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RED BLOOD CELL DISTRIBUTION WIDTH AN INFLAMMATORY BIOMARKER RELATED TO PROLIFERATIVE DIABETIC RETINOPATHY

AMPLITUD DE DISTRIBUCIÓN ERITROCITARIA UN BIOMARCADOR INFLAMATORIO RELACIONADO A RETINOPATIA DIABETICA PROLIFERATIVA

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ABSTRACT

Objective: The objective of this study was to determine the association between red blood cell distribution width and proliferative diabetic retinopathy in patients with type 2 diabetes. **Methods:** We conducted a case-control study in a hospital. Adult patients (≥ 18 years) with a diagnosis of Diabetic Retinopathy who underwent medical controls at the Ophthalmology service where they were enrolled in our study. We selected a total sample size of 262 patients, of which 131 cases had proliferative diabetic retinopathy and 131 controls had nonproliferative diabetic retinopathy. Data on age, sex, body mass index, history of hypertension, diabetic nephropathy, congestive heart failure, hemoglobin, and HbA1c were recorded for individuals who met the inclusion criteria. An odds ratio model was used to test the relationship between red blood cell distribution width and proliferative diabetic retinopathy. **Results:** The mean red blood cell distribution width +/- SD of the cases was 14.41 +/- 0.84 and the controls were 13.49 +/- 1.26. According to the bivariate analysis, an association was found between red blood cell distribution width and proliferative diabetic retinopathy (OR 3.79, P = 0.000, CI = 2.12-6.78). Multivariate logistic regression analysis indicated that red blood cell distribution width (OR 2.15, P = 0.037, CI = 1.05-4.43) was an independent risk factor for the development of proliferative diabetic retinopathy. **Conclusion:** Elevated red blood cell distribution width values were related to proliferative diabetic retinopathy, suggesting the possible application of red blood cell distribution width as an accessible predictive biomarker of disease progression in patients with diabetic retinopathy.

Key words: Erythrocyte indices; Diabetic retinopathy; Neovascularization; Pathologic; Diabetes mellitus; Glycated hemoglobin A. (source: MeSH NLM).

RESUMEN

Objetivo: El objetivo de este estudio fue determinar la asociación entre el ancho de distribución de glóbulos rojos y la retinopatía diabética proliferativa en pacientes con diabetes tipo 2. **Métodos:** Realizamos un estudio de casos y controles en el hospital. Pacientes adultos (≥ 18 años) con diagnóstico de retinopatía diabética que se sometieron a controles médicos en el departamento de oftalmología donde se inscribieron en nuestro estudio. Seleccionamos un tamaño de muestra total de 262 pacientes, de los cuales 131 casos tenían retinopatía diabética proliferativa y 131 controles tenían retinopatía diabética no proliferativa. Se registraron datos sobre edad, sexo, índice de masa corporal, antecedentes de hipertensión, nefropatía diabética, insuficiencia cardíaca congestiva, hemoglobina y HbA1c para individuos que cumplían con los criterios de inclusión. Se utilizó el modelo de odds ratio para evaluar la relación entre el ancho de distribución de glóbulos rojos y la retinopatía diabética proliferativa. **Resultados:** El ancho medio de distribución de glóbulos rojos +/- DE de los casos fue 14,41 +/- 0,84 y los controles fueron 13,49 +/- 1,26. Según el análisis bivariado, se encontró una asociación entre el ancho de distribución de glóbulos rojos y la retinopatía diabética proliferativa (OR 3,79, P = 0,000, IC = 2,12-6,78). El análisis de regresión logística multivariante indicó que el ancho de distribución de glóbulos rojos (OR 2,15, P = 0,037, IC = 1,05-4,43) fue un factor de riesgo independiente para el desarrollo de la retinopatía diabética proliferativa. **Conclusión:** Los valores elevados del ancho de distribución de glóbulos rojos se relacionaron con la retinopatía diabética proliferativa, lo que sugiere la posible aplicación del ancho de distribución de glóbulos rojos como un biomarcador predictivo accesible de la progresión de la enfermedad en pacientes con retinopatía diabética.

Palabras clave: Índices de eritrocitos; Retinopatía diabética; Neovascularización patológica; Diabetes mellitus; Hemoglobina A glucada (fuente: DeCS BIREME).

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INTRODUCTION

Diabetic retinopathy (DR), with a prevalence of 34%, is the most frequent microvascular complication among patients with type 2 diabetes mellitus. the third cause of blindness worldwide and the first in the economically active population[1,2]. Its pathophysiology with cyclical events, in a crescendo of inflammation and oxidative stress generated by toxic levels of glucose in the retinal capillary, are crucial factors in its genesis and evolution[3,4]; Neoangiogesis is a critical point for the early stages, nonproliferative diabetic retinopathy (NPDR), and late, proliferative diabetic retinopathy (PDR)[4,5,6].

The erythrocyte distribution width (RDW) is the coefficient of variation of the erythrocyte corpuscular volume, which represents in percentage terms the variability in erythrocyte size[7,8], which at present has been recognized as an inflammatory biomarker being found an association with inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate[9]; this has been found associated with both infectious and non-infectious, acute and chronic inflammatory pathologies[10]; and elevated in pathologies associated with neovessels, that is, neoplastic pathologies and associated with granulomas[11,12,13].

Recent studies have found an association between RDW and chronic complications associated with diabetes mellitus[14,15], especially diabetic nephropathy[16], a microvascular complication that has also been found associated with DR[17,18,19]. In our bibliographic review, few studies have been carried out to date for the RDW and RD relationship, finding discrepancies in results[14,15,20]. No studies were found for the RDW and PDR relationship.

The following article set out to determine the association between RDW and RDP in patients with type 2 diabetes mellitus. Determining this relationship will be essential for future preventive, prognostic, and therapeutic measures regarding this microvascular complication.

METHODS

Design and study area

The present study had an unpaired case-control analytical design, prepared at the Research Institute in Biomedical Sciences of the Ricardo Palma University and carried out in the Ophthalmology department of the Hospital National Edgardo Rebagliatti Martins, in Lima, Peru, during the year 2017 between January to December.

All patients with diabetic retinopathy who had complete blood count and glycosylated hemoglobin exams updated within the last 3 months, an evaluation by the cardiology service, and an evaluation by the nephrology service to rule out complications to end organs were included. Those patients with type 1 diabetes mellitus, acute or chronic infections, systemic and/or ocular collagen disease, chronic obstructive pulmonary disease, history of cancer and/or treatment with radiation or chemotherapy were excluded from the study.

Procedures and variables

The diagnosis of diabetic retinopathy was made using a fundus examination using a slit lamp after pupillary dilation. RD was described and classified in RDNP and RDP according to the American Academy of Ophthalmology according to the eye in the most severe state. The diagnoses of congestive heart failure, arterial hypertension, and diabetic nephropathy were taken from the evaluation given by the cardiologist and endocrinologist. The RDW is the coefficient of variation of the corpuscular volume of the red blood cell, represented as a percentage, which allows us to determine its degree of variation, its cut-off point is 14.5% when it is high, anisocytosis is reported. HbA1c is the percentage representation of glucose bound to hemoglobin through non-enzymatic glycosylation, 6.5% is the cut-off point for the diagnosis of diabetes mellitus by the American Diabetes Association (ADA).

The complete blood count, glycosylated hemoglobin, history of arterial hypertension, diabetic nephropathy, and heart failure were obtained retrospectively, taking the medical history as a source of information. The weight and height data for the calculation of the body mass index were taken during the consultation with the patient, using a scale calibrated in kilograms using up to one decimal place and a standardized 1.99-meter wooden height rod using up to two decimal places for its measurement. respectively.

Population and sample

The OpenEpi statistical package was used to calculate the sample size of unpaired case-control type design, with a statistical power of 80%, a 95% confidence interval, a percentage of exposed controls of 50%, a case-control ratio of 1: 1 and an expected Odds Ratio of 2.1. A sample size of 131 cases with PDR and 131 controls with PNR was obtained using the Fleiss

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formula with continuity correction.

Ethical Issues

It was approved by the ethics committee of the Hospital National Edgardo Rebagliatti Martins, the approval of the head of the retina service of said hospital, and the acceptance of INICIB to carry out the data collection.

Statistical analysis

The STATA statistical package was used for the univariate analysis of relative frequencies of the qualitative variables and the mean and standard deviation for quantitative variables. In the bivariate analysis for qualitative variables, the chi-square test was used for sample homogeneity between cases and controls, in turn for quantitative variables, the Shapiro-France normality test was used for normality, the non-parametric test for the difference of medians U of Mann Whitney, for these tests a critical value of 0.05 was taken; To determine the strength of association, the statistical model Odds ratio was used. In the multivariate analysis, an Odds ratio adjusted for confounding variables was performed.

RESULTS

The total sample consisted of 262 participants, of which 131 were cases with PDR and 131 controls with NPDR. The medical records of each of the study subjects were found when data collection was carried out, so there was no missing data.

In the quantitative analysis, the RDW test was of 14.41% ± 0.84% found for the cases and 13.49% ± 1.26% for the controls, a significant statistical difference of P = 0.0000 was found, at its Once the HbA1c had 6.88 +/- 0.55 for the cases and 6.53 +/- 1.12 for the controls, and one found a statistically significant difference of P = 0.0002, table 1.

For the qualitative analysis, a high RDW of 80.15% for the cases and 53.44% for the controls, finding a statistically significant difference (P = 0.000), in turn, a high HbA1c of 83.97% for the cases and 58.02% for the controls with a statistically significant difference (p = 0.000), table 2.

In the bivariate analysis, a statistically significant association was found between RDW and RDP (OR 3.79 P = 0.000 CI = 2.12-6.78), HbA1c and RDP (OR 3.52 P = 0.000 IC = 2.03-6.10), NFD (OR 2.19 P = 0.002 IC 1.33-3.61), Age Group (OR 2.29 P = 0.010 IC 1.22-4.30), in Table 3 shows these and other results obtained.

Finally, a multivariate analysis was performed obtaining an adjusted OR with a statistically significant relationship for the RDW variables (OR 2.15 P = 0.037 IC = 1.05-4.43) and HbA1c (OR 2.28 P = 0.026 IC = 1.10-4.69) in relation to the PDR, Table 4 shows these other results obtained.

Table 1. Quantitative univariate analysis.

Variables	Cases	Controls	Normality test	Statistical test
			Shapiro France	Mann Whitney U
Age	64.58+/-5.02	61.67+/-6.16	0.00007	P=0.0001
RDW	14.41+/-0.84	13.49+/-1.26	0.00001	P=0.0000
Hemoglobin	13.44+/-1.23	13.56+/-1.03	0.03321	P=0.3782
Hba1c	6.88+/-0.55	6.53+/-1.12	0.00001	P=0.0002
BMI	29.34+/-1.97	28.74+/-2.22	0.00001	P=0.0023

Abbreviations: RDW. Red cell distribution width, HbA1c. Glycated hemoglobin, BMI. Body mass index.

Table 2. Quantitative univariate analysis.

Variable	Case	Control	Statistical test
Hb1Ac			
≥6.5 %	110(83.97%)	Si: 76 (58.02%)	P= 0.000
<6.5 %	21(16.03%)	No: 55 (41.98%)	
RDW			
≥ 14.5%	Yes: 105(80.15%)	Yes: 70 (53.44%)	P=0.000
< 14.5%	No: 26(19.85%)	No: 61 (46.56%)	
Anemia			
Hb <11 gr/dL	11(8.40%)	8 (6.11%)	P=0.475
Hb ≥ 11 gr/dL	120 (91.60%)	123 (93.89%)	
Gender			
Male	67 (51.15%)	71(54.20%)	P=0.621
Female	64 (48.85%)	60 (45.80%)	
Age group			
≥ 60 years	113 (83.26%)	96 (73.28%)	P=0.009
< 60 years	18 (13.74%)	35 (26.72%)	
Congestive heart failure			
Yes	26 (19.85%)	22 (16.79%)	P=0.523
No	105 (80.15%)	109 (83.21%)	
Hypertension			
Yes	79 (60.31%)	70 (53.44%)	P=0.262
No	52 (39.69%)	61 (46.56%)	
Diabetic nephropathy			
Yes	86 (65.65%)	61 (46.56%)	P=0.002
No	45 (34.35%)	70 (53.44%)	
Obesity			
BMI ≥30	77 (58.78%)	64 (48.85%)	P=0.107
BMI <30	54 (41.22%)	67 (51.15%)	

Abbreviations: Hb1Ac. Glycated hemoglobin, RDW. Red cell distribution width, Hb. Hemoglobin, BMI. Body mass index.

Table 3. Bivariate analysis.

Variables	OR	p	IC
HbA1c	3.52	0.000	2.03-6.10
RDW	3.79	0.000	2.12-6.78
Age group	2.29	0.010	1.22-4.30
NFD	2.19	0.002	1.33-3.61

Abbreviations: HbA1c. Glycated hemoglobin, RDW. Erythrocyte distribution width, NfD. Diabetic nephropathy.

Table 4. Multivariate analysis.

Variable	OR	p	IC 95%
HbA1c	2.28	0.026	1.10-4.69
RDW	2.15	0.037	1.05-4.43
Age group	1.65	0.142	0.84-3.23
NFD	0.97	0.925	0.50-1.86

Abbreviations: HbA1c. Glycated hemoglobin, RDW. Erythrocyte distribution width, NFD. Diabetic nephropathy.

DISCUSSION

Our study is the first to find an association for an RDW> 14.5% for PDR Both by bivariate and multivariate analysis, the main limitations of our study are that it was uni-centric, the data were collected only from one hospital, it was not possible to quantify other inflammatory markers such as C-reactive protein, fibrinogen, sedimentation rate for its Compared with RDW, the study design is not prospective and does not allow a causal relationship to be established.

DR is the most common microvascular complication of diabetes mellitus, and this complication is the leading cause of blindness in the economically active population[1,2,3]. Its pathogenesis, not yet clarified, involves intermittent and sustained toxic levels of glucose in the retinal capillary, deleteriously affecting the retinal vascular lesions, that is, endothelium, pericytes, glia, and retinal neurons. By altering its function and predisposing to a retinal environment in favor of inflammation, thickening of the basement membrane and extracellular matrix increased capillary permeability, advanced glycosylation products, the formation of free radicals, thrombosis, necrosis and/or apoptosis of cells that make up said unit, chemotaxis of nuclear polymorphs and hypoxia. this will have a breaking point when this microenvironment begins to generate high values of pro-angiogenic and chemotactic molecules for fibroblasts[3,4,5,8,21]. This induces the deposition of granulation tissue and the formation of neovessels, giving way to the late stage of diabetic retinopathy, the proliferative state[5,21], this inflammatory progression related to glycemia could explain why HbA1c and RDW are higher in PDR patients, the advanced stage.

An association has been reported between the microangiopathic complications of DR and diabetic nephropathy (DN)[18], finding DN as a risk factor in the development and progression of DR[16], which could be due to the deleterious effect of elevated systemic glucose levels that damage the hemiretinal and glomerular barriers[4,19], our study did not find a relationship for PDR and DN, which could be explained by its retrospective design.

In our bibliographic review, a discrepancy of results was found in the authors who have searched for the relationship between RD and RDW; Magri et al in 2013[15] reported an absence of statistically significant association when relating these variables, as did Malandrino et al.[14] who divided the variable of the red cell distribution width into quartiles, finding no association in the 3rd quartile (OR 1.09 CI 0.61-1.97) or in the 4th quartile (OR 1.06 CI 0.37-3.03), unlike Kurtul et al.[20] who in 2016 found a statistically significant association for RD and RDW (p = 0.036 OR 1.69 CI 1.036 -2,763), we propose that this discordance of results may be due to the presence of both stages of DR, the RDNP and RDP in the same group of analyzes, based on the fact that our results find a relationship between the RDW and RDP, taking the RDNP as control.

We recommend future prospective, multicenter studies, with higher statistical power, with the ability to confirm our results. The next step is to evaluate the possible relationship of RDW with angiogenic processes and biological markers of angiogenesis.

Our study suggests that RDW would not only be a strong predictor of diabetic retinopathy, but also a marker of microvascular progression, showing the transition from NPDR to PDR, serving the clinician as an additional factor in the progression of the disease.

CONCLUSION

This is the first study to establish a statistically significant relationship between RDW and RDP. A

relationship was found for elevated levels of RDW and RDP by bivariate and multivariate analysis. We can conclude that the red cell distribution width could be a predictive biomarker for PDR and should be taken into account when evaluating patients with NPDR. We recommend prospective studies for the RDW and RDP relationship.

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