

Original Research

## The frequency of C609T polymorphism in the NQO1 gene and its relation to cytogenetic abnormalities in patients with myelodysplastic syndrome

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**Abstract:** The aim of the present study is to evaluate the frequency of C609T polymorphism in the NQO1 (NAD(P)H) quinon oxydoreductase gene and its relation to cytogenetic abnormalities in patients with Myelodysplastic Syndrome (MDS). The study group consisted of 80 patients MDS with 13 of them in the pediatric age group. The frequency of the NQO1 gene polymorphism was compared with a healthy control group involving 423 individuals. Cytogenetic abnormalities were detected in 43 patients (54%). In patients with MDS the overall frequency of the C609T polymorphism was not different than controls. Also, although the frequency of the C609T polymorphism was higher in patients with secondary MDS (sMDS) (OR: 1.893, 95% CI: 0.840-4.265, p=0.238), 5/del(5q) (OR:1.298, 95% CI: 0.331-5.086, p=0.124), +21 (OR:1.817, 95% CI:0.429-7698, p=0.124) and t(8;21) (OR:3.028, 95% CI: 0.604-15.172, p=0.137) groups, the difference did not reach statistical significance. Our results do not support the view that the C609T polymorphism has a role in the pathogenesis of MDS. Also the frequency of the C609T allele did not seem to be associated with cytogenetic abnormalities.

**Key words:** MDS, NQO1 gene, Polymorphism, Cytogenetic abnormalities.

### Introduction

Myelodysplastic Syndrome (MDS) involves heterogeneous groups of stem cell malignancies characterized by dysplastic and ineffective blood cell production (9). MDS has been classified according to criteria proposed by FAB (French-American-British) and WHO and also on a clinical basis (7, 13).

Leukomogenic chemicals, chemotherapeutics, benzen and derivatives, irradiation, viral infections, dietary factors, smoking and alcohol have all been implicated in the occurrence of MDS. MDS developing as result of such etiology is termed secondary, while those developing in the absence of any identifiable factors are termed primary MDS (pMDS) (1, 3, and 4). It has been reported that polymorphism in genes encoding enzymes involved in detoxification and metabolic activation may play a role in the pathogenesis of therapy-related leukemia and sMDS (8, 15,16).

One of these enzymes, NQO1 is protective enzyme against benzen toxicity due to its quinon-detoxifying effect and polymorphisms in this gene may lead to suboptimal enzymatic activity (5). The cells which are homozygous for the mutant allele are defective with respect to NQO1 induction and therefore vulnerable to the toxic effects of benzen metabolites (15). In a large retrospective study, Rothman et.al. reported that individuals who carry the C609T mutation in homozygous state are at a 2-6 fold increased risk of developing acute non-lymphoblastic leukemia or MDS (20). Larson et al also found that in patients with therapy-related AML (Acute Myeloid Leukemia) (tAML), the frequency of the NQO1 C609T mutation was 1.4 fold increased compared to controls (12). The NQO1 activity also displays ethnic diversity (11).

Cytogenetic evaluation is of great importance in MDS with respect to diagnosis, classification and choice

of therapy (21). Complex cytogenetic abnormalities are associated with a poor prognosis (24). Treatment with topoisomerase II inhibitors have been implicated in the etiology, especially those involving 11 (q23) (4,10)

In the present study, cytogenetic abnormalities were evaluated in conjunction with NQO1 gene polymorphism.

### Materials and Methods

#### Materials

The study group involved 80 patients with 13 of them being pediatric age range (mean age 43±5 years, male/ female 49/31). The frequency of the NQO1 gene polymorphism was compared with 423 healthy individuals. Among them, 286 individuals were in the adult (mean age:19±5 years) and 137 were in the pediatric (mean age 8 ±4 years) age range.

As MDS classified is into 3 subgroups on a clinical basis (24), we divided the patients into 3 groups:

1-Patients with pMDS : (subtypes RAEBI And II (Refractory Anemia with excess blast I and II), RA (Refractory Anemia), RARs (Refractory Anemia with ring Sideroblast), RCUD (Refractory Cytopenia with Unilineage Dysplasia) ,RCMD (Refractory Cytopenia with Multilineage Dysplasia with or without Ring Sideroblasts), MDS-U (MDS-Unclassifiable) , MDS with isolated del (5q) (n=51)

2-Patients with a history of occupational benzen expo-

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sure (sMDS) (**n=13**)

3-Patients with a MDS in the pediatric age range (Juvenile Myelomonocytic Leukemia) (JMML) (**n=13**)

### Cytogenetic Method

Chromosome was isolated from bone marrow aspiration material. For chromosome analysis, HRB (High Resolution binding) was performed following a short term bone marrow culture according to standart technique as described previously (14). Slides was stained standart G-banding method and was karyotyping with Applied Imaging Analyse System. Karyotyping was performed according to ISCN (19).

### Molecular Method

DNA was extracted by standard techniques (High Pure PCR Template Preparation Kit, REF 11796828001, Roche, Diagnostics GmbH, Mannheim, Germany) from peripheral blood (18). Detection of gene polymorphism was performed as previously described (8,12). The PCR reaction mixture contained 0.1-0.5 µg DNA 25 pmol of each primer, 50 mM KCL, 10mM Tris HCL (pH 8.3), 2, 5 pmol of each dNTP, % 5 dimethyl sulfoxide (DMSO), and 0.25 U Taq DNA polymerase in total volume of 50 µl that was subjected to 40 cycles (94 °C for 50 sec, 56 ° for 50 sec, and 72°C for 30 sec) followed by extension at 72 ° for 10 min. The sense primer NQO1 F ( 5'-AAG CCC AGA CCA ACT TCT-3'), and antisense primer DT-2 ( 5'-TCT CCT CAT CCT GAT CCT CT-3') amplified a 304 bp region including the NQO1 C609T substitution. The PCR products were digested with 5 U of restriction enzyme HinfI (MBI Fermentas) at 37 C for 3 hr. The Genotype pattern for each sample was detected by electrophoresis on a 4% agarose gel containing 0.5 µg/ ml etidium bromide. One Hinf I restriction site at PCR products cuts a 33 bp fragment in all samples, providing the control for digestion. The presence of the polymorphic Hinf I restriction site yields 151,120, and 33 bp fragments indicating the presence of the C609T polymorphic allele (8,20,22).

### Statistical Analysis

The allelic frequency of C609T polymorphism and NQO1 genotype frequencies in MDS groups and the controls were assessed by using Pearson  $\chi^2$  test. Statistical analysis was performed by using SPSS (Statistical package for social sciences, version 10.0) program. Allelic frequencies of the third group (consisting of pediatric cases) were compared with age matched controls (n=137).

## Results

### Cytogenetics Results

Clonally abnormalities were found in 53.75 % of patients with MDS. The most frequent cytogenetic abnormalities were complex abnormalities (18.75 %), followed by -7/del(7q)(10 %),-5/del (5q)/der(5q)(8.75 %),+mar (6.25%),+21 (5%),-17(5%), -14(5%),+8 (3,75 %),t(8;21)(q22;q22) (3.75%),-8 (3.75 %),-21(3.75 %) and +22(3,75 %).

### Statistically Results

The overall frequency of the C609T polymorphism was not different between adult [25.3% vs. 25%;

OR=1.030, 95% CI=0.668-1.587; p=0.259] and pediatric cases [19.2% vs. 22.6%; OR=1.228, 95% CI=0.455-3.391 p=0.134] and age-matched controls. Also, the overall frequency (adult and pediatric patients combined) was not different than controls (adult and pediatric controls combined) [24.4% vs. 24.1% OR=0.976; 95%CI: 0.649-1.467p=0.151]. There was no difference in the frequencies of NQO1 genotypes (Figure1) (C/C C/T and T/T) between the subgroups and controls (Table 1).

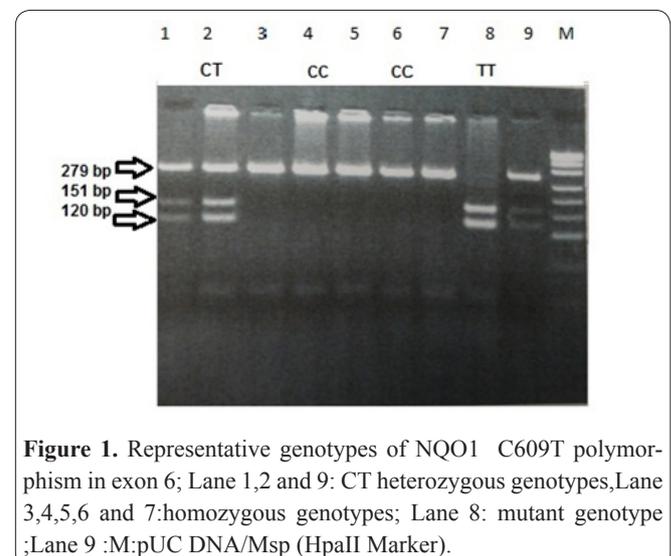
With respect to cytogenetic abnormalities, the frequency of the C609T polymorphism was found to be increased in particular chromosomal abnormalities relatively:

-5/del(5q)/der(5q) [(OR:1.212, 95% CI: 0.374-3.922,p=0.293.)], -5/del(5q) [(OR:1.298, 95% CI: 0.331-5.086,p=0.124)] , translocation [(OR:1.514, 95% CI: 0.449-5.104, p=0.482)] ,+21 [(OR:1.817, 95% CI:0.429-7.698,p=0.124)] and t(8;21) [(OR:3.028, 95% CI: 0.604-15.172,p=0.137)] compared to the control group. But there is no statistical correlation between cytogenetic abnormalities and C609T polymorphism (Table 2) (figure 2,3 and 4).

## Discussion

Cytogenetic analysis is of great importance with respect to diagnosis, prognosis and choice of treatment modality in MDS. It has been reported that 48-64 % of patients with MDS have a chromosomal abnormality either in the peripheral blood or bone marrow on conventional cytogenetic analysis. In the present study, we found cytogenetic abnormalities in 43 patients out of 80 (54 %). The most frequently encountered cytogenetic aberrations in MDS are complex abnormalities (15-60 %) including -7 del(7q) (10-50 %), -5/ del (5q) (20 %), +8 (10-15%), del (5q) (3-4 %), and -Y (3-4 %) (21, 23) (figure 2,3 and 4).

The most frequently observed complex abnormalities in the present study (19 %) were -7/ del(7q) (10%) , -5 /del (5q)/ der (5q) ( 8.75 %), + mar (6.25 %), +21 (5 %), - 17( 5%) , -14 (5%),+8 (3.75 %), t(8;21) (q22;q22) (3.75 %), -8 ( 3.75 %), -21 ( 3.75 %), +22 (3.75 %). Less frequently observed abnormalities were +2 ,+11, -16, -20, +20, -22, -X, and -Y , while -4, +5,+7, der (11q), +12, -12, -13 r (14 ),+14, +16, +17, r (17), -18,



**Figure 1.** Representative genotypes of NQO1 C609T polymorphism in exon 6; Lane 1,2 and 9: CT heterozygous genotypes,Lane 3,4,5,6 and 7:homozygous genotypes; Lane 8: mutant genotype ;Lane 9 :M:pUC DNA/Msp (HpaII Marker).

**Table 1.** NQO1 C609T genotypes, allelic frequency and statistically correlation in MDS groups and Control. ( $p < 0.05$  statistically significant.) (Me; Median).

Groups	Med Age	n	NQO1 C609T			T Allel freq	$\chi^2$ p	O.R. CI (95%)	T/T Genotype Freq . p
			C/C	C/T	T/T				
Control Group (Adult)	19	286	154(53.8%)	122(42.7%)	10(3.5%)	0.2482			
Group 1 p (MDS)	47.5	54	34(62.96%)	16(29%)	4(7.4%)	0.2222	0.564	0.865 0.529-1.415	0.118
Group 2 s (MDS)	60	13	4(30.76%)	8(61.53%)	1(7.69%)	0.3846	0.118	<b>1.893</b> 0.840-4.265	0.238
Control Group (Pediatric)	8.29	137	77(56.2%)	58(42.33%)	2(1.45%)	0.2262			
Group 3 JMML	4	13	9(69.23%)	3(23.07%)	1(7.69%)	0.1923	0.455	<b>1.228</b> 0.445-3.391	0.134
Group 1+2+3 Total	43	80	47(58.75%)	27(33.75%)	6(7.5%)	0.2437	0.907	0.976 0.649-1.467	0.151
Group 1+2 (Adult)	48	67	38(56.71%)	24(35.82%)	5(7.46%)	0.2537	0.895	<b>1.030</b> <b>0.668-1.587</b>	0.259

**Table 2.** The Frequency of NQO1 C609T genotypes, allelic frequencies and their comparison between patients and control groups and various cytogenetic abnormalities.

Cytogenetic Abnormalities	n	Homo. Normal Genotype	Hetero. Mutant Genotype	Homo. Mutant Genotype	T allel Freq.	$\chi^2$ p	O.R. CI (95%)	T/T Genotype Freq . p
		C/C n	C/T n	T/T n				
<b>Controls</b>	286	154	122	10	0.2482			
<b>Total Cytogenetic Abnormalities</b>	43	24	17	2	0.2441	1.00*	0.978 0.577-1.658	0.883
<b>Complex Karyotype</b>	15	10	5	0	0.166	0.387*	0.606 0.228-1.612	0.53
<b>-7/del(7q)</b>	8	5	3	0	0.1875	0.772**	0.699 0.196-2.487	0.804
<b>-5/del(5q)/der(5)</b>	7	4	2	1	0.2857	0.757*	<b>1.211</b> 0.374-3.922	0.293
<b>Translocation</b>	6	2	4	0	0.333	0.506*	<b>1.514</b> 0.449-5.104	0.482
<b>-5/del(5q) (solely)</b>	5	3	1	1	0.3	0.716*	<b>1.298</b> 0.331-5.086	0.124
<b>der(3)</b>	5	2	3	0	0.3	0.716	<b>1.817</b> 0.429-7698	0.707
<b>+21</b>	4	1	3	0	0.375	0.419	<b>1.817</b> 0.429-7698	0.692
<b>t(8;21)</b>	3	0	3	0	0.5	0.170	<b>3.028</b> 0.604-15.172	0.137

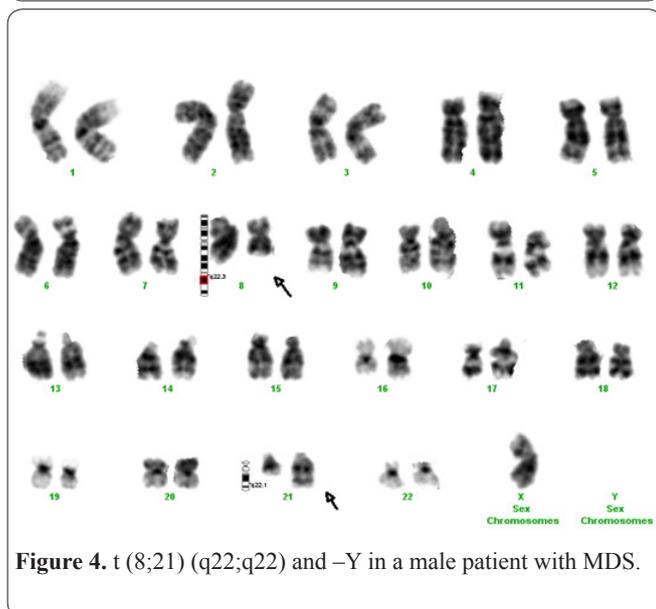
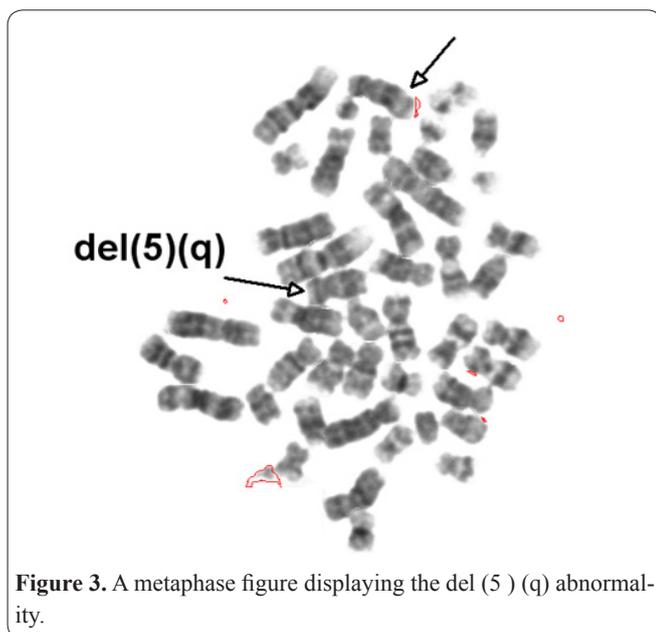
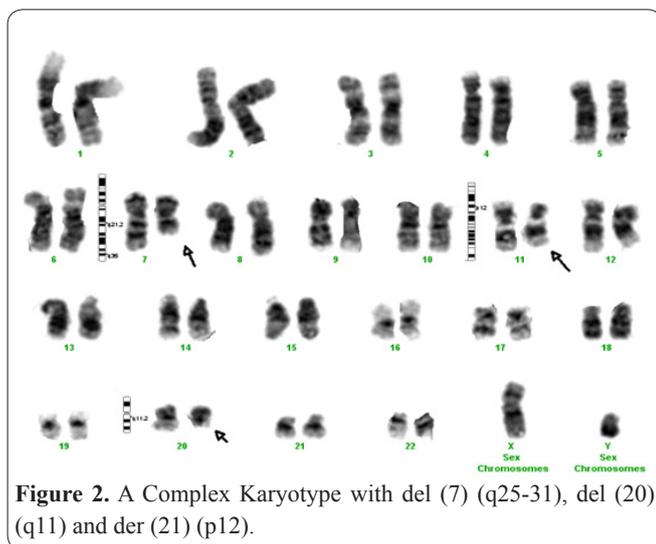
Fisher Analyses method ( $n < 5$ ), Pearson  $X^2$  method ( $n > 25$ ), \*\* Continue Correction Analyses method ( $n \geq 5-25$ ), ( $p < 0.05$  statistically significant).

+18 and +19 were observed uncommonly. Among patients with a cytogenetic abnormality, %35 had complex chromosomal aberrations. These findings and their frequencies are in agreement with other studies (7,13). HK Wu et al. Reported that -7,+8,+21 and -X are associated with hypodiploidy and such patients are at risk of their MDS evolving into a more severe form with reduced survival or leukemia (23).

It has been reported that polymorphism in genes encoding enzymes involved in detoxification and metabolic activation may play a role in the pathogenesis of therapy-related leukemia and MDS In the pres-

ent study we evaluated NQO1 gene polymorphism (C609T) in patients with MDS and its relation to cytogenetic abnormalities (4,6,10,25).

Our results do not support the view that the NQO1 C609T polymorphism in MDS. Although statistically insignificant, it is remarkable that in patients in the second group, the frequency of the C609T allele is increased 1.8 times compare to controls. Further, in the former group, however this difference did not reach statistical significant. ( $p = 0.238$ ). These results may be reflecting a possible effect of C609T polymorphism in the pathogenesis of MDS, especially sMDS.



Likewise, it is probable that the C609T polymorphism is related with the chromosomal aberrations including t (8;21), +21, der (3) and -5/del (5q) abnormalities.

In a previous study involving patients with MDS, the relation of detoxifying genes with chromosomal abnormalities were analyzed and it was found that GSTT1 (Glutathione S-transferase (GST) theta 1) and

GSTM1( Glutathione-S-transferase (GST) M1) null genotypes were found to be significantly increased compared to controls; furthermore GSTM1 null genotype was more frequent in patients with chromosomal abnormalities. Although Young *et al.* reported similar results, there was no relation between MDS, NQO1 polymorphism and MDS in their study (25). However, the frequency of the C609T polymorphism in the NQO1 gene, 187-Ser was found to be associated with chemotherapy-related AML, implying that NQO1 alterations may have a role in leukomogenesis (2,4).

Although numerous studies suggest an association between many types of cancer and C609T polymorphism, in the present study we were not able to show that it is directly related with MDS development (17). Further, in the second group (s MDS) there was no difference with respect to C609T frequency in patients and controls. This is surprising because in particularly this group one expects a higher rate of C609T mutation. Our findings also do not indicate a relation between cytogenetic abnormalities and the C609T polymorphism. However, it's a possible that studies with a larger MDS patient group with chromosomal abnormalities would yield different results.

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