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Cardiac isoform of alpha-2 macroglobulin—A new biomarker for myocardial infarcted diabetic patients

Ponnambalam Annapoorani [a,*], Perundurai S. Dhandapany [a], Sakthivel Sadayappan [a,1], Subbiah Ramasamy [a], Andiappan Rathinavel [a,b], Govindan Sadasivam Selvam [a,**]

[a] *Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India*
[b] *Department of Cardio Thoracic Surgery, Madurai Medical College and Government Rajaji Hospital, Madurai 625020, Tamil Nadu, India*

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Abstract

Cardiac isoform of alpha-2 macroglobulin [cardiac α2M] has been shown to be an early marker in cardiac hypertrophy and left ventricular mass in humans. We, here, for the first time report its presence in myocardial infarcted humans and tried to explore the possibility of using this protein as a novel diagnostic marker for myocardial infarcted diabetic patients. A total of 260 samples were analyzed in this study for the presence of cardiac α2M. These include 55 patients of diabetic with post myocardial infarction [PMI], 45 diabetic patients without PMI, 60 patients of PMI alone and 100 controls without any ailments. Levels of cardiac α2M present in the sera of diabetic patients with PMI are significantly higher than that of normal human sera and diabetic patients without PMI but not with PMI alone group, suggesting this protein as a marker for PMI itself. However, our results reveal that cardiac α2M could be a valuable marker for the diagnosis of myocardial infarcted diabetic patients and differentiating them from diabetic patients without myocardial infarction by sandwich ELISA.

Keywords: Cardiac isoform of alpha-2 macroglobulin; Serum protein; Diagnostic marker; Diabetes; Post myocardial infarction [PMI]

1. Introduction

Myocardial infarction is the leading cause of morbidity and mortality in people with diabetes, accounting for about 50% of all deaths, especially in developing countries [1]. Diabetic patients who suffer from post myocardial infarction [PMI] have higher initial case fatality and a worse prognosis with 22% of male and 46% of female survivors being disabled by cardiac failure within 6 years [2]. Determining the potential risk factors for such patients and delineating the best approaches to treating them are important health care priorities. Earlier studies from this laboratory has identified a novel high molecular weight [182 kDa] serum protein as cardiac isoform of α2 macroglobulin [cardiac α2M] suggested to be involved in development of cardiac hypertrophy in rats [3]. This protein plays an indispensable role in the development of cardiac hypertrophy in experimental rats and may be an isoform of liver macroglobulin family [4]. The level of this protein could be an early marker identifying the stages of increase in left ventricular mass [LVM] [5]. Recently, direct injection and expression in vivo of full-length cDNA of the cardiac α2M was shown to induce cardiac hypertrophy in the rat heart [6]. In the present study, we explored the possibility of using this protein as a biomarker for myocardial infracted diabetic patients. We sought to specifically determine the diagnostic value of cardiac α2M in discriminating diabetic patients with and without post myocardial infarction for a better risk management.

* Corresponding author.
** Corresponding author. Tel.: +91 452 2458223; fax: +91 452 2458223.
E-mail addresses: diapoorni@gmail.com (P. Annapoorani), biochemselvam@rediffmail.com (G.S. Selvam).
[1] Present address: Division of Molecular Cardiovascular Biology, The Cincinnati Children's Hospital Medical Centre, Cincinnati, OH 45229, USA.

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2. Materials and methods

2.1. Human samples

A total of 260 samples were analyzed in this study for the presence of cardiac α2M. Fifty-five patients from Government Rajaji hospital in Madurai between 2003 and 2004 for diabetic with post myocardial infarction, 45 diabetic without PMI, 60 patients with PMI alone and 100 controls without any ailments made up the study group. The Madurai Kamaraj University and Rajaji Government Hospital Ethical Committee have cleared the animal experiments and human samples used in this study and informed consent was obtained. Criteria for diabetes followed The American Diabetes Association Guidelines [7] and PMI was defined according to the WHO report [8] with the mean duration of PMI being 18 months. Patients who presented any manifestation of atherosclerosis without a prior PMI were not eligible for the study. Exclusion criteria were renal failure [creatinine > 2 mg/dL], hepatic insufficiency, severe chronic heart failure [NYHA functional class III or IV] and acute cardiovascular events [within the previous 90 days before inclusion in the study]. The mean illness of diabetics with and without PMI was 5 years.

2.2. Animal model

Albino rats of a Wistar derived strain weighing between 120 and 130 g were used for this study for aortic constriction. Animals were fed ad libitum with Gold Mohur rat feed [Hindustan Lever Limited, Bangalore, India].

2.3. Western blot analysis of patient's sera with anti-rat cardiac α2M antibody

The cardiac α2M from the blood serum of aorta-constricted rats were purified and raising of anti-rat cardiac α2M antisera was done as previously described [5,9,10]. In order to look for the immuno-cross reactivity between human cardiac α2M and anti-rat cardiac α2M antibody, Western blot analysis was carried out as detailed by Prabhakar and Rajamanickam [10]. Briefly, 200 μg of the total patients serum was electrophoresed on 10% SDS-polyacrylamide gel. The proteins were electrophoretically transferred onto a nitrocellulose membrane. After transfer, the membrane filter was treated with 5% defatted milk powder in phosphate buffered saline [PBS]-Tween 20 and incubated at 37 °C for 2 h for blocking. Treatment with first antibody was carried out at 1:500 ratio of the polyclonal anti-rat cardiac α2M antibody in 2% milk powder in PBS-Tween 20 at room temperature for 2 h. The second antibody treatment was at a 1:1000 ratio horseradish peroxidase [HRP]-conjugated goat anti-rabbit IgG in 2% milk powder in PBS-Tween 20, performed at room temperature for 3 h. The filter was washed extensively in PBS-Tween 20 and developed with 4-chloronaphthol substrate solution [0.006% 4-chloronaphtal, 0.0002% of 30% H_2O_2 at 20% methanol in PBS].

2.4. Sandwich ELISA

The cardiac α2M serum in the sera of normal human and patients with cardiac and non-cardiac diseases were quantified by sandwich ELISA using anti-rat cardiac α2M antibody raised in rabbit [5]. The optimal concentration of antigens and antibody for coating was determined by checkerboard titrations [BIORAD model 450 micro plate reader, Bio-Rad Laboratories, Hercules, CA, USA].

2.5. Statistical analysis

All values were given as means ± S.E.M. Repeated measures ANOVA, Kruskal–Wallis one-way analysis of variance and post hoc test using pair wise multiple comparison using Dunn's method was performed to compare our data.

3. Results

The cardiac α2M was identified and quantified in study participants using Western blot [Fig. 1] and sandwich ELISA. The characteristics of study participants and cardiac α2M levels in human samples are given in Table 1. Sex and age were well matched and did not influence the levels of protein among varied study participants. [Repeated measures ANOVA, $F_{2,198} = 0.598$, $P > 0.05$]. Kruskal–Wallis one-way analysis of variance for the differences in the means of cardiac α2M levels among diabetic with and without PMI, controls and PMI alone were observed significantly [Fig. 2, $H = 190.781$, d$f = 3$, $P < 0.001$]. Further analysis with post hoc test using pair wise multiple comparison using Dunn's method showed a significant difference on diabetes with PMI when compared to diabetes without PMI, controls and PMI alone. However, there is no significant difference between the diabetic with PMI and PMI alone group [Table 2].

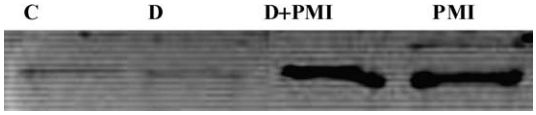

Fig. 1. Western blot analysis of serum from control [C], diabetic patients without and with PMI [D, D + PMI] and PMI alone for cardiac α2M.

Table 1
Characteristics of study participants and CA2M level

Characteristics	Control	Diabetic	Diabetic + PMI	PMI alone
Sex [male %]	65	62	63	63
Age [Years]	47 ± 5.36	46 ± 2.5	45 ± 9.8	51 ± 10.4
CA2M level [mg/dL]	47 ± 4.2	68 ± 12	131 ± 27.14	119 ± 31.6

Values are given as mean ± S.E.M. or frequencies (%).