

Reticulocyte Count and its Immature Fractions as an Early Indicator of Bone Marrow Alteration in Gas Station Workers

doi: <https://doi.org/10.32635/2176-9745.RBC.2023v69n2.3555>

Contagem de Reticulócitos e suas Frações Imaturas como Indicador Precoce de Alteração Medular em Trabalhadores de Postos de Combustíveis

Recuento de Reticulocitos y sus Fracciones Inmaduras como Indicador Temprano de Alteración Medular en Trabajadores de Gasolineras

Alain Ferreira Vilhena¹; Jamille Douahy Rebelo²; Rubens Corrêa de Souza Neto³; Ana Maria Almeida Souza⁴; Lacy Cardoso de Brito Junior⁵

ABSTRACT

Introduction: Prolonged occupational exposure to toxic components of gasoline such as benzene for a long time is recognized as an important factor in hematological changes. **Objective:** Evaluate the count of reticulocytes and their immature fractions (IRF) in samples of gas station workers tested at a private laboratory in Belém, Pará, from January to December 2021. **Method:** Forty-seven peripheral blood samples were analyzed from male gas station workers, regardless of the time of work, who submitted to periodic tests at a private laboratory in Belém (study group), and 47 samples from male customers who underwent routine blood counts at the same laboratory without previous hematological disease (control group). **Results:** In the control group, 46/47 (97.9%) of the samples had a mean reticulocyte count of 62,828/mm³ and a relative IRF of 3/47 (6.4%), whereas in the study group, the mean reticulocyte count was of 100,628/mm³ and IRF of 5/47 (10.8%). Of these, 34/47 (72.3%) samples had a reticulocyte count and an IRF higher than the reference values. **Conclusion:** The results suggest that the increase in the reticulocyte count and its IRF in the study group may be important early risk markers for medullary intoxication by benzene.

Key words: reticulocyte count; benzene; hydrocarbons; occupational exposure; anemia, aplastic.

RESUMO

Introdução: A exposição ocupacional prolongada a componentes tóxicos da gasolina, como o benzeno, há muito tempo é reconhecida como um importante fator de alterações hematológicas. **Objetivo:** Avaliar a contagem de reticulócitos e suas frações imaturas (IRF) em amostras de trabalhadores de postos de combustíveis ("frentistas") atendidos em um laboratório particular de Belém, Pará, no período de janeiro a dezembro de 2021. **Método:** Foram analisadas 47 amostras de sangue periférico de trabalhadores de postos de combustíveis do sexo masculino (grupo do estudo), independentemente do tempo de atividade laboral, e que realizam exames laboratoriais periódicos em um laboratório particular de Belém, e 47 amostras de clientes, também do sexo masculino, que realizaram hemograma de rotina no mesmo laboratório e não apresentavam doença hematológica prévia (grupo controle). **Resultados:** No grupo controle, 46/47 (97,9%) das amostras apresentavam média de contagem de reticulócitos de 62.828/mm³ e de IRF relativas de 3/47 (6,4%), já nos trabalhadores do grupo de estudo a média de contagem de reticulócitos foi de 100.628/mm³ e de IRF de 5/47 (10,8%). Destas, 34/47 (72,3%) amostras apresentavam contagem de reticulócitos e de IRF superiores aos valores de referência. **Conclusão:** Os resultados sugerem que o aumento da contagem de reticulócitos e suas IRF em frentistas de postos de combustíveis podem ser importantes marcadores de risco precoce de intoxicação medular pelo benzeno.

Palavras-chave: contagem de reticulócitos; benzeno; hidrocarbonetos; exposição ocupacional; anemia aplástica.

RESUMEN

Introducción: La exposición ocupacional prolongada a los componentes tóxicos de la gasolina, como el benceno, se ha reconocido durante mucho tiempo como un factor importante en los cambios hematológicos. **Objetivo:** Evaluar conteos de reticulocitos y sus fracciones inmaduras (IRF) en muestras de trabajadores en gasolineras atendidos en un laboratorio privado en Belém, Pará, de enero a diciembre de 2021. **Método:** Se analizaron 47 muestras de sangre periférica de individuos de sexo masculino, independientemente del tiempo de actividad laboral, que son trabajadores de gasolineras y que realizan exámenes de laboratorio periódicos en un laboratorio privado en Belém (grupo estudio), así como 47 muestras de clientes, también de sexo masculino, a quienes se les realizaron hemogramas de rutina en el mismo laboratorio y no presentaban enfermedad hematológica previa (grupo control). **Resultados:** En el grupo control, 46/47 (97,9%) de las muestras tenían un recuento promedio de reticulocitos de 62.828/mm³ y un IRF relativo de 3/47 (6,4%), mientras que en los trabajadores del grupo estudio el recuento promedio de reticulocitos fue de 100.628/mm³ y un IRF de 5/47 (10,8%). De estas, 34/47 (72,3%) muestras tenían recuentos de reticulocitos e IRF superiores a los valores de referencia. **Conclusión:** Los resultados sugieren que el aumento de los recuentos de reticulocitos y sus IRF en los trabajadores de las gasolineras pueden ser marcadores importantes del riesgo temprano de intoxicación medular por benceno.

Palabras clave: recuento de reticulocitos; benceno; hidrocarburos; exposición profesional; anemia aplásica.

¹Laboratório Amara Costa Medicina Diagnóstica. Belém (PA), Brazil. E-mail: vilhenaalain@gmail.com. Orcid iD: <https://orcid.org/0000-0001-6783-1149>

^{2,3}Faculdade Integrada Brasil Amazônia (FIBRA). Belém (PA), Brazil. E-mails: jamilledrebelo@gmail.com; r.neto27@hotmail.com. Orcid iD: <https://orcid.org/0000-0002-9681-9138>; <https://orcid.org/0000-0003-2596-858X>

⁴Universidade Federal do Pará (UFPA), Instituto de Ciências da Saúde, Faculdade de Medicina. Belém (PA), Brazil. E-mail: anamas@ufpa.br. Orcid iD: <https://orcid.org/0000-0001-7609-3133>

⁵UFPA, Instituto de Ciências Biológicas, Laboratório de Patologia Geral – Imunopatologia e Citologia. Belém (PA), Brazil. E-mail: lcardbrito2@gmail.com. Orcid iD: <https://orcid.org/0000-0001-9102-5817>

Corresponding author: Lacy Cardoso de Brito Júnior. UFPA, Instituto de Ciências Biológicas, Laboratório de Patologia Geral – Imunopatologia e Citologia. Av. Augusto Corrêa, 1 – Guamá. Belém (PA), Brazil. CEP 66075-900. E-mails: lcardbrito@ufpa.br; lcardbrito@bol.com.br



INTRODUCTION

Exposure to hydrocarbons and their derivatives, such as benzene, present in detergents, plastics and gasoline has generated several alerts by the International Agency for Research on Cancer (IARC) about their potential carcinogenic risk¹⁻⁸.

Many of these substances are absorbed by the human body through inhalation, dermal contact or even by ingestion. Especially in relation to benzene, after its absorption, its toxic effects can be detected in the liver (primary metabolism), bone marrow (secondary metabolism) and kidneys (excretion)^{2,4,5,7,9,10}.

The primary hematological changes due to this harmful exposure to benzene are often observed only when the blood count already shows anemia, leukopenia, thrombocytopenia or pancytopenia associated with bone marrow aplasia or, in more severe cases, when leukemias appear^{6-8,10,11}.

Naoum et al.¹² and Ruiz et al.¹¹ already suggested that the functionality and integrity of the bone marrow in these individuals could be estimated through the quantification, of the concentration of reticulocytes¹³⁻¹⁶ in peripheral blood. More recently, with the automated counting of reticulocytes^{13,16,17} and immature reticulocytes fractions (IRF), these parameters have gained even greater accuracy for the evaluation of erythropoiesis activity¹⁴⁻¹⁶.

This study aimed to evaluate the count of reticulocytes and IRF in gas station workers as a possible method of identifying early bone marrow injury.

METHOD

Peripheral blood samples from 47 male workers (study group) of a company that manages gas stations in the metropolitan region of Belém, Pará were included, regardless of the time in the work function and who performed blood counts as routine exams at a private laboratory in the same city (study group) from January to December 2021. The control group was randomly selected and consisted of 47 male clients who performed routine blood counts in the same laboratory and period. As a criterion for inclusion in the study, all individuals in both groups should be non-smokers, did not present qualitative-quantitative alterations or previous disease of the red series.

The samples were collected as usual and submitted to automated processing in the Sysmex XN-1000 equipment and, after analyzing the results, selected for counting reticulocytes and their IRF in the same equipment, which uses fluorescent dyes as the technology that bind to the RNA of reticulocytes and IRF^{16,17}.

Values between 25,000 and 85,000/mm³ were considered as reference values for the absolute reticulocyte count in people aged over 30 days of life. However, as there are no absolute reference values for the IRF in the literature, these were analyzed only as research parameters¹⁷.

As the study was based only on secondary databases, the investigators signed a Term of Commitment to Use Data (TCUD) of the institution responsible for providing the data, in compliance with Resolution number 466¹⁸ of December 12, 2012, and supplemented by Article 17, item VII of Resolution 510/2010¹⁹.

Descriptive and parametric analyzes were performed using the ANOVA test through the StatPlus software version 8.0 and considering $p < 0.05$.

RESULTS

The mean age of the workers in the study group was 35 years, while the means of reticulocyte count was 100,628/mm³ and the IRF was 10.8%. For this group, it was observed that, even with blood cell counts (erythrocytes, leukocytes and platelets) within normal limits according to criteria of the World Health Organization (WHO)²⁰, reticulocyte counts and IRF in 34/47 (72.3%) of the samples analyzed were higher than the reference values established in this study.

On the other hand, the analysis of the results of the samples of the 47 individuals of the control group (Table 1), who underwent routine hemogram and did not present previous alterations in the erythrocytes, revealed that the average age was 37 years, the reticulocyte count was 62,828/mm³ and the IRF was 7.4%. Of these, 46/47 (97.9%) samples showed reticulocytes count values within the reference values established in this study and also blood counts with erythrogram, leukogram and platelet count indicators within normal limits.

Parametric tests were performed to compare the results between the control and study groups, with a statistical difference ($p < 0.05$) found in the results of reticulocytes ($p = 0.000001$) counts and IRF ($p = 0.00308$) being observed between groups, suggesting that the samples of gas station attendants present higher reticulocytes and IRF counts than those observed in individuals who did not have this occupation.

DISCUSSION

Figueiredo et al.⁶, studying the concentration of substances utilized to produce gasoline at gas stations in the city of Rio de Janeiro, Brazil, noticed that benzene concentrations were above the levels recommended

Table 1. Concentration of reticulocytes and IRF in the study group and control group who were submitted to routine blood count at a private laboratory in Belém - Pará, from January to December 2021

		Age (years)	Concentration of reticulocytes (mm ³)	IRF (%)
Study Group	Max - Min	20 - 56	45.765 - 223.630	4.7 ± 25.3
	X ± SD	35 ± 8.9	100.628 ± 31.124	10.8 ± 4.5
Control Group	Max - Min	18 - 62	34.086 - 85.158	3.2 ± 17.4
	X ± SD	37 ± 12.3	62.828 ± 11.640	7.4 ± 2.9

Captions: IRF = immature reticulocytes fractions; Max-Min = maximum and minimum values; X ± SD = mean and standard deviation.

by the current legislation and that these places were potentially dangerous for gas station workers who refuel on daily bases due to the carcinogenic potential of this substance²¹.

Poça et al.⁷, in their studies on chronic exposure to benzene from gasoline, showed that genotoxic effects (DNA damage assessed by the comet assay) and immunotoxic effects (assessed by low levels of helper T lymphocytes and naive T lymphocytes) were greater among individuals who worked directly in the supply of gasoline compared to what was observed among office workers at the same filling stations.

It is also clear that the chronic effects of exposure to benzene, even in small concentrations and for a long period, may be related to hematological changes ranging from depression of blood cell production, mild (anemia, leukopenia or thrombocytopenia) and even severe conditions (pancytopenia), which can lead to irreversible medullary aplasia^{10,22} and even alterations in the appearance of leukemias and lymphomas⁸.

The investigation revealed that the gas station attendants of the gas stations had indicators of erythrogram, leukogram and blood platelet count within the normal limits, however, with reticulocyte and IRF counts higher than those found in individuals who did not have this occupation (control group).

These findings suggest that the continuous and daily exposure of these workers to benzene can be evaluated apparently through the determination of the absolute concentrations of reticulocytes and IRF, which seem to reflect the early bone marrow injury.

The study data, however, could not be compared with other studies of the literature, since it is the first one to address this relationship between changes in reticulocyte and IRF concentration with exposure to benzene. Similar studies carried out by Naoum et al.¹² and Ruiz et al.¹¹, over a decade ago, also associated reticulocytosis as an important finding in individuals with benzene intoxication.

CONCLUSION

The results suggest that the increase in the reticulocytes count and its immature fractions in the study group may be important early risk markers for bone marrow injury by benzene.

ACKNOWLEDGEMENTS

To the Directors of the *Laboratório Amaral Costa – Medicina Diagnóstica* for authorizing the utilization of the data for this study.

CONTRIBUTIONS

Alain Ferreira Vilhena contributed to the study design, acquisition and analysis of the data. Jamille Douahy Rebelo and Rubens Correa de Souza Neto contributed to the acquisition and analysis of the data. Ana Maria Almeida Souza and L. C. Brito Junior contributed to the study design, analysis and interpretation of the data, wording and critical review. All the authors approved the final version for publication.

DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interests to declare.

FUNDING SOURCES

Universidade Federal do Pará. Programa de Pós-Graduação em Análise Clínica (PPGAC).

REFERENCES

1. Fonseca ASA, Costa DF, Dapper V, et al. Classificação clínico-laboratorial para manejo clínico de trabalhadores expostos ao benzeno em postos de revenda de combustíveis. *Rev Bras Saúde Ocup.* 2017;42(Suppl 1):e5s. doi: <https://doi.org/10.1590/2317-6369000127115>

2. Moura-Correa MJ, Larentis AL. Exposição ao benzeno no trabalho e seus efeitos à saúde. *Rev Bras Saude Ocup.* 2017;42(Suppl 1):e14s. doi: <https://doi.org/10.1590/2317-6369ED0000117>
3. Maciel LA, Feitosa SB, Trolly TS, et al. Avaliação dos efeitos genotóxicos da exposição ocupacional em frentistas atuantes em postos de gasolina no município de Santarém, Pará. *Rev Bras Med Trab.* 2019;17(2):247-253. doi: <https://doi.org/10.5327/Z1679443520190382>
4. Silva VM, Medeiros RC, Modesto CSP, et al. Exposição ao benzeno em trabalhadores de postos de gasolina: uma revisão integrativa da literatura. *Res Soc Dev.* 2021;10(6):e14610615649. doi: <https://doi.org/10.33448/rsd-v10i6.15649>
5. Pádua KM, Silva LRO, Souza MN, et al. Análise da exposição ocupacional aos combustíveis automotivos em frentistas de um posto de revenda de Luz-MG. *Braz J Develop.* 2021;7(8):81969-88. doi: <https://doi.org/10.34117/bjdv7n8-418>
6. Figueiredo VO, Carvalho LVB, Borges RM, et al. Avaliação da exposição a BTEX em postos de revenda de combustíveis no Rio de Janeiro, Brasil, e os riscos à saúde do trabalhador. *Cad Saúde Pública.* 2021;37(11):e00351520. doi: <https://doi.org/10.1590/0102-311X00351520>
7. Poça KS, Giardini I, Silva PVB, et al. Gasoline-station workers in Brazil: benzene exposure; genotoxic and immunotoxic effects. *Mutat Res Genet Toxicol Environ Mutagen.* 2021;865:503322. doi: <https://doi.org/10.1016/j.mrgentox.2021.503322>
8. Tolosa Rodríguez MA, Rojas Becerra MR, Vaca Alvarado LJ, et al. Actividades económicas, laborales, ambientales y aditivas con exposición a benceno y el desarrollo de leucemia mieloide aguda. *Rev Repert Med Cir.* 2022;31(1):42-51. doi: <https://doi.org/10.31260/RepertMedCir.01217372.1059>
9. Santos MVC, Figueiredo VO, Arcuri ASA, et al. Aspectos toxicológicos do benzeno, biomarcadores de exposição e conflitos de interesses. *Rev Bras Saude Ocup.* 2017;42(supl 1):e13s. doi: <https://doi.org/10.1590/2317-6369NOTA00017>
10. Tunsaringkarn T, Soogarun S, Palasuwan A. Occupational exposure to benzene and changes in hematological parameters and urinary trans, trans-muconic acid. *Int J Occup Environ Med.* 2013;4(1):45-9.
11. Ruiz MA, Vassallo J, Souza CA. Alterações hematológicas em pacientes expostos cronicamente ao benzeno. *Rev Saúde Pública.* 1993;27(2):145-51. doi: <https://doi.org/10.1590/S0034-89101993000200011>
12. Naoum PC, Mourão CA, Ruiz MA. Alterações hematológicas induzidas por poluição industrial em moradores e industriários de Cubatão, SP (Brasil). *Rev Saúde Pública.* 1984;18(4):271-7. doi: <https://doi.org/10.1590/S0034-89101984000400002>
13. Piva E, Brugnara C, Spolaore F, et al. Clinical utility of reticulocyte parameters. *Clin Lab Med.* 2015;35(1):133-63. doi: <https://doi.org/10.1016/j.cll.2014.10.004>
14. Er I, Cetin C, Baydemir C. Can immature reticulocyte fraction be an inflammatory biomarker in late-preterm infants diagnosed with congenital pneumonia? *Ann Med Res.* 2020;27(7):1918-23. doi: <https://doi.org/10.5455/annalsmedres.2020.02.161>
15. Das J, Khonglah Y, Tiewsoh I, et al. Utility of reticulocyte indices in the diagnosis of pancytopenia. *J Family Med Prim Care.* 2022;11(4):1335-40. doi: https://doi.org/10.4103/jfmpc.jfmpc_1121_21
16. Arneth BM, Menschikowki M. Technology and new fluorescence flow cytometry parameters in hematological analyzers. *J Clin Lab Anal.* 2015;29(3):175-83. doi: <https://doi.org/10.1002/jcla.21747>
17. Małecka M, Ciepła O. A comparison of Sysmex-XN 2000 and Yumizen H2500 automated hematology analyzers. *Pract Lab Med.* 2020;22:e00186. doi: <https://doi.org/10.1016/j.plabm.2020.e00186>
18. Conselho Nacional de Saúde (BR). Resolução nº 466, de 12 de dezembro de 2012. Aprova as diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos. *Diário Oficial da União, Brasília, DF.* 2013 jun 13; Seção 1:59.
19. Conselho Nacional de Saúde (BR). Resolução nº 510, de 7 de abril de 2016. Dispõe sobre as normas aplicáveis a pesquisas em Ciências Humanas e Sociais cujos procedimentos metodológicos envolvam a utilização de dados diretamente obtidos com os participantes ou de informações identificáveis ou que possam acarretar riscos maiores do que os existentes na vida cotidiana, na forma definida nesta Resolução. *Diário Oficial da União, Brasília, DF.* 2016 maio 24; Seção 1:44.
20. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* 2016;127(20):2375-90. doi: <https://doi.org/10.1182/blood-2016-01-643569>
21. Geraldino BR, Nunes RFN, Gomes JB, et al. Risks related to the domestic laundering of filling station attendant uniforms: advances and uncertainties. *Rev Bras Med Trab.* 2021;19(2):240-8. doi: <https://doi.org/10.47626/1679-4435-2020-590>
22. Costa DF, Goldbaum M. Contaminação química, precarização, adoecimento e morte no trabalho: benzeno no Brasil. *Ciênc Saúde Colet.* 2017;22(8):2681-92. doi: <https://doi.org/10.1590/1413-81232017228.31042016>

Recebido em 8/12/2022
Aprovado em 8/3/2023