No association between \textit{GSTT1}, \textit{GSTM1}, and \textit{GSTP1} gene polymorphism and risk of non-Hodgkin lymphoma in a population from Romania

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\textbf{ABSTRACT:} Polymorphisms of the glutathione S-transferases (GTSs), which are involved in the cellular oxidative and antioxidant mechanisms of the xenobiotic substances and carcinogens, represents a factor that increases the risk of developing cancer. We aimed to determine in a case-control study (82 patients and 152 controls) a possible association between the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms and susceptibility to non-Hodgkin lymphoma (NHL) in a Romanian population. GTSs genotypes were obtained using the multiplex polymerase chain reaction (PCR) and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Increased frequencies of the \textit{GSTM1} null genotype were observed in the patients (51.22%) with NHL and in controls (56.58%). No associations were observed between \textit{GSTP1} Ile/Val + Val/Val and \textit{GSTM1} null genotypes and risk of NHL, while an increased risk for \textit{GSTT1} null genotype was noticed without statistical significance. We did not find differences for the combined \textit{GST} gene polymorphisms and risk of NHL between patients and controls. Also, no differences between patients’ demographic and clinical characteristics and GTSs genotypes were detected (p>0.05, for all comparisons). Therefore, our research suggests that \textit{GSTM1}, \textit{GSTT1}, and \textit{GSTP1} genotypes do not contribute to the risk of developing NHL.


\section{INTRODUCTION}

In Romania, approximately 1566 new cases of non-Hodgkin lymphoma (NHL) are diagnosed every year according to GLOBOCAN [1]. It has been suggested that xenobiotic substances and environmental carcinogen exposure, particularly exposure to nitrates, pesticides, herbicides and solvents, is a susceptibility factor for NHL [2]. Therefore, responsible for the biotransformation and the detoxification of many different xenobiotic substances and carcinogens are glutathione S-transferases (GTSs) [3].
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GTSs, a phase II xenobiotic-metabolizing enzymes, are a major group of multifunctional enzymes involved in the regulation of the cellular oxidative and antioxidant pathways by catalyzing conjugation to glutathione and neutralizing free radicals, respectively. In humans, were identified 8 main classes of GTSs, such as alpha, mu, kappa, omega, pi, sigma, theta, and zeta [3].

The most commonly reported polymorphisms in the genes that encoded GTSs associated with low or lack of enzyme activity are GSTT1, GSTM1, and GSTP1 [4]. A deletion of GSTT1 gene (located on chromosome 22q11.2) is associated with risk of malignant lymphomas and other types of cancer (cancer of bladder, lung, colon, stomach and skin). A similar deletion was found in the GSTM1 gene, located on chromosome 1p13.3 and in the GSTP1 gene, located on chromosome 11q13 responsible for inactivation of toxins and carcinogens [5]. Thus far, are only a few studies that associated GTSs polymorphisms with risk of NHL, and results are not consistent [6], [7], [8], [9], [10], [11].

The frequencies of GTSs polymorphisms is different among populations. Therefore GSTT1 polymorphism is more frequent in Asians and Caucasians, 35-52% and 13-26%, respectively, and is higher in Chinese (58.3%) and in French (43%) than in Nigerians (21.7%) in the case of the GSTM1 polymorphism [12]. It is found that approximately 30% of the Caucasian population carries the GSTP1 polymorphism and it is less frequent in Asians [13].

The aim of the study was to determine if there is an association between the common GST gene polymorphisms and susceptibility to NHL in a Romanian population and to correlate the GTSs genotypes to patients’ clinical characteristics.

2 MATERIALS AND METHODS

2.1 STUDY POPULATION

Our patients’ group study involved 82 Caucasian adults with confirmed diagnoses of NHL according to WHO classification [14] admitted to the Hematology Clinic I from Emergency Clinical County Hospital, Tîrgu Mureș, Romania. Patients’ clinical and laboratory data such as a histopathological classification of the NHL, performance status (according to The Eastern Cooperative Oncology Group - ECOG), Ann Arbor stage (the most often used staging system to present the extent of NHL), LDH level, treatment outcome, and the survival rate was collected from the medical records.

The control group consists of 152 healthy participants with no hematologic or other malignancies. Controls were randomly selected from the same geographical area, Transylvania region, and with a similar ethnic background as the cases.

All participants provided written informed consent in accordance with the Declaration of Helsinki. The study was conducted with the approval of the Ethics Committee from the University of Medicine and Pharmacy Tîrgu Mureș, Romania (no. 116/14.12.2015).

2.2 GENOTYPING

Two milliliters of fresh peripheral blood were collected into EDTA vacutainers from both patients and control group and used for rapid purification of genomic DNA by using Quick-gDNA MiniPrep kit (Zymo Research, USA).

Genotyping of the GSTT1 and GSTM1 polymorphisms were conducted in the Department of Genetics of our institution by using the multiplex polymerase chain reaction (PCR) method as previously reported by Sharma A. et al. [15]. For the GSTP1 polymorphism genotyping was carried out by the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) described by Hohaus S. et al. [13].

2.3 STATISTICAL ANALYSIS

The statistical analysis was performed with the GraphPad InStat software (GraphPad, San Diego, CA, USA). All statistical tests were two-sided and differences were considered significant when the p-value was less than 0.05. The strength of the association of the GSTT1, GSTM1 and GSTP1 genotype polymorphisms between patients with NHL and controls was examined with calculated odds ratios (OR) and their 95% confidence interval (95% CI).

3 RESULTS

In NHL patients, the frequencies of the GSTT1 null and GSTM1 null genotypes were 26.83% and 51.22%, respectively. We observed increased frequencies of the GSTM1 null genotype in the patients (51.22%) with NHL and in controls (56.58%). An
association was found between \textit{GSTT1} null genotype and risk of NHL (p = 0.4454; OR = 1.273, 95% CI = 0.6846-2.366) but was not statistically significant. Also, an association was found between \textit{GSTT1} and \textit{GSTM1} double null genotype and risk of NHL (p = 0.836; OR = 1.15, 95% CI = 0.508-2.61). There was a higher percentage of females than males.

Regarding the \textit{GSTP1 Ile105Val} polymorphism, Ile/Ile genotype was more frequent in patients and controls (71.95% and 65.13%, respectively) and Ile/Val frequency in NHL patients was 25.61% and in controls 30.92% while Val/Val genotype was less frequent in both groups.

Table 1 shows the frequencies of the \textit{GSTT1}, \textit{GSTM1}, and \textit{GSTP1} genotypes. The frequency distribution of the \textit{GSTM1}, \textit{GSTT1}, and \textit{GSTP1} genotypes did not differ between NHL patients and controls.

There was a decreased risk of NHL for \textit{GSTP1 Ile/Val + Val/Val} genotypes between patients and controls (p = 0.2879, OR = 0.279, 95% CI = 0.4052-1.309) without statistical significance.

Moreover, we examined the association between the combined GSTs genotypes (null \textit{GSTT1}, null \textit{GSTM1} and variant genotype of \textit{GSTP1 Ile105Val}) and NHL and there was no increased risk of developing NHL (p > 0.05).

The distribution of the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms in NHL patients stratified by gender, age, Ann Arbor stage, LDH level, ECOG performance status, treatment outcome and survival rate are shown in Table 2.

We did not detect significant differences between demographic characteristics of the patients and \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} genotypes (p > 0.05).

In the case of Ann Arbor stage according to the NHL group, it was not associated with variant \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} polymorphisms (p = 0.2476, p = 1.000, and p = 0.7816, respectively). Also, we investigated if there is an association between performance status (ECOG) and the risk group and we found a nearly significant difference (p = 0.0517) for \textit{GSTT1} null genotype.

However, we observed that the frequencies of the NHL patients were similar according to the clinical stage and ECOG status, namely 14 and 13 for the \textit{GSTT1} null genotype and 4 and 4 for the \textit{GSTM1} null genotype. Similar results were found in the case of Ile/Val and Val/Val genotypes for \textit{GSTP1 Ile105Val} gene polymorphism.

No differences were found regarding the LDH level (>190 UI/L) in NHL group and \textit{GST} gene polymorphisms. Also, no association was observed regarding patients' treatment outcome (resistant, partial remission and complete remission to treatment) and \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} genotypes (p>0.05).

Furthermore, we analyzed how the \textit{GSTT1}, \textit{GSTM1}, and \textit{GSTP1} genotypes influenced the outcome in patients group. Our data revealed that the lowest mean time survival was 41.935 months in the \textit{GSTM1} null genotype comparing to the 60.336 months in the case of \textit{GSTT1} null genotype and 17 months in Val/Val variant of the \textit{GSTP1 Ile105Val} polymorphism. The \textit{GSTT1} null and \textit{GSTM1} null genotypes had no significant association according to 5-year survival (p = 0.0683; OR = 0.2688, 95% CI = 0.07287 – 0.9913 and p = 1.000; OR = 1.217, 95% IC = 0.1162 – 12.759, respectively). In case of the \textit{GSTP1 Ile/Val}
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genotype a positive association was found in 5-year survival (p = 0.2289; OR = 2.100, 95% IC = 0.6717-6.566) but no statistical significant.

In addition, we investigated the frequency of histological subtypes of NHL patients. Of the 82 cases, 35 had diffuse large B-cell lymphoma (DLBCL), 12 had follicular lymphoma (FL), 16 had marginal zone B-cell lymphoma (MZBL), 7 cases had T-cell lymphoma (T-cell), mucosa-associated lymphoid tissue lymphoma (MALT) was found in 2 cases, and 10 cases of primary lymphoma. Taking into account the increased frequency of the DLBCL, we tested whether there is any association between investigated gene polymorphisms and risk of developing DLBCL. Moreover, lack of associations was found between GST gene polymorphisms and DLBCL patients (p>0.05, for all genotypes).

\textbf{Table 2. Patients' demographic and clinical characteristics of the GSTs genotypes}

<table>
<thead>
<tr>
<th></th>
<th>\textit{GSTT1} no. (%)</th>
<th>\textit{GSTM1} no. (%)</th>
<th>\textit{GSTP1 Ile105Val} no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present</td>
<td>null</td>
<td>p</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>19 (61.29)</td>
<td>19 (65.52)</td>
<td>Ref.</td>
</tr>
<tr>
<td>male</td>
<td>12 (38.71)</td>
<td>10 (34.48)</td>
<td>0.793</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60 years</td>
<td>17 (54.84)</td>
<td>14 (48.28)</td>
<td>Ref.</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>14 (45.16)</td>
<td>15 (51.72)</td>
<td>0.8027</td>
</tr>
<tr>
<td>Ann Arbor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>14 (58.33)</td>
<td>9 (39.13)</td>
<td>Ref.</td>
</tr>
<tr>
<td>III-IV</td>
<td>10 (41.67)</td>
<td>14 (60.87)</td>
<td>0.2476</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;190UI</td>
<td>4 (12.50)</td>
<td>7 (25.00)</td>
<td>Ref.</td>
</tr>
<tr>
<td>&gt;190UI</td>
<td>28 (87.50)</td>
<td>21 (75.00)</td>
<td>0.3176</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2</td>
<td>25 (80.65)</td>
<td>16 (55.17)</td>
<td>Ref.</td>
</tr>
<tr>
<td>3,4</td>
<td>6 (19.35)</td>
<td>13 (44.83)</td>
<td>0.0517</td>
</tr>
<tr>
<td>Treatment outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>resistant</td>
<td>16 (55.17)</td>
<td>10 (45.46)</td>
<td>0.6892</td>
</tr>
<tr>
<td>partial remission</td>
<td>9 (31.04)</td>
<td>8 (36.36)</td>
<td>1</td>
</tr>
<tr>
<td>complete remission</td>
<td>4 (13.79)</td>
<td>4 (18.18)</td>
<td>Ref.</td>
</tr>
<tr>
<td>Survival, 5-years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5-years</td>
<td>4 (14.81)</td>
<td>11 (61.11)</td>
<td>Ref.</td>
</tr>
<tr>
<td>&lt;5-years</td>
<td>23 (85.19)</td>
<td>17 (38.89)</td>
<td>0.0683</td>
</tr>
</tbody>
</table>

Ref. – Reference, no – number

4 DISCUSSIONS

The current case-control study is first to evaluate a possible association of the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms with risk of NHL, conducted in a Romanian population from Transylvania region.

Considering that GSTs have a significant role in the regulation antioxidant defense mechanism and in the mediation of xenobiotics and several carcinogens, we hypothesized that genetic polymorphisms in genes encoding glutathione S-transferase T1, M1, and P1 previously reported for other studies may modulate the risk of developing several types of cancer \cite{5}, \cite{6}, \cite{7}, \cite{8}, \cite{16} including malignant lymphomas.

The recent findings showed that the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms have been involved in carcinogenesis, but the results remain inconsistent. For example, in a meta-analysis performed by Fang J. et al \cite{6} in 2013 on 506 case-control studies, the \textit{GSTT1} null and \textit{GSTM1} null genotypes were associated with an increased risk of cancer in Caucasians and Asians (\textit{GSTM1} had an OR = 1.17; 95%CI = 1.14-1.21 and \textit{GSTT1} had an OR = 1.16; 95%CI = 1.11-1.21, respectively), but no association in Africans regarding \textit{GSTM1} gene polymorphism. Some of the cancer types analyzed were
prostate cancer [16], colorectal cancer [17], breast cancer [6], bladder cancer [18], lung cancer [19], ALL [20] and gastric cancer [21]. Another study conducted by Lourenço G.J. et al. [22] showed that GSTP1 polymorphism had an increased risk of HL.

In our study, we did not find an association between GSTT1 null genotype and risk of NHL (p = 0.4454; OR = 0.1273, 95% CI = 0.6846-2.366). Similar findings were reported by Li Y. et al. [7] in a female study, but there was an association of the GSTs, NATs and cytochrome P450s polymorphisms in the relationship between alcohol consumption and risk of DLBCL. No association was observed between GSTs polymorphisms and tobacco smoking in a population of NHL (1,115 females) [23], but significant change was found for DLBCL.

Other studies that revealed no association of the GSTT1 null and GSTM1 null genotypes and risk of acute lymphoblastic leukemia (ALL) were described in Turkish children [4] and in an Argentinian population [20]. In a meta-analysis from 2015, Xuly et al. [24] found a significant association between GSTT1 null genotype and childhood ALL in Asians and no association in Caucasians and Africans.

In contrast, several studies reported a significant association of the GSTT1 null genotype and NHL risk. For instance, Bin Q. et al. [8] suggested that GSTT1 deletion may significantly increase the risk of NHL (p = 0.02, OR = 2.75, 95% CI = 1.17-6.45) and the same effect remains in females, but no association in HL. According to Ruiz-Cosano J. et al. [9] GSTT1 null genotype had a role in the development of lymphomas in relation to smoking and occupational exposures.

In the case of GSTM1 null genotype, we observed a decreased risk of NHL in our cohort without statistical significance. In a meta-analysis conducted by Bin Q. et al. [8] and performed on 1626 patients and 2892 controls with malignant lymphomas, the GSTM1 null, and GSTP1 null genotypes were unrelated to lymphoma risk, but the double null GSTT1 and GSTM1 genotype was significant positive associated with risk of lymphoma. In another study described by Wu M. et al. [25] in 2004 the GSTM1, GSTP1, IL-1beta and IL-1RN genes did not differ between MALT lymphoma patients and controls in a Chinese population. In the study of Ruiz-Cosano J. et al. [9] no significant association of the GSTM1 null genotype was found between patients and controls in malignant lymphomas.

An increased frequencies of the GSTT1 null, GSTM1 null, and double null genotypes in DLBCL (47.9, 52.1, and 23.9 % respectively) was reported by Abdel Rahman et al. in 2012 [26] in Egypt. Moreover, we found a decreased risk of NHL in our cohort with regard to GSTP1 Ile/Val and GSTP1 Val/Val genotypes, but no significant (p = 0.3517 and p = 0.7116, respectively). Our results are in line with a study reported by Chiu et al. [27] who found a low risk of DLBCL for Val/Val variant genotype (OR = 0.2, 95% CI = 0.1-0.96) and no significant excessive risk for MZBL and other B-cell lymphomas. An association with a decreased risk of NHL of the GSTP1 polymorphism was demonstrated in Korea by Kim H.N. et al. [28] in the large case-control study (713 cases and 1700 controls). In comparison to our findings, Li Y. et al. [7] showed a 2-fold increased risk of DLBCL in females regarding alcohol consumption with GSTP1 Ile/Val and Val/Val genotypes. Similar results were observed in non-smokers with DLBCL by Kilfoy B.A. et al. [23].

We analyzed possible associations between combined GSTs genotypes and patients’ clinical characteristics, including histological subtype, stage of disease, performance status (ECOG), and LDH level. There were no differences between patients’ clinical characteristics and combined GSTs genotypes (p>0.05, for all comparisons).

No particular association with treatment outcome was observed for the investigated GSTs genotypes. In contradiction, Cho H. et al. [29] reported that GSTT1 deletion may significantly increase the risk of drug toxicity after combined chemotherapy in Korean patients with DLBCL. Similar findings were found by Yri O. et al. [10] in 2013, and in addition GSTM1 gene polymorphism was associated with an inferior outcome in DLBCL patients with the low prognostic score (p=0.004).

The association between GSTs genotypes and overall survival was not significant (p>0.05), therefore, 5-year survival was not influenced by them. However, Hohaus S. et al. [30] and Han X. et al. [31] reported that GSTT1 null and GSTP1 genotypes were associated with worse FL survival. The same decreased event-free survival was also described by Cho H. et al. [29].

In addition, we determined the distribution histological subtypes of the NHL patients. We found that DLBCL, MZBL, and FL were most frequent in our patients and there were no differences regarding GSTs genotypes. Our findings, of no significant association between GSTs genotypes and histological subtypes of the NHL, are not consistent with previous reports [7, 11, 23, 25, 32-33]. [7] [11] [23] [25] [32] [33]

Also, we analyzed the association between the combined GSTs genotypes (null GSTT1, null GSTM1 and variant genotype of GSTP1 Ile105Val) and NHL and there was no increased risk of developing NHL in our study (p > 0.05, for all comparisons). Similar results with no association between GST variant genotypes separately or in combination were described in Turkish
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children with ALL [4]. There are no previously reported studies regarding any association between combined variants of the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms and risk of NHL.

According to the literature, there is only one study which investigates the association between ECOG performance status and \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} genotypes in NHL patients. Similar to our results, Sarmanová J. et al. [5] found no association between GSTs studied genotypes and clinical stage, performance status in a case-control study consisted of 219 patients with HL and NHL.

However, we presume that our relatively small group of patients could explain some of the discrepancies between our results and those of previous studies. The lack of environmental exposure data is an important limitation of our study, therefore we want to consider these risk factors for future studies.

5 CONCLUSIONS

In summary, the \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} gene polymorphisms were more frequent in DLBCL and our findings have not revealed associations between the GSTs genotypes and risk of NHL.

Therefore, our research suggests that \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} genotypes do not contribute to the risk of developing NHL.

Further investigations are required to explore the association between GSTs polymorphisms and risk of NHL and to a better understanding, the role of the detoxification enzymes in the xenobiotic metabolism.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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