

Analysis of the C609T Polymorphism of NQO1 Gene in South Croatian Patients with Hematological Malignancies

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ABSTRACT

In this study we analyzed the effect of polymorphic variation of NAD(P)H: quinone oxidoreductase1 (NQO1) gene that encode enzyme which detoxifies harmful quinines and protect hematopoietic stem cells against oxidative stress. C609T polymorphism of NQO1 gene leads to loss of enzyme activity, which may be a risk factor in the etiology of specific types of hematopoietic malignancies. We analyzed C609T polymorphism in NQO1 gene in the group of 82 patients (56 adult and 26 children) with different type of hematopoietic malignancies and 99 healthy participants (61 adult and 38 children) using PCR and the RFLP method. We confirmed that the polymorphism C609T in NQO1 gene was more frequent in the adult patients' group with myeloid disorders, ($p=0.0267$) compared with adult controls. We could not confirm the association C609T polymorphism with recurrent chromosome translocations (clonal karyotype changes) neither in the adult nor in pediatric group of patients.

Key words: hematological malignancies, polymorphism, NQO1, clonal chromosomal abnormalities

Introduction

The World Health Organization (WHO) classifies hematological malignant diseases according to their lineage (myeloid, lymphoid, histiocytic/dendritic) and distinguishes neoplasm of precursor cells from those comprised of functionally mature cells. Within each category, distinct diseases are defined according to a combination of morphology, immunophenotype, genetic features and clinical syndromes¹. Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in early childhood while primary myeloid disorder (acute or chronic) is the most common type of leukemia in the elderly².

From a clinical point of view it is very important to understand myeloid and lymphoid classification of neoplasm³. Hematopoietic malignancies are clonal diseases that arise due to genetic changes in one hematopoietic

stem cell in the bone marrow or lymph tissue⁴. Such altered cell divides forming abnormal clone of cells, which can be determined by cytogenetic findings of the same or similar changes in three metaphases⁵. Series of low-penetrance genes can be involved in the development of hematological malignant diseases. An individual's risk of complex disease development presents a cumulative effect of genetic and environmental factors. It seems that polymorphisms of certain genes play a role in leukemogenesis⁶.

Therefore, in this study we analyzed the polymorphism of the NQO1 gene that encode protein responsible for detoxification of endogenous and exogenous mutagens and may influence susceptibility to leukemia development⁶⁻¹¹.

NAD(P)H: quinone oxidoreductase1 (NQO1) is a detoxification enzyme that protects cells against oxidative stress and toxic quinines (11). NQO1 catalyze a 2-electron reduction of quinines, causing prevention of their participation in redox cycling and thus in oxidative stress⁶.

Individuals with a lack of NQO1 activity are more susceptible to the toxic and carcinogenic effects of quinines (reactive metabolites of benzene) and have increased risk of hematotoxicity and leukemia^{6,12}. The human NQO1 gene is located on chromosome 16q22.1 and has six exons (13). Polymorphism C to T at position –609 causes proline to serine substitution in codone 187 in the NQO1 protein. Homozygous T/T show the loss of enzyme activity, and heterozygous C/T show low to intermediate NQO1 activity. It has been reported that children are particularly more vulnerable than adults to oxidative stress and toxic quinines because their metabolism is less developed/matured, and they have a higher level of cell division and growth^{6,12}.

In this case-control study, we analysed whether the NQO1 variation increase risk for an individual's susceptibility to hematological malignancies. Our study was conducted in Southern Croatian population, which can be considered relatively homogenous from both genetic and socio-cultural points of view.

Material and Methods

In this study we investigated 82 patients (34 females and 48 males) and 99 healthy participants (45 females and 54 males). Investigated group of patients was divided according to the age in two groups. Most of the patients (56 individuals) were of adult age (median age 58 years; range 34–82 years) with different types of myeloid neoplasms. The rest of them were pediatric patients (26 individuals) (median age 8.5 years; range 0.3–17 years) with prevalence of lymphoid neoplasms (20 individuals). The group of healthy participants (61 adult and 38 pediatric individuals) has been adjusted in age and sex distribution with patient group. The cases had been diagnosed in the Department of Pediatric and Internal Medicine of Split University Hospital between 2002 and 2007. The diagnosis was established according to standard rules of the WHO classification. All patients were karyotyped using previously described methods of cytogenetic analysis on pretreatment bone marrow cells¹⁴.

In our study we investigated C609T polymorphism of the NQO1 gene (rs1800566) using previously described standard methods¹⁵. For detection of C609T polymorphism, we performed the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) after digestion with restriction enzyme HinfI. The digested fragments were separated and visualized on agarose or polyacrylamide gels. Fragments of 151, 120, and 33 bp on gels confirmed the presence of the polymorphic HinfI restriction site yields.

NQO1 genotype frequencies in leukemia subgroups and controls were compared by the χ^2 -test. P-values less

than 0.05 were considered nominally significant. Statistical analysis was performed using statistical package MedCalc (Mariakerke, Belgium).

Results

The distribution of C609T genotypes of the NQO1 gene, in the adult patient group with myeloid disorder and adult controls are reported in Table 1. Genotype frequencies showed statistically significant difference between those two groups ($p=0.0267$).

A heterozygote (C/T) genotype was most frequent in the group of unclassifiable myeloproliferative neoplasms (MPN) (7/15).

Recurrent chromosome abnormalities (clonal abnormalities) detected in the leukemia cells of the adult patient group showed a wide spectrum of clonal karyotype aberrations, and were found in 25/56 patients (12 of them were C609T heterozygous). The most frequent clonal rearrangement was reciprocal translocation t(9;22) observed in 14 patients exclusively with chronic myelogenous leukemia BCR-ABL positive (CML), (5 heterozygous). Heterozygote genotypes were also found in patients with other different clonal abnormalities: deletion in chromosome 20q (2/2) and different abnormalities which include chromosome 17q (3/3). However, no association was found between specific chromosomal abnormalities and C609T genotypes of the NQO1 gene.

The distribution of genotypes in the NQO1 gene, in the pediatric leukemia patient group compared with pediatric control group is reported in Table 2 and statistical significant difference was not found ($p=0.1282$).

A heterozygote (C/T) genotype was most frequent in the group of the acute lymphoblastic leukemia (8/20). The C609T genotypes of NQO1 gene also were not related to specific clonal abnormalities in pediatric patient group.

TABLE 1
NQO1 C609T GENOTYPES AMONG ADULT MYELOID NEOPLASM SUBTYPES IN THE 56 INVESTIGATED AND 61 CONTROL SUBJECTS

| GROUPS | C/C | C/T | T/T | Chi square | p value |
|------------------------------------|-------|-------|------|------------|---------|
| Control group | 48/61 | 12/61 | 1/61 | | |
| Patient group | 31/56 | 23/56 | 2/56 | 7.25 | 0.026 |
| Subtypes of adult myeloid neoplasm | | | | No/Total | |
| MPN (unclassifiable) | 7/15 | 7/15 | 1/15 | | 15/56 |
| CML | 9/14 | 5/14 | 0/14 | | 14/56 |
| PV | 5/9 | 3/9 | 1/9 | | 9/56 |
| MDS | 5/8 | 3/8 | 0/8 | | 8/56 |
| AML | 7/10 | 3/10 | 0/10 | | 10/56 |

MPN=Myeloproliferative neoplasm; CML = Chronic myelogenous leukemia; BCR-ABL positive; PV = Polycythemia vera; MDS = Myelodysplastic syndromes; AML = Acute myeloid leukemia.

TABLE 2
NQO1 C609T GENOTYPES AMONG CHILDHOOD LEUKEMIA
TYPES IN THE 26 INVESTIGATED AND 38 CONTROL SUBJECTS

| GROUPS | C/C | C/T | T/T | Chi square | p value |
|--------------------------------|-------|-------|------|------------|---------|
| Control group | 30/38 | 8/38 | 0/38 | | |
| Patient group | 16/26 | 10/26 | 0/26 | 2.31 | 0.1282 |
| Subtypes of childhood leukemia | | | | No/Total | |
| ALL | 12/20 | 8/20 | 0/20 | | 20/26 |
| AML | 1/3 | 2/3 | 0/3 | | 3/26 |
| CML juvenile | 1/1 | 0/1 | 0/1 | | 1/26 |
| MDS | 2/2 | 0/2 | 0/2 | | 2/26 |

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CML = chronic myelogenous leukemia juveniles BCR-ABL positive; MDS = myelodysplastic syndromes.

Discussion

We confirmed that homozygote T/T and heterozygote C/T genotypes of NQO1 C609T polymorphism were significantly more frequent in the investigated group of adult patients with different myeloid disorders. These results suggest that presence of a polymorphic T allele represent a risk factor for the development of primary myeloid disorder in adults, which is associated with a reduced amount or lack of enzyme involved in the detoxification of quinone carcinogens.

NQO1 C609T allele frequency varies between different ethnic groups. The frequency of allele T of the NQO1 C609T polymorphism in white populations of Europe and America is lower (17% in the United Kingdom, 19% in Germany and 16% USA) compared with Asia and Mexican Hispanic populations where it is significantly higher (42% in Korea, 47% in China, 38% in Japan, and 43% in Mexico)^{12,16}. The allele T frequency among healthy individuals (adults and children), in our Southern Croatian population was 11% what is the expected frequency for the Caucasian population.

Larson et al as well as Yamaguti et al in their studies of different ethnic groups, mostly Caucasians, showed a significant difference in frequency of heterozygous (C/T) and homozygous (T/T) between patients with myeloid disorders and controls what is in concordance with our results^{15,17}.

Study performed in a Japanese population, showed a similar frequency of C/T and T/T genotypes in patient group but they could not reach statistical significance because of a higher frequency of the T allele in the healthy Japanese population¹⁸. An Israel study, conducted by Malik et al, showed that the frequency of C/T allele did

not differ between AML patients and control subjects because of more frequent T allele in healthy population¹⁹.

The study conducted in the Franch-Canadian province of Québec, Canada showed a significantly more frequent polymorphic T allele in the group of pediatric patients with acute lymphoblastic leukemia (ALL)⁶. In contrast to those, our results did not show association of NQO1 C609T polymorphism with pediatric leukemia. Sirma et al in Turkish population also found that NQO1 C609T polymorphism is not associated with de novo pediatric acute leukemia in their study group¹². Recently a meta-analysis of seven studies that analyzed the risk of de novo pediatric leukemia was conducted. Pooling data from seven studies, authors provided no evidence of a relationship between NQO1 C609T genotype and risk for pediatric leukemia²⁰. Yeoh AE et al in Asians population as well as Silveira Vda S et al in Brazilian population showed that carriers of NQO1 CT genotype have a lower risk of developing ALL compared to those carrying wild-type^{21,22}. This finding was in contrast to nearly all previous reports in the Caucasians. Probably racial and socio-cultural heterogeneity of populations can cause such divergent results.

We could not confirm association of C609T polymorphism with specific cytogenetic abnormalities in a karyotype. Karyotype was not found to be associated with a heterozygote (C/T) genotype in Israeli group of patients what is in concordance with our results¹⁹. However, Larson et al found that the T allele frequency was a 1.6 fold higher in the patients with chromosomal aberrations (chromosome 5 and 7), while Smith et al showed the same in patients with inversion of chromosome 16^{15,10}.

In summary, the frequency of an inactivating polymorphism of NQO1 gene appears to be increased among primary myeloid disorders in adults, while we did not provide evidence for the statistically significant effect of this polymorphism in pediatric leukemia. No evidence was found in association of cytogenetic findings and inactivating polymorphism of NQO1 gene in both groups of patients. These results suggests that inactivating polymorphism of functional genetic marker involved in the metabolic pathway of carcinogens may be a risk factor in leukemogenesis of specific types of hematopoietic malignancies in a certain age. Further research should include a larger number of genetically homogenous subjects.

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ANALIZA POLIMORFIZMA C609T GENA ZA NQO1 U BOLESNIKA S HEMATOLOŠKIM MALIGNIM BOLESTIMA U JUŽNOJ HRVATSKOJ

SAŽETAK

U ovoj studiji analizirali smo utjecaj polimorfnih varijanti gena za NAD(P)H: kvinon oksidoreduktaza1 (NQO1) koji kodira enzim za detoksikaciju štetnih kvinona i štiti hematopoetske matične stanice od oksidativnog stresa. Polimorfizam C609T gena za NQO1 dovodi do gubitka enzimske aktivnosti što može biti rizični čimbenik u etiologiji specifičnih tipova hematopoetskih malignih bolesti. Analizirali smo polimorfizam C609T gena za NQO1 u grupi od 82 bolesnika (56 odraslih i 26 djece) s različitim tipovima hematopoetskih malignih bolesti te u 99 zdravih ispitanika (61 odrasli i 38 djece) koristeći metode PCR i RFLP. Potvrdili smo da je polimorfizam C609T gena za NQO1 učestaliji u skupini odraslih s mijeloidnim poremećajima, ($p=0,0267$) u usporedbi s kontrolnom skupinom odraslih ispitanika. Nismo mogli potvrditi povezanost polimorfizma C609T s određenim kromosomskim translokacijama (promjene klonalnog kariotipa) ni u odrasloj kao ni u pedijatrijskoj skupini oboljelih.