Assessment of circulating PPAR-γ Level as a risk and diagnostic biomarker in Acute Coronary Syndrome

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Abstract

Acute coronary syndrome (ACS) is a term that encompasses both unstable angina and myocardial infarction (MI) with or without ST-segment elevation. It is a life-threatening disorder that remains a source of high morbidity and mortality despite advances in treatment. Risk assessment done by using risk factors and risk markers. One of the emerged risk markers is high circulating level of PPAR-γ (a potentially important transcription factor). This study represents a try to evaluate the role of this analyte as a risk biomarker for future cardiovascular events, and evaluation of PPAR-γ as a possible diagnostic biomarker for MI. It was a case –control study enrolled 160 subjects; 80 patients diagnosed as ACS patients by expert physicians. An equivalent age and sex matched population without coronary disease with similar risk factors considered a control group. Serum levels of PPAR-γ were measured by using ELISA technique, cTpI was investigated by qualitative membrane- based immunoassay. Independent sample t-test was used to compare means between two groups. ANOVA were used to compare means between different groups, p value ≤ 0.05 is significant. There were significant differences in mean serum levels of PPAR-γ, by patients and control. There was a significant difference in PPAR- γ serum levels between positive and negative cTpI groups. There was an insignificant difference in PPAR- γ level among different ECG finding groups. Circulating level of PPAR- γ seems to be used as a risk biomarker for ACS, it is suggested that it could be used as a diagnostic biomarker for MI.

Introduction

The term acute coronary syndrome (ACS) is used to describe the continuum of myocardial ischemia (unstable angina pectoris) or infarction (with or without concomitant ST segment elevation)[1]. Many large epidemiological studies have examined the association between the incidence of coronary artery disease (CAD) and a variety of risk factors . However, only a small number of risk factors have consistently shown an association with increased risk of CAD. Assessment of risk factors to identify individuals at high risk for developing CAD plays a central role in the strategy of prevention of the disease. Risk assessment done by using risk factors and risk biomarkers[2][3].

One of the emerged risk biomarkers is the circulating level of Peroxisome proliferator-activated receptor-γ(PPAR-γ). It is a ligand-dependent transcription factor belonging to the nuclear hormone receptor superfamily[4]. Through its role in atherosclerosis and cardiovascular disease it is suggested that its circulating level can be used as a predictor to CAD as it is a potentially important transcription factor that modulates the inflammatory response of monocytes and anti-inflammatory response in other cells[5]. In addition to that its rule in ischemia-reperfusion reaction may contribute to its predictive ability [6]. Because the PP-AR-γ gene is expressed in mononuclear phagocytes, which respond to hypoxia or ischemia with an exuberant early growth response-1(Egr-1)-dependent inflammatory response, physiologically, PPAR-γ expression may serve as an endogenous mechanism to dampen this pathological response to ischemia triggered by zinc finger transcription factor Egr-1 induction[6]. In addition to this role, its presence in cardiomyocytes and atherosclerotic plaque [7]may make it a diagnostic biomarker for MI specifically. This study aimed to estimate the significance of PPAR- γ serum levels in ACS patients, and assess their predictive ability as risk biomarker. Investigate the possibility of using circulating level of PPAR- γ as a diagnostic biomarker for MI.

Materials and methods

Subjects

Between 1st December 2014and 31stMarch 2015,160 subjects were recruited to this study, 80 consecutive patients aged ≥ 40 years old, diagnosed by expert physicians to have ACS from coronary care units (CCU) in Merjan Medical City and Al-Hilla Teaching Hospital in Babylon Province, Hilla City, in addition to age and sex matched (80 subjects) without coronary disease with similar risk factors considered as control group. This study was performed in the laboratories of Biochemistry Department in collage of Medicine /University of Babylon. The overall mean age of patients with ACS and control were (60.28±12.02) and (58.21±11.61) years old, respectively. This study was matched for gender, the ratio

of male: female was 2:1 for both sexes. ECG changes were estimated by doing electrocardiograph by expert nursing staff using apparatus by G.E.Helthcare Co. (USA).

Result: Patients' readings were: ST-elevation 50%, ST-depression 18.7%, T-inversion 26.3%, and normal ECG 5%. Of them 68.75% presented as MI, and 31.25% presented as UA.

Exclusion criteria

Patients having the following conditions were excluded: Diabetes mellitus, renal failure, patients on hormones, or glucocorticoids regimen, age<40 years, patients coming from outside the governorate, pregnant women, incapacity to provide informed consent, prior inclusion in the present study, patients with known history of thyroid, hepatic, or malignant disease, drug abusers and anemic patients.

Ethical Issues: included

a- Approval of scientific committee of the Clinical Biochemistry Department in Babylon Medical College/ University of Babylon/ Iraq.

b- Approval of Babylon Health Directorate/Ministry of Health & Information Center for Research & Development of Babylon Province.

c- The objectives and methodology were explained to all participants in the current study and their verbal consent was gained.

Sample collection:- Five ml of blood were obtained from each subject by vein puncture in sitting or lying position, and then pushed slowly into disposable tubes containing separating gel. Blood in the gel containing tubes was allowed to clot at room temperature for 2 hours and then centrifuged at 1000 ×g for approximately 15 minutes, then the supernatant were obtained and stored at -20˚C until analysis [8].

Materials

1. Human PPAR-γ ELISA Kit (Elabscience / China)Cat. No.: E-EL-H1361

2. Human cTnI qualitative membrane- based immunoassay (AbonBiopharma/ China) Lot No. CTN 3100076.

Methods

PPAR-γ circulating serum level assayed by Elabscience (China) ELISA kit. cTnI assayed by using a qualitative membrane- based immunoassay for the detection of cTnI in whole blood, serum, or plasma.

Statistical analysis

The collected data were tabulated and analysed by using the Statistical Package for Social Sciences (SPSS) for Windows version 20th version. Data were expressed as (mean ± SD).Independent sample t-test was used to compare means between two groups. Chi square ($X2$) test and Fisher exact test were used to find the significance of the categorical variables. ANOVA were used to compare between different groups. P values less than (0.05) were considered significant.

Results

a. **Differences of Patients with Acute Coronary Syndrome and control by Socio-Demographic Characteristics:**

The overall mean age of patients with ACS and control were (60.28±12.02) and (58.21±11.61) years old, respectively. There was no significant mean difference between the mean age of patients and control. This age matching helps to eliminate differences in parameters' results. This study was matched for gender, the ratio of male: female was 2:1 for both sexes.

b. **Differences of patients and control by PPAR-γ serum level**

There were significant differences of **PPAR-γ** serum level by patients and control subjects as shown in table (3.1).

Table (3.1): Mean differences of patients and control by PPAR-γ serum level

Variable	Group	N	Mean	S.D	P value
PPAR-γ	case	80	12.78	3.95	**<0.001***
	control	80	3.33	1.81	

*p value ≤ 0.05 is significant

There is a significant mean difference between PPAR-γ serum level and cTnI outcomes as a diagnostic biomarker for AMI. (P<0.05) as shown in Table (3.2)

Table (3.2): Mean difference between PPAR-γ serum level and different troponin findings.

	cTpI	No.	Mean	S.D	t-test	p value
PPAR-γ	positive	39	14.26	3.68	**3.487**	**0.001***
	negative	41	11.38	3.71		

*P<0.05 is significant

There is a non-significant difference among different ECG findings of the patients and mean PPAR-γ serum level (p>0.05) as shown in table (3.3).

Table (3.3): Mean difference between PPAR-γ serum level and different ECG findings:

ECG	**No.**	**Mean**	**S.D**	**ANOVA**	**p- value**
ST-elevation	**40**	**13.70**	**4.29**	**2.064**	**0.112**
ST-depression	**15**	**12.60**	**3.50**		
T-inversion	**21**	**11.12**	**3.01**		
Normal	**4**	**12.99**	**4.71**		
Total	**80**	**12.78**	**3.95**		

P<0.05 is significant

Discussion

Although, PPARγ genotypes considered as modulator factor in CHD risk in previous studies [9-12], but there was not any reports regarding to association between circulating PPARγ and susceptibility to CHD. Accordingly, we design current study to assess the possible influence of circulating PPARγ on risk prediction of CHD. This study is devoted to analysis of the *PPARγ in the systemic circulation*, but information about the presence of this protein in the circulation in human diseases is limited in the literatures according to our knowledge.

The difference in serum PPAR-γ concentration between controls and ACS patients presented in table (3.1) is far greater (mean serum conc. for patients was 12.75±3.95, while that for control was3.33±1.81) revealed that it is not the atherosclerotic background subsided behind it, because patients and control subjects were matched in risk factors except history of IHD, but ischemia-reperfusion reaction may be the cause as PPAR-γ acts to dampen this reaction which is proposed by Abdelrahman M [9] and IvanovaEA[10]. As the increment

in concentration of PPAR-γ observed in this study seems to be insufficient for preventing ischemic events progression in ACS patients as a part of its role as anti-inflammatory transcription factor so it is possible that the atherogenic effects of PPAR-γ itself may add to the disease, which agree with Ahmadian M *et al*[13], Takano H *et al* [14], Kapadia R *et al* [15], Cheng Q *et al*[16], and Gao X *et al* [17] .

In a pilot study done by Lin Q *et al* [18] who revealed that PPAR-γ receptor protein concentration was negatively correlated with hs-CRP and IL-6 concentrations. As ACS patients had high level of inflammatory markers (hs-CRP and IL-6, etc.) proved by Armstrong EJ *et al* [19], so it is supposed that these patients had low level of PPAR-γ, but as that was not the case, it seems that the isoform activated here acts as pro-inflammatory rather than anti-inflammatory factor, or the increment of serum PPAR-γ concentrations noticed in patients may be associated with, activation of its synthesis in tissues, or increased its loss by cells. Hence the elevation of PPAR-γ seems to be related more to ischemic events rather than the anti-inflammatory/pro-inflammatory controversial role in atherosclerosis reported by Takano H *et al*[12].

PPAR-γ as a diagnostic biomarker:- Patients with either STE- or NSTE-AMI have more persistent occlusion of a coronary artery leading to infarction, whereas patients with UA have ischemia with either transient or incomplete occlusion, so the predictive ability of this marker for MI seems to be related to necrosis of myocytes rather than other causes. Cardiac injury resulted in releasing of cardiac cells' contents to the circulation in a manner relevant to that of cardiac troponins [20]. The PPAR-γ has an isoform concentrated in cardiac cells (PPAR-γ1) as implicated by Azhar S [21], so its significant high serum level often may be related to the release of this isoform to the circulation. Another source of elevated PPAR-γ may be the necrotic core of the ruptured plaque which is a rich source of PPAR-γ4 isoform [21]. In table (3.3) where it appears that PPAR-γ level of 14.26±3.68 accompanied the cases of cTpI +ve, while a level of 11.38±3.71 accompanied the cases of cTpI –ve. The novelty of our hypothesis is that PPAR-γ receptor protein might be a circulating necrosis marker in MI. This observation may lead to the probability of using this marker as a diagnostic biomarker in the future for MI. As it was reported by Mehrabia MR *et al* [22] that in addition to its presence in the vasculature, PPAR-γ is also expressed in adult human hearts, where the transcription factor might regulate metabolic events important for cell survival and induction of growth.Mehrabia MR *et al* [22] showed that PPAR-γ expression is higher in normal left ventricles compared to the aorta and coronary arteries. In contrast to the results from healthy specimens, it was found that higher PPAR-γ levels in the coronary arteries of both coronary heart disease (CHD) and dilated cardio-myopathic (CMP) patients, compared to the aorta. Therefore, it was speculated that PPAR-γ is up-regulated

in coronary arteries of CHD and CMP patients. This might be explained partly by atherosclerotic lesions in the vessel wall, which highly express PPAR-γ. The observation of this study[22] lead us to a suggestion that high serum level of PPAR-γ seen in the present study in MI patients significantly is related more to necrotic myocytes which had primarily high expression of PPAR-γ in addition to atherosclerotic plaque ruptured in coronary arteries enriched with this transcription factor. To our knowledge, no other publications have suggested this possibility.

The non-significant difference between PPAR-γ serum level and different ECG outcomes presented in table (3.4) revealed inability of using this analyte to differentiate between different anatomic infarcted sites in myocardium as it was presented by ECG. But it seems clearly from table (3.4) that the highest PPAR-γ serum level accompanied the ST-elevation cases which represent transmural (full-thickness) MI that is associated with atherosclerosis involving a major coronary artery[20]. In ST-segment depression and T-wave inversion groups which accompanied with lowest levels of PPAR-γ as noticed in table (3.4), there is partial occlusion of a major vessel or complete occlusion of a minor vessel, causing unstable angina or partial-thickness (subendocardial) MI [20]. This observation strengthen our suggestion about the source of circulating PPAR-γ noticed in the present study which may be mostly due to larger infarct size of the heart muscle rather than the ruptured plaque. The understanding of the role of PPAR-γ in ACS gives promise for more forthcoming progress in this field and can be used to quantify risk and to guide preventive care in addition to its suggestive value as a diagnostic biomarker .

Conclusion

PPAR-γ represents a risk biomarker of M.I. in ACS patients among the other biomarkers. There is a possibility that circulating level of PPAR-γ to be a project of diagnostic biomarker for MI in addition to its predictive ability.

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الخلاصة

متلازمة الشريان التاجي الحاد: هو مصطلح يشمل كلا من الذبحة الصدرية غير المستقرة واحتشاء عضلة القلب. وهو اضطراب يهدد الحياة ويظل مصدراً لارتفاع معدلات المراضة والوفيات على الرغم من التقدم في العلاج....

تقييم المخاطر باستخدام عوامل الخطر وعلامات المخاطر, واحد من علامات الخطر الناشئة هو ارتفاع مستوى تعميم –يتم عامل النسخ محتمل الهامة PPAR–γ .

تمثل هذه الدراسة محاولة لتقييم دور هذه التحليلات كمؤشر مرتبط بالمخاطر للأحداث القلبية الوعائية المستقبلية، وتقييم PPRA–Y كمؤشر تشخيصي جديد محتمل لــمتلازمة الشريان التاجي. تم اخذ 80 مريض وتم تشخيصهم بمتلازمة الشريان التاجي ودراسة عامل النسخ المذكور انفا عليهم وتبين وجود صلة ذات اهميه مع متلازمة الشريان التاجي.

الكلمات المفتاحية:متلازمة الشريان التاجي الحاد، عامل النسخ المحتمل، الخطورة البايولوجية، التشخيص الحيوي.