RESEARCH ARTICLE

Aldose reductase gene polymorphism rs752010122 and retinopathy in type 2 diabetics

Muntaha Maqsood,[1] Palvasha Waheed,[2] Amir Rashid,[3] Asifa Majeed,[4] Ahsan Mukhtar[5]

Abstract

Objective: To investigate the association of polymorphism in rs752010122 in aldose reductase gene with the pathogenesis of diabetic retinopathy, and to determine the association and allelic frequency between the variant and the disease.

Method: The cross-sectional study was conducted from June 2021 to March 2022 at Centre for Research in Experimental and Applied Medicine (CREAM) Laboratory, Department of Biochemistry and Molecular Biology, Army Medical College, in collaboration with the Armed Forces Institute of Ophthalmology, Rawalpindi, Pakistan, and comprised blood samples from subjects of either gender aged 40-70 years. The samples were divided into group I having diabetic retinopathy patients, group II having diabetics without retinopathy, and group III having healthy controls matched for age and gender. The samples were subjected to molecular analysis. Gene sequence was downloaded from the Human Genome Database and Ensemble. Data was analysed using SPSS 22.

Results: Of the 150 subjects, there were 50(33.3%) in each of the 3 groups. Variants of aldose reductase rs752010122 polymorphism were significantly associated with a lower risk of diabetic retinopathy ($p<0.05$). An odds ratio of 1 was noted for both heterozygous and homozygous genotypes (95% confidence interval: 1).

Conclusion: Aldose reductase was associated with lower risk of the disease.

Key Words: AKR1B1, Diabetes mellitus, Diabetic retinopathy, Single nucleotide polymorphism.

(JPMA 73: 978; 2023) **DOI: 10.47391/JPMA.6382**

Submission completion date: 14-04-2022 **— Acceptance date:** 09-12-2022

Introduction

Diabetes mellitus (DM) is one of the most attention-seeking health problems across the world. It is amongst the most demanding health issues and poses a significant economic burden on healthcare systems. It is a syndrome of heterogeneous complex and polygenic factors, principally characterised by hyperglycaemia because of relative insulin deficiency.[1] In 2019, an estimated 4.2 million DM deaths were documented around the globe in adults. This toll is projected to rise to 9.2 million by 2030. According to the latest report of the International Diabetes Federation, worldwide DM prevalence presently is 8.3% and is predicted to rise to 9.6% over the next 25 years.[2] DM has been documented among the 4 major non-communicable diseases that need imperative attention from all key stakeholders globally in an attempt to deal with its prevalence and connected complications.[3] Non-modifiable risk factors, like age, race, genetics and ethnicity, and modifiable factors, like diet, smoking and physical activity, are associated with its aetiology.[1] Retinopathy, neuropathy and nephropathy are some of its microvascular complications, while coronary artery disease, stroke and hypertension are among the macrovascular complications.[4] Damage to various organ systems secondary to chronic hyperglycaemia can cause various life-threatening and disabling health complications. In the first 10 years post-DM, most patients of type 1 DM (T1DM) and more than 60% of patients with T2DM can more likely develop some kind of retinopathy. Diabetic retinopathy (DR), also known as diabetic eye disease, is a medical condition in which DM damages the retinal vessels. It is a micro-angiopathy presenting with age ranging 20-64 years, and is characterised by increased vascular permeability, neovascularisation and vitreous haemorrhage.[5] DR is the leading cause of blindness in adults. Although regular screening is critical in preventing visual problems, the expected increase in diabetic patients from 415 million in 2015 to a predicted 642 million in 2045 means that the burden of screening and follow-up represents a substantial challenge.[6] Pakistan, with a population of over 200 million people, has been predicted to have a sizable amount of DR patients with no adequate mechanism to manage the spike.[7] Poor glycaemic control results in capillary damage, leading to enhanced vascular permeability and retinal ischaemia.

[1-4]Department of Biochemistry and Molecular Biology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan, Department of Vitero Retinal Surgery, Armed Forces Institute of Ophthalmology, Rawalpindi, Pakistan.

Correspondence: Muntaha Maqsood. Email: muntahatahir786@gmail.com

ORCID ID. 0009-0009-9895-8009

Retinal neovascularisation results in the progression of non-proliferative DR to proliferative DR which subsequently may cause vitreous haemorrhage, resulting in blindness.[8] Excess nicotinamide adenine dinucleotide+hydrogen (NADH) generation supplies NADH enzyme substrate to release reactive oxygen species (ROS) that cause damage to the anti-double-stranded deoxyribonucleic acid (dsDNA) molecule. Inside the retina, the main contributor of ROS is the polyol pathway, while sorbitol accumulation is involved in retinopathy in diabetic complications.[3] Aldose reductase (AR) is the first and rate-limiting enzyme in the polyol pathway. The enzyme is responsible for converting glucose into sorbitol with reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. AR enzyme is implicated in the aetiology of DR by way of polyol pathway. A case-control study in India was conducted to see the association of Aldo-keto reductase family 1, member B1 (AKR1B1) gene polymorphism in rs759853 (-106C>T) with DR. The study having 926 participants showed a prominent association of AKR1B1 (-106C>T) polymorphism with the development of DR.[9] In another study in Australian population, a different polymorphism, rs9640883 of this single nucleotide polymorphism (SNP) was found to be associated with early onset of DR.[10] Furthermore, a study with 1290 Slovenian T2DM patients analysed the association between rs11984041 and rs2107595 factor polymorphisms and DR. The T allele and TT genotype frequencies of rs11984041 polymorphism were higher compared to the controls,[11] indicating an association of rs11084041 polymorphism with the disease.

Renalase (RNLS) is a catalyst with monoamine oxidase activity involved in the metabolism of catecholamines. The RNLS factor Asp37 Glu missense polymorphism (rs2296545) is related to hypertension, cardiac disease, cardiac myopathy and stroke.[12] A study analysed the possible involvement of this polymorphism with the microangiopathic complications of T2DM. The methodology involved genotyping of the polymorphism in 860 patients with T2DM and 400 healthy controls. The genotype and allele distribution was compared in subgroups of patients with DR versus those without DR. In distinction, the DR subgroup showed a significantly higher frequency of G allele and GG genotype compared to the group without DR. The impact of RNLS Glu37Asp polymorphism on DR remained vital once adjustments were made for age, gender, body mass index (BMI), and duration of T2DM.[12] In Jordanian T2DM patients, T allele of aldose reductase (ALR2) was found to be related to DR. However, there was no evidence of an association between severity of DR and this polymorphism.[13]

The current study was planned to scrutinise the link of polymorphism rs752010122 in AR gene in the pathophysiology of DR, and also to determine the association and allelic frequency between the variant and the disease

Patients and Methods

The cross-sectional study was conducted from June 2021 to March 2022 at CREAM Laboratory, Department of Biochemistry and Molecular Biology, Army Medical College (AMC), in collaboration with the Armed Forces Institute of Ophthalmology (AFIO), Rawalpindi, Pakistan. After approval from the AMC etyhics review committee, the sample size was calculated using the World Health Organisation (WHO) sample size calculator by using population prevelance proportion of DR among diabetic patients as 30% and precision required as 10% with confidence level 95%.[10,13,14]

The sample was raised using non-probability purposive sampling technique, and comprised blood samples from subjects of either gender aged 40-70 years regardless of DM duration. Those excluded were patients with T1DM, gestational DM (GDM), patients with ocular diseases other than DR, like papillopathy, cataract, glaucoma and ocular surface diseases, retinal vein occlusion, retinal macroaneurysm, etc, patients with retinopathy due to causes other than diabetes, such as hypertension, atherosclerosis, systemic vasculitis, blood dyscrasias, systemic infections, radiations, etc, patients with history of ocular surgeries or any retinal laser therapy and all the subjects with media opacities that may hinder fundus visualisation.

The samples were collected after taking written informed consent from the subjects. The samples were divided into group I having DR patients, and group II having DM without DR, while group III comprised healthy controls matched for age and gender. The samples were subjected to molecular analysis. Gene sequence was downloaded from the Human Genome Database and Ensemble. AKR1B1 genome and protein information was obtained from Online Mendelian Inheritance in Man (OMIM).[15] Primers (wild and mutant) of exon 10 of the AKR1B1 were designed using WASP primer software. DNA extraction was performed by phenol-chloroform-isoamylachol method.[16] Confirmation of extracted DNA quality and quantity were analysed by 1% agarose gel electrophoresis. The DNA bands were then visualiSed using Gel Documentation System (BioRad Inc., USA) and images were taken using a high-resolution digital camera. Polymerase chain reaction (PCR) test was then performed. In order to check for polymorphism in AKR1B1 gene, exon

of interest and the exon-intron boundaries were amplified by PCR technique on Thermal Cycler (Corbet Inc) machine. The PCR master mix recipe for mutant and wild primers was Taq Buffer 2µl, Magnesium chloride (Mgcl2) 1.6µl, deoxynucleotide triphosphates (dNTPs) 0.4µl, common forward primer 0.7µl, reverse and mutant primer 1µl, Taq polymerase 1µl and nuclease-free water (NFH2O) 12.5µl. PCR conditions, including protocol and reagent, were optimised through PCR technique. The amplification parameters were optimised for individual primer, template and cycler. Hot start programmed was started at 95°C for 5 minutes. Denaturation of the strand was done at 95°C for 30 seconds. Step of annealing of primers of the strands was done at 63°C for 30 seconds. Extension of the product was achieved by heating at 72°C for 30 seconds and then final extension at 72°C for 7 minutes. Total cycles were 35 and were carried out at 8°C for 2 minutes. Horizontal gel electrophoresis for checking the amplified products was performed on 1% agarose gel (Figure).

Data was analysed using SPSS 22. Chi-square test was used for testing Hardy-Weinberg equilibrium by HW-Test software for the analysis of molecular data. Disease susceptibility was determined by calculating relative risk (RR) and odds ratio (OR) by binary logistic regression through Fisher's exact ratio test. An Association of SNP with the disease was studied through Chi-square test of independence. After applying SNP stat, the models were derived when SNP association was seen with age, gender and blood sugar fasting (BSF). The best model was selected on the basis of Akaike information criterion (AIC).[17]

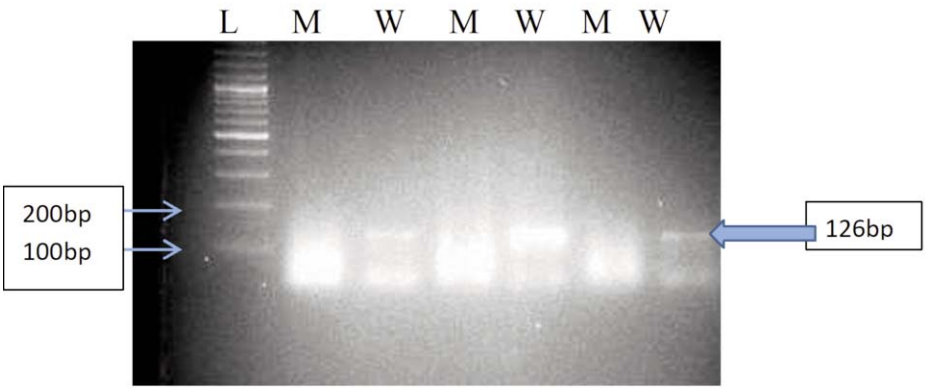

Figure: Gel image of allele-specific polymerase chain reaction (AS-PCR) in the 1st well 100bp ladder was loaded, and wild bands can be seen in the 3rd, 5th and 7th well.

Results

Of the 150 subjects, there were 50(33.3%) in each of the 3 groups. A/A genotype of AKR1B1 gene was found in 50(100%) group I cases,=. It was found in 34(68%) cases of group II which had A/T genotype 12(24%) and T/T 4(8%) as well. Group III had 32(64%) A/A genotype, followed by 7(14%) A/T allele and 11(22%) T/T allele subjects. The three AIC models were compared across the three study groups (Tables 1-2). Association of SNP with age and

Table-1: SNP association with response status between Control (C) and Diabetic Retinopathy (DR) groups

SNP association with response status (n=100, adjusted by Sr..no+gender+age+BSF)							
Model	**Genotype**	**status=C**	**status=DR**	**OR (95% CI)**	**p-value**	**AIC**	**BIC**
Codominant	A/A	32 (64%)	50 (100%)	1.00NA	1	18	41.4
	A/T	7 (14%)	0 (0%)	(0.00-NA)			
	T/T	11 (22%)	0 (0%)	NA (0.00-NA)			
Dominant	A/A			1.00	1	16	36.8
	A/T	32 (64%)	50 (100%)	NA (0.00-NA)			
	T/T	18 (36%)	0 (0%)				
Recessive	A/A			1.00	1	16	36.8
	A/T	39 (78%)	50 (100%)	NA (0.00-NA)			
	T/T	11 (22%)	0 (0%)				
Overdominant	A/A				1	16	36.8
	T/T	43 (86%)	50 (100%)	1.00			
	A/T	7 (14%)	0 (0%)	NA (0.00-NA)			
Log-additive	—-	—-	—-	955.01	1	16	36.8
				(0.00-NA)			

SNP: Single nucleotide polymorphism, AIC: Akaike information criterion, BIC: Bayesian Information Criterion, OR: Odds ratio, CI: Confidence interval, BSF: Blood sugar fasting.

Table-2: SNP association with response status between Diabetic (D) and Diabetic Retinopathy (DR) groups.

SNP association with response status (n=100, adjusted by Sr..no+gender+age+BSF)							
Model	**Genotype**	**status=D**	**status=DR**	**OR (95% CI)**	**p-value**	**AIC**	**BIC**
Codominant	A/A	34 (68%)	50 (100%)	1.00	1	18	41.4
	A/T	12 (24%)	0 (0%)	2.48 (0.00-NA)			
	T/T	4 (8%)	0 (0%)	NA (0.00-NA)			
Dominant	A/A			1.00	1	16	36.8
	A/T	34 (68%)	50 (100%)	NA (0.00-NA)			
	T/T	16 (32%)	0 (0%)				
Recessive	A/A			1.00	1	16	36.8
	A/T	46 (92%)	50 (100%)	NA (0.00-NA)			
	T/T	4 (8%)	0 (0%)				
Overdominant	A/A			1.00	1	16	36.8
	T/T	38 (76%)	50 (100%)	2.44			
	A/T	12 (24%)	0 (0%)	(0.00-NA)			
Log-additive	—-	—-	—-	5.04 (0.00-NA)	1	16	36.8

SNP: Single nucleotide polymorphism, AIC: Akaike information criterion, BIC: Bayesian Information Criterion, OR: Odds ratio, CI: Confidence interval, BSF: Blood sugar fasting.

Table-3: SNP within sex (n=100, adjusted by Sr..no+age+BSF)

	Genotype	**status=C**	**status=DR**	**OR (95% CI)**
Female	A/A	5	0	1.00
	A/T	0	0	—-
	T/T	0	0	—-
		status=C	status=DR	OR (95% CI)
Male	A/A	8	0	1.00
	A/T	0	0	—-
	T/T	0	0	—-
		status=C	status=DR	OR (95% CI)
Female	A/A	6	22	1.00
	A/T	3	0	—-
	T/T	5	0	—-
		status=C	status=DR	OR (95% CI)
Male	A/A	13	28	1.00
	A/T	4	0	—-
	T/T	6	0	—-

SNP: Single nucleotide polymorphism, C: Control, DR Diabetic retinopathy, AIC: Akaike information criterion, BIC: Bayesian Information Criterion, OR: Odds ratio, CI: Confidence interval, BSF: Blood sugar fasting.

gender was also explored (Table 3), while the Hardy-Weinberg equilibrium was found to be significant (p<0.0001) (Table 4).

Table-4: Genotypic, allelic frequencies present in the three study groups.

Genetic Variation	**Genotype**	**Group I (Diabetic Retinopathy) N= 50**	**Group II (Diabetics without retinopathy) N=50**	**Group III (Control) N=50**
	A / A	50 (100%)	34 (68%)	32 (64%)
	A / T	-	12 (24%)	7 (14%)
	T / T	-	4 (8%)	11 (22%)
Allele	A	100	92	78
	T	-	8	22
SNP exact test for Hardy-Weinberg equilibrium (n=100)	1	0.083	< 0.0001	

SNP: Single nucleotide polymorphism.

Discussion

AKR1B1 gene was found to have a strong association with DM complications. The gene has two alleles; A, that is wild, and T, which is mutant. Most of the samples analysed in the current study had the A allele and a few samples had the T allele. A link between specific AKR1B1 polymorphism and susceptibility to DR may be established. The association between the studied gene polymorphism and disease will help in identifying the genetic marker for the disease incidence. The current study adopted a unique approach to track the genetic expression in retinopathy and type 2 diabetics in Pakistani population by tracking the allele frequency by genotypic and phenotypic characterisation. SNP rs752010122 is associated with lower risk of DM. The genotyping of variants did not show deviation from the principles of Hardy Weinberg's assumptions. A study in Han Chinese population regarding DR suggested that variants of ALR2 rs759853 polymorphism were associated with a higher DR

risk, whereas variants of sorbitol dehydrogenase (SDH) rs2055858 polymorphism were associated with a lower risk.[18] A study suggested there was a protective role of CC/CC genotype among Indonesian patients.[19] A study in Egypt found that C106T rs759853 was not associated with DR.[20] A case-control study in Iran found that aldose reductase n gene polymorphism was associated with type 2 diabetic microangiopathy.[21] This shows that the association of various SNPs with DR is variable, and this variation follows a different pattern in different populations. The current study found rs752010122 to have a protective role rather than an exacerbating role in DR development.

Conclusion

Polymorphism rs752010122 of aldose reductase gene was found to be associated with lower risk of developing retinopathy.

Acknowledgment: We are grateful to the AFIO staff members who provided support.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Sami W, Ansari T, Butt NS, Ab Hamid MRB. Assessment of a dietary consultation model for effective diabetes care in Saudi population using partial least squares estimation. Pak J Stat Oper Res. 2018; 14:435-50. doi.org/10.18187/pjsor.v14i2.2249
2. International Diabetes Federation. Idf.org. [Online] [Cited 2021 December 2]. Available from: URL: https://www.idf.org. NOT FOUND
3. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019; 11:45-63.
4. Jialal I, Singh R. Oral pharmacotherapy as alternative treatment for type 2 diabetes mellitus in a 61 year old ethnic Filipino man with insulin allergies. Lab Med. 2019; 50:93-5. doi: 10.1093/labmed/lmy049.
5. Anne R, Sonia P. Role of inflammation in diabetic retinopathy. Int J Mol Sci. 2018; 19:942-73. doi: 10.3390/ijms19040942
6. Bora A, Balasubramanian S, Babenko B, Virmani S, Venugopalan S, Mitani A, et al. Predicting the risk of developing diabetic retinopathy using deep learning. Lancet Digit Health. 2021; 3:e10–9. doi: 10.1016/S2589-7500(20)30250-8.
7. Mumtaz SN, Fahim MF, Arslan M, Shaikh SA, Kazi U, Memon MS. Prevalence of diabetic retinopathy in Pakistan: A systematic review. Pak J Med Sci Q. 2018; 34:493-500. doi: 10.12669/pjms.342.13819.
8. Kim J, Park IW, Kwon S. Factors predicting final visual outcome in quiescent proliferative diabetic retinopathy. Sci Rep. 2020; 10:17233. doi: 10.1038/s41598-020-74184-9.
9. Kaur N, Vanita V. Association of Aldose reductase gene (AKR1B1) polymorphism with diabetic retinopathy. Diabetes Res Clin Pract. 2016; 121:41-8. doi: 10.1016/j.diabres.2016.08.019.
10. Priščáková P, Minárik G, Repiská V. Candidate gene studies of diabetic retinopathy in human. Mol Biol Rep. 2016; 43:1327-45. doi: 10.1007/s11033-016-4075-y.
11. Cilenšek I, Lapuh V, Petrovič MG, Petrovič D. HDAC9 rs11984041 polymorphism is associated with diabetic retinopathy in Slovenian patients with type 2 diabetes mellitus. Gene. 2021; 796-797:145802. doi: 10.1016/j.gene.2021.145802.
12. Buraczynska M, Gwiazda-Tyndel K, Drop B, Zaluska W. Renalase gene Glu37Asp polymorphism affects susceptibility to diabetic retinopathy in type 2 diabetes mellitus. Acta Diabetol. 2021; 58:1595–602. doi: 10.1007/s00592-021-01740-8.
13. Abu-Hassan DW, Al-Bdour MD, Saleh I, Freihat M, El-Khateeb M. The relationship between Aldose reductase gene C106T polymorphism and the severity of retinopathy in Type 2 diabetic patients: A case-control study. J Res Med Sci. 2021; 26:2. doi: 10.4103/jrms.JRMS_250_20.
14. WHO Sample size calculator Calculator.net. [Online] [Cited 2021 May 20]. Available from: URL: https://www.calculator.net/sample-size-calculator.html?type=1&cl=95&ci=10&pp=30&ps=&x=44&y=21
15. Online Mendelian Inheritance in Man. Apolipoprotein A-1. [Online] [Cited 2021 January 20]. Available from: URL: https://omim.org/entry/107680.
16. Green MR, Sambrook J. Purification of labeled oligonucleotides by precipitation with ethanol. Cold Spring Harb Protoc. 2022; 2022:1. doi: 10.1101/pdb.prot100701.
17. Snpstats. [Online] [Cited 2021 December 2]. Available from: URL: https://www.snpstats.net/start.htm.
18. Li W, Chen S, Mei Z, Zhao F, Xiang Y. Polymorphisms in Sorbitol-aldose reductase (polyol) pathway genes and their influence on risk of diabetic retinopathy among Han Chinese. Med Sci Monit. 2019; 25:7073-8. doi: 10.12659/MSM.917011.
19. Wihandani DM, Suastika K, Agus Bagiada IN, Malik SG. Polymorphisms of Aldose reductase (ALR2) regulatory gene are risk factors for Diabetic Retinopathy in type-2 diabetes mellitus patients in Bali, Indonesia. Open Ophthalmol J. 2018; 12:281-8. doi: 10.2174/1874364101812010281.
20. Shawki HA, Elzehery R, Abo-hashem EM, Shahin M, Youssef MM. Gene polymorphism of C106T "rs759853" is not associated with diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. Gene Rep. 2020; 21:100865. doi.org/10.1016/j.genrep.2020.100865
21. Hashemi-Soteh MB, Ahmadzadeh Amiri A, Sheikh Rezaee MR, Ahmadzadeh Amiri AA, Olfat S, Amiri AA. Aldose reductase (AC)n gene polymorphism in Iranian patients with type 2 diabetic microangiopathy; a case-control study. Diabetol Int. 2021; 12:101-7. doi: 10.1007/s13340-020-00446-6.