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Article

Altered erythrocyte morphology in Mexican adults with prediabetes and type 2 diabetes mellitus evaluated by scanning electron microscope

Alejandra Loyola-Leyva[1], Juan Pablo Loyola-Rodríguez[2,*], Yolanda Terán Figueroa[3], Francisco Javier González[4], Marco Atzori[5], and Simón Barquera Cervera[6]

[1]School of Medicine, Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí), Avenida Venustiano Carranza 2405, Los Filtros, 78210, San Luis Potosí, S.L.P, Mexico, [2]Laboratory of Nanobiotechnology, Faculty of Medicine, Autonomous University of Guerrero (Universidad Autónoma de Guerrero), Acapulco, Guerrero, Mexico, [3]Faculty of Nursing and Nutrition, Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí), Av. Niño Artillero 130. Zona Universitaria, 78240, San Luis Potosí, S.L.P, México, [4]Coordination for Innovation and Application of Science and Technology (CIACyT), Avenida Sierra Leona 550, Lomas 2ª sección, 78210, San Luis Potosí, S.L.P, México, [5]Faculty of Sciences, Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí), Lateral Av. Salvador Nava, Lomas, 78290, San Luis Potosí, S.L.P, México, and [6]National Institute of Public Health (Instituto Nacional de Salud Pública), Av. Universidad No.655 Col Sta. Ma. Ahuacatitlán. Cuernavaca, Morelos, México

[*]To whom correspondence should be addressed. E-mail: juanpablo.loyola8@gmail.com

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Abstract

Aim: To evaluate the erythrocyte morphology in people with prediabetes, T2DM and healthy subjects in a Mexican population and its association with biochemical parameters.

Methods: Cross-sectional study consisted of three groups: healthy (HG), people with prediabetes (PG) and with T2DM (DMG). A blood sample was obtained from all participants to assess the erythrocyte morphology, and levels of HbA1c, glucose and lipid profile. Anthropometrical parameters were also evaluated.

Results: It was observed that compared with healthy individuals, people with prediabetes presented a significant decrease in the diameter (−0.08 μm, $P = 0.014$) and height (−0.07 μm, $P = 0.004$), as well as people with T2DM (−0.33 μm, $P < 0.001$ in diameter; and −0.36 μm, $P < 0.001$ in height). Besides, it was found a significant difference in diameter (−0.25 μm, $P < 0.001$) and height (−0.29 μm, $P < 0.001$) between the PG and DMG. No significant differences in the axial ratio between groups. Also, HbA1c, glucose, triglycerides, cholesterol, LDL cholesterol, systolic blood pressure, weight, BMI, waist and hip circumference were significantly associated with diameter and height.

Conclusions: Erythrocyte morphological alterations can serve as an indicator of early diagnosis of T2DM and a factor implicated in the course of the clinical condition, so the correction of these alterations could serve as a treatment for prediabetes and T2DM. It is essential to promote constantly checkups of biochemical and anthropometrical parameters, as well as erythrocyte morphological alterations to prevent the onset of prediabetes and T2DM and possible clinical complications.

Key words: erythrocytes, morphology, type 2 diabetes mellitus, prediabetes, scanning electron microscopy

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most significant public health problems and a risk factor for cardiovascular disease (CVD) [1]. In 2016, T2DM and CVD were the leading causes of death in Mexico [2]. In that same year, Mexico had a prevalence of 9.4% for T2DM (previous diagnosis), 28% for hypercholesterolemia and 25.5% for hypertension [3]. Prediabetes also presents a high prevalence in the Mexican population (33% in the 18-year population [4]) and is associated with an increased risk for T2DM and CVD [5].

On the other hand, erythrocyte alterations have been reported in people living with T2DM. During chronic inflammation, erythrocyte can suffer biochemical, biophysical or rheological changes. These alterations can be used to determine the severity of the disease or to follow the treatment progress of each patient [6].

The biochemical changes of erythrocytes are produced in the molecular arrangement (e.g. loss in the asymmetry of phospholipids of the membrane, an increase in cholesterol and cytoskeleton proteins degradation). Most of these changes are due to the rise in the intracellular Ca^{2+} concentration (due to oxidative stress, hyperosmotic shock or ATP depletion). These biochemical changes produce biophysical erythrocyte morphology alterations, such as cell shrinkage, membrane blebbing and scrambling [6,7]. These biophysical alterations, create modifications in the structural cell deformability (capacity of the erythrocyte to change shape to pass the microcirculation) and in the rheology and hemorheology, which plays an essential role in the pathogenesis of diseases like T2DM [8,9], hypertension [10] and CVD [11].

Therefore, these erythrocyte morphological alterations may be an indicator of disease and a factor implicated in the course of the clinical condition. Besides, it is essential to identify if biochemical or anthropometrical factors could be involved in these erythrocyte morphological alterations, and then the correction of these factors could serve to prevent or as an indicator of clinical follow-up of treatment for T2DM and CVD [12].

The light microscope has been the most frequent method for the evaluation of the erythrocyte morphology. However, this technique does not supply sufficient magnification to detect all of the alterations; therefore these cells may end up being erroneously classified as unaltered, while actually, they possess distorted shapes. For this reason, the scanning electron microscope (SEM) has been used to assess some of the erythrocytes abnormalities in different pathologies [13,14]. Some advantages that make the SEM a better morphology analysis tool are: (1) the erythrocyte preparation does not involve staining the samples and it is probably easier than focusing a light microscope; (2) it has a broad area view (several mm) of the variations in surface structure that can be acquired all at once, and (3) it has a considerable depth of field responsible for the three-dimensional appearance of the specimen. However, a limitation of this instrument is that it is not widely available [15,16].

There are few studies in the world that had compared qualitative information of the erythrocyte morphology of T2DM people with healthy subjects using SEM [17,18]. These studies observed that erythrocytes membranes of people with T2DM are smoother, presented projections and had elongated morphology compared to healthy participants. Besides, to our knowledge, it has not been determined the erythrocyte morphology of people with prediabetes. Therefore, this study aimed to assess the erythrocyte morphology by scanning electron microscope (SEM) in people with prediabetes, T2DM and healthy subjects in a Mexican population, and to evaluate the association between erythrocyte morphology changes and biochemical parameters.

Methods

This cross-sectional and analytical study was conducted from March 2018 to July 2018 at the Faculty of Nursing and Nutrition at the Autonomous University of San Luis Potosí (UASLP) in San Luis Potosí, Mexico.

Participants and recruitment

The study comprised three study groups: healthy (HG), people with prediabetes (PG) and people with T2DM (DMG). Participants were females and males between 18 and 60 years old selected from the Laboratory of Clinical Analysis at the Faculty of Nursing and Nutrition, UASLP and from a Mexican company that manufactures domestic appliances and has a health campaign every year with their workers by non-probabilistic consecutive sampling.

The inclusion criteria for HG was having a healthy body mass index (BMI) (18.5–24.9 kg/m^2), a HbA1c value of <5.7% and Finnish Diabetes Risk Score (FINDRISC) score ≤7 points; for PG a HbA1c value between 5.7% and 6.4% and a FINDRISC score ≥14 points; for DMG a T2DM diagnosis of >1 year, a HbA1c value of ≥7%, without clinical complications. The exclusion criteria for the three groups were: having Alzheimer diagnosis, a recent infection, smokers, being pregnant or taking insulin.

Study design

First, a clinical record and the FINDRISC (Finnish Diabetes Risk Score) questionnaire were completed. This questionnaire was used to identify people with prediabetes because it is a validated risk assessment tool to predict T2DM within the next 10 years. The score value can be classified as low (<7), slightly elevated (7–11), moderate (12–14), high (15–20) and very high risk (21–26) [19].

As well, standardized personnel evaluated anthropometric measurements and blood pressure. The height was assessed with the subject barefoot and recorded to the nearest 0.1 cm (Torino Persona Plus), and the weight was evaluated with the subject wearing minimal clothing and recorded to the nearest 0.1 kg (Omron HBF 514 C). Then, the BMI was calculated with the height and weight (kg/m^2) and classified in normal weight (18.5–24.9), overweight (25–29.9) and obesity (≥30) [20]. Also, waist circumference was measured with a metallic diameter tape (Lufkin executive thinline) directly on the skin at the middle point between the iliac crest and the last rib edge at the end of a normal exhalation; the obtained values were classified as normal and abdominal obesity (>85 cm for women and >90 cm for men) [21]. As well, the hip circumference located around the widest portion of the buttocks was measured. With the waist and hip measurements the cardiovascular risk was calculated (waist cm/hip cm) and classified as low risk and high risk (>0.85 in women and >90 in men) [21]. The systolic and diastolic blood pressure was assessed after participants had rested in a seated position for 5 min using a digital sphygmomanometer (Omron HEM 7320). All measurements were taken twice, and the mean of the two valid measurements was used in the analysis.

Finally, if the participant fulfilled the inclusion criteria, a blood sample was obtained in EDTA tubes and tubes without anticoagulant at the subject´s appointment at the Clinical Analysis Laboratory.

Informed written consent was obtained from all subjects before collecting data and blood samples. The Ethics Committee of Research of the Faculty of Nursing and Nutrition of the UASLP (CEIFE-2018-258) approved the study.

Sample preparation

Each EDTA blood sample was separated into two parts: one part was used to evaluate the levels of HbA1c (Glycohemoglobin Reagent Set, Teco Diagnostics) and the other part was used to assess the erythrocytes morphology. The blood from the tube without anticoagulant was used to evaluate glucose, cholesterol, triglycerides, LDL cholesterol and HDL cholesterol (Spinreact).

For the erythrocyte morphology analysis, the sample preparation consisted of centrifuge 1 ml of whole blood from the EDTA tube. Then, the remaining supernatant was discarded (plasma, platelets and white blood cells) and the remaining pellet (erythrocytes) was fixed in 2.5% of glutaraldehyde for 30 min and washed three times with PBS. Then, it was fixed with 1% osmium tetroxide, rinsed with distilled water [17] and a drop was placed in silicon oxide and air-dried. Finally, the sample was sputtered with gold for 15 s.

SEM imaging

For each study participant, 30 erythrocytes were assessed to compare the morphological changes between study groups. In all of the erythrocytes, it was measured the diameter (longest axis from the erythrocyte) and the height (the minor axis length, which is the perpendicular line drawn in the center of the major axis). Then the axial ratio was calculated by dividing the diameter by the height, which is an indicator of the roundness of the cell (a value of 1 indicates a perfect circle) [18].

Therefore, 1050 cells were scanned to assess the morphological changes. All samples were viewed with the SEM Inspect F50 (FEI Company, Eindhoven, Netherlands) at the Terahertz Science and Technology National Lab (LANCYTT, San Luis Potosí, Mexico). The conditions to obtain the images were 30.0 kV and a spot of 3.0.

Statistical analysis

The primary outcomes were the changes in the erythrocytes morphology (diameter, height, axial ratio) compared

by study groups (HG, PG and DMG). Shapiro–Wilk tests were used to verify normality and homogeneity of all variables. Statistical tests such as Fisher exact test (categorical variables), and Kruskal–Wallis test (continuous variables without normal distribution or with small samples) were performed. Also, multiple linear regression models were run to evaluate the association between erythrocytes height (dependent variable) and risk factors for T2DM and CVD.

All data are expressed as a mean and standard deviation or as frequency and proportion. All analyses were performed using STATA version 13 (Stata Corp., College Station, Texas).

Results

In this cross-sectional study, 88 participants were eligible, but only 35 met all the inclusion criteria. Therefore, the final sample was $n = 15$ in the HG, $n = 12$ in the PG and $n = 8$ in the DMG (Fig. 1). All of the participants of the PG were diagnosed during the study (prediabetes prevalence of 25%).

The mean age of the total sample was 31.20 ± 12.9, and 80% were women. People with T2DM had an average time of diagnosis of 4.4 ± 4.1 years (one participant was diagnosed during the study), and all of them took medication such as metformin, glibenclamide, pravastatin or a combination of drugs. However, 50% ($n = 4$) of the DMG had uncontrolled values of HbA1c (>7%) with a mean of 8.0 ± 0.46.

In Table 1 it can be observed a significant difference in age, HbA1c, glucose, triglycerides, diastolic and systolic blood pressure, weight, BMI, waist, hip and waist-to-hip ratio between the study groups. However, compared to the healthy group, people with T2DM had the highest values in the biochemical parameters, and the PG had the highest values in almost all the anthropometric parameters.

Erythrocyte morphological changes

In Fig. 2 it can be observed qualitative information of the erythrocyte morphology, multiple morphologies were found in the blood sample of each study group. Besides, it can be seen that erythrocytes with abnormal morphologies lost their concavity or change its location in the PG and DMG, and some of them presented a smoother membrane. In all of the study groups different morphologies such as discocytes, spherocytes and acanthocytes were observed. However, only the PG and DMG presented abnormally shaped erythrocytes (poikilocytosis) that could not be classified conventionally into discocytes, spherocytes or acanthocytes.

In Table 2 is shown quantitative information regarding erythrocyte morphology alterations. It can be observed that erythrocytes in the DMG compared with HG have a decreased diameter (−0.33 µm, $P < 0.001$) and height (−0.36 µm, $P < 0.001$). Also, PG compared with the HG had a significant decrease in diameter (−0.08 µm, $P = 0.014$) and height (−0.07 µm, $P = 0.004$). Besides, it was found a

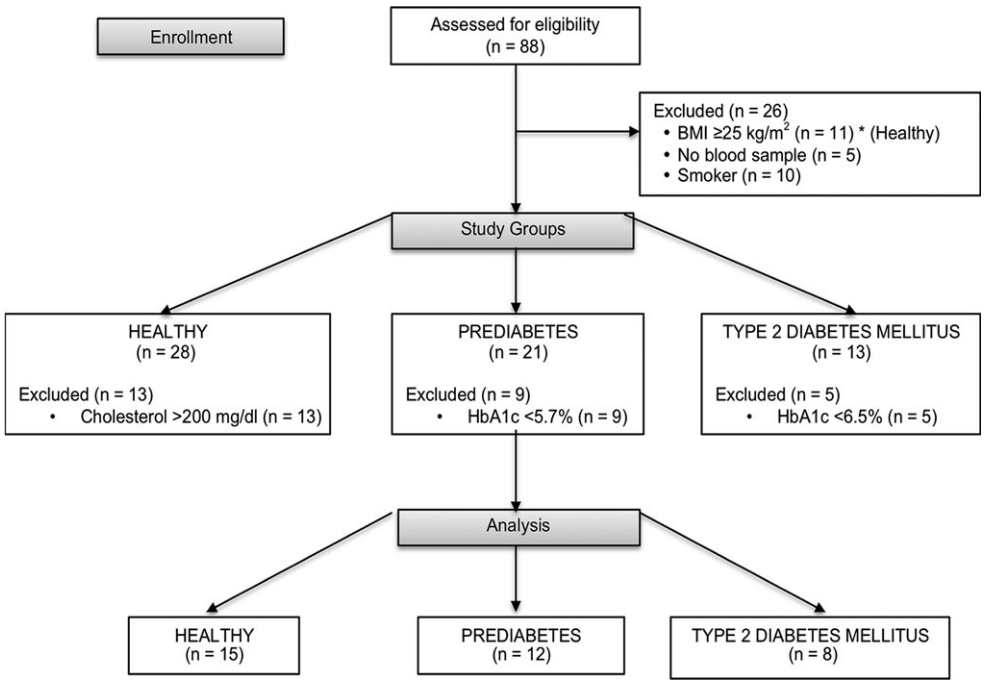

Fig. 1. Flowchart of participants' eligibility.

Table 1. Characteristics of the study sample

Variables	Healthy group	Prediabetes group Mean ± SD Proportion (*n*)	T2DM group	*P* value
Sex				0.649[d]
Female	87 (13)	75 (9)	75 (6)	
Male	13 (2)	25 (3)	25 (2)	
Age (years)	21.9 ± 2.8[a,b]	33.9 ± 10.7	46.6 ± 13.5	**0.000**[e]
FINDRISC points	3.9 ± 2.3	11.5 ± 6.6	–	**0.000**[f]
T2DM risk			–	**0.002**[d]
Low risk	87 (13)	36 (4)		
Slightly elevated	13 (2)	0 (0)		
Moderate risk	0 (0)	36 (4)		
High risk	0 (0)	28 (3)		
HbA1c (%)	4.6 ± 0.6 [a,b]	5.9 ± 0.2[c]	7.4 ± 0.7	**0.000**[e]
Glucose (mg/dl)	87.5 ± 11.1[b]	82.5 ± 9.4[c]	131.4 ± 49.4	**0.008**[e]
Cholesterol (mg/dl)	155.8 ± 31.0[b]	156.5 ± 36.4	193.8 ± 38.4	0.070[e]
Triglycerides (mg/dl)	89.2 ± 33.3[b]	119.3 ± 62.7[c]	231.4 ± 97.7	**0.003**[e]
HDL (mg/dl)	51.6 ± 21.9	52.8 ± 15.7	53.9 ± 16.5	0.892[e]
LDL (mg/dl)	87.3 ± 20.4	80.1 ± 32.4	93.9 ± 40.6	0.586[e]
Creatinine (mg/dl)	0.75 ± 0.18	0.70 ± 0.17	0.90 ± 0.40	0.349[e]
Urea (mg/dl)	30.6 ± 10.2	28.5 ± 7.4	25.3 ± 8.7	0.365[e]
BUN (mg/dl)	14.3 ± 4.8	13.4 ± 3.6	11.7 ± 4.1	0.375[e]
Uric acid (mg/dl)	4.6 ± 1.4	5.1 ± 1.1	5.4 ± 1.3	0.379[e]
Blood pressure (mmHg)				
Systolic	101.3 ± 7.5[b]	104.7 ± 13.0[c]	120.5 ± 12.0	**0.015**[e]
Diastolic	67 ± 4.3[a,b]	74.6 ± 8.2	76.5 ± 4.2	**0.016**[e]
Weight (kg)	54.7 ± 8.2[a,b]	80.0 ± 18.1	71.9 ± 13.8	**0.001**[e]
BMI (kg/m^2)	20.5 ± 2.3[a,b]	29.4 ± 6.7	28.2 ± 4.3	**0.000**[e]
Waist (cm)	67.3 ± 5.7[a,b]	90.4 ± 16	88.3 ± 12.5	**0.000**[e]
Hip (cm)	91.9 ± 5.6[a,b]	106.4 ± 11.4	98.5 ± 5.9	**0.002**[e]
Waist-to-hip ratio	0.73 ± 0.03[a,b]	0.86 ± 0.09	0.89 ± 0.12	**0.001**[e]
Abdominal obesity				**0.000**[d]
No	100 (15)	33 (4)	25 (2)	
Yes	0 (0)	67 (8)	75 (6)	
Cardiovascular risk				**0.000**[d]
Low	100 (15)	67 (8)	25 (2)	
High	0 (0)	33 (4)	75 (0)	

FINDRISC, Finnish Diabetes Risk Score; T2DM, type 2 diabetes mellitus; BUN, blood urea nitrogen; BMI: body mass index.
Bold values, $P < 0.05$.
[a]Significant difference between healthy group and prediabetes group.
[b]Significant difference between healthy group and T2DM group.
[c]Significant difference between prediabetes group and T2DM group.
[d]Fisher test.
[e]Kruskal–Wallis test.
[f]Student-*t* test.

significant difference in diameter (−0.25 μm, $P < 0.001$) and height (−0.29 μm, $P < 0.001$) between the PG and DMG. However, no differences were observed in the axial ratio between groups.

Biochemical and anthropometrical parameters associated with morphological changes

About the variables that were correlated with the morphological parameter, it was observed a significant negative correlation between diameter and HbA1c, glucose, triglycerides, systolic blood pressure, weight, BMI, waist, hip and waist-to-hip ratio. Besides, a significant negative correlation was found between height and HbA1c, glucose, triglycerides, systolic and diastolic blood pressure, BMI, waist and hip. Also, glucose had a positive correlation with the axial ratio (Table 3).

In the linear regression analysis, variables such as glucose, triglycerides, systolic blood pressure, weight, BMI,

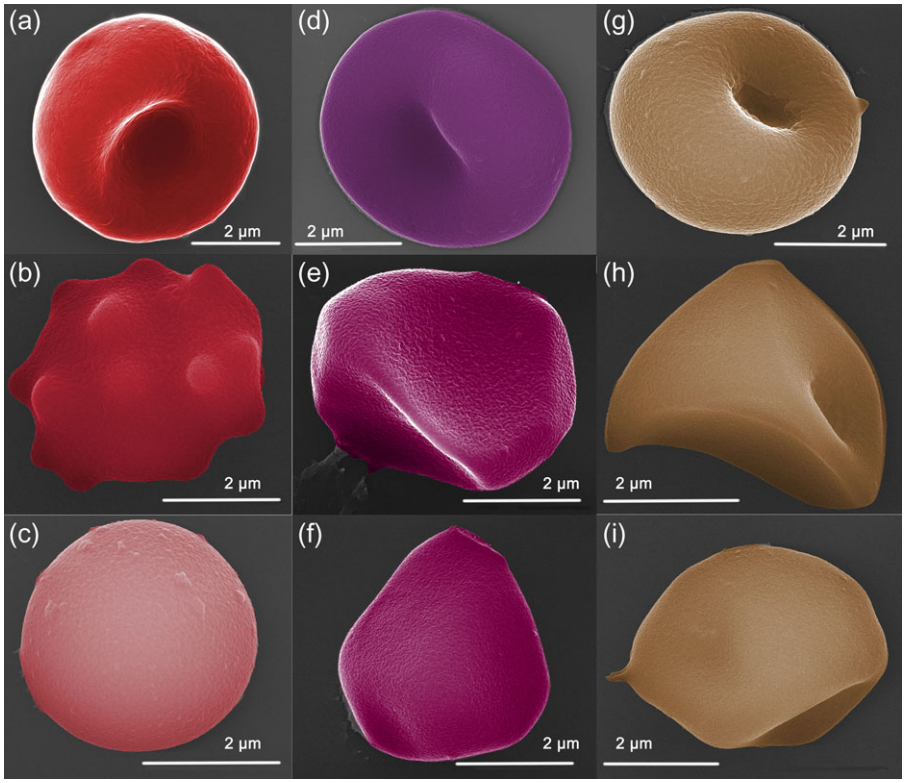

Fig. 2. SEM images of erythrocytes morphology by study group, a to c are erythrocytes found in the HG, d to f are erythrocytes found in the PG group, g to i are erythrocytes found in the DMG. (a, d and g) are discocytes, (b) is an acanthocyte, (c) is a spherocyte, (e, f, h and i) are rare morphologies found in some of the blood samples of the PG and DMG group.

Table 2. Erythrocyte morphological parameters by study group

Variables	Healthy group	Prediabetes group	Type 2 diabetes group	*P* value
	Mean ± SD			
Diameter (µm)	4.98 ± 0.53[a,b]	4.90 ± 0.55[c]	4.65 ± 0.46	**0.0001**[d]
Height (µm)	4.37 ± 0.45[a,b]	4.30 ± 0.47[c]	4.01 ± 0.38	**0.0001**[d]
Axial ratio	1.12 ± 0.08	1.13 ± 0.09	1.14 ± 0.09	0.171[d]

Bold values, $P < 0.05$.
[a]Significant difference between healthy group and prediabetes group.
[b]Significant difference between healthy group and T2DM group.
[c]Significant difference between prediabetes group and T2DM group.
[d]Kruskal–Wallis.

waist, and hip statistically predicted erythrocyte´s diameter and these variables accounted from 9.3% to 19.9% of the explained variability (data not shown). Similar findings were observed in height due to age, HbA1c, triglycerides, systolic blood pressure, weight, BMI, waist and hip statistically predicted from 8.4% to 17.7% of the explained variability (data not shown).

Table 4 illustrates different multiple linear regression models run to predict the effect of biochemical and anthropometric parameters in the diameter and height. The adjusted models explained significantly 43.5–63.7% of the total variance in the erythrocyte's diameter and height values. Variables such as age, HbA1c, glucose, cholesterol, triglycerides, LDL cholesterol, BMI, waist and hip were significantly associated with the diameter (Model 1D and 2D) and height (Model 1 H and 2 H) in the different regression models. However, some variables such as age, cholesterol, LDL cholesterol and systolic blood pressure (that were or were not significantly correlated with diameter and height) showed a different effect when adjusted with other variables in the regression analysis. These results suggest that those variables could have an impact in the erythrocytes morphology alterations when adjusted by other variables.

Table 3. Correlation between biochemical and anthropometric parameters with erythrocyte morphological parameters

	Diameter (μm)		Height (μm)		Axial ratio	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	−0.186	0.292^{s}	−0.271	0.121^{s}	−0.156	0.378^{s}
HbA1c (%)	−0.370	**0.026**^{p}	−0.513	**0.002**^{s}	0.233	0.179^{p}
Glucose (mg/dl)	−0.472	**0.004**^{s}	−0.391	**0.020**^{s}	−0.426	**0.013**^{s}
Cholesterol (mg/dl)	0.051	0.770^{p}	0.019	0.913^{s}	0.130	0.455^{p}
Triglycerides (mg/dl)	−0.337	**0.048**^{s}	−0.357	**0.035**^{s}	0.064	0.714^{s}
HDL (mg/dl)	0.176	0.311^{s}	0.169	0.332^{s}	−0.045	0.798^{p}
LDL (mg/dl)	0.150	0.388^{p}	0.154	0.377^{s}	0.102	0.559^{p}
Creatinine (mg/dl)	−0.269	0.118^{s}	−0.185	0.288^{s}	−0.292	0.089^{s}
Systolic blood pressure (mmHg)	−0.037	**0.050**^{p}	−0.414	**0.029**^{s}	0.064	0.746^{p}
Diastolic blood pressure (mmHg)	−0.352	0.066^{p}	−0.443	**0.018**^{s}	−0.121	0.538^{p}
Weight (kg)	−0.547	**0.000**^{s}	−0.432	**0.011**^{s}	−0.162	0.360^{s}
BMI (kg/m^2)	−0.545	**0.000**^{s}	−0.528	**0.001**^{s}	0.027	0.879^{s}
Waist (cm)	−0.498	**0.003**^{s}	−0.438	**0.009**^{s}	−0.079	0.658^{s}
Hip (cm)	−0.521	**0.002**^{s}	−0.467	**0.005**^{s}	−0.147	0.408^{s}
Waist-to-hip ratio	−0.342	**0.048**^{s}	−0.310	0.075^{s}	0.019	0.912^{s}

BMI, body mass index.
Bold values, $P < 0.05$.
s: Spearman correlation; p: Pearson correlation.

Table 4. Multiple linear regression with diameter and height

	Diameter				Height			
	Model 1D		Model 2D		Model 1H		Model 2H	
	β_1	*P*	β_1	*P*	β_1	*P*	β_1	*P*
Age (years)	−0.007	0.294	−0.004	0.393	−0.002	0.703	−0.009	**0.028**
HbA1c (%)	0.025	0.744	–	–	−0.132	**0.038**	–	–
Glucose (mg/dl)	−0.009	**0.045**	−0.007	0.067	–	–	−0.004	0.196
Cholesterol (mg/dl)	–	–	0.006	**0.003**	0.002	0.168	–	–
Triglycerides (mg/dl)	−0.001	0.240	−0.002	**0.044**	−0.000	0.547	−0.001	0.189
HDL (mg/dl)	0.003	0.283	–	–	–	–	0.002	0.283
LDL (mg/dl)	0.008	**0.002**	–	–	–	–	0.005	**0.001**
Systolic blood pressure (mmHg)	−0.006	0.373	−0.006	0.365	−0.004	0.384	−0.007	0.161
BMI (kg/m^2)	0.027	0.431	0.020	0.534	−0.050	**0.033**	0.007	0.763
Waist (cm)	0.018	0.122	0.019	0.091	0.020	**0.042**	−0.024	**0.010**
Hip (cm)	−0.051	**0.007**	−0.047	**0.006**	–	–	−0.040	**0.003**

Bold values, $P < 0.05$.
Model 1D: $\beta_0 = 8.64$, Adjusted R^2 0.536, $P = 0.012$.
Model 2D: $\beta_0 = 8.23$, Adjusted R^2 0.533, $P = 0.004$.
Model 1H $\beta_0 = 4.92$, Adjusted R^2 0.435, $P = 0.011$.
Model 2H: $\beta_0 = 7.07$, Adjusted R^2 0.627, $P = 0.001$.

Discussion

The primary goal of this cross-sectional study was to compare the erythrocyte morphology of people with T2DM and prediabetes with healthy subjects using the SEM. SEM qualitative evaluation showed that erythrocytes from the PG and DMG presented anisocytosis (unequally sized RBC), as well as very irregular shapes that could not be classified conventionally (acanthocytes, echinocytes, stomatocytes, etc.). Also, as reported by Buys *et al.*, some of the erythrocyte membranes in the DMG presented a smoother appearance compared to healthy participants [17].

On the other hand, SEM quantitative evaluation of the erythrocyte diameter and height in people living with prediabetes and T2DM demonstrated a significant reduction compared with healthy individuals. Also, it was found a significant difference in the diameter and height between

PG and DMG. This information suggests that erythrocytes morphological changes appear since the prediabetic stage, which could lead to micro and macro vascular complications. Unfortunately, not many people presenting prediabetes is aware of its condition as seen in this study, where all participants of the PG (prediabetes prevalence of 25%) and one of the DMG were diagnosed during the study. Besides, due to alterations in the erythrocyte's diameter and height in people with prediabetes were slightly higher than in healthy subjects, it is essential to promote public health activities (education campaigns, self-care) for the early detection of prediabetes and to delay the onset of T2DM.

Besides, some studies have reported quantitative erythrocyte morphological alterations in people living with T2DM, such as a decreased diameter (−0.42 μm) and an increased axial ratio (+0.11 μm) [22]. However, those reported values were higher than the findings of the present study. This difference might be due to multiple causes; one is that participants in our DMG were younger and had lower values of HbA1c and LDL cholesterol [18,23], and on average they had controlled values of glucose (<130 mg/dl), cholesterol (<200 mg/dl), LDL (<100 mg/dl), HDL (>40 mg/dl), blood pressure (<130 mmHg) compared to other studies [24]. Another one is the difference in the instrument used to measure the erythrocytes. Most studies used the atomic force microscopy (AFM), and in the present study, the SEM was used. It has been reported that AFM images result from the convolution of the shape of the probe and the shape of the sample, thus making protruding features to appear wider than in reality [25]. And finally, it could be due to different cell preparation. For example, the cell preparation for AFM analysis is easier (glutaraldehyde and PBS) than SEM preparation, which involves more steps and reagents (glutaraldehyde, PBS, osmium tetroxide, etc.).

Furthermore, these morphological alterations (poikilocytosis and anisocytosis) observed in the PG and DMG produces hemorheology changes, such as lower deformability and higher aggregation, which leads to micro and macro vascular complications. For example, lower deformability causes a deterioration in the function of oxygen transportation system at several levels (heart, vascular flow, and oxygen-transporting blood function) [26]. Besides, higher aggregation due to plasma protein changes (fibrinogen) may have a role in the pathogenesis of diabetic angiopathy [27]. Also, the shape and elasticity of the erythrocyte are essential determinants of blood viscosity. Abnormalities in blood viscosity have been associated with decreased tissue perfusion, and with the development of atherosclerosis and arterial hypertension [28].

On the other hand, the second aim of this cross-sectional study was to assess the association between morphological alterations in the erythrocyte and biochemical parameters. It is essential to know if the biochemical profile and the anthropometrical parameters of the individual could be related to erythrocyte morphological alterations produced by biochemical changes (lipids, band 3 and spectrin). In this study, the results indicate that age, HbA1c, glucose, cholesterol, triglycerides, LDL cholesterol, systolic blood pressure, weight, BMI, waist and hip circumference were significantly associated (correlations and regression analysis) with the changes in the diameter or height of the erythrocyte. This information can be used to perform an early diagnosis or individualized medicine for the treatment of T2DM and CVD [6]. For example, if people with T2DM perform exercise and improve their diet, the erythrocyte aggregation can decrease [29].

There are few studies in humans where the relation between erythrocyte morphology, and biochemical and anthropometrical parameters of the patient has been evaluated. We compared our results with studies that assessed the association of biochemical or hemorheology changes because those changes are related to morphological alterations (as described above).

Regarding biochemical changes, in a study performed with 50 women with healthy weight and 50 with obesity, it was found a decrease in the phospholipids and the n-3 fatty acid of the membrane. Also, an increase in the cholesterol/phospholipid ratio (as a consequence of oxidative damage) that could modify the membrane fluidity and be part of the pathogenetic mechanism for atherosclerosis and hypertension [30]. In another study, it was described that spectrin (the major component of the membrane cytoskeleton), ankyrin and protein 4.2 of people with T2DM were heavily glycosylated, and the erythrocytes presented less deformability compared to healthy subjects [31].

In relation to hemorheology changes, a study found that predicted blood viscosity [blood viscosity = (0.12 × hematocrit in %) + {0.17 × (plasma protein concentration in g/dl −2.07)}] was positively correlated with age, waist circumference, blood pressure, cholesterol, triglycerides and HbA1c in people with prediabetes (n = 1136) [32]. Also, it has been observed a hyper-aggregation in people living with T2DM with a poor metabolic control [33].

One limitation of the study is that the PG and DMG were older than the HG, and it has been reported that age is a factor that can alter the erythrocytes morphology [23]. Therefore, we performed regression analysis adjusting by age. It was observed that age affected height only when adjusted by glucose, lipid profile and anthropometrical variables. Another possible limitation is that the alteration in the components of the erythrocyte membrane (spectrin, lipids, etc.) or hemorheology parameters (deformability, blood viscosity, aggregation) was not assessed. However,

biochemical membrane changes (molecular arrangement) as a result of oxidative stress cause biophysical shape changes including cell elasticity or rigidity and this translates to structural cell deformability and alterations in rheology [6].

Conclusion

In this study was observed that erythrocyte morphology evaluated by SEM in people living with prediabetes and T2DM presented a decreased diameter and height, poikilocytosis and a smoother membrane compared to healthy subjects. The present study suggests that micro and macro vascular complications of T2DM can appear since the prediabetic stage because these erythrocyte morphological alterations produce hemorheology changes (lower deformability, higher aggregation and blood viscosity). Therefore, erythrocyte morphological alterations can serve as an indicator of early diagnosis of T2DM and a factor implicated in the course of the clinical condition, so the correction of these alterations could serve as a treatment for prediabetes and T2DM.

Besides, erythrocyte morphological alterations could be explained by higher values of HbA1c, glucose, triglycerides, cholesterol, LDL cholesterol, systolic blood pressure, weight, BMI, waist and hip circumference, which are risk factors for developing T2DM and cardiovascular diseases. For all these reasons, it is essential to promote constantly checkups of biochemical and anthropometrical parameters to prevent the onset of prediabetes and T2DM, as well as clinical complications in the future due to erythrocyte morphological alterations.

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Conflicts of interest

None.

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