

Evaluation of serum fucose and protein bound fucose on myocardial infarction patients in Erbil city

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Abstract

Background and objectives: L-fucose is a methyl pentose similar to L-galactose, except for the loss of the alcohol group on carbon No.6. It is found in human serum bound to proteins by covalent bonding and it is a member of a large group of compounds known as glycoproteins. Glycoproteins determine and steer up the vital functions of plasma membranes, e.g. cell-cell recognition, growth control, information uptake mediated via hormones or toxins and the attachment of antibodies. The aim of the current study is to measure the serum concentrations of these parameters on both the healthy individuals and the myocardial infarction patients.

Methods: The existing study has been initiated and implemented in earnest over the period commenced from February 2009 to October 2010 materialised on 100 healthy volunteer individuals countered to 110 (newly diagnosed myocardial infarction) patients. Serum total fucose and protein bound fucose were measured by UV/VIS spectrophotometry.

Results: The mean concentrations of serum fucose and protein bound fucose were significantly being higher on myocardial infarction patients versus those of the control individuals.

Conclusion: in order to envisage the negative impact on the heart muscle can be judged by the increase in serum level of fucose and the subsequent protein bound fucose on the myocardial infarction patients, thus, they can be labelled as the biomarkered for the myocardial infarction.

Key words: TF, PBF and MI.

Introduction

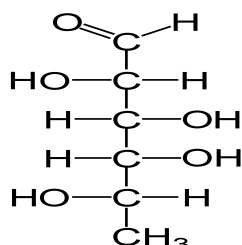
Myocardial infarction (MI) signifies the death of part of the heart muscle due to the interruption blood supply most commonly due to occlusion (blockage) of coronary artery causing the heart muscle to die. Without prompt immediate treatment, this can cause damage to the affected part of the heart, and leads eventually to heart attack. An MI is sometimes called a heart attack or a coronary thrombosis.¹ A heart attack occurs when one or more of the coronary arteries that supply blood to the heart are completely blocked and blood to the heart muscle is cut off^{2,3}, therefore it occurs when myocardial cells have

reached the threshold for ischemia; which in turn causes the body's myocardial cell turnover mechanism to be overwhelmed and completely fail. When this mechanism fails, myocardial tissue necrosis causing irreparable tissue/cell death occurs.⁴ About one fifth of all heart attacks are silent, that is, the victim does not know when the attack has been occurred. Although the victim feels no pain, silent heart attacks can still damage the heart, so infarction occurs in the absence of physical signs.^{2,5} L-fucose is a methyl pentose similar to L-galactose, except for the loss of the alcohol group on carbon No.6. It is found in human serum bound to proteins by covalent bonding and it is a member of

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a large group of compounds known as glycoproteins. In a typical plasma glycoprotein, oligosaccharide side chains are attached to an oligosaccharide core, which, in turn, is bound by a glycopeptides bond and in every case involves carbon 1 of the most internal sugar ⁶.



Structure of L-fucose.

Fucose is one of the eight essential sugars (Glucose, Galactose, Mannose, Fucose, Xylose, N-Acetylglucosamine, N-Acetylgalactosamine, and N-Acetylneuraminic acid). The body requires this sugar for optimal function of cell-cell communication. The L-form is the only common form of the sugar, while the D-form is a synthetic galactose analogue. ⁷ Reports of the research work done in this field, revealed that the serum concentrations of serum total fucose (TF) and protein bound fucose (PBF) were being increased and it has been suggested that the serum total fucose and protein bound fucose can be used as laboratory biomarkers in a variety of pathological conditions such as breast cancer⁸, leukemia⁹, diabetes mellitus¹⁰, gastric ulcers¹¹, and cirrhosis^{11,12}. Glycoproteins determine characteristic functions of plasma membranes, e.g. cell-cell recognition, growth control, information uptake mediated via hormones or toxins and the attachment of antibodies. ¹³ Fucose is a type of sugar that may be external or internal in both N- and O-linked glycoproteins, linked to the N-acetylglucosamine (GlcNAc) residue, attached to asparagine (Asn) in N-linked species. Can also occur internally attached to the -OH of serine (e.g. certain clotting factors). ¹⁴ The plasma membranes of eukaryotic cells contain carbohydrates that are covalently linked to both lipid and

protein components. Depending on the species and cell type, the carbohydrate content of the plasma membrane ranges between 2 and 10 percent by weight. More than 90 percent of the membrane's carbohydrate is covalently linked to proteins to form glycoproteins; the remaining carbohydrate is covalently linked to lipids to form glycolipids. All of the carbohydrate of the plasma membrane faces outward into the extracellular space. ¹⁵

Methods

Subjects: This study was conducted from February 2009 to October 2010 at the department of medical biochemistry/ college of medicine/ Hawler medical university/ Erbil/ Iraq. The current survey has been carried out on (210) volunteers, which were divided into two groups:

Group I (Control group): One hundred randomly selected subjects (70 males and 30 females) were served. All are apparently healthy volunteers.

Group II (MI patient group): One hundred and ten (76 males and 34 females) MI patients (diagnosed by consultants) were participated in the study. Details concerning both groups are shown in Table 1:

Table 1: Details of number and age of both groups.

Groups	Number	Age	
		range (years)	Mean±SE
Normal	100	35-75	53.28±5.97
MI patients	110	38-80	65.31±6.03

2-Samples: Eight to ten milliliters of peripheral blood samples were drawn in the morning from controls and MI patients using disposable syringes. The samples were transferred into glass tubes, quiting for (30) minutes for clotting and centrifuged at 3000 rpm for (15) minutes). The separated serum was used for measurement of serum TF and PBF.

3-Methods: Serum TF was estimated according to the method of Dische and Shettels.¹⁵

Principle: The principle depends on the formation of a chromogen after addition of concentrated H₂SO₄ and cysteine reagent into the tube containing the sample and the color product measured at (396-430 nm). The difference in absorbance was directly proportional to α-L-fucose content of the sample.

Procedure:

1. To 0.1ml ice cold serum, 4.5ml of chilled solution (83% H₂SO₄) was added slowly with constant shaking in ice bath (to prevent rise in temperature).
2. The tubes were transferred to a water bath at room temperature for few minutes, and then to vigorously boiling for exactly three minutes, the tubes were then placed in water bath at room temperature for 5 minute.
3. One-tenth ml of 3% cysteine hydrochloride was added to each tube then mixed. After two hours the absorbance was read at 396nm and 430nm.
4. The same procedure was applied for standard fucose solution (10mg/ml).

Calculations:

$$\text{Serum TF (mg/dl)} = \frac{\text{Ax at 390nm} - \text{Ax at 430nm}}{\text{Ast at 390nm} - \text{Ast at 430nm}} \times 12$$

Where Ax = Absorbance of the unknown.

Ast = Absorbance of the standard.

12 = Dilution factor.

Serum concentration of PBF was also determined for the two groups according to the method of Dische and Shettels¹⁵.

Principle: After precipitating the protein by ethanol, a color product was formed by fucose in strong acid medium, which combines with color developer (cysteine hydrochloride). The color product with cysteine is measured at 396 and 430nm.

Procedure:

1. One ml of 95% ethanol was added to 0.1ml of serum for protein precipitation, centrifuged for 20min, at 3000rpm, to get a clear solution.

2. The supernatant was decanted and washed with 1ml of 95% Ethanol then centrifuged again.
3. The supernatant decanted, the precipitate was re-suspended in 3ml of 0.1N NaOH to re-solubilize the proteins.
4. One ml of the above solution was placed into two test tubes, 4.5ml of concentrated H₂SO₄ was added, then the mixture was boiled in water bath for three minute. A colored product was obtained.
5. Duplicate test tubes were cooled using tap water and 0.1ml of 3% cysteine hydrochloride was added to one of the test tubes and mixed.
6. The absorbance was read against distilled water at 396nm and 430nm respectively.

$$\text{Serum PBF (mg/dl)} = \frac{(\text{Ax at 390nm} - \text{Ax at 430nm}) - (\text{Ay at 390nm} - \text{Ay at 430nm})}{\text{Ast at 390nm} - \text{Ast at 430nm}} \times 12$$

Where Ax = Absorbance of the unknown sample with cysteine.

Ay = Absorbance of the unknown sample without cysteine.

Ast = Absorbance of the standard.

12 = Dilution factor.

Results

Table 2: provides the mean serum TF concentrations in both groups. The results obtained reveal that the mean serum TF was (30.817 ± 6.79 mg/dl in MI patients, these values exceed significantly (P < 0.01) than those obtained in normal group, (mean = 14.772 ± 3.341 mg/dl). On the other hand the mean serum level of TF in MI patients was two folds more than that of normal subjects, and serum TF concentration showed an increase in about 52 % of MI patients. However there was no significant difference (P > 0.01) between males and females in both groups. Table (2) shows also the mean serum PBF concentrations in both groups. The data

obtained indicate that the mean serum PBF was (13.51 ± 3.76 mg/dl), (Mean \pm SD) in MI patients. This value exceeds significantly ($P < 0.01$) that obtained in normal group (9.932 ± 1.69 mg/dl). The mean serum level of PBF in MI patient was 1.3 folds more than that of normal subjects and serum PBF showed an increase in about 26.5 % of MI patients. However there was no significant difference ($P > 0.01$) between males and females in both groups.

Table 2: Details of serum TF and PBF (Mean \pm SD) in normal and MI groups

Groups	No.	Serum fucose (mg/dl)	Serum PBF (mg/dl)	P
		Mean \pm S.D	Mean \pm S.D	
Normal	100	14.772 \pm 3.341	9.932 \pm 1.69	
MI group	110	30.817 \pm 6.79	13.51 \pm 3.76	<0.01

Discussion

The mean serum TF concentration in MI patients was significantly higher than that of control subjects ($P < 0.01$). This result is in agreement with that of Lindberg *et al.*¹⁶ who studied the serum level of sialic acid and fucose in atherosclerotic patients and found an increase in MI patient. The serum TF comes mostly from the liver, and many researchers showed the possibility of using serum TF as a biomarker of many diseases such as breast cancer⁸, leukemia⁹, diabetes mellitus¹⁰, gastric ulcers¹¹, and cirrhosis.^{11,12} Elevated serum TF might result either from the shedding or secreting of fucose from the cell membrane surface, or releasing of cellular fucose from the cell into the blood stream due to cell damage after myocardial infarction.¹⁷ The mean serum PBF in MI patients was significantly higher than that of control subjects ($P < 0.01$). Similar results were obtained by Snyder *et al.*¹⁷ The level of serum glycoproteins has been shown to correlate

with coronary atherosclerosis, and with atherosclerosis-related conditions such as peripheral vascular disease, diabetes and hypertriglyceridemia¹⁶. Thus, it is suggested that the increase in serum levels of PBF may be due to glycosylation of proteins or may be due to biosynthesis of glycoproteins in liver in MI patients¹⁸.

Conclusion

As a result of this study, the following conclusions are drawing:

1. Estimation of the serum TF and PBF are easy and reliable methods.
2. The mean serum TF and PBF in MI patients were significantly higher than that of controls. So their measurements may be used as a biomarker in the diagnosis of MI.

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