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RESEARCH ARTICLE

The Usefulness of Serum Sorbitol and Vascular Endothelial Growth Factor A in Predicting Diabetic Retinopathy as Compared to Optical Coherence Tomography

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Abstract:

Background:

No specific and sufficient diagnostic biomarkers are currently available for predicting diabetic retinopathy (DR).

Objectives:

This study was conducted to investigate the validity of serum sorbitol and Vascular Endothelial Growth Factor A (VEGF-A) in diagnosing DR and differentiating it from diabetes without retinopathy (DNR). The study also investigated the diagnostic efficiency of these biomarkers when compared to optical coherence tomography OCT.

Methods:

A cross-sectional study included 164 diabetes mellitus patients: 30 patients with no retinopathy (the control group), 86 patients with non-proliferative diabetic retinopathy (NPDR), and 48 patients with Proliferative Diabetic Retinopathy (PDR). Patients were referred to the Layla Qasim Diabetic Center between November 2016 and October 2017 and an ophthalmologist established a DR diagnosis using OCT. Serum sorbitol and serum VEGF-A were measured for all patients.

Results:

By using study biomarkers, the cut-off values of VEGF-A (124.7 ng/ml) and sorbitol (0.3112 mg/ml) were established, and their validity parameters. For sorbitol, the values were as follows: specificity was 75.4, the sensitivity was 80 and 68.3% of observed agreement with the results of the OCT technique. For VEGF-A, the specificity was 73.1 the sensitivity was 80 and 76.2% of the observed agreement. The combined parallel test was applied as negative if both the tests were negative or as positive if either of the tests was positive: a highly significant statistical agreement (Kappa test p <0.001) was found with the gold standard diagnosis (OCT), with 85.4% of observed agreement.

Conclusion:

A combination of serum sorbitol and VEGF-A for diagnosing DR and for differentiating DR from DNR patients exhibits a significant agreement with an OCT diagnosis.

Keywords: Diabetic retinopathy, Optical coherence tomography, Serum sorbitol, Specificity, Sensitivity, Vascular endothelial growth factor.

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1. INTRODUCTION

Diabetic retinopathy is one of the significant inconveniences in patients with diabetes: the retina becomes progressively damaged, causing visual impairment and blindness due to the long-term cumulative damage to small blood vessels in the retina. The pathogenesis of diabetic retinopathy, including diverse, cells, molecules and diverse factors [1 - 3]. Hyperglycaemia harms retinal microvascular cells and causes different changes in retinal tissues, (for example, encourage vascular penetrability) due to pericytes damage, trailed by microvascular plug in the retina [4]. Pericytes are extended cells of mesodermal source that fold over and along endothelial cells of small vessels.

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Advanced Glycation End Products (AGEs) has a major role in the occurrence of microvascular disease in diabetes. Throughout diabetes, retinal pericytes collect AGEs, which detrimentally affect pericytes survival and task [1 - 5]. AGEs cause apoptosis of retinal pericytes and instigate vascular endothelial growth factor (VEGF). VEGF is an encouraging factor that is studied in association with diabetic retinopathy; it induces angiogenesis (which causes a breakdown of the blood-retinal barrier), enhances endothelial cell development and neovascularization (production of new blood vessels), and increments vascular porousness in the ischaemic retina [6, 7]. A high intraocular concentration of VEGF corresponds to increased vascular penetrability, which progresses to haemorrhage, exudates and vascular seepage, prompting non-proliferative diabetic retinopathy (NPDR). Angiogenesis and vasculogenesis lead to proliferative diabetic retinopathy (PDR) [4, 8 - 11].

At the point when glucose level in the crystalline lens reaches a very high level, aldose reductase reduces the glucose to sorbitol, which thus collects in the lens. Changes in the body glucose from a hyperglycaemic to a hypoglycaemic condition, an abundant amount of glucose in the lens moves out into the aqueous humour, however, the sorbitol stays in the lens. The osmotic pressure gradient produced results in the movement of water from the aqueous humour into the lens, causing lenticular swelling with hyperopic refractive change. Indeed, even a mild decline in the refractive index of the crystalline lens delivers a critical transient hyperopic alteration [12]; this is a conceivable clarification for the event of a refractive change in diabetic patients.

Optical Coherence Tomography (OCT) is a device made recently for the diagnosis of DR and diabetic macular oedema in ophthalmology centres. It gives an assessment of quantitative rather than qualitative by using macular biomicroscopy or fundus photography [13 - 16].

Likewise, the OCT can be used with an incorporated retinal camera qualifying both assessments in a single picture catch. When the referable retinopathy (pre-proliferative, proliferative, and macular oedema) is predicted, the patient is sent to the ophthalmology clinic to conduct diagnostic test using OCT. The validity of this device as a diagnostic method for retinopathy and macular oedema in diabetic patients was studied by Azrak *et al.* [17]. However, among the diverse biochemical pathways involved in the pathogenesis of DR, no certain and sufficient indicative serum biomarkers are now available for predicting DR. Therefore, this study set out to investigate the validity of serum sorbitol and serum VEGF-A in diagnosing DR and differentiating it from Diabetic Non-Retinopathy (DNR). Additionally, the study compared the diagnostic efficiency of these biomarkers to OCT.

2. MATERIALS AND METHODS

2.1. Study Groups

This study was a cross-sectional study that comprised of 164 diabetic patients: 30 DNR patients (the control group), 86 NPDR patients and 48 PDR patients. All the patients were referred to Layla Qasim Diabetic Center between November 2016 and October 2017, given a questionnaire **and signed it**. They were all aged between 21 and 75 years, and both genders were included.

All of the study groups were diagnosed and evaluated by an ophthalmologist and classified according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria [18]. The Ethics Committee of the College of Pharmacy at Hawler Medical University approved the study, and verbal consent was obtained from all study participants.

2.2. Exclusion Criteria

The exclusion criteria were as follows: intravitreal injections within three months prior to the start of the study, history of renal or liver impairment, malignancy, cardio-vascular disease (recent myocardial infarction, stroke, peripheral artery disease), uncontrolled hypertension (≥140/90 mm Hg), deep vein thrombosis and pulmonary embolism, eyes that had undergone vitrectomy, and chorioretinal abnormalities.

2.3. Sample Collection

Five milliliters of venous blood with minimal stasis were collected from each subject. Blood without anticoagulant was allowed to clot, then centrifuged for 15 minutes at 3000 rounds per minute. Thereafter, the obtained sera were dispensed into Eppendorf tubes and immediately frozen at -20 °C for later determination of VEGF-A and sorbitol by Enzyme Linked-Immuno-Sorbent Assay (ELISA) (MyBioSource; Serum VEGF-A ELISA Kit, San Diego, CA 92195-3308, USA) and by Sorbitol Microplate Assay Kit (MyBioSource; Sorbitol Microplate Assay Kit, San Diego, MBS8243206, U.S.A), respectively.

2.4. Study Protocol

Detailed information about each patient, including age, gender, duration of diabetes in years, type of diabetes, type of diabetic treatment, smoking, hypertension, and dyslipidaemia, was recorded.

2.5. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 22 was used for data analysis. The comparison between the means of the three groups was done by a one-way analysis of variance (ANOVA). A post hoc test (LSD) was used to determine significant differences between the groups. A chi-square test of association was used to compare the proportions between groups.

The correlation coefficient was used to measure the strength of a linear association between the two variables.

Receiver Operating Characteristic (ROC) curve was plotted in order to estimate the cut-off value that provides the highest sensitivity and the highest specificity (knowing that when sensitivity increases the specificity decreases). The highest Youden's index value (J = sensitivity + specificity - 1) was used to estimate the cut-off value [19]. A discriminant analysis was then used to determine which variables were the best predictors: a Kappa statistic was used to determine a statistically significant agreement between the test (the results

of VEGF-A and sorbitol as screening tests, whether positive or negative) and the golden standard diagnosis (OCT) beyond chance. A *p*-value of ≤ 0.05 was considered statistically significant.

3. RESULTS

Table **1** illustrates the baseline characteristics of the study patients. Of 164 diabetic patients, 30 were DNR patients: 18 (60%) of these patients were female and 12 (40%) were male, and 15 (50%) patients were type I diabetic. Furthermore, 17 patients from this group (56.7%) received insulin as a treatment, and the remaining 13 (43.3%) received an oral hypoglycaemic. The second group consisted of 134 patients with diabetic retinopathy: 85 (63.4%) were female and 49 (36.6%) were male, 45 (33.6%) patients were type I diabetic and 68 (50.7%) patients received insulin as a treatment. There was no statistically significant difference in the distribution of dyslipidaemia and smoking habits between the two groups ($p > 0.05$).

Only age ($p < 0.001$), duration of diabetes ($p < 0.001$) and hypertension ($p = 0.002$) exhibited a statistically significant difference between the two groups. In the DNR group, 10 (33.3%) patients were under 30 years of age and 29 (96.7%) had diabetes for less than 15 years; in the DR group, 5 (3.7%) patients were under 30 years of age, and 68 (50.7%) patients had diabetes for less than 15 years.

Table **2** demonstrates that there was a statically significant difference ($p < 0.001$) in the means of age, VEGF-A and sorbitol between the DNR group (38.9 ± 17.9 years, 96 ± 59.9 ng/ml VEGF-A and 0.29 ± 0.031 mg/L sorbitol) and the entire DR group (55.5 ± 10.0 years, 320.9 ± 259.6 ng/ml VEGF-A and 0.387 ± 0.103 mg/L sorbitol). Additionally, the mean duration of diabetes in the DNR group (5.9 ± 3.8 years) was significantly lower ($p = 0.003$) than the mean duration in the entire DR group (14.2 ± 6.2 years).

Table **3** illustrates that there was a statistically significant difference ($p < 0.001$) in the mean age, duration of diabetes, serum VEGF-A and sorbitol between all the three study groups; however, there was no statistically significant difference between the mean age of NPDR patients (55.5±10.0 years) and PDR patients (54.3 ± 11.4) ($p = 0.604$).

The mean of the duration of diabetes in the PDR group (15.2 ± 6.9 years) was higher than the mean duration in the NPDR group (13.6 ± 5.7 years) but the difference was not significant ($p = 0.120$). The VEGF-A mean of the PDR group (413.7 ± 301.4 ng/ml) was significantly different ($p = 0.001$) from the VEGF-A mean of the NPDR group (269.1 ± 218.3 ng/ml).

Table **3** also shows the absence of a statistically significant difference ($p = 0.429$) in the mean percent of glycosylated hemoglobin HbA1c (%) between study groups: the mean of the DNR group was 9.03 ± 1.9%, the mean of the NPDR group was 9.27 ± 2.1%, and the mean of the PDR group was 9.6 ± 2.4%.

The results of the correlation analysis, presented in Table **4**, indicate that the higher the age of the DNR patients, the higher the level of sorbitol ($r = 0.426$, $p = 0.019$). No significant correlation between age and level of sorbitol was found in the NPDR and PDR groups. The table also shows a significant moderate positive association between serum VEGF-A and sorbitol levels in the NPDR group ($r = 0.317$, $p = 0.003$). A significant moderate negative linear correlation was detected between the duration of diabetes and VEGF-A level in the PDR group ($r = - 0.332$, $p = 0.021$).

Table **5** clearly indicates that the duration of diabetes and age are more important for predicting retinopathy than the study biomarkers. In addition, sorbitol has a higher validity than VEGF-A in predicting retinopathy.

In diagnosing any type of DR by differentiating it from DNR, the area under the curve of VEGF-A (0.806) and the area of sorbitol (0.849) differ significantly from the area under the mid line (0.5) with $p < 0.001$.

Table **6** shows that regarding the screening for NPDR and differentiating it from DNR, the area under the curve of VEGF-A (0.768) and the area of sorbitol (0.824) differ significantly from the midline area ($p < 0.001$). Similarly, when screening for PDR to differentiate it from NPDR, the area under the curve of VEGF-A (0.656) and the area of sorbitol (0.73) differ significantly from the midline area ($p = 0.003$ and $p < 0.001$, respectively).

Table **7** shows the cut-off values of VEGF-A and sorbitol when screening for DNR, NPDR and PDR, as well as the validity parameters of these tests. The cut-off value of sorbitol (0.3112 mg/ml) showed higher sensitivity (75.4) than VEGF-A at a cut-off value of 124.7 ng/ml (sensitivity 73.1) for predicting DRP, with both having the same specificity value of 80. On the other hand, for screening NPDR and differentiating it from DNR, sorbitol cut-off value (0.4065 mg/ml) showed both higher sensitivity and specificity (68.8 and 75.6) than the VEGF-A cut-off value of 285.9 ng/ml (sensitivity value of 66.7 and specificity value of 60.5). Both biomarkers demonstrated the same sensitivity (79.2) for predicting PDR and differentiating it from NPDR with higher specificity for sorbitol (100) than for VEGF-A (93.3).

Table **8** shows that in a serial tests combination of VEGF-A and sorbitol (negative if either test was negative and positive if both tests were positive), the area under the ROC curve (0.774) differed significantly ($p < 0.001$) from the equivocal area (0.5). Furthermore, it was larger than the area of the parallel tests combination (negative if both the tests were negative and positive if either test was positive**)**, which was 0.768 with $p < 0.001$.

Table **9** shows the cut-off values of VEGF-A and sorbitol (>=124.7 ng/ml and >= 0.3112 mg/L, respectively) when used to predict DR and differentiate it from DNR: the specificity of both was 80.0, whereas their respective sensitivity values were 73.1 and 75.4. In a parallel tests combination, the sensitivity increased to 90.3, while specificity decreased to 63.3; on the other hand, in a serial tests combination, the specificity increased to 96.7, whereas sensitivity decreased to 58.2.

Table **10** shows that there was a statistically significant agreement ($p < 0.001$) for using the cut-off values of the study biomarkers: the percentages of observed agreement for serum VEGF-A (124.7 ng/ml) and sorbitol (0.3112 mg/ml) when used

to screen for DR were 76.2% and 68.3% respectively. The percentage of observed agreement when a parallel tests combination applied was 85.4%, which was higher than the percentage of observed agreement when a serial tests combination was applied (65.2%).

Table 1. Age, gender, type of diabetes mellitus (DM), duration and type of treatment distribution of the study sample.

Study Variables	No Retinopathy		Retinopathy (Any Type of Retinopathy)		
	N	%	N	%	*P*
Age (years)					
<30	10	33.3	5	3.7	<0.001
30-44	5	16.7	10	7.5	
45-59	13	43.3	67	50.0	
60+	2	6.7	52	38.8	
Total	30	100.0	134	100.0	
Gender					
Female	18	60.0	85	63.4	0.73
Male	12	40.0	49	36.6	
Total	30	100.0	134	100.0	
Type-II DM (compared to type-I)					
Type-I	15	50.0	45	33.6	0.09
Type-II	15	50.0	89	66.4	
Total	30	100.0	134	100.0	
Duration of DM (years)-categories					
<5	15	50.0	9	6.7	<0.001
5-9	8	26.7	27	20.1	
10-14	6	20.0	32	23.9	
15-19	1	3.3	32	23.9	
20+	0	0.0	34	25.4	
Total	30	100.0	134	100.0	
Treated with insulin (solo or in combination with oral hypoglycaemics) compared to oral hypoglycaemics					
Oral hypoglycaemic agent	13	43.3	66	49.3	0.56
Insulin	17	56.7	68	50.7	
Total	30	100.0	134	100.0	
Hypertension					
Negative	23	76.7	60	44.8	0.002
Positive	7	23.3	74	55.2	
Total	30	100.0	134	100.0	
Dyslipidaemia					
Negative	19	63.3	64	47.8	0.12
Positive	11	36.7	70	52.2	
Total	30	100.0	134	100.0	
Smoking habit					
Negative	29	96.7	117	87.3	0.14
Positive	1	3.3	17	12.7	
Total	30	100.0	134	100.0	

Table 2. (Mean ± SD) for age, duration of diabetes, VEGF and sorbitol by the presence of retinopathy.

Study Variables	No Retinopathy	Retinopathy (Any Type. of Retinopathy)	*p*
Age (years)	(38.9 ± 17.9)	(55.5 ± 10.0)	<0.001
Duration (years)	(5.9 ± 3.8)	(14.2 ± 6.2)	0.003
VEGF-A (ng/ml)	(96 ± 59.9)	(320.9 ± 259.6)	<0.001
Sorbitol (mg/L)	(0.29 ± 0.031)	(0.387 ± 0.103)	<0.001

Table 3. (Mean ± SD) for age, duration, VEGF-A and sorbitol by study groups.

	Mean ± SD	*p* (ANOVA)	LSD (Groups)	*p* (LSD)
Age (years)				
A) DNR	(38.9 ± 17.9)		A X B	<0.001
B) NPDR	(55.5 ± 10.0)	<0.001	A X C	<0.001
C) PDR	(54.3 ± 11.4)		B X C	0.604(NS)
Duration (years)				
A) DNR	(5.9 ± 3.8)	<0.001	A X B	< 0.001
B) NPDR	(13.6 ± 5.7)		A X C	< 0.001
C) PDR	(15.2 ± 6.9)		B X C	0.120(NS)
HbA1c (%)				
A) DNR	(9.03 ± 1.9)	0.429	A X B	0.570
B) NPDR	(9.27 ± 2.1)		A X C	0.213
C) PDR	(9.6 ± 2.4)		B X C	0.347
VEGF-A (ng/ml)				
A) DNR	(96.0 ± 59.9)	< 0.001	A X B	<0.001
B) NPDR	(269.1 ± 218.3)		A X C	<0.001
C) PDR	(413.7 ± 301.4)		B X C	0.001
Sorbitol (mg/L)				
A) DNR	(0.290 ± 0.031)	<0.001	A X B	<0.001
B) NPDR	(0.356 ± 0.065)		A X C	<0.001
C) PDR	(0.442 ± 0.133)		B X C	<0.001

Table 4. Linear correlation coefficients between study biomarkers for all study groups.

Study Variables	DNR	NPDR	PDR
VEGF-A (ng/ml) x sorbitol (mg/L)	r = 0.243 *p* = 0.19[NS]	r = 0.317 *p* = 0.003	r = 0.187 *p* = 0.2[NS]
VEGF-A (ng/ml) x duration(years)	r = 0.174 *p* = 0.36[NS]	r = - 0.019 *p* = 0.86[NS]	r = - 0.332 *p* = 0.021
VEGF-A (ng/ml) x age (years)	r = 0.128 *p* = 0.5[NS]	r = 0.109 *p* = 0.32[NS]	r = -0.233 *p* = 0.11[NS]
Sorbitol (mg/L) x duration (years)	r = 0.211 *p* = 0.26[NS]	r = 0.173 *p* = 0.11[NS]	r = -0.282 *p* = 0.05[NS]
Sorbitol (mg/L) x age (years)	r = 0.426 *p* = 0.019	r = 0.058 *p* = 0.59[NS]	r = 0.029 *p* = 0.84[NS]
Sorbitol (mg/L) x duration (years)	r = 0.211 *p* = 0.26[NS]	r = 0.173 *p* = 0.11[NS]	r = -0.282 *p* = 0.05[NS]

Table 5. Discriminant analysis with retinopathy as the dependent outcome variable and a set of explanatory variables (VEGF-A, Sorbitol, Age, Gender, Duration of DM.

Study Variables	Order of Importance	Canonical Discriminant Function Coefficients
Duration of DM (years)	1	0.090
Age (years)	2	0.036
Sorbitol (mg/L)	3	4.431
VEGF-A (ng/ml)	4	0.002

Table 6. ROC area for VEGF-A and sorbitol when used as tests to diagnose any type of DR.

Study Groups and Variables	ROC Area	*P*
DNR X DR		
VEGF	0.806	< 0.001
Sorbitol	0.849	< 0.001
DNR X NPDR		
VEGF	0.768	< 0.001

(Table 6) contd.....

Study Groups and Variables	ROC Area	*P*
Sorbitol	0.824	< 0.001
NPDR X PDR		
VEGF	0.656	0.003
Sorbitol	0.73	< 0.001

Table 7. Validity parameters for VEGF-A and sorbitol when used as tests to diagnose and determine the type of DR.

Study groups and variables	Optimum cut-off value	Sensitivity	Specificity	Accuracy	PPV at pretest probability		NPV at pretest probability = 10%
					50%	90%	
DNR X DR							
VEGF (ng/ml)	124.7	73.1	80	74.4	78.5	97.1	96.4
Sorbitol (mg/ml)	0.3112	75.4	80	76.2	79	97.1	96.7
DNR X NPDR							
VEGF (ng/ml)	285.8	66.7	60.5	62.7	62.8	93.8	94.2
Sorbitol (mg/ml)	0.4065	68.8	75.6	73.1	73.8	96.2	95.6
NPDR X PDR							
VEGF (ng/ml)	173.9	79.2	93.3	84.6	92.2	99.1	97.6
Sorbitol (mg/ml)	0.3363	79.2	100	87.2	100	100	97.7

Table 8. ROC area for VEGF-A and sorbitol tests (solo or combined) for diagnosing any type of DR by differentiating it from DNR.

	ROC area	*p*
Positive VEGF-A test (at optimum cut-off value of >=124.7 ng/ml)	0.766	<0.001
Positive Sorbitol test (at optimum cut-off value of >= 0.3112 mg/L)	0.777	<0.001
Parallel tests combination	0.768	<0.001
Serial tests combination	0.774	<0.001

Table 9. Validity parameters for VEGF-A and sorbitol tests (solo or combined) for diagnosing any type of DR and differentiating it from DNR

Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy	PPV at pretest probability		NPV at pretest probability = 10%
				50%	90%	
Positive VEGF-A test (at optimum cut-off value of >=124.7 ng/ml)	73.1	80.0	74.4	78.5	97.1	96.4
Positive sorbitol test (at optimum cut-off value of >= 0.3112 mg/L)	75.4	80.0	76.2	79.0	97.1	96.7
Parallel tests combination	90.3	63.3	85.4	71.1	95.7	98.3
Serial tests combination	58.2	96.7	65.2	94.6	99.4	95.4

Table 10. The results of a Kappa test (agreement beyond chance) reflecting a statistically significant agreement between the cut-off values of the study biomarkers, used either separately or in combination, and OCT when screening for DR.

	DNR		Retinopathy (Any type of retinopathy)		*p* (Kappa)	Kappa
	N	%	N	%		
Positive VEGF test (at optimum cut-off value of >=124.7 ng/ml)					<0.001	0.383
Negative	24	80.0	33	24.6		
Positive	6	20.0	101	75.4		
Total	30	100.0	134	100.0		
Percent observed agreement=76.2						

(Table 10) contd.....

	DNR		Retinopathy (Any type of retinopathy)		*p* (Kappa)	Kappa
	N	**%**	**N**	**%**		
Positive Sorbitol test (at optimum cut-off value of >= 0.3112 mg/L)					<0.001	0.41
Negative	24	80.0	36	26.9		
Positive	6	20.0	98	73.1		
Total	30	100.0	134	100.0		
Percent observed agreement=68.3%						
Parallel tests combination					<0.001	0.523
Negative (both tests negative)	19	63.3	13	9.7		
Positive (either test positive)	11	36.7	121	90.3		
Total	30	100.0	134	100.0		
Percent observed agreement=85.4%						
Serial tests combination					<0.001	0.321
Negative (either test negative)	29	96.7	56	41.8		
Positive (both tests positive)	1	3.3	78	58.2		
Total	30	100.0	134	100.0		
Percent observed agreement=65.2%						

4. DISCUSSION

The data presented have been obtained from a relatively large and diverse sample of patients of both genders with type 1 and type 2 diabetes. The diabetic duration and patient age are the more effective factors in the development of DR and other microvascular complications [20 - 22]; It is confirmed that DR develops in about 75% of patients with diabetes for more than 15–20 years. Our study demonstrates that the duration of diabetes and age are more important for predicting retinopathy than the study biomarkers (sorbitol and VEGF-A) (Table **5**). The greatest risk state (PDR) was observed among the oldest patients with the longest duration of diabetes: in this, our findings are consistent with an earlier study by Zoungas *et al.* [23], which showed that diabetes duration is independently associated with microvascular events.

We found an obvious number of DR patients who had diabetes for less than 15 years: of 134 DR patients in our study, 6.7% had been diabetic for less than 5 years, 20.1% for less than 10 years, and 23.9% for less than 15 years (Table **1**). These findings may have implications for the development of DR in Iraq, and they indicate that most individuals with diabetes rarely achieve consistent euglycaemia.

It has been established that proper glycaemic control has a distinct role in the prevention of retinopathy [24 - 27]. Semeraro *et al.* and Kohner *et al.* have shown that stricter glycaemic control at HbA1c <7% reduces the risk of retinopathy in both type 1 and 2 diabetes mellitus [28, 29]. A study by Kumamoto found that the glycaemic threshold to prevent the onset and diagnosis of microvascular complications in diabetes was HbA1c ≤ 6.5% [30]. In the present study, the HbA1c% was high in the all groups, indicating the poorest glycaemic control with no significant difference ($p = 0.49$) in the association of HbA1c% in the NPDR and PDR groups as compared to the DNR group (Table **3**), as all the three groups lacked proper glycaemic control for a long period. Our results confirm the need for more intensive awareness and advice for glycaemic control to avoid major microvascular complications in younger people living with diabetes (the mean age of the DNR group was 38.9±17.9 years and HbA1c was 9±1.9%).

There is a strong correlation between diabetic complications including DR and increased oxidative stress [31], with activation of polyol pathway, sorbitol being its main outcome, known to contribute to oxidative stress [7]. The present study demonstrates that sorbitol is significantly higher in DR patients in the more progressive stage of the disease (PDR) than in DNR patients (Table **3**). This is highly consistent with the findings of Reddy *et al.* [32].

Interestingly, the higher serum sorbitol in the DR groups in the present study was not correlated with diabetes duration and patient age (Table **4**). This finding suggests that serum sorbitol might serve as an independent risk identification factor for DR, regardless of the duration of diabetes and age of the patient; this is also confirmed by the ROC area of 0.849 (Table **6**) for differentiating DR from DNR.

The presence of a significant positive moderate correlation between serum VEGF-A and sorbitol in the NPDR group only indicates that the behaviour of these biomarkers varies in different stages of disease: these biomarkers may be involved in the initial but not later stages of DR.

In the present study, a high level of serum VEGF-A was observed in DR patients and it was significantly higher in PDR patients (Table **3**): this indicates that VEGF-A is an effective factor which induces neovascularization in more progressive forms of retinopathy, which is in agreement with findings from others studies [33, 34].

However, no data are available on the usefulness of serum sorbitol as a specific and sufficient diagnostic biomarker, either when used on its own or in combination with VEGF-A, for predicting DR and differentiating it from DNR. The current study obtained the cut-off value for serum sorbitol of 0.3112 mg/ml with a reasonably associated validity (a specificity value of 75.4 and a sensitivity value of 80) (Table **7**) and with 68.3% of observed agreement with the results of the OCT technique

(Table **10**). For predicting any type of DR and differentiating it from DNR, the cut-off value for serum VEGF-A is 124.7 ng/ml, with the associated specificity value of 73.1 and the sensitivity value of 80 (Table **7**), and 76.2% of observed agreement (Table **10**). On the other hand, when the ophthalmological examination of the retina using OCT was completed to detect retinopathy and macular oedema, the validity factors showed sensitivity of 91.67% and specificity of 93.18% [17].

When the cut-off values of the study biomarkers were used (serum sorbitol 0.3112 mg/ml, VEGF-A 124.7 ng/ml) to screen for DR among diabetic patients, the surprise finding was that there was a highly significant statistical agreement (Kappa test $p < 0.001$) between the parallel tests combination and the gold standard diagnosis (OCT). The percentage of observed agreement for this approach was 85.4% (Table **10**). However, when each study biomarker was used separately, despite the presence of a statistically significant agreement with the gold standard diagnosis (OCT) (Kappa $p < 0.001$), the percent of observed agreement was 68.3% for sorbitol and 76.2% for VEGF-A, less than that of the combined approach (Table **10**).

Along with clinical investigation, the combined approach of serum sorbitol and VEGF-A is reliable discriminant of DR from DNR and is more easily applied in areas with low socioeconomic status because it is less costly than the OCT technique.

CONCLUSION

1-A combination of serum sorbitol and VEGF-A demonstrates a significant agreement with OCT for diagnosing and differentiating DR from DNR patients.

2- An absence of a correlation between serum sorbitol, and the duration of diabetes and patient's age, indicate that serum sorbitol may serve as an independent biomarker for predicting DR; this is further confirmed by its high specificity and reasonable sensitivity in predicting DR.

LIST OF ABBREVIATIONS

DR	=	Diabetic Retinopathy
OCT	=	Optical Coherence Tomography
VEGF	=	Vascular Endothelial Growth Factor
AGEs	=	Advanced Glycation End Products
NPDR	=	Non-Proliferative Diabetic Retinopathy
DNR	=	Diabetic Non-Retinopathy
PDR	=	Proliferative Diabetic Retinopathy

AUTHORS' CONTRIBUTIONS

Sanaa Gadbaan Hama Almandlawi designed the research. Sanaa Almandlawi with Muhanad Salah Mawlood carried out the experiments, who analyzed the data. Sanaa Gadbaan Hama Almandlawi wrote the manuscript. All authors are in agreement with the content of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the College of Pharmacy at Hawler Medical University approved the study.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all the participants prior to data collection.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICTS OF INTEREST

The authors declare there is no conflict of interest, financial or otherwise.

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