SERUM LEVELS OF TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN TYPE 1 DIABETIC CHILDREN AS APREDICTOR OF METABOLIC CONTROL

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ABSTRACT

Background*: Type 1 diabetes mellitus (T1DM) is the most common metabolic disease in childhood. In the course of diabetes, the main clinical problem to be faced is the development of micro- and macro-angiopathies. It has been suggested that hyperglycemia may lead to the activation of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12)]that are crucial for development and progression of microangiopathy. TNF-α production is thought to play a role in the generation of microvascular complications associated with diabetes. In addition to triggering acute and chronic inflammation, TNF-α regulates glucose and lipid metabolism and inhibits insulin production in pancreatic beta cells.*

Aim of the study: *To estimate the tumor necrosis factor alpha (TNF-α) levels as one of immunologic markers of microangiopathies in children with type 1 DM.*

and the correlation of its level with other inflammatory markers as CRP and ESR as well as with other important factors as microalbuminuria and Glycosylated hemoglobin (Hb A1c).

Patients and methods: *The study was a cross-sectional study included 60 children with T1DM recruited from the regular attendants of the Diabetes Clinic, Children's Hospital, Assiut University. They were 28 males (46.7%) and 32 females (53.3%), Their ages ranged between 1-16 years with a mean ± SD 9.46 ± 4.09 years. T1DM, was defined in accordance with the criteria of the American Diabetes Association* ***(ADA 2007)*** *All patients were on regular insulin treatment in dose of (0.99 ± 0.28 unit/kg/day).The control group consisted of 20 apparently healthy children of matched age, sex and nutritional status.*

Results: *Our results demonstrated that; there were significant differences between type 1 diabetic patients with normal and abnormal TNF-α regarding mean levels of blood urea , serum lipid profile (total cholesterol, triglycerides , LDL & HDL) , HbA1c concentrations and mean levels of inflammatory markers and microalbuminuria. While, there was no significant difference regarding CBC and serum creatinine between both groups. Also there was a significant difference between diabetic patients with abnormal TNF group and diabetic patients with normal TNF group regarding weight and body mass index (BMI). While there was no significant difference between*

both groups regarding age, height, systolic as well as diastolic blood pressure measurements, duration of diabetes and insulin dose.

Conclusion: *Type 1 diabetes is associated with elevation of serum TNF- α levels. This association is more evident in both newly diagnosed and poorly controlled patients indicating a relevant role of TNF- α on the pathogenesis of the disease. TNF- α can be used as a marker of severity in T1DM as it is significantly increased in those with poor glycemic control.*

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the most common metabolic disease in childhood **(Ross, 2003).** As DM is a serious chronic disorder of childhood and represents a major public health problem, type 1 diabetes is the predominant form of diabetes during this period, accounting for about 90% of cases ***(Craig et al., 2009).***

Incidence rate varies greatly between different countries, within countries, and between different ethnic populations. The global incidence of T1DM is increasing worldwide, at an annual rate of 3-5%, particularly in children under the age of 5 years and this trend leads to a significant health burden (***Soltesz et al., 2007).***

In the course of the diabetes, the main clinical problem to be faced is the development of micro- and macro-angiopathies. Nephropathy, retinopathy and arterial hypertension are present in children already 5 years before the onset of the disease ***(Fong et al., 2003).***

The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive glycemic control reduces the long-term vascular complications of hyperglycemia in T1DM. Unfortunately, diabetic complications continue to be a major cause of morbidity and mortality in persons with T1DM ***(Libby et al., 2005***).

By using predictors of metabolic control based on data collected at diagnosis or shortly after, closer monitoring of at-risk patients would be possible. Early intervention and monitoring in these patients could potentially reduce the likelihood of severe negative health outcomes associated with sustained hyperglycemia ***(Frey et al., 2007)***.

Regardless numerous studies carried out worldwide, albumin excretion rate (AER) remains the best available non-invasive predictor for diabetic nephropathy ***(Unnikrishnan et al., 2007)***, while ophthalmologic examination standard for diagnosis of retinopathy but both after damage development ***(Giorgino et al., 2004).***

Therefore; researches have been focused on the search for markers of diabetic damage to kidneys and eye apparatus already at the early phase of the disease, when the widely used parameters of function of these organs remain still within the range of norm ***(Browning et al., 2006).***

It has been suggested that hyperglycemia may lead to the activation of proinflammatory cytokines that are crucial for development and progression of microangiopathy ***(Yokoi et al., 2005).*** One of the glucose toxic mechanisms, the protein glycation, is associated with cytokines: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12) that are substantial factors in the development of diabetic microangiopathy so TNF-α production is thought to play a role in the generation of microvascular complications associated with diabetes. In addition to triggering acute and chronic inflammation, TNF-α regulates glucose and lipid metabolism and inhibits insulin production in pancreatic beta cells ***(Devaraj et al., 2007).***

THE AIM OF THE STUDY

(1) To estimate the tumor necrosis factor alpha (TNF-α) levels as one of predictors of metabolic control and as one of immunologic markers of microangiopathy in children with T1DM.

(2) To study the correlation of these levels with other inflammatory markers as CRP and ESR levels as well as with other important factors as Glycosylated hemoglobin (Hb A1c) and microalbuminuria.

PATIENTS AND METHODS

Patients:

This study included 60 children with T1DM recruited from the regular attendance of the Diabetes Clinic, Children's Hospital, Assiut university. They were 28 males (46.7%) and 32 females (53.3%) Their ages ranged between 1-16 years with a mean ± SD of 9.46 ± 4.09 years. T1DM was defined in accordance with the criteria of the American Diabetes Association ***(ADA, 2007).*** All patients were on regular insulin treatment in dose of 0.99 ± 0.28 unit/kg/day. The control group consisted of 20 apparently healthy children of matched age, sex and nutritional status.

Inclusions criteria:

- T1DM patients.
- Their ages ranged between 1-16 years.
- Recently diagnosed or after treating of DKA attack.
- On regular insulin treatment.

Exclusions criteria:

- Age less than one year or more than 16 years.

- Patients during DKA attack.
- Patients with systemic diseases as TB, malignancy, hematological, cardiac, respiratory, hepatic, etc.
- Patients who receive any drug other than insulin.

Methods:

All children were subjected to the following:

I. **Careful history taking**: A questionnaire was planned to fulfill the following data:

1. Demographic data; name, age, sex and socio-economic class. Age at onset of diabetes, disease duration was calculated.
2. Insulin therapy; regarding, type, dose and frequency, mean insulin dose was calculated.
3. History suggestive of acute metabolic complications:
   - History suggestive of hypoglycemic attacks (sweating, headache, blurring of vision, tremors, convulsions, coma) and number of hospital admissions due to severe attacks.
   - History suggestive of hyperglycemia (Polyuria, polyphagia, polydipsia, loss of weight, coma due to DKA) and number of hospital admissions due to DKA.
4. History suggestive of chronic diabetic complications:
   - Ocular manifestations: blurring of vision or flashes of light.
   - Peripheral neuropathy (PNP) manifestations: All patients were questioned regarding the presence or otherwise of symptoms, either positive or negative suggesting the presence of neuropathy. The questionnaire was the Diabetic Neuropathy Symptom DNS Score ***(Meijer et al., 2002).*** The questions should be answered 'yes' (positive: 1 point) if a symptom occurred more times a week during the last 2 weeks or 'no' (negative: No point) if it did not.

1. Symptoms of unsteadiness in walking?
2. Do you have a burning, aching pain or tenderness of your legs or feet?
3. Do you have pricking sensations at your legs and feet?
4. Do you have places of numbness on your legs or feet?

Maximum score: 4 points; 0 points- PNP absent; 1-4 points - PNP present.

- Renal manifestations: polyuria, oliguria, dysuria, loin pain, or hematuria.

II- **Examination:** Thorough clinical examination with particular emphasis on:

1. Anthropometric measures; height in cm and weight in kg were plotted against percentiles for age and sex according to Egyptian growth charts.
2. Body mass index was calculated by applying the following formula:

$$BMI = \frac{\text{weight in kg}}{(\text{height in m}^2)}$$

Ambulatory blood pressure; measured by conventional mercurial sphygmomanometer.

3. Full neurological examination to detect evidence of peripheral neuropathy.

III- **Investigations:**

- Complete blood picture.
- Complete urine analysis.
- Liver function tests (SGOT & SGPT) for exclusive causes.
- Estimation of kidney function (B.urea & S. creatinine).
- Estimation of fasting & post-prandial blood glucose levels.
- Estimation of Glycosylated hemoglobin (HbAlc); using quantitative colorimetric determination of glycohaemoglobin in whole blood.
- Estimation of urinary micro-albumin. Micro-albuminuria defined as value more than 30mg albumin/g creatinine.
- Serum total cholesterol, serum triglycerides and HDL .

  And LDL calculated by (total cholesterol- 1/5 triglycerides)
- Estimation of erythrocyte sedimentation rate (ESR) by Weatergren method.
- Estimation of C-reactive protein (CRP) by latex test.
- Estimation of serum TNF-α level using an ELISA test.

Statistical analysis:

The data were collected, coded and entered to the computer. The data were analyzed using SPSS (statistical package for social science) version ten. Data were presented as median if nonparametric and mean and standard deviation when data were parametric. Qualitative data was presented as number and percent. Comparison between two means was estimated by independent t test and testing association between qualitative variables was done by Chi-square test and Fisher's Exact test which is recommended when expected cell is less than five. Probability ≤ 0.05 is considered significant. Probability ≤ 0.001 is considered highly significant.

RESULTS

Table (1): Demographic characteristics and clinical data of the studied groups.

Parameters		*Type 1 diabetic patients (n=60)*	*Control group (n=20)*	*p-value*
Sex n (%)	*Male*	*28 (46.7%)*	*10(50%)*	*NS*
	Female	*32 (53.3%)*	*10(50%)*	
Residence n(%)	*Urban*	*24 (40%)*	*8(40%)*	*NS*
	Rural	*36 (60%)*	*12(60%)*	
Age (years)	*Mean± SD*	*9.46 ±4.09*	*7.5±4.41*	*NS*
Weight (kg)		*27.075 ±11.34*	*24.5 ±11.66*	*NS*
Height (cm)		*124 ±19.68*	*117.1 ±26.13*	*NS*
Body mass index (kg/m2)		*17.54 ±3.43*	*17.89 ±3.61*	*NS*
Systolic blood pressure (mmHg)		*104.45 ±21.52*	*96.25 ±9.58*	*NS*
Diastolic blood pressure (mmHg)		*67.42 ±11.52*	*65.92 ±8.73*	*NS*
Duration of diabetes (years)		*2.55 ±2.61*	*----*	*----*
Season at diagnosis n (%)	*Autumn*	*13 (21.6%)*	*----*	*----*
	Spring	*8 (13.3%)*	*----*	*----*
	Summer	*9 (15%)*	*----*	*----*
	Winter	*30 (50%)*	*----*	*----*
Family History of diabetes n (%)	*T1DM*	*5 (8.3%)*	*----*	*----*
	T2DM	*15 (25%)*	*----*	*----*

n = number (%) = percentage NS= non significince (p >0.05)

There was no significant differences between type 1 diabetic patients and control group regarding all demographic characteristics and clinical data of the studied groups.

Table (2): Comparison between type 1 diabetic patients and control group regarding biochemical results.

Parameters	*Diabetic patients (n=60)*	*Control group (n=20)*	*P-value*
Complet blood count (CBC)			
Haemoglobin (gm/dl)	*11.4±1.3*	*12.14±0.73*	*NS*
White blood cells (.10^3/mm^3)	*6.84±2.27*	*7.03±1.37*	*NS*
Platelet count (.10^3/mm^3)	*304.87±78.5*	*330.9±78.06*	*NS*
Kidney function tests			
Blood urea (mg / dl)	*25.27 ± 8.32*	*23.6±2.9*	*NS*
Serum Creatinine (mg / dl)	*0.67±0.18*	*0.67±0.18*	*NS*
Microalbuminuria(mg/g.cr.)	*35.65±22.05*	*9.47±2.08*	*<0.001***
Serum lipid profile			
Serum Cholesterol (mg/ dl)	*175.65±54*	*130.15±25.6*	*<0. 01***
Serum Triglycerides (mg/ dl)	*135.32±73.6*	*76.35±21.8*	*<0.001****
HDL Cholesterol (mg/dl)	*56.63±18.2*	*45.2±12*	*<0. 05**
LDL Cholesterol (mg/ dl)	*125±41.2*	*84.95±19.24*	*<0.001****
Glycosylated haemoglobin			
HbA1c (%)	*10.1±2.27*	*5.3±0.57*	*<0.01***
Blood levels of inflammatory markers			
ESR (1 st. hour)	*14.2±8.61*	*5.55±2.14*	*<0.01***
CRP (mg/L)	*8.34±4.54*	*4.4±0.83*	*<0.01***
Serum TNF-α (pg/ml)	*35.9±24.26*	*5.6±2.63*	*<0.001***

N.S =non significant (P > 0.05) *** significant (P < 0.05)**
**** Highly significant (p < 0.01)**

There were highly significant differences between type 1 diabetic patients and control group in both groups regarding mean levels of microalbuminuria, serum lipid profile (triglycerides, total cholesterol, LDL & HDL), HbA1c concentrations and mean levels of informatory markers . While there was no significant difference regarding blood urea, serum creatinine and CBC between both groups.

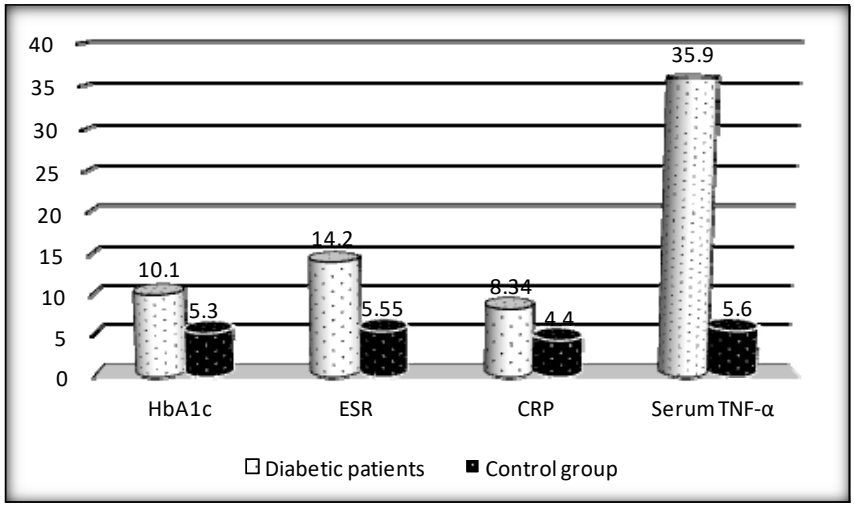

Figure (1): Mean levels of *glycosylated haemoglobin (HbA1C) and inflammatory markers (ESR, CRP and TNF-- α)* in studied groups.

Table (3): Comparison between normal and abnormal serum TNF- α results regarding demographic characteristics and clinical data in diabetic children.

Parameters		*Normal TNF-α group (serum Level<10 pg/ml) (no=12)*	*Abnormal TNF –α group (serum Level >10 pg/ml) (no=48)*	*p-value*
Sex n (%)	*Male*	*3(25%)*	*25(52.1%)*	*NS*
	Female	*9(75%)*	*23(47.9%)*	
Residence n (%)	*Urban*	*6(50%)*	*18(37.5%)*	*NS*
	Rural	*6(50%)*	*30(62.5%)*	
Age (years)	*Mean± SD*	*11.33±3.02*	*8.98±4.2*	*NS*
Weight (kg)		*30.25±12.7*	*32.3±10.3*	*<0.05**
Height (cm)		*133±16.3*	*132±19.9*	*NS*
Body mass index (kg/m2)		*17.1±4.6*	*19.12±2.9*	*<0.05**
Systolic blood pressure(mmHg)		*110±15.14*	*102±22.7*	*NS*
Diastolic blood pressure(mmHg)		*68.7±9.5*	*67.2±12.01*	*NS*
Insulin Type	*long-acting & Regular*	*0(0)*	*14(29.2)*	*NS*
	Intermediate & Regular	*9(75)*	*25(52.1)*	
	Mixed	*3(25)*	*9(18.8)*	
Insulin Dose (unit/kg/day)	*Mean± SD*	*1.12±0.3*	*0.95±0.26*	*NS*
Duration of diabetes (years)		*2.75±0.96*	*2.5±0.88*	*NS*

N.S : non significant (P > 0.05) **** significant (P < 0.05)***

There was significant differences between diabetic patients with abnormal TNF group and diabetic patients with normal TNF group regarding weight and body mass index (BMI). While there was no significant difference between both groups regarding age, height, systolic as well as diastolic blood pressure measurements, duration of diabetes and insulin dose.

Table (4): Comparison between normal and abnormal TNF regarding the laboratory data of the studied patients.

Parameters	*Normal TNF-α group (serum Level<10 pg/ml) (no=12)*	*Abnormal TNF –α group (serum Level >10 pg/ml) (no=48)*	*P-value*
Complet blood count (CBC)			
Haemoglobin (gm/dl)	*12.6±1.2*	*11.8±1.3*	*NS*
White blood cells (.10^3/mm^3)	*6.7±2.3*	*6.8±2.28*	*NS*
Platelet count (.10^3/mm^3)	*319±51.1*	*301±84.03*	*NS*
Kidney Function tests			
Blood urea (mg / dl)	*24.06±7.08*	*30.08±11.2*	*<0.05**
Serum Creatinine (mg / dl)	*0.67±0.19*	*0.67±0.18*	*NS*
Microalbuminuria(mg/g.cr.)	*25.21±3.63*	*33.52±24.26*	*<0.05**
Lipid Profile			
Serum Cholesterol (mg / dl)	*165±48.5*	*215±58.4*	*<0.01***
Serum Triglycerides(mg /dl)	*132±74.5*	*146±71.8*	*<0.05**
HDL (mg/dl)	*47.7±14.9*	*62.16±25.1*	*<0.05**
LDL (mg/ dl)	*80.9±23.21*	*129±37.2*	*<0.001***
Glycosylated haemoglobin			
HbA1c (%)	*7.6±0.4*	*12.7±2.1*	*<0.001***
Blood levels of inflammatory markers			
ESR(1 st. hour)	*10.16±5.95*	*18.95±9.87*	*<0.05**
CRP (mg/L)	*5.7±0.92*	*7.8±3.9*	*<0.05**

N.S =non significant (P > 0.05) **** significant (P < 0.05)***
***** Highly significant (p < 0.01)***

There were significant differences between type 1 diabetic patients with normal and abnormal TNF-α regarding mean levels of blood urea, microalbuminuria, serum lipid profile (triglycerides, total cholesterol, LDL & HDL), HbA1c concentrations and mean levels of inflammatory markers. While, there was no significant difference regarding serum creatinine and CBC between both groups.

Figure (2): Mean levels of Kidney function tests of studied diabetic patients according to TNF- α results.

Figure (3): Mean levels of Serum lipid profile of studied diabetic patients according to TNF- α results.

Figure (4): Mean levels of *glycosylated haemoglobin(HbA1C) and inflamatory markers (ESR, CRP)* of studied diabetic patients according to TNF- α results.

Table (5): Comparison between diabetic patients with Peripheral neuropathy and patients without Peripheral neuropathy regarding biochemical results

Parameters	*Diabetic patients with Peripheral neuropathy (n=15)*	*Diabetic patients without Peripheral neuropathy (n=45)*	*P-value*
Complet blood count (CBC)			
Haemoglobin (gm/dl)	*10.97±1.2*	*11.87±0.32*	*NS*
White blood cells (.10^3/mm^3)	*5.96±1.84*	*6.98±1.12*	*NS*
Platelet count (.10^3/mm^3)	*300.32±76.5*	*312.7±43.03*	*NS*
Kidney Function tests			
Blood urea (mg / dl)	*27.3±8.07*	*24.75±8.4*	*NS*
Serum Creatinine (mg / dl)	*0.7±0.2*	*0.6±0.17*	*NS*
Microalbuminuria(mg/g.cr.)	*35.12±14.56*	*26.31±2.86*	*<0.05**
Lipid Profile			
Serum Cholesterol (mg / dl)	*191.2±55.2*	*179.2±74.2*	*<0.05**
Serum Triglycerides (mg /dl)	*156.6±68.5*	*132.4±75.2*	*<0.05**
HDL (mg/dl)	*58.6±18.3*	*46.6±17.7*	*<0.05**
LDL (mg/ dl)	*139.5±45.6*	*122.6±40.2*	*<0.05**
Glycosylated haemoglobin			
HbA1c (%)	*12.2±1.4*	*9.6±2.1*	*<0.001***
Blood levels of inflamatory markers			
ESR (1 st. hour)	*18±5.7*	*11.75±9.17*	*<0.05**
CRP (mg/L)	*10.4±3.5*	*4.9±0.62*	*<0.05**
TNF-α (pg/ml)	*45.5±22.1*	*32.72±23.94*	*<0.05**

N.S : non significant (P > 0.05) ** significant (P < 0.05)*
*** Highly significant (p < 0.01)*

There were significant differences between type 1 diabetic patients with peripheral neuropathy and those without peripheral neuropathy regarding mean levels of microalbuminuria , serum lipid profile (triglycerides , total cholesterol, LDL & HDL), HbA1c concentrations and mean levels of inflammatory markers. While there was no significant difference regarding blood urea serum creatinine and CBC between both groups.

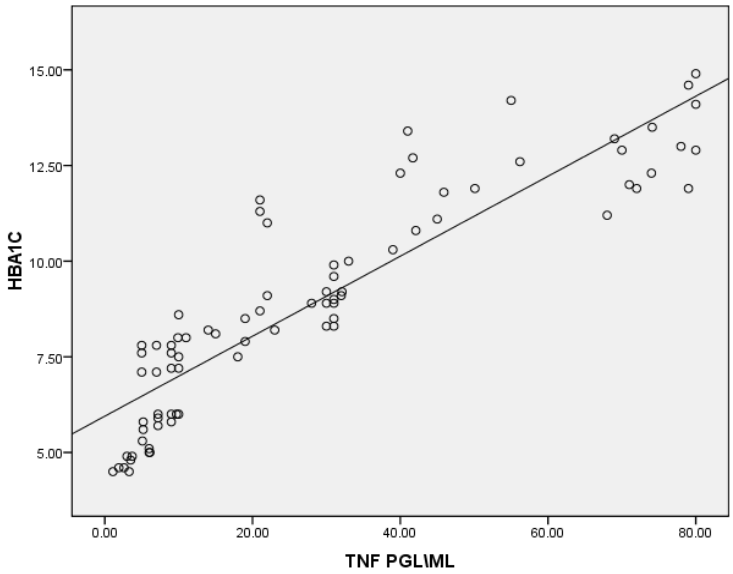

Figure (5): Positive correlation between serum levels of TNF- α and levels of HbA1c in studied type 1 diabetic patients

DISCUSSION

The present study was carried out to determine the role of TNF-α on 60 diabetic children with T1DM and 20 apparently healthy children as controls.

Regarding the anthropometric measurements like weight, height and body mass index (BMI) of TIDM children, they were found to be non-significant compared to controls in the current study, these results were in agreement with ***Jaleel et al., 2013*** who reported the same results.

Regarding biochemical results; the diabetic children were characterised by significantly higher mean levels of HbA1c, CRP, ESR as well as mean level of microalbuminuria than the healthy control group, these results are consistent with ***Zorena et al., 2007***. These results may reflect poor glycemic control, infections

and renal affection in our studied diabetic children.

As well as mean levels of serum lipid profile (triglycerides, total cholesterol, LDL & HDL) were significantly higher in diabetic children than the healthy control group, these results are consistent with ***Abu Shady et al., 2013 and Saud, 2014.*** The pathophysiology of these lipid abnormalities is not totally explained, but the increased Lipoprotein Lipase activity observed in these patients due to hyperglycemia and peripheral hyperinsulinemia, caused by the subcutaneous route of insulin administration, are likely to play a role ***Zorena et al., 2007.***

The etiology of diabetic neuropathy is poorly understood and is likely complex. Hyperglycemia is implicated in the development of defects in peripheral nerve conduction, and improved glycemic control reduces the risk of developing neuropathy. However, subclinical neuropathy is common even among youths with good glycemic control from the time of their diabetes diagnosis ***(Nordwall et al., 2009).***

Among potential biological mechanisms that have been proposed to cause neuropathy independently of hyperglycemia are excess sorbitol and fructose deposition in nerve cells, and increased inflammatory cytokines leading to nerve degeneration ***(Nordwall et al., 2009).*** This is supported by our results, where the level of TNF-α in the group with neuropathy was significantly higher 45.5±22.1pg/ml as compared with those without neuropathy 32.72±23.94pg/ml ($p< 0.05$). These results are consistent with ***Doupis et al., 2009*** and ***Gonzalez-Clemente et al., 2005.***

The level of TNF-α appeared to be independent marker of neuropathy as it increased the risk of neuropathy in children. The serum concentrations of CRP levels and the mean levels of microalbiminuria were also significant but weaker risk factors. The concentration of serum TNF-α in children with neuropathy by far exceeded that in the diabetic children without complications. This finding points to the significance of TNF-α and other proinflammatory mediators as the early diabetic neuropathy risk factors. Thus, TNF-α was placed at the top of inflammatory risk markers. This implies that diabetic children with the detectable serum TNF-α but without neuropathy should be provided with careful neurological assessment ***(Nordwall et al., 2009).***

Regarding The proinflammatory cytokine TNF-α (table 3&4). There was significant differences between diabetic patients with abnormal TNF group and diabetic patients with normal TNF group regarding weight and body mass index (BMI). While there was no significant difference between both groups regarding age, height, systolic as well as diastolic blood pressure measurements, duration of diabetes and insulin dose.

The proinflammatory cytokine TNF-α plays an important role in the pathogenesis of diabetes mellitus **.** It is well documented that TNF- α can be cytotoxic and cytostatic since it inhibits insulin synthesis and secretion ***(Rabinovitch et al., 2002).***

Additionally TNF- α mediated damage to micro-and macro-vascular tissues, altered insulin secretion directly or through stimulation of free fatty acids production and altered glucose homeostasis ***(Haller & Schatz, 2008).***

This is supported by the present study which revealed that serum cytokine TNF- α level was significantly higher in diabetic patients than healthy controls. These results were in agreement with those of ***Azza et al., in 2010 and Wasmuth et al., in 2004*** who reported that the inflammatory activity is increased in individuals with type-1 diabetes, may be due to hyperglycemia and the formation of advanced glycation end products. Another report indicated that TNF-α initially increases and then inhibits, the activity of a number of key enzymes involved in energy metabolism and major histocompatibility (MHC) class I molecule expression. These enzymes include protein-tyrosine kinase (PTPase). Enzymes involved in energy metabolism, cell proliferation and stimulation of MHC class I molecule pathway and concomitant destruction of pancreatic beta cells. So, TNF- α can be implicated as indicator of continuing autoimmune aggression against beta-cells before the development of extensive beta cell destruction ***(Haller & Schatz, 2008).***

There is a discrepancy between the data obtained by our work and that of ***Haller and Schatz, (2008)*** who found non significant change in serum TNF-α levels of diabetic children when compared with age-matched apparently healthy controls. This difference may be attributed to racial and environmental influences.

Despite all studied diabetic patients had non-significant

differences in weight and (BMI) compared to controls in the current study, those with abnormal TNF-α had significant differences in weight and BMI compared with those with normal TNF-α group. Furthermore ***Yaturu et al., 2013, and Saud, 2014*** demonstrated positive correlation between TNF-α and BMI. While there is no significant differences between diabetic patients with abnormal TNF- and those with normal TNF-α group regarding age, height, systolic as well as diastolic blood pressure measurements, duration of diabetes and insulin dose.

However our results showed a statistically significant differences between type 1 diabetic patients with abnormal TNF- α and diabetic patients with normal TNF-α regarding mean levels of blood urea, microalbuminuria, HbA1c concentrations and mean levels of inflammatory markers (ESR & CRP). Same results were found by ***Haller and Schatz, (2008).***

Additionally, in the current study there were significant statistical differences between type 1 diabetic patients with abnormal TNF- α and diabetic patients with normal TNF-α regarding mean levels of serum lipid profile (total cholesterol, triglycerides, HDL & LDL). Similar to the finding by ***Snell-Bergeon et al. 2010.***

Diabetic children with longer duration of the disease had, higher mean levels of serum lipid profile. In ***2008 Edge et al.,*** demonstrated positive correlation between mean levels of serum lipid profile and duration of diabetes, which were in agreement with our results.

In addition, our work showed that diabetic children with longer duration of the disease had higher mean levels of microalbuminuria. These findings were similar to the results of the studies done by**,** ***Lutale et al., 2007, Raile et al., 2007, Razavi et al., 2009.***

Also our study demonstrated that diabetic children with a duration of disease more than five years had higher TNF-α and other inflammatory markers (ESR & CRP) compared with diabetic patients with a shorter duration of diabetes (< 1 yr) and those with a manifested diabetes for 1- 5 yr, same was found by ***Blandino et al. 2008***.In the present study, it was found that the newly diagnosed (<1yr) diabetics had higher TNF- α and other inflammatory markers (ESR & CRP) levels compared with longer standing T1DM (1- 5 yr). Similar results were detected by ***Azza et al., 2010.*** However we have found insignificant correlation between

TNF-α levels and duration of disease in all patients.

In the present study according to glycemic control regarding mean levels of HbA1c there is significant differences in serum lipid profile (total cholesterol, triglycerides, HDL & LDL) between different groups of diabetic patients (good, fair & poor controlled). These results are consistent with ***Edge et al., 2008***, ***Guy et al., 2009, and Marcovecchio et al., 2009,*** who reported positive correlation between HbA_{1c} and mean levels of serum lipid profile, indicating that these disorders were mostly observed in patients with poor glycemic control.

In agreement with other studies we also found positive correlation between HbA_{1C} level (glycemic control index) and microalbumiuria in the present study. For instance, ***Roy et al., 2012*** showed correlation between higher rate of microalbuminuria and poor glycemic control (high level of HbA_{1C}).

Also, the present study demonstrated positive significant correlation between HbA_{1c} and TNF- α. These results are in agreement with ***Azza et al. ,in 2010*** who demonstrated positive significant correlation between TNF- α and HbA_{1c} with higher levels of TNF-α in patients with $HbA_{1c} \geq 7$ % than in those with $HbA_{1c} < 7$ %.

CONCLUSION

1- Type 1 diabetes is associated with elevation of serum TNF-α levels. This association is more evident in both newly diagnosed and poorly controlled patients incriminating a relevant role of TNF- α on the pathogenesis of the disease. TNF-α plays a significant role in both complicated and non-complicated diabetes mellitus type 1.

2- The inflammatory cytokine TNF- α can be used as a marker of severity in T1DM as it is significantly increased in those with poor glycemic control.

3- TNF- α could be used as predictor of complications in T1DM such as peripheral diabetic neuropathy and diabetic nephropathy as evident in our study by increment of mean levels of serum TNF-α in type 1 diabetic patients either with peripheral neuropathy or microalbuminuria.

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تحديد مستويات عامل نخر الورم – ألفا في مصل الأطفال الذين يعانون من مرض السكري من النوع الأول كعامل ضابط في التحكم في مستوى سكر الدم

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يتزايد مرض السكري من النوع الأول في جميع أنحاء العالم وخاصة في الأطفال الذين تقل أعمارهم عن 5 سنوات وهذا الأمر يؤدي إلى عبءٍ صحى كبير.

إن المضاعفات التى تحدث فى الأوعية الدموية هي المشكلة الرئيسية التي نواجهها في سياق هذا المرض.

لذا، كان الهدف من هذه الدراسة هو تحديد مستويات عامل نخر الورم ألفا باعتباره واحد من المؤشرات التى تدل على مدى انتظام سكر الدم وباعتباره واحد من العلامات المناعية التى تنبئ عن اعتلال الأوعية الدقيقة في الأطفال الذين يعانون من مرض السكري من النوع الأول، ودراسة العلاقة بين هذه المستويات مع علامات الالتهابات الأخرى عامل البروتين المتفاعل ومستويات سرعة الترسيب فى الدم وكذلك مع عوامل أخرى مهمة مثل نسبة الألبيومين الدقيق فى البول ومستوى الهيموجلوبين السكرى فى الدم.

وقد تضمن هذا البحث 60 طفلا من الأطفال الذين يعانون من مرض السكري من النوع الأول تم اختيارهم من الحضور المنتظم لعيادة سكر الأطفال– بمستشفى الأطفال– جامعة أسيوط و 20 طفلا أصحاء ظاهريا يتشابهون مع المجموعة الأولى فى العمر والجنس والحالة الغذائية كمجموعة ضابطة .

– أجريت الفحوصات المعملية الآتية لجميع الأطفال:

- **صورة دم كاملة .**
- **سكر عشوائى بالدم.**
- **الهيموجلوبين السكرى بالدم**
- **وظائف الكلى**

- **تحليل بول كامل.**
- **الألبيومين الدقيق فى البول.**
- **اختبارات وظائف الكبد.**
- **تقدير معدل الترسيب فى الدم.**
- **تقدير البروتين المتفاعل.**
- **حساب مجموعة مصل الكوليسترول والدهون الثلاثية في الدم.**
- **تحديد مستويات عامل نخر الورم ألفا.**
- **اختبار درن،أشعة عادية على الصدر وتخطيط القلب عند اللزوم.**

وكشفت هذه الدراسة عن النتائج التالية:

- **عدم وجود فروق ذات دلالة إحصائية بين الأطفال الذين يعانون من مرض السكري من النوع الأول – موضع الدراسة – والمجموعة الضابطة فيما يتعلق بجميع الخصائص الديموجرافية والفحص الطبى.**
- **ارتفاع نسبة السكر فى الدم المصحوب بزيادة حموضة الدم كانت الصورة الأولى في (41.6٪) من الأطفال الذين يعانون من مرض السكري من النوع الأول – موضع الدراسة– وكان شرب كميات كبيرة من الماء مع تبول كميات كبيرة هى الأعراض الأولى في (48.3٪) من الحالات.**
- **تبين من خلال الدراسة أن (50٪) من الأطفال الذين يعانون من مرض السكري من النوع الأول تمت إصابتهم بهذا المرض في فصل الشتاء و (21.6٪) تمت إصابتهم به فى فصل الخريف.**
- **في هذه الدراسة وجد أنه من أصل خمسة عشر طفلا من أطفال مرضى السكري الذين يعانون من اعتلال الأعصاب الطرفية وجد عشرة أطفال يعانون أيضا من زيادة نسبة الألبيومين الدقيق فى البول. في حين أن كل أطفال مرضى السكري الذين لا يعانون من اعتلال الأعصاب الطرفية كانت نسبة الألبيومين الدقيق فى بولهم فى مستواها الطبيعى.**
- **مستويات عامل نخر الورم ألفا فى مصل الأطفال الذين يعانون من مرض السكري من النوع الأول يزيد بشكل ملحوظ عن الأطفال الأصحاء الذين تم أخذهم كمجموعة ضابطة.**
- **الأطفال الذين يعانون من مرض السكري من النوع الأول ومستويات عامل نخر الورم ألفا فى مصلهم أعلى من الطبيعى يزيد لديهم بشكل كبير وزن ومؤشر كتلة الجسم، ومستويات البولينا في الدم ومستويات عامل البروتين المتفاعل و سرعة الترسيب و الهيموجلوبين السكرى ومستوى الدهون في الدم وكذلك نسبة الألبيومين الدقيق فى البول مقارنة بالأطفال**

الذين يعانون من مرض السكري من النوع الأول ومستويات عامل نخر الورم ألفا فى مصلهم فى الإطار الطبيعى.

- **زيادة مستويات عامل نخر الورم ألفا بشكل كبير في الأطفال الذين يعانون من مرض السكري من النوع الأول ويرتبط مستواه بشكل كبير بمدى السيطرة على السكر فى الدم.**
- **زيادة مستويات عامل نخر الورم ألفا بشكل كبير في مرضى السكري الذين يعانون من اعتلال الأعصاب الطرفية الناتجة عن مرض السكرى.**
- **زيادة مستويات عامل نخر الورم ألفا بشكل كبير في مرضى السكري الذين يعانون من زيادة الألبيومين الدقيق فى البول الناتجة عن مرض السكرى.**

ويمكننا أن نستخلص من هذا البحث الآتى:

- **يلعب عامل نخر الورم ألفا فى مرض السكري من النوع الأول دورا مهما سواء في حدوث المرض نفسه أو في حدوث مضاعفات الأوعية الدموية الدقيقة الناشئة عن هذا المرض.**
- **من الممكن استخدام عامل نخر الورم ألفا كعلامة مفيدة فى الكشف المبكر عن مرض السكري من النوع الأول في الأطفال و كعلامة دالة على مدى انتظام وفاعلية العلاج فى ضبط نسبة السكر فى الدم فى هؤلاء الأطفال الذين يعانون من هذا المرض.**
- **كما أنه من الممكن أن يستخدم عامل نخر الورم ألفا كمؤشر للتنبؤ على حدوث مضاعفات في الأطفال الذين يعانون من مرض السكري من النوع الأول مثل اعتلال الأعصاب الطرفية واعتلال الكلية الناتج عن هذا المرض.**