RESEARCH ARTICLE

Association of C3435T, C1236T and C4125A Polymorphisms of the MDR-1 Gene in Egyptian Children with Acute Lymphoblastic Leukaemia

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Abstract

   Background: P-glycoprotein (P-gp), a membrane transporter encoded by the multidrug resistance-1 (MDR1) gene, influences pharmacokinetics and metabolism of anticancer drugs and contributes to multidrug resistance phenotype in acute lymphoblastic leukemia (ALL). Genetic variation ofMDR1 in ALL patients is increasingly recognized as a factor influencing response to treatment. **Aim:** To investigate the possible role of MDR-1 gene polymorphisms (C3435T, C1236T and C4125A) as risk factors for the development and clinical outcome of ALL in Egyptian children. **Materials and Methods:** Genotyping of MDR-1 C3435T, C1236T and C4125A single nucleotide polymorphisms (SNPs) was accomplished using a polymerase chain reaction–restriction fragment length polymorphism (RFLP-PCR) assay with 120 childhood ALL patients and 100 healthy controls. **Results:** Homozygous T with the C3435T SNP showed a protective effect as compared to homozygous C (OR=0.748) while heterozygous CT correlated with a poor outcome (high risk, drug unresponsiveness, relapse and high percentage of death). Additionally, the T allele of the C1236T SNP showed a significant relation with ALL risk (OR=1.6). However, there were no significant differences in the genotype and allele frequencies of MDR-1 SNPs between patients and controls. Only one genotype (CC) and one allele of MDR-1 (C4125A) were seen. Neither CA/AA genotypes nor A alleles were present in ALL patients and normal controls. TC was the predominant haplotype in both groups, while CT proved to be minor. The cumulative incidence of relapse was higher with the CC genotype of C1236T as compared with TT. **Conclusion:** From our preliminary data, the CT genotype of C3435T is associated with a poor ALL outcome while the CC genotype of C1236T is related with an increased incidence of relapse. Although our results provide assistance for oncologist choice of individual therapeutic strategy taking the patient genetic repertoire into consideration, further investigations with larger sample size should be conducted to validate our results.

   Keywords: ALL- MDR-1- polymorphism- Egyptian

Introduction

   Acute lymphoblastic leukemia (ALL) is still one of the most common childhood malignancies worldwide (Mei et al., 2017). It is characterized by uncontrolled proliferation of hematopoietic cells in the bone marrow (Bektaş-Kayhanet al., 2012). The current chemotherapy protocols cure almost 80% of patients who remain in the remission stage for 10 years, and 20% of children suffer from recurrence, making the final cure rate around 25-40% (Bartram et al., 2012). Several side effects including relapse, resistance, and death remain a problem facing chemotherapeutic treatment of ALL. Thus, a considerable portion of patients cannot permanently be cured (Ramírez-Pacheco et al., 2016). One major reason is the development of drug resistance (Efferth et al., 2003).

   The human multi drug resistance gene (MDR-1), located on chromosome 7q21, comprises 28 exons and encodes for P-glycoprotein (P-gp), a 170-kDa member of adenosine triphosphate- binding cassette (ABC) superfamily of membrane transporters (Rao et al., 2010). P-gp has a role as a membrane efflux pump transporting several amphipatic molecules through lipid membranes. It is involved in the metabolism or membrane transport of a variety of cancer chemotherapeutic agents and has been implicated in drug resistance. MDR-1 expression has been shown to be up-regulated in various types of chemo-resistant tumors like hepatocellular carcinoma, renal cell, adrenocortical, and colon cancers suggesting that it is a poor prognosis marker (Zhai et al., 2012).

   Single nucleotide polymorphisms (SNPs) are the most frequently inherited genetic variations among

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people and occur every 100-300 bp (Cheok et al., 2009). Polymorphisms in genes participating in drug transport and drug metabolism might play an important role in variability of enzyme activity and consequently on survival after cancer therapy (Ekhart et al., 2009). Choi et al., (1988) were the first to document a mutation in MDR-l gene associated with an altered pattern of cross resistance to chemotherapeutic drugs. Subsequently, a number of SNPs in the MDR-l gene have been identified in different populations (Hoffmeyer et al., 2000) including C3435T polymorphism (rs1045642) in exon 26 and C1236T (rs128503) in exon 12; although; conflicting results have been obtained (Samanian et al., 2011).

A number of studies have been performed on MDR-1 gene polymorphism in Egyptian chronic myeloid leukemia (CML) patients (Elghannam et al., 2014; Ghallab et al., 2015). In the present investigation, we analyzed the C3435T, C1236T and C4125A SNPs of MDR-l gene in 120 ALL patients and 100 healthy donors. Our study aimed to shed some light about the influences of these SNPs on the risk of childhood ALL development and whether they affect on the clinical outcome of the disease. To our knowledge, one study has been performed on C3435T polymorphism in childhood ALL in Egyptians (El-hoseiny et al., 2015).

Materials and Methods

Patients and controls

In this study, 120 pediatric ALL (66 males and 54 females) were enrolled. One hundred unrelated healthy blood donors free of any chronic diseases, living in the same geographical area and have the same ethnic origin as patients were recruited as normal healthy controls (43 females and 57 males). Pediatric ALL were evaluated by Pediatric Oncology Department at South Egypt Cancer Institute, Assiut University, Egypt. The age and sex of two groups were matched.

Patients were categorized by risk depending on assignment protocol (Smith et al., 1996). In general, disease risk was defined by initial white blood cells (WBCs) count, age at diagnosis and Bone marrow smears. Patient's clinical data like sex, WBC count, blast percentage, platelet (PLT) count, haemoglobin (Hb), Lactate dehydrogenase (LDH) and immunophenotype were performed in clinical pathology department with the help of pediatric oncologist during follow up. Children with ALL were classified into low, moderate/high risk groups and treatment based on Total XIIIB regimen (Dervieux et al., 2002; Relling et al., 2003; Kishi et al., 2004). All protocol investigations were done in accordance to Human Ethical Clearance Committee guidelines for Clinical Researches at South Egypt Cancer Institute and Hospital, Assiut University. Informed consent was obtained from all the study subjects or their parents.

DNA isolation

Blood was collected by withdrawal of ≅ 3 ml venous blood from each individual involved in this study into sterile vacutainer tubes containing tri-potassium ethylene diaminetetraacetic acid (EDTA.K3). Genomic DNA was extracted from whole blood-EDTA

samples by using Genomic DNA Purification Kit (QIAGEN, Germany) according to manufacturer's instructions (Plasschaert et al., 2004). Extracted DNA was applied to 1% agarose gel electrophoresis to confirm the presence and integrity of purified DNA. The quality and concentration of DNA in all samples was measured by using nanodrop (Thermo Scientific).

Genotyping

Primer sequences and PCR conditions of SNPs in MDR-l gene (C3435T, C1236T and C4125A) are presented in Table 1. All polymerase chain reactions (PCR) were performed in the 2720 thermal cycler (Applied Biosystems).

MDR-1C3435T, C1236T and C4125A were genotyped by restriction fragment length-PCR (RFLP-PCR) as previously described (BadrulHishamet al., 2006; Rüstemoglu et al., 2011; Ren et al., 2012); respectively. A 25 µl of PCR reaction mixture contained MyTaqTM Red Master Mix (2x) (Maridian life Science Company, USA), 10pmol of each primer and 150ng of DNA template. PCR products for MDR-1(C3435T, C1236T and C4125A) were visualized on 2% agarose gel electrophoresis (344bp, 370bp and 221bp; respectively). PCR products were subjected to digestion with MboI (New England Biolabs), BsuRII (Fermantas; Thermoscientific) and RsaI (Fermantas) restriction enzymes; respectively. DNA fragments characterizing each genotype/allele were summarized in Table 1.

Statistical analysis

Statistical analyses were performed by SPSS statistical package version 19 (SPSS, IBM Corporation, USA). Comparisons between pediatric ALL and controls were made by using independent T-test and results were presented as mean ±SE. Chi-squared tests were performed to examine the differences in the allele frequency and genotype distribution between different groups. Odds ratios [with 95% confidence interval (CI)] were calculated to measure the relative risks in both control and pediatric ALL patients. The online tool SNPstats (http://bioinfo.iconcologia.net/SNPstats) performed the haplotype analyses and calculated the Linkage disequilibrium (LD) parameters (D′and r^2). Correlation between variables was determined using Spearman's correlation test. The Overall survival (OS) was calculated as the period from diagnosis to death or last contact. Probabilities of OS, and proportional hazards of relapse were estimated using Kaplan–Meier method (Kumar, et al., 2010). Differences between survival curves were tested for significance using the log-rank test. All values were two-tailed, and P values <0.05 were considered to be statistically significant.

Results

Patients and controls

The characteristics of the 120 ALL patients and 100 control subjects were displayed in Table 2. The ALL patients and the controls had similar distribution of sex and age. The mean age of pediatric ALL were 6.40±3.99

Table 1. Primers and PCR Conditions that Used to Detect Polymorphisms of MDR-1 Gene in Pediatric ALL Patients and Controls

MDR SNPs	Primers	Restriction Enzyme	PCR product Length	Length of expected Bands (bp)
Exon 26 (3435C/T)	Forward: 5'-GAT CTG TGAACT CTT GTT TTCA-3' Reverse: 5'-GAA GAG AGACTT ACA TTA GGC-3'	MboI	244bp	CC:172, 72 CT:244, 172, 72 TT:244
Exon 12 (1236 C/T)	Forward: 5'- TATCCTGTGTCTGTGAATTGCC-3' Reverse: 5'- CCTGACTCACCACACCAATG -3'	BsuRI	370bp	CC:307, 63 CT:370, 307, 63 TT:370
Exon 28 (4125C/A)	Forward: 5'- CCCAATTTAAATTCTTACCTGT -3' Reverse: 5'- GCTGTTAGAACTTTACTTTCAGTTC-3'	RsaI	221bp	AA:199, 22 AC:221, 199, 22 CC:221

(ranged from 1 to 15 years) while the mean age of normal controls was 5.91±3.45 (ranged from 1 to 15 years).All clinical parameters of ALL patients (sex, immufinophenotypes, white blood cell number …..etc) were summarized in Table 3.

The association between C3435T, C1236T and C4125A of MDR-1 polymorphisms and ALL susceptibility

Table 4 shows the frequency of the analyzed genotypes and alleles for C3435T, C1236T and C4125A of MDR-1in childhood ALL patients and healthy control subjects. With regards to the MDR-1 exon 26 (C3435T) genotypes; patients heterozygous or homozygous for the T allele did not differ with respect to the risk of ALL.
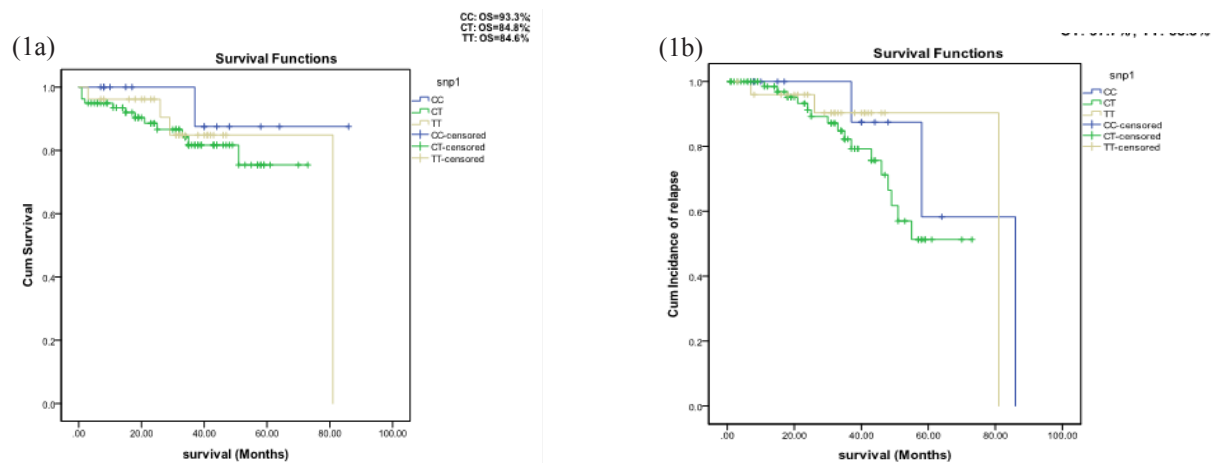

Figure 1.Overall Survival According to C3435T (1a) and C1236T (1b) Genotypes in Pediatric ALL.

Table 2. Demographic and Biochemical Characteristics of ALL Patients and Healthy Controls

Characteristics	Control group N= 100	Patient group N= 120	P	Correlation with the disease
Demographic Data				
Age	5.91±3.45	6.40±3.99	NS	NS
Gender (Male ♂/ Female ♀)	57/43	66/54		
Biochemical Data				
HB (g/dl)	10.68 ± 0.076	8.16 ± 0.23	P<0.001	r=-0.586 P<0.001
PLT (10^9/L)	326.16 ± 5.63	81.83 ± 8.52	P<0.001	r= -0.793 P<0.001
WBCs (10^9/L)	8.06±0.15	49.33±8.98	P<0.001	r=0.273 P<0.001
Neutrophils (%)	3.10±0.075	4.15±0.71	NS	r=0.11 P=NS
Blast (%)	0.00±0.00	50.43±3.12	P<0.001	r=-0.902 P<0.001
LDH (U/L)	194.80±4.48	1443.42±212.17	P<0.001	r=0.37 P<0.001

All data are presented as mean ± SE; HB, haemoglobin; PLT, platelet count; WBCs, white blood cells; LDH, lactate dehydrogenase.

(2a)

CC: OS=80%;
CT: OS=97.7%;
TT: OS=88.5%

(2b)
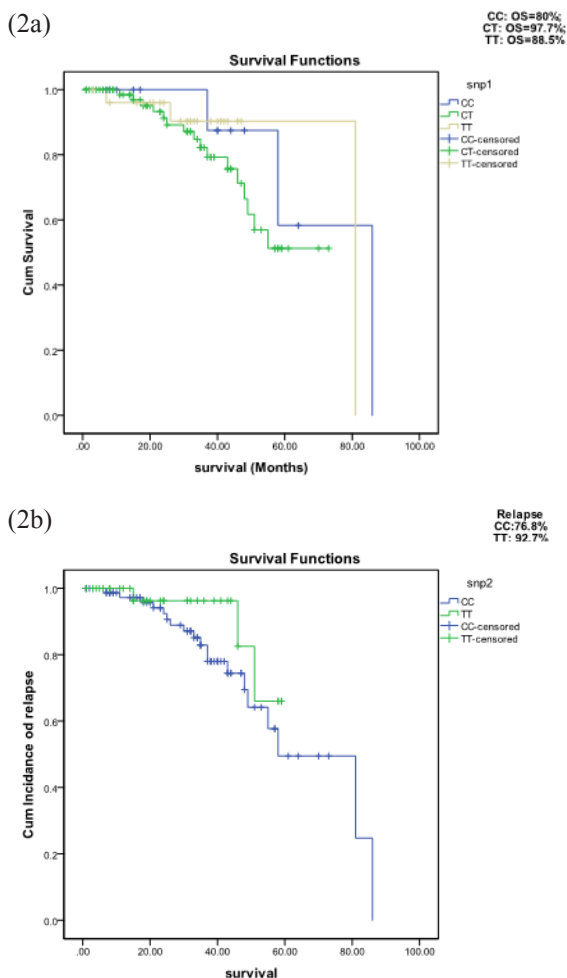

Relapse
CC:76.8%
TT: 92.7%

Figure 2. Disease Free Survival According to C3435T (2a) and C1236T (2b) Genotypes in Pediatric ALL

Fifteen patients had C/C genotype, 79 C/T and 26 T/T genotypes (Table 4). When ORs were calculated for the overall case-control group, no significant associations were observed between the 3 investigated genotypes (CC, and CT) and the risk of ALL. A protective effect from ALL was detectable for patients homozygous for the MDR-1T allele (OR= 0.748) in comparison with those homozygous for the MDR-1 3435C allele. Of interest was that, higher T allele frequency in both groups when compared with C allele was observed, although this difference was not significant.

There was no significant difference in the distribution of C1236T genotypes between ALL patients and controls (68.3% CC, and 31.7% TT versus 77% CC, and 23% TT; respectively) with complete disappearance of CT genotype. Analysis of MDR-1C1236T SNP revealed that there was a significant increase in the frequency of Tallele (P<0.01, OR= 1.610 and 95%CI= 1.049-2.471) in ALL patients coincides with increase in the frequency of C allele (P<0.01, OR= 0.610 and 95%CI= 0.404-0.952) in controls. We found significant effect of 1236Tallele causing 1.6 fold increased in ALL risk compared to carriers of alternative 1236C allele. Considering C4125A SNP, only one genotype (CC) and one allele of MDR-1(C4125A) were seen. Neither CA/AA genotypes nor A allele were present in ALL patients and normal controls.

Possible risk haplotypes were performed from two SNPs (C3435T and C1236T) localized on the same chromosome Table 5. TC is the predominant haplotype in both groups, while CT is the minor ones, although; we found statistically insignificant differences in haplotypes distribution between ALL patients and controls. The LD pattern between C3435T and C1236Tshowed a non significant LD with a D' value of 0.0807 and r^2 value of -0.0445.

The association between C3435T, C1236T and C4125A of MDR-1 polymorphisms and ALL clinical characteristics

The genotype frequency of each polymorphism was compared between patients with different clinical outcome (Sex, age at diagnosis, risk group, response to treatment, immunophenotype, survival status and presence or absence of relapse). No significant differences in C3435T and C4125Apolymorphism of MDR-1 involvement in

Table 3. Clinical Characteristics of ALL Patients

Parameter	N (%)
Sex	
Male	66 (55)
Female	54 (45)
Age at diagnosis	
1-10 years	97 (80.8)
>10 years	23 (19.2)
Risk	
Low Risk (WBC<50,000 cell/μl)	88 (73.3)
High Risk (WBC >50,000/ cell/μl)	32 (26.7)
Response for treatment	
Responders	100 (83.3)
Non responders	20 (16.7)
Immunophenotype	
B-ALL	100 (83.3)
T-ALL	20 (16.7)
Clinical outcome	
Alive	103 (85.8)
Death	17 (14.2)
Relapse	
No relapse	98 (81.7)
Relapse	22 (18.3)
Bone marrow aspiration (blast cells)	% (M±SE)
Day 15 post treatment	4.924 ± 0.778
Day 36 post treatment	4.165 ± 0.254
Week 16 post treatment	4.326 ± 0.471
Week 29 post treatment	6.814 ± 1.530
Week 55 post treatment	3.527 ± 0.461
Week 67 post treatment	4.646 ±.0.649
Week 79 post treatment	4.046 ± 0.623
Week 95 post treatment	2.827 ± 0.209
Week 120 post treatment	2.927 ± 0.187

N, Number; M, Mean, SE, Standard error

Table 4. The Genotype and Allele Frequencies of MDR1 Gene Polymorphisms (C3435T, C1236T and C4125A) in ALL Patients and Controls

Polymorphisms	Control (N= 100) N (%)	Patients (N= 120) N (%)	95% Confidence Interval OR (lower-upper)	p-value
MDR-1 C3435T (Genotype Frequency)				
CC	11 (11)	15 (12.5)	1.156 (0.505-2.645)	NS
CT	62 (62)	79 (65.8)	1.181 (0.680-2.052)	NS
TT	27 (27)	26 (21.7)	0.748 (0.403-1.389)	NS
Allele Frequency				
C	84 (42)	109 (45)	1.071 (0.732-1.565)	NS
T	116 (58)	131 (55)	0.933 (0.638-1.364)	NS
MDR-1 C1236T (Genotype Frequency)				
CC	77 (77)	82 (68.3)	0.645 (0.352-1.179)	NS
CT	--	--	--	--
TT	23 (23)	38 (31.7)	1.551 (0.848-2.832)	NS
Allele Frequency				
C	154 (77)	164 (68)	0.610 (0.404-0.952)	P<0.01
T	46 (23)	76 (32)	1.610 (1.049-2.471)	P<0.01
MDR-1 C4125A (Genotype Frequency)				
CC	100 (100)	120 (100)	--	--
CA	--	--	--	--
AA	--	--	--	--
Allele Frequency				
C	200 (100)	240 (100)	1.199 (0.023-60.72)	NS
A	--	--	--	

NS, not significant

clinical outcome of ALL (Table6 and 8); respectively. Although, presence of CT was coincides with bad outcome. Twenty patients (out of 32) belong to high risk group. Most of non responders (13 out of 20), suffering from relapse (16 out of 22) and even died children (12 out of 17) are CT carriers. With respect to the association of C1236TMDR-1 genotypes with clinical parameters (Table 7), our data showed a significant correlation (P<0.05) with relapse occurrence (19 out of 22 with relapse harbor CC genotype).

Survival analyses of various genotypes are presented in Figures 1a and 2a. As seen in these Figures we analyzed whether the polymorphisms of two SNPs (C3435T and C1236T) were associated with OS of ALL patients. OS did not differ significantly between C3435T and C1236T genotypes in pediatric ALL. Cumulative incidence of relapse was higher in CC of C1236T (23.2%) genotypes as compared with TT (79%), although it was not statistically significant. Moreover relapse incidence did not differ significantly between C3435T genotypes in pediatric ALL (Figure 1b and 2b).

Discussion

The physiological expression of P-gp in tissues is one of the determinants for drug detoxification in various cells, and thus provides a cellular defense mechanism against potentially harmful compounds. Moreover; elevated production of P-gpcan result in an increased efflux of the cytotoxic drugs from the cancer cells, thus; lowering their intracellular concentrations (Bektaş-Kayhanet al., 2012). In various cancer types, including AML, breast cancer, various childhood tumors, over-expression of P-gp encoding MDR-1 gene has been found to correlate with poor outcome in patients treated with chemotherapy (Illmer et al., 2002; Siegsmund et al., 2002; Bektaş-Kayhanet al., 2012). P-gp expression and function are modified by genetic polymorphisms of the MDR-1 gene. In recent years, many articles have reported about the MDR-1polymorphism and susceptibility to ALL, but the results are still controversial. Therefore, we examined the association of the development of childhood ALL and gene polymorphisms, including three polymorphisms

Table 5. Haplotype Frequencies of MDR Genes (C3435T, C1236T and C4125A) Polymorphisms in ALL Patients and Controls

Haplotype	Control (N= 100) N (%)	Patients (N= 120) N (%)	95% Confidence Interval OR (lower-upper)	p-value
TC	44.58	35.12	1.00	NS
CC	32.42	32.35	1.33 (0.81 - 2.21)	NS
TT	13.59	19.67	1.44 (0.86 - 2.55)	NS
CT	9.41	12.86	1.38 (0.67 - 2.84)	NS

NS, not significant.

Table 6. Comparison between C3435T Genotypes in Pediatric ALL Patients Related to Demographic, Clinical and Laboratory Data

Parameter	ALL N (%) (n=120)	MDR1 (C3435T)			
		CC (n=15)	CT (n=79)	TT (n=26)	p-value
Sex					
Male	66 (55)	6 (40)	43 (54.4)	17 (65.4)	NS
Female	54 (45)	9 (60)	36 (46.6)	9 (34.6)	
Age at diagnosis					
1-10 years	97 (80.8)	14 (93.3)	64 (81)	19 (73.1)	NS
>10 years	23 (19.2)	1 (6.7)	15 (19)	7 (26.9)	
Risk					
Low Risk (WBC<50,000 cell/μl)	88 (73.3)	10 (66.7)	59 (74.7)	19 (73.1)	NS
High Risk (WBC >50,000/ cell/μl)	32 (26.7)	5 (33.3)	20 (25.3)	7 (26.9)	
Response for treatment					
Responders	100 (83.3)	12 (80.0)	66 (83.5)	22 (84.6)	NS
Non responders	20 (16.7)	3 (20.0)	13 (16.5)	4 (15.4)	
Immunophenotype					
B-ALL	100 (83.3)	14 (93.3)	67 (84.8)	19 (73.1)	NS
T-ALL	20 (16.7)	1(6.7)	12 (15.2)	7 (26.9)	
Survival status					
Alive	103 (85.8)	14 (93.3)	67 (84.8)	22 (84.6)	NS
Death	17 (14.2)	1 (6.7)	12 (15.2)	4 (15.4)	
Relapse					
No relapse	98 (81.7)	12 (80)	63 (79.7)	23 (88.5)	NS
Relapse	22 (18.3)	3 (20)	16 (20.3)	3 (11.5)	

Table 7. Comparison between C1236T Genotypes in Pediatric ALL Patients Related to Demographic, Clinical and Laboratory Data

Parameter	ALL N (%) (n=120)	MDR1 (C1236T)			
		CC (n=82)	CT	TT (n=38)	p-value
Sex					NS
Male	66 (55)	44 (53.7)	--	22 (57.9)	
Female	54 (45)	38 (46.3)	--	16 (42.1)	
Age at diagnosis					NS
1-10 years	97(80.8)	67 (81.7)	--	30 (78.9)	
>10 years	23 (19.2)	15 (18.3)	--	8 (21.1)	
Risk					NS
Low Risk (WBC<50,000 cell/μl)	88 (73.3)	61 (74.4)	--	27(71.1)	
High Risk (WBC >50,000/ cell/μl)	32 (26.7)	21 (25.6)	--	11 (28.9)	
Response for treatment					NS
Responders	100 (83.3)	67 (81.7)	--	33 (86.8)	
Non responders	20 (16.7)	15 (18.3)	--	5 (13.2)	
Immunophenotype					NS
B-ALL	100 (83.3)	70 (85.4)		30 (78.9)	
T-ALL	20 (16.7)	12 (14.6)		8 (21.1)	
Survival status					NS
Alive	103 (85.8)	69(84.1)	--	34(89.5)	
Death	17(14.2)	13(15.9)	--	4(10.5)	
Relapse					P<0.05
No relapse	98 (81.7)	63 (76.8)	--	35 (92.1)	
Relapse	22 (18.3)	19 (23.2)	--	3 (7.9)	

Table 8. Comparison between C4125A Genotypes in Pediatric ALL Patients Related to Demographic, Clinical and Laboratory Data

Parameter	ALL N (%) (n=120)	MDR1 (C4125A)			
		CC (n=120)	CA	AA	p-value
Sex					
Male	66 (55)	66 (55)	--	--	NS
Female	54 (45)	54 (45)	--	--	
Age at diagnosis					
1-10 years	97 (80.8)	97(80.8)	--	--	NS
>10 years	23 (19.2)	23 (19.2)	--	--	
Risk					
Low Risk (WBC<50,000 cell/µl)	88 (73.3)	88 (73.3)	--	--	NS
High Risk (WBC >50,000/ cell/µl)	32 (26.7)	32 (26.7)	--	--	
Response for treatment					
Responders	100 (83.3)	100 (83.3)			NS
Non responders	20 (16.7)	20 (16.7)			
Immunophenotype					
B-ALL	100 (83.3)	100 (83.3)			NS
T-ALL	20 (16.7)	20 (16.7)			
Survival status					
Alive	103(85.8)	103(85.8)			NS
Death	17(14.2)	17(14.2)			
Relapse					
No relapse	98(81.7)	98(81.7))			NS
Relapse	22(18.3)	22(18.3)			

C3435T, C1236T and C4125Aof the MDR-1gene.

We found that MDR-1 C3435T CT genotype is the most frequent genotype, but we could not find any statistically significant association between MDR-1 C3435T genotypes or alleles and the risk of ALL. The elevation of heterozygous MDR-1 CT genotype was previously documented by Bektasx-Kayhan et al., (2012) in a Turkish population. Allele frequencies of the MDR-1C3435T polymorphism have been evaluated around the world, and significant inter-population differences have been detected (Leal-Ugarte et al., 2008). Our observation supports previous reports of childhood ALL who notified no significant difference in C3435T gene MDR-1 polymorphism between ALL patients and controls in Hispanic (Urayama et al., 2007), Mexican (Leal-Uqarte et al., 2008), Hungarian (Semsei et al., 2008), Chinese (Zhai et al., 2012) and Latvia population (Kreile et al., 2014). In addition to these studies, C3435T MDR-1polymorphism was not significantly associated with the development of ALL in Thai children (Pongstaporn et al., 2015). On the contrary, many lines of evidences from Poland (Jamroziak et al., 2004), Japan (Hatori et al., 2007), Iran (Miladpour et al., 2009), India (Rao et al., 2010), Turkey (Bekas-kayan et al., 2012), and Egypt (El-hoseiny et al., 2015) indicate that these genotypes are involved in the risk of childhood ALL. A research made by Hattori et al., (2007) who genotyped 4 MDR-1SNPs in 157 pediatric ALL patients and 92 controls, also indicates an association between 3,435T-allele and risk of ALL. This apparent inconsistency of results between different

populations of ALL patients may be due to divergence in disease prevalence and ethnic group differences, as well as possible limitations due to small sample size with limited number of patients investigate, and heterogeneity of ALL (Wang et al., 2012; Wang et al., 2013).

With respect to clinical variables, CT genotype was increased in ALL patients with poor prognosis (high risk and/or suffering from relapse). Moreover, most of non responders and dead subjects were harboring CT genotype. Thus, CT genotype is correlated with bad outcome of the disease. We can hypothesized that ALL patients with CT might be eliminating the anti-leukemic drugs(such as anthracyclines, Daunorubicin, Vincristeine, Mitoxanthrone) which are P-gp substrates more effectively leading to low intracellular drug concentration and poor prognosis.

There were no differences in C1,236T genotype frequency between ALL patients and controls. We found a 1.6-fold increase in the risk of developing ALL in mutant T-allele compared with the carriers of other alleles. In accordance to our results, Urayama et al., (2002) did not detect any significant differences in MDR-1 polymorphism (C1,236T) genotype frequency between patients and controls. Another study of Pongstaporn et al., (2015) found in significantly association of C1,236T MDR-1polymorphisms with the development of ALL in Thai children. Our ALL childhood patients were classified by risk-based assignment protocol into low and high-risk groups and results indicated to an increase in frequency of T alleles in ALL patients at high risk. This result is in line

was that of Pongstaporn et al., (2015) which indicates that both C3435T and C1236T MDR-1genotypes are significantly associated with high-risk groups for childhood ALL.

It was demonstrated that the novel C4,125A polymorphism of MDR-1is associated with susceptibility to hepatocellular carcinoma in Chinese population (Ren et al., 2012) but there still not known the role of this novel polymorphism. Accordingly, we have examined the frequency of this SNP in ALL patients. Only wild genotype/allele (CC/C) was detected with total disappearance of CA or AA genotypes.

In conclusion, we found that the no particular genotype/allele increased the risk of developing ALL in Egyptians. CT genotype of C3435T might be associated with bad outcome of the disease. On another hand, CC genotype carriers of C1,236T were associated with increased incidence of relapse and poor prognosis in pediatric ALL. Knowing that ALL is one of the complex diseases, genetic heterogeneity is somehow hard to be analyzed. The sample size of the present association study is likely insufficient for detecting all the associations between MDR-1 SNPs and ALL and larger sample size is required for further evaluation.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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