International Journal of Retina (*IJRETINA*) 2023, Volume 7, Number 1.
P-ISSN. 2614-8684, E-ISSN.2614-8536

IJRETINA
International Journal of Retina

EVALUATION OF ENDOTHELIN-1 AND GLYCATED HAEMOGLOBIN IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT RETINOPATHY

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Abstract

Introduction: Diabetic retinopathy (DR) can lead to blindness and therefore requires early diagnosis and careful monitoring.

Method: The present cross-sectional analytical survey assessed endothelin-1 (ET-1) and glycated haemoglobin (HbA1C) levels, two DR markers, in type 2 diabetic patients with and without DR in the National Obesity Centre of the Yaoundé Central Hospital in Cameroon, from February 1 to July 31, 2021. It was carried out on DR and non-DR patients, and non-diabetic patients. Various clinical parameters related to diabetes were collected, and a comprehensive ophthalmological examination as well as an analysis of HbA1C and ET-1 in participants' blood samples were performed.

***Results*:** A total of 12 DR participants, 32 non-diabetic participants, and 45 diabetic participants without DR were enrolled. The majority of diabetic patients did not have a good HbA1C level, without a statistical difference (p-value = 0.702) between DR (75%) and non-DR (80%) patients. For ET-1, it was recorded high serum levels in diabetic patients (10.9 pg/mL [10.00 – 19.50]) compared (p-value = 0.04) to non-diabetic patients (10.6 pg/mL [6.3 – 13.5]). Moreover, DR patients recorded serum levels of ET-1 greater than (p=0.03) non-DR patients (13.1 pg/mL [10.89-21.42] vs 10.8 pg/mL [10.00-18.9]).

Conclusion: These biomarker levels are a warning in DR evolution of diabetic patients. ET-1 could be a better control biomarker than HbA1c in the clinician management of DR according to this study. Thus, it is necessary to improve the description of these biomarkers in order to reinforce their use.

Keywords: diabetes, diabetic retinopathy, endothelin-1, glycated haemoglobin.

Cite This Article: DOMNGANG, Christelle et al. EVALUATION OF ENDOTHELIN-1 AND GLYCATED HAEMOGLOBIN IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT RETINOPATHY. International Journal of Retina, [S.l.], v. 7, n. 1, p. 39, feb. 2024. ISSN 2614-8536. Available at: <https://www.ijretina.com/index.php/ijretina/article/view/255>. Date accessed: 27 feb. 2024. doi: https://doi.org/10.35479/ijretina.2024.vol007.iss001.255..

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INTRODUCTION

Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus which has become a global health problem with prevalence expected to increase to 366 million worldwide by 2030.[1] Diabetic retinopathy is characterized in clinical practice by two main stages, non-proliferative diabetic retinopathy, and proliferative diabetic retinopathy. Characteristic microvascular alterations of DR include microvascular endothelial cell dysfunction, vascular permeability, increased tissue ischemia, and angiogenesis.[2] Because DR may lead to the loss of vision, diabetics must be well followed and clinicians must have a good knowledge of the pathology's progression. Endothelin-1 (ET-1) is a biomarker used in monitoring DR .[2-4] Its biological concentrations may indicate cases of advanced diabetic retinopathy.[2,4] Another biomarker, glycated hemoglobin A1c (HbA1c) usually helps to monitor diabetes balance. Diabetic patients must have a good glycemic control, as weak glycemic controls increase the risk of DR. HbA1c level is a recommended indicator of the average blood glucose concentrations over the preceding 2 – 3 months and values less than 7% are appropriate. [3,5,6,7] The goal of this study was to evaluate ET-1 and HbA1c levels in type 2 diabetic patients with and without retinopathy to assess their simultaneous pertinence in a Cameroonian population.

METHODS

Ethical consideration

For the present study, an ethical approval [No 2021/058 /UdM/PR/CIE] was obtained from the institutional Ethical Committee of *Université des Montagnes* and an administrative authorization was obtained from the Yaounde Central Hospital.

Design and study setting

A cross-sectional analytical study was carried out with a prospective recruitment strategy, at the National Obesity Centre (NOC) of the Yaoundé Central Hospital from February 1 to July 31, 2021. The target population was diabetic patients consulting in the NOC. Biological analyses were performed in the laboratory of the NOC.

Patients with type 2 diabetes were included in the study. On the other hand, type 2 diabetic patients affected with chronic inflammatory disease of the eye like open-angle glaucoma and uveitis of diverse etiologies, type 2 diabetic patients with an autoimmune or neoplastic disease, and pregnant women were excluded.

A group of non-diabetic, apparently healthy participants frequenting the NOC were included; here, all potential participants with any ocular, autoimmune, systemic inflammatory affection as well as any neoplastic disease were excluded from the study. Pregnant women were equally excluded.

Diabetic participants were divided into two groups based on whether the patient had or did not have a retinopathy.

Thus, considering all the selection criteria, the participants were separated into 3 groups as follows: a group of diabetic patients with retinopathy, a second one of diabetic patients without retinopathy, and lastly, a group of non-diabetic individuals.

Data collection

After obtaining their informed consent, patients completed a pretested questionnaire. Sociodemographic and anthropometric data were collected. Biological data related to diabetes were also collected from the medical records.

A comprehensive ophthalmological examination was carried out.

Analysis of samples

Ophthalmological examination

A comprehensive ophthalmological examination was carried out and included a visual acuity test with a Monoyer scale 5 meters away from the patient, a slit lamp examination and a dilated examination of the fundus carried out with a biomicroscope Topcon® and 90 Dioptrics lens. A detailed description of the papillae, macula, and retinal vessels in search of diabetic retinopathy lesions (microaneurysms, exudates, haemorrhages, neovascularization as well as macular oedema. The stage or severity of DR could then be determined.

Biological sampling

About 5 ml of venous blood was collected in a labelled dry EDTA sterile and vacutainer tube. The sample in the dry tube was centrifugated for 10 min at 3000 g and the serum was immediately aliquoted and stored at -20°C within 30 min until analysis.

Glycated Haemoglobin analysis

This analysis was performed by a fluorescence immunoassay commercial kit, Finecare™ HbA1c Rapid Quantitative Test (Finecare™, Guangzhou Wondfo Biotech Co.). A total of 10 µL of whole blood in the EDTA tube was added to a detection buffer tube. Then, the sample with buffer was mixed well for 1 min by inverting the tube. Thereafter, 75 µL of the mixture was loaded onto the sample well of the manufacture test cartridge and left on the workstation. Five minutes after, a reading was performed with Finecare™ FIA Meter [GuangzhouWondfo Biotech Co., Ltd].

Endothelin-1 assay with ELISA method

The quantitative sandwich enzyme-linked immunosorbent assay (ELISA) was used for the analysis of Endothelin 1 in patient serum. The test was performed with a commercial kit, Human Endothelin 1 ELISA KIT (96T) by Melsin Medical Co. Briefly, 10 µL of samples and 50 µL of standards (concentrations 160, 80, 40, 20, 10, and 0 pg/mL) were added into 96-well plates pre-coated with antibody specific for human ET-1. A blank test was also included in the analysis. Diluted sera, standards and blank well were manipulated in accordance with the manufacturer's requirements. Colour intensity was measured using the multi-well plate reader RAYTO RT 2100C [Rayto Life and Analytical Sciences Co., Ltd.] and ET-1 serum concentrations were calculated on the basis of a standard curve generated by the same reader. Just before analysis, the frozen sample and kit were allowed to gradually thaw at room temperature and stirred gently.

Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 21.0 for Windows. Quantitative variables were presented as means ± standard deviation or as the median with interquartile ranges. Qualitative variables were expressed as frequencies and percentages. The association between qualitative variables was evaluated using chi-square and Fischer tests. In addition, the association between qualitative and quantitative variables was evaluated using Kruskal-Wallis test. The threshold of significance of the tests was fixed at a probability of $p < 0.05$.

RESULTS

A total of 89 participants were included in the present study. The population comprised 57 diabetic participants and 32 non-diabetic ones. In the diabetic population, 21.05% [n=12/57] had diabetic retinopathy [Table 1].

Table 1: Sociodemographic data of participants

Variables	Diabetic patients [N= 57]	Non-diabetic patients [N= 32]
Male	30	18
Female	27	14
Mean age ± Standard Deviation	56 ± 10	57 ± 10
[min-max] years	[36-74]	[37-73]
Diabetic retinopathy	12	-
Non-diabetic retinopathy	45	-

The set of tables below presents the distribution of diabetic retinopathy according to anthropomorphic parameters, glycated haemoglobin and comorbidities [Tables 2 & 3].

As reported in Table 2, the level of glycated haemoglobin was similar for patients with diabetic retinopathy (median: 8 [7.1-8.3]; min-max: 6.8-12) compared to patients without diabetic retinopathy (median 8[7-9]; min-max: 4.0-14.6).

We observed from the table 3 that over half of diabetic patients had had diabetes for more than 5 years. However, although the majority came from patients without DR, patients with DR recorded the highest rate of diabetes duration over 5 years [DR: 75% (9/12) vs non-DR: 46.6% (21/45)]. Almost one-third of diabetic patients had high blood pressure [DR: 41.6%(5/12) vs non-DR: 28.8%(13/45)]. Also, 14% had a weak glomerular filtration flow and the majority came significantly from patients without DR [DR: 50% (6/12) vs non-DR: 4.4% (2/45)].

Table 2 : Clinical and paraclinical biomarkers levels according to diabetes retinopathy status

	Diabetic retinopathy N=12	No diabetic retinopathy N=45	p-value
Abdominal circumference [cm]			
Mean ± SD	99.9 ± 18	94.6 ± 15.3	0.307
[min-max]	[76-133]	[69-160]	
BMI [kg/m2]			
Mean± SD	31.1 ± 7.79	26.5 ± 4.9	**0.014**
[min-max]	[20.8 – 46.6]	[17.1-38.3]	
Hb A1C [%]			
Median [IR]	8 [7.1; 8.3]	8 [7.0; 9.0]	0.336
[min-max]	[6.8 – 12]	[4 – 14.6]	

Table 3 : Clinical and paraclinical factors according to diabetes retinopathy status

Variables	Diabetic retinopathy N=12 [%]	No diabetic retinopathy N=45 [%]	Total N=57	p-value	OR
Duration of diabetes > 5 years	9 [75]	21 [46.6]	30	0.089	3.4 [0.8-14.3]
Comorbidities [HBP]	5 [41.6]	13 [28.8]	18	0.489	1.8 [0.71- 6.55]
Hb A1C > 7%	9 [75]	36 [80.0]	45	0.702	0.75 [0.16 - 3.6]
GFR [ml/min] < 90	6[50.0]	2[04.4]	8	0.001	21.5[3.5-131.9]

HBP: high blood pressure GFR: glomerular filtration rate

Endothelin 1 biomarker level was statistically different (p=0.03) among diabetic participants according to diabetic retinopathy presence as seen in Table 4.

Table 4 : Endothelin-1 level according to diabetes and diabetic retinopathy status

Endothelin 1 [pg/mL]	Diabetes	No diabetes	P-value
Median	10.9	10.6	**0.04**
Interquartile	[10.00 – 19.5]	[6. 3 – 13.5]	
Min - max	5.6 – 213.3	2.7 – 213.3	
Endothelin-1 [pg/mL]	**Diabetic retinopathy**	**No diabetic retinopathy**	
Median	13.1	10.8	**0.03**
Interquartile	[10.8-21.4]	[10-18.9]	
Min - max	10.8 -42.7	[5.6 – 213.3]	

DISCUSSION

Diabetes is associated with a range of vascular diseases, including atherosclerosis, myocardial infarction and heart failure resulting from endothelial dysfunction.[8] Since diabetes affects small as well as large vessels, diabetic complications are globally classified as microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (heart disease, stroke, and peripheral arterial vasculopathy).[9] Research has revealed the role of endothelin in the pathogenesis of diabetic complications, particularly in the regulation of the capillary flow, which is affected in the course of retinopathy. [10] Endothelins (ETs), pro-inflammatory and vasoconstrictor peptides, are thought to contribute to vascular endothelial dysfunction.[11] ET-1 is the main cardiovascular isoform of the ET system produced primarily in the endothelium, although it can also be produced in vascular smooth muscle cells (VSMCs) of the arterial wall, macrophages, leukocytes, cardiomyocytes, and fibroblasts.[10] ET-1 is a strong vasoconstrictor with mitogenic, pro-oxidative, and proinflammatory properties.[10]

In the present study, the levels of glycated haemoglobin and Endothelin-1 were compared in type 2 diabetics with and without diabetic retinopathy. This study found high serum levels of Endothelin-1 in type 2 diabetics patients with

diabetic retinopathy (13.1 pg/mL [10,89-21,42]) as compared to non-retinopathy diabetics patients (10.8 pg/mL[10-18,9]) with a significant difference (p=0.03). The majority of diabetic patients (75% in DR *vs* 80% in non-DR) showed poor glycemic control.

Glycated haemoglobin and Diabetic retinopathy

About four-fifths of diabetic patients had a high level of HBA1c without significant difference between DR and non-DR diabetic patients. These results suggest that HBA1c or glycemic control was not associated with DR in this study. However, several authors have concluded HBA1c or glycemic control is associated with DR and poor glycemic control (HbA1c>7%) and that increases the risk of DR.[3,56,7] This difference could be a consequence of other issues such as a long duration of diabetes, high blood pressure, and dyslipidemia, which are also known risk factors of DR.[3,7] In this study context, DR could be affected not individually by the known risk factors for DR but collectively.[12] Also considering the research methodologies in the literature, [7] the small size of the study group in the current research could be a reason for this non-association. Future studies may be done to confirm these hypotheses. It is advisable to consider these HbA1c results as warnings, and they are also related to a population for which diabetes monitoring and management must be strengthened. [13]

Endothelin-1 and Diabetes and Diabetic retinopathy

Hyperglycemia induces oxidative stress and upregulation of ET-1, [14] that leads to the augmentation of the capillary permeability and the overproduction of extracellular matrix with the progression of vascular complications.[15]

In the present study, there was a difference in ET-1 levels between diabetic patients and controls [p=0.04]; its level was also higher in patients with diabetic retinopathy compared to patients without retinopathy. In contrast to HbA1c, ET-1 is shown to be proportionally dependent on the condition of the patients in agreement with the already known literature.[2,4] According to Schneider et al., plasma ET-1 concentrations were significantly higher in patients with type 1 diabetes (0.28 ± 0.34 fmol/mL, P = 0.001), type 2 diabetes (0.31 ± 0.32 fmol/mL, P < 0.0001), and hypertension (0.35 ± 0.26 fmol/mL, P < 0.0001) compared to controls (0.08 ± 0.13 fmol/mL). Non-significant associations were found between ET-1 levels and age or vascular complications and a weak association was found between plasma ET-1 levels and glycemic control.[16] Plasma ET-1 level is not a marker of endothelial dysfunction but changes in plasma ET-1 levels may precede vascular complications associated with hypertension and diabetes.[16] These ET-1 findings also highlight a warning and suggest enhanced management of patients in this population as high Endothelin-1 (ET-1) concentrations may reflect the pathogenesis of more advanced diabetic retinopathy (Lam et al., 2003).[4] In their study, Kang et *al*. (2002) found the mean aqueous ET-1 level was significantly higher in the eyes with advanced DR (severe non-proliferative DR or proliferative DR) than those with early DR (no gross DR, mild non-proliferative DR, or moderate non-proliferative DR) and the control group (patients who underwent uncomplicated cataract surgery, had no other concomitant ocular disease, and had no history of diabetes mellitus). Also, they found the mean aqueous ET-1 level was significantly reduced after intravitreal anti-VEGF injections in the advanced DR group (Kang et al., 2022). [2]

Comorbidities

In the present study, although arterial hypertension was found in 41.6% and 28.8% of diabetic retinopathy and non-diabetic retinopathy patients respectively, the difference was not significant. According to the literature, arterial hypertension is an aggravating factor in diabetes. Arterial

hypertension, especially poor control and/or lack of treatment, are associated with the presence of the diabetic retinopathy. [17,18] Our results could be explained by the small sample size.

The decline in renal function was greater in the group with diabetic retinopathy [p=0.001]. It was found in 50% and 4.4% of patients with and without diabetic retinopathy respectively. According to the literature, glomerular filtration rate is an indicator of the deterioration of renal function. Both retina and kidney are susceptible to diabetes-related microangiopathy. Authors have reported that reduced glomerular filtration rate is associated with diabetic retinopathy [19] and its progression[20]. Moreover, a strong association between nephropathy and diabetic retinopathy has been reported by many authors [21, 22]

Despite the small sample size, this study provides data related to ET-1 in an African population. The findings in this work show a significant difference between ET-1 levels in diabetic patients with retinopathy and those without retinopathy. This suggests that ET-1 level could be a better biomarker than HbA1c level in the clinical management of DR in diabetic patients. However, without the same categorization of the DR as various authors, [2,4] this hypothesis remains to be investigated. Thus, further studies with a larger sample size are needed to assess these two markers to better guide and frame their utility in relation to other risk factors for DR and to the different stages of DR in the Cameroonian population. [2,3, 12, 23]

CONCLUSION

In the present investigation, glycated haemoglobin levels were higher in the majority of diabetic patients independently of DR presence or absence. Endothelin-1 levels were significantly higher in diabetic patients and especially in patients with diabetic retinopathy. These levels highlight the potential relevance of Endothelin-1 as a biomarker in the management of diabetic patients. These data encourage further studies to clearly describe the variability of these biomarkers in order to improve their use.

CONFLICT OF INTEREST

No conflict of interest.

FUNDING

None.

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