Detection of Common Deletional of α-Thalassemia 3.7 Kb from Metropolitan Region of Manaus, Amazonas, Brazil

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Abstract. Background: Alpha Thalassemia (α-thal) is a heterogeneous group of hereditary alterations caused by deletions that affect alpha regulatory genes, and the 3.7Kb deletion is the most frequent worldwide. The prevalence ranges from 20% and 35% in Brazil, depending mainly on race, predominant in Afro-descendants.

Purpose: The aim was to determine α-thal-α3.7Kb and -α4.2Kb deletions, estimating their frequency in individuals from six regions of Amazonas State.

Methods: Volunteers age between 18-59 years old of both genders participated in the study. Blood was collected from March 2014 to September 2017 at the health centers of each participant city. α-thal3.7kb was performed by GAP-PCR, while α-thal4.2kb by Multiplex-PCR. The total samples collected from each city were: Manaus (capital), 356 (19.7%); Iranduba 232 (12.8%); Manacapuru, 287 (15.9%); Presidente Figueiredo, 370 (20.5%); Itacoatiara, 301 (16.6%); and Coari, 263 (14.5%).

Results: The average age among males was 35.3±14.8, while for females, it was 36.7±14.9 years old. Microcytosis (MCV <80fL) was found in 158 individuals (8.46%) and α-thal diagnosed in 143 individuals (7.9%), and all of these individuals carried the 3.7Kb deletion 5.95% in heterozygous and 1.95% in homozygous. α-thal4.2kb was not found in any volunteer. The association analyses to the α-thal3.7kb genotypes were statistically significant for all hematological parameters (p<.001), except serum iron and serum ferritin analyses.

Conclusion: This study highlights α-thal 3.7kb deletion as an important public health problem, especially in a population not yet characterized about this disease. Thus, epidemiological studies using molecular tools become relevant in regions where the disease is underestimated, contributing to a better understanding of thalassemia incidence and iron deficiency anemias incidence of the participating cities. We reinforce that future molecular studies in North Region from Brazil can be utilized to describe other genetic anemias as structural hemoglobinopathies that have already proven to be highly prevalent in Brazil.

Keywords: Alpha Thalassemia; Iron Treatment; Amazonas; Brazil.

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Introduction. Alpha thalassemia (α-thal) is characterized by the reduction or absence of α-chain production. Widely distributed, alpha globin's chains deletion is often found in tropical regions and is especially common in Southeast Asia, the Indian Subcontinent, Africa, and the Middle East, with frequencies rising from 70% to 90%.³ The degree of severity varies according to the number of involved genes and may range from an individual asymptomatic to a life-incompatible condition.³

The most common α-thal-1 forms found are α−SEA, α−FIL, α−MED and α−THAI. However, the most frequent form of deletion is the α-thal-2 (α+), that affects only one out of the four α-globin genes and whose alterations α−3.7Kb and α−4.2Kb are the most prevalent throughout the world.⁴,⁵

The clinical severity of heterozygous carriers individuals is low; they usually present milder symptoms; however, their red blood cell deficiencies have to be differentiated from subtle anemia, microcytic-hypochromic anemia, refractory or iron deficient. On the other hand, the homozygous form accompanies signs ranging from moderate to severe forms, such as hemolytic anemia.⁶,⁷

Widely distributed, the frequency of α-thal is directly linked to the world's constant migratory waves in recent centuries. For example, Africans were taken to North and South America during the European colonization, or among Vietnamese refugees, or in the latest crisis in Syria - which has sent about a million people from the Middle East to Europe and the Americas through Turkey. All these individuals come from areas with a high incidence of thalassemia, and consequently, there is a genetic flux between the country of origin and country of destination. The new genetic background can lead to thalassemia at all levels of the disease and favor the shuffling of mutations that are not commonly seen in their local population.⁸,⁹

The aim of this study was to determine the frequency of thalassemia alpha α−3.7Kb and α−4.2Kb deletions in individuals living in Manaus, capital of the State of Amazonas, and from cities within the metropolitan region of Manaus. Besides, to characterize the hematological parameters, serum ferritin, and serum iron to each population and evaluate its association.

Materials and Methods. The studied population was composed by volunteers (> 18 years old), naturals from the State of Amazonas, of both genders, from outpatient units in six cities: Manaus (N=356); Coari (N=263); Manacapuru (N=287); Iranduba (N=232); Presidente Figueiredo (N=370) and Itacoatiara (N=301). All samples were recruited of the outpatients randomly at six cities. Subjects under 18 years old, pregnant, transfused in the last three months, and patients with onco-hematological and/or hospitalized conditions were not included in this study.

A total of 1809 peripheral blood samples were collected in three years, from 2014 to 2017. The hematological analyses were performed at the respective outpatient units of the respective study cities. These analyses were performed in the automated hematology analyzers of the new generation impedance technique and always calibrated before every test: BC-5800 (Mindray, Shenzhen, China), Pentra XL (ABX 80 Horiba®, France), and ADVIA 120 Hematology (Siemens Healthineers Brasil). For serum ferritin and serum iron analyses were used Bioclin® KIT by immunoturbidimetry and colorimetric assays, respectively, carried out in a Bioclin 3000 (Quibasa-Belo Horizonte, Brazil).

The genomic DNA was prepared using the BIOPUR Mini Spin Plus® extraction kit, following the manufacturer's recommendations. The integrity and DNA quantification were evaluated by Nanodrop™ 2000 (Thermofisher®).

The α-thalassemia 3.7Kb deletion was executed as by a previous study,¹⁰ and 4.2Kb deletion by Multiplex- PCR technique adapted from the previous study using only primers of wild type alpha genes and 4.2Kb deletion.¹¹ The PCR products were submitted to electrophoresis (Bio-Rad, EUA) in 1.5% agarose gel and visualized under ultraviolet light in ENDURO™ GDS Gel Documentation System (Labnet International, New York, USA).

This project was approved by the Ethics in Research Committee (CEP) from Universidade Federal do Amazonas and Fundação Hospitalar de Hematologia e Hemoterapia, based on the Brazil Platform in three projects: Nº 834.086, CAEE 30668114.0.0000.5020; Nº 213.167, CAEE: 01193312.4.0000.0009; and Nº 1.178.117, CAEE: 46020315.4.0000.5020.

The distribution of variables analysis was performed using the Kolmogorov-Smirnov test. The parameter values were presented as mean and standard deviation. The One Way ANOVA parametric test was used to analyze the distribution of the means of quantitative variables with normal distribution within categories. As independent variables, the groups were divided into α-thalassemia genotypes, gender, and cities. As dependent variables, the continuous data were age in years, Hematological parameters, and iron serum and ferritin values. Contingency table chi-square tests were performed comparing the incidence of α-thalassemia between Cities. p<0.05 was considered significant. Statistical analyses were performed using SPSS version 19.0 (Chicago, EUA) and GraphPad Prism version 5.0
Table 1. Age and gender distribution by cities included in the study.

| City            | Gender | N (%) | Age Mean/SD |
|-----------------|--------|-------|-------------|
| Manaus (356)    | Male   | 195 (54.8) | 32.8 ± 11.4 |
|                 | Female | 161 (45.2) | 33.7 ± 12.0 |
| Iranduba (232)  | Male   | 89 (38.4)  | 31.9 ± 13.7 |
|                 | Female | 143 (61.6) | 32.4 ± 11.4 |
| Manacapuru (287)| Male   | 121 (42.2) | 34.0 ± 12.3 |
|                 | Female | 166 (57.8) | 35.3 ± 13.0 |
| Presidente Figueiredo (370) | Male | 135 (36.5) | 43.9 ± 16.2 |
|                 | Female | 235 (63.5) | 41.7 ± 17.0 |
| Itacoatiara (301) | Male | 111 (36.9) | 37.2 ± 19.8 |
|                 | Female | 190 (63.1) | 42.3 ± 17.5 |
| Coari (263)     | Male   | 109 (41.4) | 31.1 ± 10.8 |
|                 | Female | 154 (58.6) | 30.9 ± 10.3 |
| Total (1809)    | Male   | 760 (41.0) | 35.3 ± 14.8 |
|                 | Female | 1049 (58.0) | 36.7 ± 14.9 |

N: Volunteers, SD: Standard Deviation

(San Diego, EUA) software.

Results. The studied population was composed predominantly of females (N=1049, 58%), against 760 (42%) males. The average age among females was 36.7±14.9 years old and 35.3±14.8 years old for males (Table 1). The alpha thalassemia screening found 143 individuals (7.9%) harboring the -α^{3.7Kb} deletion: 108 (6%) in heterozygous (-α/αα) and 35 (1.9%) in homozygous (-α/-α). The prevalence in males was 7.9% (95% CI 6.0-9.9) and females 8.0% (CI 6.4-9.8) (Fisher test, p = 0.92). The frequency in Manaus was 7.9% (95% CI 5.1 - 10.7); Iranduba 7.3% (95% CI 3.9 - 10.8), Manacapuru 4.5% (95% CI 2.4 - 7.0), Presidente Figueiredo 10.3% (95% CI 7.3 - 13.2), Itacoatiara 9.6% (95% CI 6.3 - 13.3), Coari 7.2% (95% CI 4.2 - 10.3). The 4.2Kb deletion was not found in our studied population.

The Leukocytes counts (WBC), erythrocytes (RBC), Hemoglobin (Hgb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Anisocytosis Index (RDW), serum iron and serum ferritin were analyzed following stratification for the α-thal genotype, i.e., one wild type group (αα/αα) (Supplementary Table 1A); one group composed only of heterozygous (-α/αα) individuals (Supplementary Table 1B); and one group composed only of homozygotes (-α/-α) individuals (Supplementary Table 1C). When analyzing only wild types genotypes (αα/αα) between cities, the association were statistically significant among all hematological parameters, including for the serum iron and serum ferritin analyzes between (p<0.001). Although statistically different (p <.001), the hematological indexes and parameters for all cities are within the normal reference values.12

Hematological levels and iron test values were higher in men than women, according to the literature. Hemoglobin, hematocrit, and erythrocytes values were corrected for sex (Supplementary Table 2). As expected, a higher frequency of α-thal was observed in those with microcytosis (40.68%), against 4.65% in normocytic (Figure 1). We demonstrated that eight individuals have concomitantly α-thalassemia and iron deficiency, representing 4% of the total number of α-thal carriers.

By the contingency table chi-square tests, significant differences were found when comparing the lowest and highest prevalence of α-thal with other cities; Manacapuru vs. Presidente Figueiredo (p=.010 ), vs. Manaus (p=.119), vs. Iranduba (p=.242), vs. Itacoatiara (p=.025), vs. Coari (p=.332) and Presidente Figueiredo vs. Manaus (p=.318), vs. Iranduba (p=.283), Itacoatiara

![Figure 1](https://example.com/image1.png) Frequency of the -α^{3.7Kb} genotypes between individuals with microcytic and normocytic erythrocytes.
Moreover, finally, we create a map of the -α^{3.7}kb frequencies found in the Amazon region. The results showed that -α^{3.7}kb allele frequency was the highest in the Presidente Figueiredo City (10.3%) located 98 km from Manaus and lowest followed in Itacoatiara City (4.5%) located 128 km from Manaus (Figure 2).13

Discussion. The α-thal occurrence in the city of Manaus-AM is still based only on screening methods, such as hematological indices (MCV and MCH) and supravital staining to detect Hb H inclusions (denatured β4 tetramers). However, none of these approaches is entirely reliable or sensitive to detect α-thalassemia trait (-α/αα). This problem is easily overcome by the molecular tools applied to the alpha thalassemia's genotyping's various genetic determinants.14-16

Although our group has already shown the 5.35% frequency -α^{3.7}kb is the deletion in blood donors from Manaus,17 this disease has never been studied in Manaus metropolitan region using molecular methods. Thus its real prevalence is unknown and probably underestimated.18

In this study, the -α^{3.7}kb deletion was uniquely found in 7.9% of our population, consistent with the high incidence in all States from Brazil.19,20 In most studies, including Brazil, -α^{3.7}kb is the deletion more frequently reported, ranging from 70% to 90% in the regions of Melanesia and Nepal; reaching out 70.7% in Iran, 72.8% in the Middle East, 35.2% in India, 16.3% in Thailand, 40% in African countries and from 5 to 20% in Brazil.21-25

We believed that our study shows some limitations since not all population was included to make the prevalence estimation. However, we do not have selection bias once individuals were free to participate in the study. None showed any onco-hematological diseases, hospitalizations and surgical procedures recent, blood transfusions, or any visible comorbidity during the interview and signing of the consent form. However, the low frequency of -α^{3.7}kb (4.5%) found in Manacapuru perhaps can be explained because this city has been formed by a unique indigenous ethnicity known as "Mura," and currently, this city has smaller racial miscegenation then others investigated in this study.26,27

During the First Rubber Cycle in Brazil, an industry that demands many workers, Manaus received a high number of immigrants from several Latin American, European, and African countries.28-31 Besides, the state of Amazonas has indigenous communities with the same hierarchical bases of the past centuries. Thus, the neo-Brazilian population's formation took place from social and geographic colonization, which mixed with Spaniards, English, French, Dutch, Portuguese, and Irish, Arab-Turkish, Italian Japanese, Scandinavian and Jewish.32,33

The population's characterization through the laboratory analysis, including its hematological data and the serum iron and ferritin dosages, in this study, allowed to differentiate and individualize the populations. The results of the observations and comparisons of hematological indices among alpha thalassemia genotypes showed subtle reductions in the normal ranges and were statistically significant, corroborant with the literature.
A high number of individuals with hypochromic microcytic anemia, without iron deficiency, were diagnosed with alpha thalassemia, reinforcing the importance of the molecular techniques used in this study. Despite the technical improvement currently offered and the constant training of human resources, alpha thalassemia in its heterozygous form continues to represent diagnostic difficulties for the analyst of the conventional clinical laboratory, as well as for hematologist, who, for the most part, are unaware of such genetic alteration, confusing it frequently with other microcytic and hypochromic anemias. Thus, it is essential to increase personal training and information about these changes in our population.

Furthermore, we believe that the main advantage of alpha thalassemia's molecular identification is the correct distinction from iron deficiency anemia, which avoids the possible administration of iron and other unnecessary metals to these patients.

This study highlights thalassemia as an important public health problem, especially in a population not yet characterized by this disease, and reinforces the importance of assessing its frequency.

Conclusions. The present study demonstrates the importance of alpha thalassemia diagnosis in this region. The prevalence results of α-αβ were relatively high in the majority of cities, (exception for Manacapuru), in which many people are unaware of their genetic anemia.

Future molecular studies might be used to describe other genetic anemias as the pieces of beta-thalassemia or structural hemoglobinopathies as S, C, and D that have already proven to be highly prevalent in Brazil but not yet fully described in the northern region of Brazil, and not studied in this paper.

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**Supplementary table 1.** Hematologic parameters characterization and levels of serum ferritin and serum iron among alpha thalassemia 3.7<sup>th</sup> deletion in metropolitan region of Manaus.

|                  | Manaus          | Iranduba        | Manacapuru      | Presidente Figueiredo | Itacoatiara | Coari         | p-value |
|------------------|-----------------|-----------------|-----------------|-----------------------|--------------|----------------|---------|
| **Wide type (aa / aa)** (A) |                 |                 |                 |                       |              |                |         |
| RBC (x10<sup>12</sup>/mm L)   | 4.67 ± 0.48     | 4.35 ± 0.57     | 4.71 ± 0.61     | 4.71 ± 0.48           | 4.75 ± 0.57  | 4.43 ± 0.56 | <.001   |
| Hg (g/dL)         | 13.95 ± 1.42    | 13.08 ± 1.43    | 13.63 ± 1.45    | 13.56 ± 1.48          | 13.79 ± 1.42 | 13.03 ± 1.62 | <.001   |
| Hct (%)           | 42.41 ± 4.3     | 39.03 ± 4.41    | 41.03 ± 4.28    | 41.52 ± 4.21          | 40.75 ± 4.25 | 40.33 ± 4.96 | <.001   |
| MCV (fL)          | 91.07 ± 4.53    | 90.11 ± 7.62    | 87.49 ± 6.28    | 88.39 ± 4.94          | 85.25 ± 4.89 | 91.26 ± 5.63 | <.001   |
| MCH               | 29.97 ± 1.97    | 30.20 ± 2.32    | 29.11 ± 2.19    | 28.87 ± 1.10          | 28.83 ± 1.25 | 29.48 ± 1.93 | <.001   |
| RDW (%)           | 32.90 ± 1.28    | 33.57 ± 1.64    | 33.28 ± 1.76    | 32.67 ± 1.68          | 33.87 ± 1.32 | 32.32 ± 1.39 | <.001   |
| Serum Iron (μg/dL)| 101.69 ± 52.74  | 93.77 ± 25.62   | 85.46 ± 25.74   | 83.32 ± 32.59         | 90.60 ± 32.61 | 96.20 ± 32.37 | <.001   |
| Ferritin (μg/dL)  | 13.727 ± 52.69  | 110.16 ± 33.28  | 94.92 ± 25.31   | 96.30 ± 26.66         | 112.09 ± 47.01 | <.001   |         |
|                  |                 |                 |                 |                       |              |                |         |
| **Heterozygous (-a / aa)** (B) |                 |                 |                 |                       |              |                |         |
| RBC (x10<sup>12</sup>/mm L)   | 4.46 ± 0.41     | 4.52 ± 0.44     | 5.03 ± 0.51     | 4.52 ± 0.62           | 4.98 ± 0.62  | 4.72 ± 0.88 | .017    |
| Hg (g/dL)         | 12.17 ± 1.11    | 12.01 ± 1.50    | 13.77 ± 1.40    | 12.41 ± 1.52          | 13.49 ± 1.20 | 12.86 ± 3.38 | .002    |
| Hct (%)           | 38.30 ± 2.72    | 35.29 ± 3.85    | 42.27 ± 5.78    | 38.70 ± 4.88          | 38.81 ± 3.76 | 38.09 ± 7.86 | .002    |
| MCV (fL)          | 86.19 ± 5.77    | 78.31 ± 7.87    | 83.91 ± 4.94    | 86.09 ± 8.51          | 78.24 ± 5.29 | 80.60 ± 5.25 | <.001   |
| MCH               | 27.36 ± 2.25    | 26.68 ± 3.29    | 27.40 ± 2.13    | 27.62 ± 2.59          | 27.19 ± 1.62 | 25.16 ± 2.20 | <.001   |
| MCHC (pg)         | 31.75 ± 1.67    | 34.02 ± 1.59    | 32.65 ± 1.32    | 32.16 ± 2.07          | 34.81 ± 1.53 | 31.20 ± 1.57 | <.001   |
| RDW (%)           | 12.41 ± 1.14    | 12.64 ± 1.17    | 13.89 ± 0.51    | 13.67 ± 0.74          | 13.41 ± 0.76 | 12.51 ± 1.46 | <.001   |
| Serum Iron (μg/dL)| 76.88 ± 5.56    | 76.48 ± 31.54   | 87.0 ± 32.21    | 66.54 ± 36.12         | 81.78 ± 35.46 | 81.65 ± 46.95 | .647    |
| Serum Ferritin (μg/dL)| 101.81 ± 40.91 | 101.42 ± 45.05 | 104.56 ± 32.63 | 102.95 ± 41.58       | 97.18 ± 33.39 | 114.07 ± 60.70 | .656    |
|                  |                 |                 |                 |                       |              |                |         |
| **Homzygous (-a / -a)** (C) |                 |                 |                 |                       |              |                |         |
| RBC (x10<sup>12</sup>/mm L)   | 4.84 ± 0.45     | 5.23 ± 1.16     | 5.44 ± 0.20     | 5.19 ± 0.51           | 5.55 ± 1.03  | 4.96 ± 0.16 | .517    |
| Hg (g/dL)         | 12.22 ± 1.96    | 10.62 ± 2.52    | 12.95 ± 1.04    | 12.91 ± 1.58          | 12.46 ± 1.91 | 10.49 ± 0.78 | .098    |
| Hct (%)           | 38.44 ± 5.41    | 35.02 ± 5.33    | 40.72 ± 1.16    | 38.27 ± 4.19          | 37.26 ± 5.88 | 32.45 ± 1.96 | .196    |
| MCV (fL)          | 79.17 ± 5.22    | 67.08 ± 6.11    | 75.21 ± 6.67    | 73.06 ± 5.54          | 67.74 ± 7.27 | 65.34 ± 3.83 | .006    |
| MCH               | 25.17 ± 2.58    | 20.67 ± 4.28    | 23.92 ± 2.18    | 24.95 ± 2.85          | 22.68 ± 2.76 | 21.13 ± 1.66 | .050    |
| MCHC (pg)         | 31.75 ± 1.39    | 30.67 ± 4.42    | 31.81 ± 0.63    | 33.72 ± 3.29          | 33.51 ± 1.48 | 32.31 ± 0.7  | .222    |
| RDW (%)           | 11.25 ± 0.78    | 12.85 ± 0.69    | 12.99 ± 0.91    | 13.42 ± 0.84          | 13.70 ± 0.77 | 12.94 ± 1.22 | <.001   |
| Serum Iron (μg/dL)| 70.25 ± 5.41    | 53.22 ± 19.61   | 101.03 ± 16.84  | 94.37 ± 3.82          | 92.45 ± 39.74 | 79.98 ± 21.10 | .176    |
| Serum Ferritin (μg/dL)| 86.55 ± 49.20  | 96.21 ± 47.82  | 88.52 ± 35.47  | 101.69 ± 52.74        | 85.88 ± 24.20 | 81.59 ± 9.42 | .011    |

RBC: Red Blood Cell Count; Hb: Haemoglobin; Hct: Haematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hb; MCHC: Mean Corpuscular Hb Concentration; RDW: red blood cell distribution width; SD: standard deviation.
### Supplementary Table 2. Hematological data and levels of serum ferritin and serum Iron among study participants.

|                | Manaus | Iranduba | Manacapuru | Presidente Figueiredo | Itacoatiara | Coari |
|----------------|--------|----------|------------|-----------------------|-------------|-------|
|                | N=195  | N=89     | N=143      | N=121                 | N=135       | N=190 |
| Mean ± SD      | Male   | Female   | Male       | Female                | Male        | Female |
|                |        |          |            |                       |             |       |
| RBC (x10^6/mm L) | 4.85 ± 0.42 | 4.41 ± 0.41 | 4.61 ± 0.57 | 4.23 ± 0.54          | 4.87 ± 0.66 | 4.42 ± 0.54          | 4.96 ± 0.53 | 4.36 ± 0.38          | 5.12 ± 0.60 | 4.41 ± 0.47          | <.001 |
| SD             |        |          |            |                       | <.001       | <.001 |
| Hb (g/dL)      | 14.46 ± 1.29 | 13.02 ± 1.32 | 13.70 ± 1.31 | 12.45 ± 1.52         | 13.92 ± 1.42 | 13.41 ± 1.40         | 14.44 ± 1.71 | 12.8 ± 1.0           | 14.04 ± 1.36 | 13.35 ± 1.39         | <.001 |
| Hct (%)        | 44.01 ± 3.53 | 39.77 ± 3.59 | 40.98 ± 3.96 | 37.26 ± 4.48         | 42.23 ± 4.57 | 40.04 ± 4.24         | 43.69 ± 4.66 | 39.7 ± 3.4           | 42.99 ± 4.27 | 39.07 ± 3.68         | <.001 |
| MCV (fL)       | 90.72 ± 4.57 | 90.34 ± 5.52 | 89.46 ± 9.31 | 88.54 ± 8.37         | 87.26 ± 7.55 | 86.77 ± 7.00         | 88.23 ± 5.75 | 88.1 ± 6.4           | 84.27 ± 6.31 | 84.71 ± 5.16 | .945 |
| MCH (pg)       | 29.28 ± 1.97 | 29.59 ± 2.41 | 29.92 ± 2.92 | 29.65 ± 3.04         | 28.82 ± 2.84 | 29.03 ± 2.16         | 29.16 ± 2.31 | 28.5 ± 2.3           | 28.29 ± 1.60 | 28.70 ± 1.53         | .010 |
| MCHC (pg)      | 32.86 ± 1.15 | 32.74 ± 1.53 | 33.49 ± 1.62 | 33.59 ± 2.03         | 33.08 ± 1.98 | 33.57 ± 2.04         | 33.07 ± 1.83 | 32.4 ± 1.6           | 33.63 ± 1.35 | 33.93 ± 1.27         | .059 |
| RDW            | 13.35 ± 1.33 | 13.83 ± 0.97 | 13.08 ± 1.38 | 13.01 ± 0.87         | 13.66 ± 0.83 | 13.65 ± 1.02         | 13.5 ± 0.70 | 13.5 ± 0.7           | 14.0 ± 0.8 | 14.01 ± 0.7          | .680 |
| Iron (μg/dL)   | 81.8 ± 10.2 | 80.70 ± 9.44 | 85.89 ± 25.34 | 82.22 ± 25.42        | 89.21 ± 24.57 | 83.22 ± 26.27        | 88.23 ± 36.41 | 78.24 ± 30.70        | 95.09 ± 35.35 | 87.08 ± 31.10        | .005 |
| Ferritin (μg/L)| 177.7 ± 114.1 | 83.9 ± 35.1 | 114.0 ± 31.8 | 105.9 ± 30.7         | 114.0 ± 31.8 | 107.4 ± 30.7         | 89.8 ± 30.9 | 96.7 ± 24.9          | 104.6 ± 30.7 | 91.8 ± 23.5          | .020 |

RBC: Red Blood Cell Count; Hb: Haemoglobin; Hct: Haematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hb; MCHC: Mean Corpuscular Hb Concentration; RDW: red blood cell distribution width; SD: standard deviation.