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Short Communication

Assessment of *BCL2*/J(H) translocation in healthy individuals exposed to low-level radiation of 137CsCl in Goiânia, Goiás, Brazil

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ABSTRACT. Healthy radio-exposed individuals who received low levels of Cesium-137 radiation during the accident that occurred in Goiânia in 1987, their families and controls were tested for the detection of t(14;18)-rearranged B cells in peripheral blood by using a highly sensitive, real-time quantitative PCR method. The chromosomal translocation t(14;18)(q32;q21) is characteristic of follicular lymphoma and is a frequent abnormality observed in other types of non-Hodgkin's

lymphoma. This translocation leads to constitutive activation of the *BCL2* oncogene by the enhancers of the immunoglobulin heavy-chain locus. In healthy individuals, the same translocation may also be found in a small fraction of peripheral blood lymphocytes, and positive cells might serve as an indicator for environmental exposure to carcinogens and possibly correlate with the cumulative risk of developing t(14;18)-positive non-Hodgkin's lymphoma. Twenty healthy radio-exposed individuals, 10 relatives and 10 non-exposed healthy individuals were tested for the detection of this translocation. Only 1 non-exposed individual was positive for the chromosomal translocation, and healthy radio-exposed individuals presented lower levels of cells bearing the BCL2/J(H) rearrangement when compared to the levels of the patients with follicular lymphoma before treatment. However, evaluation of more cells would be required to confirm the total absence of circulating cells bearing *BCL2*/J(H) rearrangement.

Key words: Cesium-137; Translocation; Lymphoma; Rearrangement; *BCL2*/J(H); Radiation

INTRODUCTION

Radiological accidents such as those that occurred in Chernobyl, Three Mile Island in the United States, and the accident with Cesium-137 (137Cs) in Brazil allow for the evaluation of the stochastic effects of ionizing radiation (IR). In 1987 in Goiânia, Brazil, individuals were accidentally exposed to IR in one of the most serious radiological accidents of the southern hemisphere when an abandoned radiotherapy unit was dismantled and sold as scrap. The source containing 137CsCl (50.9 TBq or 1375 Ci) was partially dispersed over 2000 m^2, causing serious social, economic and medical consequences, including 4 fatalities. Over 100,000 people were screened and more than 150 people were exposed, both externally and internally, to doses ranging from 0.1 to 7 Gy (da Cruz et al., 2008).

Some IR can induce chromosomal translocations in human cells *in vitro*, for example, t(9;22) and t(8;21) (Deininger et al., 1998). Based on this observation, studies were initiated to investigate whether exposure to low-level radiation could influence the frequency as well as the total number of circulating t(14;18)-positive cells in healthy individuals (Dölken et al., 2002), that is, the clonal expansion or progression as well the generation of the translocation.

In the t(14;18) cells, analysis of the breakpoint regions has shown that the *BCL2* gene on chromosome 18 is fused to one of the JH gene segments from the immunoglobulin (Ig) H locus on chromosome 14 (Clearly and Sklar, 1985). The involvement of the Ig DH and JH gene segments in the recombination process, along with the presence of N regions at the breakpoints, prompted early interpretations of the t(14;18) translocation as a mistake in the normal mechanism of V(D)J recombination (Jager et al., 2000).

There has been extensive investigation of t(14;18) at the molecular level. Nearly 70% of the translocations are clustered within a 150-bp segment at the 3'-untranslated end of *BCL2* exon 3, designated the major breakpoint region (MBR; Clearly and Sklar, 1985*)*. This translocation is not, however, sufficient for the development of follicular lymphoma (FL; Jager et al., 2000), suggesting that additional genetic events are required (McDonnell and Korsmeyer, 1991).

Very little is known about the molecular mechanism of this translocation. Analysis of the breakpoints revealed the involvement of at least 2 mechanisms: 1) V(D)J recombination, mediating the breaks at the JH locus on chromosome 14, and 2) an additional, yet unknown, mechanism that is distinct from V(D)J recombination, responsible for creating the breaks at the *BCL2* MBR locus on chromosome 18 (Jager et al., 2000).

FL accounts for approximately 25% of adult non-Hodgkin's lymphomas (NHL) in western countries (Armitage and Weisenburger, 1998). The characteristic chromosomal translocation of FL, the t(14;18)(q32;q21), had also been detected at high prevalence and frequency in circulating B cells in healthy individuals (Dölken et al., 1996). The t(14;18)-positive cells can also be found in up to 60% of healthy individuals by very sensitive polymerase chain reaction (PCR) techniques (Limpens et al., 1991). The translocations found in healthy individuals are indistinguishable from those found in FL, based on nucleotide sequence analysis. The association of the *BCL2* gene with the heavy-chain locus leads to the formation of chimeric mRNA transcripts and a deregulated constitutive expression of the translocated *BCL2* gene. Its product, the BCL2 protein, is involved in the inhibition of apoptosis (Dölken et al., 1996).

Peripheral blood PCR is uninformative regarding relapse in patients whose tumors are undetectable. In summary, there is much to learn about the evolution of t(14;18) and its relationship to NHL. Chromosomal translocations are critical molecular events in the development of hematopoietic malignancies. A better understanding of the unique biology of t(14;18)-positive cells in healthy individuals could provide insight into their potential role as tumor precursors that may, in some instances, become clonogenic founders of NHL (Rabkin et al., 2008). Therefore, the number of circulating t(14;18)-positive cells might serve as an indicator for environmental exposure to carcinogens and possibly correlate to the cumulative risk of developing t(14;18)-positive NHL (Fuscoe et al., 1996).

In this study, the incidence and frequency of PCR-detectable MBR *BCL2*/J(H) rearrangement were evaluated in individuals accidentally exposed to 137CsCl and compared to a control group. There is a possibility of evaluating the implications for risk of development of malignancies involving B cells in the population exposed to ionizing radiation, as the importance of the detection of rearranged cells in peripheral blood can indicate physical and psychological health of each individual that is tested.

MATERIAL AND METHODS

Study population and sample collection

This study involved a total number of 40 individuals from the Goiânia municipality, located in Central Brazil. Blood samples were collected from 2008 to 2009. Fifty percent of the individuals were exposed to 137CsCl (mean age: 39.7 ± 16.6 years), and only 10 individuals were relatives of the exposed group, including spouses and children (mean age: 25.6 ± 18.5 years); 10 control individuals were included from Goiânia in the Goiás State (mean age: 37.8 ± 10.6 years). All individuals answered a questionnaire that included standard demographic data (age, gender, etc.) as well as questions related to medical issues, lifestyle (smoking and alcohol consumption) and occupation. In all groups, individuals who smoked more than 5 cigarettes per day for at least 1 year were considered smokers (exposed smokers = 5). Each individual exposed to the accident stated different times and frequencies, although they were included in the same group that was classified as receiving maximum doses of 0.2 Gy.

The individuals included in the exposed group received low levels of radiation (≤0.2 Gy). The control group consisted of employees living in the same region as the exposed individuals, none of whom was exposed to ionizing radiation. Blood samples were collected during the same period for both groups. All blood samples were collected by venipuncture and were transported to the laboratories at or below 8°C and processed within 8 h of collection.

Genomic DNA was purified from 100 µL blood with a commercial DNA extraction kit (Easy® DNA Purification Kit, Invitrogen, Carlsbad, CA, USA) according to manufacturer specifications. The DNA concentration (ng/µL) in each sample was measured by a spectrophotometric method (GeneQuant™ Amersham Biosciences, Amersham, UK and QUBIT, Invitrogen).

Real-time quantitative PCR

PCR was performed using the ABI PRISM 7500 Sequence Detection System (PE, Applied Biosystems, Foster City, CA, USA). Primers and fluorogenic Probes (Sigma Aldrich, St. Louis, MO, USA), hybridizing between the forward and reverse primers, were labeled at the 3'-end with the quencher dye, Black Hole Quencher and at the 5'-end with the reporter dye, 6-carboxy-fluorescein. PCR was performed for the MBR *BCL2*/J(H) rearrangement, together with the *KRAS* gene as endogenous references, to allow for quantification of DNA in each sample. For the *BCL2*/J(H) assay, primers for the JH consensus, *BCL2* MBR region and probe were used as previously described (Cleary and Sklar, 1985). A 20-µL reaction mixture was used, which included the TaqMan Universal PCR Master Mix (PE, Applied Biosystems), 300 nM primers, 150 nM probe, and between 100-400 ng DNA for each individual tested and 0.1-1000 ng for the standard curve. The DNA was subjected to 50 cycles of 15 s at 95°C and a 1-min combined annealing/extension step at 61°C. Standard curves were constructed with the KARPAS 422 cell line (kindly provided by Dr. Martin Dreyling, CCG-Leukemia, Munich) that carries the t(14;18) rearrangement. KARPAS 422 DNA was diluted 5-fold to generate standards ranging from 0.1 to 10^3 ng. These dilutions were used to generate standard curves for both the *BCL2*/J(H) rearrangement and the *KRAS* endogenous reference gene. The quantity of target (*BCL2*/J(H), and *KRAS*) in a test sample was derived from where it lay on the KARPAS standard curve. The standard assay procedure for every DNA sample consisted of 2 tests with 0.4 µg DNA for the detection of t(14;18)-MBR translocations and 2 tests with 0.1 µg DNA for the *KRAS* wild-type gene. Approximately 0.8 µg DNA in 2X 0.4-µg reactions (equivalent to 1.3 x 10^5 cells; 1 cell = 6 pg) from each of the 20 individuals was screened for the presence of the *BCL2*/J(H) rearrangement. Since only a few portions of peripheral blood cells of this population were used for DNA extraction, only a quarter of this quantity of cells could be used for the quantification of the endogenous gene, based on spectophotometric measurements. Standard curves were made in triplicate and were used in every reaction.

RESULTS

The t(14;18)-positive KARPAS 422 cell line was used to generate standard curves for real-time PCR of the *BCL2*/J(H) MBR and the *KRAS* endogenous reference gene. The slope value of the standard curve represented the number of cycles corresponding to 1 log difference in the starting copy number. The variable slope values were indicative of the difference in efficiency of the PCRs.

Sample quantification

Each sample contained the quantity of cells with viable DNA as determined using an endogenous reference gene, *KRAS*, based on spectrophotometric measurements. Each reaction for the amplification of the endogenous gene contained 100 ng DNA; however, the quantity observed in the amplification plot showed differences between the samples (Figure 1). This could be explained by the fact that some samples were frozen for a few years after DNA extraction (frozen aliquots of DNA conserved for long periods at -20°C deteriorate; some of them were stored for 2 or more years). All samples were tested differently in relation to the number of cells; however, they were usable in the assay for detection of the *BCL2*/J(H) rearrangement, which requires a minimum of 1000 cells for the validated amplification of 10 target cells bearing the rearrangement. Figure 2 shows the amplification plot of this quantitation assay.

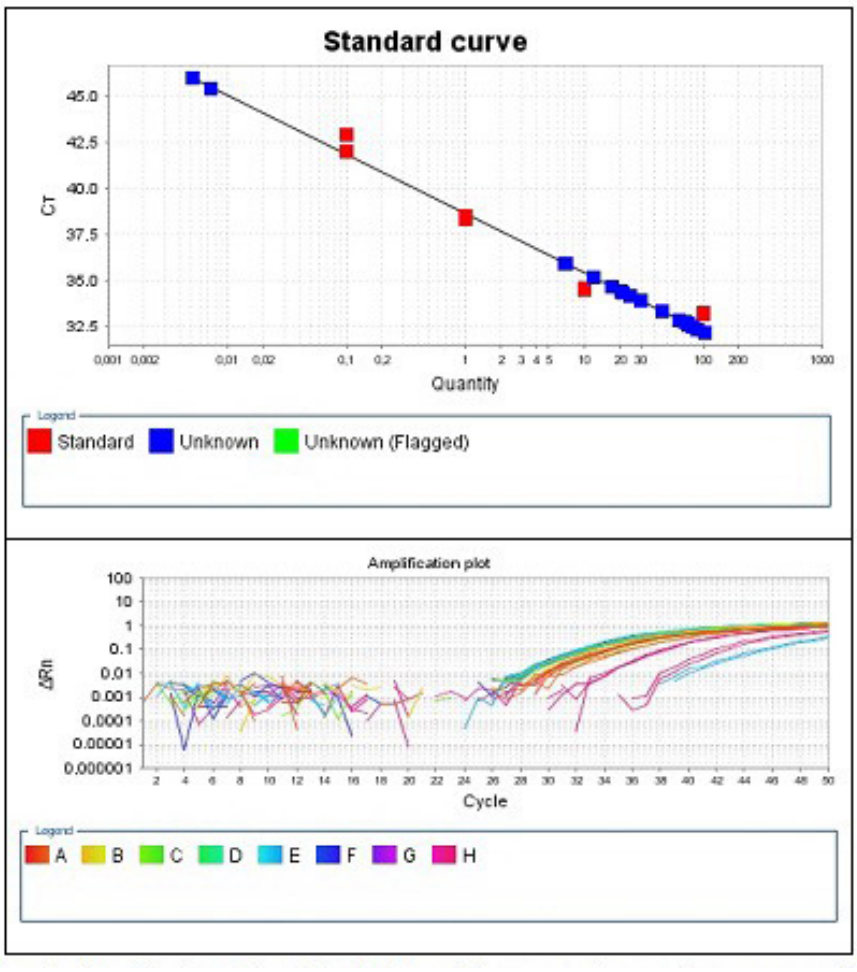

Figure 1. Amplification plot and standard curve from assay using an endogenous gene *KRAS* using 4 standards ranging from 0.1 to 100 ng.

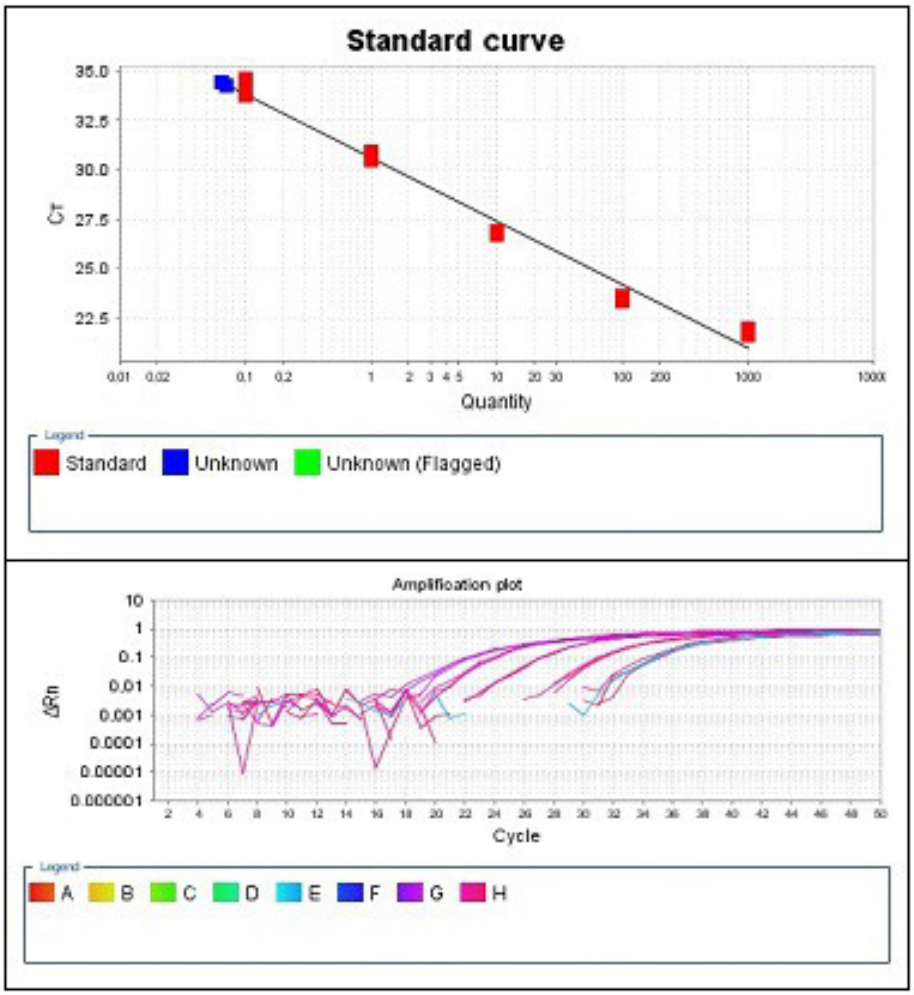

Figure 2. Amplification plot and standard curve in the assay for detection of t(14;18); positive KARPAS 422 were used to generate 5 standards ranging from 0.1 to 1000 ng.

Frequencies of *BCL2*/J(H)

Of the 40 peripheral blood DNA samples analyzed, only 1 sample contained the *BCL2*/J(H) MBR rearrangement. This sample was derived from a non-exposed man and was determined to be positive in each of 2 reactions. The rearrangement was confirmed with electrophoresis on a 2% agarose gel. The number of cells bearing the *BCL2*/J(H) rearrangement was determined using a standard curve, and for this individual, it was obtained by the median between the 2 positive reactions - 67 pg (11 cells). The *BCL2*/J(H) MBR real-time reaction had a sensitivity of 1 in 10,000 cells. However, to obtain highly reproducible results, the presence of a minimum of 10 target copies of the rearrangement was necessary.

DISCUSSION

The presence of the *BCL2*/J(H) rearrangement in the peripheral blood of healthy and NHL-positive individuals detected by very sensitive PCR is an informative test that presents diverse implications. The correlation between low levels of ionizing radiation and the new malignant event in the B-rearranged cells remains undefined (Dölken et al., 2002). Our study showed that radio-exposed individuals presented low levels of cells bearing the *BCL2*/JH rearrangement; the lower incidence of this rearrangement in healthy individuals compared with that observed in other studies (Schüler et al., 2003; Mahfouz et al., 2007) likely reflects the smaller amount of DNA analyzed for each individual. Nevertheless, this study provided an important observation regarding the frequency levels of t(14;18) cells in radio-exposed individuals. Considering the amount of cells observed, the absence of cells bearing the rearrangement in the assays could lead us to determine that these individuals presented lower levels than the median observed for individuals with FL, since the median frequency of cells bearing t(14;18) in peripheral blood cells of normal individuals is more than 3 times lower than that of individuals with FL before treatment (1 per 200,000 cells *vs* 1 per 100 cells, respectively; Schüler et al., 2003).

Whether individuals with high numbers of circulating t(14;18)-positive cells and/or multiple t(14;18)-positive clones are at an increased risk of developing FL is an open question. Presently, it is difficult to relate the findings of circulating t(14;18)-positive B cells in healthy individuals with the development of FL cells, and it is unclear as to the type of B-cell subset that carries the translocation (Dölken et al., 2008). Furthermore, nothing is known regarding the fate and evolution of these t(14;18)-positive cells apart from long-term clonal persistence.

The t(14;18)+ clones, which are particularly prominent in farmers exposed to pesticides, constitute *bona fide* FL precursors occurring at various stages of tumor progression (Agopian et al., 2009). Together with the recent findings indicating that pesticide exposure is specifically associated with a higher risk of t(14;18)+ NHL development, the first direct connection between t(14;18) frequency in blood and malignant progression data was provided (Agopian et al., 2009).

Previous studies have shown an increasing incidence of the *BCL2*/J(H) rearrangement with age that suggested a link between the incidence of FL and the occurrence of the *BCL2*/J(H) rearrangement (Dölken et al., 2008). A correlation between *BCL2*/J(H) translocation frequency and sunlight record has been reported in a UK population (Bentham et al., 1999), thus potentially strengthening the suspected link between NHL and sunlight (Adami et al., 1999).

Several investigators have postulated that the prevalence and/or frequency of t(14;18)-positive cells in healthy individuals may be related to the environmental exposure to carcinogens (Fuscoe et al., 1996), pesticides (Agopian et al., 2009) and excessive smoking (Bell et al., 1995). In our study, the unexposed individual that carried the *BCL2*/J(H) MBR rearrangement stated that they smoke more than 30 cigarettes/day, which could be responsible for the presence of this translocation in the peripheral blood. In the exposed group, 25% were smokers, which related a median consumption of lesser than 6 cigarettes/day ($X = 1.3$; $SD \pm 1.3$).

In different human populations, the median of t(14;18) cells is significantly related to the incidence and development of FL bearing the translocation (Mahfouz et al., 2007). In Japanese individuals, the frequency of the *BCL2*/J(H) translocation increased dramatically at around 20 years of age (Yasukawa et al., 2001). In addition, previous reports demonstrated that

the frequency of the *BCL2*/J(H) translocation rises with age in white individuals (Dölken et al., 2002). This study is part of the constant genetic biomonitoring of individuals accidentally exposed to 137Cs in Goiânia and their offspring. However, only a few of those radio-exposed individuals agreed to participate in this and other studies that aim to identify mutations such as cytogenetic assays and the evaluation of mutation in short-tandem repeat loci.

Although low levels of radiation as described in other studies (Dölken et al., 2002) have no significant relation to the increase of the t(14;18) cells and do not display evidence of being the causative event of other genetic mutations that culminate in neoplastic development, age, heavy smoking and exposure to pesticides were described as factors increasing the frequency of t(14;18) cells. However, for almost all subjects in this study, no significant relationship between the presence of t(14;18) and alcohol consumption or/and heavy smoking was expected. Still, few studies proposed to evaluate the relationship between exposure to low-level radiation and the increase of *BCL2*/J(H) rearrangement in peripheral blood, even though radiation can induce genetic mutation. No relationship with age was expected, since there are no data for the Brazilian population that relate this observation. Understanding the risks of low doses of radiation still has social importance in relation to issues as varied as screening tests for cancer.

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