PHYSICO-CHEMICAL AND ENZYMATIC STUDY IN TYPE1 DIABETES MELLITUS[+]

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Abstract:

This study included 130 Type 1 diabetic patients who were admitted to the Center of Endocrinology and Diabetes near AL-Kindy Teaching Hospital ; from Nov. 2006 to April 2007, in addition to 40 apparently healthy subjects used as control.

Fasting blood glucose (FBG) , C-Peptide, HbA1c , Lipid profile and body mass index (BMI) were performed for all..

This study revealed that the disease is prevalent among male (58.5%) , majority of them (55.4%) at age of (11-17) years with highly significant difference in comparison with other age groups (P<0.001) .

It was clear that the genetic factor has a crucial role in susceptibility for the disease with previous family history (87.7%) (P<0.001).

The majority of diabetic patients (43.8%) had a disease duration range (1-5 years). Most of patients (79.8%) were students (P,0.001).

The mean of FBG among diabetic patients was highly significantly elevated (13.58±3.39 m mole / L) in comparison with healthy individuals (4.427± 0.72 m mole/L) (P=0.001) .HbA1c mean has been increased significantly among diabetic (11.62% ± 2.86) in comparison with control group (4.66% ± 0.72) (P =0.001).

No significant variation was noticed between BMI value of diabetic patients type 1 (17.44 Kg/m² ± 2.83), in comparison with control group (16.72 Kg/ m² ±3.40) (P=0.184).

Lipid profile determination showed that there was significant elevation in serum cholesterol of DM type 1 (4.626 ± 1.272 m mole / L) and triglycerides (1.441 ± 0.741 m mole/ L) in comparison with healthy controls (3.975 ± 0.994 & 0.970 ± 0.389) .

There was a strong correlation between disease duration and most of the above parameters such as C-peptide , FBG , LDL, HDL and triglyceride , while the latter was associated with BMI (P = 0.011).

المستخلص:

شملت هذه الدراسة ١٣٠ مريضا" مصابا" بالسكري النمط (١) من مراجعي المركز التخصصي لامراض الغدد الصم والسكري المجاور لمستشفى الكندي التعليمي للفترة من تشرين الثاني ٢٠٠٦ ولغاية نيسان ٢٠٠٧ . وقد اضيف للدراسة ٤٠ سليما" ظاهريا" كفئة مقارنة.

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تم فحص جميع الاشخاص لفحوص الدم للكلوكوز والببتيد- C وخضاب الدم المسكر HbA1c ونمط الدهون اضافة الى معامل كتلة الجسم Body mass index(BMI) .

اظهرت النتائج مايلي:

ان غالبية المرضى (58.5%) هم من الذكور واغلب المرضى (55.4%) هم من الفئة العمرية ١١-١٧ سنة وبدلالة معنوية عالية (P<0.001) مقارنة بباقي الفئات العمرية.

للعامل الوراثي دورا" هاما" ، فاغلبهم (87.7%) ذو تاريخ عائلي للاصابة (P<0.001) ان مدة المرض ل(43.8%) تتراوح بين ١-٥ سنة.

واغلب المرضى (79.8%) هم من الطلبة مقارنة بالشرائح الاخرى(P<0.001)

ان معدل كلوكوز الدم عند المرضى (13.58±3.39 m mole/L) مقارنة بالسويين (4.427±0.72 m mole /L)

(P=0.001) . وضوح ارتفاع معدل خضاب الدم المسكرHbA1c لدى المرضى (11.62%± 2.86) عما هو عليه عند الاصحاء(4.66%±0.72) (P<0.001)

لم تظهر أي فوارق معنوية (P=0.184) بين معامل كتلة الجسم BMI (17.44Kg/m²±2.83) عند المرضى عن نظيرها لدى الاصحاء (16.72Kg/m²±3.40) بينما هناك ارتفاعا" ملحوظا" في معدل الكوليستيرول (4.626±1.272 m mole/L) والكلسيريد الثلاثي (1.441±0.741 m mole/L) عند المرضى عما هو عليه عند مجموعة المقارنة(0.970±0.389 m mole/L, 3.975±0.994 m mole/L) على التوالي.

لوحظ وجود علاقة معنوية قوية بين مدة المرض ومعالم السكر مثل الببتيد – C ومستوى الكلوكوز وLDL و HDL و والكلسيريد الثلاثي . فيما اظهر الاخير ارتباطا" معنويا" مع معامل كتلة الجسم فقط (P=0.011) .

Introduction :

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion ,action or both [1] .

Type 1 DM was known as Insulin Dependent Diabetes Mellitus (IDDM) or juvenile – onset diabetes , accounts for 5-10% of all cases of primary DM [2].It is an autoimmune disease [3], leading to progressive exhaustion and destruction of β-cells , probably by auto- reactive T lymphocyte culminating in hypo- insulinemia and hyperglycemia[4] . The destruction is associated with HLA- DR3 / DR4 hetrozygote [5].Dietary modification and oral hypoglycemic agent are inadequate therapy[6].Insulin is therefore necessary for survival [7] .

Assessment of Glycemic Control:

1-Blood testing :

Fasting blood glucose (FBG) ≥ 126 mg/dl (7.0 m mol/L) or randomly or oral glucose tolerance test ≥ 200mg/dl (11.1 m mol /L) when observed twice , the person is considered DM according to WHO.The classical symptoms include polyuria, polydipsia and unexplained weight loss [8].

2-Glycated hemoglobin (GHb or HbA1c) test:

It is a blood test that measures the amount of GHb provides an accurate and objective measure of glycemic control over a period of weeks to months which is the erythrocyte life span .When exceeds 8% , the person is considered DM[9] .

The amino acid valine at N- terminal of hemoglobin β- chain combines with glucose to form stable ketoamine GHb (HbA1c) due to long term DM [10].

C – peptide :

C- peptide (Connecting) , C –shaped polypeptide with a molecular weight 3600, containing 31-36 amino acids ; derived from pro- insulin produced in pancreatic β-cells . Pro – insulin is converted into insulin and C- peptide , which are then secreted into portal blood in an equimolar quantity [11]. C-peptide replacement has been shown to partially prevent diabetic polyneuropathy in rats and human . C-peptide assay , so , is more reliable indicator of insulin level and presence ; hence distinguishes type 1 from 2 [12].

Body Mass Index (BMI):

Body mass index (BMI) is a relative indicator of body fatness. Over-weight and obese individuals are at increased risk of many diseases [13].

Lipid profile :

Lipids (free cholesterol, cholesterol esters , phospholipids , and triglycerides) are transported in the plasma as lipoproteins ,which include chylomicron (located normally at the intestinal mucosa only) ,very low density lipoprotein (VLDL) , intermediate-density lipoprotein (IDL) , low density lipoprotein (LDL), and high density lipoprotein (HDL) [14].

Triglyceride (TG) & esterified cholesterol (EC) are transported by lipoprotein molecules. VLDL & LDL lipoproteins are filled by TG & EC in the liver , transported by blood plasma into tissues , drop most of their contents of TG & EC in the tissues and return back to the liver via plasma , in form of HDL. Same molecule when releases it's contents becomes smaller & thus the ratio of protein to lipid becomes high (HDL) , while before releasing TG & EC the ratio was low (LDL & VLDL) .

Lipid profile when shows high HDL means low lipid transportation , i.e., normal lipidemia and vice versa applied for LDL & VLDL.

Lipid disorders in type 1 diabetes:

DM is , as compared with non-diabetic population, associated with a much mortality of ischemic heart disease and other cardiovascular diseases mostly due to diabetic dyslipidemia [15].

Lipoprotein pattern is anti- atherogenic in individuals with type 1 DM and have optimal glycemic control. In type 1 diabetic patients man had normal lipid and lipoprotein values. Abnormal values , including elevated total and LDL cholesterol and decreased HDL cholesterol ,were observed in younger female patients with higher HbA1c levels[16] .In type 1 DM, lipid values usually become normal when the blood glucose level becomes normal , however qualitative changes of lipoprotein frequency persist . Increased glycation and oxidation of lipoproteins is a common sign of both types of diabetes [17].

Subjects, Materials & Methods:

Subjects (Patients & controls):

One hundred and thirty selected type 1 diabetic patients were enrolled in the study , who attend the Specialized Center for Endocrinology and Diabetes (AL-Kindy Hospital) during the period from November 2006 to April 2007, age range (

2.2 – 18) years. Various parameters were studied : age , sex, BMI, FBG , lipid profile,C-peptide and HbA1c. As a control, forty apparently healthy persons,(3-18) years, were included ,with no history of systemic disease .Normal BMI & normal family history.

Blood sampling :

About 8 ml blood was drown from all ; 2 ml in an EDTA tube for HbA1c measurement. The rest was put in an Eppendrof disposable plain tube , centrifuged (10 min., 3000 rpm) , serum used for the biochemical determinations and serological assy of C-peptide.

Measurement of body mass index (BMI):

BMI is a mathematical formula based on a person's weight (kilograms) divided by height (meters) (BMI= Kg/m^2) [18].

BMI= 18.5 to 24.9 normal weight
25 to 29.9 over weight
≥ 30 obese

Materials:

Used from highest performance and origins when possible, as follows (table 1):

Equipment	Manufacturing company	Country
Spectrophotometer	Cecil CE 7200	England
Variant gamma counter	Bio-rad LKB(127 Rock gamma	U.S.A
^{125}C-Peptide [I] IRMA Kit	REF-RK-84CT	Bodapest
Glucose Kit	Biomegreb	France
HDL-Cholesterol Kit		
Total – Cholesterol Kit		
Triglyceride Kit		
Variant Hemoglobin AC Reorder pack	B 10 Rad	U.S.A

Methods:

1-Determination of serum glucose by enzymatic colorimetric (GOD-PAP):

Following the procedure provided by the manufacturing , Biomeghreb, (France).

Glucose is oxidized by glucose-oxidase to gluconate and by hydrogen peroxide according to the following equation [19].

Normal Range = 70- 105 mg/dl
3.89 – 5.84 m mole/L

2-Determination of glycated hemoglobin HbA1c%:

Using the variant hemoglobin A1c program developed by BIO-Red . The variant hemoglobin A1c program utilizes the principles of ion exchange high performance liquid chromatography (HPLC) for the automatic and accurate separation of hemoglobin A1c (HbA1c) ; without interference from Schiff base, lipemia or temperature fluctuation. The separated HbA1c then passed through the flow cell of the filter photometer at (415 nm) to be measured. A chromatogram of the changes in the absorbance was plotted versus the retention time. Preceding analysis ,

samples were diluted with hemolysis reagent and then incubated at 8-28 ºC for a minimum of 15 minutes [9,20] .
Normal Range : Up to 6% (6-8% critical).

3- Determination of C-Peptide [I^{125}] IRMA:

Uses two high affinity monoclonal antibodies in an immuno-radiometric assay

(IRMA) system .The I^{125}labeled signal-antibody binds to an epitope of the C-peptide molecule, which is different from that recognized by the unlabelled capture- antibody .

The two antibodies react simultaneously with the antigen present in standards or samples to the formation of a capture antibody-antigen- signal antibody complex "Sandwich " .

The radioactivity is measured in gamma counter . The concentration of antigen is directly proportional to the radioactivity measured. By constructing a calibration curve plotting binding values against a series of standards containing known amount of C-peptide , the unknown concentration of C-peptide in patient's samples could be determined [21].

Tests were duplicated .

Normal range = (1.07 – 3.5) ng/ml

4-Determination of serum HDL – Cholesterol (HDL-C):
As performed by the manufacturers [22].
Normal range = 0.9 – 1.4 m mole / L

5-Determination of triglyceride(TG):
As performed by the manufacturers [23] .
Normal range = 0.9 – 2.4 m mole/ L

6- Determination of Low – Density Lipoprotein Cholesterol (LDL-C):
Could be calculated mathematically using Fried Wald's formula: [23]
Normal range : 1.8 – 4.3 m mole / L

7- Determination of Very Low Density Lipoprotein (VLDL) :
Could be estimated [23] :
Normal range : < 1 m mole / L

8-Determination of total Cholesterol (TC):
As performed by the manufacturers [24].
Normal range : 3.9 – 6.5 m mole / L

Statistical Analysis **:**

SPSS statistical methods were used. [25].

Results and Discussion:

Demographical picture of patients:

This search (table 2) revealed that males are slightly dominant (58.5%) , no significancy (P= 0.054) . The majority of patients (55.4%) lie at age 11-17 years, high frequency (41.5%) at age 2-10 years (P<0.01). Agrees with Rooman *et al* [26] who stated that type 1 largely restricted to boys , particularly less than 10 years.

The explanation for these results suggests that an environmental factor may preferentially accelerate the sub – clinical disease process in young boys.

The majority of type 1 (87.7%) are with a previous family history (P=0.001) . These data coincide with that of Freire *et al* [27] who denoted to an inherited pre dispositional factor.

The observed data reveal that most of patients (43.8%) complained from DM for 1-5 years ; while the least (3.1%) were suffering for 11-13 years (P= 0.001) .

This table (2) shows that the students (79.8%) exposed for type 1 DM development are the highest group (P= 0.001) , which resemble other authors [26 & 28].

The explanation of these data could be that the students are nearer to the educational culture than other groups.

Table (2) Demographical Picture of Patients :

Parameter	Group	Percentage%	Significancy
Gender	Boys	58.5	Non sig. P> 0.05
	Girls	41.5	
Age groups (years)	2-10	41.5	H.S.P<0.01
	11-17	55.4	
	18-25	03.1	
Family history	Positive	87.7	H.S P=0.001
	Negative	12.3	
Disease duration (years)	<1	30.8	H.S. P=0.001
	1-5	43.8	
	6-10	22.3	
	11-13	3.1	
Occupation	Childhood	11.6	H.S. P=0.001
	Student	79.8	
	Housewife	4.7	
	Worker	3.9	

Estimation of Some Biochemical Parameters & Body Mass Index:

Fasting blood glucose (FBG) and HbA1c have been estimated ; the results reveal that there is a highly significant elevation in the level of F.B. G. among the patient's samples (13.58 ±3.39 m mole/L) in comparison with control group (4.427± 0.72 m mole / L) (P = 0.001) . Furthermore , there is a highly significant increment in the level of HbA1c among the diabetic cases (11.62%± 2.86) in comparison with control cases (4.66% ± 0.72) (P = 0.001).

The BMI observed to be (17.44 Kg / m² ± 2.83) for DM patients in comparison with (16.72 Kg /m² ± 3.40)for control with no significant difference between them (P = 0.184).

Regarding blood glucose , it is well accepted that patients are suffering from significant elevation in FBG which rises due to carbohydrate consumption . Insulin is released into blood stream by Beta cells of the pancreas , subsequently this principal hormone regulates the uptake of glucose into most cells . Deficiency of insulin due to β-cells destruction as in type1 DM or insensitivity of its receptors as in type 2 DM results in elevated blood glucose levels . The results of the current study are compatible with Rother [29] .

Table (3) Mean of FBG , HbA1c% & BMI levels among studied groups.

Parameters	Studied group	N	Mean	Std. Deviation	Std. Error	Comparison of significant	
						P-value	Sig.
F.B.G. m mole/L	Control	40	4.427	0.72	0.11	0.00	HS
	Patient	130	13.58	3.93	0.34		
	Total	170					
HbA1c %	Control	40	4.66	0.72	0.11	0.00	HS
	Patient	130	11.62	2.86	0.25		
	Total	170					
Body mass index Kg/ m²	Control	40	16.72	3.40	0.53	0.184	NS
	Patient	130	17.44	2.85	0.25		
	Total	170					

The high level of glycated hemoglobin (HbA1c) represented poor glucose control . Levels of HbA1c are not influenced by daily fluctuation in the blood glucose conc. but reflect the average glucose level over the prior six to eight weeks . Therefore is a useful indicator of how well the blood glucose level has been controlled ,which may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in DM patients.

Recent studies referred to use HbA1c as an indicator for accumulative effect of type 1 DM [30] . The present results demonstrated the same finding .

Considering BMI , the data disagree with some studies [31] which referred to decline in BMI of type 1 DM from the average , and the patients often have a thin body –type . On the other hand , another study [32] denoted elevation of BMI which is consistency with the current study .

Most people affected by type 1 DM are otherwise healthy and a healthy weight when onset occurs . Diet and exercise can not reverse or prevent type 1 DM , sensitivity or responsiveness to insulin treatment usually is normal especially in early stages [33].

Our current study , however , shows that the weight increment after intensive therapy was moderate and patient's BMI remained approximately in the same control value.

Estimation of Lipid Profile among the studied groups;

Lipid profile tests , show that there is a highly significant elevation in the levels of cholesterol (4.626± 1.272 m mole / L) and triglycerides (1.441± 0.741 m mole/L) in the sera of DM patients in comparison with control group (3.975± 0.994 & 0.970 ± 0.389) (P= 0.003 & 0.001) respectively .

Moreover , there is a significant elevation in the level of LDL among patients
(2.679 ± 1.189 mg / dl) in comparison with controls (2.217 ± 0.972 mg / dl) (P=0.027).

Table (4) Mean distribution of DM patients lipid profile tests levels among studied groups.

Lipid profile	Studied Group	N	Mean	SD	SE	Comparison of significant	
						P-value	Sig;
S – Cholesterol m mole/L	Control	40	3.975	0.994	0.157	0.003	HS
	Patient	130	4.626	1.272	0.111		
	Total	170					
S- triglycerides m mole/L	Control	40	0.970	0.389	0.061	0.00	HS
	Patient	130	1.441	0.741	0.065		
	Total	170					
HDL m mole/L	Control	40	1.313	0.254	0.040	0.653	NS
	Patient	130	1.347	0.463	0.043		
	Total	170					
LDL m mole/L	Control	40	2.217	0.972	0.153	0.027	S
	Patient	130	2.679	1.189	0.104		
	Total	170					
VLDL m mole/L	Control	40	0.456	0.435	0.068	0.111	NS
	Patient	130	0.773	1.226	0.107		
	Total	170					

Little information has been published about the lipid profile among type 1 DM. The present study reveals a high prevalence of dyslipidemia in type 1 DM and is in agreement with previous study [34].

The individuals with poorly controlled insulin- treated type 1 DM show normal or mildly increased triglyceride , total cholesterol and LDL-C levels . These finding suggest that the lipid profile of type 1 DM is highly dependent on glycemic control.

The current lipid profile picture is similar to that of [35].

Correlation between lipid profile test & glucose test parameters:

The correlation between all the parameters are shown in table (5) . There is a good correlation between disease duration and other parameters except for C-peptide, HDL and VLDL . Body mass index , triglycerides , & cholesterol correlate significantly with the disease duration (P = 0.001 for each) beside F.B.G. and HbA1c% (P = 0.006 , 0.005 respectively) . While LDL reveals significant association with duration of DM (P = 0.015).

Body mass index doesn't associate significantly with any of the other parameters except for triglyceride (P = 0.011) . These facts have been proved by the others work [36].

Regarding F.B.G. , it shows highly significant correlation (P = 0.001) between it and HbA1c. Highly sig. correlation observed between HbA1c & C – peptide. Both are structural parameters [10 & 11].

Table (5) Correlation between parameters (Duration of disease, Lipid profile tests , BMI, C-peptide , F.B.G. ,& HbA1c %) of diabetic patients.

Pearson Correlation		BMI	F.B.G	HbA1c	C-Peptide	Tri. G	Cholesterol	HDL	LDL	VLDL
Duration (years)	r	0.385	0.241	0.245	-0.03	0.424	0.376	-0.02	0.213	0.04
	Sig.	0.00	0.006	0.005	0.665	0.00	0.00	0.802	0.015	0.648
	C.S	HS	HS	HS	NS	HS	HS	NS	S	NS
BMI	r		0.098	0.028	0.047	0.222	0.082	-0.04	0.033	-0.005
	Sig.		0.268	0.752	0.595	0.011	0.352	0.581	0.706	0.952
	C.S		NS	NS	NS	S	NS	NS	NS	NS
F.B.G	r			0.338	-0.08	0.025	0.125	-0.12	0.149	-0.038
	Sig.			0.00	0.372	0.777	0.155	0.148	0.090	0.666
	C.S			HS	NS	NS	NS	NS	NS	NS
HbA1c	r				-0.48	0.103	0.174	-0.16	0.123	-0.029
	Sig.				0.00	0.243	0.047	0.056	0.164	0.744
	C.S				HS	NS	S	NS	NS	NS
C-Peptide	r					0.001	-0.06	0.115	-0.10	0.155
	Sig.					0.995	0.481	0.194	0.240	0.077
	C.S					NS	NS	NS	NS	NS

r = Pearson correlation coefficient CS= comparison of significance

It is well- known that the long duration of disease is accompanied with many complications such as a triglyceride and total cholesterol elevation . These results may be due to the accumulation effects of decline in insulin and continuous eating of patients due to hunger felling of cells , as a result bad intake of glucose takes place[37].

Blood glucose was observed to be strongly correlated with HbA1c (P = 0.001) this result is in a harmony with the fact that there is an amazing relationship between blood glucose control and HbA1c , which is also demonstrated by Rohlfing *et al* [38].

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