

Serum sialic acid and its lipid and protein bounds as possible biomarkers of acute myocardial infarction in erbil city

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Abstract

Backgrounds and objectives: Myocardial infarction (MI) usually occurs when coronary blood flow decreases abruptly after a thrombotic occlusion of a coronary artery previously affected by atherosclerosis. Sialic acid (SA) is attached to non reducing residues of the carbohydrate chains of glycoproteins and glycolipids. An elevation in serum TSA, LBSA and PBSA concentrations has been observed in a number of pathological conditions. The aim of the study was to determine the serum TSA and its LBSA and PBSA in patients with acute myocardial infarction (AMI).

Methods: Serum TSA, LBSA and PBSA concentrations were evaluated by UV/VIS spectrophotometry in (100) apparently healthy individuals and (100) newly diagnosed AMI patients.

Results: The mean levels of serum TSA, LBSA and PBSA in AMI patients were significantly higher ($P < 0.05$) than those of apparently healthy individuals.

Conclusion: The results indicate that the serum values of TSA, LBSA and PBSA appeared to be of a value in diagnosis of AMI.

Key words: AMI, TSA, LBSA, PBSA

Introduction

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the world. The most common cause of AMI is atherosclerotic ischemic heart disease (IHD) with fissuring or ulceration of a plaque causing clotting and complete arterial occlusion¹. Several risk factors for AMI have been well documented, including hypertension, hyperlipidaemia, diabetes, a positive family history, smoking, obesity and inactivity. However, these factors explain only part of attributable AMI². Sialic acid (SA) an important component of glycolipids and glycoproteins, is found in negatively charged surface polyanions on various cell membranes and play an important role in the antigenic characterization of cells³.

From over 30 derivatives of neuraminic acid, N-acetylneuraminic acid (NANA, SA) is the most common in humans, glucose is the precursor and the biosynthesis takes place in Golgi apparatus⁴. Reports of the research work done in this field since the last few years, reveals that the serum concentrations of total sialic acid (TSA), lipid bound sialic acid (LBSA) and protein bound sialic acid (PBSA) were increased and have been used as laboratory biomarkers in a variety of pathological conditions⁵⁻⁷. Lipids are common conjugates of serum lipoproteins, and SA is also present in combination with lipids and proteins as glycolipids and glycoproteins respectively⁸. The serum or plasma TSA concentration is a marker of the acute-phase response, since many of the acute-phase proteins (e.g. alpha-acid glycoprotein, fibrogen

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and haptoglobin) are glycoproteins with SA as the terminal sugar of the oligosaccharide chain. An elevated serum TSA level is a strong predictor of cardiovascular mortality in general population⁹. A prospective study conducted by Kario and Matsuo¹⁰ showed that mortality due to cardiovascular disease was increased in people with high serum TSA concentration. Another study showed that the level of serum TSA increases in proportion with the numbers of the coronary artery diseases(attack times) and the concentration of serum LBSA was related to the severity of coronary atherosclerosis¹¹. Van Dijk *et al*¹² reported that this increase in the serum levels of TSA and LBSA may occur via changes in the biosynthesis and post-translational glycosylation processing of the acute-phase glycoproteins in the liver .Recently, in a 17 year –follow up study serum TSA has been proposed to be a long-term predictor of IHD in adults, especially in women¹³. The role of serum TSA , LBSA and PBSA values in AMI promoted us to investigate their serum levels in AMI patients and to know whether we can use such parameters in diagnosis on AMI. To the best of our knowledge this is the first study in this field in our region.

Methods

1-Subjects :

This study was carried out during the period from July 2008 to September 2009 at the dept. of Clinical Biochemistry / College of medicine / Hawler Medical University / Erbil / Iraq .

The present investigation was conducted on (200) subjects , which were divided into two groups :

Group 1 (control group): Included 100 apparently healthy individuals (30 females and 70 males), the average age was (54.85 ±0.94) years, and the range of age was (35—76) years .

Group 11 (patient group): Included 100 newly diagnosed AMI patients (30 female-sand 70 males), the average age was (59 ± 1.02) years and the range of age was (36-80) years .

All these patients had been admitted to the coronary care unit (CCU)/ Erbil teaching hospital and the diagnosis by internal medicine consultants was based on a history of prolonged ischemic chest pain, characteristic electrocardiogram (ECG) changes , elevated serum creatine kinase iso-enzyme (CK-MB) and troponin T within 12 hours after the onset of pain. All AMI patients were carefully screened to exclude evidence of congestive heart failure, hepatic and renal failures , endocrinal disorders. Patients who had fasting plasma glucose ≥ 140 mg / dl were excluded.

2-Sampling: Eight—ten mls of peripheral blood was drawn from each subject using disposable syringes. The samples were transferred into glass tubes, quiting for 30 minutes for clotting and centrifuged for 15 minutes at 3500 rpm. The separated serum was used for measurements of: TSA , LBSA and PBSA .

3-Methods:

A-Determination of serum TSA:

Serum TSA was estimated according to the method of Katapoids *et al*¹⁴.

Principle :The principle of this method depends on the formation of a chromogen after addition of resorcinol reagent into the tube containing the sample. The chromogen formed was extracted by butyl acetate: methanol (85 :15 v / v) reagent and measured at 580 nm .

Procedure :To 20 µls of serum sample or standard solutions of SA, 980 µls of de-ionized water was added. The assay tubes were vortexed and placed on ice. To each assay tube, 1 ml of resorcinol was added, then the tubes were placed in a boiling water bath for exactly 15 minutes, then for 10 minutes on ice.

Two mls of butylacetate: methanol reagent (85:15 v/v) were added, then the assay mixtures were vortexed and centrifuged for 10 minutes at 3000 rpm. The extract chromophore was read at 580 nm against de-ionized water .

Caculation: A standard curve was obtained by plotting the absorbance at 580 nm against the corresponding concentrations of standard SA solutions (5,10, 15,20,25,30,35,40,45,50 mg/dl) and from this curve the concentration of TSA in the sample was determined, (Figure 1) .

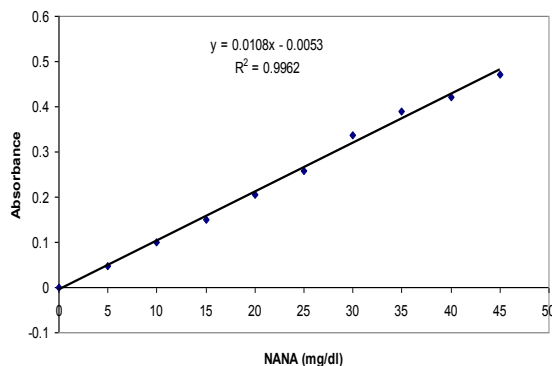


Figure1: Standard curve for determination of serum TSA .

B-Determination of serum LBSA:

Serum LBSA was also estimated according to the method of Katapodis *et al* ¹⁴

Principle :The principle depends on the formation a colored complex resulted from the reaction of phosphotugistic acid with the separated layer of the lipid using a mixture of chloform: methanol 2: 1 (v/v) .

Procedure :

Serum LBSA was measured according to the following steps:

1-One hundred and fifty μ ls of de-ionized water was added to 50 μ ls of serum sample, the tubes were vortexed for 5 minutes, and placed on ice.

2-Three mls of cold (4° C) chloroform: methanol 2:1 (v/v) solution was added, then 0.5 ml of cold de-ionized water was added, the tubes were centrifuged for 5 minutes at 3000 rpm at room temperature.

3-One ml of the resulting upper layer was transferred to another tube, and 50 μ ls of phosphotungistic acid was added, mixed and allowed to stand at room temperature for 5 minutes .

4-The assay tubes were centrifuged for 5 minutes at 3000rpm. The supernatant fluid

was removed and the remaining precipitate was dissolved in 1 ml of de-ionized water.

5-To each assay tube, 1 ml of resorcinol reagent was added, then the tubes were placed in a boiling water bath for exactly 15 minutes, then for 10 minutes on ice .

6-Two ml of butylacetate: methanol reagent (85 : 15 v/v) was added, the assay mixtures were vortexed and centrifuged for 10 minutes at 3000 rpm. The extracted chromophore was read at 580 nm against de-ionized water .

C-Determination of serum PBSA:

Serum PBSA values were calculated according to the method of Guzel *et al* ¹⁵ by subtracting of LBSA from serum TSA.

4-Statistical analysis :Statistical analysis were performed by SPSS version 18.0, t-test was used for data analysis and the results were expressed as mean \pm SE. The significance level was set as $P < 0.05$ ¹⁶ .

Results

The mean serum level of TSA was (65.03 ± 1.50 mg/ dl) in AMI patients which is significantly increased as compared to the control group (40.05 ± 0.39 mg / dl), ($P < 0,05$). There is no significant difference in the serum levels of TSA between males and females in both groups, ($P > 0.05$) (Table 1) .

The mean serum level of LBSA in AMI patients was (37.84 ± 0.85 mg/dl) which is significantly higher than that obtained in control group (21.08 ± 0.66 mg / dl), $P < 0,05$. There is also no significant difference in serum level of LBSA between males and females in both groups ($P > 0.05$), (Table 2)

Table (3) shows the mean serum levels of PBSA in both groups. The data obtained in AMI patients (27.20 ± 1.50 mg / dl) indicate a significant difference in the mean serum level of PBSA when compared with that of control group (18.89 ± 0.81), ($P < 0.05$). There is also no significant difference in serum level of PBSA between males and females in both groups , ($P > 0.05$) .

Table 1 : The mean \pm SE of serum TSA in both groups

Groups	Sex	No.	TSA(mg/dl) (Mean \pm S.E)	Statistical evaluation
Control	Males	70	39.72 \pm 3.89	P > 0.05
	Females	30	40.81 \pm 4.09	P > 0.05
	Both	100	40.05 \pm 0.39	
AMI	Males	70	65.52 \pm 8.09	P > 0.05
	Females	30	63.90 \pm 7.99	P > 0.05
	Both	100	65.03 \pm 1.50	P < 0.05

Table 2 : The mean \pm S.E. of serum LBSA in both groups

Groups	Sex	No.	LASA(mg/dl) (Mean \pm S.E)	Statistical evaluations
Control	Males	70	20.30 \pm 6.80	P > 0.05
	Females	30	22.90 \pm 5.91	P > 0.05
	Both	100	21.08 \pm 0.66	
AMI	Males	70	37.78 \pm 9.37	P > 0.05
	Females	30	37.98 \pm 6.08	P > 0.05
	Both	100	37.84 \pm 0.85	P < 0.05

Table 3 : The mean \pm S.E. of serum PBSA levels in both groups

Groups	Sex	No.	PBSA(mg/dl) (Mean \pm S.E)	Statistical evaluations
Control	Males	70	19.41 \pm 3.76	P > 0.05
	Females	30	17.91 \pm 3.07	P > 0.05
	Both	100	18.97 \pm 0.81	
AMI	Males	70	27.74 \pm 7.56	P > 0.05
	Females	30	25.92 \pm 5.81	P > 0.05
	Both	100	27.20 \pm 1.50	P < 0.05

Discussion

An increase in mortality was observed by AMI in elderly. Age is a powerful predictor of short term outcome in AMI. In the present study, the average age of AMI patients was (59.49) years, this is in agreement with the results obtained by Majid *et al* ¹⁷, who found that the prevalence of AMI was in the mean age of (57) years. The results obtained reveal also that the incidence of AMI was more in males than females and

the ratio was(2.4:1). This finding is similar to that reported by Lovlien *et al* ¹⁸. On the other hand the mean serum TSA level increases with age in women, but not in men and this increase seems to be due to menopause. This finding is similar to that of Pönniö *et al* ⁵, who showed that the mean serum concentration of TSA start to increase in women at about (45) years of age, which is close to the mean age of menopause. The mean of serum TSA level was significantly higher (P<0.05) in AMI

patients compared to controls. This finding is similar to those obtained by Nigam *et al*⁷, Zafar and Iqbal⁸, Majid *et al*¹⁷, Inayat *et al*¹⁹, and Gokmen *et al*²⁰. Several other studies have confirmed that serum TSA concentrations are elevated in patients with coronary heart disease CHD and MI and there is a positive correlation between increased serum TSA level and the severity of coronary lesions, Nigam *et al*⁷ Lindberg *et al*²¹, Allain *et al*²², Gokmen *et al*²³. Knuiman *et al*¹³ suggested that the association between serum TSA and CHD reflects an atherosclerotic process. The same authors showed that the increase in serum TSA may reflect increased sialylation of glycolipids and glycoproteins due to increased sialyltransferase activity. Recently, serum TSA has been shown to be a novel marker that could be particularly useful in assessing myocardial cell damage in patients undergoing cardiac surgery²⁴. Also it has been shown that an increased output of serum proteins by the liver due to some type of acute-phase reactions may be one of the possible sources of an increased serum TSA concentration in patients with MI²⁰. An increase in the activity of sialidase, which cleaves the terminal SA residues from oligosaccharides, glycoproteins and glycolipids, may play an important role in the elevation of serum TSA in MI²⁵. The mechanistic aspect of the raised serum level of TSA in cardiac patients is still not very clear, though several possibilities have been suggested such as reduction in desialylation of plasma glycoproteins⁷, increase in acute-phase reactants containing SA²⁶, or increased sialylation of serum proteins²⁷. Gokman *et al*²⁰ suggested that elevated serum TSA might also result either from the shedding or secreting of SA from the cell membrane surface, or releasing of cellular SA from the cells into the blood stream due to cell damage after MI. Gokman *et al*²³ in another study also reported that the shedding or secretion of SA from the cells or cell membrane may be responsible to a greater degree for the elevated serum TSA levels on the first day

after MI than the increased output of SA-rich inflammation sensitive proteins. Reganon *et al*²⁷ reported that serum TSA levels were significantly increased in post-infarction with and without recurrent coronary ischemia, compared to control subjects.

The results obtained in this study showed also a significant higher value of serum LBSA ($P < 0.05$) in AMI patients compared to controls. Similar findings were obtained by Gokmen *et al*¹¹ and Sonmez *et al*²⁵. The mean serum level of PBSA was also significantly higher ($P < 0.05$) in AMI patients compared to controls. Guzel *et al*¹⁵ demonstrated that the TSA, LBSA and PBSA concentrations in cattle with theileriosis or anaplasmosis were higher than those of healthy cattles and suggested that these elevations are due to the host immune response to the parasites. The same authors reported also that this may alter receptor-ligand interactions, which are known to play an important role in inflammation and immune response. The results obtained showed also a positive correlation between serum TSA, LBSA and PBSA in AMI patients. Similar results were obtained by Guzel *et al*¹⁵. Karagenc *et al*²⁸ reported that the release of SA from glycolipids and glycoproteins of the lysed cell membrane causes elevated LBSA and PBSA levels in theileriosis or anaplasmosis in cattles. Finally Breen²⁹ suggested that the possibility of auto-polysialylation may be responsible for the elevated serum LBSA in MI patients.

Conclusion

The results obtained indicate that the mean serum TSA, LBSA and PBSA were significantly higher in AMI patients than those of controls, so these parameters may be considered as possible biomarkers in AMI patients.

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