INFLUENCE OF HEMATOLOGIC, BIOCHEMICAL AND NUTRITIONAL VARIABLES ON THE KINETICS OF OSMOTIC LYSIS OF ERYTHROCYTES IN WOMEN BREAST CANCER SURVIVORS

INFLUÊNCIA DE VARIÁVEIS HEMATOLÓGICAS, BIOQUÍMICAS E NUTRICIONAIS SOBRE A CINÉTICA DA LISE OSMÓTICA DE ERITRÓCITOS EM MULHERES SOBREVIVENTES DE CÂNCER DE MAMA

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RESUMO: O câncer é um problema de saúde pública mundial. Entre as mulheres, o câncer de mama é o mais frequente. As abordagens terapêuticas podem causar alterações sanguíneas, necessitando de acompanhamento dos sobreviventes desta doença. **Objetivo:** Verificar as possíveis correlações entre a cinética da lise osmótica eritrocitária com variáveis hematológicas, bioquímicas, nutricionais e antropométricas em mulheres sobreviventes ao câncer de mama. **Métodos:** Após 12 horas de jejum e restrição de atividade física, foram coletadas amostras de sangue de 24 mulheres, para contagem de células sanguíneas, determinação de lipidograma e cinética de lise hiposmótica de eritrócitos. A composição corporal e a ingestão nutricional foram avaliadas por bioimpedância elétrica e por análise de recordatório nutricional, respectivamente. A correlação entre cada par de variáveis estudadas foi investigada por meio dos testes de *Pearson* ou *Spearman*. **Resultados:** A amostra apresentou níveis sanguíneos indesejáveis de triglicerídeos, colesterol *LDL*, colesterol total e glicose em 45,8%, 50,0%, 83,3% e 50,0% dos voluntários, respectivamente. A contagem de reticulócitos teve correlação positiva significativa (r = 0,5) com a ingestão estimada de folato. O tempo necessário para promover metade da hemólise osmótica, apresentou correlação negativa significativa com a contagem de reticulócitos (r = -0,5) e com a ingestão estimada de folato (r = -0,6). **Conclusão:** Nas sobreviventes de câncer de mama, a frequência de dislipidemia foi bastante elevada, sugerindo que a dislipidemia observada está atendendo a um aumento na demanda hematológica de colesterol, a fim de garantir a estabilização da membrana dos eritrócitos, preservando assim a contagem dessas células sanguíneas.

Palavras-chave: Neoplasias mamárias; Eritrócitos; Membrana; Fragilidade Osmótica.

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ABSTRACT: Cancer is a worldwide public health problem. Among women, breast cancer is the most frequent. Therapeutic approaches can cause blood changes, requiring monitoring of survivors of this disease. **Objective:** To verify the possible correlations between the kinetics of erythrocyte osmotic lysis with hematological, biochemical, nutritional and anthropometric variables in women surviving breast cancer. **Methods:** After 12 hours of fasting and physical activity restriction, blood samples were collected from 24 women, for blood cell counting, determination of lipidogram and kinetics of hyposmotic lysis of erythrocytes. Body composition and nutritional intake were assessed by electrical bioimpedance and by nutritional recall analysis, respectively. The correlation between each pair of studied variables was investigated using the Pearson or Spearman tests. **Results:** The sample had undesirable blood levels of triglycerides, LDL-cholesterol, total cholesterol and glucose in 45.8%, 50.0%, 83.3% and 50.0% of the volunteers, respectively. The reticulocyte count had a significant positive correlation ($r = 0.5$) with the estimated folate intake. The time required to promote half of the osmotic hemolysis showed a significant negative correlation with the reticulocyte count ($r = -0.5$) and with the estimated folate intake ($r = -0.6$). **Conclusion:** In breast cancer survivors, the frequency of dyslipidemia was quite high, suggesting that the observed dyslipidemia is attending to an increase in the hematologic demand of cholesterol, in order to guarantee stabilization of the membrane of erythrocytes, thus preserving the count of these blood cells.

Keywords: Breast neoplasms; Erythrocytes; Membrane; Osmotic Fragility.

1 INTRODUCTION

According to the World Health Organization, factors such as access to information, basic sanitation and the nutritional standard have caused deaths from infectious diseases to decline. In contrast, the current lifestyle adopted by a large part of the population is associated with an increase in the number of deaths from chronic diseases. These diseases are responsible for about 60% of all deaths in the world, thus representing the main cause of mortality (WHO, 2005).

One of the most prominent chronic diseases is cancer, which has been considered a serious public health problem worldwide (COUGHLIN; EKWUEME, 2009). In this context, breast cancer has been identified as the most frequent type of this disease among women, regardless of socioeconomic factors, such as residing in developed countries or in developing countries (DESANTIS; LIN; MARIOTTO; SIEGEL *et al.*, 2014; SAÚDE; (INCA), 2015). Estimates related to breast cancer for 2016 showed more than 240,000 new cases of the disease in the United States (SIEGEL; MILLER; JEMAL, 2016) and about 58,000 new cases in Brazil (SAÚDE; (INCA), 2015).

Concomitant to this high number of cases, advances in the treatment of the disease have also been achieved, with consequent increase in the number of breast cancer

survivors (BCS) (DI LASCIO; PAGANI, 2017). In Brazil, survival increased between the periods 1995 to 1999 and 2005 to 2009, from 78% to 87% (SAÚDE; (INCA), 2015).

In the clinical history of BCS, interventions with chemotherapy and radiotherapy, which are important approaches in the fight against cancer, are common but are not free of side effects. The general impact caused by the intervention to combat this disease affects the hematologic parameters (DOLAN; GELMON; COURNEYA; MACKEY *et al.*, 2010), and considering that the blood cells are immersed in a microenvironment directly influenced by the treatment, this condition can also alter the stability of the erythrocyte membrane.

The relationship between the action of radiotherapy and chemotherapy with the osmotic stability of the erythrocyte membrane is still not well established in the literature. Selim *et al*. (2009) reported an increase in the rate of hemolysis of red blood cells by an increase in the osmotic fragility of the membrane as the radiation dose increases (SELIM; DESOUKY; ALI; IBRAHIM *et al.*, 2009). In contrast, Khoshbian *et al*. (2015) did not observe alterations in the osmotic membrane fragility of erythrocytes, at any stage of treatment, of women with breast cancer undergoing chemotherapy and radiotherapy (KHOSHBIN; MOHAMADABADI; VAFAEIAN; BABANIA *et al.*, 2015). Often, the analysis of the osmotic stability parameters of the red blood cell membrane verifies the influence of agents that modulate its formation and destruction equilibrium, but does not investigate the influences of those blood variables that interfere with the kinetics of cell membrane lysis (CUNHA; BERNARDINO-NETO; GARROTE-FILHO; AVELAR *et al.*, 2014). In addition, no research was conducted after the main breast cancer treatments to determine the existence of adverse effects in the recovery and/or the adjuvant treatment period in BCS.

In this sense, it is evident the need to expand the scientific knowledge about the type of treatment and the stability of the erythrocyte membrane in this population. Thus, the present study aimed to verify the possible correlations between the kinetics of the osmotic fragility of the erythrocyte membrane with hematologic, biochemical, nutritional and anthropometric parameters in women who survived breast cancer after the initial treatments with radiotherapy and/or chemotherapy.

2 MATERIAL AND METHODS

2.1 STUDY POPULATION

This study was approved by the Ethics Committee on Human Research of the Federal University of Uberlândia, under number 57837416.5.0000.5152. All 24 participants signed a free and informed consent form before blood collection and anthropometric assessments.

Inclusion criteria were: previous submission to lymphadenectomy, completion of chemotherapy and/or radiotherapy at least 6 months prior to study initiation, with no history of smoking and chronic alcohol abuse. Twenty-four breast cancer survivors who volunteered in this study met these criteria.

2.2 BLOOD COLLECTION

Blood samples were taken by intravenous puncture on the contralateral side of lymph node removal surgery after 12 hours of fasting and restriction of physical effort. Blood samples were collected in 2 sterile 4-mL tubes (Vacutainer™, Becton-Dickinson, Juiz de Fora, MG, Brazil), one containing 1.8 mg/mL K_3EDTA as an anticoagulant, for hematologic and kinetics analyzes, and another without anticoagulant, for the biochemical analyzes.

2.3 ANTHROPOMETRIC ASSESSMENTS

The body composition was evaluated by electric bioimpedance analysis (BIA), after 10 hours of fasting, using a four-pole device (InBody230™, Biospace, Seoul, Korea), with estimates of absolute body mass (BM), fat free mass (FFM), lean mass (LM), fat mass (FM) and percentage of body fat (%BF). The height was evaluated using a 2 meter long stadiometer (Personal Caprice, Sanny™, São Bernardo do Campo, SP, Brazil) with 0.1 cm precision. BM and height measurements were used to calculate body mass index (BMI), using the formula: BMI = mass (kg) ÷ height (m) x height (m) (GARROW; WEBSTER, 1985).

2.4 KINETICS OF OSMOTIC LYSIS OF ERYTHROCYTES

The preparation of the saline solution was performed using NaCl (Labsynth™, Diadema, SP, Brazil) with a purity of 99.5%, which was duly corrected. Volume measurements were made on refractory glass burettes and / or with the aid of automatic

pipettes (Labsystems™, Finnpipette Digital model, Helsinki, Finland). For the mass measurements a precision digital scale (AND™, model 870, Japan) was used.

The kinetics of hyposmotic lysis of the erythrocyte membrane (CUNHA; BERNARDINO-NETO; GARROTE-FILHO; AVELAR *et al.*, 2014) was performed in a duplicate series of 14 microtubes (Eppendorf ™, Hamburg, Germany) of 2 mL volume. Initially, microtubes with 1 mL of 0.40 g/dL NaCl solution were preincubated in a thermostated water bath (Marconi™, model MA 184, Piracicaba, SP, Brazil) for 10 min at 37 °C. Then, after addition of 20 μL of whole blood to each microtube, the whole set of microtubes was homogenized and incubated at 37 °C. The osmotic lysis in each microtube was interrupted by the addition of 1 mL of hypertonic solution of 5 g/dL NaCl, in the time range of 0 to 30 minutes. After interruption of the osmotic lysis by hypertonic shock, the solutions were further incubated for 30 min at 37 °C and then centrifuged for 10 min at 1,600 x *g* and 37 °C (Hitachi Koki™, model CF15RXII, Hitachinaka, Japan) for separation of the supernatant and subsequent reading of absorbance at 540 nm in a UV-VIS spectrophotometer (Shimadzu™, model UV1650TC, Japan) using the UV Probe 2.21 software.

The kinetic curve of osmotic lysis of erythrocytes was determined by statistical adjustment to a hyperbola given by equation.

$$A = \frac{A_{max} . t}{t_{1/2} + t} \quad (1)$$

Where, A is the absorbance at 540 nm obtained at each time interval (t) considered in the test, Amax is the maximum absorbance value reached in the plateau of the curve, which represents the total lysis of erythrocytes, and t1/2 is the time elapsed for half of the total hemolysis (Amax/2).

2.5 HEMATOLOGIC AND BIOCHEMICAL ANALYZES

The hematologic variables and their indices were acquired using an automated system of analysis (Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, IL, USA). The reference values for these variables were: hemoglobin (Hb), 12.0-16.0 g/dL; hematocrit (Ht), 35.0-46.0%; mean corpuscular volume (MCV), 80.0-100.0 fL/cell; mean corpuscular hemoglobin (MCH), 26.0-34.0 pg/cell; mean corpuscular hemoglobin concentration (MCHC), 31.0-36.0 g/dL; erythrocytes (RBC), 4.0-5.2 million cells/mm^3; red-cell distribution width (RDW), 11.5-15%; and platelets (Plt), 150,000-450,000

cells/mm^3 (BAIN; BATES; LAFFAN, 2016; HOFFMAN; BENZ; SILBERSTEIN; HESLOP *et al.*, 2017).

Biochemical variables were obtained using an automated analyzer (Architect C 8000, Abbott Diagnostics, Abbott Park, IL, USA). The reference values for the lipid profile were: total cholesterol (t-C), desirable <190 mg/dL; high-density lipoprotein cholesterol (HDL-C), desirable > 40 mg/dL; triglycerides (TGC), desirable < 150 mg/dL (FALUDI; IZAR; SARAIVA; CHACRA *et al.*, 2017); low-density lipoprotein cholesterol (LDL-C), optimal < 100 and high > 160 mg/dL (XAVIER; IZAR; FARIA NETO; ASSAD *et al.*, 2013). The reference value for fasting blood glucose was < 100 mg/dL (MILECH; ANGELUCCI; GOLBERT; MATHEUS *et al.*, 2016).

2.6 EVALUATION OF FOOD CONSUMPTION

The recording of food intake was verified by means of an individual interview, performed by nutritionists, in two non-consecutive moments, one in the middle of the week and the other at the weekend (SLATER; PHILIPPI; MARCHIONI; FISBERG, 2003). Data on the consumption of macronutrients (carbohydrates, lipids and proteins) and daily energy consumption were analyzed using the DietPro 5.7i^{TM} (Viçosa, MG, Brazil) application.

2.7 STATISTICAL ANALYSIS

Data normality was verified by the Shapiro-Wilk test. Data with normal distribution were expressed as mean and standard deviation of the average variation. However, the data that did not show normal distribution were described as median and interquartile range. The kinetics analysis of erythrocyte lysis was performed using an integrated kinetic model described by the non-integrated equation of the Michaelis-Menten model. Correlation analyzes were performed using the Pearson or Spearman tests, when the data presented normal and non-normal distribution, respectively. Statistical tests were performed using the SPSS 21.0 (*IBM* Corporation, Armonk, NY, USA) or Origin 9.0 (Microcal Inc., Northampton, Massachusetts, USA) software packages.

3 RESULTS

The age range of the 24 volunteers who participated in the study varied between 33 and 70 years of age. According to a classification based on BMI (WHO, 2006), 54.2% of the studied population was overweight or obese. In addition, 79.2% had a fat

percentage greater than 33%. In relation to drug use as adjuvant therapy, two of the volunteers were not taking any drugs, three were using aromatase inhibitors and 19 were under tamoxifen. The number of lymph nodes removed surgically ranged from 1 to 26. The time interval after surgery until participation in the study ranged from 4 to 78 months. The time of adjuvant treatment of the 22 women who were under anti-hormonal therapy ranged from 3 to 70 months. The study population was statistically described, according to the data distribution pattern, as shown in Table 1.

Table 1. Characterization of the study population (n=24).

Variables	Mean ± SD or Median (Q1-Q3)
Age (years)	49.8 ± 9.4
Time after surgery (months)	22 (16.0 – 29.5)
Number of lymph nodes removed	9 (1.5 - 12.0)
Time of medication use (months)	17 (9.5 - 27.3)[a]
Weight (kg)	64.3 ± 11.1
Height (m)	1.6 ± 0.1
Body Mass Index (kg/m^2)	25.9 ± 4.7
Fat mass (%)	38.4 ± 7.2

[a]n=22. **Source:** Prepared by the authors.

The body composition, in kg, of the water mass, mineral mass, body fat mass, fat free mass and lean mass of the studied population was 28.7 ± 3.1, 2.8 ± 0.1, 25.3 ± 8.6, 39.1 ± 4.3, and 41.8 ± 4.6, respectively. The respective percentages of mineral mass, fat-free mass and lean mass were 4.3 ± 0.5, 61.6 ± 7.2 and 66.0 ± 7.7.

Table 2. Hematologic profile of study volunteers (n=24).

Variables	Mean ± SD or Median (Q1-Q3)
Leukocytes (10^3/uL)	4.9 (4.4 - 5.7)[a]
Red Blood Cells (10^6/uL)	4.7 ± 0.4
Hemoglobin (g/dL)	13.7 ± 0.8
Hematocrit (%)	40.8 ± 2.8
Mean Corpuscular Hemoglobin (pg/celula)	29.1 ± 1.5
Mean Corpuscular Volume (fL)	86.5 ± 3.9
Mean Corpuscular Hemoglobin Concentration (g/dL)	33.7 ± 0.8[a]
Red Cell Distribution Width (%)	13.3 (12.9 - 13.9)
Reticulocytes (/mm^3)	37.5 ± 10.3
Platelets (10^3/uL)	216.0 ± 50.6
Neutrophils (10^3/uL)	2.6 (3.2 - 2.9)[a]
Lymphocytes (10^3/uL)	1.7 ± 0.4
Monocytes (10^3/uL)	0.4 ± 0.1
Eosinophils (10^3/uL)	0.09 (0.06 - 0.2)
Basophils (10^3/uL)	0.02 (0.02 - 0.04)[b]
Serum Iron (µg/dL)	109.3 ± 26.4

[a]n=22; [b]n=21. **Source:** Prepared by the authors.

The percentages of reticulocytes, neutrophils, lymphocytes, eosinophils and basophils of the studied population, with their respective interquartile ranges, were 0.8 (0.7-0.8), 54.3 (50.1-63.1), 33.9 (27.7-37.1), 2.2 (1.4-4.3) and 0.4 (0.3-0.9), respectively. The percentage of monocytes presented a mean of 7.8 with a standard deviation of 1.73. Twenty-five percent of the population had erythrocyte counts lower than the reference range. The complete hematologic profile of volunteers is presented in Table 2.

According to the reference values adopted in the present study, 83.3%, 45.8%, 8.3%, 50.0% and 50.0% of the population had no desirable values of total cholesterol, triglycerides, HDL-C, LDL-C and fasting glycemia, respectively. The averages or medians of the biochemical variables of the volunteers are presented in Table 3.

Table 3. Biochemical profile of the study volunteers (n = 24).

Variables	Mean ± SD or Median (Q1-Q3)
Total Cholesterol (mg/dL)	200.8 ± 22.4
Triglycerides (mg/dL)	151.1 ± 65.1
High Density Lipoprotein Cholesterol (mg/dL)	60 (47.5-123.5)
Low Density Lipoprotein Cholesterol (mg/dL)	91.9 ± 35.5
Very Low Density Lipoprotein Cholesterol (mg/dL)	30.2 ± 13.0
Fasting Blood Glucose (mg/dL)	99.5 (95-107.5)

Source: Prepared by the authors.

The food intake variables, calculated from the food recall, are presented in Table 4. In the present sample, the estimated dietary intake of folic acid was lower than the daily recommendation, which is 400 μg.

Table 4. Daily nutritional intake estimated from food recall (n = 19).

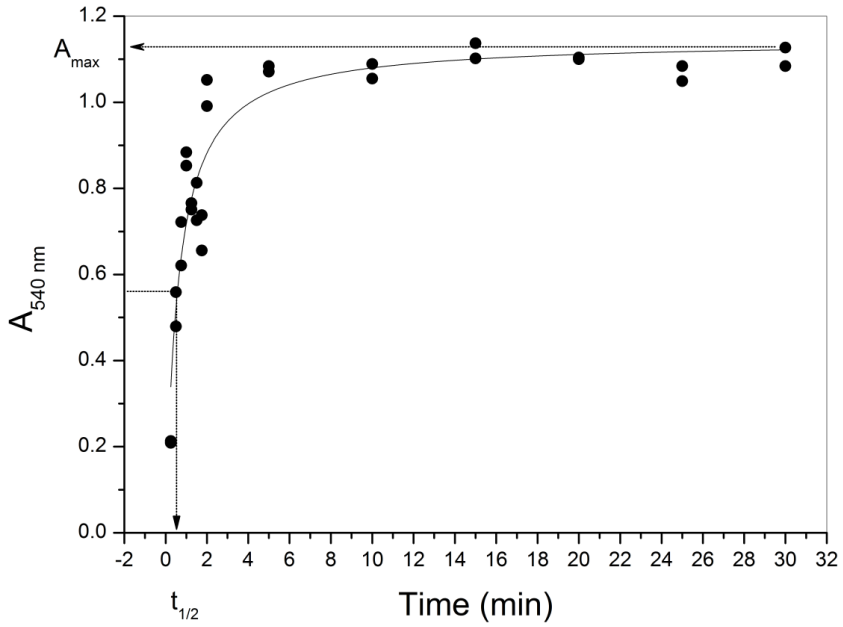
Variables	Mean ± SD or Median (Q1-Q3)
Energy (kcal)	1758.5 ± 344.7
Carbohydrates (g)	200.2 ± 47.2
Proteins (g)	78.5 ± 24.9
Lipids (g)	67.5 ± 18.3
Cholesterol (mg)	226.0 ± 118.7
Saturated fats (g)	21.5 ± 6.6
Monounsaturated fats (g)	27.8 ± 9.1
Fibers (g)	19.8 ± 7.3
Iron (mg)	11.1 ± 3.4
Folic acid (μg)	47.2 ± 25.6
Vitamin B6 (μg)	1.4 (1.3 - 1.7)[a]

[a]n=20. **Source:** Prepared by the authors.

Figure 1 shows a typical kinetic curve of osmotic lysis of erythrocytes from one of the volunteers of the present study. The study population presented a median of 1.0,

with an interquartile range of 0.8-1.1 for A_{max}, and a median of 0.6, with an interquartile range of 0.4-1.3 for $t_{1/2}$.

Figure 1. Typical curve of the osmotic lysis kinetics of erythrocytes from a study volunteer



Source: Prepared by the authors.

4 DISCUSSIONS

The mean BMI of the population of this study (25.9 ± 4.7 kg/m^2) exceeded the cutoff point of 24.9 kg/m^2 that characterizes overweight (WHO, 2006). Indeed, the majority of the population in this study (54.2%) was overweight or obese. This percentage is close to that found by Yeo *et al*. (2017), who reported a frequency of overweight or obesity in 52.1% of the 280 BCS who participated in their study, according to their BMI values (YEO; MO; PANG; SUEN *et al.*, 2017). In addition, on average, the volunteers in the present study tended to be obese, as they had a percentage of fat higher than 33% (PITANGA, 2008). These findings are worrisome because excess adipose tissue or increased body fat mass are associated with increased risk of recurrence of breast cancer and development of comorbidities that may lead to decreased survival of BCS (CAAN; KWAN; HARTZELL; CASTILLO *et al.*, 2008; PLAYDON; BRACKEN; SANFT; LIGIBEL *et al.*, 2015).

Regarding the hematologic variables, the values obtained in this study were within the reference range, which is consistent with the results reported by Shahid (2016), except for the Ht values, for survivors of all types of cancer treated by radiotherapy and/or

Table 5 presents the correlation coefficients between each pair of variables considered in this study.

Table 5. Coefficients of correlation between pairs of studied variables

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	Age	1																					
2	Weight	0.0	1																				
3	BMI	0.0	0.9§	1																			
4	A_{max}	0.2	-0.1	-0.2	1																		
5	$t_{1/2}$	0.2	-0.1	-0.2	-0.2	1																	
6	RBC	0.0	0.0	0.0	0.5§	0.0	1																
7	Hb	0.2	-0.3	-0.2	0.5§	0.0	0.8§	1															
8	Ht	0.3	-0.2	-0.1	0.5§	0.0	0.8§	0.9§	1														
9	MCH	0.2	-0.3	-0.2	-0.1	0.0	-0.7§	0.0	-0.2	1													
10	MCV	0.3	-0.3	-0.1	-0.1	0.1	-0.5§	0.1	0.1	0.9§	1												
11	MCHC	-0.3	-0.1	-0.2	-0.1	0.1	-0.3	0.1	-0.4	0.5*	0.0	1											
12	RDW	-0.2	0.5*	0.5*	-0.0	-0.1	0.2	0.0	0.1	-0.4	-0.3	-0.2	1										
13	Plt	0.1	0.1	0.2	0.2	-0.4	0.1	0.1	0.2	-0.1	0.0	-0.2	-0.3	1									
14	Rtc	0.3	0.2	0.1	0.1	-0.5§	0.2	0.2	0.2	-0.2	-0.1	-0.4	0.4	0.1	1								
15	Iron	0.2	-0.0	-0.1	-0.1	0.0	-0.2	0.0	0.0	0.3	0.3	0.0	-0.1	0.1	0.2	1							
16	t-C	0.1	0.6§	0.5*	0.2	-0.1	0.3	0.2	0.2	-0.2	-0.2	-0.1	0.3	0.4*	0.2	-0.1	1						
17	TGC	0.4*	0.2	0.2	0.3	0.0	0.3	0.2	0.3	-0.2	-0.1	-0.1	-0.1	0.5*	0.2	-0.2	0.4*	1					
18	HDL-C	0.0	-0.1	-0.0	-0.3	0.1	-0.5*	-0.2	-0.3	0.3	0.5*	-0.1	-0.2	0.1	0.0	0.4	0.3	-0.3	1				
19	LDL-C	-0.1	0.3	0.2	0.4	-0.2	0.6§	0.3	0.4*	-0.5*	-0.4*	-0.1	0.2	0.0	0.1	-0.4	0.3	0.4	-0.9§	1			
20	VLDL-C	0.4*	0.2	0.2	0.3	0.0	0.3	0.2	0.3	-0.2	-0.1	-0.1	-0.1	0.5*	0.2	-0.2	0.4*	1.0*	-0.3	0.4	1		
21	Glucose	0.5*	0.3	0.3	0.4	-0.2	0.1	0.3	0.2	0.2	0.2	0.0	0.0	0.3	0.3	0.1	0.4*	0.7§	-0.3	0.3	0.7§	1	
22	Vitamin B9	-0.1	-0.2	-0.2	0.6§	-0.6§	0.7§	0.5*	0.5*	-0.4*	-0.4*	-0.1	0.1	0.1	0.5*	-0.1	0.2	0.0	-0.3	0.4	0.0	0.1	1

Abbreviations: BMI, Body Mass Index; A_{max}, Maximum Absorbance; $t_{1/2}$, time for half of the total lysis; RBC, Red Blood Cells; Hb, Hemoglobin; Ht, Hematocrit; MCH, Mean Corpuscular Hemoglobin; MCV, Mean Corpuscular Volume; MCHC, Mean Corpuscular Hemoglobin Concentration; RDW, Red cell distribution width; Plt, Platelet count; Rtc, Reticulocyte Count; t-C, total Cholesterol; TGC, Triglycerides; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very-Low-Density Lipoprotein Cholesterol. *$p<0.05$; §$p<0.01$. **Source:** Prepared by the authors.

chemotherapy, but not for BCS, which presented values below the reference range for Hb, Ht and MCH (SHAHID, 2016). An important determinant of this hematological difference in BCS appears to be menopause. In postmenopausal BCS, lower values of RBC and Hb and higher values of RDW were observed in relation to premenopausal BCS, although there was no difference between these two groups in relation to leukocyte, lymphocyte, monocyte and neutrophil counts (DEZAYEE; AL-NIMER, 2016).

Occurrence of dyslipidemia is a common finding in breast cancer survivors (YEO; MO; PANG; SUEN *et al.*, 2017), as also shown in the present study. Regarding LDL-C, blood levels were elevated in 56.1% of volunteers in that study, a frequency very close to that found in this study (50.0%). The same can be said of the frequency of low HDL-C values, which was 6.6% in that study compared to 8.3% in this study. However, for frequencies of t-C and TGC, frequencies higher than desirable were much higher in this study (83.3% and 45.8%, respectively) than in that study (34.5% and 22.9%, respectively), which could be due to the use of tamoxifen (YEO; MO; PANG; SUEN *et al.*, 2017).

Regarding the correlation analyzes, some of the results found were expected, since there are conceptual relations between the variables. This is the case of BMI and BM (r = 0.93) (GARROW; WEBSTER, 1985), RBC e Hb (r = 0.75), RBC and Ht (r = 0.82), RBC and MCH (r = -0.65), RBC and MCV (r = -0.53), Hb and Ht (r = 0.93), MCH and MCV (r = 0.89), and MCH and MCHC (r = 0.47) (GEORGE-GAY; PARKER, 2003), but also of t-C and TGC (r = 0,41), t-C e VLDL-C (r = 0,41) e TGC e VLDL-C (r = 1.0) (FRIEDEWALD; LEVY; FREDRICKSON, 1972).

In relation to A_{max}, a parameter obtained in the osmotic lysis kinetics of erythrocytes and representing the maximum absorbance found in the osmotic lysis curve of these patients, their values showed significant positive correlations with erythrocytes (r = 0.53), Hb (r = 0.53) and Ht (r = 0.52). These correlations are obvious, since the absorbance measured at 540 nm represents the measure of a hemoglobin property (CUNHA; BERNARDINO-NETO; GARROTE-FILHO; AVELAR *et al.*, 2014).

In relation to $t_{1/2}$, a parameter also obtained in the osmotic lysis kinetics of erythrocytes, which represents the time required to promote lysis of half of the erythrocyte population used in the test, their values showed a significant negative correlation with the percentage of reticulocytes (r = -0.5). This means that volunteers with a higher percentage of reticulocytes have kinetically weaker erythrocytes. Usually, the percentage of reticulocytes increases when there is an increase in erythropoiesis rate. An increase in

erythropoiesis demands elevation in the action of folic acid coenzyme on nucleotide biosynthesis to meet the increased demand for cell division (GREEN; DATTA MITRA, 2017). Therefore, a deficiency of folic acid in the diet is associated with elevation in the release of reticulocytes, which are precursors of mature erythrocytes, into the bloodstream. Therefore, the reticulocyte (Rtc) index was expected to increase with a decrease in ingested folate. However, the positive correlation observed between Rtc and folate (Table 5) indicates the opposite, i.e., the reticulocyte index decreased with the decrease in the intake of folate. In addition, the inverse correlation observed between $t_{1/2}$ and folic acid feed intake ($r = -0.6$) should mean that erythrocytes were kinetically more fragile (lower $t_{1/2}$) the higher the folic acid intake, when it would be expected that a higher intake of folic acid would generate more mature and more stable erythrocytes. It is possible that these changes in the trend are due to changes in cancer and/or its treatments, together with the low intake of folic acid in the study population in relation to the recommended daily intake of this vitamin (ORGANIZATION.; ORGANIZATION., 2001).

An important aspect of the correlation analysis that deserves to be highlighted is the positive correlation observed between the RBC counts and the LDL-C levels, indicating that the elevation of lipidemia in this population may be attending to the need for elevation of erythropoiesis, in the sense that the increase in reticulocyte population could allow the generation of a larger number of stable mature erythrocytes as a result of cholesterol transfer to red blood cells. This makes sense, especially in view of the also positive correlation observed between Ht and LDL-C.

The main limitation of the present study was the small size of the population due to recruitment difficulties. The variable adherence of the volunteers to the blood tests represents another important limitation, certainly related to the psychological aspects of these women, even after they have already undergone the main treatment of the disease.

5 CONCLUSIONS

A parameter obtained in the osmotic lysis kinetics of erythrocytes, $A_{max,}$ which represents the maximum absorbance found in the osmotic lysis curve of erythrocytes from BCS, presented significant positive correlations with RBC counts, Hb concentrations and Ht values; these correlations were expected, since Amax represents a spectral property of the hemoglobin released on lysis.

The other parameter also obtained in the osmotic lysis kinetics of erythrocytes, $t_{1/2}$, which represents the time required to promote lysis of half of the erythrocyte population used in the test, showed a significant negative correlation with the percentage of reticulocytes; this correlation means that volunteers with a higher percentage of reticulocytes have kinetically weaker erythrocytes. This suggests that breast cancer survivors still preserve some hematological changes normally associated with the therapeutic approaches used.

Undesirable levels of triglycerides, LDL-cholesterol and total cholesterol found in 45.8%, 50.0% and 83.3% of the volunteers, respectively. However, as erythrocyte counts showed a significant positive correlation with LDL-C and negative levels with HDL-C levels, this suggests that the observed dyslipidemia is attending to an increase in the hematological demand for cholesterol, to guarantee stabilization of the membrane of erythrocytes, thus preserving the count of these blood cells.

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