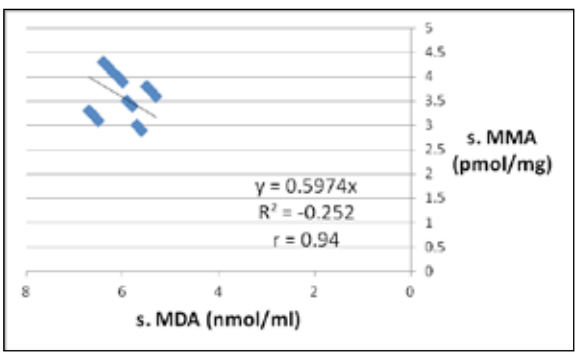

Figure (2): Correlation between serum (MDA & MAA) in G1: ACS patients with UA. (n=84; r = 0.94; P< 0.001).

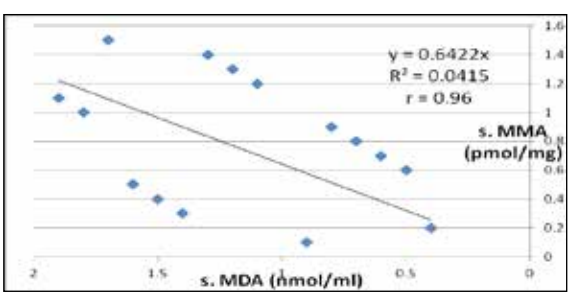

Figure (3): Correlation between serum (MDA & MAA) in G1: ACS patients with UA. (n=90; r = 0.96; P< 0.001).

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