STUDIES ON THE ASSOCIATION OF URINARY 8-EPI-PROSTAGLANDIN F₂A WITH METABOLIC SYNDROME AMONG PATIENTS OF MYOCARDIAL REPERFUSION

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ABSTRACT

OBJECTIVES: To evaluate the urinary 8-epi-Prostaglandin F₂α, fasting blood glucose, serum total cholesterol, serum HDL-c, serum LDL-c and prothrombin time in myocardial reperfusion patients and control subjects. Emphasis was given on correlation of urinary 8-epi-Prostaglandin F₂α with biophysical and biochemical parameters of blood.

STUDY DESIGN: This study was carried out in the Department of Biochemistry, Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Centre (JPMC) Karachi in collaboration with National Institute of Cardiovascular Diseases (NICVD) Karachi.

SUBJECT AND METHODS: A total of 200 subjects were included in this study. Written consent was taken from all the subjects. Patient group comprised of 100 subjects which were diagnosed cases of angina and myocardial reperfusion who visited the NICVD, Karachi. 100 healthy subjects having no any cardiovascular symptoms, age range 30 to 60 years of either sex were from general population. Their blood and urine samples were collected.

RESULTS: The result of our study showed that all the biochemical parameters in patients increased significantly when compared to control except HDL-c which was significantly decreased. When these parameters were correlated with urinary 8-epi PGF₂α it was found that the fasting blood glucose (FBG), Cholesterol and LDL-c were positively correlated with highly significances. However the HDL-c and PT showed no significant correlation vs urinary 8-epi-PGF₂α.

CONCLUSION: In the present study urinary 8-epi-prostaglandin F₂α, fasting blood glucose, total cholesterol, LDL-c, HDL-c and prothrombin time were analyzed in patients of myocardial reperfusion. We found significant changes when values of these parameters were compared between patient and control groups. We concluded that not only urinary 8-epi-prostaglandin F₂α but also lipid and lipoprotein in addition to prothrombin time were also disturbed in patients with myocardial reperfusion.

KEYWORDS: 8-epi-prostaglandin F₂α, myocardial reperfusion, metabolic syndrome, prothrombin time, lipids, lipoproteins.

INTRODUCTION

Myocardial ischemia is becoming an increasingly important public health problem and is now the most commonly

diagnosed disease in middle and old age population. Reperfusion is essential for salvage of the ischemic heart. It is essential to restore coronary flow to the ischemic

myocardium by intervention such as thrombolytic treatment, angioplasty or coronary bypass surgery¹. If the coronary flow is not restored within a critical period of time, reperfusion itself may cause a wide variety of harmful effect in the ischemic heart, phenomenon referred to as reperfusion injury².

Myocardial reperfusion is the restoration of blood supply to heart tissue which has become ischemic due to decrease blood supply. The decrease may result from any source including atherosclerotic obstruction, narrowing of the artery, or surgical clamping. Reperfusion can be induced to treat ischemia. Methods to restore blood supply include chemical dissolution of an occluding thrombus, administration of vasodilator drugs, angioplasty, catheterization, and artery bypass graft surgery. However, it is thought that reperfusion can itself further damage the ischemic tissue, causing myocardial reperfusion injury³. Reperfusion of ischemic tissue may intensify pathological processes that contribute to the generation of oxy-radicals, disturbances in cation homeostasis, and depletion of cellular energy stores, which may elicit arrhythmias, contractile dysfunction, and ultra-structural damage, in addition to endothelial dysfunction and coronary vasoconstriction. These paradoxical effects of reperfusion become evident quite rapidly upon the restoration of perfusion⁴. It is therefore essential to restore coronary flow to the ischemic myocardium. However, if the coronary flow is not restored within a critical period of time, reperfusion itself may cause a wide variety of harmful effects in the ischemic heart – a phenomenon referred to as “reperfusion injury”⁵.

F2-isoprostanes a family of oxygenated arachidonic acid is a component of membrane phospholipid. They are cleaved presumably by a phospholipase A2 appear in plasma and urine as 8-epi-prostaglandin F2 α (8-epi-PGF2 α). 8-epi-PGF2 α presents a chemically stable end product of lipid per oxidation. Urinary determination of 8-epi-PGF2 α excretion may be a sensitive index of oxidant stress⁶.

Oxidative stress is recognized to be a prominent feature of many acute and chronic diseases

including cancer, cardiovascular disease, neurodegenerative disease, lung disease and even the normal aging process⁷. In recent years, additional related compounds, derived from various polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been discovered to be formed as products of the iso-prostane pathway⁸.

8-epi-PGF2 α is a potent vasoconstrictor, potentiate platelet aggregation, smooth muscle cell mitogen and a mediator of pulmonary oedema in addition to a weak platelet thromboxane/endoperoxide receptor agonist⁹. The powerful proliferative and vasoconstrictory action of the most prominent member so far, 8-epi-PGF2 α makes it an interesting parameter for both pathophysiological understanding and eventual diagnostic assessment of oxidation injury in tissue extracts, plasma and/or urine¹⁰. 8-epi-PGF2 α can be synthesized both enzymatic and nonenzymatic processes and was initially to be formed in smash into seminal vesicle microsomes during the synthesis of prostaglandin¹¹.

Metabolic syndrome is a cluster of the most dangerous heart attack risk factors which includes diabetes mellitus, raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure¹². It is estimated that around 20-25% of the world's adult population have the metabolic syndrome. The clustering of cardiovascular disease (CVD) risk factors that typifies the metabolic syndrome is now considered to be the driving force for a new CVD epidemic¹³. The most widely accepted by the World Health Organization (WHO), the European group of the study of insulin resistance (EGIR) and the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP ATP III) on the core component of metabolic syndrome: obesity, insulin resistance, dyslipidemia and hypertension¹⁴.

The aim of the present study was to study the parameters urinary 8-epi-Prostaglandin F2 α and levels of fasting blood glucose, serum total cholesterol, serum HDL-c, serum LDL-c and prothrombin time in metabolic syndrome with

myocardial reperfusion patients. Then we had to compare these parameters with the control group and to correlate these biochemical parameters between patient group and control group. We emphasize specially the relationship of urinary 8-epi-Prostaglandin $F_2\alpha$ with other biochemical parameters of blood.

MATERIAL AND METHODS

This proposed study was carried out in the Department of Biochemistry, BMSI, JPMC Karachi with collaboration of NICVD Karachi. The study was approved by Karachi University. A total 200 subjects were included in this study. Patient group comprised of 100 subjects which were diagnosed cases of angina and myocardial reperfusion patients who attended the National Institute of Cardiovascular Diseases (NICVD), Karachi.

100 healthy subjects having no any cardiovascular symptoms age range 30 to 60 years of either sex were selected from general population. Their blood and urine samples were collected for biochemical parameters like urinary 8-epi-prostaglandin $F_2\alpha$, fasting blood glucose (FBG), serum total cholesterol low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and prothrombin time. The blood sample was collected from each study subject (control and patient) after an overnight fast (12–14 hours).

About 5 ml blood was taken by vein puncture using plastic disposable syringe under all aseptic measures and was transferred to a clean centrifuged tube, allowed to clot at 37°C. After 30 to 60 minutes the blood was centrifuged for 10 minutes at a speed of 2500–3000 rpm. The serum was separated and transferred to a proper, clean and dry plastic container. The container were properly covered, labelled and stored at 20°C till analyzed.

The urinary 8-epi-PGF $_2\alpha$ was done by enzyme-linked immunosorbent assay (ELISA) method, while FBG, serum level of Total cholesterol, LDL-c, HDL-c were analyzed by enzymatic kit method of Randox UK and plasma prothrombin time was

determined by kit plasma scan (Spain).

RESULTS

Table 1 shows the comparison of mean $\pm$ s.e.m levels of urinary 8-epi-PGF $_2\alpha$ and fasting blood glucose level between myocardial reperfusion patients and control group. The values of urinary 8-epi-PGF $_2\alpha$ and fasting blood glucose were 115.64 ± 2.85 vs 91.10 ± 1.04 respectively. When these values were compared to each other, both parameters were highly significant ($P < 0.001$).

Table 2 shows the comparison of mean $\pm$ s.e.m levels of serum total cholesterol, serum low density lipoprotein cholesterol (LDL-c), serum high density lipoprotein cholesterol (HDL-c) and prothrombin time (P.T) between myocardial reperfusion patients and control subjects. The serum values in patients and control groups of total cholesterol 207.44 ± 1.53 and 184.22 ± 1.34 ; LDL-c 98.59 ± 0.82 and 91.44 ± 0.83 respectively. Similarly the values of serum HDL-c was 37.62 ± 2.87 and 45.75 ± 0.47 and prothrombin time 17.13 ± 0.63 and 12.94 ± 0.14 respectively were observed in both groups ($P < 0.001$). Figure 1 showed the correlation coefficient of low density lipoprotein cholesterol versus 8-epi-PGF $_2\alpha$ which was positively correlated with significantly high ($P < 0.001$).

Table 3 shows urinary 8-epi-prostaglandin $F_2\alpha$ VS fasting blood glucose level, total cholesterol, low density lipoprotein cholesterol (LDL-c) in patient group were positively correlated with highly significance ($p < 0.005$), while high density lipoprotein cholesterol (HDL-c) VS 8-epi-prostaglandin $F_2\alpha$ and prothrombin time VS 8-epi-prostaglandin $F_2\alpha$ in patient group were not significantly correlated ($p > 0.01$).

Table - 1

Comparison of 8-epi-PGF₂α and fasting blood glucose between myocardial reperfusion patients and control.

Parameters	Control (n = 100)	Patients (n = 100)	P-value
	---	Mean ± SEM	
Urinary 8-epi ₂ α (pmol/mmol)	90.39 ± 0.66	268.60 ± 3.03	0.001
Fasting Blood Glucose (mg/dl)	91.10 ± 1.04	115.54 ± 2.85	0.001

p<0.001 Significant when compared

Table - 2

Comparison of serum total cholesterol, serum LDL-c, serum HDL-c and prothrombin time between myocardial reperfusion patients and control subjects

Parameters	Control (n = 100)	Patients (n = 100)	p-Value
	Mean ± SEM	Mean ± SEM	
Serum Total Cholesterol (mg/dl)	184.22 ± 1.34	207.44 ± 1.53	0.001
Serum LDL-c (mg/dl)	91.44 ± 0.83	98.59 ± 0.81	0.001
Serum HDL-c (mg/dl)	45.75 ± 0.47	37.62 ± 2.87	0.001
Prothrombin Time(Sec)	12.94 ± 0.14	17.13 ± 0.63	0.001

P<0.001, highly significant

Table 3

Cor relations of urinary 8-epiprstaglndin F_{2α} VS fasting blood glucose, total cholesterol, LDL-c, HDL-c and prothrombin time in patient group

Correlation	Correlation coefficient (r)	p value
8-epi-PGF 2α vs FBG	.605**	.001
8-epi-PGF 2α vs total chol	.896**	.0001
8-epi-PGF 2α vs LDL-c	.947**	.0001
8-epi-PGF 2α vs HDL-c	.061	.549
8-epi-PGF 2α vs PT	.059	.558

Significant p<.01, Highly significant p<.005, **Highly significant

Figure - 1

Correlation coefficient of LDL-c versus urinary 8-epi-PGF 2α

DISCUSSION

As in myocardial reperfusion oxidative stress occurs, this may alter the biochemical and biophysical parameters. This study was aimed to observe the comparison of urinary 8-epi-PGF₂α and other biochemical parameters in myocardial reperfusion patients with control subjects and to correlate the 8-epi-PGF₂α with biochemical parameters in patient group.

There are various biochemical parameters that can be altered in myocardial reperfusion patients including urinary 8-epi-PGF₂α, fasting blood glucose, total cholesterol, low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and prothrombin time (PT). In this study we observed that urinary 8-epi-PGF₂α and FBS were increased in myocardial reperfusion patients, and in accordance with the study of Delanty *et al.*, (1996)¹⁵ and Lawloret *et al.*, (2007)¹⁶. Our study also showed significant increased LDL-c and significant decreased HDL-c in myocardial reperfusion patients in comparison with the control group, Shinde *et al.*, (2005)¹⁷ and Executive Summary of NCEP, 2001 also showed the same results. The PT was also increased in our study similar to Schafer *et al* (2007)¹⁸.

In this proposed study we also calculated the correlation of urinary 8-epi-PGF₂α with fasting blood sugar, total cholesterol LDL cholesterol were positively significantly high in myocardial reperfusion patients, Urakawa *et al.*, (2003)¹⁹ also found the same results. In our study the urinary 8-epi-PGF₂α were about three fold increased in myocardial reperfusion patients with metabolic syndrome.

In the present study the significant increase of 8-epi-PGF₂α in myocardial reperfusion patients in comparison with the control group indicate that reperfusion of ischemic myocardial tissues may intensify pathological processes that contribute to the generation of oxy-radicals. These free oxy-radicals were responsible for increased formation of 8-epi-PGF₂α and disturbances in cations homeostasis and depletion of cellular energy stores in addition to endothelial dysfunction and

coronary dysfunction. These paradoxical effects of reperfusion become evident quite rapidly upon the restoration of reperfusion.

CONCLUSION

We concluded that:

1. The urinary 8-epi-prostaglandin $F_2\alpha$, fasting blood glucose, total cholesterol, LDL-c, and prothrombin time were increased highly significant whereas HDL-c were decreased significantly among patients of myocardial reperfusion.
2. In myocardial reperfusion patients the 8-epi-prostaglandin $F_2\alpha$ was positively correlated with highly significance.

So we recommend that not only the urinary 8-epi-prostaglandin $F_2\alpha$ is the biochemical marker for diagnosis of myocardial reperfusion but also metabolic syndrome: lipid and lipoproteins in addition to prothrombin time may be analyzed.

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