Mediterranean Journal of Hematology and Infectious Diseases

Original Article

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

Fernanda Cozendey Anselmo[1,2], Abdou Gafar Soumanou[2], Cleidiane de Aguiar Ferreira[4], Flora Maia Viga Sobrinha[4], Ana Caroline Santos Castro[2], Rafael Oliveira Brito[2], Adolfo José da Mota[2], Marilda de Souza Gonçalves[3] and José Pereira de Moura Neto[1,2].

[1] Universidade do Estado do Amazonas - Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas, Manaus, Amazonas, Brasil.
[2] Universidade Federal do Amazonas, Faculdade de Ciências Farmacêuticas, Manaus, Amazonas, Brasil.
[3] Fundação Oswaldo Cruz - Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brasil.
[4] Secretaria do Estado do Amazonas – SUSAM.

Competing interests: The authors declare no conflict of Interest.

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 -$^{\alpha 3.7Kb}$ and -$^{\alpha 4.2Kb}$ 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-thal$^{3.7Kb}$ was performed by GAP-PCR, while α-thal$^{4.2Kb}$ 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 3.7^{Kb} deletion 5.95% in heterozygous and 1.95% in homozygous. α-thal$^{4.2kb}$ was not found in any volunteer. The association analyses to the α-thal$^{3.7kb}$ 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.

Citation: Anselmo F.C., Soumanou A.G., Ferreira C.A., Sobrinha F.M.V., Castro A.C.S., Brito R.O., Mota A.J., Souza Gonçalves M., Moura Neto J.P. Detection of common deletional of α-thalassemia 3.7 kb from metropolitan region of Manaus, Amazonas, Brazil. Mediterr J Hematol Infect Dis 2021, 13(1): e2021001, DOI: http://dx.doi.org/10.4084/MJHID.2021.001

Published: January 1, 2021 **Received: April 21, 2020** **Accepted: December 7, 2020**

Correspondence to: José Pereira de Moura Neto, Universidade Federal do Amazonas, Faculdade de Ciências Farmacêuticas, Avenida General Rodrigo Otávio Jordão Ramos, 6200 - Coroado I, Manaus, Brasil. Tel. + 55-92-3305-1181- R:2007. E-mail: jpmn@ufam.edu.br or jp-mn@hotmail.com. ORCID: https://orcid.org/0000-0003-2177-7292

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%.[1,2] 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 $--^{SEA}$, $--^{FIL}$, $--^{MED}$ and $--^{THAI}$. 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 $-\alpha^{3.7Kb}$ and $-\alpha^{4.2Kb}$ 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 $-\alpha^{3.7Kb}$ and $-\alpha^{4.2Kb}$ 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 of 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 3.7^{Kb} deletion was executed as by a previous study,[10] and 4.2^{Kb} deletion by Multiplex- PCR technique adapted from the previous study using only primers of wild type alpha genes and 4.2^{Kb} 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: N° 834.086, CAEE 30668114.0.0000.5020; N° 213.167, CAAE: 01193312.4.0000.0009; and N° 1.178.117, CAAE: 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 analyzes 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 (N)	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 -$\alpha^{3.7Kb}$ 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 4.2^{Kb} 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 a-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 a-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

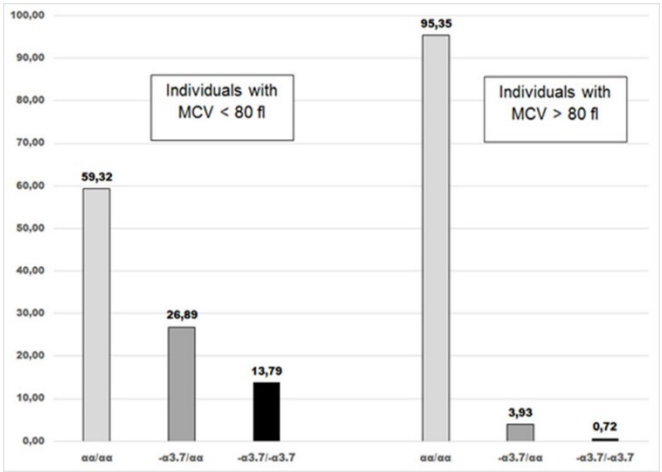

Figure 1. Frequency of the -$\alpha^{3.7Kb}$ genotypes between individuals with microcytic and normocytic erythrocytes.

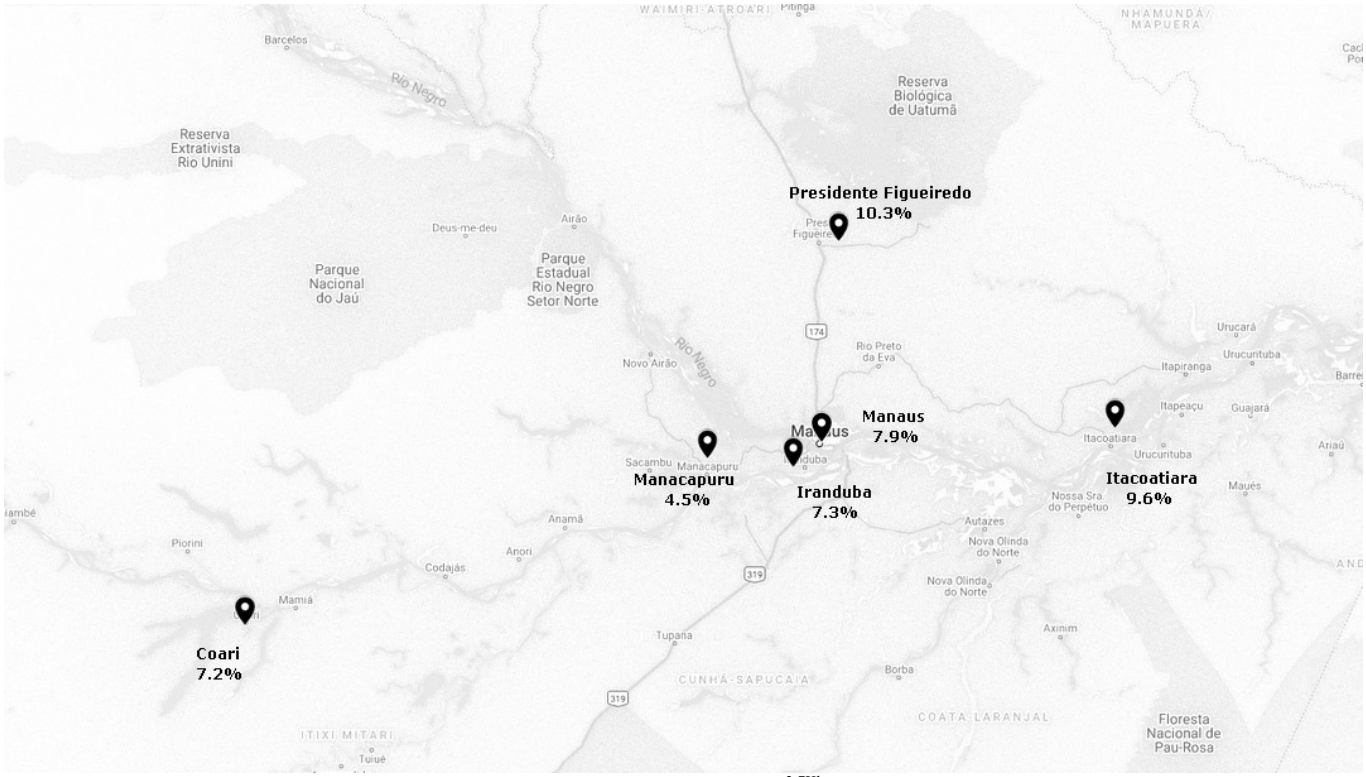

Figure 2. Map of Metropolitan Region of Manaus-Amazonas - Frequencies of -$\alpha^{3.7Kb}$ deletion.

(p=.886), Coari (p=.176).

Moreover, finally, we create a map of the -$a^{3.7Kb}$ frequencies found in the Amazon region. The results showed that -$\alpha^{3.7Kb}$ 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 -$\alpha^{3.7Kb}$ 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 -$\alpha^{3.7Kb}$ 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, -$\alpha^{3.7Kb}$ 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 -α3.7Kb (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 $-\alpha^{3.7Kb}$ 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.

Acknowledgments. Sponsorships: Financial support was provided by grants from:

- Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) – Protocol Number: 1094/2013-FAPEAM.
- Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
- The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

References:

1. Sakai Y, Kobayashi S, Shibata H, Furuumi H, Endo T, Fucharoen S, Hamano S, Acharya GP, Kawasaki T, Fukumaki Y. Molecular analysis of alpha-thalassemia in Nepal: correlation with malaria endemicity. J Hum Genet. 2000;45(3):127-32. https://doi.org/10.1007/s100380050198 PMid:10807536
2. Sabath DE. Molecular Diagnosis of Thalassemias and Hemoglobinopathies: An ACLPS Critical Review. Am J Clin Pathol. 2017;148(1):6-15. https://doi.org/10.1093/ajcp/aqx047 PMid:28605432
3. P. Ponka, M.J. Koury, A.D. Sheftel. Erythropoiesis, hemoglobin synthesis, and erythroid mitochondrial iron homeostasis. G.C. Ferreira, K.M. Kadish, K.M. Smith, R. Guilard (Eds.), Handbook of Porphyrin Science, World Scientific Co., Singapore (2013), pp. 41-84. https://doi.org/10.1142/9789814407755_0011
4. Goh SH1, Lee YT, Bhanu NV, Cam MC, Desper R, Martin BM, Moharram R, Gherman RB, Miller JL. A newly discovered human globin gene. Blood. 2005 Aug 15;106(4):1466-72. Epub 2005 Apr 26. https://doi.org/10.1182/blood-2005-03-0948 PMid:15855277 PMCid:PMC1895206
5. Higgs DR, Weatherall DJ. The Alpha Thalassaemias. Cell Mol Life Sci. 2009;66(7):1154-62. https://doi.org/10.1007/s00018-008-8529-9 PMid:19020805
6. Kasper, Dennis L., Anthony S. Fauci, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, and Joseph Loscalzo. eds. Harrison's Principles of Internal Medicine, 18e. New York, NY: McGraw-Hill; 2015.
7. Spier C. Wintrobe's Atlas of Clinical Hematology. Am J Surg Pathol. 2008;32:1428. https://doi.org/10.1097/PAS.0b013e31816955c5
8. Hardison RC. (2012) Evolution of hemoglobin and its genes. Cold Spring Harb Perspect Med. 2:a011627. https://doi.org/10.1101/cshperspect.a011627 PMid:23209182 PMCid:PMC3543078
9. Li CK. New trend in the epidemiology of thalassaemia. Best Pract Res Clin Obstet Gynaecol. 2017 Feb;39:16-26. https://doi.org/10.1016/j.bpobgyn.2016.10.013 PMid:27847257
10. Baysal E. Huisman TJH. Detection of common deletional α-thalassemia-2 determinants by PCR. Am J Hematol. 1994;46(3):208-13. https://doi.org/10.1002/ajh.2830460309 PMid:8192150
11. Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for a-thalassemia. Blood, vol. 98, no. 1, pp. 250-251, 2001. https://doi.org/10.1182/blood.V98.1.250 PMid:11439976
12. Rosenfeld LG, Malta DC, Szwarcwald CL, et al. Reference values for blood count laboratory tests in the Brazilian adult population, National Health Survey. Valores de referência para exames laboratoriais de hemograma da população adulta brasileira: Pesquisa Nacional de Saúde. Rev Bras Epidemiol. 2019; 22 Suppl:E190003.SUPL.2. https://doi.org/10.1590/1980-549720190003.supl.2 PMid:31596374
13. GOOGLE, INC. Google Maps. Available in: http://code.google.com/apis/maps/documentation/directions/ Accessed on: March 2020.
14. Higgs DR, Goodbourn SE, Lamb J, Clegg JB, Weatherall DJ, Proudfoot NJ. Alpha-Thalassemia caused by a polyadenylation signal mutation. Nature. 1983 Nov 24-30;306(5941):398-400. https://doi.org/10.1038/306398a0 PMid:6646217
15. Cürük MA1, Kilinç Y, Evrüke C, Ozgünen FT, Aksoy K, Yüreğir GT. Prenatal diagnosis of Hb H disease caused by a homozygosity for the α2 polyA mutation. Hemoglobin. 2001 May;25(2):255-8. https://doi.org/10.1081/HEM-100104034 PMid:11480787
16. Karen DF. Clinical evaluation of hemoglobinopathies: Part I. Thalassemia. The Warde Medical Laboratory Article Archives. 2003, Volume 14, Number 2.
17. Anselmo FC, Ferreira NS, da Mota AJ, et al. Deletional Alpha-Thalassemia Alleles in Amazon Blood Donors. Adv Hematol. 2020;2020:4170259. https://doi.org/10.1155/2020/4170259 PMid:32351571 PMCid:PMC7178540
18. Foglietta E, Deidda G, Graziani B, Modiano G, Bianco I. Detection of alpha-globin gene disorders by a simple PCR methodology. Haematologica. 1996 Sep-Oct;81(5):387-96. Erratum in: Haematologica 1996 Nov-Dec;81(6):XVI. PMID: 8952150.
19. Wagner SC, Silvestri MC, Bittar CM, Friedrisch JR, Silla LMR, Para C. Prevalence of thalassemias and variant hemoglobins in patients with nonferropenic anemia. bras hematol hemoter. 2005;27(1):37-42.

https://doi.org/10.1590/S1516-84842005000100010
20. WHO. Thalassaemia and other haemoglobinopathies. 2006. http://apps.who.int/gb/archive/pdf_files/EB118/B118_5-en.pdf (Acesso em 20/01/2019).
21. de Medeiros Alcoforado GH, Bezerra CM, Araújo Moura Lemos TM, et al. Prevalence of α-thalassemia 3.7 kb deletion in the adult population of Rio Grande do Norte, Brazil. Genet Mol Biol. 2012;35(3):594-598. https://doi.org/10.1590/S1415-47572012005000049 PMid:23055797 PMCid:PMC3459408
22. de Souza RA, Carlos AM, de Souza BM, Rodrigues CV, Pereira Gde A, Moraes-Souza H. A-Thalassemia: Genotypic Profile Associated with Ethnicity and Hematological Differentiation of Iron Deficiency Anemia in the Region of Uberaba, Minas Gerais, Brazil. Hemoglobin. 2015;39(4):264-9. https://doi.org/10.3109/03630269.2015.1037890 PMid:26182338
23. Sankar VH, Arya V, Tewari D, Gupta UR, Pradhan M, Agarwal S. Genotyping of alpha-thalassemia in microcytic hypochromic anemia patients from North India. J Appl Genet. 2006;47(4):391-5. https://doi.org/10.1007/BF03194650 PMid:17132905
24. Lois R. Manning, J. Eric Russell, Julio C. Padovan, Brian T. Chait, Anthony Popowicz, Robert S. Manning, and James M. Manning. Human embryonic, fetal, and adult hemoglobins have different subunit interface strengths. Correlation with lifespan in the red cell. Protein Sci. 2007 Aug; 16(8): 1641-1658. https://doi.org/10.1110/ps.072891007 PMid:17656582 PMCid:PMC2203358
25. Karamzade A, Mirzapour H, Hoseinzade M, Asadi S, Gholamrezapour T, Tavakoli P, Salehi M. α-Globin Gene Mutations in Isfahan Province, Iran. Int. J. Hemoglobin. Res. 2014;38(3). https://doi.org/10.3109/03630269.2014.893531 PMid:24826792
26. RUIS, Josué Ferreira. Manacapuru e sua história. Manacapuru: Shirley Pinheiro, 2000.
27. IBGE, Biblioteca. Manacapuru Amazonas-AM: Histórico. Disponível em:<http://biblioteca.ibge.gov.br/visualizacao/dtbs/amazonas/manacapuru.pdf >. Acesso em: 29 de junho de 2020.
28. Amaz RV. Revista Veredas Amazônicas - Nov - no 01, vol i, 2011. issn: 2237- 4043. 2011;I(V).
29. Jakob AAE. International migration in the Brazilian Amazon. Toledo Vol. 15, Ed. 3, (2011): 422-442.
30. Xavier FCC. Migrações Internacionais na Amazônia Brasileira: Impactos na Política Migratória e na Política Externa. 2012. http://repositorio.unb.br/bitstream/10482/10739/1/2012_Fernando%20Cesar%20Costa%20Xavier.pdf
31. Jakob AAE. The recent international migration in the Brazilian Amazon. REMHU, Rev. Interdiscip. Mobil. Hum. vol.23 no.45 Brasília July/Dec. 2015. https://doi.org/10.1590/1980-858525031988000451 3
32. Osório, Rafael Guerreiro. O Sistema Classificatório de Cor ou Raça do IBGE. Brasília, nov.2003. Disponível em: http://www.ipea.gov.br/portal/index.php?option=com_content&view=article&id=4212 Acesso em março 2019
33. Petruccelli, José Luís & Saboia, Ana Lucia(org.). Características Étnico-Raciais da População. IBGE, 2013.

Supplementary table 1. Hematologic parameters characterization and levels of serum ferritin and serum iron among alpha thalassemia 3.7^{kb} deletion in metropolitan region of manaus.

	Manaus	Iranduba	Manacapuru	Presidente Figueiredo	Itacoatiara	Coari	p-value
Wide type (αα / αα)							
(A)							
RBC (x10^6/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 ± 4.41	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 ± 2.41	28.87 ± 2.10	28.83 ± 1.25	29.48 ± 1.93	<.001
MCHC (pg)	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
RDW (%)	13.20 ± 1.18	13.07 ± 0.78	13.67 ± 0.97	13.51 ± 0.71	14.04 ± 0.68	12.90 ± 0.87	<.001
Serum Iron (µg/dL)	81.97 ± 4.38	93.77 ± 25.62	85.46 ± 25.74	83.32 ± 32.59	90.60 ± 32.61	96.20 ± 32.37	<.001
Ferritin (µg/dL)	137.27 ± 52.69	110.16 ± 33.28	109.87 ± 31.31	94.92 ± 25.31	96.30 ± 26.66	112.09 ± 47.01	<.001
Heterozygous (-α / αα)							
(B)							
RBC (x10^6/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	.062
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	.049
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
Homozygous (-α / -α)							
(C)							
RBC (x10^6/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 ± 5.35	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	73.96 ± 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=161	p-value	N=89	N=143	p-value	N=121	N=166	p-value	N=135	N=235	p-value	N=111	N=190	p-value	N=109	N=154	p-value
	Mean ± SD			Mean ± SD			Mean ± SD			Mean ± SD			Mean ± SD			Mean ± SD		
	Male	Female		Male	Female		Male	Female		Male	Female		Male	Female		Male	Female	
RBC ($x10^6$/mm L)	4.85 ± 0.42	4.41 ± 0.41	<.001	4.61 ± 0.57	4.23 ± 0.54	<.001	4.87 ± 0.66	4.42 ± 0.54	<.001	4.96 ± 0.53	4.36 ± 0.38	<.001	5.12 ± 0.60	4.41 ± 0.47	<.001	4.70 ± 0.56	4.24 ±1 0.58	<.001
Hb (g/dL)	14.46 ± 1.29	13.02 ± 1.32	<.001	13.70 ± 1.31	12.45 ± 1.52	<.001	13.92 ± 1.42	13.41 ± 1.40	<.001	14.44 ± 1.71	12.8 ± 1.0	<.001	14.04 ± 1.36	13.35 ± 1.39	<.001	13.71 ± 1.66	12.35 ± 1.52	<.001
Hct (%)	44.01 ± 3.53	39.77 ± 3.59	<.001	40.98 ± 3.96	37.26 ± 4.48	<.001	42.23 ± 4.57	40.04 ± 4.24	.002	43.69 ± 4.66	39.7 ± 3.4	<.001	42.99 ± 4.27	39.07 ± 3.68	<.001	42.67 ± 5.18	38.2 ± 4.4	<.001
MCV (fL)	90.72 ± 4.57	90.34 ± 5.52	.232	89.46 ± 9.31	88.54 ± 8.37	.431	87.26 ± 7.75	86.77 ± 5.18	.200	88.23 ± 5.19	88.1 ± 6.4	.945	84.27 ± 6.31	84.71 ± 5.16	.612	90.76 ± 5.62	89.91 ± 7.55	.506
MCH (pg)	29.82 ± 1.97	29.59 ± 2.41	.286	29.92 ± 2.92	29.65 ± 3.04	.559	28.82 ± 2.84	29.03 ± 2.16	.644	29.16 ± 2.13	28.5 ± 2.3	.010	28.29 ± 1.60	28.70 ± 1.53	.022	29.18 ± 1+97	29.07 ± 2.67	.898
MCHC (pg)	32.86 ± 1.15	32.74 ± 1.53	.762	33.49 ± 1.62	33.59 ± 2.03	.806	33.08 ± 1.98	33.57 ± 2.04	.525	33.07 ± 1.83	32.4 ± 1.6	.000	33.63 ± 1.35	33.93 ± 1.27	.059	32.16 ± 1.32	32.21 ± 1.39	.516
RDW	13.35 ± 1.33	12.83 ± 0.97	<.001	13.08 ± 0.87	13.01 ± 0.80	.862	13.66 ± 0.83	13.65 ± 1.02	.789	13.5 ± 0.7	13.5 ± 0.7	.504	14.0 ± 0.8	14.01 ± 0.68	.680	12.8 ± 0.9	12.94 ± 0.94	.373
Iron (µg/dL)	81.8 ± 4.4	80.70 ± 5.03	.037	102.19 ± 25.34	85.89 ± 25.42	<.001	89.21 ± 24.57	83.22 ± 26.27	.052	88.23 ± 36.41	78.24 ± 30.70	.005	95.09 ± 35.35	87.08 ± 31.10	.042	100.15 ± 28.45	91.69 ± 35.95	.040
Ferritin (µg/L)	177.7 ± 27.4	83.9 ± 19.5	<.001	114.1 ± 35.1	105.9 ± 33.8	.076	114.0 ± 31.8	107.4 ± 30.7	.082	89.8 ± 30.9	96.7 ± 24.9	.020	104.6 ± 30.7	91.8 ± 23.5	<.001	156.3 ± 34.0	80.1 ± 24.9	<.001

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.