Evaluation the Level of a-L-fucose and protein Bound Fucose (PBF) in diabetic patients

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ABSTRACT

Biochemical changes occurring in two types of diabetic patients have been investigated to identify the correlations between these changes and type 1 and type 11 of diabetic patients, in comparison with that of normal healthy, sera of 68 diabetic patient with type 1 and type 11 were used to estimate the L-fucose level and other related parameters. These parameters measured throughout this project are: TF, PBF, TP, TFYTP ratio and PBF\TP. As final approach, the author concluded that fucose level together with its related parameters could be a biochemical markers for early assessment of response to the therapy served.

الخلاصة

تم دراسة بعض التغيرات البايوكيمياوية التي تحدث في مصل دم المرضى المصابين بداء السكري لكلا النوعين ، وقد تم الاشارة الى توضيح العلاقة بين هذه التغيرات والنوع الاول والثاني من داء السكري وعند مقارنتها بالمصل الطبيعي للاصحاء(العينة الضابطة).

اجريت الدراسة على 68 عينة من مرضى داء السكري لتقدير الفا – ل – فيوكوز وبعض الدوال المرافقة لهذا **TF** السكر.ولقد اجريت الفحوصات المدرجة : **,PBF, TP,TF/TP ratio and PBF/TP**

ونستنتج من خلال هذه الدراسة يمكن اعتماد مستويات الفيوكوز كدالة متابعة لتشخيص داء السكري مكملة للكلوكوز المستخدم كدليل عمل تشخيصي.

INTRODUCTION

Diabetes mellitus is a complex disease or a group of metabolic diseases characterized by high blood sugar(hyperglycemia) levels which result to absolute or relative deficiencies in insulin secretion and \ or insulin action. In [1] sulin is a hormone that is produced by specialized cells (beta cells) in Langerhans of the pancreas. diabetes mellitus is typically classified in to two main sub type -I- or insulin dependent diabetes (IDDM) and -II- non insulin dependent diabetes (NIDDM) insu[2] lin deficiency or resistance to the action of insulin results diabetes mellitus About 90% of patients with diabetes have non-insulin depended (NIDDM). Type -II-such patients are usually obese, have elevated plasma insulin , and have down - regulated insulin receptors . The other 10% have insulin -depended (IDDM) tyupe-I- -L- [3] fucose is mono saccharide which is present in low concentrations in normal serum pub[4]lished works show that serum fucose is elevated in diabetes, cancer. and in flammocory diseases.

L - fucoce is a 6-carbon deoxy hexose, a 6 - deoxy -L-galactose, or methyl pentose similar to ga-lactose except for the loss of alcohol group on carbon -6 [6]. Fucose is a monosaccharide found in gly-coproteins and cell, also poly saccharide, such as xyloglucon and thamno gala cturonams I and II. [7] L_fucose is commonly in corporated in to human glycoproteins and glycolipids fucose (TF), it is found in two forms D and L. fucose, D. fucose is generally limited to plant products and microbial antibiotic substances plant poly saccharides and animal glycans contain the L. enantiomer. Fucose [8] may present in the human body in three forms. [9]

1- freefucose: present in trace amount in serum.
2- lipid - bound fucose: (LBF) in glycolipids.
3- protein .boundfucose: (PBF) :asterminal pre-terminal suger of glyco protein in the cellmemberance and other cell components .

These three forms of fucose represent the total serum. The serum level of proteins depends on the balance between their syntheis and their catabolism from the body. [10]

The protein contained large amount of dicarboxylic amino acids, free glutamic and aspartic acids were poorly utilized the svnthssis of this protein. People with type (1) or type (2) diabetes who are in poor meta bolic control may have increased protein requirement [11]. However, the usual amount of protein consumed by people with diabetes compensates for the increase protein catabolism [12]. Because insulin has aglobal effect on protein metabolism, increasing the rate of protein degradation. Thus in-sulin deficiency will lead to increased catabolism of protein. The increased rate of proteolysis lead to elevated concentration in plasma amino acids.

METHODOLOGY

Patient samples

Sixty eight samples of blood were taken from males, (40 samples) and females (28 samples) with type I DM and type II DM , aged between (25 - 55)years . The normal healthy donors were of 24 samples.

Blood sampling

Blood (5mls) of both normal and patients were collected by venous arm puncture , and was allowed to clot and

settle at room temperature for one hour and was centrifuged at (3000 rpm) for (20 minutes), the sera were removed with a peasteurpiptte and stored frozen at (-20 C) until assayed .

Biochemical test

Biochemical test include TF, PBF and TP were determined in diabetic patient and normal healthy control. The study was comprehensive determination of the ration (TF/TP), (PBF/TP), for diabetics and control.

Statistical analysis Z-test was used to determine if the mean value for the biochemical tests were significantly different in diabetics from that of normal healthy control P. value < (0.05), was considered significant. [13]

Determination of total fucose (TF) Principle

The principle of this method depends on a direct reaction of concentrated sulfuric scid with serum components .the reactants combine with cysteine, and the color product measured at (396 - 430 nm). the difference in absorbance were directly proportional to methyl pentose content of the solutions . this protocol of determination was adopted of that of dische and shettels method [14].

Determination of protein - bound fucose (PBF) Principle

Protein - bound fucose was determined according to dische and shettles method a color product (chromophor) was formed by fucose in stroung acid medium, which combine with color develops (cysteine hydrochloride). the color product with cystein measured at 396 nm. [15]

Determination of serum total protein (TP) Principle

The principle of this method depends on the reaction of peptide bond of the protein with cupric ion (Cu^{*2}) in alkaline medium to form colored products whose absorbance was measured at (540 nm). This method is called Biuret method (15).

DISCUSSION

Serum Total Fucose

Fucose is normally present in the serum and is the only levorotary sugar synthesized and utilized by mammalian system .it is a normal component of glycoproteins. [4]

Fucose levels widely investigated in different specimens. It is found in plant and bacteria, abundant in human breast milk and certain mushrooms, and in amniotic fluid. [6]

Data obtained Table 1 showed the mean ± SX of TF. Showed higher level of total fucose in type I (mean X = 25.42 mg/dl) and type II (mean X=25.26 mg/dl) compared with controls (mean X= 12.05 mg/ dl).

TF is found to be significantly higher than that of control group, the result was consistent with the finding reported [3]. Those studies were reflect the increase level of L-fucose in diabetic patients. Figure 1 illustrates the distribution of TF level in sera of control group, type I and type II. The reason for the elevation in TF level due to an increase in fucose moiety, from glycol—conjugates (mostly protein , and lipid) by the increase in fucose transferase activity, that liberate fucose from the carbohydrate chain in to sera, plasma and urine. [16]

Table 2 and Figure 2 show the (TF/TP) ratio for type I and type II of diabetic patient. This ratio can be used as a good parameter during development of the cases studies which is also increased in type I and type II.

Total protein PBF, glycoprotein

Table 3 and Figure 3 show the (mean ± SX) of control group (6.28 ± 6.ol), type I (6.01 ± 0.25) and type II (6.24±0.37) expressed as g /dl.

Table 4, 5 show the predictive values of TP. The results show no significant elevation in type I and type II, protein (as total protein) remain constant.

It had been assumed that, in people with type II diabetes, abnormalities of protein metabolism are less sensitive to insufficient insulin action than those of glucose .howevergougcon *et .al* [17] have demonstrated that moderate hyper glycemia can cotribute to an increased turnover of protein in subjects with type 2 diabetes compared with an obese - control group . previous studies have suggested that smaller amounts of circulating insulin are sufficient to prevent protein loss in type 2 diabets . In [18] people with type Idiabetes, the effect of protein on glycemia will be dependent on the state of insu-linization and the degree of glycemiacontrol .protein require insulin for metabolism as dose carbohy-drate and fat, but has minimal effects on blood glucose levels. [19]

Studying levels of protein - bound fucose (PBF) these levels were revealed highly significant elevation of PBF LEVEL (P<0.0005) in type I and type II when compared to control groups. The level of PBF become high as shown in Figure 4. The presence of higher ratio (PBF / TP) in type I and type II when compared with control group as in table 6. shows that are highly significant differences (P< 0.0005) in the level of this ratio.

Table 4, 5 considered the increase in PBF level goes back to the increase in the activity of silyl transfers (sialidase) on silyl glycoprotein may release the terminal sugar of the glycoprotein chain (sialic acid) in to serum [20], this agreed with report of that fucose and sialic acid are terminal sugars in carbohydreate chain of glycoprotein[8] .

REFERENCES

[1] Edward , C.R.W; Boncher , I.A.D and Hasslet, **C**. Davidson's principles of medicine 17th ed**.** In-ternational student edition. Diabetes mellitus pp.72-746.1996.

[2] Sheruin , R.S. Cecil text book of medicine 20th ed. U.S.A, Vol. 2: 1258-1278.1996.

[3] Granner , D.K; Robert, K.M; Peter , A.M . and Victor , W.R. Harpers biochemistry , 24th ed. international edition.1996.

[4] Raymond .Adwek . chem. Rev. 96(2) : 683-720, 1996.

[5] Yorek, M.A ; Conner . C.E ;spenheimer, R.G. cell phsiol. 165: 658-666 .1995.

[6] Hassan, H.G . Ibn al-haithem .for pure and appl.sci, 17(8) :71-79 .2004.

[7] Dr. Michael, W.king .science magazine vol.291 p-5591 Jan.26 .2001.

[8] Al-Talbany NSH " an msc thesis " supervised by Hamid G.hasan , university of sulaomania , collage of science , Iraq .2001.

[9] Jay , J. listiusky ; Gene , P. seigal and Catherine. M.ListinskyAm.j.clin. Pathol . 110: 425-440.1998.

[10] Harris ,RJf biochemistry, 32: 639-6547.1993.

[11] Jonase . Richmond metabolism of plasm glycoproteins vol. 2 ,no.4 , July .any .1963.

[12] Marion J.Frans ,Ms, RD, LD, CDE, diabetes spectrum vol .(13), no.(3), 132.2000.

[13] Daniel W.W. biostatistics : A foundation for analysis in the health science by page 127-139. 4th ed.1987.

[14] Disch Z. and shettles L.B.J Biol. Chem. 175: 595-603.1948.

[15] Nishimura , H.J takao , T.; Hase , S.; shimonishi, Y.; Lwanaga, H.J. Biol.Chem. 276: 17520-17525.1995.

[16] Doumes , B.T.B ; Borner, K.C, and scahfferclin.chem. 27: 1642.1981.

[17] Al-Dorri , M.S ; Athesis, M.Se - Al-mustansiryah university by collage of medicine , biochemistry (2000).

[18] Gougeon, R; Marliss .E.B; Jones .P.J ; penchar, P.Bandmoris, J.A. effect of exogenous insulin on protein metabolism with differing non-protein energy in takes in type 2 diabetes , Int . jobes relate metbodisord 22: 250-61, 1998.

[19] Welle , S.L ; Nair, K.S . failure of glyburide and insulin treatment to decrease leuinoflut in obese type 11 diabetic patient Intabes 14: 701-710,1990.

[20] Mao , J ;prica, D.D ; meyer, DJ; Hayo , R.J, brain res. 14; 588(1): 144-149, Aug,1992.

Appendix-A

Table 1. comparison of TF, TP values in sera of control and different Types of DM

Group description	No.	TF(mg/dl)	PBF (mg/dl)	TP(g/dl)
Control	24	12.05 ±0.21	7.13 ±0.37	6.29 ±0.1 6
Type I	30	25 .42 ±1.25	11.57 ±0.57	6,01 ±0.24
Type II	38	25.26 ± 0.63	13.57 ±0.20	6.23±0.27

Table 2. Biostatistical calculation &Z - test for (TF/TP) level in sera of control and different type of DM

Serum TF/TP	Control	Type I	Type II
Sample size	N=24	N=30	N=38
Mean	X=1.72	X = 3.57	X=3.67
Standard deviation	SD = 0.06	SD=1.54	SD=1.20
Standard error of the mean	SX-0.01	SX = 0.20	SX = 0.07
Confidance interval of the mean	1.70-1.72	3.25-4.14	3.57-4.02
Z - test		6.60	18.75
Probability		P<0.0005	p<0.0005

Table 3, Biostatical calculation & Z - test for TP level in sera of control and different type of DM

Serum TP	Control	Type I	Type II
Sample size	N = 24	N=30	N=38
Mean	X=6.28	X = 6.01	X =6.24
Standard deviation	SD= J.10	SD=2.03	SD=4.9
Standard error of the mean	SX=0.15	SX = 0.25	SX = 0.37
Confidance interval of the mean	6.05 - 6.73	5.47-6.51	5.45 - 7.02
Z – test		1.11	0.22
Probability		N.S	N.S

Table 4. the predictive values for the overall biochemical makers in the type I DM

Biochemical makers	Sensitivity	Specificity	Positive productivity	Negative productivity	Efficiency
TF	69.30%	90%	90.87%	75%	80.61%
PBF	46%	85%	79%	56%	65%
TP	41.62%	45%	65%	40.6%	50.01%
TF/TP	81.37%	54.86%	75.30%	80.65%	88.03%
PBF/TP	81.86%	55%	85.70%	60.11%	81.60%

Table 5. The predictive values for the overall biochemical markers in the type II of DM

Biochemical makers	Sensitivity	Specificity	Positive productivity	Negative productivity	Efficiency
TF	90.22%	90%	95.76%	80.50%	90.30%
PBF	63,20%	95%	93.92%	40.70%	75.40%
TP	43.16%	51%	75.15%	16.24%	44.66%
TF/TP	97.81%	60.83%	90.80%	82.30%	90.80%
PBF/TP	80.3%	81.80%	90.10%	51%	80.12%

Table 6. Biostatistical calculation & Z - test for (PBF/TP) level in sera of control and different type of DM

Serum (PBF/TP)	Control	Type I	Type II
Sample size	N=24	N=30	N=38
Mean	X=1.21	X=1.83	X =2.04
Standard deviation	SD= 0.031	SD = 0.33	SD = 0.63
Standard error of the mean	SX= 0.041	SX = 0.03	SX= 0.03
Confidance interval of the mean	1.1-1.32	1.73-1.93	1.94-2.14
Z – test		6.28	8.77
Probability		P<0.0005	P<0.0005

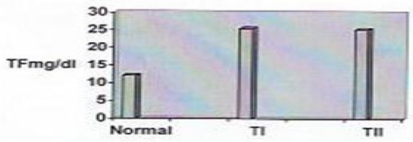

Fig.1: Median serum TF level in sera of normal, and type I,II of diabetic patient

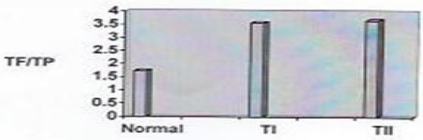

Fig.2: Median serum (TF/TP) level in sera of normal and type I,II of diabetic patient

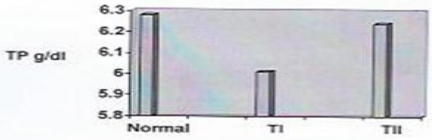

Fig.3: Median serum TP level in sera of normal, normal, and type I,II of diabetic patient

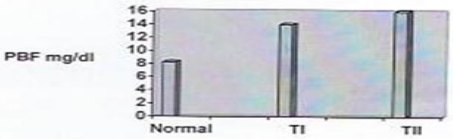

Fig.4: Median serum PBF level in sera of and type I,II of diabetic patient

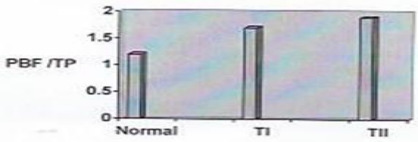

Fig.5: Median serum (PBF/TP) level in sera of normal, and type I,II of diabetic patient