

## TRIGLYCERIDES PREDICT PLASMA FIBRONECTIN IN CHILDREN WITH TYPE I DIABETES MELLITUS RATHER THAN DIABETES

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**Summary:** Fibronectins are adhesive proteins considered as markers of endothelial activation. Plasma fibronectin levels in diabetes mellitus (DM) have been found to be associated with atherosclerotic risk factors. This study was carried out to investigate plasma fibronectin and its relation with serum lipids, apolipoproteins AI, B100 and lp(a) in diabetic children. 35 children (19F/16M) with type I DM and 30 non-diabetic age and gender-matched controls were enrolled. Apolipoprotein and fibronectin concentrations were determined with nephelometric methods. Plasma fibronectin levels of the children with type I DM and the control group are not statistically different. HbA1c and triglycerides concentration are found to be significant predictors of plasma fibronectin in diabetic children, while effect of plasma cholesterol, apolipoprotein AI, B100 and lp(a) are insignificant. Diabetic children with triglycerides 1.13 mmol/l have elevated plasma fibronectin (median, 25<sup>th</sup>-75<sup>th</sup> percentiles; 29.6, 8.3-40.8 mg/dL) compared to the diabetic  $\geq 19.9$ , 8.6-30.7 mg/dL,  $p < 0.05$ ) and non-diabetic children (16.6, 12.7-32.4 mg/dL,  $p < 0.01$ ) with triglycerides  $< 1.13$  mmol/L. On the other hand plasma fibronectin concentrations of diabetic and non-diabetic children with high triglycerides are not significantly different. In conclusion our data does not support the concept that plasma fibronectin is elevated in type I diabetes mellitus at least in children, but high plasma triglycerides secondary to diabetes or not is associated with higher FNp concentrations which may have implications on atherogenesis. Plasma cholesterol, apolipoproteins AI, B100 and lp (a) are not significant determinants of FNp in type I diabetic children.

**Key words:** Apolipoprotein; Children; Fibronectin; Lipoprotein(a); Triglycerides; Type I diabetes mellitus

Non-standard abbreviations: FNp: Fibronectin in plasma. FNc: Cellular fibronectin. ED: Extra domain. DM: Diabetes mellitus. BMI: Body mass index. HDL: High density lipoprotein. LDL: Low density lipoprotein. CHD: Coronary heart disease. Apo AI: Apolipoprotein AI. Apo B100: Apolipoprotein B100. Lp(a): Lipoprotein(a). CRP: C-reactive protein. CV: Coefficient of variation. CI: Confidence interval.

### Introduction

Fibronectins are large glycoproteins found in plasma, in extracellular matrix and cell surfaces. They promote cell-cell and cell-matrix interactions and thus play a role in tissue construction and reconstruction (5). A single gene encodes for various distinct fibronectin moieties, with differences in primary structure resulting from alternative splicing of the primary mRNA transcript (6). The predominant fibronectin in plasma (FNp) is secreted by hepatocytes and lacks extra domain (ED) segments. Cellular fibronectin (FNc) usually  $< 1-2$  % of total fibronectins in plasma is an endothelium derived protein involved in subendothelial matrix assembly and often contains an extra type III structural domain called ED-A (8,13,23).

Elevated plasma FNc have been described in clinical syndromes with vascular damage like, rheumatoid vasculitis, preeclampsia, collagen vascular disease, acute trauma and thrombotic thrombocytopenic purpura. It is considered as a marker of endothelial activation (8,21). Results regarding plasma FN levels in diabetes have been inconclusive and are reported to be increased in type I and type II diabetes mellitus (DM) for FNc, increased in type II DM and increased or unchanged in type I DM for FNp (8,18,22,25). Some studies reported no differences in FNc concentrations with regard to type of diabetes (8). Although an association of FN and chronic complications of DM has not been observed, variables that may have implication on macroangiopathic complications of diabetes mellitus (i.e.; high density lipoprotein-cholesterol (HDL-cholesterol), tri-

glycerides, body mass index (BMI), age, treatment for hypertension and smoking) were found to be associated with plasma FN levels (8). Other studies revealed type of diabetes, age, BMI, triglycerides and HbA<sub>1c</sub> as significant predictors of FNp levels in patients with type I or type II DM (18,25). In healthy subjects an association between circulating FNc and age has been observed, for each year increase in age, the mean increase in plasma FNc was 0.014 µg/mL (8). However these study groups included adult patients and plasma FNp levels are not assessed in children with type I DM.

Apolipoproteins may offer analytical and clinical advantages over lipoproteins for prediction of Coronary Heart Disease (CHD) risk assessment. Apo AI and apo B100 are the major proteins in HDL and low density lipoprotein (LDL) respectively. These apolipoproteins are somewhat better discriminators of people with CHD than the cholesterol concentrations of the corresponding lipoprotein. Increased serum apo B100 and decreased apo AI concentrations were also found in children of parents with premature atherosclerotic disease (14). Lipoprotein(a) (Lp(a)) is an independent lipoprotein risk factor for CHD. It is distinguished from LDL in that, in lp(a), attached to the LDL particle is another apoprotein, termed apoprotein(a). Analysis of apo(a) cDNA reveals close homology with plasminogen but it is devoid of enzyme activity. Although the intra individual variability in lp(a) is small (15 %) the interindividual variability is large (1000 fold). Plasma concentrations appear to be highly heritable. Factors that do not appear to markedly affect lp(a) include gender, age, weight, moderate exercise and most lipid lowering medications (7). Although inconsistent yet, significant elevations of lp(a) concentrations and differences in lp(a) phenotypes in patients with DM were reported (3,17). Increased lp(a) levels and increased glycation of lp(a) is suggested to contribute micro- and macro-vascular complications (3,9).

In this cross sectional study we investigated plasma FNp levels and possible implication of serum lipid, apolipoprotein AI, B100 and lp(a) concentrations on plasma FNp levels in children with type I DM and compared with age/gender and serum triglycerides matched non-diabetic control group.

## Material and method

### Subjects

This study was conducted at Gaziantep University, Faculty of Medicine, Departments of Pediatrics and Department of Biochemistry and Clinical Biochemistry in 2001. Informed consent was obtained from all subjects according to the Helsinki declaration as revised in 1996. Thirty-five children (19 girls, 16 boys) with type I DM were recruited. Mean age ( $\pm$ SEM) was 11.9 $\pm$ 0.7 years. Mean diabetes duration was 3.9 $\pm$ 0.5 years. All patients were treated with daily regular doses of insulin (1 IU/kg/day). Thirty children without diabetes (14 girls, 16 boys) recruited the control group. Baseline characteristic of the groups are pre-

sented in Table 1. All participants were monitored and were excluded if symptoms of infection or systemic somatic illness other than DM were present. Type I DM was diagnosed according to the World Healthy Organisation criteria (15). Prior medical histories and personal characteristics were obtained from participants via a questionnaire.

Hypertension was defined as a resting arterial blood pressure >95<sup>th</sup> percentile. Children with triglycerides >1.13 mmol/L were considered hypertriglyceridemic. Microalbuminuria or macroalbuminuria (urinary albumin/ creatinin ratio <30 µg/mg normoalbuminuria, 30–300 µg/mg microalbuminuria,  $\geq$ 300 µg/mg macroalbuminuria) on at least two different early morning specimens were defined as diabetic nephropathy. Retinopathic evaluation of the patients was performed with fundoscopy and fluorescein fundus angiography when necessary. According to these criteria none of the diabetic children were with hypertension, diabetic nephropathy or diabetic retinopathy. Seventeen of the diabetic patients and 13 of the non-diabetic children had elevated triglycerides, 18 diabetic and 17 non-diabetic children were with triglycerides  $\leq$ 1.13 mmol/L.

**Tab. 1.** Baseline characteristics, lipid, apolipoprotein and fibronectin concentrations in children with type 1 DM and the control group (Mean  $\pm$  SEM).

	Type 1 DM n:35	Healthy controls n:30	p
Age (year)	11.9 $\pm$ 0.7	10.8 $\pm$ 0.5	NS
Gender	19F/16M	14F/16M	NS
BMI (kg/m <sup>2</sup> )	19.3 $\pm$ 0.6	16.9 $\pm$ 0.5	<0.005
Diabetes duration (years)	3.9 $\pm$ 0.5	-	<0.001
HbA <sub>1c</sub> (%)	10.7 $\pm$ 0.5	5.4 $\pm$ 0.0	<0.001
T Cholesterol (mmol/L)	5.21 $\pm$ 0.15	4.55 $\pm$ 0.11	<0.001
Triglycerides (mmol/L)	1.21 $\pm$ 0.10	1.32 $\pm$ 0.14	NS
ApoA-I (g/L)	1.30 $\pm$ 0.05	1.24 $\pm$ 0.03	NS
ApoB-100 (g/L)	0.95 $\pm$ 0.03	0.77 $\pm$ 0.02	<0.001
Lp(a) (mg/dL)	11.0 $\pm$ 1.6	16.4 $\pm$ 6.2	NS
Fibronectin (mg/dL)	23.7 $\pm$ 1.3	21.0 $\pm$ 1.4	NS

NS: not significant

### Experimental

Blood samples were collected using standard venipuncture technique between 9:30–11:00 am after 12 h fast. Serum and plasma samples were separated immediately after centrifugation at +4°C, 2000 g for 10 minutes and stored at -20°C until analysis that was performed in the same run. Serum apo AI, apo B100, lp(a) and plasma FNp concentrations were determined using a BN II nephelometer (Behring Diagnostics GmbH, Marburg, Germany) and Dade Behring reagents according to the manufacturer's instructions. Control sera were included in each analytical run. Intraassay and interassay precision studies obtained from the quality control data of the laboratory yielded CVs between 1.7 and 6.8 %.

Total cholesterol and triglycerides concentrations were determined on an Olympus AU 800 automated analyser (Olympus Diagnostica GmbH, Hamburg, Germany) with Olympus reagents.

### Statistical

Data are presented as mean  $\pm$  SEM or median (25<sup>th</sup>-75<sup>th</sup> percentiles) when  $n \leq 30$ . Comparison of variables was performed with the unpaired student t-test or with Mann Whitney U test when necessary. Case-control differences in nominal data were evaluated with the  $\chi^2$  test. Two tailed p values  $< 0.05$  were considered significant. SPSS 9.0 (SPSS Inc, Chicago, USA) and MedCalc (MedCalc software, Mariakerke, Belgium) programs were used for statistical analyses and illustrations.

### Results

The baseline characteristics of the study participants are presented in Table 1. No statistically difference was noted in age and gender. Children with type I DM have higher HbA1c levels and BMI than the healthy controls ( $p < 0.001$  and  $p < 0.005$ ).

**Tab. 2:** Univariate regression analysis in children with type I DM, plasma fibronectin as the dependent variable.

	B	95 % CI	p
Age (years)	0.44	(-0.38/1.26)	0.28
BMI (kg/m <sup>2</sup> )	-0.84	(-1.80/0.12)	0.08
Sex (F:0 M:1)	5.05	(-0.72/10.82)	0.08
Diabetes Duration (years)	0.04	(-1.13/1.20)	0.95
HbA1c (%)	-2.11	(-3.18/-1.04)	0.001
ApoA (g/L)	17.80	(-21.55/57.15)	0.354
ApoB (g/L)	18.84	(-16.27/53.95)	0.274
Lp(a) (mg/dL)	-0.19	(-0.42/0.19)	0.44
T Cholesterol (mmol/L)	-2.64	(-8.49/3.21)	0.36
Triglycerides (mmol/L)	7.31	(1.90/12.72)	0.01

NS: not significant

**Tab. 3:** Fibronectin concentrations of the children with type I DM with respect to triglycerides concentration in comparison with the non-diabetic children. Median (25<sup>th</sup>-75<sup>th</sup> percentiles).

Fibronectin (mg/dL)	Triglycerides $< 1.13$ mmol/L	Triglycerides $\geq 1.13$ mmol/L
Type 1 DM	n:18 19.9 (8.6-30.7)	n:17 29.6(8.3-40.8)*
Non-diabetic controls	n:17 16.6 (12.7-32.4)	n:13 22.0 (11.1-45.5)

\*  $p < 0.05$  versus children with type 1 DM and triglycerides  $< 1.13$  mmol/L,  $p < 0.01$  versus non-diabetic children with triglycerides  $< 1.13$  mmol/L.

Total cholesterol and apo B100 concentrations are higher in children with type I DM ( $p < 0.001$ ) where triglycerides, apo AI, lp(a) and fibronectin concentrations are not statistically different (Tab. 1). Results of univariate regression analysis in children with type I DM, plasma FNp as the dependent variable are shown in Table 2. In the model that included age, BMI, gender, diabetes duration, HbA1c %, apo B100, apo AI, lp(a), cholesterol and triglycerides concentrations, only HbA1c and triglycerides were significant predictors of plasma FNp levels. Relations with gender and BMI did not reach significance level ( $p: 0.08$ ). Plasma levels of FNp decreased by  $2.11 \pm 0.51$  mg/dl for each unit increase in HbA1c % ( $p < 0.001$ ) and increased by  $7.31 \pm 2.75$  mg/dl for each increase in triglycerides mmol/L ( $p < 0.01$ ). This model explains 64.8 % of the variation in plasma FNp concentration. Diabetic children with triglycerides concentration  $\geq 1.13$  mmol/L had higher fibronectin levels compared to the diabetic and non-diabetic children with triglycerides  $< 1.13$  mmol/L ( $p < 0.01$  and  $p < 0.05$ ) (Tab. 3).

### Discussion

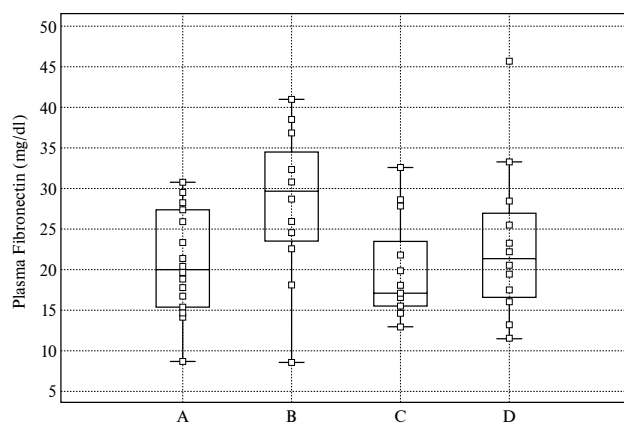
Previous studies about FNp levels in diabetes are contradictory. Authors reported that FNp levels were elevated in type I and type II DM or were elevated in type II but unchanged in type I DM (18,22,25). However in childhood type I DM may involve different pathological mechanisms that influence FNp metabolism compared to the later decades. The half-life of fibronectin is estimated to  $\sim 70-72$  h (16). For several proteins and enzymes a slowdown of their turnover was observed in aging organisms as the most plausible explanation of the accumulation of partially or totally inactive proteins (4). This phenomenon of 'molecular aging' appears to result from post-synthetic modifications of proteins due to the increase of their residence time in cell or tissue compartments before removal and replacement by newly synthesised molecules (10). Therefore this discrepancy may be at least partially explained by ages of the study participants and confounding factors that may imply on plasma fibronectin levels as we enrolled type I diabetic children without hypertension and diabetic microvascular complications.

In the present study circulating levels of plasma fibronectin in diabetic children with type I DM were comparable with the non-diabetic controls. However in our control group 12 children had elevated serum triglycerides and comparable FNp levels with hyperlipidemic diabetic children. On the other hand diabetic children with elevated serum triglycerides had higher FNp than diabetic and non-diabetic children with lower triglycerides suggesting triglyceride metabolism as a significant predictor of FNp rather than type I DM itself.

The mechanism, which makes triglycerides, an important determinant of plasma fibronectin is not exactly known, but it's possible association with future complicati-

ons is considerable in diabetes. Diabetes-induced metabolic abnormalities are responsible for hyperlipidemia as well as hyperglycemias. Hyperlipidemia is often comprised of marked elevations of serum LDL and triglycerides. These serum abnormalities are due to the formation of w-6 free fatty acids contributing to the formation of LDL/triglycerides. Abnormal fatty acid metabolism and hiperlipidemia are thought to be responsible for many diabetic complications (2). In diabetes associated microangiopathies and atherosclerosis there are alterations of the extracellular matrix in the intima of small and large arteries. High concentrations of triglycerides might alter the basement membrane composition of endothelial cells. In arteries, smooth muscle cells are major producers of proteoglycans and glycoproteins in the intima and this is the site of lipoprotein deposition and modification, key events in atherogenesis (11). Large amounts of fibronectin have been detected in atherosclerotic plaques. In animal models hypertension has been shown to rapidly increase fibronectin expression in arterial walls. Elevated FNp levels in patients with CHD and its relation with hypertension was observed suggesting pathogenic implications of plasma fibronectin in ischemic heart disease (19).

Obesity related metabolic disturbances are suggested to influence FNp levels. Several studies reported a positive correlation of BMI and plasma fibronectin in diabetic or non-diabetic subjects (1,18). A low-level chronic inflammatory state reflected by levels of IL-6, TNF- $\alpha$  and CRP, induced by adipose tissue is related with elevated FNC levels (24). However in our study implication of BMI on fibronectin did not reach significance level.



**Fig. 1.** Scatter diagram of plasma fibronectin concentrations in diabetic and non-diabetic children with regard to triglycerides. A: diabetic children with triglycerides <1.13mmol/l, B: diabetic children with triglycerides  $\geq$ 1.13mmol/l, C: controls with triglycerides <1.13mmol/l, D: controls with triglycerides  $\geq$ 1.13mmol/l. The central box represents the median, 25<sup>th</sup>-75<sup>th</sup> percentiles. A line extends from the minimum to the maximum value excluding extreme values, which are shown as separate points.

An interaction of lp(a) and fibronectin in atherogenesis is suggested. The apo(a) binds to the carboxyterminal heparin binding domain of fibronectin. Lp(a) bound to fibronectin is internalised through the fibronectin receptor pathway and thereby causes increased accumulation of lipid and foam cell formation (20). However a relation of fibronectin with lp(a) is not observed in our study.

## Conclusion

Our data does not support the concept that plasma fibronectin is elevated in type I DM, at least in children, but high plasma triglycerides secondary to diabetes or not is associated with higher FNp concentrations. Plasma cholesterol, apolipoproteins AI, B100 and lp(a) are not significant determinants of FNp in type I diabetic children.

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