Effect of smoking on serum xanthine oxidase and malondialdehyde levels in patients with acute myocardial infarction

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Abstract

Objective: To evaluate the effect of smoking on blood levels of xanthine oxidase and malondialdehyde in acute myocardial infarction patients.

Methods: The cross-sectional study was conducted from June to November 2013 and comprised myocardial infarction in-patients at Liaquat University of Medical and Health Sciences Hospital, Jamshoro, and Isra University Hospital, Hyderabad. Serum samples from age and gender matched smoking and non-smoking patients were investigated for the levels of xanthine oxidase and malondialdehyde by kit methods.

Results: Of the 88 serum samples investigated, 68(77.3%) belonged to men and 20(22.7%) to women. Among the 44 smokers, 35(79.5%) were men and 9(20.5%) were women. The overall mean age of the group was 49.6±8.0 years. Among the 44 non-smokers, 33(75%) were men and 11(25%) women. The overall mean age of the group was 50.9±8.4 Years. Smokers compared to non-smokers had significantly raised mean serum xanthine oxidase (0.31±0.05 vs 0.28±0.03mg/dl; $p<0.05$) and malondialdehyde (32.29±3.30 vs 30.30±2.87 µmol/L; $p<0.04$) levels.

Conclusion: Smokers as against the non-smokers were at higher risk of developing acute myocardial infarction owing to increased level of oxidative stress caused by smoking.

Keywords: Xanthine oxidase, Malondialdehyde, Myocardial infarction, Smoking. (JPMA 65: 39; 2015)

Introduction

The effects of smoking on human health are detrimental and, in many cases, lethal. Cigarette smoke carries approximately 4000 chemicals, including numerous toxic metals, poisonous gases and free radicals.[1] These ingredients in smoke when inhaled regularly can cause oxidative stress (OS) via biotransformation or by macrophage activation.[2] Smoking is now recognised as a major risk factor of acute myocardial infarction (AMI), especially in men.[3] AMI is the leading cause of death in the developed countries.[4] The prevalence of AMI increases progressively with age and peaks between the ages 45 and 65 years. Men are four to five times more likely to develop MI than women.[4]

Different biomarkers have been identified for the detection of myocardial damage. These include aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and troponins I and T. In the assessment of myocardial injury, these markers are of not much value because of certain limitations.[5] In human beings xanthine oxidase (XO) is normally present in liver and in the myocardium, but is absent in the blood. During MI, XO is released from the myocardium into the blood, so a blood assay for XO can be used to assess AMI patients. XO is important as it has been shown to be a major source of free radicals generation under ischaemic conditions.[6,7]

Malondialdehyde (MDA) is one of the final products of lipid peroxidation by reactive oxygen species (ROS) generated in cells.[8] The production of MDA is used as a biomarker to measure OS level in an organism.[9,10] In the current study, serum XO and MDA levels were measured in smoking and non-smoking AMI patients in order to see the effect of smoking on the levels of these blood parameters.

Patients and Methods

The cross-sectional study was conducted from June to November 2013 and comprised myocardial infarction in-patients at Liaquat University of Medical and Health Sciences Hospital, Jamshoro, and Isra University Hospital, Hyderabad. Initially, 115 MI patients were interviewed regarding their smoking habits. The responses thus obtained were recorded on a pre-designed proforma. Informed written consent was obtained from all patients and the approval was granted by the Ethical Committee of Isra University. From among the respondents, two age and gender matched groups of smokers and non-smokers were evaluated for serum XO and MDA levels which were measured by Bio Vision kit and oxiselect TM MDA Adduct enzyme-linked immunosorbent assay (ELISA) kit respectively.

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XO oxidises xanthine to hydrogen peroxide (H_2O_2) which reacts stoichiometrically with OxiRed™ Probe to generate colour which is measured at 570nm. As for the reagents, OxiRed™ Probe Preparation was prepared by dissolving the probe in 220µl of dimethylsulfoxide (DMSO); XO Enzyme Mix was prepared by dissolving XO enzyme mixture in 220µl deionised water; XO Substrate Mix was prepared by dissolving XO substrate mixture in 220µl deionised water; and XO Positive Control was prepared by diluting XO positive control with 92µl deionised water.

XO assay protocol was followed and 10mM H_2O_2 standard was prepared by diluting 4 µl 0.88mM H_2O_2 standard with 348µl deionised water; 0.1mM H_2O_2 standard was prepared by diluting 10 µl of 10mM H_2O_2 standard with 990 µl deionised water. For the colorimetric assay, 0, 10, 20, 30, 40 and 50µl of 0.1mM H_2O_2 standard were transferred into 96 well plate in duplicates and deionised water was added to make the final volume to 50µl in each well. Thus, working standards of 0, 1, 2, 3, 4, and 5nM H_2O_2 were generated.

For sample and positive control preparations, the serum was directly added to sample wells and the final volume was adjusted to 50µl/well with deionised water. Positive control was prepared by putting 5µl control solution to wells and adjusting the volume to 50µl/ well with deionised water.

After adding 50 µl/well reaction mix in H_2O_2 standards, test samples and positive controls, the contents in wells were mixed and immediately read for absorbance at 570nm. Results were determined via calibration curve.

For the measurement of MDA by OxiSelect™ MDA Adduct ELISA kit,

Bovine serum albumin (BSA) standards and protein samples (10µg/ml) were adsorbed onto a 96-wellplate for 2 hours at 37ºC. The MDA-protein adducts present in the sample or standard were probed with an anti-MDA antibody, followed by a horseradish peroxidase (HRP) conjugated secondary antibody. The MDA protein adducts content in an unknown sample was determined by comparing with a standard curve that was prepared from pre-determined MDA-BSA standards.

For reagents preparation, 1X wash buffer was prepared by diluting 10X wash buffer concentrate to 1X with deionised water and stirred to homogeneity; anti-MDA antibody and secondary antibody was diluted 1:1000 each with assay diluent and was used freshly.

The 10µg/ml standard curve of reduced BSA was freshly prepared by diluting the 1mg/ml BSA standard in 1X phosphate buffered saline (PBS), and the 0.5µg/ml of MDA-BSA standard curve was freshly prepared by diluting the 1mg/ml MDA-BSA standard in 10 µg/ml of reduced BSA.

The MDA assay protocol was followed. Each serum sample and MDA-BSA standard were assayed in duplicate.

In the 1st incubation, 100µl of the serum samples or reduced/MDA-BSA standards were put in 96 well protein-binding plates and incubated at 37ºC for 3 hours. The wells were then washed twice with 250µl 1X PBS per well. After the last wash, wells were emptied and tapped to remove excess wash solution.

In the 2nd incubation, 200µl of assay diluents/well were added and incubated for 1-2 hours at room temperature on an orbital shaker. The wells were then washed thrice with 250µl of 1X wash buffer with thorough aspiration between each wash. After the last wash, the wells were emptied to remove excess 1X wash buffer.

In the 3rd incubation, 100µL of the diluted anti-MDA antibody was added to all the wells and incubated for 1hr at room temperature on an orbital shaker. This was followed by washing step as in the 2nd incubation.

In the 4th incubation, 100µL of the diluted secondary antibody-HRP conjugate was added to all the wells and incubated for 1hr at room temperature on an orbital shaker.

Additional 100µl of warmed substrate solution was added to each well, including the blank well, and the contents were incubated at room temperature on an orbital shaker. The enzyme reaction was stopped by adding 100µL of stop solution to each well. Absorbance of each well was then immediately measured on a microplate reader using 450nm as the primary wavelength and reduced BSA standard as the absorbance blank.

All data was presented as mean± standard deviation (SD). Comparison of mean values between smokers and non-smokers with MI were made by using unpaired student t test. Differences between the two groups were considered significant at $p < 0.05$.

Results

Of the 115 AMI patients initially approached, 60(52.2%) were smokers and 55(47.8%) were non-smokers. Of the 88(76.5%) subjects who formed the main study sample, 68(77.3%) were men and 20(22.7%) were women. Among the 44 smokers, 35(79.5%) were men and 9(20.5%) were women. Men irrespective of whether they were smokers or non-smokers, were more than three times likely to have

Table-1: Gender distribution.

MI patient Group	Gender		Male to Female ratio
	Male	**Female**	
Smokers (n=44)	35 (79.5%)	09 (20.5%)	3.9:1
Non-smokers (n=44)	33 (75%)	11 (25%)	3:1
Total	68	20	3.4:1

MI: Myocardial infarction.

Table-2: Comparison between smokers and non-smokers.

Serum Variable	Smokers with AMI (n=44) Mean±S.D.	Non-smokers with AMI (n=44) Mean±S.D.	P Value	Significance level
XO (mg/dl)	0.308±0.05	0.281±0.027	P=0.05	P = 0.05
MDA (µmol/L)	32.29±3.30	30.30±2.87	P=0.04	is significant

AMI: Acute myocardial infarction
XO: Xanthine oxidase
MDA: Malondialdehyde.

AMI compared to women (Table-1).

The overall mean age of the group was 49.6±8.0 years. Among the 44 non-smokers, 33(75%) were men and 11(25%) women. The overall mean age of the group was 50.9±8.4years.

Smokers compared to non-smokers had significantly raised mean serum xanthine oxidase (0.31±0.05 vs 0.28±0.03mg/dl; p<0.05) and malondialdehyde (32.29±3.30 vs 30.30±2.87 µmol/L; p<0.04) levels (Table 2).

Discussion

AMI is the predominant cause of death in both developed and developing countries. It is a multi-factorial disease, and cigarette smoking is one of the major contributing factors for atherosclerosis and, hence, of AMI.

The finding of the current study that males are more than three times more likely to develop AMI than the age-matched females is in full agreement with literature,[11] and supports the notion that males are four to five times more prone to developing MI than females.[4]

A study[12] reported higher levels of XO in MI patients, irrespective of their smoking status. XO levels are usually raised in ischaemia, reperfusion injuries, anoxia and inflammation. In the present study the effect of smoking, which is an important source of oxygen free radicals, on the serum levels of XO, which is a potential source of free radicals generation in ischaemic conditions, and MDA in AMI patients were investigated. Both serum XO and MDA, which is a marker of OS, levels were significantly raised in

smokers with AMI than in non-smokers with AMI. This finding suggests that significantly higher levels of XO in smokers with AMI has increased the generation of reactive oxygen species (ROS) which in turn has increased the lipid peroxidation manifested by high levels of MDA.

Our finding that MDA levels are significantly higher in smoking than in non-smoking AMI patients is in line with earlier reports[13,14] that found that regular smokers had a slightly higher average concentration of MDA than the non-smokers.

Since the concentration of free radicals in smoke is extremely high, smoking by inducing superoxide radical production in vascular endothelial cells could cause endothelial dysfunction owing to conversion of nitric oxide into peroxynitrite.[15] Smoking-induced OS also results in increased low-density lipoprotein (LDL) oxidation which in turn facilitates the formation of the atheromatous plaque.[16] In this way smokers are at higher risk of developing AMI and chronic heart diseases (CHD) than the non-smokers.

Regarding the finding of high levels of XO in serum samples of smokers as against the non-smokers, it is not clear at this stage as to why and how the level of XO increases more in smoking AMI patients, and this aspect needs to be thoroughly investigated by future researchers.

Conclusion

Smoking AMI patients were at a higher risk of developing OS, and, hence, of AMI than the non-smokers.

Acknowledgment

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