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Relation between Fibrinogen Gene Polymorphisms and Microvascular Complications in Patients with Type 1 Diabetes: A Cross-Sectional Study

ABSTRACT

Objective: The present work aimed at genotyping *fibrinogen beta* (*FGB*) gene rs1800790 polymorphism and studying its relation to plasma fibrinogen (FG) level and microvascular complications in patients with type 1 diabetes (T1D).

Materials and methods: The study included 100 patients with T1D attending outpatient clinic. Full history taking and physical examination were done. Routine biochemical parameters and plasma FG level were measured. Genotyping of rs1800790 *FGB* gene polymorphism was done.

Results: The study included 52 females and 48 males with T1D with mean diabetes duration of 7.75 ± 2.95 years. Their mean age was 14.71 ± 3.24 years. Plasma FG level was significantly higher in patients with diabetic peripheral neuropathy (DPN) (p = 0.024) and in patients with diabetic kidney disease (DKD) (p = 0.036). No significant relation was found between plasma FG level and rs1800790 *FGB* gene polymorphism. The GA genotype of gene polymorphism was associated with 6 times increased risk of DPN. The dominant mode GA + AA was associated with a 4 and 7 folds increased risk of DPN in univariate and multivariate analysis respectively. A cut-off values of plasma FG > 348 mg/dL and > 358 mg/dL were able to differentiate patients with DPN and DKD respectively.

Conclusions: In patients with T1D, the GA and the GA + AA genotypes of rs1800790 *FGB* gene polymorphism were significantly associated with DPN while plasma FG level was associated with DPN and DKD but not with rs1800790 *FGB* gene polymorphism.

Keywords: type 1 diabetes, microvascular complications, fibrinogen, rs1800790 gene polymorphisms

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Introduction

Type 1 diabetes (T1D) is considered a multifactorial disease with a background of autoimmunity. Its prevalence is rising among children and adolescents [1]. About 35 million persons worldwide have T1D with annual increase 3–5% [2]. It is one of the commonest chronic diseases among children and strongly associated with increased risk of microvascular complications, including diabetic peripheral neuropathy

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(DPN), diabetic retinopathy (DR), and diabetic kidney disease (DKD) [3].

Diabetic peripheral neuropathy is associated with significant morbidity through its role in the pathogenesis of diabetic foot ulceration which may progress to irreversible tissue damage that may end with lower limb amputation [4]. Moreover, DR remains the leading cause of loss of vision in the working-age group in western world population [5]. Furthermore, DKD represents the most common cause of end stage renal disease requiring dialysis in developed countries. Its prevalence rises with increasing age and diabetes duration [6].

Chronic hyperglycemia is the initiating factor of microangiopathy complex etiology. It depends mainly on long diabetes duration and poor glycemic control [4]. Additional factors affecting microangiopathy include: hypertension, dyslipidemia, inflammation, glycemic variability and genetic predisposition [7, 8]. Moreover, dysfunction of hemostasis is considered another risk factor for diabetic microvascular complications through impairment of vascular flow.

Fibrinogen (FG) is the major protein of the coagulation system which is a soluble, homodimeric glycoprotein [9]. It is made up of 3 polypeptide chains in 2 pairs (alpha, beta, and gamma) where their genes are located on chromosome 4q28. Fibrinogen exhibits pleiotropic biological functions owing to its various binding sites. Moreover, it has the capability of binding to a range of adhesion molecules or receptors presented on immune, hematopoietic, and neuronal cells. Being an acute phase reactant, FG is proved to have a pivotal role in inflammation [10].

Recent studies has focused on *FG beta* (*FGB*) gene polymorphism (e.g., rs1800790, –455G/A) as its synthesis is the rate limiting process for FG synthesis. Previous data highlighted the association of *FGB* polymorphisms with stroke (ischemic and cardioembolic) and abdominal aortic aneurysm [11, 12].

There is paucity of data regarding the relation between *FGB* gene polymorphism and different microvascular complications in T1D.This invited us to conduct the present study. The aim of our study was to genotype *FGB* (rs1800790) polymorphism in patients with T1D and to determine its relation to microvascular complications in young subjects with T1D.

Materials and methods

Study design

The present study is a cross-sectional study.

Study participants

The study included 100 patients with T1D, aged 6–19 years with diabetes duration ≥ 3 years. T1D was diagnosed according to the criteria of the International Diabetes Federation/International Society for Pediatric and Adolescent Diabetes [13]. Patients were recruited from diabetes outpatient clinic of Alexandria Main University Hospital during routine visits. Patients with diabetes duration less than 3 years, type 2 diabetes, pregnancy, severe renal or hepatic impairment and patients with history of cardiovascular disease were excluded from the study. Included subjects were informed regarding the aim of the study. An informed consent was signed by all adult patients and by their legal representatives, in case of children.

Ethical approval

This study was performed in accordance with the ethical standards of the Declaration of Helsinki. The approval of the local Ethical Committee of Faculty of Medicine, Alexandria University was obtained.

Data collection

All selected cases were subjected to: thorough history taking including age, gender, diabetes duration and medications.

Full physical examination was done including: measuring anthropometric parameters (body weight and height). Body mass index was calculated by dividing weight in kg by height in m^2). Blood pressure also was measured. Neurological examination for detection of DPN was done using Michigan Neuropathy Screening Instrument (MNSI) score, MNSI questionnaire and MNSI examination were used for the overall evaluation of neuropathy severity. MNSI questionnaire score ≥ 4 is considered abnormal and MNSI examination score > 2 was considered abnormal [14]. Fundus examination was performed by an ophthalmologist through dilated pupils using a 90-diopter Volk lens and biomicroscope to diagnose DR. The presence and grading of DR was based on the International Clinical Diabetic Retinopathy and Macular Edema Disease Severity Scales [15].

For each patient, venous blood samples were collected into plain vacutainer tubes after an overnight fast as follows: 2 mL in a vacutainer blood collection tube containing K2EDTA (di-potassium ethylenediamine tetra-acetic acid) for complete blood count (CBC), another 2 mL in a vacutainer blood collection tube containing K2EDTA for FG genotyping of rs1800790 polymorphism, 3 mL in plain vacutainer blood collection tube left to clot for 30 minutes at room temperature then centrifuged for 10 minutes at 5000 rpm to obtain serum for chemical testing and the rest 2 mL in a vacutainer blood collection tube containing sodium citrate for plasma FG measurement.

Complete blood count was performed on differential automated cell counter XN-1000 (Sysmex, USA), chemical tests were done using fully automated chemistry analyzer ADVIA1800 (Siemens, Germany), they included: fasting plasma glucose (FPG), hemoglobin A1C (HbA1c), lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C) and serum creatinine. The one-year mean HbA1c was calculated for each patient from multiple readings (3 or 4) during the last year. Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI equation. [16] Spot urine sample was obtained (second morning sample in a 6 ml plain urine tube) for measurement of urinary albumin to creatinine ratio (UACR). DKD was diagnosed in the presence of albuminuria (UACR≥ 30 mg/g) and/or eGFR < 60 mL/min/1.73 m2) [17]. Plasma fibrinogen level was measured using fully automated coagulation analyzer (Sysmex, Germany).

Genotyping of FGB gene rs1800790 polymorphism

Genomic DNA was extracted from peripheral blood leucocytes using QIAamp DNA Mini Blood DNA extraction kit (Cat. No. 51104, QIAGEN, Germany). DNA concentrations were measured using NanoDrop 2000 spectrophotometer (ThermoScientific, USA) and the purity was assessed by the absorbance ratios of A260/ /A280 and A260/A230. After that, all DNA samples were stored at −80 °C until use. The extracted DNA was amplified by TaqMan 5′ nuclease allele discrimination technique using TaqMan® SNP Genotyping Assay for genotyping of rs1800790 (Cat No. 4351379, Life Technologies Corporation, USA). PCR reaction mix with total reaction volume 20 μL for each sample was prepared as follows: 10 μL of TaqMan™ Master Mix, 1 μL of the genotyping assay, 2μl of DNA template (with concentration 5 ng/μL) and 8μl nuclease free water. Then, PCR reaction mixture was subjected to thermal cycling on Rotorgene Q PCR system (Qiagen, USA) using the following Q-PCR thermal cycling protocol; 95°C for 10 minutes for initial denaturation, 40 cycles: 95°C for 1 minute for denaturation and then 60°C for annealing and extension. Allelic and genotypic identification is performed using the plotted fluorescence signals as follows: homozygous for A allele with VIC dye fluorescence, heterozygous for A and G alleles with VIC/FAM-dye fluorescence and homozygous for G allele with FAM dye fluorescence.

Statistical analysis

Data were analyzed using IBM SPSS version 20.0 software package (Armonk, NY: IBM Corp). Number and percent were used to describe qualitative data. The Kolmogorov-Smirnov test was used to test the normality of distribution. Mean and standard deviation were used to describe quantitative data. Chi-square test was used to compare between groups for categorical variables. Comparing between the studied groups for abnormally distributed quantitative variables was done using Mann-Whitney and Kruskal-Wallis tests for 2 and more than 2 studied groups respectively.

However, for normally distributed quantitative variables, Student t-test and F-test (ANOVA) were used to compare between 2 and more than 2 studied groups respectively. Correlation between quantitative variables was done using Spearman coefficient. Receiver operating characteristic (ROC) curve was generated by plotting specificity (FP) on X axis versus sensitivity (TP) on Y axis at different cut off values. Area under the curve > 50% gives acceptable performance. Univariate and multivariate regression analysis were performed to detect factors affecting diabetic microvascular complications.

Sample size calculation

Sample size was calculated using Power Analysis and Sample Size Software (PASS 2020) "NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass". Thus, a minimal total hypothesized sample size of 100 eligible subjects with T1D, aged 6–19 years, admitted to Alexandria Main University Hospital was needed to determine the *FGB* gene rs1800790 polymorphism and evaluate its relation to microvascular complications in patients with T1D; taking into consideration 95% level of confidence, an effect size of 0.4 and 5% margin of error using Z- test [18–20].

Results

The study included a total of 100 patients with T1D (52 females and 48 males), age ranged from 6.0–19.0 years with a mean of (14.71 ± 3.24). Regarding diabetes duration, it ranged from 5.0–18.0 years with a mean of (7.75 ± 2.95).The laboratory parameters of the study participants are shown in Table 1.

Regarding microvascular complications, 25(25%) patients had DPN, 3 (3%) patients had DR, 15 (15%) patients had DKD and 31 (31%) patients had at least one microvascular complication. The one-year mean HbA1c was significantly higher in patients with DKD than patients without DKD. DR and microvascular complications showed similar results while regarding DPN, it showed no significant relation with the one-year mean HbA1c (Tab. 2). The total sample was subdivided according to one-year mean HbA1c into 3 groups [< 7%: good control (n = 5); 7–9%: moderate control (n = 48) and > 9%: poor control (n = 47)]. However,

Table 1. Descriptive Analysis of the Studied Cases According to Laboratory Parameters (n = 100)

Laboratory parameters	Mean ± SD
FPG [mg/dL]	180.7 ± 69.7
HbA1c % [mmol/mol]	9.5 ± 2.3 (80 ± 2)
One-year mean HbA1c % [mmol/mol]	9.4 ± 2 (79 ± 2)
Cholesterol [mg/dL]	190.1 ± 46.4
HDL-C [mg/dL]	61.9 ± 9.0
LDL-C [mg/dL]	113.5 ± 45.5
Triglycerides [mg/dL]	99.2 ± 42.7
Creatinine [mg/dL]	0.6 ± 0.1
eGFR [mL/min/1.73 m^2]	140.8 ± 17.7
UACR [mg/g creatinine]	35.3 ± 117.3
ALT [units/L]	20.7 ± 16.0
Fibrinogen [mg/dL]	374.1 ± 274.6

ALT — alanine transaminase; eGFR — estimated glomerular filtration rate; FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; SD — standard deviation; UACR — urinary albumin to creatinine ratio

no significant relation was found between subgroups according to microvascular complications.

Plasma FG level was significantly higher in females (p = 0.008).Moreover, it was significantly higher in patients with DPN (p = 0.024) and patients with DKD (p = 0.036).

There was a statistically significant positive correlation between FG and UACR (r = 0.217, p = 0.030) but no other significant correlations were found between FG and other studied parameters.

Distribution of the studied participants according to FGB gene polymorphisms

The genotype frequencies of *FGB* polymorphisms rs1800790 in the studied participants were presented under different genetic models. The general model (GG n = 50, GA n = 42 and AA n = 8) puts each genotypes in a separate category.

On the other hand, the dominant model (GG n = 50, and GG + GA n = 50) considers both heterozygotes and homozygotes for polymorphic allele are at higher risk so there is increased risk of the disease even if only one polymorphic allele is present.

However, the recessive model (GG + GA n = 92 and AA n = 8) considers that if both alleles are polymorphic there is increased risk of the disease while the multiplicative model (G n = 142 and A n = 58) studies the association of alleles with increased disease risk.

Fibrinogen gene polymorphism and plasma fibrinogen level

There were no statistically significant differences between the groups of genotypes of rs1800790 gene polymorphism (GG, GA, AA) regarding FG level (p = 0.270). Moreover, in the dominant model there were no statistically significant differences between GG and GA + AA genotypes and FG level (p = 0.290).

Fibrinogen gene polymorphisms and microvascular complications

In the general model, relation between the genotype frequencies of the polymorphisms rs1800790 in

Table 2. Relation between One-Year Mean HbA1c and Microvascular Complications (n = 100)

	N	One-year mean HbA1c Mean ± SD % [mmol/mol]	p
Diabetic peripheral neuropathy			
No	75	9.2 ± 1.9 [76 ±2]	0.087
Yes	25	10.0 ± 2.3 [86 ± 2]	
Diabetic retinopathy			
No	97	9.3 ± 1.9 [78 ± 2]	0.017*
Yes	3	12.8 ± 2.6 [116 ± 5]	
DKD			
No	85	9.2 ± 1.8 [76 ± 1]	0.033*
Yes	15	10.6 ± 2.4 [92± 3]	
Micro vascular complications			
No	69	9.1 ± 1.9 [76 ± 2]	0.015*
Yes	31	10.1 ± 2.1[87± 1]	

*Statistically significant at p ≤ 0.05; DKD — diabetic kidney disease; p — p-value for comparing between No and Yes

Table 3. Relation between Gene Polymorphism and Microvascular Complications (n = 100)

	Gene polymorphism						P
	GG (n = 50)		GA (n = 42)		AA (n = 8)		
	No.	%	No.	%	No.	%	
Diabetic peripheral neuropathy							
No	44	88.0	24	57.1	7	87.5	0.002*
Yes	6	12.0	18	42.9	1	12.5	
Sig. bet.Grps			$p_1 = 0.001$*, $p_2 = 1.000$, $p_3 = 0.134$				
Diabetic retinopathy							
No	48	96.0	41	97.6	8	100.0	$^{MC}p = 1.000$
Yes	2	4.0	1	2.4	0	0.0	
DKD							
No	44	88.0	34	81.0	7	87.5	0.628
Yes	6	12.0	8	19.0	1	12.5	
Microvascular complications							
No	39	78.0	23	54.8	7	87.5	0.028*
Yes	11	22.0	19	45.2	1	12.5	
Sig. bet.Grps			$p_1 = 0.018$*, $p_2 = 1.000$, $p_3 = 0.123$				

*Statistically significant at $p \leq 0.05$; DKD — diabetic kidney disease; p — p-value for comparing between the studied categories (p_1 — p-value for comparison between GG and GA; p_2 — p-value for comparison between GG and AA; p_3 — p-value for comparison between GA and AA); Sig. bet.Grps — significance between groups

the studied participants and microvascular complications are shown in Table 3.

In the dominant model, a statistically significant difference was found between GA + AA and GG (38% vs. 12%) genotypes of rs1800790 gene polymorphism in patients with DPN only (p = 0.003) while there were no significant relations between the dominant model and DKD, DR or microvascular complications (p = 0.401, 1.0 and 0.052 respectively).

However, in the recessive model, There were no statistically significant differences between GG + + GA and AA genotypes in patients with DPN, DR and DKD groups (p = 0 0.675, 1.000 and 1.000 respectively).

Regression analysis of factors affecting microvascular complications

As regards DPN, univariate regression analysis showed that age, diabetes duration, insulin dose, mean systolic BP, FPG, HbA1c, log. FG, GA genotype and GA + AA genotype were the independent risk factors for DPN. However, in multivariate analysis only diabetes duration, log FG and GA + AA genotype were the independent risk factors affecting DPN (Tab. 4).

The GA genotype was associated with 6 folds increased risk of DPN. OR (95% CI) 5.50 (1.926–15.706). The dominant mode GA + AA was found in 19 patients (76%) out of 25 with DPN and was associated with a 4 folds increased risk of DPN. OR (95% CI) 4.495 (1.610–12.545) in univariate analysis while in multivariate analysis it was associated with 7 folds risk of DPN (p = 0.004 OR (95% CI) 7.414 (1.892–29.049).

On the other hand, univariate regression analysis to detect factors affecting DKD showed that HbA1c (%) and one-year mean HbA1c were the only independent risk factor for DKD (p = 0.026 OR (95% CI) 1.305 (1.032–1.651) for HbA1c and p = 0.017 OR (95% CI) 1.380 (1.058–1.798) for one-year mean HbA1c). However, none of them were found to be independent factor affecting DKD in multivariate analysis.

Furthermore, studying the factors affecting microvascular complications (in patients with at least one microvascular complications (n = 31)) revealed that age, diabetes duration, HbA1c, one-year mean HbA1c, UACR and the GA genotype were the independent risk factors affecting microvascular complications in univariate analysis. However, in multivariate analysis, diabetes duration, UACR and the GA genotype were the only independent factors affecting microvascular complications.

ROC curves for cut off values of plasma FG level detecting microvascular complications

Regarding DPN, a cut-off value of plasma FG > 348 mg/dL significantly differentiated patients with from those without DPN with AUC 0.651, p = 0.024 with good sensitivity and specificity (64.0 and 70.67 respectively).

Table 4. Univariate and Multivariate Analysis for the Parameters Affecting Diabetic Peripheral Neuropathy (DPN) Cases

	Univariate		Multivariate#	
	P	OR (95% CI)	P	OR (95% CI)
Age [years]	**0.025***	1.214* (1.025–1.438)	**0.713**	1.070 (0.748–1.530)
Diabetes duration [years]	**0.002***	1.284* (1.094–1.507)	**0.006***	1.388* (1.099–1.754)
Insulin dose [units]	**0.042***	1.018* (1.001–1.035)	**0.503**	1.010 (0.982–1.039)
Mean blood pressure				
Systolic [mm Hg]	**0.025***	1.055* (1.007–1.106)	**0.864**	0.992 (0.910–1.083)
FPG [mg/dL]	**0.030***	1.007* (1.001–1.014)	**0.279**	1.005 (0.996–1.014)
HbA1c % [mmol/mol]	**0.028***	1.249* (1.024–1.524)	**0.076**	1.277 (0.975–1.674)
One-year mean HbA1c % [mmol/mol]	**0.062**	1.238 (0.990–1.548)		
Log fibrinogen [mg/dL]	**0.045***	8.384* (1.051–66.867)	**0.038***	20.35* (1.181–350.513)
Dominant				
GG®	**1.000**	–	**1.000**	–
GA + AA	**0.004***	4.495* (1.610–12.545)	**0.004***	7.414*(1.892–29.049)

*Statistically significant at $p \leq 0.05$; #All variables with $p < 0.05$ was included in the multivariate CI — confidence interval; FPG — fasting plasma glucose; OR — odds ratio

Moreover, a cut-off value of plasma FG > 358mg/dL significantly differentiated patients with from those without DKD with ACU 0.671, p = 0.036 with good sensitivity and specificity (66.67 and 70.59 respectively).

Discussion

T1D is an autoimmune disease with genetic predisposition. Hemostasis dysfunction is a possible risk factor for diabetic microangiopathy. The present work studied plasma FG level in patients with T1D. It also aimed at genotyping *FGB* (rs1800790) polymorphism and its relation to microvascular complications in subjects with T1D.

In the present study, the one-year mean HbA1c was significantly higher in patients with DKD than those without DKD. This is also applied to DR and microvascular complications but not DPN. In agreement with the results of the present study, Giordano C et al [21] found a higher probability to develop albuminuria and DR in patients with higher mean HbA1c. Moreover, a real world study by Pettus et al. [22] showed that microvascular complications are more common in patients with suboptimal diabetes control. Additionally, Anderzén et al. [23] results showed significantly higher albuminuria and DR in teenagers with higher HbA1c. The one-year mean HbA1c is an emerging indicator of long term glycemic control along with glycemic variability. They are used recently as better measures affecting the development of microvascular complications.

The present study showed that plasma FG level was significantly higher in females. In agreement with the results of the present study, Klein et al. [24] found a significantly higher plasma fibrinogen level in females with T1D.

In the present study, plasma FG level was significantly higher in patients with DKD and it also showed a significant positive correlation with UACR. Similarly, previous studies have shown an association between increased FG level and microalbuminuria in T1D [25–27]. Additionally, Klein et al. [24] found a positive correlation between plasma FG level and urinary albumin excretion rate in patients with T1D although this relation was significant in men only. It is not clear, whether hyperfibrinogenemia occurs secondary to the onset of DKD or is a primary factor that precedes microalbuminuria. However, the present study showed that HbA1c and one-year mean HbA1c were the only independent risk factor for DKD in univariate analysis confirming the role of hyperglycemia in development of microvascular complications.

The current study also found a significantly higher plasma FG level in patients with DPN compared to those without DPN. Moreover, log FG level was identified as an independent risk factor for DPN in univariate and multivariate analysis. In disagreement with the results of the present study, Vojtková et al. [20] found no significant difference between patients with DPN compared to patients without DPN regarding plasma FG level.

Fibrinogen gene polymorphisms and microvascular complications

After thorough literature review, very few studies were available about the relation between *FGB* (rs1800790) gene polymorphism and microvascular complications in subjects with T1D. One study by Klein et al. (the DCCT/EDIC study) [24] studied the relation

between *FGB* (rs1800790) polymorphism and DKD in subjects with T1D, another study by Vojtková et al. [20] studied the relation between *FGB* (rs1800790) gene polymorphism and DPN in subjects with T1D.

The present study showed that the GA genotype of rs1800790 *FGB* polymorphism (–455 G/A) was significantly higher in patients with DPN with more than 6 folds increased risk for DPN in the general model. Moreover, in the dominant model, the GA + AA genotype was significantly higher than the GG genotype in patients with DPN with 7 folds increased risk of DPN in multivariate analysis [p = 0.004, OR 95% CI = 7.414 (1.892–29.049)]. However in the recessive model there was no relation between genotypes and DPN. In discordance with the results of the present study, Vojtková et al. [21] found that the AA genotype of rs1800790 *FGB* polymorphism (–455 G/A) had 4-fold increased risk for DPN in the general and recessive model. This difference in studies results may arise from the use of different study cohorts.

The mechanism by which rs1800790 gene polymorphism may affect neuropathy is not completely understood. Previous results showed that *FGB* –455 G/A polymorphism stimulates *FGB* gene transcription. The A allele has a significant increase in promoter activity [28].

In the AIRGENE study, there was no significant elevation of IL6 or CRP upon proinflammatory stimulation in the study participants [29].

Plasma fibrinogen level and FGB gene polymorphism

The current study showed no significant difference in plasma FG concentration (p = 0.270) between different genotypes of studied gene polymorphisms in patients with T1D. Results from the DCCT/EDIC study showed similar results as they showed no significant correlation between plasma FG concentration and –455 G/A *FGB* gene polymorphisms [24]. In agreement with the results of the present study, Vojtková et al didn't find significant differences between different genotypes regarding FG concentration [20]. Tybjaerg et al. [30] also reported that only 1% of variation in plasma FG concentration could be explained by –455 G/A polymorphisms. Plasma FG level is influenced by various polymorphisms. The linkage disequilibrium between them may explain this association.

In discordance with the results of the present study, Canseco et al studied a cohort of Mexican population and concluded that elevated plasma FG levels and the *FGB* gene polymorphisms –455 G/A are association with major adverse cardiovascular events and coronary disease [31].

This discrepancy between findings in the literature [32], suggests the presence of other factors such as inflammation or disease state that contributes to modification of this relationship. The variability in the findings may reflect differences in the cohorts regarding their age, genetic heterogeneity, or may be a result of differences in study designs, methodologies and statistics or in methods detecting FG levels. Gene-environment interaction also may contribute to these differences [33, 34].

However, the present study has some limitations. First, very few patients had DR (n = 3). This led to difficulties in studying the relation between *FGB* gene polymorphism and DR so further studies including more participants with DR are needed to verify this relation. Moreover, it is a single center study where further multicenter studies on different cohorts could generalize results.

Conclusions

The one-year mean HbA1c was significantly higher in patients with DKD, DR and microvascular complications but not in patients with DPN than patients without these complications. The present study found a significantly higher plasma FG level in females. It also showed significantly higher FG level in patients with DPN and patients with DKD. FG was significantly positively correlated with UACR. The GA genotype was the commonest genotype in patients with DPN, DKD and patients with microvascular complications. This was statistically significant in DPN and microvascular complications but not in DKD. In the dominant model the relation of genotypes was statistically significant only in patients with DPN while the recessive model failed to show significant relations with any of the microvascular complications. Additionally, diabetes duration, log FG and GA + AA genotype were the independent risk factors affecting DPN in multivariate analysis while regarding DKD, HbA1c and one-year mean HbA1c were the only independent risk factors affecting DKD in univariate analysis. To the best of our knowledge, this is the first study to set a cut-off value of plasma FG level that significantly differentiates patients with DPN (FG > 348 mg/dL) and patients with DKD (FG > 358 mg/dL) with good sensitivity, specificity and AUC in young patients with T1D.

Article information

Data availability

The datasets generated during and/or analyzed during the current study are available through the corresponding author on reasonable request.

Ethics statement

This cross-sectional study was performed in accordance with the ethical standards of the Declaration of Helsinki. The approval of the local Ethical Committee of Faculty of Medicine, Alexandria University was obtained.

Author contribution

Eman Y Morsy and Nahla A M Hamed designed the study and defined the aim of work. They participated in the interpretation of data and revised the manuscript. Mona M Tahoun performed laboratory investigations and gene polymorphism analysis. She participated in study design, data analysis and revised the manuscript. Salwa H Mohamed collected study data, participated in study design and wrote the manuscript. Heba S. Kassab participated in conception, data collection and analysis and co-drafted the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Ahmed AEA, Sakhr HM, Hassan MH, et al. Vitamin D receptor rs7975232, rs731236 and rs1544410 single nucleotide polymorphisms, and 25-hydroxyvitamin D levels in Egyptian children with type 1 diabetes mellitus: effect of vitamin D co-therapy. Diabetes Metab Syndr Obes. 2019; 12: 703–716, doi: 10.2147/DMSO.S201525, indexed in Pubmed: 31190930.
2. Mostofizadeh N, Hashemipour M, Roostazadeh M, et al. The impact of poor glycemic control on lipid profile variables in children with type 1 diabetes mellitus. J Educ Health Promot. 2019; 8: 6, doi: 10.4103/jehp.jehp_194_17, indexed in Pubmed: 30815477.
3. Barrett EJ, Liu Z, Khamaisi M, et al. Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. J Clin Endocrinol Metab. 2017; 102(12): 4343–4410, doi: 10.1210/jc.2017-01922, indexed in Pubmed: 29126250.
4. Dahlin LB, Elgzyri T, Löndahl M, et al. Improved metabolic control using glucose monitoring systems leads to improvement in vibration perception thresholds in type 1 diabetes patients. Acta Diabetol. 2020; 57(4): 433–438, doi: 10.1007/s00592-019-01450-2, indexed in Pubmed: 31705298.
5. Sivaprasad S, Prevost AT, Vasconcelos JC, et al. CLARITY Study Group. Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): a multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial. Lancet. 2017; 389(10085): 2193–2203, doi: 10.1016/S0140-6736(17)31193-5, indexed in Pubmed: 28494920.
6. Klein BE, Moss SE, Klein R, et al. Blood pressure and hypertension in diabetes. Am J Epidemiol. 1985; 122(1): 75–89, doi: 10.1093/oxfordjournals.aje.a114088, indexed in Pubmed: 4014203.
7. Pop-Busui R, Ang L, Holmes C, et al. Inflammation as a Therapeutic Target for Diabetic Neuropathies. Curr Diab Rep. 2016; 16(3): 29, doi: 10.1007/s11892-016-0727-5, indexed in Pubmed: 26897744.
8. Picconi F, Parravano M, Ylli D, et al. Retinal neurodegeneration in patients with type 1 diabetes mellitus: the role of glycemic variability. Acta Diabetol. 2017; 54(5): 489–497, doi: 10.1007/s00592-017-0971-4, indexed in Pubmed: 28238189.
9. Xu X, Teng X. Effect of fibrinogen on blood coagulation detected by optical coherence tomography. Phys Med Biol. 2015; 60(10): 4185–4195, doi: 10.1088/0031-9155/60/10/4185, indexed in Pubmed: 25955503.
10. Arbustini E, Narula N, D'Armini AM. Fibrinogen: a circulating factor in search of its genetic architecture. Circulation. 2013; 128(12): 1276–1280, doi: 10.1161/CIRCULATIONAHA.113.005125, indexed in Pubmed: 23969697.
11. Luo H, Li X, Jiang A, et al. Associations of -Fibrinogen Polymorphisms with the Risk of Ischemic Stroke: A Meta-analysis. J Stroke Cerebrovasc Dis. 2019; 28(2): 243–250, doi: 10.1016/j.jstrokecerebrovasdis.2018.09.007, indexed in Pubmed: 30503677.
12. Hu X, Wang J, Li Y, et al. The -fibrinogen gene 455G/A polymorphism associated with cardioembolic stroke in atrial fibrillation with low CHADS-VaSc score. Sci Rep. 2017; 7(1): 17517, doi: 10.1038/s41598-017-17537-1, indexed in Pubmed: 29235504.
13. Mayer-Davis EJ, Kahkoska AR, Jefferies C, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018; 19 Suppl 27(Suppl 27): 7–19, doi: 10.1111/pedi.12773, indexed in Pubmed: 30226024.
14. Herman WH, Pop-Busui R, Braffett BH, et al. DCCT/EDIC Research Group. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. Diabet Med. 2012; 29(7): 937–944, doi: 10.1111/j.1464-5491.2012.03644.x, indexed in Pubmed: 22417277.
15. Wilkinson CP, Ferris FL, Klein RE, et al. Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003; 110(9): 1677–1682, doi: 10.1016/S0161-6420(03)00475-5, indexed in Pubmed: 13129861.
16. Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis. 2010; 55(4): 622–627, doi: 10.1053/j.ajkd.2010.02.337, indexed in Pubmed: 20338463.
17. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int. Suppl. 2013; 3(1): 1–150, doi: 10.1038/kisup.2012.73.
18. Enas N. PASS: A Quick and Easy Power and Sample-Size Calculation Program. The American Statistician. 1988; 42(3): 229, doi: 10.2307/2685020.
19. Grimes DA, Schulz KF. Determining sample size and power in clinical trials: the forgotten essential. Semin Reprod Endocrinol. 1996; 14(2): 125–131, doi: 10.1055/s-2007-1016320, indexed in Pubmed: 8796935.

20. Vojtková J, Kolková Z, Motyková K, et al. An association between fibrinogen gene polymorphisms and diabetic peripheral neuropathy in young patients with type 1 diabetes. Mol Biol Rep. 2021; 48(5): 4397–4404, doi: 10.1007/s11033-021-06455-1, indexed in Pubmed: 34061326.
21. Giordano C, Amato MC, Ciresi A, et al. Predictors of microvascular complications in type 1 diabetic patients at onset: the role of metabolic memory. Eur J Intern Med. 2011; 22(3): 266–274, doi: 10.1016/j.ejim.2011.02.009, indexed in Pubmed: 21570646.
22. Pettus JH, Zhou FL, Shepherd L, et al. Differences between patients with type 1 diabetes with optimal and suboptimal glycaemic control: A real-world study of more than 30 000 patients in a US electronic health record database. Diabetes Obes Metab. 2020; 22(4): 622–630, doi: 10.1111/dom.13937, indexed in Pubmed: 31789439.
23. Anderzén J, Samuelsson U, Gudbjörnsdottir S, et al. Teenagers with poor metabolic control already have a higher risk of microvascular complications as young adults. J Diabetes Complications. 2016; 30(3): 533–536, doi: 10.1016/j.jdiacomp.2015.12.004, indexed in Pubmed: 26775554.
24. Klein RL, Hunter SJ, Jenkins AJ, et al. DCCT/ECIC Study Group. Fibrinogen is a marker for nephropathy and peripheral vascular disease in type 1 diabetes: studies of plasma fibrinogen and fibrinogen gene polymorphism in the DCCT/EDIC cohort. Diabetes Care. 2003; 26(5): 1439–1448, doi: 10.2337/diacare.26.5.1439, indexed in Pubmed: 12716802.
25. Myrup B, de Maat M, Rossing P, et al. Elevated fibrinogen and the relation to acute phase response in diabetic nephropathy. Thromb Res. 1996; 81(4): 485–490, doi: 10.1016/0049-3848(96)00021-7, indexed in Pubmed: 8907298.
26. Coonrod BA, Ellis D, Becker DJ, et al. Predictors of microalbuminuria in individuals with IDDM. Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Care. 1993; 16(10): 1376–1383, doi: 10.2337/diacare.16.10.1376, indexed in Pubmed: 8269796.
27. Knöbl P, Schernthaner G, Schnack C, et al. Thrombogenic factors are related to urinary albumin excretion rate in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993; 36(10): 1045–1050, doi: 10.1007/BF02374497, indexed in Pubmed: 8243853.
28. Siegerink B, Rosendaal FR, Algra A. Genetic variation in fibrinogen; its relationship to fibrinogen levels and the risk of myocardial infarction and ischemic stroke. J Thromb Haemost. 2009; 7(3): 385–390, doi: 10.1111/j.1538-7836.2008.03266.x, indexed in Pubmed: 19143925.
29. Jacquemin B, Antoniades C, Nyberg F, et al. Common genetic polymorphisms and haplotypes of fibrinogen alpha, beta, and gamma chains affect fibrinogen levels and the response to proinflammatory stimulation in myocardial infarction survivors: the AIRGENE study. J Am Coll Cardiol. 2008; 52(11): 941–952, doi: 10.1016/j.jacc.2008.06.016, indexed in Pubmed: 18772067.
30. Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, et al. A common mutation (G-455--> A) in the beta-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. J Clin Invest. 1997; 99(12): 3034–3039, doi: 10.1172/JCI119499, indexed in Pubmed: 9185528.
31. Canseco-Avila LM, Lopez-Roblero A, Serrano-Guzman E, et al. Polymorphisms -455G/A and -148C/T and Fibrinogen Plasmatic Level as Risk Markers of Coronary Disease and Major Adverse Cardiovascular Events. Dis Markers. 2019; 2019: 5769514, doi: 10.1155/2019/5769514, indexed in Pubmed: 31354890.
32. Friedlander Y, Kark JD, Sinnreich R, et al. Combined segregation and linkage analysis of fibrinogen variability in Israeli families: evidence for two quantitative-trait loci, one of which is linked to a functional variant (-58G > A) in the promoter of the alpha-fibrinogen gene. Ann Hum Genet. 2003; 67(Pt 3): 228–241, doi: 10.1046/j.1469-1809.2003.00016.x, indexed in Pubmed: 12914575.
33. Voetsch B, Loscalzo J. Genetic determinants of arterial thrombosis. Arterioscler Thromb Vasc Biol. 2004; 24(2): 216–229, doi: 10.1161/01.ATV.0000107402.79771.fc, indexed in Pubmed: 14615395.
34. Thomas AE, Green FR, Humphries SE. Association of genetic variation at the beta-fibrinogen gene locus and plasma fibrinogen levels; interaction between allele frequency of the G/A-455 polymorphism, age and smoking. Clin Genet. 1996; 50(4): 184–190, doi: 10.1111/j.1399-0004.1996.tb02623.x, indexed in Pubmed: 9001796.