Translocation t(14;18) in healthy individuals: Preliminary study of its association with family history and agricultural exposure

O. Paltiel, A. Zelenetz, I. Sverdlin, L. Gordon & D. Ben-Yehuda

Departments of Social Medicine, Hematology, Hadassah Medical Center, Jerusalem, Israel; Lymphoma Unit, Memorial Sloan-Kettering Hospital, New York, NY, USA

Summary

Background: The t(14;18) translocation, present in 90% of follicular non-Hodgkin’s lymphomas (NHL), has been found to exist in low levels in healthy persons. Its clinical/prognostic significance in healthy populations is unknown, and risk factors for its development have not been determined. Our objectives were to assess the prevalence of t(14;18) in individuals without NHL, comparing residents of agricultural settlements (kibbutzim) with city dwellers, as well as first degree relatives of NHL cases.

Patients and methods: Residents of kibbutzim and members of two control groups: 1) Jerusalem residents – randomly selected hospital administrative workers and 2) first degree family members of lymphoma patients were interviewed extensively regarding exposures and had blood drawn for t(14;18) determination. The translocation was detected after B-cell purification of blood samples with CD-19 microbeads (Mini-Macs®) using nested PCR. The method detects the translocation in a BCL2 positive cell line after dilutions of up to 1:10⁵ with normal peripheral blood lymphocytes.

Results: Nineteen of two hundred thirty healthy individuals (8.3%) tested were found to be positive for t(14;18). No statistically significant differences in the prevalence of t(14;18) were detected among the rural and urban populations. Five of thirty-four (11.9%) family members tested positive for t(14;18). No age or sex differences between t(14;18) positive and negative individuals were found. No significant association with exposure to specific agricultural or other chemicals was found.

Conclusions: The presence of the t(14;18) translocation in healthy individuals was not associated with agricultural residence in this preliminary study. Whether relatives of patients with NHL are at increased risk will require further study in larger populations. Specific exposures affecting the onset of this translocation have not been ruled out. The significance of this translocation in healthy individuals remains unknown.

Key words: agriculture, BCL-2, non-Hodgkin’s lymphoma, pesticides, translocation t(14;18)

Abbreviations: NHL – non-Hodgkin’s lymphoma; PCR – polymerase chain reaction; SD – standard deviation

Introduction

The increase in the incidence of non-Hodgkin’s lymphoma (NHL) which has been observed world-wide over the last 30 years [1] has occurred in Israel as well [2]. This increase cannot be fully accounted for by improved diagnosis or by tumors associated with the acquired immunodeficiency syndrome. Hence environmental causes have been sought, including occupation, hair dyes, solvents, viruses and others [3]. Lymphomas are more frequently observed in agricultural as compared to urban populations [4, 5], especially in the US.

Although pesticides are considered to contribute minimally to the overall risk of cancer [6], an association between NHL and pesticide exposure has been observed repeatedly [7–9], but not consistently [10–12]. Other lymphoproliferative disorders have also been associated with pesticides [13]. Factors other than pesticide exposure may also be responsible for the increase in NHL observed in agricultural communities [3].

The t(14;18) translocation occurs in 85%–90% of follicular lymphomas and 25% of diffuse B-cell lymphomas [14]. This translocation juxtaposes the BCL2 locus on chromosome 18 with the immunoglobulin heavy chain joining (J) region gene on chromosome 14 (BCL-2-JH), resulting in over-expression of the BCL-2 gene. This leads to an inhibition of apoptosis (programmed cell death) and consequently to a prolongation of survival of affected B cells. The presence of the t(14;18) translocation is probably not sufficient for the development of neoplasia since only 11% of mice transfected with this gene developed follicular tumors or other lymphomas [15]. A model of neoplasia resulting from this translocation has been suggested [16]. According to this model the t(14;18) translocation results in a long-lived B cell, progressing to a long-lived B-cell clone after antigen stimulation, and after further antigen stimulation or mutation of other oncogenes results in a B-cell tumor. BCL-2 rearrangement has been observed in patients in remission for early follicular lymphoma for more than 10 years following curative radiation without any evidence of disease progression [17].
Table 1 Characteristics of subjects found to be positive for the t(14:18) translocation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>t(14:18)+ (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>7 (10.8)</td>
<td>65</td>
</tr>
<tr>
<td>Kibbutz</td>
<td>8 (6.2)</td>
<td>130</td>
</tr>
<tr>
<td>First degree relatives</td>
<td>4 (11.4)</td>
<td>35</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (7.9)</td>
<td>114</td>
</tr>
<tr>
<td>Female</td>
<td>10 (8.6)</td>
<td>116</td>
</tr>
<tr>
<td>Ethnic origina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia–Africa</td>
<td>2 (5.0)</td>
<td>40</td>
</tr>
<tr>
<td>Europe–America</td>
<td>15 (10.6)</td>
<td>142</td>
</tr>
<tr>
<td>Israel</td>
<td>2 (4.3)</td>
<td>47</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>2 (8.3)</td>
<td>24</td>
</tr>
<tr>
<td>12 years</td>
<td>6 (7.9)</td>
<td>76</td>
</tr>
<tr>
<td>&gt; 12 years</td>
<td>6 (5.6)</td>
<td>107</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>9 (7.4)</td>
<td>122</td>
</tr>
<tr>
<td>Past</td>
<td>6 (10.5)</td>
<td>57</td>
</tr>
<tr>
<td>Current</td>
<td>4 (7.8)</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>19 (8.3)</td>
<td>230 (100)</td>
</tr>
</tbody>
</table>

* Continent of birth or father’s continent of birth for Israeli born.

work has shown that $BCL-2$-JH can be detected in peripheral blood of normal blood donors [18], and in hyperplastic lymphoid tissue [19]. It has been suggested that the quantity of t(14:18) in the lymphocytes of healthy adults can be used as a possible biomarker for environmental exposures to carcinogens [20], but work of this kind has not previously been reported.

We report here a study of the prevalence of t(14:18) in an agricultural population in Israel living in kibbutzim (collective agricultural settlements), compared to two control groups with correlation to exposures experienced by these subjects obtained by in-depth interview.

Subjects and methods

1. Agricultural workers

Kibbutzim situated in northern, central and southern regions in the country in which at least one member had been diagnosed and treated for NHL at our hospital were approached for participation. Within these kibbutzim the nurse approached four to six members currently employed in the agricultural sector, two laundry workers, four to six members chosen randomly according to their identity number and using a table of random numbers, and family members of affected NHL cases if living on kibbutz. Subjects were interviewed on the kibbutz by two trained hematology nurses using a modification of the FARM questionnaire [8], which contains extensive questions on places of residence, work performed, exposure to specific insecticides, herbicide and fungicides as well as medical history and hobbies. Blood was drawn for molecular studies.

2. Urban controls

Employees of Hadassah hospital who were Jerusalem residents and were employed in administrative occupations (not exposed to patients, laboratories or medication as part of their job description) were chosen at random using the hospital computer system or by visiting their place of employment. They were interviewed by the study nurses and blood was drawn for molecular analysis.

3. Family members of patients with NHL

First degree relatives of patients with follicular and other NHL were approached to participate in the study. They were interviewed by the study nurses and blood was drawn for molecular analysis.

The study was approved by the ethics committee of the hospital and the health committees of all participating kibbutzim, and all participants gave signed informed consent.

Laboratory methods

Isolation of mononuclear cells

The mononuclear cell fraction of peripheral blood specimens obtained from study participants were isolated on a Ficoll-Hypaque® density gradient (Pharmacia, Piscataway, NJ) as directed by the manufacturer. A portion of the samples was frozen as viable cells in 90% fetal calf serum and 10% DMSO. An inventory of available material has been maintained as part of the database.

Isolation of B cells

In normal individuals the level of t(14:18) positive cells is very low; in fact, it is below the level of detection in peripheral blood lymphocytes. Therefore, the assay must be performed on purified B-cell populations. Purification of B cells was achieved by selection on MiniMacs® CD19 Microbeads (Miltenyi Biotec GmbH, Gladbach Germany). This method is rapid, allows for the isolation of up to $10^7$ B cells, and can be used to process numerous samples in parallel. With this method we were able to yield cell populations consisting of approximately 85% CD19 positive cells.

$t(14;18)$ polymerase chain reaction (PCR)

Because of the low frequency of the t(14:18) translocation in lymphoid tissues and peripheral blood B cells of normal individuals, very sensitive techniques are necessary. We used nested PCR as described by Liu et al. [16]: The first stage of PCR uses 1 pmol of outer primers for 30 cycles P1 (AGTTATATGGCTATACACTATTTGT) and P4 (ACCTGAGGAGACGGTGACCAGGGT). The second stage of PCR is reamplification of a 0.4 ml of this first PCR product in a reaction containing 50 pmol of the

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inner primers for 30 cycles P2 (TTGTGAGCAAGGTTGATCGT) and P3 (CAGGGTCTCTGGGCACCCAG). The PCR products were resolved in agarose gels. Primers P1 and P2 are homologous to the 5' sequence of the major breakpoint region (MBR). Primers P4 and P3 are homologous to the consensus sequence of J1–J6. Using this method we could detect the t(14;18) translocation in BCL-2 positive cell line after dilutions of up to 1:10^5 with normal peripheral blood lymphocytes.

Statistical analysis

Data were entered on EXCEL for Windows and data analysis was performed using SPSS for Windows (SPSS Inc, Chicago, IL). Categorical variables were compared using chi-square and differences between means were compared using the t-test. A two-sided P-value of 0.05 was considered statistically significant.

Results

Fourteen kibbutzim took part in the study. Only subjects for whom there are both interview and molecular testing results are included in the following analysis. In all, 130 kibbutz residents participated including agricultural workers, laundry workers, and residents chosen at random. The urban control group consisted of 65 administrative hospital workers chosen randomly. Thirty-five family members of lymphoma patients (representing 14 families) represented the second control group. Kibbutz residents who were also first degree relatives of lymphoma patients were considered in the group of family members. The distribution of t(14;18) translocation positivity according to the three groupings is shown in Table 1.

The differences between the prevalence of the t(14;18) translocation among the populations was not statistically significant. When we compared t(14;18) positive vs. negative subjects in terms of residence in an agricultural settlement for at least 1 year at any point in their lives after age 18, we found that 15 of 19 (78.9%) of the positive individuals reported past agricultural residence, compared to 66% of the translocation-negative subjects (odds ratio 1.94, 95% confidence interval (95% CI): 0.6–8.3, P = 0.37). There were no differences in the age and sex distribution of the t(14;18) positive and negative individuals. The mean age of t(14;18)+ individuals was 48.6 (SD 13.2) versus 44.2 (SD 12.9) for t(14;18)– individuals (P = 0.5). Of the 19 t(14;18) positive subjects 52.6% were women compared to 50.2% of the t(14;18) negative group (P = 0.5). Other characteristics are shown in Table 1.

We measured exposure to various chemical agents, including agricultural and industrial chemicals. We elicited exposure to 183 agents including 68 insecticides and crop insecticides, 40 herbicides, 31 fungicides, 1 biological agent and 43 other chemicals. Table 2 compares the pattern of exposure to categories of agents among the groups. No discernable differences were detected when comparing maximum, mean or median number of agents.

In addition we examined all agents to which at least 10% of the study population had been exposed. These included 4 insecticides, 13 crop insecticides, 8 herbicides, 3 fungicides and 29 other chemicals. The distribution of exposure among these agents was similar comparing t(14;18) positive and negative subjects except for three agents: glyphosate (Roundup) – a phosphononylmethyl glycine to which 53.8% of t(14;18)+ were exposed compared to 32.6% of t(14;18) negative subjects; 2,4 D to which 23.1% of t(14;18)+ compared with 12.0% of t(14;18) negative subjects were exposed, and metal dust to which 38.5% of translocation-positive individuals were exposed compared to 19% of negative subjects. None of these differences was statistically significant. When we grouped the insecticides into chemical groupings such as carbamates, organochlorines, organophosphates, nitrogen compounds, etc., we again found no evidence of increased exposure among t(14;18) positive individuals. This was also the case with respect to chemical groupings of herbicides including triazines, carbamates, benzoic acid, thiocarbazates, urea and others. We investigated specific personal exposures to pesticides and found no difference in t(14;18) positive and negative individuals in terms of having been present in a field at the time of aerial spraying, having been a flagger for an aerial spray plane, or having been involved in irrigation of fields.

We did not find an association between raising livestock in general, or particular animals specifically and the presence of the t(14;18) translocation.

Due to the small numbers of BCL-2 positive subjects we did not perform an analysis based on length and/or intensity of exposure.
Table 3: Comparison of studies investigating t(14;18) in normal populations using peripheral blood.

<table>
<thead>
<tr>
<th>Author, year [reference]</th>
<th>Method</th>
<th>DNA quantity</th>
<th>Sensitivity</th>
<th>t(14;18) positive</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu, 1994 [16]</td>
<td>Rapid nested PCR</td>
<td>2 µg</td>
<td>1 in 10^6</td>
<td>29 of 55 (55%)</td>
<td>Increase with age</td>
</tr>
<tr>
<td>Fuscoe, 1996 [20]</td>
<td>Nested PCR, secondary PCR</td>
<td>2.5 µg (40–50 cc of blood)</td>
<td>1 in 4 × 10^3</td>
<td>30 of 34 (88%)</td>
<td>Up to 100-fold difference of t(14;18) concentration</td>
</tr>
<tr>
<td>Dolken, 1996 [26]</td>
<td>Two step PCR</td>
<td>Increasing quantities 0.001 µg to 10 µg</td>
<td>1 in 10^7 (if 0.1 µg) and 1 in 10^6 if 10 µg</td>
<td>22 of 26 (84%) t(14;18)+ if &gt; 2.5 µg DNA used</td>
<td>Healthy blood donors, 6 had &gt; 1 clone</td>
</tr>
<tr>
<td>Ji, 1995 [27]</td>
<td>Seminested PCR</td>
<td>7, 14, or 28 µg, 2–5 ml of peripheral blood</td>
<td>1 in 5 × 10^6</td>
<td>79 of 132 (60%) including cancer patients and normal donors</td>
<td>One donor had much higher levels</td>
</tr>
<tr>
<td>Current study</td>
<td>Two step nested PCR</td>
<td>5–20 cc peripheral blood, B-cell separation</td>
<td>~ 1 in 10^5</td>
<td>19 of 230 (8.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

This study is the first that we are aware of which attempted to determine whether there is an association between a acquired genetic translocation specifically related to NHL and agricultural exposures. We were unable to demonstrate a statistically significant association with current nor past residence in an agricultural settlement. Furthermore we investigated whether there was a higher prevalence of this translocation among family members of lymphoma patients. Once again we found no statistically significant association. Preliminary findings raise the hypothesis that family members may be at increased risk of carrying this translocation in their B lymphocytes. This will have to be pursued in further studies. Although specific exposures were not correlated to NHL, exposures to 2,4 D and metal dust were more common among t(14;18)+ individuals. Both of these agents have been previously associated with NHL [8, 21].

Several possible explanations for the negative findings with respect to agricultural residence exist.

Firstly, it is possible that no true association exists between current agricultural exposure and the presence of t(14;18). While it is generally accepted that lymphoma incidence is higher in agricultural than in rural populations, the mechanism for this increased risk is not necessarily via an increase of BCL-2 expression through the t(14;18) translocation. Lipkowitz et al. have found hybrid antigen-receptor genes resulting from V(D)J rearrangements to be more common in agricultural workers than in healthy controls [22]. Chromosome breaks have been found to be common among fumigant workers; these changes were found to be reversible after exposure had stopped [23]. Other investigators hypothesize that pesticides increase the risk of NHL via T-cell immunosuppression [24]. The plausibility of a biological association between agricultural chemicals and the development of non-Hodgkin’s lymphoma has been further strengthened by a recent finding of a strong dose-response relationship between serum polychlorinated biphenyls (PCB) concentrations and subsequent risk of NHL, up to 20 years later [25]. Our (albeit, nonsignificant) finding that lifetime residence in an agricultural settlement was indeed more common among subjects harboring this translocation in their lymphocytes suggests that a) further refinement of our measure of exposure is necessary and that b) further sampling of clearly exposed and unexposed populations, perhaps with serum markers of extent of exposure would be required to characterize this association.

Secondly, the sample size in this study may not have been large enough to detect a difference in prevalence, even if one exists (i.e., beta-error may have occurred). We could not estimate the required sample size when we first proposed this project since there were very few preliminary data on the prevalence of t(14;18) in normal individuals. Specifically, we will need to increase the size of the control groups in order to draw meaningful conclusions. Future projects could utilize our preliminary findings as a starting point for calculating the required sample size.

Thirdly, our study may have differed from others investigating t(14;18) in healthy subjects in terms of technique and sensitivity of the assay. Different studies have found varying frequencies of t(14;18) among healthy subjects. Table 3 summarizes findings in the literature concerning the presence of t(14;18) in healthy populations. It is clear that there is a wide variety of methods and a wide range of sensitivities. Our assay was relatively insensitive, compared with others which have detected the translocation in up to 88% of subjects tested when large quantities of DNA were used [20]. Clearly, if the majority of the population harbors this translocation it cannot be used as an instructive or discriminating marker. In the future it will be important to determine standards as to the amount of DNA required for the assays and to establish cut-offs or threshold values for the concentration of cells bearing the translocation and try to correlate this clinically.

Given the obvious variability in techniques, quantity of DNA, and populations studied it is difficult to draw
conclusions and compare the studies. All studies confirm that the t(14;18) translocation exists in healthy subjects, that it is detectable using PCR techniques, and that there is a wide range of concentrations of this translocation even in subjects free of lymphoma. None of these findings serve to clarify the clinical significance of the translocation. Follow-up of affected individuals is necessary.

Conclusions

This study has confirmed that the t(14;18) translocation can be found in healthy individuals. We did not find an association with current rural residence nor with specific occupational exposure. Furthermore we have shown that performing molecular epidemiologic studies on kibbutzim is feasible. The level of co-operation was high. The study was underpowered to detect subtle differences in t(14;18) positivity among the groups. Larger numbers of family members of NHL cases as well as urban controls are required in order to verify if there are differences among the groups. Careful sampling procedures will be required to further test the hypothesis that the t(14;18) translocation is differently distributed among population groups. Furthermore the finding that 15 of 19 translocation-positive individuals had resided in agricultural settings at some point in their adult life obliges us to further explore this association.

Like other researchers, we found the multiplicity of exposures and the imprecise measures of exposure difficult to analyse. Further basic knowledge on the effects of specific pesticides on lymphopoietic tissues would help to strengthen future studies.

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References


Correspondence to:
Dr O. Paltiel, MD
Department of Social Medicine
Hadassah Medical Center
P.O Box 12000, Jerusalem
Israel 91120
E-mail: ora@vms.huji.ac.il