Benzene poisoning, clinical and blood abnormalities in two Brazilian female gas station attendants: two case reports

Fábio Santiago1,2,3, Simone Lima1, Tayná Pinheiro1, Rafaele Tavares Silvestre1,2,3, Ubirani Barros Otero4, Marianne Medeiros Tabalipa4, Nadezda Kosyakova5, Maria Helena Ornellas1,2,6*, Thomas Liehr5 and Gilda Alv es1,2,3

Abstract

Background: Brazilian gas station workers are chronically exposed to benzene, toluene, xylene (BTX) during their working time. Describe below two cases of Latin female gas station workers with benzene poisoning symptoms and miscarriage history.

Case presentation: In both cases were identified complex chromosomal rearrangements (CCR) with fluorescence in situ hybridization, applied to whole chromosome paints by chromosomes 1, 2 and 4. The lower natural killer cell (NK) cells have also been observed in cases correspondents, especially the rare type of NK (NKbright) in their peripheral blood cells.

Conclusions: It is known that acquired chromosomal aberrations are positively correlated with cancer and reproductive risk. In concordance, lower NK cytotoxicity increases the risk for cancer, as well. Thus, this is the first study providing hints on a possible causative relation of lower NK cytotoxicity and increase rates of chromosomal rearrangements including CCRs.

Keywords: Benzene, Toluene, Xylene, Cytogenetic, Painting chromosome, Natural killer

Background

Brazilian gas station workers are chronic exposed to benzene, toluene and xylene (BTX), mainly benzene, during the working time [1]. Chronic exposure to benzene may lead to progressive degeneration of bone marrow, aplastic anemia and/or leukemia. According to the U.S. Department of Labor only a detailed history and appropriate investigative procedures will enable a physician to rule out or confirm the benzene poisoning [2]. To assist the examining physician the cytogenetic tests with fluorescence in situ hybridization (FISH) shown to be a new and valuable tool to determine the workers in risk [3]. Genetic damages caused by benzene include sister chromatid exchanges, DNA cross linking agents, DNA adduct formations, and impairment of DNA repair mechanisms [4]. Since 2010, 115 gas station attendants have been monitored in Rio de Janeiro city, Brazil applying FISH, using whole chromosome painting (wcp) probes for chromosomes 1, 2 and 4. Among the study group, the medical inquiry identified two female gas station attendants with signs and symptoms of acute benzene intoxication, associated with history of abortions. Added to the cytogenetic tests an large hematological evaluation by cytometry were made and found a down regulation of the natural killer (NK) cells association an acquired complex chromosomal rearrangements (CCRs).

Case presentation

Case 1
A 25-year-old Latin woman, working 8 h per day, 6 days a week, for the last 4 years as a gas station attendant...
had one gestation with miscarriage in the first half of pregnancy. Headache, dizziness irritability, asthenia and normal menstrual cycle were reported. A physical examination showed changes in the thyroid gland, nodules in the right lobe, and a nonspecific pulmonary auscultation. The attendant also reported being a former smoker and not having a family history of cancer. Was found in 1/100 metaphases a CCR involving 8 chromosomal breakpoints described as: 46,XX,der(1)t(1;4),der(4)t(1;4;?),ace(1),ace(1), (Fig. 1). Hemogram showed mild neutopenia (1470 cells/mm$^3$) and biochemistry tests revealed no changes compared to normal values as described in Table 1. On the other hand, the immunophenotypic analysis confirmed neutropenia (33.00%), with a lower NK cell count (2.28%), with all NK CD56+/CD16− (Table 2; Fig. 1).

Case 2
A 40-year-old latin woman, who was not a smoker or drug addict, working 48 h a week for the last 9 years as a gas station attendant had a pathological history of one miscarriage in the first half of pregnancy. Anxiety, dizziness, cramps, asthenia and normal menstrual cycle were reported. No physical examination alteration and no family history of cancer were observed. For case 2, Fig. 1 shows one CCR, which was found in 1/100 metaphases, described as der(4)ins(2;4), which was due to a 3 breakpoint event. Complete hemogram and biochemistry tests

![Fig. 1](image-url)
showed no abnormalities in comparison with normal values, as described in Table I. Like case 1, the immunophenotypic analysis of case 2 resulted in a lower NK cell count (1.51%), with all NK CD56+/CD16− (Table 2; Fig. 1).

Conclusions
We characterized cytogenetic, hematological, and immunophenotypic status in two female gas station attendants, who working in gas station with a proved harmful enviromental concentration of BTX. The following abnormalities were found: CCRs, a decrease in NK cells with abnormal CD16 expression, and early pregnancy loss.

It is well known that gas station workers are exposed to potentially harmful chemicals including BTX. However, benzene is considered the main carcinogetic agent (group 1 according to IARC) and studies associate this compound with acquired cytogenetic alterations [5–7]. Among various forms of benzene-induced genetic alterations, aneuploidy and chromosomal breakage are the most studied [4]. Chromosomal aberrations in peripheral blood lymphocytes of chronically benzene-exposed patients were previously documented [5–7].
Zhang et al. [5] reported dose-dependent chromosomal aneuploidies (mono- and trisomies) in the peripheral blood lymphocytes of workers exposed to benzene. In our study, we analyzed CAs only in three pairs of chromosomes, which make up 22.8% of the human genome. It’s a cheaper and faster test to estimate the DNA damage when compared to whole genome CA screening. Chromosomal aberrations (CAs) of high complexities could be detected in 1 out of 100 metaphases per patient (i.e. 1%). The rate of CCRs in normal controls lies between 0 and 0.5%, determined in 1000 metaphases, each [8]. In the present study, only 100 metaphases could be analyzed per case. Thus, the finding of one meta-phase with a CCR among 100 cells is at least noteworthy.

Even though CCR detection in peripheral blood is not directly correlated with enhanced cancer risk, it should be kept in mind that such CAs may indicate increased radio- and/or chemosensitivity. As tumors may be induced by environmental factors in combination with a special genetic susceptibility, the two cases reported may be at risk of acquiring malignancies [8].

As is well known, meiosis is a complex process controlled by different checkpoints, but males and females respond differently to meiotic disturbances [9]. During oogenesis, meiosis is generally pursued leading to the formation of aneuploid gametes or with single gene mutations.

Thus, in gametes, acquired genetic changes can be passed on to the next generation. Several epidemiological studies support the idea that genotoxic and nongenotoxic events following benzene exposure may be initiators of childhood leukemia in utero [10]. Another study on AML has shown that disease is usually initiated in utero because the leukemic translocations and other genetic changes are present in blood spots collected at birth [10, 11]. Also interesting is the fact that the majority of the CCR cases are reported in females ascertainment through repeated spontaneous abortions or the birth of a malformed child [11].

Besides the detected CCRs pointing towards enhanced chemosensitivity, these two female workers had hematological and immunological abnormalities characterized by mild leukopenia (case 1) and NK abnormalities. There are some studies concerning benzene with hematological and immunological abnormalities in humans [12–16]. The effects of immunotoxicity induced by benzene are depression and alteration of both the immune system mediated by cells and the humoral system [15]. Lan et al. [13] observed that leucocytes, B and CD4+/-T cell counts, were significantly decreased in workers exposed to benzene compared to the controls. In another study, the number of T lymphocytes, lymphocytes T CD4 and T CD8, and NK cells was reduced in the percentages and absolute numbers, and an increase in the monocyte count in workers during the period of exposure was found [15]. Thus, it was suggested that the depressive effect of benzene on the T and NK cells may be a factor of the probable carcinogenic activity of benzene through the immune system.

Natural killer (NK) cells are immune effector cells that recognize both virally infected and malignant target cells. Surprisingly, the results of the immunophenotypic analysis revealed NK CD56 positive (normal fluorescence) and CD16 negative in both cases, suggesting the presence of the rare subtype NK bright in the peripheral blood, which has low cytotoxic action [15]. It is possible that the action of BTX on the immune system had blocked the transition of immature CD56 bright cells into CD56 dim cells. In agreement with this finding, an 11 year follow up study showed that low NK cytotoxicity of peripheral blood lymphocytes correlates with an increased risk for cancer [16].

The identifications of chromosomal abnormalities and NK downregulation in the blood may be a new indicator for effective follow up of workers exposed to BTX, preventing diseases mainly important for females and their offspring. Further studies with a larger number of workers are necessary to confirm the results found.

Abbreviations
AML: acute myeloid leukemia; BTX: benzene, toluene and xylene; CAs: chromosomal aberrations; CCR: complex chromosomal rearrangements; FISH: fluorescence in situ hybridization; NK: natural killer.

Authors’ contributions
GA, UBO and MHO designed the study and applied for Research Ethics Board approval. MMT, TP, RTS and FS recruited the workers. The cytogenetic and immunophenotypic data were analyzed by NK, SL and FS. FS prepared the manuscript draft with important intellectual input from TL and MHO. All authors approved the final manuscript and had complete access to the study data.

Author details
1 Laboratório de Marcadores Circulantes, Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil. 2 Pós-graduação em Ciências Médicas (PGCM), Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil. 3 Coordenação de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, Brazil. 4 Unidade Técnica de Exposição Ocupacional, Ambiental e Cáncer, Coordenação de Prevenção e Vigilância, Instituto Nacional de Câncer, Rio de Janeiro, Brazil. 5 Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Kollegiengasse 10, D-07743 Jena, Germany. 6 Departamento de Patologia Gerai, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Avenida Professor Manuel de Abreu 444, 4º andar, Vila Isabel, Rio de Janeiro 20551-030, Brazil.

Acknowledgements
We thank the subjects who volunteered in the study.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All the data supporting our findings is contained within the manuscript.
Consent for publication
Written informed consents were obtained from the patients for publication of this Case Report and any accompanying images. A copy of the written consents are available for review by the Editor-in-Chief of this journal.

Ethics approval
This study was approved by the local ethics committees (Instituto Nacional de Cancer—INCA (121/09) and Universidade do Estado do Rio de Janeiro—UERJ (758.647), Brazil.

Grant support
Programa de Oncobiologia, Rio de Janeiro, Brazil, and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro Brazil.

Received: 18 September 2016   Accepted: 30 December 2016
Published online: 18 January 2017

References
1. Otero UB, Ornellas MH. Health risk related to the exposition to benzene and other chemicals present in gasoline. In: Alves G, editor. The health and environmental threats related to gas stations. Germany: Lambert; 2015. pp. 9–25. ISBN-13: 978-3-659-81445-7.
2. Occupational Safety and Health Administration, US Department of Labor. Medical surveillance guidelines for Benzene. In: Occupational Safety and Health Standards. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10045. Accessed 25 Oct 2016.
3. Verderfer I, Neubauer S, Letzel S, Angerer J, Arutyunyan R, Martus P, et al. Chromosome painting for cytogenetic monitoring of occupationally exposed and non-exposed groups of human individuals. Mutat Res/Genet Toxicol Environ Mutagenesis. 2001;49:97–109.
4. Smith MT. Advances in understanding benzene health effects and susceptibility. Annu Rev Public Health. 2010;31:133–48.
5. Zhang L, Lan Q, Guo W, Hubbard AE, Li G, Rappaport SM, et al. Chromosome-wide aneuploidy study (CWAS) in workers exposed to an established leukemogen, benzene. Carcinogenesis. 2011;32:605–12.
6. Santiago F, Alves G, Otero UB, Tabalipa MM, Scherner LR, Kosyakova N, et al. Monitoring of gas station attendants exposure to benzene, toluene, xylene (BTX) using three-color chromosome painting. Mol Cytogenet. 2014;7:15.
7. Zhang L, Lan Q, Ji Z, Li G, Shen M, Vermeulen R, et al. Leukemia-related chromosomal loss detected in hematopoietic progenitor cells of benzene-exposed workers. Leukemia. 2012;26:2494–8.
8. Neubauer S, Dunst J, Gebhart E. The impact of complex chromosomal rearrangements on the detection of radiosensitivity in cancer patients. Radiother Oncol. 1997;43:189–95.
9. Madan K. Balanced complex chromosome rearrangements: reproductive aspects. A review. Am J Med Genet. 2012;158:947–63.
10. Mchale CM, Wienells JL, Zhang L, Ma X, Buffle PA, Feusner J, et al. Prenatal origin of childhood acute myeloid leukaemia harboring chromosomal rearrangements t(15;17) and inv(16). Blood. 2013;101:4640–1.
11. Greaves MF, Wienells J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3:639–49.
12. Brândao MM, Rêgo MAV, Pugliese L, Clarencio J, Bastos CM, Ferreira J, et al. Phenotype analysis of lymphocytes of workers with chronic benzene poisoning. Immunol Lett. 2005;101:65–70.
13. Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, et al. Hematotoxicity in workers exposed to low levels of benzene. Science. 2004;306:1774–6.
14. Luan FJ. A study on lymphocyte subpopulations and immunologic status of female workers exposed to benzene. Zhonghua Yu Fang Yi Xue Za Zhi. 1992;26:77–9.
15. Viver E, Tomasiello E, Baratin M, Walzer T, Ugolini S, et al. Functions of natural killer cells. Nat Immunol. 2008;9:503–10.
16. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K, et al. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow up study of a general population. Lancet. 2000;356:1795–9.