Toxicology Reports 3 (2016) 225–229

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Occupational exposure to pesticides and occurrence of the chromosomal translocation t(14;18) among farmers in Jordan

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ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form
23 November 2015
Accepted 4 January 2016
Available online 13 January 2016

Keywords:
Pesticides
Translocation t(14;18)
Follicular lymphoma

ABSTRACT

Background: An increased incidence of non-Hodgkin's lymphoma (NHL) has been reported in farmers and other occupational groups working with pesticides. In these individuals, an increased prevalence of the chromosomal translocation t(14;18)(q32;q21), one of the most common chromosomal abnormalities in NHL, has been detected in peripheral blood lymphocytes. This translocation juxtaposes the antiapoptotic BCL2 protein to the immunoglobulin heavy chain gene locus (IGH) leading to overexpression of BCL2. This causes an increase in cell survival, paving the way for malignant transformation.
Aim of the study: The present study aimed to evaluate the association between the occurrence of the chromosomal translocation t(14;18) and occupational exposure to pesticides among a group of Jordanian farmers.
Methods: A total of 192 male subjects including 96 agricultural workers and 96 control subjects participated in this study. BCL2-IGH t(14;18) fusions were detected by a nested polymerase chain reaction (PCR) assay targeting the major breakpoint region (MBR).
Results: We found that occupational exposure to pesticides in open-field farming and insecticide used on animals increased the frequency of the chromosomal translocation t(14;18). Farmers occupationally exposed to pesticides and insecticide were 13.5 times more likely to harbor t(14;18). 63.5% (61 of 96) of farmers compared to 11.5% (11 of 96) of controls carried the translocation (odds ratio: 13.5; 95% confidence interval (CI) = 6.3–28.6). We ruled out the influence of possible confounding factors such as age, duration of sun exposure, alcohol intake, smoking, and use of personal protective equipment.
Conclusion: Our results indicate that pesticides increased the frequency of chromosomal translocation in the 14q32 region. Accordingly, the presented data agrees with previous suggestions from the literature that pesticides might be involved in the development of NHL through the t(14;18) pathway.

1. Introduction

Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of Lymphoproliferative malignancies that can arise from B- or T-lymphocytes. B-cell NHLs are primarily most abundant in adulthood. Follicular lymphoma is one of the most common types of B-cell derived NHL in western countries [1]. However, in Jordan, it only accounts for 8.1% of cases of diagnosed B-cell lymphoma, with the majority being attributed to Diffuse Large B-cell lymphoma [2].

The chromosomal translocation t(14;18)(q32;q21) is one of the most common chromosomal abnormalities in NHL, which occurs in 70–90% of cases of follicular lymphoma (FL), 20–30% of diffuse large B-cell lymphoma, and 5–10% of other less common subtypes [3]. This translocation involves 2 specific loci; the immunoglobulin heavy chain (IgH) locus on chromosome 14q32 and the B-cell leukemia/lymphoma-2 (BCL-2) locus on chromosome 18q2l [4]. During the typical translocation process, the BCL2 gene located on chromosome 18 is juxtaposed to the transcriptionally active IgH gene on chromosome 14 resulting in over expression of the former. Consequently, the heightened anti-apoptotic function of BCL2 increases cell survival, which represents an early step in the malignant process of NHL [4–6].

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http://dx.doi.org/10.1016/j.toxrep.2016.01.002

Pesticides are substances or mixture of substances used for preventing, destroying, repelling, or mitigating pests, which include insects, rodents, weeds, as well as other unwanted organisms. Most commonly, they are classified relying on the target species they act on such as insecticides, herbicides, or fungicides [7]. Pesticides may cause a variety of acute and delayed adverse health effects in those who are exposed. Acute effects range from simple skin and eyes irritation to general malaise, while chronic long-term effects encompass cancer and adverse reproductive outcomes [8]. Carcinogenic pesticides exist among insecticides, herbicides, and fungicides as well as in several chemical classes such as triazines, organophosphates and organochlorines [8–10]. Pesticides may exert their carcinogenic effects through a variety of mechanisms including genotoxicity [9].

An increased incidence of NHL has been reported among farmers and other occupational groups working with pesticides [9]. Furthermore, an increased prevalence of the chromosomal translocation t(14;18)(q32;q21) has been detected in peripheral blood lymphocytes from individuals occupationally exposed to pesticides [11]. To date, the relationship between pesticide exposure and occurrence of t(14;18) chromosomal translocation in farmers from Jordan, where FL is not common, has not been investigated.

The aim of this study is to evaluate if occupational exposure to pesticides among farmers in Jordan may contribute as a risk factor to the occurrence of the chromosomal translocation t(14;18)(q32;q21). We detected the translocation t(14;18) by a nested polymerase chain reaction (PCR) assay targeting the major breakpoint region (MBR) from peripheral blood lymphocytes of a group of farmers. We assessed pesticide exposure in detail taking into account potential confounding factors including smoking, sunlight, and age. To the best of our knowledge, this is the first study in Jordan and the Middle East region to address this issue.

2. Materials and methods

2.1. Study subjects

Study subjects were selected from the Jordanian Ghor region, the northern part of the Jordan Valley, a key agricultural area in the country. 96 farmers occupationally exposed to pesticides and 96 control individuals not exposed to pesticides were recruited from the town of Deir Alla. All subjects signed informed consent forms to participate in this study. Two farms were included: The Jordan University Agricultural Research Center Farm and The National Center for Agricultural Research and Extension (NCARE). In Both farms, agricultural activities consisted of open field farming, green house farming as well as cattle and poultry breeding. Inclusion criteria for cases were as follows: Jordanian males, age $\geq$ 18 years, direct exposure to pesticides including one or more of the following: using pesticides on open field crop, using insecticides on animals, or using herbicides. Agricultural workers with less than 6 months of pesticide exposure, or indirect exposure, were excluded. Inclusion criteria for controls were as follows: Jordanian males, age $\geq$ 18 years, never undertaken work related to farming. Subject were interviewed face-to-face and given a comprehensive questionnaire detailing age, tobacco use, alcohol consumption, medical history—especially haemolymphatic cancer, agricultural practices, personal use pesticides, type(s) of pesticides used, years of exposure, use of protective equipment, and sunlight exposure.

2.2. Sample collection and DNA preparation

Peripheral blood samples (10 ml/subject) were collected in Vacutainer tubes EDTA (K2) collection tubes (BD Bioscience). DNA was extracted from buffy coat peripheral blood lymphocytes using Wizzard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. DNA concentration and purity was measured using UV spectrophotometry (Bio-Rad, SmartSpec plus, USA). All samples had OD260/OD280 ratio values of 1.7–2.0. A working dilution for DNA samples of 100 ng/μl was prepared in nuclease-free water and stored at −20 °C for analysis.

Table 1
Primer sequences.

ß-Globin primers	
ß-Globin F	Forward: 5′-CAA CTT CAT CCA CGT TCA CC-3′
ß-Globin R	Reverse: 5′-GAA GAG CCA AGG ACA GGT AC-3′
t(14;18) MBR, first round primers	
MBR-1	Forward: 5′- CAGCCTGAAACATTGATGG-3′
JH-1	Reverse: 5′- ACCTGAGGAGACGGTGACC-3′
t(14;18) MBR, second round primers	
MBR-2	Forward: 5′-TATGGTGGTTTGACCTTTAG-3′
JH-2	Reverse: 5′- ACCAGGGTCCCTTGGCCCCA-3′

2.3. PCR and nested-PCR

The ß-globin gene was used an internal gene control for DNA integrity (primer sequences are shown in Table 1) [12]. The PCR mixture contained 300 ng of genomic DNA, 1X Green GoTaq® Reaction Buffer (Promega, USA), 2.5 U GoTaq® DNA Polymerase (Promega, USA), 10 mM dNTPs Mix (Promega, USA) and 20 pmol oligonucleotide primer (Invitrogen, USA) in a final volume of 50 μl. The PCR conditions were an initial denaturation step at 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 57 °C for 45 s and 72 °C for 60 s, then a final extension step at 72 °C for 5 min. A negative control was included in each run, whereby no DNA template was added.

For BCL2-IGH t(14;18) fusions nested PCR amplification, we used specific primers for the major breakpoint region (MBR) in combination with JH consensus primers as described in Gribben et al. [12,13] (Table 1). For each individual, 2–3 technical replicates were utilized in both rounds of the nested-PCR. Almost all replicates were homogeneous with respect to the observed PCR products, showing either a band of the expected size or not displaying a PCR product. Where replicates did not show that same result, be it positive or negative, the reaction was repeated. The PCR mixture for each sample contained 1 μg of genomic DNA, 1X Green GoTaq® Flexi Buffer (Promega, USA), 2.5 U GoTaq® Hot Start Polymerase (Promega, USA), 2.5 mM $MgCl_2$ (Promega, USA), 10 mM dNTPs Mix (Promega, USA) and 20 pmol oligonucleotide primer (Midland oligos, USA) in a final volume of 50 μl. The reaction conditions for the first round of nested PCR were as follows: an initial denaturation step at 94 °C for 7 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 60 s, with a final extension step at 72 °C for 5 min. One micro liter of the first round PCR product was re-amplified under the same conditions for 30 additional cycles in the second round. Both negative (no template) and positive controls were added in each PCR round. The positive controls used were plasmid DNA (Nanogen Advance Diagnostic, Italy) containing the MBR region within the BCL2-IGH rearrangement. Amplified PCR products, t(14;18) fusions, were analyzed on 3% agarose gel electrophoresis containing ethidium bromide (1 μg/ml) and visualized under UV light, using gel documentation system (UVP, USA).

2.4. Statistical analysis

Data were summarized as average ± SD and range for continuous variables (age, duration of pesticides use and sunlight exposure) or counts (percentages) for categorical variables (smoking, alcohol consumption, pesticides use, insecticides use, herbicides use and protective clothing). Normality was checked