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N-Acetyl-β-D-glucosaminidase in acute myocardial infarction

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Abbreviations: AMI, acute myocardial infarction; MI, myocardial infarction; NAG, *N*-Acetyl-β-D-glucosaminidase

Abstract

The objective of the study was to investigate whether the lysosomal enzyme, *N*-Acetyl-β-D-glucosaminidase (NAG) activity is increased in plasma of patients with acute myocardial infarction (AMI) and to determine if there is any association between plasma levels of NAG and severity of myocardial infarction (MI). NAG activity in plasma was monitored in 69 patients with AMI and 135 normal healthy subjects using a spectrofluorimetric method. A modified Aldrich ST elevation score was used to gauge the severity of MI in terms of size of the infarct. Plasma NAG levels in AMI patients and normal healthy subjects were found to be 10.92±7.5 U/l and 6.8±2.2 U/l, respectively. These two mean value when compared by Student's *t*-test were significantly different *P* = 0.0001. No statistically significant differences in NAG activity were observed in patients in terms of gender, age, location of infarct, time from onset of chest pain to blood sampling in the hospital and size of the infarct.

Keywords: acetylglucosaminidase; biological markers; inflammation; lysosomes; myocardial infarction

Introduction

N-Acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme, which is normarlly present in human plasma (Price and Dance, 1972). However, its levels have been found to be increased in a number of diseases, such as diabetes mellitus (Whiting *et al.*, 1979; Severini *et al.,* 1988), liver disease (Severini *et al.,* 1990) and cancer (Reglero *et al.,* 1980; Severini and Aliberti, 1994; Iqbal *et al.,* 1999). NAG activity has also been reported to be elevated in inflammatory conditions like arthopathies (Iqbal *et al.,* 1999) and chronic obstructive pulmonary diseases (Javed *et al.,* 1996). Since coronary artery disease and myocardial infarction (MI) are now also seen as an inflammatory conditions and markers like c-reactive protein are considered to be strong predictors of risk for future cardiovascular events (Libby, 2002), it is imperative that the role of other markers of inflammation, such as NAG should be studied in acute myocardial infarction (AMI).

The present study was undertaken to investigate whether or not NAG activity is increased in AMI patients compared to normal healthy subjects and to see if there is any association between plasma levels of NAG and size of the infarct.

Materials and Methods

Sixty nine consecutive Pakistani patients with AMI were included in this study. All these patients were admitted to the coronary care unit of the Aga Khan University Hospital between June 1998 to October 1999. The study had been approved by the Ethical Committee of the Aga Khan University. Demographic and clinical characteristics of the patients have been given in Table 1. Diagnosis of AMI was on the basis of clinical history, electrocardiogram (ECG) and biochemical data.

Blood collection

Ten ml blood was collected from the patients immediately after admission and transferred to a tube containing EDTA as anticoagulant. Plasma was immediately removed from it by centrifugation at 1,500 *g* for 15 min and stored frozen at -60°C until the time of analysis of NAG.

Analysis of NAG

NAG activity was determined in the plasma samples by the method of Whiting *et al.* (1979) as described in a previous publication (Iqbal *et al.,* 1998). Briefly, 0.1 ml of plasma was diluted with 0.4 ml of distilled water. To this was added 4.5 ml of 20 mM citrate-phosphate buffer, pH 4.4, containing 0.2 mM methyl-umbelliferyl-glucosaminidine at 37°C. One ml from this was then withdrawn at various time intervals (0 min,

5 min, 10 min, 15 min) and transferred to separate tubes containing 2 ml of 0.5 M glycine-NaOH buffer, pH 10.4, to stop the reaction.

The fluorescence of 4-methylumbelliferone (MU) released in each reaction was monitored spectrofluorimetrically at excitation and emission wavelengths of 368 and 448 nm, respectively, using JASCO FP-770 spectrofluorimeter. One unit of NAG activity was defined as that which released 1 μmole of 4-methylumbelliferone per minute under assay conditions.

Statistical analysis

Mean values have been presented as means±SD. Comparison of the two mean values was carried out by using Student's *t*-test. One way ANOVA followed by Tukey's HSD test was used for comparison of the mean values of more than two groups. *P* value of $<$ 0.05 was considered significant.

Severity of MI

Severity of MI in terms of size of the infarct was carried out by using criteria for modified Aldrich ST elevation score (Porela *et al.,* 1999) as outlined below:

Anterior

$3 \times [1.5 \times (\text{number of leads with ST elevation} \geq 0.1 \text{ mV}) - 0.4]$

Inferior

$3 \times [0.6 \times (\sum \text{ST elevation in mV}) + 2.0]$

Leads 1, aVL, V5, and V6 were included in the anterior and inferior scores according to the primary site of epicardial injury: Anterior V1 through V4; Inferior II, aVF and III.

A score of $\leq$15 was arbitrarily assigned to the small infarct; a score between 15.1-25 was assigned to the medium size infarct, a score $>$25 was ascribed to large infarct.

Controls

One hundred and thirty five normal healthy subjects with the mean age of 48±11.4 years were included as controls. They had no evidence of coronary artery disease, diabetes mellitus, hypertension, obesity, hypercholesterolemia, liver disease, uremia and malignancy. Those using any medication during the last 24 h were also excluded.

Results

Table 1 shows the demographic and clinical characteristics of patients. Male to female ratio was 4:1, and 6 of them had prior history of MI.

Figure 1 shows the means±SD plasma NAG activity in AMI patients and normal healthy subjects. Mean plasma levels of NAG in AMI patients and in normal healthy subjects were found to be 10.92±7.5 U/l and 6.8±2.17 UI, respectively. The mean NAG values in these two groups when analyzed by student's t-test were found to be significantly different (*P* = 0.0001).

Table 2 shows mean value of NAG in AMI patients relative to their sex, age, location of infarct and time from onset of symptoms to blood sampling for NAG. Comparison of the mean NAG values reveals no statistically significant difference between male and female groups, older and younger patients groups, and groups of patients in whom the time between onset of chest pain and blood sampling was $\leq$90 min, or 91-180 min, or $>$180 min (as commonly practiced).

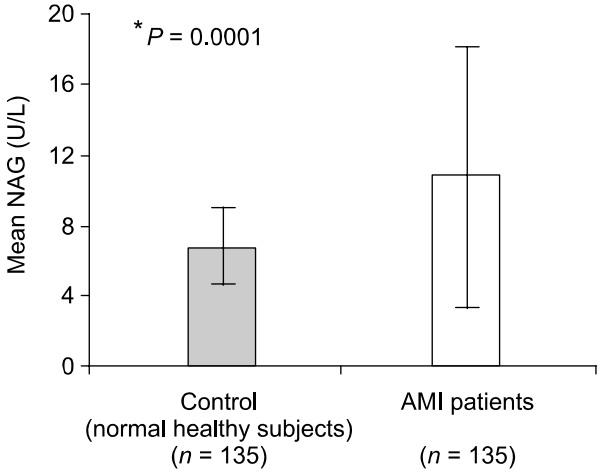

Figure 1. Plasma NAG activity in a group of AMI patients and normal control group. Comparison of the distribution of NAG values in two groups was carried out using Student's *t*-test.

Table 1. Demographic and clinical characteristics of patients with AMI.

Variable	Value	Frequency (%)
Age	56.8±12.3 years (range 40-79 years)	
Gender	55 males 14 females	80 20
Prior history of MI	6	9
Interval between onset of chest pain and blood sampling	3.06±2 h[a]	
MI location:		
-Anterior	33	48
-Inferior	36	52

[a]Means±SD.

Table 3 shows the relationship between the NAG activity in plasma of AMI patients and the size of infarct on the basis of modified ST elevation Aldrich score. Comparison of the mean NAG values in the 3 groups reveals no statistically significant difference suggesting that there was no apparent relationship between the plasma levels of NAG and the size of infarct in AMI patients.

Discussion

Plasma level of an enzyme reflects a balance between rate of its release in extracellular compartment and clearance or uptake from the extracellular space (Goldberg, 1986). The observed increase of NAG activity in plasma of AMI patients appears to be due to its release from cardiac lysosomes. This is supported by the observation that drugs known to stabilize the lysosomes during experimental myocardial anoxia cause reduction in plasma NAG levels (Welman *et al.,* 1980).

Our results are in agreement with those reported by Welman *et al*. (1980) and Inoue *et al*. (2000) who have also shown increased levels of NAG in AMI patients and in patients with coronary artery disease. Though, plasma NAG can arise from other tissues as well, due to complications of MI, yet the elevated activity observed in AMI patients is less likely to be due to that because we monitored NAG activity within just few hours after MI. The mean time from onset of chest pain to blood sampling in our patients was nearly 3 h.

In our patient populations, we did not find any significant difference in NAG values in terms of gender, age, location of infarct, time from onset of chest pain to blood sampling in the hospital and size of the infarct. Although Inoue *et al*. (2000) have reported a relationship between the activity of NAG and severity of coronary artery disease in terms of multi-vessel disease and single-vessel disease, yet our data did not show any relationship between NAG activity and size of the infarct. It may be because of the reason that our classification regarding severity of MI was based on modified Aldrich ST elevation score, which may not be as precise as would be the angiographic assessment (Inoue *et al.,* 2000). Moreover, another factor for the observed lack of association between NAG activity and severity of MI could be the relatively small number of patients in our study.

Our data, however, clearly show that plasma NAG activity is elevated in AMI patients. For evaluation of its role as a nonspecific diagnostic maker for MI, a prospective study comprising a large number of patients would be required.

Table 2. Plasma NAG activity in AMI patients relative to gender, age, position of infarct and time between onset of pain to blood sampling.

Variable	Number (n)	NAG(U/l)	*P*-value[a]
Gender:			
- Male	55	11.11 ± 8.21	0.68
- Female	14	10.17 ± 3.94	
Age:			
- ≤ 57 years	37	12.46 ± 9.47	0.067
- > 57 years	32	9.13 ± 3.72	
Location of Infarct:			
- Anterior	33	10.77 ± 7.92	0.88
- Inferior	36	11.04 ± 7.25	
Time (onset of pain to sampling):			
0-90 min	19	12.1 ± 8.6	0.388
90-180 min	26	11.6 ± 9.3	
>180 min	24	9.2 ± 3.3	

[a]*P*-value compares the groups within that variable.

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Table 3. Comparison of the distribution of NAG values in 3 groups of AMI patients (representing small, medium and large size infarcts) on the basis of modified Aldrich ST elevation score.

Group	Modified aldrich score	Classification based on size of infarct	No. (n)	NAG (U/l)	*P*-value[a]
1	≤15	Small	16	11.9 ± 7.3	NS
2	15.1-25	Medium	40	10.3 ± 7.3	NS
3	>25	Large	13	11.7 ± 8.8	NS

[a]*P*-value compares the mean values in the 3 groups using one way ANOVA.
NS, Not significant.

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