RESEARCH ARTICLE

Is the MDR1 C3435T Polymorphism Responsible for Oral Mucositis in Children with Acute Lymphoblastic Leukemia?

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Abstract

Background and Aim: Although the functional consequences of MDR-1 polymorphisms have been the subject of numerous studies, to the best to our knowledge, associations with clinical side effects of anticancer drugs have yet to be assessed. Our aim was to clarify any role of the C3435T polymorphism of the MDR1 gene in oral mucositis and its relation with elevated reactive oxygen species (ROS) levels, in children with acute lymphoblastic leukemia (ALL). Materials and Methods: The distribution of the MDR-1 C3435T polymorphism in 47 patients with ALL was determined by RFLP and compared with that of 68 healthy controls. Results: There were no association in distribution of genotypes of MDR-1 C3435T polymorphism and the risk of ALL. Oral mucositis were detected in 78.7% (n=37) of the patients and significantly related to the MDR-1 CT genotype (p=0.042), as confirmed by logistic regression analysis. Conclusion: Our preliminary data suggest that children carrying the CT genotype are more prone to develop oral mucositis, which might mean that the heterozygous genotype leads to accumulation of more reactive oxygen species. Since a limited number of patients was investigated, further studies are needed to confirm these findings.

Keywords: MDR-1 C3435T polymorphism - acute lymphoblastic leukemia - oral mucositis - reactive oxygen species

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Introduction

Acute leukemia, the main subtype of childhood leukemia which is characterized by the uncontrolled proliferation of hematopoietic cells in the bone marrow, is the major pediatric cancer all over the world with an approximately 20% mortality rate in each year (Pui et al., 2004; Greaves, 2006). Dysregulation of immune responses may be a cause of ALL. Although epidemiological data showed that the transplacental carcinogen exposure such as ionizing radiation as a basis for infant leukemia associated with MLL gene fusion, the role of environmental carcinogenesis as postnatal high-dose radiation, chemotherapeutic agents in ALL is currently undefined. In addition environmental risk factors may have different effects on the risk of childhood leukemia depending on the timing of exposure and individual genetic susceptibility (Sandler and Ross, 1997; Greaves, 2006).

The treatment outcome in malignant hematologic diseases such as ALL is poor due to the resistance to chemotherapeutic drugs (Aladjidi et al., 2003; Chessells et al., 2003). To date only 30-35% of all cancer patients can be cured with chemotherapy alone because of the emergence of malignant cell populations, which are resistant to all clinically available anticancer drugs (Bradley et al., 1988). The existence of so-called pleiotropic drug resistance (multidrug resistance, MDR) has been identified (Goldstein et al., 1989) The human multidrug resistance 1 (MDR1 or ABCB1) gene encodes P-glycoprotein (P-gp), a 170-kDa ATPase-dependent integral membrane protein which belongs to the ATP binding cassette (ABC) super family of transporters and responsible for the efflux of a wide variety of lipophilic compounds, including multiple chemotherapeutic agents used in leukemia therapy, naturally occurring xenobiotics, pesticides, and cellular metabolites (Lin and Yamazaki, 2003). MDR1 is expressed primarily in the gastrointestinal tract, blood-tissue barrier, liver, kidney, testis, placenta (Schinkel, 1997) and interestingly in several subclasses of bone marrow and peripheral leukocytes (Hitzl et al., 2001; Sakaeda et al., 2002; Elliott et al., 2004). Experimental studies have shown that suppression of MDR1 results in a decrease in natural killer and CD8+ T-cell activity (Gupta et al., 1992; Chong et al., 1993). Furthermore, some other studies demonstrated that P-glycoprotein involves in the release of interleukin-2, interleukin-4, and IFN-g from lymphocytes (Drach et al., 1996; Pawlik et al., 2005).

MDR1 gene is located on chromosome 7q21, comprises 28 exons (Lin and Yamazaki, 2003). Several

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polymorphisms have been identified in MDR1, including silent C3435T polymorphism (rs1045642) in exon 26 at position 3435 associated with the altered expression and function of P-gp (Hoffmeyer et al., 2000; Cascorbi et al., 2001; Kim et al., 2001; Tanabe et al., 2001; Kroetz et al., 2003; Vijayakrishnan and Houlston, 2010). The results of these studies are differed from each other but a case-control study from Poland (Jamroziak et al., 2004) reported a significant association between the risk of ALL and 3435TT genotype compared with the CC and CT genotypes. Although the functional consequences of MDR1 polymorphisms have been the subject of numerous studies, none have assessed the association with clinical side effects of the anticancer drugs. Oral mucositis remains one of the most common and troubling side effects of antineoplastic therapy (Sonis, 2011). Due to a recent work P-gp-mediated MDR is abolished under conditions of elevated reactive oxygen species (ROS) levels suggesting that the MDR phenotype can be circumvented by modest increase of intracellular ROS generation (Wartenberg et al., 2005). In the light of this data we aimed to reveal the role of C3435T polymorphism of MDR1 gene in oral mucositis formation, which is known with high ROS expression, in children with acute lymphoblastic leukemia (ALL).

Materials and Methods

Study participants and clinical investigation

The study was in accordance with the declaration of Helsinki, and informed consent was obtained from the parents of all the subjects. Protocol was approved by Local Ethics Committee of İstanbul University Medical Faculty. Between years 2008-2010 all volunteered patients who were under maintance therapy or routine follow ups enrolled the study. A total of 47 patients (13 girls and 34 boys) diagnosed with ALL and 68 healthy volunteers (25 girls and 43 boys) without any symptoms of ALL were included as the control group. The patients records were examined to fill the survey about diagnose of the disease, protocol of therapy and about the highest score of oral mucositis, according to WHO classification, detected during the whole therapy. Furthermore an intraoral examination was carried out to find out dental needs of this paediatric population. The patients' prognoses were also noted in this period of time. One patient was excluded from mucositis classification due to conflicting data.

Polymerase chain reaction (PCR)-based detection of MDR1 C3435T polymorphism

Blood specimens were collected in tubes containing EDTA, and genomic DNA samples were extracted from whole blood with a salting-out procedure (Miller, 1998).

Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine MDR1 C3435T. After amplification of the isolated DNA with PCR, the MDR1 C3435T mutation was detected by cutting the PCR product with the restriction endonuclease DpnII (New England Biolabs, Hitchin, U.K.) (Sills et al., 2005). The digested DNAs were separated on 3% agarose gel in 1XTris borate EDTA buffer followed by staining with ethidium bromide solution. The genotypes were typed by visualization under ultraviolet light.

Statistical methods

Statistical analysis was performed by using SPSS software package (revision 13.0 SPSS Inc., Chicago, IL, USA). Clinical laboratory data are expressed as mean±SD. Mean values were compared between patients and controls by unpaired Student's t-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the Chi-square statistic. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

The characteristics of the study groups were summarized in Table 1. The ALL patients and the controls had similar distribution of sex and age. The mean age of patients and controls were 8.68 and 10.14, respectively. Oral mucositis were detected 80.4% (n=37) of the patients and 31.9% (n=15) of those had severe type.

MDR1 C3435T genotype and allele frequencies in the patient and control groups were also presented in Table 1. There were no significant differences in distribution of genotypes of MDR-1 gene between the patient and control groups. The distribution of MDR1 C3435T polymorphism according to presence of oral mucositis in ALL patients were summarised in Table 2. CT genotype was found related with higher incidence of oral mucositis.

To investigate the diagnose age in ALL patients according to the MDR1 C3435T polymorphism chi-square test was performed. The patients carrying TT genotype and under the age 3 have a higher ratio of ALL diagnosis when compared with those over 3 years (p=0.057). In addition the C allele carriers of over 3 years age have a higher ratio of ALL diagnosis (p=0.057).

Table 1. Characteristics of the Study Population

		GROUPS		P value
		Control (n=68)	ALL (n=47)	
Age (years) (X±SD)		10.14±3.86	8.68±4.90	0.080
Sex (Girls/Boys) (n)		25/43	13/34	0.308
Oral Mucositis (Yes, No)(%)		0	80.4/19.6*	
MDR1 C3435T Polymorphism:				
Genotypes:	CC	11 (16.2%)	11 (23.4%)	0.333
	TT	13 (19.1%)	10 (21.3%)	0.776
	CT	44 (64.7%)	26 (55.3%)	0.311
Alleles:	C	66 (48.52%)	48 (51.06%)	0.776
	T	70 (51.47%)	46 (48.93%)	0.333

*Oral mucositis was evaluated for 46 patients, n: number of individuals

Table 2. Distribution of MDR1 C3435T Polymorphism According to Presence of Oral Mucositis in ALL Group

MDR1 C3435T		Oral mucositis		P value	Odd Ratio
polymorphism		Presence n (%)	Absence n (%)		(95% CI)
Genotypes	CC	7 (18.9%)	4 (44.4%)	0.186*	2.349 (0.874-6.317)
	TT	7 (18.9%)	3 (33.3%)	0.384*	
	CT	23 (62.2%)	2 (22.2%)	0.059*	2.80 (0.103-1.245)
Alleles	C	30 (81.1%)	6 (66.7%)	0.384	0.384
	T	30 (81.1%)	5 (55.6%)	0.186	0.186

*Fisher's exact test, n: number of individuals, Data are shown as %

Table 3. Survival of ALL Patients Stratified by MDR 3435C/T Genotypes and Alleles

MMDR 3435C/T mutation	No of patients	Alives/Death
CC	11	11/-
TT	10	10/2*
CT	26	26/-

*p=0.043 (Fisher's exact test), Odds Ratio=5.50 (95% CI:2.939-10.294)

Table 4. Logistic Regression Analysis for Presence of Oral Mucositis

	p value	Odd Ratio	95% CI*
MDR3435T CT genotype	0.042	0.168	0.030-0.938
Gender (male)	0.683	1.462	0.236-9.047
ALL diagnosis age ≤ 3	0.878	0.880	0.170-4.544

*CI: Confidence interval

Overall survival of the ALL patients were evaluated and correlated with MDR 3435C/T genotypes and alleles as shown in Table 3.

Furthermore, the risk of oral mucositis was evaluated by logistic regression analysis. Presence of oral mucositis was included as the dependent variable. The CT genotype of the MDR1 C3435T polymorphism, male gender and ALL diagnosis age ≤3 were included in the model as categorical variables. There was a significant difference in the risk of oral mucositis among subjects with CT genotype (p=0.042) (Table 4). In the logistic regression analysis, we confirmed the presence of a relationship between the CT genotype and the increased risk of oral mucositis in children with ALL.

Discussion

The oral mucositis is one of the most deliberating complications of cancer chemotherapy. It is a complex condition resulting from the interaction between antineoplastic agents and epithelial cells, the action of proinflammatory cytokines, oral microbiata, overlapping oral trauma, unsatisfactory oral hygiene conditions, and the deficient immune status of the patient (Pinto et al., 2006). A recent work suggested that MDR phenotype can be circumvented by modest increase of intracellular ROS generation (Wartenberg et al., 2005). Previously the initiation phase of oral mucositis was described along with clonogenic cell death and production of ROS by injured cells. It was proposed that by understanding its genetic control we can predict mucositis risk (Sonis, 2011). In this study the impact of MDR1 C3435T polymorphism on P-gp activity which could determine intracellular concentrations of drugs and /or ROS leading to formation of oral mucositis was investigated.

The previous data was concentrated more on risk of developing ALL and MDR1 polymorphism. We investigated the relation but could not find any statistically significant association between MDR1 C3435T genotypes or alleles and the risk of ALL (p>0.05). First report was from Poland indicating that mutant homozygous TT genotype was found to be associated with risk of developing ALL and the occurrence, whereas CC genotype was associated with worse prognosis (Jamroziak et al., 2004). Another study from Iranian supported the association between homozygous TT and also CT genotype and incidence of ALL (Miladpour et al., 2009). Rao et al. (2010) also investigated MDR1 C3435T polymorphism in ALL and AML patients in Indian population and, parallel with findings of Poland study, ALL incidence was found associated with homozygous TT genotype and poor prognosis was related with CC genotype. Urayama et al. (2007) conversely, notified no significant difference in C3435T gene MDR1 polymorphism between patients and controls that supported our results. Recently two meta-analysis were published investigating the relation of C3435T polymorphism and cancer risk. Sheng et al. found that individuals with the 3435TT genotype showed an increased risk of cancer, compared with those with the 3435CC genotype. They also reported, in the stratified analyses, significantly elevated risks were more pronounced among hematologic malignances, breast cancer, and renal cancer. However, C3435T polymorphism had no effects on gastrointestinal cancer and lung cancer risk (Sheng et al., 2012). The other meta-analysis from China supported, the C3435T polymorphism might be a risk factor in the susceptibility of breast cancer, renal cancer, and in Caucasian individuals, but it did not show significant role in Asian population and colorectal cancer, gastric cancer, and acute lymphoblastic leukemia (Wang et al., 2012). Lack of association in both studies may be due to ethnic differences or may be related with limited number of patients investigated.

The drug resistance in cancer cells often results from elevated expression of particular proteins, such as P-glycoprotein, which can result in an increased efflux of the cytotoxic drugs from the cancer cells, thus lowering their intracellular concentrations (Liscovitch and Lavie, 2002). In various cancer types, including acute myeloid leukaemia, breast cancer, various childhood tumors, over-expression of P-gp encoding MDR1 gene has been found to correlate with poor outcome in patients treated with chemotherapy (Illmer et al., 2002; Siegsmung et al., 2002). In addition to physiological and environmental factors, P-gp expression and function are modified by genetic polymorphisms of the MDR1 gene. Hoffmeyer et al. (2000) was described, which was associated with significantly reduced intestinal P-glycoprotein contents in subjects with the TT genotype in comparison to subjects homozygous for the wild type allele. The patient group in our study had high survival rate (95.4%) and only 2 subjects with TT genotype could not survive. In other studies poor prognose of ALL patients were associated with CC genotype. They suggested that higher concentrations of P-gp expression would lower the intracellular drug concentration therefore decreasing the benefit of the chemotherapy (Miladpour et al., 2009; Rao et al., 2010). Our number is below the correlation limits and predicting outcome in ALL patients, which is not the of first priority aim of this work.

This study investigated a possible link between MDR1 polymorphism and existence of oral mucositis in children with ALL. High expression of ROS by ulcerated tissues is a well- known issue and the control of oral

mucositis formation is closely related with the elevated ROS levels. Our preliminary data suggests that children carrying the CT genotype are more prone to develop oral mucositis which might mean heterozygous genotype leads accumulation of more ROS. This data must be confirmed with future studies in larger samples but still an interesting finding in order to predict this side effect and moreover might light a way to solve the genetic predisposition to develop oral mucositis.

In conclusion, these preliminary data in Turkish population suggested that children carrying the CT genotype are more prone to develop oral mucositis and might mean being more susceptible to the side effects ofchemotherapy. As low incidence of childhood cancer and wide range in types causes methodologic problems limited numbers of the patients were investigated, multi-institutional collaborative future sudies are needed to confirm these findings.

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References

Aladjidi N, Auvrignon A, Leblanc T, et al (2003). Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of pediatric hematology and immunology. *J Clin Oncol*, **21**, 4377-85.

Bradley G, Juranka PF, Ling V (1988). Mechanisms of multidrug resistance. *Biochim Biophys Acta*, **948**, 87-128.

Cascorbi I, Gerloff T, Johne A, et al (2001). Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in White subjects. *Clin Pharmacol Ther*, **69**, 169-74.

Chessells JM, Veys P, Kempski H, et al (2003). Long term follow-up of relapsed childhood acute lymphoblastic leukemia. *Br J Haematol*, **123**, 396-405.

Chong AS, Markham PN, Gebel HM, Bines SD, Coon JS (1995). Diversemultidrugresistance-modification agents inhibit cytolytic activity of natural killer cells. *Cancer Immunol Immunother*, **36**, 133–9.

Drach J, Gsur A, Hamilton G, et al (1996). Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4, and interferon-g in normal human T lymphocytes. *Blood*, **88**, 1747-54.

Elliott JI, Raguz S, Higgins CF (2004). Multidrug transporter activity in lymphocytes. *Br J Pharmacol*, **143**, 899-907.

Goldstein LJ, Galski H, Fojo A, et al (1989). Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst*, **81**, 116-24.

Greaves M (2006). Infection, immune responses and the aetiology of childhood leukaemia. *Nat Rev Cancer*, **6**, 193-203.

Gupta S, KimCH, Tsuruo T, Gollapudi S (1992). Preferential expression and activity of multidrug resistance gene 1 product (P-glycoprotein), a functionally active efflux pump, in human CD8+ T cells: a role in cytotoxic effector function. *J Clin Immunol*, **12**, 451–8.

Hitzl M, Drescher S, Van der Kuip H, et al (2001). The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics*, **11**, 293-8.

Hoffmeyer S, Burk O, Von Richter O, et al (2000). Functional polymorphisms of the human multidrug resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA*, **97**, 3473-8.

Illmer T, Schuler US, Thiede C, et al (2002). MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res*, **62**, 4955-62.

Jamroziak K, Mlynarski W, Balcerczak E, et al (2004). Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur J Haematol*, **72**, 314-21.

Kim RB, Leake BF, Choo EF, et al (2001). Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther*, **70**, 189-99.

Kroetz DL, Pauli-Magnus C, Hodges LM, et al (2003). Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics*, **13**, 481-94.

Lin JH, Yamazaki M (2003). Role of p-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet*, **42**, 59-98.

Liscovitch M, Lavie Y (2002). Cancer multidrug resistance: a review of recent drug discovery research. *IDrugs*, **5**, 349-55.

Miladpour B, Shokouhi AM, Shirdel A, et al (2009). Association of acute lymphoblastic leukemia and MDR1 gene polymorphism in an ethnic Iranian population. *IJBC*, **2**, 63-7.

Miller SA, Dykes DD, Polesky HF (1998). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Nakamura T, Sakaeda T, Horinouchi M, et al (2002). Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther*, **71**, 297-303.

Pawlik A, Baskiewicz-Masiuk M, Machalinski B, Kurzawski M, Gawronska-Szklarz B (2005). Involvement of C3435T and G2677T multidrug resistance gene polymorphisms in release of cytokines from peripheral blood mononuclear cells treated with methotrexate and dexamethasone. *Eur J Pharmacol*, **528**, 27-36.

Pinto LP, Souza LB, Gordon-Nunez MA, et al (2006). Prevention of oral lesions in children with acute lymphoblastic leukemia. *Int J Pediatric Otorhinolaryngol*, **70**, 1847-51.

Pui CH, Schrappe M, Ribeiro R, Niemeyer CM (2004). Childhood and adolescent lymphoid and myeloid leukemia. *Hematology Am Soc Hematol Educ Program*, **1**, 118-45.

Rao DN, Anuradha C, Vishnupriya S, et al (2010). Association of an MDR1 gene (C3435T) polymorphism with acute leukemia in India. *Asian Pac J Cancer Prev*, **11**, 1063-66.

Sakaeda T, Nakamura T, Okumura K (2002). MDR1 genotype-related pharmacokinetics and pharmacodynamics. *Biol Pharm Bull*, **25**, 1391-400.

Sandler DP, Ross JA (1997). Epidemiology of acute leukemia in children and adults. *Semin Oncol*, **24**, 3-16.

Schinkel AH (1997). The physiological function of drug-transporting P-glycoproteins. *Semin Cancer Biol*, **8**, 161-70.

Sheng X, Zhang L, Tong N, et al (2012). MDR1 C3435 T polymorphism and cancer risk: a meta-analysis based on 39 case-control studies. *Mol Biol Rep*, **39**, 7237-49.

Siegsmung M, Brinkmann U, Scheffeler E, et al (2002). Association of the P-glycoprotein transporter MDR1 (C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol*, **13**, 1847-54.

Sills GJ, Mohanraj R, Butler E, et al (2005). Lack of association

between the C3435T polymorphism in the human multidrug resistance (MDR1) gene and response to antiepileptic drug treatment. *Epilepsia* **46,** 643-47.

Sonis ST (2011). Oral mucositis. *Anticancer Drugs*, **22**, 607-12.

Tanabe M, Ieiri I, Nagata N, et al (2001). Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther,* **297**, 1137-43.

Urayama KY, Wiencke JK, Buffler PA, et al (2007). MDR1 gene variants, indoor insectisid exposure, and the risk of childhood acute lymphoblastic leukemia. *Cancer Epidemiol Biomarkers Prev*, **16**, 1172-7.

Vijayakrishnan J, Houlston RS (2010). Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. *Haematologica*, **95**, 1405-14.

Wang J, Wang B, Bi J, Li K, Di J (2012). MDR1 gene C3435T polymorphism and cancer risk: a meta-analysis of 39 case-control studies. *J Cancer Res Clin Oncol*, **138**, 979-89.

Wartenberg M, Hoffmann E, Schwindt H, et al (2005). Reactive oxygen species-linked regulation of the multidrug resistance transporter P-glycoprotein in Nox-1 overexpressing prostate tumor spheroids. *FEBS Lett*, **579**, 4541-9.