Research Article

A Study of Serum Betatrophin levels in Children with Type 1 Diabetes Mellitus

Basma A. M Ali, Doaa M. Mahrous and Mai M. khalaf
Department of Pediatrics, Faculty of Medicine, Minia University, Minia, Egypt.

Abstract
Introduction: Diabetes is reaching an epidemic stage worldwide with International Diabetes Federation (IDF) estimating that around 415 million adults are diabetic, and this number will increase to approximately 640 million by the year 2040. **Aim of the work:** The aim of this study were to evaluate the levels of betatrophin in children with T1D, and to study their correlations with different clinical and laboratory parameters. **Patients and methods:** This study was conducted upon 90 children who were randomly selected ; 60 children with Type 1 Diabetes (T1D) who had regular follow up in the pediatric endocrinology outpatients' Clinic, Maternity and Children University Hospital . Another 30 children were taken as a control group apparently healthy age and sex matched to the diabetic group. The study was conducted during the period from December 2016 to May 2017. **Results:** Diabetic groups had higher levels of betatrophin than the control. On the other hand ,the newly diagnosed diabetic children had significantly higher level of betatrophin than long standing diabetics and the control groups where (p =0.001) for both. The long standing T1D children had higher levels of lipid profile (TC, LDL, TG), ACR and lower level of LDL than the newly diagnosed and the control groups. **Conclusion:** Our study found increasing of serum betatrophin levels in newly diagnosed as well as in the long standing diabetic children, which reflects that there is already a potential stimulus for beta cell proliferation present in type 1 diabetes. In addition, there were differences between the newly diagnosed and the long standing diabetic children as regard serum betatrophin levels which had a significant positive moderate correlations with HbA1c,TG levels and a significant fair correlations with group I and a significant strong positive correlation with HbA1c levels in group II. This results may suggest that the duration of T1D affects the betatrophin levels.
Key words: Betatrophin, Type 1 diabetes (T1D).

Introduction
Diabetes is reaching an epidemic stage worldwide with International Diabetes Federation (IDF) estimating that around 415 million adults are diabetic, and this number will increase to approximately 640 million by the year 2040**(1).**

Generally, diabetes develops from the inabil- ity of the pancreas to cope with the increased insulin demand in type 2 diabetes (T2D) 3-7 or the inability of β-cells to produce insulin due to a destruction of β-cells in type 1 diabetes (T1D).

Type 1 diabetes (T1D)develops due to the inability of β-cells to produce insulin due to a destruction of β-cells in T1D[(2)]**.**

Despite intensive research, there is still no treatment available to prevent loss of beta cells in T1D. At disease onset, generally 30–40% of beta cells remain. Most patients have residual B-cell function for several years, and even up to several decades later[(3)]. This suggests the possibility of ongoing renewal of beta cells in patients with T1D, or that some of these cells are resistant to immune destruction***(3)***[A].

Betatrophin (ANGPTL8, lipasin, RIFL) is a new member of angiopoietin-like protein family. ANGPTLs exhibit multiple functions, playing a role in lipid and glucose metabolism, inflam- mation, hematopoiesis, and cancer. Betatrophin is predominantly expressed in liver and adipose tissue[(4)]**.**

Betatrophin is involved in triglyceride metabolism through its interaction with ANGPTL3 and regulation of lipoprotein lipase activity[5].

Patients and Methods

This study was conducted upon 90 children who were randomly selected; 60 children with Type 1 Diabetes (T1D) who had regular follow up in the pediatric endocrinology outpatients' Clinic, Maternity and Children University Hospital. Another 30 children were taken as a control group apparently healthy age and sex matched to the diabetic group. The study was conducted during the period from December 2016 to May 2017.

These children were diagnosed with T1D by as regard to (ADA 2018) criteria:-

Fasting plasma glucose (FPG) ≥ 126 mg/dl (7 mmol/L). Fasting is defined as no caloric intake for at least 8h. OR

2hr plasma glucose post prandial after 75 mg oral glucose tolerance test (OGTT) ≥ 200 mg/dl (11.1 mmol/L). OR

In patients with classic symptoms of hyperglycemia or hyperglycemic crisis, random plasma glucose (RPG) ≥ 200 mg/dl (11.1 mmol/L). OR

HbA1C ≥ 6.5%.

The participents of this study were be divided into 3 groups:

Group I: (30 child) newly diagnosed with T1D (within one year)

Group II: (30 child) with long standing T1D (diagnosed for more than five year).

Group III: (30 child) apparently healthy, age and sex matched to the diabetic children as a control group .

Inclusion criteria:

• Both sexes were be included.

• Physically active non smokers participants.

• Co-operated patients.

Exclusion criteria:

We excluded type 2 diabetic children a

• Presence of any other diseases such as thyroid disorders.

Statistical analysis

The data were encoded, entered and processed on computer using SPSS (statistical program for social science, version 13.0).Figures were done using Microsoft Excel.

Descriptive statistics:

Continuous variables were presented as mean followed by standard deviation (SD), and categorical variables were presented as frequently and percentage.

Analytic statistics:

- Chi-square (X^2) was used to compare between more than one proportion

A significant statistical test result was considered according to the p value as follows:

- P value > 0.05 -------- non-significant.
- P value < 0.05 -------- significant.
- P value < 0.01 -------- highly significant.

Correlation was used to relate two numeral variables.

Pearson correlation was used to assess the strength of association between two variables. The correlation co-efficient, denoted symbolically (r) which defines the strength and direction of the linear relationship between two variables.

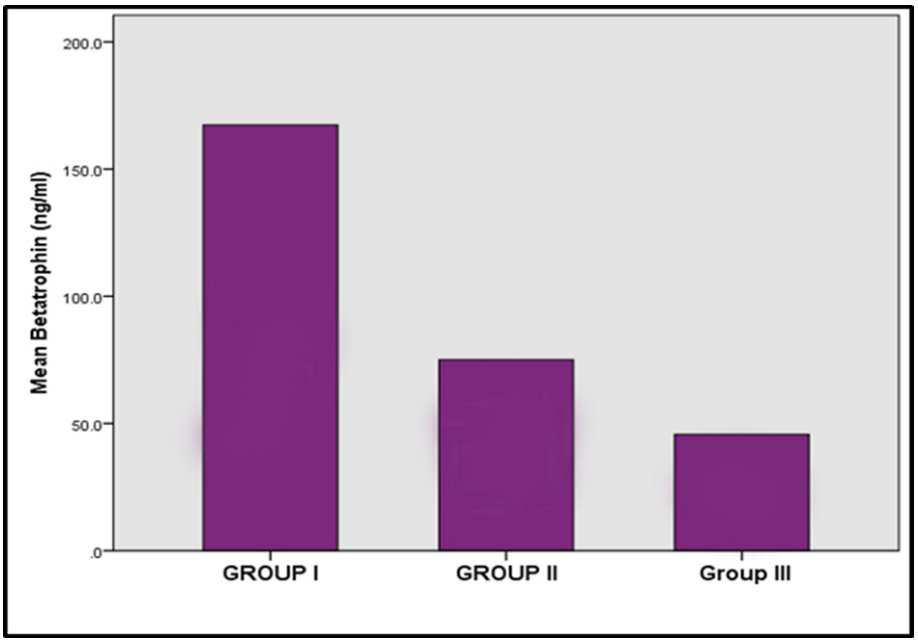
Results

Comparison between different studied groups regarding laboratory parameters :

Variable	Group I N=30	Group II N=30	Group III N=30	P-value		
	Mean ±SD	Mean ±SD	Mean ±SD	I#II	I#III	II #III
FPG (mg/dl)	148.5±21.9	148.7±25.1	83.3±10.03	1	<0.001**	<0.001***
PPPG (mg/dl)	245.4±37	244.0±31	154.2±14.4	1	<0.001**	<0.001**
HbA1c (%)	9.42±1.66	8.89±1.66	4.36±1.08	0.170	<0.001**	<0.001**
C-peptide (ng/ml)	0.26±0.07	0.27±0.06	1.47±1	1	<0.001**	<0.001**
ACR (mg/gm)	10.6±6.1	34.13±63.5	10.8±5.5	0.04**	0.988	0.04**
T.C (mg/dl)	146.6±24.4	160.03±13	131.7±21.5	0.038*	0.019*	<0.001**
LDL (mg/dl)	85.3±18.6	98.5±12.4	74.7±16.03	0.006**	0.033*	<0.001**
HDL (mg/dl)	43.3±10.1	38.5±3.5	38±5.04	0.027**	0.011*	1
TG (mg/dl)	97.8±18.5	114.9±15.3	98.6±14.2	<0.001**	0.001**	0.001**

Comparison between different groups of the study as regard betatrophin levels:

Variable	Group I N=30	Group II N=30	Group III N=30	P-value		
				I#II	I#III	II #III
Betatrophin (ng/ml)						
Range	15-640	15-223	22-62			
Mean ±SD	169.3±161.3	75.1±38.8	45.7±11.6	<0.001*	<0.001*	0.239



The newly diagnosed diabetic children had significantly higher level of betarophin than long standing diabetics and the control groups where (p =0.001) for both.

Discussion

The incidence of T1D is reported to be increasing by 3-5% per year, and the number of people with diabetes is estimated to reach 380 million by 2025 as regard[6].

Despite intensive research, there is still no treatment available to prevent loss of beta cells in type 1 diabetes. At disease onset, generally 30–40% of beta cells remain. Most patients have residual beta cell function for several years, and even up to several decades later[7]. This suggests the possibility of ongoing renewal of beta cells in patients with type 1 diabetes, or that some of these cells are resistant to immune destruction.

Betatrophin was recently described as a potent stimulator of mouse beta cell proliferation . Moreover, the secreted protein has been detected in human plasma. When overexpressed in mice, a 17-fold increase in beta cell proliferation was observed[8].

So, the aims of this study were to evaluate the levels of betatrophin in children with T1DM, and to study their correlations with different parameters.

Our study included 90 children aged from 5 -17 years. They were divided to 3 groups; Group I included 30 children with duration of T1D ≤ 1 year (newly diagnosed T1D), they were 13(43.3%) males and 17(56.7%) females with a mean age of 8.12±3.5 years; Group II included 30 children with duration of T1D ≥ 5year (long standing T1D), they were 13(43.3%) males and 17(56.7%) females with a mean age of 9.7±3.8 years; and Group III included 30 healthy children age and sex matched to diabetic children ,they were 13(43.3%) males 17(56.7%) females with a mean age of 8.01±3.1 years.

Concerning the clinical data of the T1D cases group I (newly diagnosed T1D) & group II (long standing T1D) the frequency of DKA was (63.3 & 76.7) respectively as a first presentation and it was more than presentation with classical symptoms or accidentally discovered and this was in agreement with[9] who found that the incidence of DKA as a first presentation of T1D was higher than that reported by other studies in Australia.

Moreover group I had significantly lower doses of insulin than group II and this could be explained by that Shortly after T1D diagnosis, patients may sometimes experience a brief spontaneous partial remission, called honeymoon, in which insulin requirements decrease and glycemic control improves[10]. During this stage, which has an average duration of 9 months, the remaining β-cells can still produce enough insulin to reduce exogenous insulin doses. Restored β-cell function and C-peptide secretion had been reported to last up to two years[11].

Our study showed that diabetic groups had significantly higher levels of T. cholesterol and this was in agreement with who found that T. cholesterol levels were increased T1D patients , furthermore our study showed that there were significant increase in levels of LDL and TG among diabetic than the control group and this was in agreement with who found that Mean value of total Cholesterol, serum triglyceride, LDL- Cholesterol and VLDL-Cholesterol in diabetic group were increased compared to control group. Mean value of serum HDL-Cholesterol was significantly lower in diabetic group compared to control . This could be explained by In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides in plasma may be due to the abnormal lipid metabolism[13]. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibit the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes[14]. High levels of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may be due to the obesity, increase calorie intake and lack of muscular exercise in the patients of diabetes mellitus[15].

In long standing diabetic children (group II), we found that they had significantly higher levels of ACR than group I diabetic and control group and this was in agreement with Andrzej, (1995) who reported that the risk of microalbuminuria increased with the duration of T1D .

Regarding betatrophin ,the current study found that the diabetic groups had higher levels of Betatrophin than the control and this was in agreement with Espes et al., (2014) who reported that Betatrophin concentrations were approximately doubled in patients with T1D compared with controls, Moreover, this was in agreement with Yamada et al., (2015) who reported that serum levels of circulating betatrophin were increased by 4.1 to 5.4 times in patients with T1DM compared with healthy controls. This indicates that there is already a potential stimulus for beta cell proliferation present in T1D[(16)].
Elevated betatrophin levels in T1D was not known and a further studies is needed to determine the exact changes in serum betatrophin.

Furthermore , our results showed that (group I) had significantly higher levels of betatrophin than (group II) and the control, till now there is no other studies that compare betatrophin level between the newly diagnosed and long standing T1DM children.
In our study, we found insignificant association between betatrophin levels and different age groups or sex distribution in the studied cases and this was in agreement with Espes et al., (2014)[A].

Conclusions

In conclusion, the current study found increasing of serum betatrophin levels in newly diagnosed as well as in the long standing diabetic children, which reflects that there is already a potential stimulus for beta cell proliferation present in type 1 diabetes . In addition, there were differences between the newly diagnosed and the long standing diabetic children as regard serum betatrophin levels which had a significant positive moderate correlations with HbA1c,TG levels and a significant fair correlations with group I and a significant strong positive correlation with HbA1c levels in group II.

This results may suggest that the duration of T1D affects the betatrophin levels.

References

1. Abu-Farha M, Abubaker J, Al-Khairi I (2015). Higher plasma betatrophin/ ANGPTL8 level in Type 2 Diabetes subjects does not correlate with blood glucose or insulin resistance. Sci Rep;(5):10949.
2. ADA (American Diabetes Association) (2018)[D]. Standards of medical care in diabetes. Diabetes care ; (1): S13-105.
3. Ammerpohl O, Pratschke J, Schafmayer C, Haake A, Faber W, von Kampen O, Brosch M, Sipos B, von Schonfels W, Balschun K, et al., (2011). Distinct DNA methylation patterns in cirrhotic liver and hepatocellular carcinoma. Int. J. Cancer; (130):1319–928.
4. Belo L, Caslake M , Gaffney D, Santos-Silva A, Pereira-Leite L, Quintanilha A, Rebelo I (2002). Changes in LDL size and HDL concentration in normal and preeclamptic pregnancies. Atherosclerosis; (162): 425–32.
5. Boussageon R, Bejan-Angoulvant T, Saadatian-Elahi M, Lafont S, Bergeonneau C, Kassaï B, Erpeldinger S, Wright JM, Gueyffier F, Cornu C (2011). "Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomized controlled trials". BMJ; (343): 4169.
6. Clark A, Jones LC, de Koning E, Hansen BC, Matthews DR (2001). Decreased insulin secretion in type 2 diabetes: a problem of cellular mass or function? Diabetes; (1): S169–71.
7. Espes D, Lau J, Carlsson PO (2014)[A]. Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes. Diabetologia ;(57): 50-3.
8. Duntas LH (2002). Thyroid disease and lipids. Thyroid; (12): 287-93.
9. Gerstein HC, Islam S and Anand S (2010). Dysglycaemia and the risk of acute myocardial infarction in multiple ethnic groups: an analysis of 15,780 patients from the INTERHEART study. Diabetologia.; (12): 2509-17.
10. Harjutsalo V, Forsblom C, and Groop PH (2011). Time trends in mortality in patients with type 1 diabetes: nationwide population based cohort study. BMJ ;(343):64.
11. Herold KC, Gitelman SE, Masharani U, et al (2005). A single course of anti-CD3 mono-

clonal antibody hOKT3g1 (Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes; (54): 1753-69.

12. John N and Michael A Reference ranges for laboratory tests and procedures in (2010) Nelson text book of pediatrics 19th ed. WB saunders Co. Philadilphia; (710): 2396-427.
13. Knip M, Virtanen SM, Seppa¨ K, Ilonen J, Savilahti E, Vaarala O, Reunanen A, Teramo K, Hamalainen AM, Paronen J, Dosch HM, Hakulinen T, Akerblom HK (2010). Dietary intervention in infancy and later signs of beta-cell autoimmunity. N Engl J Med; (20): 1900-8.
14. Mathieu C, Bandenhoop K (2005). Vitamin D and type 1 diabtes mellitus, state of the art. Trends Endocrinol Metab; (16):261-6.
15. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004). The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. Pediatriccs; (2): 555-76.
16. Olerud J, Mokhtari D, Johansson M (2011). Thrombospondin-1: an islet endothelial cell signal of importance for {beta}-cell function. Diabetes; (7):1946-54.