Original Article
Comparison of side effects of chemotherapy between the elderly Uygur patients and the Han patients with non-Hodgkin lymphoma

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Abstract: Purpose: This study is to detect whether the different side effects of the same amount chemotherapy drugs administered in elderly Uygur patients with non-Hodgkin lymphoma (NHL) and aged Han NHL patients are related to the levels of cytogenetic damage in the two ethnics. Methods: Sister chromatid exchanges (SCE) rate, hypoxanthine guanine phosphoribosyltransferase (HPRT) gene mutation rate, micronucleus (MN) rate, and white blood cell (WBC) count in 20 cases of two groups before and after chemotherapy were compared. Results: Before chemotherapy (at baseline level), the average SCE rate, HPRT gene mutation rate, MN rate were lower in elderly Uygur patients than that in aged Han patients, but the difference was not significant (P>0.05). After each cycle of chemotherapy, the average SCE rate, HPRT gene mutation rate, MN rate in elderly Uygur patients were lower than that in Han patients, but this difference was not statistically significant (P>0.05). However, the three indicators described above in two ethnics were significantly higher after each cycle of chemotherapy than those before each cycle of chemotherapy (P<0.05). In addition, the decreased level of average WBC in aged Uygur NHL patients was lower than that in Han patients after each cycle of chemotherapy, but this difference was not statistically significant (P>0.05). Spearman analysis showed that there is a negative correlation between the three indicators and the decrease of WBC counts. Conclusion: The difference of side effects of chemotherapy for NHL between Uygur and Han patients is related to the levels of cytogenetic damage.

Keywords: SCE rate, HPRT gene mutation rate, MN rate, the Uygur nationality, non-Hodgkin lymphoma

Introduction

Diffuse large B-cell lymphoma (DLBCL) is one of the most common subtypes of non-Hodgkin lymphoma (NHL), accounting for 30% to 40% of NHL patients [1-4]. The incidence of DLBCL increases with age [5]. The majority of patients with DLBCL are over the age of 60 years [6], with approximately 40% of patients aged over 70 years [7-9]. Xinjiang is a multi-ethnic region, with Uygur and Han population accounting for about 47% and 38.2% of the total population by the end of 2011, respectively, according to the statistical results by XIN JIANG office of Facts and Figures, indicating that the ethnic Uygur group is the majority in Xinjiang region [10]. Chemotherapy for non-Hodgkin’s lymphoma (NHL) acts in a dose-dependent manner generally. During our clinical work, it was found that the side effects of chemotherapy are nausea, vomiting and severe bone marrow suppression (I-II degree majority). Some others were all mild in the elderly Uygur patients with NHL group after chemotherapy using the same dosage as in the elderly Han case, in comparison with the elderly Han patients with NHL. This difference may be caused by cytogenetic diversity between the Uygur and Han population.

Sister chromatid exchange (SCE) rate is the exchange rate of genetic material between two identical sister chromatids of a chromosome. SCE is required not only to understand the changes in chromosome structure at cellular level, but also the DNA damage, repair and recombination at the molecular levels. Chromosome can be broken after radiation or certain chemical mutagen, and the fragments
cannot enter mitosis with the child nucleus when cells enter the next division step, forming the round or oval micronucleus (MN) with completely unequal sizes in the cytoplasm (diameter <1/3 of main nuclear). The hypoxanthine guanine phosphoribosyltransferase (HPRT) gene is located on the X sex chromosome distal Xq26 region, encoding the HPRT protein. This protein is a membrane enzyme in the purine salvage pathway involved in cell nucleotide synthesis in vivo. Lack of or reduced activity of this protein affects nucleic acid metabolism. SCE test, HPRT gene mutation, and MN test are often used due to the sensitive detection of cytogenetic damages. Therefore, they were widely used in the fields of chemical mutagenesis, environmental monitoring, and cancer research.

In this work, we have investigated the difference of cytogenetics in elderly Uygur and Han patients with NHL using detection of SCE rate, HPRT gene mutation rate, MN rate and the decrease level of white blood cells (WBC) counts before and after chemotherapy. Therefore, this study was to detect whether there were cytogenetics differences in peripheral lymphocytes between the elderly Uygur and Chinese NHL before and after chemotherapy.

Materials and methods

Patients

A sample size calculation was performed prior to the commencement of this study. Patients including 20 Uygur ethic cases and 20 Han population cases were enrolled in this study after providing informed consent. These patients had been chemotherapy-naive patients with NHL from CancerHospital affiliated with Xinjiang Medical University in 2012-2015. They were aged 60 to 70 years old. All patients were received heparin to achieve systemic anticoagulation. Two microliters of peripheral blood samples were collected from patients before and after the end of chemotherapy within 24 hours. Blood samples were drawn into sterile tubes and stored at 4°C, inoculated within five hours. The patients who were not able to tolerate the chemotherapy showing severe adverse effects, and the patients who received the adjusted chemotherapy, were calculated by the cumulative period of CHOP (combination of cyclophosphamide, doxorubicin/hydroxydoxorubicin, vincristine and prednisone)-based chemotherapy. The patients treated with reduced CHOP-based chemotherapy were also included.

Administration of CHOP chemotherapy

CHOP is commonly administered in 4 cycles of chemotherapy, including cyclophosphamide (CTX) 750 mg/m², day 1, intravenous injection; doxorubicin/hydroxydoxorubicin 50 mg/m², day 1, intravenous injection; vincristine 1.4 mg/m² (maximum, 2 mg), 1st day intravenous injection; prednisone tablets 100 mg/m² first to the fifth day, oral, 21-days repeat. The patients were monitored for four cycles.

Inclusion criteria and exclusion criteria

The patients enrolled in this study were selected using stringent inclusion criteria as follows: 1. The new cases with pathological stage I-III of NHL proved histologically. 2. ECOG (Eastern Cooperative Oncology Group) score ≤2 points in Non-Hodgkin’s lymphoma patients. 3. No other complications, no poison, radiation exposure history. 4. Age ≥60 years old. 5. Long-term living in the Xinjiang region not less than 30 years. Exclusion criteria were as follows: 1. Age >70 years old. 2. NHL patients received chemotherapy previously. 3. Smokers.

SCE assay

Cells with complete shape and well-expanded chromosomes were investigated. Fluorescence plus giemsa (FPG) staining method was used. The diploid mitotic metaphase cells in the second period were observed and counted. Number of the stained-depth conversion in each chromatid was named as SCE frequency. Those appeared at the end of the chromatid exchanges were counted as one SCE, appeared in the middle chromatid exchange recorded as two SCEs. If it is exchanged once in parts of centromere, found not two chromatids at the centromere position reverse occurs, counted as one SCE. Using oil immersion (×1000 times), each blood sample (mid-cycle phase) was observed for 20 to 25 seconds, followed by calculation of the average SCE frequency (%) (Figure 1A)
Side effects of chemotherapy in NHL

Cytokinesis-block micronucleus (CBMN) assay

Criteria for binucleated lymphocytes were as follows: a cell with two major micronuclei of substantially equal size; no more than 6 micronuclei in a cell; nuclear material possibly connected between the two major nuclei; and possible overlap between the two major nuclei.

In the dual-nuclei cells, micronucleus diameter was one sixteenth to one third of the main nucleus with no light refraction. No nuclear materials were connected between the major nuclei. There were possible overlaps with the border of the major nucleus. However, each nuclear membrane could be observed clearly.

High magnification (×400 times) microscope was used. For each specimen, 1000 pairs of core lymphocytes were counted. The number of dual-nuclei cells was calculated to obtain the incidence of micronuclei (MNf, ‰) (Figure 1B).

HPRT gene mutation frequency examined by multinucleated cells

Cells containing two or more nuclei were considered as mutant cells. The number of samples containing a 6-Thioguanine (6-TG) in every 1000 transformed cells were divided by the number of samples without 6-TG, which gave hprt gene mutation rate (‰) (Figure 1C).

Statistical analysis

Quantitative data were given as mean ± standard deviation. SCE rates before or after each chemotherapy, HPRT gene mutation rate, MN compare rates, Uygur and Chinese indexes were indicated by paired t test. Correlation between SCE rate and WBC counts were analyzed by spearman correlation. P<0.05 was considered as statistically significant.

Results

Comparison of SCE rate, HPRT gene mutation rate, and MN rate between the Han group and the Uygur group of elderly NHL patients before chemotherapy and after four-cycle chemotherapy

To determine whether there is difference in SCE rate between the Han group and the Uygur group of elderly NHL patients before chemotherapy and after four-cycle chemotherapy, FPG method was used to evaluate the mean SCE rate in both groups. As given in Table 1, the average SCE rate was significantly lower in Uygur NHL elderly patients than in the Han patients before chemotherapy (at baseline) (P<0.05). With the increase of chemotherapy cycles, the average SCE rate was significant lower than in the aged Uygur NHL patients both before and after chemotherapy than in the Han patients (P<0.05), as indicated in Table 1. These results showed that the mean SCE rate in elderly Uygur was lower than in Han patients, indicating an ethnic-dependent pattern of NHL incidence.

To explore whether there is difference in MN rate between the Han group and the Uygur group of elderly NHL patients before chemotherapy and after four-cycle chemotherapy, the number of lymphocytes and micronuclei were counted using oil-immersion microscope to determine the mean MN rate in both groups. The average MN rate has no significant differences between the aged Uygur NHL patients and the Han patients either before or after the first cycle of chemotherapy (P>0.05). However, after the first chemotherapy cycle, it was significantly lower in aged Uygur NHL patients than the Han patients either before or after chemotherapy (P<0.05), as shown in Table 2. These
Table 1. Comparison of the average rates of SCE between the Han group and Uygur group of elderly NHL patients before chemotherapy and after chemotherapy of four cycles

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>1st cycle (n=20)</th>
<th>2nd cycle (n=20)</th>
<th>3rd cycle (n=19)</th>
<th>4th cycle (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before chemo</td>
<td>After chemo</td>
<td>Before chemo</td>
<td>After chemo</td>
</tr>
<tr>
<td>Uygur</td>
<td>14.25±3.35</td>
<td>23.25±7.66</td>
<td>34.75±8.35</td>
<td>40.25±9.39</td>
</tr>
<tr>
<td>Han</td>
<td>18.25±6.34</td>
<td>30.25±8.03</td>
<td>42.50±10.07</td>
<td>48.00±10.18</td>
</tr>
<tr>
<td>t-value</td>
<td>2.49</td>
<td>2.82</td>
<td>2.65</td>
<td>2.50</td>
</tr>
<tr>
<td>P-value</td>
<td>0.017*</td>
<td>0.008*</td>
<td>0.012*</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

Note: Two-sample t-test (large sample approximations). *Significant at 5% level, P-value corrected up to three decimal. SCE: Sister chromatid exchanges rate.

Table 2. Comparison of the average rates of MN between the Han group and Uygur group of elderly NHL patients before chemotherapy and after chemotherapy of four cycles

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>1st cycle (n=20)</th>
<th>2nd cycle (n=20)</th>
<th>3rd cycle (n=19)</th>
<th>4th cycle (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before chemo</td>
<td>After chemo</td>
<td>Before chemo</td>
<td>After chemo</td>
</tr>
<tr>
<td>Uygur</td>
<td>17.55±5.10</td>
<td>32.10±7.96</td>
<td>32.30±7.90</td>
<td>43.20±6.73</td>
</tr>
<tr>
<td>Han</td>
<td>20.15±11.44</td>
<td>30.15±9.29</td>
<td>37.75±8.57</td>
<td>48.15±7.18</td>
</tr>
<tr>
<td>t-value</td>
<td>0.93</td>
<td>-0.71</td>
<td>2.09</td>
<td>2.25</td>
</tr>
<tr>
<td>P-value</td>
<td>0.359</td>
<td>0.481</td>
<td>0.043*</td>
<td>0.031*</td>
</tr>
</tbody>
</table>

Note: Two-sample t-test (large sample approximations). *Significant at 5% level, P-value corrected up to three decimal. MN: Mircronucleus rate.

Table 3. Comparison of the average rates of HPRT between the Han group and Uygur group of elderly NHL patients before chemotherapy and after chemotherapy of four cycles

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>1st cycle (n=20)</th>
<th>2nd cycle (n=20)</th>
<th>3rd cycle (n=19)</th>
<th>4th cycle (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before chemo</td>
<td>After chemo</td>
<td>Before chemo</td>
<td>After chemo</td>
</tr>
<tr>
<td>Uygur</td>
<td>83.93±36.27</td>
<td>148.10±45.98</td>
<td>95.85±33.26</td>
<td>159.10±45.53</td>
</tr>
<tr>
<td>Han</td>
<td>84.75±62.50</td>
<td>146.65±51.16</td>
<td>98.05±60.95</td>
<td>165.35±47.92</td>
</tr>
<tr>
<td>t-value</td>
<td>0.05</td>
<td>-0.09</td>
<td>0.142</td>
<td>0.42</td>
</tr>
<tr>
<td>P-value</td>
<td>0.960</td>
<td>0.925</td>
<td>0.893</td>
<td>0.675</td>
</tr>
</tbody>
</table>

Note: Two-sample t-test (large sample approximations). HPRT: Hypoxanthine guanine phosphoribosyltransferase gene mutation rate.
results suggest that there is no significant difference in the mean MN rate between elderly Uygur and Han patients at the baseline level, however, it also denote an ethnic-dependent pattern of NHL incidence after chemotherapy.

To clarify whether there is difference in HPRT rate between the Han group and the Uygur group of elderly NHL patients before chemotherapy and after four-cycle chemotherapy, HPRT rate was calculated to determine the mean HPRT rate in both groups. There was no significant difference of the average HPRT gene mutation rate in the whole chemotherapy cycle in stages 1-4 between the two populations (P>0.05), as shown in Table 3. These results indicate that there is ethnic-independent in mean HPRT rate in both two groups.

**Comparison of the average rate of SCE, MN rate, HPRT gene mutation rate before and after each cycle of chemotherapy in elderly Uygur and Han NHL patients themselves**

To verify whether SCE, MN rate, HPRT gene mutation rate can reflect the cytogenetic damage caused by chemotherapy drugs, comparison of the average rate of SCE, MN rate, HPRT gene mutation rate before and after each cycle of chemotherapy in elderly Uygur and Han NHL patients was performed. The mean SCE rate, MN rate, and HPRT gene mutation rate were significantly higher after each cycle of chemotherapy than before each cycle of chemotherapy in all the four cycles for both elderly Uygur NHL patients and Han patients (P<0.05), as shown in Table 4. These results indicate that SCE rate, MN rates, HPRT gene mutation rate can sensitively reflect cytogenetic damage induced by anti-tumor chemotherapy, which has a good dose-effect relationship with the cumulative dose chemotherapy, further demonstrating the reliability of the Tables 1-3.

**Comparison of decrease level of WBC counts in elderly Uygur patients with NHL and Han patients**

To determine whether there is significant difference in blood toxicity caused by chemotherapy in elderly Uygur patients with NHL and Han patients, the decrease level of WBC counts was performed. The decreased level of average white blood cell count is lower in elderly patients with NHL Uighur than in Han patients after each cycle of chemotherapy for all the four cycles, but the difference was not statistically significant (P>0.05), as is shown in Table 5. These results suggested that WBC count was slightly higher in elderly Uygur NHL patients and the hematological toxicity degree due to chemotherapy was a little lower than in Han patients, showing no significant.

**Pair-wise correlation analysis of the average SCE rate, HPRT gene mutation rate, MN rate, and WBC count in the aged NHL patients**

Spearman correlation coefficient (R) and the value of probability P were shown in Figure 2. A moderately negative correlation was observed between the average SCE rate of every cycle and the correspondingly average WBC count in all the patients (R=-0.833, P=0.01, P<0.05) (Figure 2A). The linear regression equation was as follows:  
$$Y = -2.511X + 48.297$$  
We also observed a moderately negative correlation between the average MN rate of every cycle and the correspondingly average WBC count in all the patients (R=-0.833, P=0.01, P<0.05) (Figure 2B). The linear regression equation was as follows:  
$$Y = -3.062X + 50.852$$  
As shown in Figure 2C, there was a moderately negative correlation between the average HPRT gene mutation rate of every cycle and the correspondingly average WBC count in all the patients (R=-0.976, P<0.001). The linear

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Table 4. Comparison of the average rate of SCE, MN rate, HPRT gene mutation rate before and after each cycle of chemotherapy in elderly Uygur and Han NHL patients themselves

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Average SCE rate (%)</th>
<th>Average MN rate (%)</th>
<th>Average HPRT gene mutation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uygur</td>
<td>Han</td>
<td>Uygur</td>
</tr>
<tr>
<td></td>
<td>t-value</td>
<td>p-value</td>
<td>t-value</td>
</tr>
<tr>
<td>1st</td>
<td>-6.09*</td>
<td>0.00*</td>
<td>-6.69*</td>
</tr>
<tr>
<td>2nd</td>
<td>-11.00*</td>
<td>0.00*</td>
<td>-7.68*</td>
</tr>
<tr>
<td>3rd</td>
<td>-12.01*</td>
<td>0.00*</td>
<td>-10.21*</td>
</tr>
<tr>
<td>4th</td>
<td>-9.85*</td>
<td>0.00*</td>
<td>-12.04*</td>
</tr>
</tbody>
</table>

*Note: Two-sample t-test (large sample approximations).
Table 5. Comparison of the decreased levels of WBC count between elderly Uygur patients with NHL and Han patients before and after each chemotherapy cycle

<table>
<thead>
<tr>
<th>cycles</th>
<th>WBC count (10^9/l)</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Han (n_1-2 cycles=20; n_3-4 cycles=19)</td>
<td>Uygur (n_1-2 cycles=20; n_3-4 cycles=19)</td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>4.34±1.61</td>
<td>3.91±1.59</td>
<td>0.86</td>
</tr>
<tr>
<td>2nd</td>
<td>4.45±1.55</td>
<td>3.94±1.28</td>
<td>1.12</td>
</tr>
<tr>
<td>3rd</td>
<td>3.28±0.99</td>
<td>3.22±0.91</td>
<td>0.204</td>
</tr>
<tr>
<td>4th</td>
<td>3.27±1.00</td>
<td>2.99±1.08</td>
<td>0.839</td>
</tr>
</tbody>
</table>

Note: Two-sample t-test (large sample approximations).

Discussion

In this study, the statistical results showed that mean SCE rate was significantly lower in the aged Uygur patients than in elderly Han NHL patients before chemotherapy (baseline levels). However, MN rate, HPRT gene mutation rates were not significantly different between the two population. Comparative studies of SCE rate, HPRT gene mutation rate, MN rate among different ethnic groups are rarely reported.

Previous study has shown that frequency distribution of HLA alleles originated from Caucasian is higher in Xinjiang Uygur than in other ethnic groups, equivalent to the numerical of Caucasians, suggesting that Uighur groups have Caucasian blood, having different genetic
Side effects of chemotherapy in NHL

background with Han population [11]. That may be the reason why Uygur NHL patients have significant lower average SCE rate than Han patients when at baseline. However, there no significant differences of MN rate and HPRT gene mutation rate between the two populations. This finding may be due to the following reasons: (i) although the Uighur patients contain Caucasian descent, and have different genetic backgrounds from Han patients, cytogenetic characteristic is similar in the two populations. Because all patients involving in this experiment were long-term residents in the Xinjiang region for not less than 30 years, and the common area, eating customs, living conditions may contribute to the similarity. HPRT gene mutation rate and MN rate are influenced by many factors [12-17], such as certain drugs, vitamins [18], chemical materials [19] etc, and we cannot exclude the confounders above completely. (ii) The dynamics of the cell itself, leading to some mutant cell loss [20]. (iii) Although the average MN rate and HPRT gene mutation rate did not show a significant difference between the two population before chemotherapy (at baseline), however, the indicators above were lower in elderly Uighur patients than in Han patients, possibly due to the sample size. (iv) The significant difference of SCE rate and the no significant difference of HPRT gene mutation rate and MN rate between Uighur elderly patients with NHL and Han patients before chemotherapy (at baseline) may reflect the particular difference of cytogenetics in Uighur elderly NHL patients and the polymorphism of genetic material among different human ethics.

The average SCE rate was significantly higher after each cycle chemotherapy in both elderly Han patients and Uygur patients with NHL than before chemotherapy, from initial chemotherapy to the end of the fourth cycle of chemotherapy (P<0.05). This is consistent with the findings of Silva [21], Mourelatos C et al. [22], Mourelatos et al. [23] and Suspiro A [24]. The average HPRT gene mutation rate, MN rate were significantly increased after each cycle of chemotherapy, which is consistent with the reports from Tates et al. [25] and Elsendoom et al [26]. These studies again show that the chemotherapy can cause cytogenetics damage.

As shown in this study, the mean SCE rate, MN rate, HPRT gene mutation rate decreased before each cycle of the second, third and fourth cycle chemotherapy compared to after each cycle of the first, second and third cycle chemotherapy in the two populations. However, these rates did not fully restore to the baseline (before the initial chemotherapy) level, which showed the repair of chemotherapy drugs to DNA damage was incomplete [27].

The average MN rate was shown the same results with the average SCE rate since the beginning of the second cycle chemotherapy. This suggests that elderly Uygur patients with NHL showed different degree of cytogenetic damage and DNA repair mechanisms from the Han patients. The complexity and polymorphisms in the genetic materials between ethnic groups may lead to different clinical toxicity of chemotherapy drugs. spearman correlation analysis showed that the average SCE rate, MN rate, HPRT gene mutation rate were all moderately negatively correlated with WBC cells in elderly patients with NHL.

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Disclosure of conflict of interest

None.

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Side effects of chemotherapy in NHL


Side effects of chemotherapy in NHL