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Role of advanced glycation end products and sorbitol dehydrogenase in the pathogenesis of diabetic retinopathy

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Abstract

Background: Diabetic retinopathy is one of the common microvascular complications of diabetes. The formation of advanced glycation end products (AGE) exerts deleterious effects by acting directly to induce cross-linking of proteins promoting vascular damage. Hyperglycemia causes disturbance in glycogenesis pathway resulting in reduction of glucose to sorbitol which is converted to fructose by sorbitol dehydrogenase.

Methods: The levels of advanced glycation end products (AGE), lipid profile, and glycosylated Hb were estimated in 266 type I diabetic patients without retinopathy, patients with nonproliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy patients (PDR).The association between genotypes of two polymorphisms of sorbitol dehydrogenase gene (SDH) was estimated in the promoter region: a C/G transversion located at _1214 position and a G/C transversion at _888 position. This study showed allele–specific PCR for C-1214G polymorphism and restriction fragment length polymorphism (RFLP) technique for a G/C transversion at _ 888 position.

Results: Significant increase was detected in glycosylated Hb levels in diabetic group, both with retinopathy and without retinopathy. Also, a significant increase in Hb1c in PDR group compared to NPDR. Significant increase in total cholesterol, HDL, TG, and AGE in PDR group compared to the group without retinopathy. No significant change was observed in the same parameter between PDR and NPDR group. Significant increase in AGE in both PDR and NPDR group compared to the group without retinopathy. No significant change in PDR group compared to NPDR.

The results of this study showed no significant difference in genotype distribution (C/C, C/G, G/G) of the C-1214G polymorphism between the two groups of patients with and without DR A2-. There was no statistically significant difference between the three genotypes (CC, CG, and GG) of the C-1214G polymorphism in relation to DR severity in male genders. However, there was a statistically significant difference in female gender with increased frequency of CC genotype (2.7%, 21.9%, and 23.7%).

There was no significant difference in genotype distribution (C/C, G/C, and G/G) of the G-888C polymorphism between the two groups of patients with DR and without DR. However, the CC genotype occurred more frequently in patients with DR than patients without DR (6.7% vs. 3.9%), and G/G genotype occurred more frequently in

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patients without DR than patients with DR (0.70.6 vs. 0.59.7).
There was no statistically significant influence of the three genotype (GG, GC, and CC) polymorphism on DR progression in both genders. However, there was an increased frequency of **CC** genotypes polymorphism with severity in both genders (0.0%, 4.2%, 6.9%) in males and (5.6%, 6.3%, 8.8%) in females.

Conclusion: The onset of diabetic retinopathy is multifactorial, and a cascade of hyperglycemia-oxidative pathways has been involved in the initiation and progression of this disease. However, further research is required to understand the cellular and molecular mechanism of diabetic retinopathy pathogenesis. So that novel anti–diabetic retinopathy strategies can be developed, aiming to reduce the major economical and medical burden caused by diabetic retinopathy.

Keywords: Advanced glycation end products, Sorbitol dehyderogenas, Diabetic retinopathy

Introduction

Diabetes is a microvascular disorder characterized by hyperglycemia. It is mostly considered to be the major cause of diabetic complications (retinopathy, nephropathy, and neuropathy) in both types 1 and 2 diabetes (Wan et al. 2015). One mechanism linking chronic hyperglycemia with diabetic retinopathy is the formation and accumulation of advanced glycation end products (AGE) (Madonna et al. 2017).

Formation of advanced glycation end products (AGE) correlates with glycemic control. They exert deleterious effects by acting directly to induce cross-linking of long-lived proteins to promote vascular stiffness, altering vascular structure, and function and interacting with receptor for AGE to induce intracellular signaling leading to enhanced oxidative stress and elaboration of key pro-inflammatory cytokines (Kandarakis et al. 2014).

AGE effects include production of reactive oxygen species (ROS), binding to specific cell surface receptors and forming cross-links and contributing to the pathophysiology of vascular disease in diabetes. AGEs bind to specific cellular proteins changing the local concentrations of cytokines and growth factors resulting in accumulation in the vessel wall which may contribute to acceleration of diabetic retinopathy (Takeuchi and Yamagishi 2009).

Morphological and functional changes in diabetic retinopathy include basement membrane thickening, loss of pericytes, and increased permeability (Katagiri et al. 2017). Resulting in vascular leakage (Choudhuri et al. 2013) and leading to occlusion and ischemia (Warboys et al. 2009) along with induction of vascular endothelial growth factor (VEGF), result in angiogenesis and neovascularization (Prasad and Mishra 2018).

Polyol pathway (sorbitol–aldose reductase pathway) activation represents one of the processes observed under the hyperglycemia-induced oxidative stress conditions during DR pathogenesis (Pusparajah et al. 2016). Glucose is reduced to sorbitol and subsequently oxidized to fructose, with the help of two enzymes: aldose reductase, which converts glucose into sorbitol, and sorbitol dehydrogenase, which oxidize sorbitol into fructose (Kim et al. 2012). Hyperglycemia leads to an imbalance between glycogenesis and glycolysis pathway favoring the accumulation of sorbitol (Liu et al. 2016). Also, sorbitol and fructose accumulation in cells are leading to increase in osmotic pressure and membrane permeability damage.

Under hyperglycemic conditions, polyol pathway activity increases, which is followed by a decrease in the levels of NADPH that can regenerate an intracellular antioxidant, GSH (Romeo et al. 2002), leading to the accumulation of ROS, which induces oxidative stress in cells (Liu et al. 2016). Also, the reduction of NAD+ into NADH, due to hypoxia and redox imbalance, increases intracellular NADH levels, leading to cell edema, structural alterations, metabolic disorders, and microvascular lesion (Jing et al. 2012).

The aim of this study is predicting diabetic retinopathy among Egyptian patients with type I diabetes by measuring the levels of AGE products in serum of those patients together with detecting the genetic polymorphisms of sorbitol dehydrogenase. We will investigate the association between genotypes of two polymorphisms of sorbitol dehydrogenase gene (SDH) in the promoter region: a C/G transversion located at _1214 position and a G/C transversion at _888 position. So that novel anti-AGE strategies can be developed, aiming to reduce the major economical and medical burden caused by diabetic retinopathy.

Subjects and methods

Study design and sample size

A cross-sectional, observational study was undertaken in a sample of type I diabetic patients who attend diabetes polyclinic of RIO's hospital, Giza, Egypt, between October 2012 and December 2016 and who were willing to participate. The sampling procedure consisted of randomly selecting 2 days each week (Sunday and Wednesday) and recruiting all the diabetic patients (type I) who attended on those days to be the study population. A comprehensive data was collected from patients with

type I diabetes with the aim of identifying genetic and environmental risk factors for diabetic complications. In the first visit, a detailed history followed by baseline blood samples for fasting blood sugar (FBS) and glycosylated hemoglobin (HBA1c) were obtained to confirm DM. Once confirmed, all patients were referred to the Ophthalmic Department of the diabetic polyclinic. Written informed consents were obtained from all subjects in accordance with the principles of the Declaration of Helsinki. The protocol of the study was approved by the RIO Ethics Committee; a venous blood sample was collected from each subject after the consent form had been signed.

Inclusion criteria

1. Participants diagnosed to have diabetes mellitus without diabetic retinopathy were included in this study.
2. Participants diagnosed to have diabetes mellitus with diabetic retinopathy were also included.

Exclusion criteria

1. Participants with known other systemic diseases which could manifest as retinal pathological lesions such as hypertensive retinopathy.
2. Participants with very hazy ocular media which obscure the ocular fundus.
3. Gestational diabetics.
4. Participants not accepting the informed consent.

Two hundred and sixty-six type I diabetic patients were studied. (108 males, 158 females) aged 41.7 + 12.5 (mean ± SD) years. They were diabetic for > 5 years.

All patients in our study were subjected to:

1. Full medical examination
2. Full ophthalmic examination
3. Laboratory Investigations
4. Genetic examination

Medical examination

Full personal, family, and medical history include a standardized questionnaire for cardiovascular disease. The following parameters were collected: gender, age of onset of diabetes, and duration of diabetes.

Full medical examination:

- Cardiovascular examination and ECG.
- Blood pressure was measured twice in a sitting position using a mercury sphygmomanometer and after rest of at least 10 minutes.
- Investigations for other diabetic complications as neuropathy and nephropathy.
- Monthly follow-up of patients with uncontrolled blood sugar levels.

Ophthalmic examination

Complete ophthalmological examination including:

- Absolute visual acuity
- Intraocular pressure using slit lamp applanation tonometer or air buff tonometer
- Pin torch external eye examination to screen for extra ocular abnormalities
- Anterior segment biomicroscopic examination
- Fundus examination using slit lamp biomicroscopy after full dilation of the pupil using cyclopentolate 1% eye drop
- Fluorescein fundus angiography (FFA) using fundus camera (TRC50EX) and intravenous injection of 5 ml 10% sodium fluorescein solution
- The pictures were studied to categorize the different phenotypes of DR according to FFA as being mentioned in the phenotypes classification of DR (A.B.C). Also a report on the state of macular area was done for the presence or absence of edema

Laboratory investigation

Sample collection: venous blood samples (10 ml) were withdrawn from all subjects and emptied on:

- Sterile ethylene diamine-tetra-acetate "EDTA" vacutainer (2 ml) tubes used for DNA extraction and HbA1c. HbA1c was extracted using the Lobona system. DNA was extracted from blood samples and stored at − 30 °C till time of assay
- Sterile fluoride vacutainer tube for blood sugar (2 ml)
- Sterile plain vacutainer tube (6 ml) was centrifuged, and the serum was stored at − 20 °C to measure the rest of the parameters. Lipid profile [total cholesterol, high density lipoprotein (HDL-cholesterol), low density lipoprotein (LDL-cholesterol) and triglycerides] using regular commercial kits. Urea and creatinine using commercial kits and Glycation end products (GEP) using immune-sorbent assay (ELISA) kits

Biochemical methods

- HbA1c was extracted using the Lobona system (American Diabetes Association 2010)
- Lipid profile, total cholesterol, high-density lipoprotein (HDL-cholesterol), low-density lipoprotein

(LDL-Cholesterol), and triglycerides using regular commercial kits
- Glycation end products using immune-sorbent assay (ELISA) kits (Onarato et al. 2000)

Genetic study

One hundred and ninety patients were examined at the ophthalmic clinic for diabetic patients; 133 were diagnosed as having DR based on the ophthalmologist's examination. Also, a group of 57 diabetic patients without DR was taken as a control group. All were subjected to molecular genetic analysis to evaluate its association with two selected candidate genes proposed to be related to DR pathogenesis. We investigate the association between genotypes of two polymorphisms of sorbitol dehydrogenase gene (SDH) in the promoter region: a C/G transversion located at _1214 position (the C_1214G polymorphism) and a G/C transversion at _888 position (the G_888C polymorphism).

Full medical history includes name, age, sex, family history, parental consanguinity and complaints; also, the family pedigree was constructed, and a complete medical genetic examination was done for all participants.

Blood samples, 2 ml peripheral blood on ethylene diamine tetra acetic acid (EDTA) tubes, were collected from all participants.

Extraction of DNA was done and stored at − 30 °C.

Allele-specific PCR for C-1214G polymorphism

The genotypes of the C 1214G polymorphism were determined by using the following primers:

The two allele-specific:

C variant 5′-TGTTGCCCAGGCTGGTGTTC-3′

G variant 5′-TGTTGCCCAGGCTGGTGTTG-3′

The two control primers:

Forward 5′-TTTGGGCAGAAACTCTG-3′

Revers 5′-ACGCAGCGTCCACGTC-3′

PCR conditions

Thermo-Scientific kit was used for the PCR reactions. This includes the polymerase enzyme *Taq* DNA polymerase, dNTPs, and PCR Green buffer. Where each 20 μl of the PCR reaction contained Genomic DNA 6 ng (3 μl), 2.5 μl of 10× PCR buffer, 2.5 μl dNTPs of dNTP mixture stock (2 mM), 2 μl of 10.0 pmol forward primer, 2 μl of 10.0 pmol reverse primer, Taq polymerase 2.5 U (0.5 μl), and PCR water nuclease free 7.5 μl

PCR was carried out on a Biometra™ Thermal Cycler, (Model: TProfessional Basic) using the following conditions:

1. Initial denaturation step at 95 °C for 1 min.
2. Denaturation at 95 °C for 30 s.
3. Annealing temperature at 62 °C for 30 s.
4. Extension for 30 s at 72 °C.
5. Stages 2–4 carried out 35 times.
6. The final extension step was performed at 72 °C for 5 min.

PCR products were separated onto a 2% agarose gel.

PCR was carried out on a **Biometra**™ Thermal Cycler, **(TProfessional Basic)** using the following conditions table (IV): for G-888C polymorphism, each 20 μl of the PCR reaction contained:

1. Genomic DNA 6 ng (2.0 ng/μl equal to 3μl).
2. 2.5μl of 10× PCR buffer.
3. 2.5μl dNTPs of dNTPs mixture stock (2 mM).
4. 2μl of 10.0 pmol forward primer.
5. 2μl of 10.0 pmol reverse primer.
6. Taq polymerase 2.5 U (0.5μl).
7. PCR water nuclease free 7.5μl

Forward Primer 5′CGCCCGGCCTCATGTCTTTT-3′

Reverse Primer 5′TTGGGGTGGGGAATGTGAGG-3′

Each exon of interest (in the studied genes) was amplified using the suitable pair of primers via conventional Polymerase Chain Reaction (PCR) technique.

The PCR amplicons for each exon of interest were digested using the suitable restriction enzyme according to the Restriction Fragment length Polymorphism (RFLP) technique.

Horizontal agarose gel electrophoresis technique was used to detect the genotype(s) for each PCR product according to the size of DNA fragments after digestion.

The bands were detected via gel documentation system.

Excel (2016) was employed to perform the statistical analysis of the results.

Statistical analysis

All data was analyzed using the statistical package for social studies software (SPSS version 21). Descriptive analysis was conducted with the X^2 test for categorical variables. Fisher's exact test is a statistical significance test used in the analysis of contingency tables where sample sizes are small as opposed to the chi-square (X^2) test that can be used with larger samples. Normally distributed variables were presented as the mean (± SD) and non-normally distributed variables expressed as median (inter-quartile range).

Frequencies and percentages were calculated for all the qualitative data including gender, age group, and type of DR. logistic regression analyses, providing odds ratios (OR), their 95% confidence intervals (CI), p values, and Wald's chi-square estimates, were performed. P values < 0.05 were accepted as indicating statistical significance. In this study, the allele frequencies for each variant in patients and controls were tested for

agreement with expectations using Fisher's exact test as a sort of chi-square (X^2) test ($P \leq 0.05$).

Results

A total of 266 type I diabetic patients (40.6% males) were studied; male-to-female ratio was 1:1.46 (108:158). Mean age at ophthalmologic examination was 41.7 ± 12.5 years. Fifty-six percent of the studied patients had duration of DM > 10 years. Overall proportion of any DR was 71.4% (n = 190). Eighty-five patients (31.9%) had NPDR, and 105 patients (39.5%) were diagnosed to have PDR. Our study revealed that history of hypertension, family history of diabetic retinopathy (DR), and duration of diabetes were correlated to the occurrence of DR. No significant correlation could be detected of gender, education, family history of diabetes, consanguinity, ischemic heart disease, nephropathy, and neuropathy to DR (Table 1).

There was a significant increase in HbA1c between groups with and without DR and between NDPR and PDR groups. Also, significant increase in Hb1c in PDR group compared to NPDR. There was a significant increase in total cholesterol, HDL, TG, and glycated end product (AGE) in the group PDR compared to the group without retinopathy. There was a significant increase in AGE between the group in both and PDR and NPDR. No significant change in AGE in PDR group compared to NPDR (Table 2).

This study investigated the association between genotypes of polymorphisms of sorbitol dehydrogenase gene (SDH) in the promoter region: a C/G transversion located at _1214 position (the C_1214G polymorphism) (Table 3) (Fig. 1) and a G/C transversion at _888 position (the G_888C polymorphism) in relation to microangiopathies (Table 4) (Fig. 2). Table 5 was showed the distribution of genotype frequency of G-888C polymorphism of SDH gene in relation to severity (NPDR and PDR) of retinopathy in both genders.

Table 6 verified genotype frequency of C-1214G polymorphism of SDH gene in relation to severity (NPDR and PDR) of retinopathy in both genders.

Clinical and laboratory features of type I DM patients

From the above tables, significant increase is observed in the following:

- HbA1c between groups with and without DR and between NDPR and PDR groups
- Total cholesterol, HDL, TG, and GEP in the group without DR compared to the PDR group. The increase of HDL in PDR group can be explained by the fact that the main protein component of HDL is APO lipoprotein which is already high in type I DM

Table 1 Clinical characteristics of patients grouped according to the presence of DR

	Retinopathy		No retinopathy		*P* value
	No	%	No	%	
Gender					0.430
Male	80	74.1	28	25.9	
Female	110	69.6	48	30.4	
Age					**0.004**
< 30 yrs	30	56.6	23	43.4	
30–40 yrs	43	65.2	23	34.8	
41–60 yrs	104	78.2	29	21.8	
> 60 yrs	13	92.9	1	7.1	
Education					0.343
Illiterate	121	73.3	44	26.7	
Middle	37	63.8	21	36.2	
High	32	74.4	11	25.6	
Duration of diabetes					**< 0.001**
= <10 yrs	41	53.2	36	46.8	
11–20 yrs	65	77.4	19	22.6	
> 20 yrs	84	80.0	21	20.0	
Control of diabetes					**< 0.001**
Controlled	64	51.6	60	48.4	
Uncontrolled	126	88.7	16	11.3	
Family history of DR					**0.039**
Positive	37	82.2	8	17.8	
Negative	66	65.3	35	34.7	
Unknown	120 patients did not know				
History of Consang.					0.246
Positive	27	64.3	15	35.7	
Negative	163	72.8	61	27.2	
Hypertension					**0.034**
No	114	68.3	53	31.7	
Yes	58	81.7	13	18.3	
IHD					0.737
No	169	72.8	63	27.2	
Yes	4	66.2	2	33.3	
Nephropathy					0.198
No	159	71.3	64	28.7	
Yes	13	86.7	2	13.3	
Neuropathy					0.375
No	88	69.8	38	30.2	
Yes	84	75.0	28	25.0	

Clinical features of type I DM patients grouped according to the presence or absence of DR that are shown in Table 1. Patients with no DR had a shorter duration of DM and were younger than patients with DR. Gender proportion did not differ significantly among groups. The study revealed no significant correlation of gender, education, family history of diabetes, consanguinity, ischemic heart disease, nephropathy, and neuropathy to DR, whereas family history of DR and hypertension were correlated to retinopathy, and there was a highly significant correlation of duration of diabetes to retinopathy.

Table 2 Laboratory investigations of diabetics grouped according to DR types

		No.	Mean	SD	*P* value
FBS	No.	76	223.4	76.4	**0.835**
	NPDR	85	223.5	79.1	
	PDR	105	217.4	84.0	
PPBS	No.	76	286.3	81.2	**0.437**
	NPDR	85	306.2	105.1	
	PDR	105	295.8	103.1	
HbA1c	No	76	7.65	1.58	**0.003***
	NPDR	85	7.81	1.53	
	PDR	105	8.00	1.61	
AGE	No	76	22.07	18.73	**< 0.001***
	NPDR	85	26.49	16.15	
	PDR	105	24.13	14.02	
Total cholesterol	No	76	230.67	41.03	**0.001***
	NPDR	85	231.67	45.16	
	PDR	105	239.36	47.46	
Triglycerides	No	76	172.47	26.44	**0.002***
	NPDR	85	177.81	43.27	
	PDR	105	179.98	33.29	
HDL	No	76	49.18	11.72	**0.001***
	NPDR	85	47.64	11.61	
	PDR	105	51.34	10.41	
LDL	No	76	152.72	43.19	**0.028***
	NPDR	85	148.84	45.86	
	PDR	105	151.28	47.10	
Creatinine	No.	76	1.0	0.4	**0.003***
	NPDR	85	1.0	0.2	
	PDR	105	0.9	0.2	
Urea	No.	76	42.0	10.2	**0.001***
	NPDR	85	40.2	10.8	
	PDR	105	36.9	6.6	
Lab. test	Comparison A (*p* value)		Comparison B (*p* value)		Comparison C (*p* value)
HbA1c	0.017*		0.002*		0.096
GEP	>0.001*		>0.001*		0.275
Total Cholesterol	0.055		>0.001*		0.151
Triglycerides	0.018		>0.001*		0.347
HDL	0.322		>0.001*		0.014*
LDL	0.23		0.017*		0.607
Creatinine	0.08		0.001*		0.095
Urea	0.21		< 0.001*		0.015*

*According to Table 2, microangiopathy (nephropathy and neuropathy) may not be clinically detected; however, investigations are needed for further diction.
Comparison A between groups without retinopathy and that with NDPR.
Comparison B between groups without retinopathy and with PDR
Comparison C between groups with NDPR and that with PDR
*(Kruskal-Wallis Test, Mann-Whitney *U* with Bonferroni's adjustment: lower the significance of *P* value from 0.05 to 0.017)

Table 3 Genotype frequency of C-1214G polymorphism of SDH gene in patients with and without DR

Allele		With retinopathy (n = 131)		Without retinopathy (n = 52)		OR	(95% CI)	P value
Specific		No.	%	No.	%			
Genotype	GG	31	25.2	11	21.2	0.80	(0.37–1.74)	0.57
	CG	69	56.1	37	71.2	1.93	(0.96–3.88)	0.06
	CC	23	18.7	4	7.7	0.36	(0.12–1.11)	0.07
Alleles	G	131	53.3	59	56.7	1.15	(0.73–1.83)	0.55
	C	115	46.7	45	43.3	0.87	(0.55–1.38)	

There was no significant difference in the genotype distribution (C/C, C/G, G/G) of the C-1214G polymorphism between the two groups of patients with and without DR. Also, there was no significant difference of G and C allele frequencies

- Significant decrease in AGE between the groups without DR compared to DR (NPDR, PDR). No significant change in PDR group compared to NPDR (Table 2)

Discussion

The onset of diabetic retinopathy is multifactorial, and a cascade of hyperglycemia-linked pathways has been involved in the initiation and progression of this disease (Fu et al. 2016).

The results of this study revealed that the duration of diabetes is correlated to the occurrence of DR. There was a significant increase in HbA1c between groups with and without DR; also, there is a significant increase in HbA1c in PDR compared to NPDR. Highly statistically significant increase in HbA1c levels in both PDR and NPDR groups which agree with Lim (2019). Also, Aldebasi et al. (2011) detected a highly significant level of HbA1c in patients with PDR patients compared to NPDR. There was a significant increase in total cholesterol, HDL, TG, and glycated end product (AGE) in the PDR group compared to group without retinopathy. There was controversy results regarding the role of lipids in the pathogenesis of diabetic retinopathy. Chatziralli (2017). To the contrary, there was no statistically significant association of total cholesterol with DR. These results are consistent with several other studies, Ahmed et al. (2016) and Jayanthi et al. (2017). The Singapore Malay Eye study reported that higher cholesterol levels could have a good role in retinopathy, Wong et al. (2008), while CrosbyNwaobi and co-authors Crosby-Nwaobi et al. (2015) reported no lipid profile association with progression of DR.

There was a significant increase in AGE in both PDR and NPDR groups compared to the group without retinopathy.

These results agreed with many studies (Giurdanella et al. 2017) (Saker et al. 2014), who postulated that AGE were localized to retinal blood vessels in diabetics and were correlated with the degree and clinical progression of retinopathy.(Giurdanella et al. 2017) and (Saker et al. 2014) found that AGE were elevated in various ocular tissues of diabetic subjects compared to diabetics without retinopathy (Navaratna et al. 2007) (Fan and Yan 2016).This includes vitreous collagen (Fan and Yan 2016), where the AGE levels correlate with diabetic retinopathy (Kim et al.

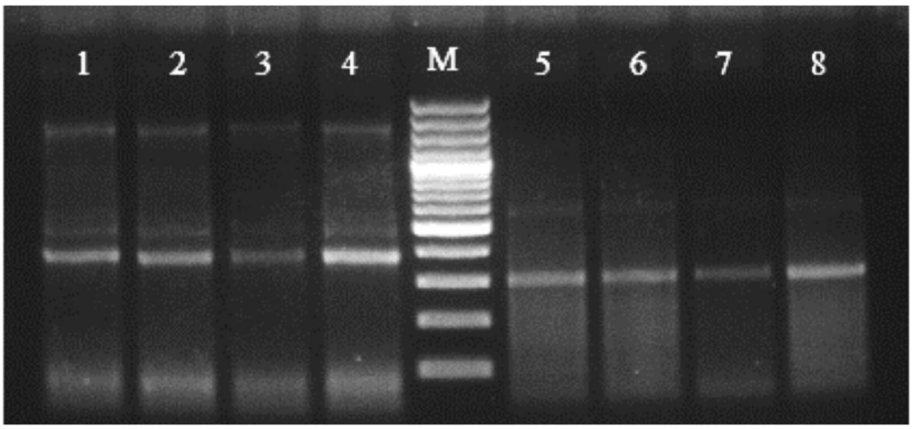

Fig. 1 Genotypes of the C/G polymorphism at the – 1214-bp SDH promoter region determined by allele-specific PCR detection (ASO-PCR) and analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide, and viewed under gel documentation system. Lane M is the (100 bp) DNA ladder; lanes 1–4 show the result of amplification with primer specific to the G allele at 494 bp and lanes 5–8 with primer specific to the C allele

Table 4 Distribution of genotype and alleles, and frequency of G-888C polymorphism of SDH gene in patients with and without DR

G888C		With retinopathy (n = 119)		Without retinopathy (n = 51)		OR	(95% CI)	P value
		No.	%	No.	%			
Genotype	GG	71	59.7	36	70.6	1.62	(0.80–3.28)	0.18
	GC	40	33.6	13	25.5	0.68	(0.32–1.41)	0.30
	CC	8	6.7	2	3.9	0.57	(0.12–2.76)	0.48
Allele	G	182	76.5	85	83.3	1.54	(0.84–2.81)	0.16
	C	56	23.5	17	16.7	0.65	(0.36–1.19)	

There was no significant difference in the genotype distribution (C/C, G/C, and G/G) of the G-888C polymorphism between the two groups of patients with DR and without DR. However, the CC genotype occurred more frequently in patients with DR than patients without DR (6.7% vs. 3.9%), and G/G genotype occurred more frequently in patients without DR than patients with DR (0.70.6 vs. 0.59.7)

2010). In the diabetic retina, AGE accumulation has been observed in vascular cells, neurons, and glia which may have pathogenic implications in retinal function (Radeva and Waschke 2018) (Prasain and Stevens 2009).

In diabetes, AGE accumulate in retinal pericytes which play an important role in the maintenance of microvascular homeostasis. Loss of pericytes could predispose the vessels to angiogenesis, thrombogenesis, and endothelial cell (EC) injury, thus leading to full clinical picture of diabetic retinopathy (Guo et al. 2009).

Wang et al. (2012) and van Hecke et al. (2005) demonstrated that the AGE–RAGE interaction induces ROS generation in cultured retinal pericytes inducing apoptotic cell death of pericytes. AGEs induced nuclear factor-κB (NF-κB) activation and decreased the ratio of Bcl-2/Bax increasing the activity of caspase-3, an enzyme responsible in the execution of apoptosis of pericytes. AGEs also upregulate RAGE mRNA levels in pericytes through the intracellular ROS generation (Joussen et al. 2004).

van der Wijk et al. (2017) suggested the involvement of AGE in the development and progression of diabetic retinopathy by inducing VEGF over expression in pericytes. VEGF level was also found co-related with the breakdown of the blood–retinal barrier, thus being involved in retinal vascular hyper-permeability (Capitao and Soares 2016) and (Kaji et al. 2007). Furthermore, AGE interact with RAGE and directly stimulate growth and tube formation of microvascular ECs, the key steps of angiogenesis (Yamagishi et al. 2006) and (Nalini et al. 2017). It was also observed in (Huang et al. 2011) and (Aveleira et al. 2010) that the angiogenic activity of AGEs was mainly mediated by autocrine VEGF production by ECs. Studies have also shown that the AGE–RAGE interaction might increase VEGF gene transcription in micro vascular ECs by NADPH oxidase-mediated ROS generation and subsequent NF-κB activation via Ras-MAPK pathway (Nalini et al. 2017).

Sorbitol dehydrogenase is the enzyme involved in polyol pathway. Li et al. (2019) had found an association of

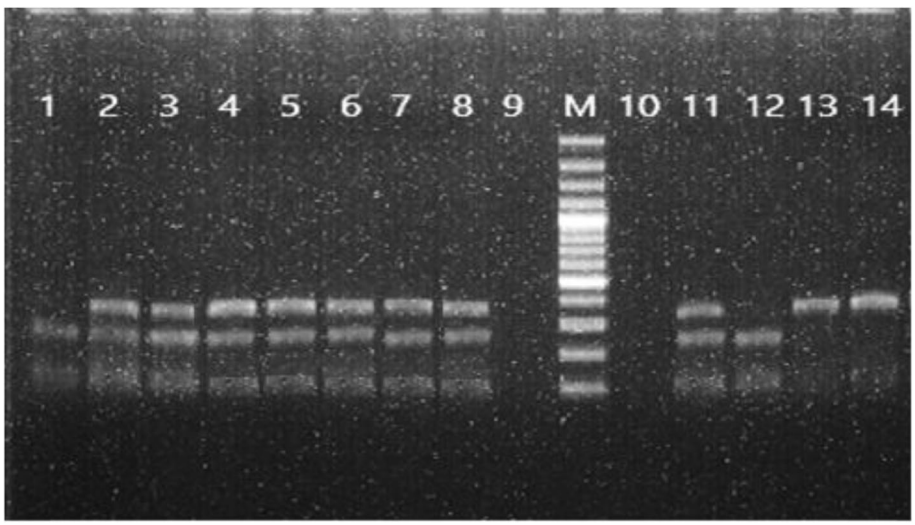

Fig. 2 Genotypes of the C/G polymorphism at the − 888-bp SDH promoter region determined by the PCR-based BseDI restriction fragment length polymorphism (RFLP-PCR) and analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide, and viewed by gel documentation system. Lanes 1 and 12 show the result of amplification of G/G homozygote; lanes 13 and 14 show homozygote C/C; lanes 2, 3, 4, 5, 6, 7, 8, and 11 show heterozygote G/C; lanes 9 and 10 are empty; lane M is (100 bp) the DNA ladder

Table 5 The distribution of genotype frequency of G-888C polymorphism of SDH gene in relation to severity (NPDR and PDR) of retinopathy in both genders

		G-888C						
Retinal state		**GG**		**GC**		**CC**		***P* value**
(Males)	No	12	80.0%	3	20.0%	0	0.0%	**0.488**
	NPDR	13	54.2%	10	41.7%	1	4.2%	
	PDR	17	58.6%	10	34.5%	2	6.9%	
(Females)	No	24	66.7%	10	27.8%	2	5.6%	**0.932**
	NPDR	19	59.4%	11	34.4%	2	6.3%	
	PDR	22	64.7%	9	26.5%	3	8.8%	

There was no statistically significant influence of the three genotypes (GG, GC, and CC) polymorphism on DR progression in both genders. However, there was an increased frequency of CC genotypes polymorphism with severity in both genders (0.0%, 4.2%, 6.9%) in males and (5.6%, 6.3%, 8.8%) in females

genotype variant of SDH_1214-C-G (rs2055858) polymorphism that were associated with lower risk to DR in diabetic patients. These results agree with the results of this study.

Two polymorphisms described in the promoter region of the SORD gene, _1214C-G (rs2055858) and _888G-C (rs3759890), are in complete linkage disequilibrium and have been associated with the SORD gene expression in retinal cells in diabetes. These results agree with that reported in Amano et al. (2003), thus playing a role in the pathogenesis of DR. There was increased frequency of G_888_C polymorphism of SDH gene, and the frequency of CC genotype polymorphism increased severity in both genders. While the genotype frequency of C_1214_G polymorphisms of SDH gene, there was an increased frequency of CC genotype in females only. Also, the genotype and allele frequency of G_888_C polymorphisms of SDH gene were insignificant among the DR patient with and without retinopathy in type 1 diabetics. To our knowledge, there was no study investigating these associations of the genotype polymorphism in type 1 diabetes. The_888 GG genotype has been more frequently observed in patients with DR than in those without this complication among Japanese patients (Amano et al. 2003). Based on these findings and that both polymorphisms are linked, we investigated whether the _888G > C polymorphism in the SORD gene is associated with the presence or severity of DR in patients with type1diabetes.

Table 6 Genotype frequency of C-1214G polymorphism of SDH gene in relation to severity (NPDR and PDR) of retinopathy in both genders

		C-1214 G of SDH						
Retinal state		**CC**		**CG**		**GG**		***P* value**
(Males)	No	3	20.0%	10	66.7%	2	13.3%	**0.386**
	NPDR	1	4.2%	16	66.7%	7	29.2%	
	PDR	6	20.7%	16	55.2%	7	24.1%	
(Females)	No	1	2.7%	27	73.0%	9	24.3%	**0.05**
	NPDR	7	21.9%	15	46.9%	10	31.3%	
	PDR	9	23.7%	22	57.9%	7	18.4%	

There was no statistically significant difference between the three genotypes (CC, CG, and GG) in relation to DR severity in male genders. However, there was a statistically significant difference in female gender with increased frequency of CC genotype (2.7%, 21.9%, and 23.7%)

Conclusion

The onset of diabetic retinopathy is multifactorial, and a cascade of hyperglycemia-linked pathways has been involved in the initiation and progression of this disease. The biochemical process of advanced glycation appears to be enhanced in diabetes due to hyperglycemia along with oxidative stress and lipids. Further research is required to understand the cellular and molecular processes and to firmly establish whether AGEs are direct contributors in the initiation and progression of diabetic retinopathy. So that novel anti-AGE strategies can be developed, aiming to reduce the major economical and medical burden caused by diabetic retinopathy.

1-Medical equipment	
Source	equipment
1-Pcr (TProfessional Basic)	Research Institute of Ophthmology (RIO)
2-Gel Electrophoresis unit used in this study was provided by Biometra, Germany (Model: Compact M).	Research Institute of Ophthmology (RIO)
3-Robonik Spectrophotometer	Research Institute of Ophthmology (RIO)
Kit	Source
Blood sample	Research Institute of Ophthmology (RIO)
Glucose	Biomerieux, ca 61-269(France).
Glycosylated Hemoglobin (HbA1c)	NS Biotec (Egypt).
4-Cholesterol	Spectrum Diagnostics 225 003 (Egypt).
5-Triglycerides	Spectrum Diagnostics 314 009 (Egypt)
6-HDL Cholesterol	Spectrum Diagnostics 267 002(Egypt).
7-LDL Cholesterol:	Spectrum Diagnostics (Egypt).
8-glycation end products	akit supplied by cell biolabs, CA 92126, San Diego, and (USA).
9-DNA extraction	(Gene JET Whole Blood Genomic DNA Purification Mini Kit) Thermo Scientific, (EU) Lithuania
10-Dream *Taq* DNA polymerase	Thermo Scientific
	Thermo Scientific
11-Agarose A and Tis-Borate-EDTA (TBE)	Biobasic, Canada
12-GeneRuler 20 bp	Thermo Scientific

Abbreviations

DR: Diabetic R etinopathy; PDR: Proliferative diabetic retinopathy; TNF-α: Tumor necrosis factor alpha; BRB: Blood–retinal barrier; HbA1c: Glycated hemoglobin; NPDR: Non–proliferative diabetic retinopathy; AGE: Advanced glycation end products; SDH: Sorbitol dehydrogenase; RFLP: Restriction fragment length polymorphism; FBS: Fasting blood sugar; FFA: Fluorescein fundus angiography; HDL: High density lipoprotein; LDL: Low density lipoprotein; ELISA: Immune-sorbent assay; EDTA: Ethylene diamine tetra acetic acid

Authors' contributions

1- Leqaa A. Moemen: Developing the research idea of scientific and legal aspects and supervising the practical part and writing the texet. 2- Mona A.Abdel Hamid: Developing the research idea of scientific and legal aspects and supervising the practical part and writing the texet. 3- Soher Abdel Wahab: supervising the practical part. 4- Mahmoud Kenawy M. Kenawy: Doing the analysis part. 5- Manal H. Abuelela: Make the statistical analysis. 6- Olfat A. Hassanin: Diagnosis of diabetic patients and those diabetic retinopathy. 7- Marwa A. Fouly: Diagnosis of diabetic patients and those diabetic retinopathy by fluorescein angiography. 8- Amira A. Abdel azeem: Supervising the part of the practical and theoretical genetic work. 9- Shahira Riad Noweir: Supervising the part of the practical and theoretical genetic work. 10- SomaiaM. Ismail: Supervising the part of the practical and theoretical genetic work. 11- Yara Hossam Eldin R.Abdel Gawad: Help make the statistical analysis. 12- Safa Refaat: Diagnosis of patients of diabetic retinopathy. 13- Azza K. Amer: Supervising routine laboratory investigations. 14- Nervana A Khalaf: Supervising the part of the practical., and help in writing. 15- Safya H Aboelmakarem: routine laboratory investigations. 16- Mehry S Elsobky: routine laboratory investigations. 17- Mona R Abdelrasool: routine laboratory investigations. 18- Sherif. S. Karawya: Diagnosis of diabetic patients and diabetic retinopathy by fluorescein angiography. 19- Zeinab M. Osman: Diagnosis of patients of diabetic retinopathy. The author(s) read and approved the final manuscript.

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Availability of data and materials

All authors declare that the data and material are available. The study protocol was performed according to the Helsinki declaration as regards human guideline.

Ethics approval and consent to participate

The study was approved by the ethical committee of the Research Institute of Ophthalmology.

Consent for publication

All authors declare that they approved the publication of this research in the Bulletin of National Research Center.

Competing interests

The authors declare that they have no competing interests.

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