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Original Article

Assessing the Impact of Aldose Reductase (*ALR2*) Regulatory Gene Polymorphism in Diabetic Retinopathy Patients

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Abstract

Diabetic retinopathy, a serious complication of diabetes, may be influenced by genetic factors, including the Aldose Reductase *(ALR_2) gene* polymorphism. There is a crucial need to understand how the polymorphism in the *ALR2 gene* contributes to diabetic retinopathy. This study evaluates the *ALR2 gene* polymorphism's impact on diabetic retinopathy and its association with inflammatory markers and antioxidant status among diabetic patients residing in Kano State, Nigeria. In this study, 40 participants were enrolled; the participants were divided into 4 groups, with three test groups (diabetes mellitus, cataract, and diabetic retinopathy groups) and the control group. The investigation encompassed and incorporated the prevalence of the SNP C(-106)T polymorphism within the ALR2 promoter region, in combination with the assessment of inflammatory markers (C-reactive protein [CRP] and high-sensitivity C-reactive protein [hsCRP]), malondialdehyde (MDA) levels, vitamin A and aldose reductase concentrations. The findings revealed a 6.7% prevalence of the SNP C(-106)T polymorphism. Fasting blood glucose and HbA1c levels were significantly ($p<0.05$) lower in the control group compared to the diabetic, cataract, and diabetic retinopathy groups. The result also revealed elevated CRP, hsCRP, and MDA levels in the study groups compared to the control group. However, weak negative correlation values were found between vitamin A levels and ALR_2 concentrations, indicating a complex relationship that warrants further investigation. Molecular analysis unveiled single nucleotide polymorphisms (SNPs) at nucleotide position-106 within two samples (DR6 and D3). This followed the successful amplification of the *AR2 gene* using Polymerase Chain Reaction (PCR). Subsequently, DNA sequencing was performed using the ABI Prism BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit on the ABI PrismTM 3730/3730XL DNA Sequencer. In addition, there was no significant ($p > 0.05$) observed correlation between ALR_2 and MDA (r= 0.026) or CRP (r= -0.077). The study identified a 6.7% prevalence of *ALR2* gene polymorphism in diabetic retinopathy patients, alongside elevated inflammatory markers and MDA levels. Moreover, in the present finding, all control group had the C (CC genotype) allele, while the study group had 90% C allele and 10% T allele. The T allele showed no significant association with DR 1.00 (OR 95% CI: 0.127-7.893; p:1.00). However, Additional research with larger sample sizes is warranted to comprehensively investigate the implications of the SNP C(-106)T polymorphism at the ALR2 promoter region.

Keywords: Diabetic Retinopathy, *ALR2* Gene Polymorphism, Inflammatory Markers, Antioxidant Status.

INTRODUCTION

Diabetes mellitus (DM) poses a significant global public health challenge, with an estimated 463 million adults currently affected worldwide [1]. In Africa, approximately 19.4 million adults aged 20–79 live with DM [2]. The disease carries the risk of severe multiorgan complications, including diabetic retinopathy (DR), which can lead to both life and sight-threatening consequences [3,4].

Globally, DR is responsible for moderate-to-severe visual impairment in 3.28 million individuals and blindness in 1.07 million people. It is a primary cause of blindness and visual impairment among working-age adults with DM [5]. DR encompasses nonproliferative (NPDR) and proliferative (PDR) forms, further categorized into mild, moderate, or severe stages. Diabetic macular edema (DMO), characterized by retinal thickening in the macula, can develop at any stage and pose a significant risk to vision [5]. PDR and DMO are particularly concerning due to their potential to cause visual impairment and blindness. However, evidence from clinical trials underscores the importance of timely treatment in preserving vision, highlighting the preventable nature of blindness resulting from PDR and DMO [6].

Aldose reductase (ALR2), encoded by the AKR1B1 gene, plays a crucial role in the polyol pathway, converting glucose to sorbitol. This pathway becomes significant under hyperglycemic conditions, such as in diabetes mellitus. Numerous studies have investigated the association between AKR1B1 gene polymorphisms and the risk of diabetic retinopathy (DR) in diabetic patients [7,8]. Recent studies have shown correlations between specific AKR1B1 gene polymorphisms and the development of DR in diabetic patients. For instance, the SNP -106C > T polymorphism, located in the promoter region of the AKR1B1 gene, has been linked to increased susceptibility to DR due to its influence on gene expression modulation [8,9]. The frequency of ALR2 genotypes and their association with DR risk factors have been extensively explored [3,10,11]. Certain alleles and genotypes of AKR1B1 are significantly associated with an elevated risk of DR development among diabetic patients, while others appear to confer protective effects [12,13].

Aldose reductase contributes to the pathogenesis of DR through multiple mechanisms. Increased sorbitol levels in the retina due to ALR2 activity lead to osmotic stress, oxidative stress, and alterations in intracellular signaling pathways, ultimately resulting in retinal microvascular damage [3,14]. Furthermore, the polyol pathway consumes NADPH, depleting the cell's antioxidant defenses and exacerbating oxidative stress [15]. ALR2 inhibition has been proposed as a therapeutic strategy to mitigate the progression of DR by reducing sorbitol accumulation and oxidative stress in the retina [16].

The AKR1B1 gene spans approximately 32 kilobases and is located on chromosome 7q35. It comprises 10 exons and encodes the aldose reductase enzyme, which consists of 315 amino acids [7,17](Petrovič et al., 2005). The promoter region of the AKR1B1 gene contains various regulatory elements that modulate gene expression, including the SNP -106C > T polymorphism, which has been implicated in DR susceptibility [11,18].

The SNP -106C > T polymorphism in the promoter region of the AKR1B1 gene has garnered considerable attention due to its association with DR development. Studies have demonstrated that this genetic variation affects AKR1B1 gene expression by altering transcription factor binding sites or promoter activity, thereby influencing aldose reductase levels and polyol pathway activity in the retina [11,18].

The complex pathogenesis of diabetes complications involves metabolic and hemodynamic disturbances, encompassing hyperglycemia, insulin resistance, dyslipidemia, hypertension, and immune dysfunction. These disturbances give rise to detrimental processes, including increased production of reactive oxygen species (ROS), inflammation, and ischemia, primarily affecting endothelial and nerve cells in densely vascularized and innervated areas like the eyes, kidneys, and nerves [19]. Clinical studies have shown that the risk of developing DR is closely correlated with blood glucose levels and diabetes

duration [20]. However, other studies suggest that factors like hemoglobin A1c (HbA1c) and disease duration account for only 11% of the retinopathy risk, and individuals with well-controlled blood sugar levels may or may not develop DR [21,22].

Understanding the underlying pathways involved in DR development is crucial, with processes such as the polyol pathway, non-enzymatic glycation, endothelial dysfunction, vascular tone maintenance, extracellular matrix remodeling, and angiogenesis being deregulated in diabetes and culminating in proliferative diabetic retinopathy [21,22]. The candidate gene approach, which has recognized and proposed multiple candidate genes related to DR pathogenesis, such as *AKR1B1*(or *ALR2*), *GRB2*, *NOX4*, and *NVL*, as well as genes like *NME3*, *LOC728699*, and *FASTK* that may have protective effects against DR, has been employed to identify DR-associated genes [18,23]. The role of inflammation in DR development has also been suggested, but the precise contribution of these factors to the pathogenesis of DR remains largely unknown. Aldose reductase is a key process in the polyol pathway that transforms harmful substances into safe alcohol. The intracellular antioxidant reduced glutathione (GSH) is maintained by NADPH, which is depleted during hyperglycemia when this enzyme also transforms extra glucose into sorbitol. Reduced GSH levels cause oxidative stress, which can cause cell death or damage [10,22].

The escalating prevalence of diabetes in Africa, with Nigeria having the highest number of diagnosed patients, emphasizes the need for extensive research to identify genes associated with DR. The use of molecular techniques has facilitated the exploration of the genetic basis of various diseases, including DR, holding promise for enhanced diagnosis and prevention [9,19,24]. While several candidate genes have been analyzed for their link with DR, few attempts have been made to investigate these genes in Nigeria, highlighting the significance of this research. Diabetic retinopathy (DR) is a severe microvascular complication that, if left unchecked, can lead to vision loss. It is one of the leading causes of blindness worldwide, particularly in adults [9,22].

The advent of molecular techniques has allowed researchers to explore the genetic basis of various diseases, including DR. Identifying these genes will play a crucial role in the diagnosis and prevention of many diseases [9,24]. The candidate gene approach has been instrumental in analyzing many genes for their link with DR [9,25]. However, very few attempts have been made to investigate these genes in Nigeria, underscoring the importance and novelty of this research. Therefore, this present study aimed to elucidate and evaluate the role of single nucleotide polymorphism (SNP) of the *AR_2 gene* in diabetic retinopathy in Kano, Nigeria.

MATERIALS and METHOD

Study Area and Participants

The study area for this research study was Makkah Specialist Eye Hospital in Kano State, Nigeria, situated at Dorayi Emir's Line Road, Jan Bulo, Kofar Dukawuya, Gwale Local Government Area. The hospital's coordinates are 11.9756765, 8.4673154 . The sample size was determined using the formula yielding 40 participants due to scarcity [23]. Ethical clearance was obtained from the Kano State Ministry of Health and Bayero University Health Ethics and Research Committee (BUHeREC) with approval number NHREC/17/03/2018, and the study complied and adhered to the principles for medical research involving human subjects outlined in the Declaration of Helsinki 1964 tenets.

A total of 40 participants were enrolled, categorized into four groups: The diabetes mellitus group, the diabetic cataract group, the diabetic retinopathy group, and a control group, each consisting of 10 participants March to May 2022. The selection criteria included diabetic patients of both sexes who were at least 18 years old and had cataracts or any other eye condition and participants who agreed to the informed consent. Patients with inflammatory conditions, individuals with additional endocrine issues, and individuals older than 18 or 65 were excluded. Adults who did not have diabetes and had no eye conditions made up the control group.

Socio-Demographic Studies

Socio-demographic data of study participants was obtained through the use of questionnaires, where information on Age, Gender, Tribe, Level of

education, Settlement, Occupation, and Marital status was obtained, participant Body weight and height were measured for all subjects using (ZT-160), Harris Medical England, and blood pressure levels were recorded using a sphygmomanometer.

Sample Collection

Each participant in the study underwent blood collection using a sterile vacutainer blood specimen bottle, holder, and needle. A total of five milliliters (5.0 mL) of venous blood was drawn from each subject. Among the collected blood, two milliliters (2.0 mL) were carefully transferred into a sterile plain vacutainer blood specimen bottle, where the specimen was then allowed to clot naturally at room temperature. Subsequently, it was centrifuged at 3000 rpm/min for 5 minutes to obtain a clear hemolyzed serum. The harvested sera were stored in sterile containers at -20◦C until required for malondialdehyde (MDA), C-reactive protein (CRP), hs-CRP, aldose reductase, and vitamin A analysis, while the remaining 3 milliliters (3 mL) of whole blood in the EDTA container were used for HbA1c, vitamin A, and DNA analysis.

Serum Biochemical Analysis

Blood samples were collected from each participant for various analyses, including estimation of fasting plasma glucose, HbA1c, malondialdehyde (MDA), C-reactive protein (CRP), aldose reductase, and vitamin A levels. The FBS was determined through the Oxidase-Peroxidase method using Accu Chek glucometer [25]. The determination of glycosylated hemoglobin (HbA1c) was based on the fluorescence immunoassay technique as described by [26], while the estimation of MDA was performed using the chemical method described by [27]. CRP, hs-CRP and aldose reductase were detected using the immunoenzymometric assay technique as described by [28] and vitamin A levels were determined according to the method.

MOLECULAR TECHNIQUE ANALYSIS

Genomic DNA Extraction from the Blood

The analysis of promoter single nucleotide polymorphism (SNPs) in the *ALR2 gene* were determined and involved various steps. Initially, DNA extraction from the human blood of each participant (a diabetic patient and a healthy person)was performed using the QIAMP Blood mini kit following the manufacturer's instructions [29,30] . The quality, integrity and quantity of each DNA extracted sample were determined by NanodropTM 1000 spectrophotometer (Thermo Scientific, USA).

Genotyping of *ALR2* Gene Polymorphism

Genotyping of C(-106)T polymorphism of the *ALR2* gene was performed using the primer set (the forward primer r 5'-TTC GCT TTC CCA CCA GAT-3' and reverse primer 5'CGC CGT TGT TGA GCA CGA GAC-3') to amplify the 263-base pair DNA fragment [31].

Polymerase Chain Reaction (PCR) and Gel Electrophoresis

Polymerase Chain Reaction (PCR) technique (a Thermocycler PCR machine) was used to amplify the targeted DNA segment of a promoter region of the *ALR2 gene* polymorphism using the designed primers. A PCR master mix was prepared in a 25µL reaction mixture, including 0.51 µL each of forward primer and reverse primer of both the *AR2 gene*. 2.0 µL of genomic DNA was added to the mix, and the PCR reaction was set up in labeled tubes. The PCR cycling conditions for the amplification of the *AR2 gene* consisted of the following steps: an initial denaturation of DNA at 96°C for 2 minutes, followed by 33 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 68°C for 2 minutes. After the 33 cycles, there was a final extension step at 68°C for 7 minutes [31]. Finally, the reaction was cooled to 4°C in a single cycle. After completing the PCR process, the ultimate PCR product obtained was examined using electrophoresis on a 1.5% agarose gel using a DNA molecular weight marker (Biolin DNA Ladder) to determine the unknown size of the DNA amplicon. The PCR product was then examined to determine whether the band was present or absent.

Sequencing and Post-Sequence Analysis

Following PCR amplification, all products underwent purification and sequencing. Polymorphisms were identified by analyzing the

electropherograms, further validated through BLAST alignment with the reference sequence in the Gene Bank (sequence ID: gb|U72619.1 |HSU72619), as outlined by Ko et al [31].

Data Analysis

The result of the research was presented using tables and charts. The data obtained was analyzed using Microsoft Office Excel 2016 and SPSS Statistical Software Version 23. The results were expressed as mean ± SD. Group comparisons was carried out using One Way Analysis of Variance (ANOVA), F Test, Chi-square test of association and Spearman correlation coefficient. Typically, a p-value equal to or less than 0.05 ($p \leq 0.05$) was considered statistically significant.

RESULTS

Socio-Demographic Characteristics of the Study Respondents

The present study was conducted to evaluate *ALR2 gene* polymorphism prevalence and its association with inflammatory markers and antioxidant status in diabetes, cataract, and diabetic retinopathy patients

Table 1 shows the socio-demographic characteristics of the study respondents. A total of 40 patients were screened; of these patients, 55% (n = 22) were found to be males, while 45% (n = 18) were found to be females.

Table 1: Demographic Characteristics of the Study Population

Variable	Group	Frequency	Percentage (%)
Sex	Male	22	55.0
	Female	18	45.0
Marital Status	Single	11	27.5
	Married	19	47.5
	Widowed	2	5.0
	Divorced	8	20.0
Tribe	Hausa	29	72.5
	Igbo	2	5.0
	Yoruba	2	5.0
	Others	7	17.5
Occupation	Farming	2	5.0
	Business	18	45.0
	Civil service	11	27.5
	Unemployed	9	22.5

n=40

The present result on the marital status of the respondents indicates that 11 (27.5%) of the respondents are still single; 19 (47.5%) are married, 2 (5%) are widowed and 8 (20%) are divorced. The present study showed that respondents of Hausa origin had the highest number of patients (72.5%) while Igbos and Yorubas had 2 (5%) respondents each. The result also showed that respondents of other tribes had percentage distribution of 7 (17.5%). The result indicates that 11 (27.5%) of the respondents are civil servants, 2 (5%) respondents are farmers, 18 (45%) respondents are businesspersons, and 9 (22.5%) respondents are unemployed.

Retrospective Clinical and Lifestyle Characteristics of the Study Population

The results revealed the Clinical and Lifestyle Characteristics of the Study Population (Table 2). From the results, 57.5% of respondents had co-morbidity while 42.5% had no co-morbidity. The results revealed that 50% (n = 20) of the study respondents had 0 years of experience with diabetes. One respondent had less than 5years of experience battling with Diabetes while 13 (32.5%) respondents have been battling the disease for 5 years.

Table 2: Clinical and Lifestyle Characteristics of the Study Population

Variable	Response	Frequency	Percentage (%)
Co-morbidity	Yes	23	57.5
	No	17	42.5
History (years)	0	20	50.0
	<5	1	2.5
	5	13	32.5
	10	3	7.5
	>10	3	7.5
Diet	Carbohydrate	20	50.0
	Proteins	8	20.0
	Modified	12	30.0
Exercise	Yes	23	57.5
	No	17	42.5
Late Dinner	Yes	11	27.0
	No	29	72.5

n = 40

In addition, the results showed that 3 (7.5%) respondents had the disease for less than 10 years

while those that had the disease for 7 years were 3(7.5%). Of the 40 respondents, 20 (50%) majorly consumes carbohydrates, 12 (30%) respondents consume modified diets while 8 (20%) consume carbohydrates. The majority of the respondents participated in exercises while 17 (42.5%) respondents do not participate in exercises. From the results, majority of the respondents 29 (72.5%) do not eat late dinner while 11 (27%) eat late dinner.

Diabetic Profile of the Study Population

The Table 3 presents and compares the diabetic profile of the study population BMI (Body Mass Index) in Kg/m2, FBG (Fasting Blood Glucose) in mmol/L, HbA1c (%) (a measure of average blood glucose levels), systolic and diastolic blood pressure in mmHg. Comparing the control group (I) with the other groups, it was observed that the Diabetic group (II) has a lower BMI (22.54±3.57 Kg/m^2) compared to the control group (23.59±2.81 Kg/m^2). However, the FBG levels (11.42±6.11 mmol/L) and HbA1c values (8.68±2.12%) in the Diabetic group are significantly ($p<0.06$) higher than those in the control group (95±1.14 mmol/L and 5.97±0.51%, respectively). The systolic and diastolic blood pressure values in the Diabetic group (129.70±17.20mmHg and 83.10±7.56mmHg) are also higher compared to the control group (122.40±10.15mmHg and 80.70±5.72mmHg). Similarly, the Diabetic retinopathy group (IV) exhibits a higher BMI (280±7.05 Kg/m^2), FBG levels (9.67±2.53 mmol/L), and HbA1c values (9.60±2.09%) compared to the control group. However, the differences in BMI, FBG, and HbA1c between the Diabetic retinopathy and the control groups are not as pronounced as in the Diabetic group. The systolic and diastolic blood pressure values in the Diabetic retinopathy group (125.40±12.47mmHg and 76.60±7.23mmHg) are also slightly different from those in the control group.

Table 3. Clinical Profile of the Study Population

Groups	BMI (Kg/m^2)	FBG (Mmol/L)	HbAc1 (%)	Systolic (mmHg)	Diastolic (mmHg)
I	23.59±2.81	95±1.14	5.97±0.51	122.40±10.15	80.70±5.72
II	22.54±3.57	11.42±6.11 [a]	8.68±2.12[a]	129.70±17.20	83.10±7.56
III	21.17±2.77	5.09±1.01	6.32±0.38	122.80±13.41	78.00±8.23
IV	280±7.05	9.67±2.53 [a]	9.60±2.09[a]	125.40±12.47	76.60±7.23

Keys: I: Control group; II: Diabetic group; III: cataract; IV: diabetic retinopathy. Data expressed as mean ± SD; n=40. Values with the same superscript along the same column are significantly ($p<0.05$) different from the control group. One way ANOVA was performed, followed by Tukey multiple comparison test.

Diabetic Profile of the Study Population by Sex

Table 4 presents the diabetic profile of a study population by sex, divided into four groups: Control, Diabetic, Cataract, and Diabetic Retinopathy. The groups were compared based on their FBG, HbA1c, and BMI values. In the control group, male participants had a higher BMI (22.67±2.40 Kg/m^2) than female participants (298±3.14 Kg/m^2). There were significant differences ($p<0.05$) in FBG and HbA1c levels between male and female participants with diabetes. Female participants with diabetes had higher FBG levels (12.31±6.91 mmol/L) than male participants (9.33±3.96 mmol/L), while male participants with diabetic retinopathy had higher HbA1c levels (9.57±2.50%) than female participants (9.64±1.65%). Additionally, the cataract group had significantly ($p<0.05$) higher FBG levels in male participants (5.60±0.52 mmol/L) compared to female participants (3.90±0.85 mmol/L). However, there were no significant sex differences in BMI values in the cataract group. When comparing the four groups, it was observed that the FBG levels and HbA1c levels in the male and female participants in the diabetic group and diabetic retinopathy group were significantly higher than the control group. The BMI of male and female participants with diabetic retinopathy was significantly lower than the control group, while the BMI of female participants in the cataract group was significantly higher than the control group.

Table 4: Diabetic Profile of the Study Population by Sex

Group	Gender	FBG (mmol/L)	HbA1c (%)	BMI (Kg/m^2)
I	Male (n=6)	5.15±1.05	5.83±0.39	22.67±2.40
	Female (n=4)	65±1.38	6.18±0.67	298±3.14
II	Male (n=3)	9.33±3.96^a	8.60±3.27	21.57±0.38
	Female (n=7)	12.31±6.91^b	8.71±1.78^b	22.96±28
III	Male (n=7)	5.60±0.52*	6.29±0.15	20.60±2.41
	Female (n=3)	3.90±0.85	6.43±0.75	22.50±3.66
IV	Male (n=6)	8.92±1.90^a	9.57±2.50^a	19.90±3.73
	Female (n=4)	10.80±3.21^b	9.64±1.65^b	25.07±03

Keys: I: Control group; II: Diabetic group; III: cataract; IV: diabetic retinopathy. Data expressed as mean ± SD. Values with the same (a) superscript along the same column under male gender are significantly ($p<0.05$) different from the control group; while Values with same (b) superscript along the same column under female gender are significantly ($p<0.05$) different from the control group all data analyzed using one way ANOVA followed by Tukey multiple comparison test. Values with superscript (*) along the same column under each group are significantly different as analyzed by independent sample t-test. n=40

Diabetic Profile of the Study Population by Physical Activity

The Table 5 demonstrate the diabetic profile of the study population by activity. In the control group, the FBG level of those who did not participate in exercise (96±1.09mmol/L) was higher than those who participated (94±1.32mmol/L). Similarly, the HbA1c level of those who did not participate in exercise (6.04±0.69%) was higher than those who participated (5.90±0.33%). In the diabetic group, the FBG level of those who did not participate in exercise (12.75±6.43 mmol/L) was higher than those who did exercise (11.09±6.44 mmol/L). The HbA1c level of patients with in diabetic group that did not participate in exercise (8.93±2.33%) was higher compared to those that participated in exercise (7.70±0.28%). Similarly, in the cataract group, the FBG level of those who did not participate in exercise (5.48±0.97mmol/L) was higher than those who participated (70±0.98mmol/L). The HbA1c level of those who did not participate in exercise (7.08±0.53%) was higher than those who did exercise (6.94±1.05%). Among patients with diabetic retinopathy, the FBG level of those who did not participate in exercise (10.40±2.37mmol/L) was higher than those who did exercise (8.94±2.72mmol/L).

Table 5: Diabetic Profile of the Study Population by Physical Activity

Group	FBG (mmol/L)		HbA1c (%)	
	No Exercise	Exercise	No Exercise	Exercise
I	96±1.09	96±1.09	6.04±0.69	5.90±0.33
II	12.75±6.43^a	11.42±2.44^a	8.93±2.33^a	8.90±2.28^a
III	78±0.97	5.48±0.98	7.08±0.53	6.31±0.15
IV	10.40±2.37^a	8.94±2.72^a	9.70±2.04^a	9.70±2.04^a

Keys: I: Control group; II: Diabetic group; III: cataract; IV: diabetic retinopathy. Data expressed as mean ± SD. Values with same superscript along the same column are significantly ($p<0.05$) different from the control group. by one way ANOVA followed by Tukey multiple comparison test. While value with * along the same row are significantly different as analysed by independent sample t-test. n=40

Aldose Reductase Concentration

The Figure 1 displays the AR level in the study population. Finding reveals, no statistical difference in the concentration of AR among the groups ($p>0.05$): control (1538.23±179.93 pg/ml); diabetes (458.13±81.11 pg/ml); cataract (521.06±78.97 pg/ml) and diabetic retinopathy (722.61±92.43 pg/ml). The results show a significant (p = 0.050) difference between the control and the cataract group.

Oxidative Stress Markers of the Study Population

Table 6 presents data on oxidative stress markers, including CRP (C-reactive protein), hsCRP (high-sensitivity C-reactive protein), and MDA (malondialdehyde), in different groups of study participants. In the Control group, the mean levels of CRP, hsCRP, and MDA were 5.8±1.12 mg/L, 1.2±0.46 mg/L, and 2±2.4 µmol/L, respectively. Comparatively, the Diabetic group showed significantly($p<0.05$)) higher levels of CRP (22.1±3.57 mg/L), hsCRP (2±1.62 mg/L), and MDA (6.6±1.32 µmol/L). These elevated levels suggest increased oxidative stress in individuals with diabetes compared to the control group. The Cataract group demonstrated slightly higher levels of CRP (6.6±1.3 mg/L), hsCRP (4±1.36 mg/L), and MDA (5.6±2.15 µmol/L) compared to the control group. Although these elevations were not as significant as in the Diabetic group, they still indicate a possible association between cataract and oxidative stress. Similarly, the Diabetic retinopathy group exhibited slightly elevated levels of CRP (6.6±1.9 mg/L), hsCRP (5.0±0.18 mg/L), and MDA (6.2±0.7 µmol/L) compared to the control group.

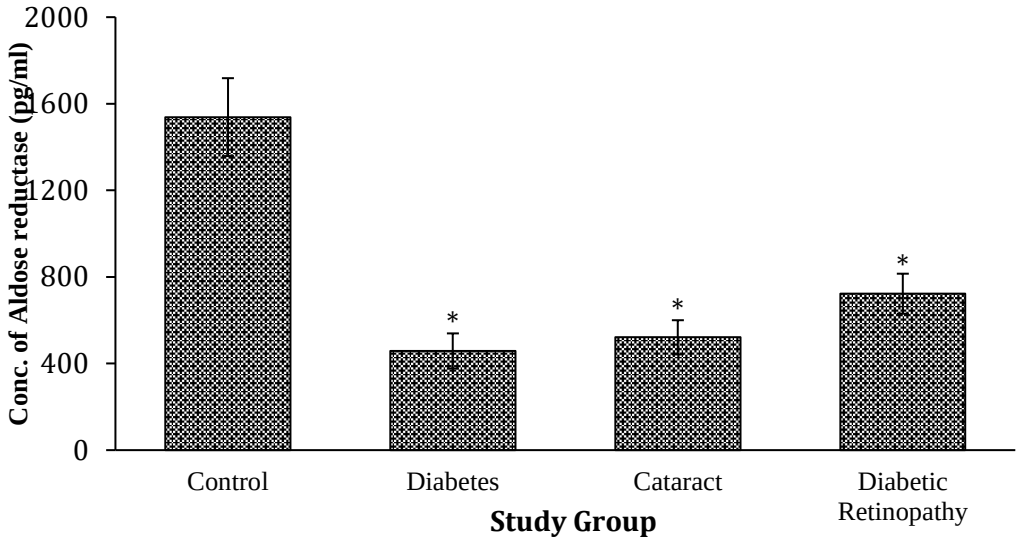

Figure 1. AR level of the Studied Population.

*Denotes statistically significant difference for AR of control patients compared with diabetic, cataract and diabetic retinopathy groups.

Table 6: Oxidative Stress Markers of the Study Participants

Groups	CRP(mg/L)	hsCRP(mg/L)	MDA(µmol/L)
I	5.8±1.12	1.2±0.46	2±2.4
II	22.1±3.57[a]	2±1.62[a]	6.6±1.32[a]
III	6.6±1.3	4±1.36[a]	5.6±2.15
IV	6.6±1.9	5.0±0.18[a]	6.2±0.7[a]

Keys: I: Control group; II: Diabetic group; III: cataract; IV: diabetic retinopathy; CRP: C-reactive protein; hsCRP: High-sensitivity C-reactive protein; MDA: Malondialdehyde. Data expressed as mean ± SD. Values with same superscript along the same column are significantly ($p<0.05$) different from the control group. One way ANOVA was performed, followed by Tukey multiple comparison test.

Vitamin A levels in the Study Populations

The Figure 2 shows the vitamin A levels in the study populations. The results revealed that control samples had the highest vitamin A level of 20.72±0.51 µM while patients with cataract had the lowest vitamin A level of 13.39±0.22 µM. The vitamin A levels of the various study populations are in the order control group (20.72±0.51 µM) > diabetic group (17.83±0.42µM) > cataract group (12.99±0.29µM) > diabetic retinopathy group (13.39±0.22µM). The results show a significant (p = 0.001) difference between the control, the cataract group and the diabetic retinopathy group.

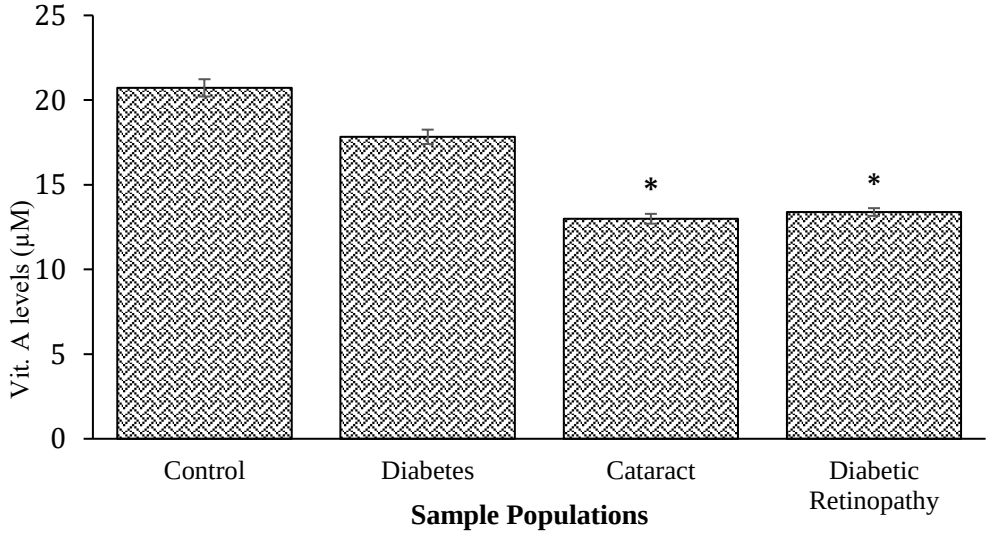

Figure 2. Vitamin A Levels in the Study Populations.

*Denotes statistically significant difference for Vit A of control patients compared with diabetic, cataract and diabetic retinopathy groups

Association Between Aldose Reductase Concentration and Oxidative Stress Level

The Table 7 shows the correlation (r) between ALR concentrations with various variables related to oxidative stress and vitamin A. The variables examined include malondialdehyde (MDA), vitamin A, and C-reactive protein (CRP). The coefficient (r) represents the strength and direction

of the correlation, while the p-value indicates the statistical significance of the correlation. In this table, none of the correlations are statistically significant ($p>0.05$). The correlation coefficient between ALR concentration and MDA is 0.285, suggesting a weak positive relationship but is not statistically significant. Similarly, the correlation coefficients between ALR concentration and vitamin A (r=-0.122) and ALR concentration and CRP (r= -0.103) indicate weak negative associations, but they are not statistically significant. Overall, the table indicates no significant correlation exists between ALR concentration and the examined variables related to oxidative stress and vitamin A in the study population.

Table 7: Correlation Coefficient (r) of Aldose Reductase (ALR) Concentration with Oxidative Stress Level and Vitamin A

Variable	Coefficient (r)	P-value	Remark
MDA	0.285	0.075	NS
Vitamin A	-0.122	0.454	NS
CRP	-0.103	0.527	NS

Key: Values are presented as coefficients of correlation (r).; NS=insignificant. * Correlation is significant at the 0.05 level

Molecular Analysis

The Figure 1 represents the gel chromatogram of the *ALR2 gene* with 263 bps. From the results, 2 control samples (lanes C7 and C8) were successfully amplified. From the first (upper) gel of Figure 2, only 3 (lanes D3, D4 and D9) of the DNA fragments for diabetics were successfully amplified. In the lower gel of Figure 2, four (lanes DR1, DR2, DR6 and DR7) of the DNA fragments for diabetic's retinopathy was successfully amplified. The result in Figure 3 exhibited that two DNA fragments (lanes TC4 and TC8) for cataract were successfully amplified. These fragments all had 263 bps.

The Figure 3 shows the sequence alignment of *ALRs gene* of the controls and study groups. From the results, DR1, DR2, DR6 and DR7 are representative of diabetic retinopathy; D4, D9, and D3 are selected representatives of the diabetic group while TC4 and TC8 are representatives of cataract samples. C1 and C2 are representatives of control group samples. ALR2 Reference Sequence is also shown as a standard to determine the polymorphism. The position of the suspected polymorphism is 106 when Cytosine (C) has been replaced by Thymine (T). At the position, there are two SNP mutations from all the candidate genes (D3 and DR6) when compared to the ALR2 Reference sequence (sequence ID: gb|U72619.1).

Single nucleotide polymorphisms (SNPs) in nucleotides 106 (sample DR6) and (sample D3) were detected in 2 samples and 1 sample for each SNP mutation (Table 8).

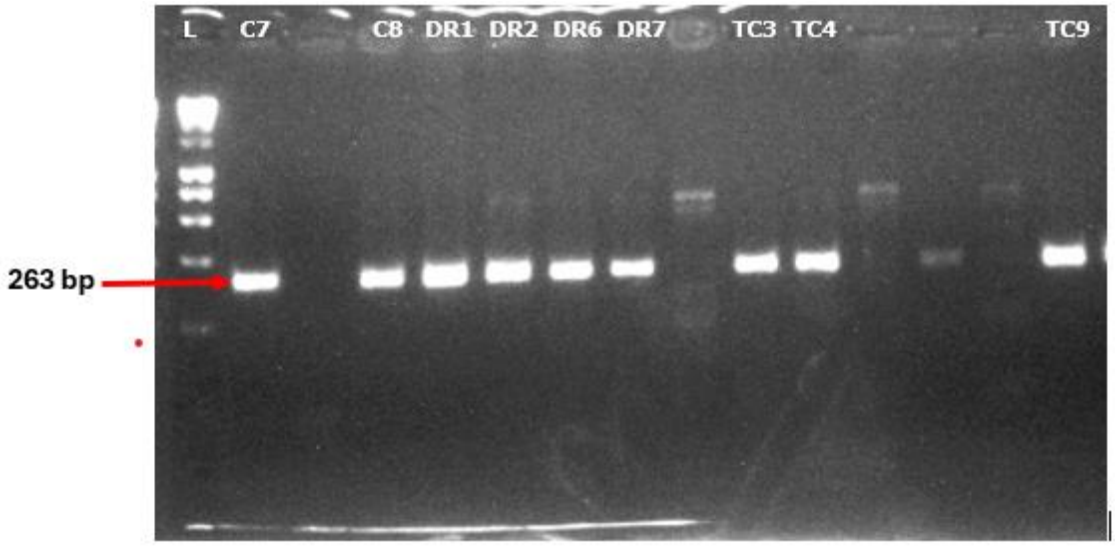

Figure 3. Agarose gel electrophoresis (1.5 %) illustrates 263 bp PCR products corresponding to amplifying the *ALR₂* gene in a normal healthy person, diabetic retinopathy (DR) and Cataract patients.

Lane L: 100 bp DNA ladder. Lane C7, C8, Lane DR1, DR2, DR6 and DR7; and Lane TC3, TC4 and TC9 demonstrate positive PCR DNA amplicon size (263 bp). Patient C1, C2, C3, C4, C5, C6, C9 and C10 correspond to healthy individual and represent negative PCR results (no amplification). Patients DR3, DR4, DR5, DR8, DR9 and DR10 correspond to diabetic retinopathy (DR) and indicate negative PCR results (no amplification). Patient TC1, TC2, TC5, TC6, TC7, TC8 and TC10 correspond to Cataract) and represent the negative PCR results (no amplification).

Genotype and allele frequency

The table presents data on the distribution of ALR2 (C/T) gene polymorphism genotypes and alleles in control individuals and those with Diabetic Retinopathy (DR). In the control group, all individuals exhibited the CC genotype, while in the DR group, 90% had the CC genotype and 10% had the TT genotype. Allele frequencies indicated that 100% of controls carried the C allele, whereas 90% of individuals with DR carried the C allele and 10% carried the T allele. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for the TT genotype and the T allele, with the TT genotype showing an OR of 1.00 (95% CI: 0.054-18.574) and the T allele showing an OR of 1.00 (95% CI: 0.127-7.893). Chi-square tests showed no statistically significant associations for either the TT genotype ($\chi^2 = 0.00$, $p = 1.00$) or the T allele ($\chi^2 = 0.000$, $p = 1.00$) with Diabetic Retinopathy.

Table 4.8. Genotype and Allele Distribution of the AKR1B1 (>106 (C/T) Gene Polymorphism in Patients with Diabetic Retinopathy and Controls

Genotypes	DC (%) (n =10)	DR (%) (n = 10)	OR	95% CI	Chi-square	p-value
CC	9 (100.00)	9 (90.00)	-	-	-	-Reference
CT	0 (0.00)	0 (0.00)	-	-	-	-
TT	1 (90.00)	1 (10.00)	1.00	0.054-18.574	0.00	1.00
Alleles						
C	18 (100.00)	18 (90.00)	-	-	-	-
T	2 (0.00)	2 (10.00)	1.00	0.127-7.893	0.000	1.00

DC: Diabetic Control, OR: Odds ratio, CI: Confidence intervals, p-values: *p<0.05and **p<0.01 refer as significant. DC: Diabetic Control; DR: Diabetic Retinopathy

DISCUSSIONS

The findings depict demographic features of the participants. The control and case groups were matched based on age, sex, and socioeconomic status. Kano, the commercial center of northern Nigeria and primarily populated by Hausa/Fulani people who follow Islam [32], explains why 72.5% of the participants were from civil service and business backgrounds. The study found that 72.5%) of the participants were Hausas, while the remaining 27.5% belonged to other tribes. In terms of gender distribution, 55% were males, and 45% were females. Regarding marital status, majority of the respondents (47.5%) were married. The current finding aligns with a similar study conducted in Jigawa by Yakubu *et al.* [33].

The study population's retrospective clinical and lifestyle characteristics were presented, indicating that 57.5% of the respondents had co-morbidities associated with diabetes such as retinopathy, ulcers, and high blood pressure, while the rest did not have any. This suggests that the disease has been present for a long time, consistent with Vasudevan *et al.* [34] and IDF [33] findings on diabetes complications. The finding further revealed that most respondents (50%) consumed starchy staple food for their meals, while only 20% primarily ingested proteinous food. The remaining 30% of participants consumed modified diabetic food as recommended by a dietician. The population's lifestyle also supports diabetes management and care, with 57.5% regularly exercising and 72.5% avoiding late dinners. According to Vasudevan *et al.* [34], this lifestyle can help with proper glucose metabolism.

The results of this study also demonstrated the clinical attributes of the individuals participating in the research. The parameters include FBG, HbA1c, BMI, and blood pressure. The data shows a significant difference in association between HbA1c and blood pressure in all the study groups ($p<0.05$). This account for genetic variation of HbA1c value in different ethnicity to be high, as well as bearing in mind that elevated HbA1c levels have been associated with an increased risk of developing cardiovascular diseases, including hypertension and renal complications [35]. moreover,

the FBG levels in the study groups were significantly higher than the control group ($p<0.05$). In agreement to the present finding, Inzucchi *et al.* [36] and American Diabetes Association [37], posited that in normal healthy individuals, the pancreas releases insulin in response to the body's glucose needs, which helps facilitate the uptake and utilization of glucose by cells, thereby maintaining blood glucose levels within a healthy range [36]. However, in diabetic patients, there is an impairment in either insulin production, insulin sensitivity, or both, which leads to the inability of cells to take up and utilize glucose effectively. As a result, glucose remains in the bloodstream at higher concentrations, causing elevated FBG levels as observed in this study.

The present findings reveal varying BMI among the study population. The variation in BMI among the study population could be attributed to multiple factors such as genetics, cultural norms, socioeconomic status, and access to healthy diet options. Dietary habits, physical activity levels, and sedentary behavior contribute to BMI variation [38,39]. Studies that revealed that high BMI in diabetic patients exacerbates insulin resistance, poor glycemic control, and increases the risk of diabetic complications such as diabetes retinopathy and cataract [40,41]. These suggest that body adiposity is related to an increase risk of cancer, metabolic syndromes, cardiovascular diseases, stroke and diabetes. Hence, maintaining a healthy body weight, adopting a balanced diet, engaging in regular physical activity, and seeking appropriate medical care are crucial for mitigating the risks associated with high body adiposity[42,43]. The systolic blood pressures of the study population were higher ($p>0.05$) than the control group. This suggests that individuals with diabetes are at a higher risk of developing high blood pressure, and having high blood pressure can increase the risk of developing diabetes-related complications. Hypertension is a significant risk factor for diabetic retinopathy, a common complication of diabetes that can lead to vision loss [44,45]. Additionally, diabetes increases the risk of developing cataracts, a clouding of the eye's lens that can cause vision impairment [45]. Surprisingly, the diastolic blood pressure was insignificant among the study groups. This could be due to the antihypertensive agent (Furosemide) taken by some of the participants. This result contradicts Kumar *et al.* [45].

Findings on the diabetic profile of the population segregated by gender revealed that there is no correlation between physiological biochemistry and the gender of the individual. Contrary to the present finding, previous studies identified an association between physiological biochemistry and the gender of the individual [46,47]. According to Guyton and Hall, [48] and Sembulingam and Sembulingam [49], this association could account for the persistent utilization of glucose for the metabolic processes in the male gender, unlike the case of females that store excess as adipose tissue. No significant difference ($p> 0.05$) was observed in the BMI distribution across genders. The females had higher BMI across the various groups than males. This could result from higher body adiposity in females than in their male counterparts [50].

The results regarding the diabetes profile of the population, with respect to physical activity, indicate that individuals engaged in exercise exhibited lower levels of FBG and HbA1c compared to those who were inactive. Physical activities play a vital role in glucose homeostasis [51]. According to Karen *et al*., [51], exercise enhances skeletal muscle glucose uptake using insulin-dependent and insulin-independent mechanisms, and regular exercise results in sustained improvements in insulin sensitivity and glucose disposal. The control group was observed also to have lower FBG and HbA1c than the diabetic, cataract, and diabetic retinopathy groups. Similarly, Stocks and Zierath [52], stated that exercise is an effective intervention to reduce glycemia and improve insulin sensitivity.

The findings on the oxidative stress markers (CRP, hs-CRP, and MDA) were analyzed, and found that the C-reactive protein (CRP), high sensitivity C-reactive protein (*hs-CRP*), and malondialdehyde (MDA) levels of the diabetic, cataract and diabetic retinopathy groups were significantly ($p < 0.05$) higher than the levels observed in the control group. However, no

significant difference ($p > 0.05$) was observed in the malondialdehyde (MDA) concentration between the diabetic and cataract groups. In agreement with the present study, Kushner *et al*. [53] and Song *et al*. [54] observed that the concentrations of CRP or HsCRP were higher in the case groups than in the control. Elevated levels of C-reactive protein (CRP), high sensitivity C-reactive protein (hs-CRP), and malondialdehyde (MDA) are significant in the context of diabetes, cataract, and diabetic retinopathy [55,56]. CRP is an acute-phase reactant that indicates systemic inflammation, and increased CRP levels have been associated with the development and progression of diabetes and its complications. Similarly, hs-CRP, a more sensitive marker of inflammation, has been linked to an increased risk of diabetic complications, including cataracts and diabetic retinopathy [25]. On the other hand, MDA is a marker of oxidative stress, which is known to be elevated in diabetes and contributes to the pathogenesis of cataracts and diabetic retinopathy [57]. Findings on the vitamin A levels across the study population revealed that vitamin A level was significantly higher ($p < 0.05$) in the control group than in the case groups (diabetes, cataract, and diabetic retinopathy). The findings suggest that reduced serum vitamin A levels may increase susceptibility to diabetes complications such as cataracts and diabetic retinopathy. The results highlight the importance of antioxidant therapies, like vitamin A supplementation, in mitigating oxidative stress in diabetic retinopathy patients. Vitamin A is a fat-soluble vitamin that plays a crucial role in maintaining vision and overall eye health. It is essential for the retina's normal functioning and the eyes' adaptation to low-light conditions [58]. Additionally, it acts as an antioxidant, protecting the eyes from oxidative stress and potential damage caused by free radicals [13]. In diabetes, there is evidence to suggest that vitamin A metabolism and its transport mechanisms may be affected. Some studies have reported lower serum levels of vitamin A in individuals with diabetes compared to non-diabetic individuals. This deficiency may contribute to the increased risk of developing diabetic complications, including cataract and diabetic retinopathy [13]. Oxidative stress and inflammation are known to play a role in cataract development, and vitamin A's antioxidant properties can help counteract these processes. Therefore, a lower level of vitamin A in individuals with cataracts may suggest a diminished protective effect against oxidative damage. Similarly, oxidative stress, inflammation, and impaired retinal microcirculation are implicated in the pathogenesis of diabetic retinopathy [59]. Vitamin A, through its role in maintaining retinal health and its antioxidant properties, may protect against the development and progression of diabetic retinopathy. Therefore, lower levels of vitamin A in individuals with diabetic retinopathy may contribute to the disease's pathophysiology [31].

The result of the molecular analysis showed the presence of amplified fragment of *ALR2 gene* at 263 bps in control and study groups. Sequencing result revealed two single nucleotide polymorphisms (SNPs) in nucleotides 106 (sample DR6) and (sample D3). A similar study by Kao *et al*. [31] and Li *et al*. [60] observed a polymorphism in the aldose *reductase gene* at the promoter region, which is strongly associated with diabetic retinopathy. This shows a 6.7% prevalence of SNP C(-106)T polymorphism at the ALR2 promoter region.

In the present study, the association of *ALR2*(106 C>T) Polymorphism with DR was evaluated, the result of the molecular analysis showed the presence of amplified fragment of *ALR2* gene at 263bps in control and study groups. Sequencing result revealed two single nucleotide polymorphisms (SNPs) in nucleotides 106 (sample DR6) and (sample D3 This shows a 6.7% prevalence of SNP C (-106) T polymorphism at the *ALR2* promoter region, the study showed no statistically significant association between the polymorphism and risk of DR. Similarly, the result was in accordance with the findings of Zhu *et al.,* [61] which revealed that under allelic, dominant recessive, homozygote and heterozygote genetic models all odd ratios showed lack of significant relationship between DR and polymorphism. Also, research conducted by Coa *et al.,* [62] is in accordance with this finding, which revealed that *ALR2* polymorphism had no significant effect on

developing DR under all contrasts in overall analysis; nevertheless, this polymorphism was significantly related to a decreased risk of DR in patients with T1DM after subgroup analysis by DM type.

The present findings showed the expression of *ALR2* in both the study groups and the control group, suggesting that the enzyme functions as a housekeeping gene, playing a constitutive role in maintaining basic cellular functions and being expressed in all cells of an organism under healthy and pathological conditions [63]. Moreover, in the present finding all control group had C (CC genotype) allele, while the study group had 90% C allele and 10% T allele. The T allele showed no significant association with DR 1.00(OR 95% CI:0.127-7.893). Contrary to the present findings Kaur *et al.,* revealed that TT genotype is linked to the risk of developing DR. Also, Abhary *et al.,* [17] reported the T allele as a protective agent. Again, in another study Katakami *et al.,* [64] reported that the C allele of *ALR2* gene polymorphism was a susceptibility allele for DR incidence in Japanese patients with T2DM. In addition, Ren *et al.,* [65] indicated that the *ALR2* polymorphism could also lower the risk of DR in patients with T2DM. However, Deng *et al*. [66] recruited 268 Chinese patients with T2DM and concluded that this polymorphism might not significantly correlate with the DR initiation. These phenomena suggest that genetic factors may significantly impact the initiation and progression of DR [62].

CONCLUSION

In conclusion, the study revealed a 6.7% prevalence of SNP C(-106)T polymorphism at the ALR2 promoter region. The control group demonstrated significantly lower glycemic level than the diabetic, cataract, and diabetic retinopathy groups, indicating the importance of glycemic control in disease management. Elevated levels of C-reactive protein (CRP), high sensitivity C-reactive protein (hsCRP), and malondialdehyde (MDA) were observed in the control groups, suggesting a potential association between inflammation and oxidative stress in the development of diabetic retinopathy. The lack of significant correlations between ALR2 and MDA or CRP suggests that other factors may contribute to the observed effects. Despite significant demographic and clinical differences observed among study groups. The analysis revealed no statistically significant association between ALR2 gene polymorphism and the risk of DR. However, Future studies with larger cohorts are necessary to confirm the role of SNP C(-106)T polymorphism in diabetic retinopathy and to explore its therapeutic implications.

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Conflict of Interest

The authors declare they have no conflicting interests.

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