Survival and risk of relapse of acute lymphoblastic leukemia in a Mexican population is affected by dihydrofolate reductase gene polymorphisms

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Abstract. Dihydrofolate reductase (DHFR) is the major target of methotrexate, a key component in childhood acute lympho-blastic leukemia (ALL) treatment. Polymorphisms in the gene coding for DHFR have been associated with adverse event treatment. This study evaluated the effect of the -A317G and C829T polymorphisms in the DHFR gene on survival and risk of relapse of ALL. Seventy patients with ALL and 100 healthy individuals were genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. An association between the polymorphisms and the risk of relapse was found ($p<0.05$); patients with the -317G/G genotype were found to have an 8.55 (95% CI 1.84-39.70) higher chance of relapse and carriers of the 829T/T genotype had a 14.0 (95% CI 1.13-172.63) higher chance of relapse. Other variables, such as age and leukocyte count, were associated ($p<0.05$) with the risk of relapse of the disease. Individuals with the G/G and T/T genotype of the -A317G and C829T polymorphisms had poorer survival compared to other genotype groups (log-rank test; $p<0.05$). Although preliminary, these data seem to suggest a role for the DHFR polymorphisms in the risk of relapse of ALL and the mortality risk in these patients.

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Introduction

In Mexico, acute leukemia is considered a public health issue; it represents the fourth leading cause of mortality of all neoplastic malignancies in children under 15 years of age (1). The mortality rate from 1996 to 2000 was 63.7 per 1 million children, one of the highest rates reported in the world (2). In 2005, leukemia was the second cause of mortality in the State of Guerrero in children less than 15 years of age, according to the National Institute of Statistics, Geography and Computing (INEGI) (3).

Methotrexate (MTX) is an antineoplastic agent used in the treatment of patients with acute lymphoblastic leukemia (ALL) and was introduced five decades ago to clinical oncology. It is presently used in the treatment of other neoplastic diseases, including osteosarcoma, breast cancer, head and neck cancer, and non-Hodgkin's lymphoma (4,5). MTX is a folic acid antagonist, and its efficacy as an antineoplastic treatment is largely attributed to its high affinity for dihydrofolate reductase (DHFR) (EC 1.5.1.3), the enzyme which is responsible to catalyze the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) (6). The major mechanism of MTX action involves competitive inhibition of DHFR, leading to the impaired regeneration of THF from DHF; essential for the biosynthesis of purines and thymidylate, thus it also blocks the novo synthesis of DNA (7,8). A subset of patients develop adverse events of resistance to MTX; however, approximately 80% of ALL children experience good clinical response (5,9,10).

The mechanisms that lead to clinical failure to MTX are DHFR overexpression, impaired intracellular transport and decreased levels of reduced folate carrier at the cell membrane (11,12). Changes in the levels of DHFR expression and consequently in the sensitivity to MTX can also be due to single nucleotide polymorphisms (SNPs), particularly those located in the regulatory elements. The C829T SNP is located at the 223 nucleotide downstream from the stop codon between the first and second polyadenylation sites in the 3'UTR of the DHFR gene, which leads to the stability of mRNA (13). A previous study reported that the -A317G SNP in the DHFR promoter region results in higher transcriptional activity (14).

The -A317G and C829T SNPs in the DHFR gene have not been studied as a factor for the risk of relapse to ALL in Mexico. In the present study, our objective was to evaluate the effect of the -A317G and C829T polymorphisms in the DHFR gene on survival and risk of relapse of ALL.

Materials and methods

Study population. A case control study was carried out in the Pediatric Oncology Service of the State Cancer Institute (SCI) from the South of Mexico (Acapulco, Guerrero), between September 1996 and May 2009. The cases consisted of 70 patients diagnosed with ALL through bone marrow aspirate based on French-American-British morphological criteria, cytochemical staining properties and immunophenotyping of blast cells.

The diagnosis of ALL was further subclassified as T-lineage ($CD3^+$, $CD7^+$ plus $CD2^+$ or $CD5^+$, or both) or B-lineage ($CD22^+$, $CD19^+$, HLA-DR$^+$, $CD10^+$). Patients were treated with VDCPM (vincristine 1.4 mg/m^2 at days 1, 8, 15 and 22, daunorubicin 45 mg/m^2 at days 1-3, cyclophosphamide 0.75 mg/m^2 at days 1 and 15, prednisone 40 mg/m^2 at days 1-28 and intrathecal methotrexate 8 mg/m^2 for 1-2 years, 10 mg/m^2 for 2-3 years, 12 mg/m^2 for 3-8 years and 15 mg/m^2 for >8 years) or VDLPM (vincristine 1.4 mg/m^2 at days 1, 8, 15 and 22, daunorubicin 45 mg/m^2 at days 1-3, asparaginase 6,000 U/m^2 at days 19-28, prednisone 40 mg/m^2 at days 1-28 and intrathecal methotrexate 8 mg/m^2 for 1-2 years, 10 mg/m^2 for 2-3 years, 12 mg/m^2 for 3-8 years and 15 mg/m^2 for >8 years) regimens (15).

Complete remission was defined by <5% blast cells in the bone marrow and normalization of peripheral blood counts at 4 weeks after starting induction therapy (16). Relapse was defined as the reappearance of >20% blast cells in the marrow, or the presence of localized leukemic infiltrates at any site after completion of induction chemotherapy (16). Worse outcome was defined as a lack of response to induction therapy, a relapse after achieving complete remission or death due to any cause (16). Risk classification: standard risk: 1-9 years of age and presenting white blood cell (WBC) count of <50,000/mm^3; high risk: <1 and >9 years of age and WBC count >50,000/ mm^3 (17). The controls were 100 healthy individuals (4-10x10^3 leukocytes/mm^3) without a family history of leukemia. Subjects in both groups in the study were 1-18 years of age, included both genders and were residence in the State of Guerrero, Mexico.

Specimen collection. A bone marrow and/or blood sample was taken from the 170 participants and placed in tubes with anticoagulant. Leukocytes were purified from the whole blood sample by a selective osmotic lysis of erythrocytes; the leukocyte genomic DNA and RNA total was extracted by the phenol-chloroform technique (18).

Genotyping of the -A317G and C829T polymorphisms in the DHFR. The -A317G polymorphism (dbSNP; rs408626) was detected using previously reported PCR primers (14): forward primer 5'-GTAGGTTCTGTCTGGGACTGG-3' and reverse primer 5'-GCAGCTTTCTTCTAGTCACCC-3'; and using previously established protocols (19). The PCR products (400 bp) were digested with 4 units of the *Hinf*I enzyme

Table I. General characteristics of the population and clinical data of patients with childhood acute lymphoblastic leukemia (ALL) and healthy individuals.

Variable	Patients with ALL (n=70)	Healthy individuals (n=100)
Age (years; mean ± SD)	7.65±4.67	9.99±5.49
No. of leukocytes/mm^3	13,000 (5,400-39,000)^a	8,000 (7,000-9,000)^a
Gender		
Male	45 (64.29)	53 (53.00)
Female	25 (35.71)	47 (47.00)
Status of participants		
Alive	30 (42.86)	100 (100)
Deceased	40 (57.14)	0
Immunophenotype		
B-lineage	66 (94.28)	0
T-lineage	4 (5.72)	0
Risk by age		
Standard (1-9 years)	18 (25.71)	0
High (<1 and >9 years)	52 (74.29)	0
Relapse during treatment		
No	22 (31.43)	0
Yes	48 (68.57)	0

Data indicate n (%); ^{a}median (percentiles 25-75).

(Invitrogen Life Technologies, USA). Individuals with the A/A genotype presented two fragments (266 and 134 bp), individuals with the A/G genotype presented four fragments (266, 134, 83 and 51 bp) and those with the G/G genotype presented three fragments (266, 83 and 51 bp).

The C829T polymorphism (ddsSNP; rs34764978) was detected using previously reported PCR primers (20): forward primer 5'-CTTCTCCAAGACCCCAACTG-3' and reverse primer 5'-CTTCCAGGTTGTTTTCAATTTTT-3'; and using previously established protocols (19), The amplified products (269 bp) were digested with 3 units of the *TspR*I enzyme (New England Biolabs, Berverly, MA, USA). The C/C genotype presented three fragments (203, 36 and 30 bp), the C/T genotype presented four fragments (239, 203, 36 and 30 bp) and the T/T genotype presented two fragments (239 and 30 bp).

Statistical analysis. Continuous data are presented as the means ± standard deviation or median, 25th and 75th interquartiles. Categorical data were compared by Chi-square or Fisher's exact test. Univariate logistic regression analysis for the association with the risk of relapse of ALL was tested first for -A317G and C829T genetic polymorphisms, gender and other clinical characteristics, and those factors were included into a second multivariate logistic analysis. The log-rank test and Kaplan-Meier curves were used to analyze the effect of the -A317G and C829T genetic polymorphisms, gender and relapse of ALL on overall survival (OS). OS was

Table II. Genotype and allele frequencies of DHFR SNPs in the childhood acute lymphoblastic leukemia (ALL) cases and controls, and association with risk of ALL.

Model[a]	Genotype	ALL cases (n=70)	Controls (n=100)	p-value[b]	OR	95% CI	p-value[c]	p-value HWE
-A317G (rs408626)								
Co	A/A	14 (20.00)	36 (35.00)	0.037[e]	1.00			0.152[d]
	A/G	33 (47.14)	42 (43.57)		2.24	1.03-4.88	0.042[e]	
	G/G	23 (32.86)	22 (21.43)		2.89	1.22-6.86	0.016[e]	
Do	A/A	14 (20.00)	36 (35.00)		1.00			
	A/G + G/G	56 (80.00)	64 (65.00)		2.47	1.19-5.11	0.015[e]	
Re	A/A + AG	47 (67.14)	78 (78.57)		1.00			
	G/G	23 (32.86)	22 (21.43)		1.74	0.87-3.45	0.116	
	Allele							
	A	61 (43.57)	114 (57.00)	0.011[e]	1.00			
	G	79 (56.43)	86 (43.00)		1.73	1.12-2.68	0.014[e]	
C829T (rs34764978)								
Co	C/C	10 (14.29)	33 (33.00)	0.016[e]	1.00			0.077[d]
	C/T	53 (75.71)	56 (56.00)		2.83	1.27-6.33	0.011[e]	
	T/T	7 (10.00)	11 (11.00)		1.97	0.60-6.46	0.261	
Do	C/C	10 (14.29)	33 (33.00)		1.00			
	C/T + T/T	60 (85.71)	67 (67.00)		2.69	1.22-5.95	0.014[e]	
Re	C/C + C/T	63 (90.00)	89 (89.00)		1.00			
	T/T	7 (10.00)	11 (11.00)		0.89	0.33-2.44	0.835	
	Allele							
	C	73 (52.14)	122 (61.00)	0.119	1.00			
	T	67 (47.86)	78 (39.00)		1.38	0.89-2.12	0.151	

Data indicate n (%). [a]Genetic model: Co, codominant, Do, dominant, Re, recessive. [b]Obtained by the Chi-square test. [c]Obtained by the logistic regression analysis, taking reference to AA and CC genotypes; [d]HWE (Hardy-Weinberg equilibrium) to controls. OR, odds ratio; 95% CI, 95% confidence interval. [e]Significant $p<0.05$.

defined as the time between surgery and either death or the time of the last follow-up. The Hardy-Weinberg equilibrium (HWE) was used to determine the genetic equilibrium in the healthy group. $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA) and STATA software, version 9.2 (StataCorp, College Station, TX, USA).

Ethics statement. The bone marrow samples of the patients and blood samples of healthy individuals used in this study were part of the samples taken for clinical diagnostic tests in the hospital. Since no extra amount of samples was collected from the study subjects, informed consent was obtained from all the individuals or their guardians, after a detailed briefing of the study purposes. The study and the informed consent procedure were approved by the Institutional Review Board of the Cancer Institute from Guerrero State, Mexico.

Results

Characteristics of the study population. The 70 ALL patients had ages ranging from 1.0 to 18 years (mean ± SD, 7.65±4.67 years). There were 45 (64.29%) males and 25 (35.71%) females. Eighteen patients (25.71%) were in the age group of 1-9 years (standard

risk). Fifty-two patients (72.29%) were <1 year and >9 years of age (high risk) at the time of initial diagnosis. Relapse of ALL occurred in 68.57% of the patients. WBC and characteristics of immunophenotype are depicted in Table I.

We also included 100 control subjets (controls). In this group, the age range was 1-18 years (mean ± SD, 9.99±5.49 years), and the leukocyte count was normal (4-10x10³ leucocytes/mm³; median 8,000). In this group, 53 healthy individuals (53%) were male and 47 (47%) were female (Table I).

Associations of -A317G and C829T SNP of DHFR with risk of ALL. The allele and genotype distributions for two SNPs of DHFR in the cases and controls are summarized in Table II. The genotype frequencies of these polymorphisms were in HWE (p>0.05) in the controls. When the genotype frequencies were compared between cases and controls, they showed a statistically significant association with the disease. The distribution of -A317G and C829T SNP genotypes was significant between the cases and controls (p=0.037 and p=0.016, respectively), and the A/G and C/T genotypes were more prevalent among the patients (47.14 and 75.71%, respectively). The homozygous variant G/G [odds ratio (OR)=2.89, 95% CI 1.22-6.86] and heterozygote variant C/T (OR=2.83, 95 %CI 1.03-4.88) were risk factors for ALL (Table II).

Table III. Genotype distribution and allele frequency of the -A317G and C829T polymorphisms of DHFR in individuals with and without relapse of childhood acute lymphoblastic leukemia.

	Without relapse n (%)	With relapse n (%)	p-value[a]
-A317G (rs408626)			
Genotypes			
A/A	9 (40.91)	4 (8.33)	
A/G	9 (40.91)	25 (52.08)	
G/G	4 (18.18)	19 (39.58)	0.014[b]
Allele			
A	27 (61.36)	34 (35.42)	
G	17 (38.64)	62 (64.58)	0.004[b]
C829T (rs34764978)			
Genotypes			
C/C	7 (31.82)	3 (6.25)	
C/T	14 (63.64)	39 (81.25)	
T/T	1 (4.54)	6 (12.50)	0.015[b]
Allele			
C	28 (63.64)	45 (46.88)	
T	16 (36.36)	51 (53.12)	0.048[b]

[a]Obtained by the Chi-square test; [b]Significant $p<0.05$.

Genotype distribution and allele frequency of SNPs in individuals with and without relapse of ALL. The A/A genotype was present in 10.42% of the patients with relapse, compared to 40.91% of those who did not relapse, whereas 50% of the patients with relapse were carriers of the A/G genotype compared to 40.91% of those who did not relapse, and 39.58% of the patients with relapse were carriers of the G/G genotype, compared to 18.18% of those who did not relapse. Regarding the distribution of the C829T polymorphism in patients with relapse, 81.25% were carriers of the C/T genotype, 12.50% had the T/T genotype and only 6.25% carried the C/C genotype. In contrast to those who did not relapse, 31.82% were carriers of the C/C genotype, 63.64% of the C/T genotype and 4.54% carriers of the T/T genotype. Genotypic and allelic frequencies of both polymorphisms were statistically significant between patients with and without relapse of ALL ($p<0.05$) (Table III).

Risk of relapse based on the -A317G and C829T SNP genotypes and other risk factors in ALL. In a logistic regression analysis, an association was found between the -A317G, C829T polymorphisms and the risk of relapse of disease ($p<0.05$). Those patients carrying the G/G genotype of the -A317G polymorphism, showed a significant increase in the risk of relapse of ALL (OR=8.55, 95% CI 1.84-39.70) compared to carriers of the A/A genotype (p=0.006) (Table IV). Carriers of the T/T genotype of the C829T polymorphism had a 14.00 greater chance of a relapse of the disease (OR=14.00, 95% CI 1.13-172.63) compared to carriers of the C/C genotype (p=0.039) (Table IV). Other variables, such as age, leukocytes,

but not gender, were associated with the risk of relapse of disease ($p<0.05$) (Table IV).

The following variables were included in a multivariate analysis: leukocytes count at diagnosis, age, -A317G and C829T polymorphism genotypes, to determine whether either or both the SNPs predict the risk of relapse independently. It was observed that A/G or G/G genotype carriers (p=0.041 and p=0.017, respectively), together with the C/T or T/T genotype carriers (p=0.015 and p=0.049, respectively), were two independent prognostic markers for the risk of relapse of ALL compared to the other variables (Table V).

Association between DHFR -A317G and C829T SNP and overall survival in the ALL patients. Kaplan-Meier analysis of OS curves showed significant results between the relapse of ALL and DHFR -A317G, C829T polymorphisms. We found no significant associations between gender and OS (log-rank test; p=0.79) (Fig. 1A). A different rate of OS was evident between the individuals with and without relapse of ALL (log-rank test; p<0.001) (Fig. 1B). In fact, 81.82% of the patients without relapse were alive, while only 25% of patients with relapse were alive (Table I). Those patients with relapse had a 13.5 greater chance of death (OR=13.5, 95% CI 3.81-47.84) compared to those without relapse (p<0.001) (data not shown).

The relationship between OS and the polymorphisms was calculated. Individuals with the G/G genotype had poorer OS compared to the A/A genotype (log-rank test; p<0.05) (Fig. 1C and D). We found no significant association for the DHFR C829T polymorphism, although we observed a reduction in survival at 11 years of follow-up among T/T-carriers with respect to that of patients with the wild-type genotype (log-rank test; p=0.069) (Fig. 1E). However, we carried out log-rank test for combined genotypes, C/T + T/T vs. C/C (Fig. 1F), showing significant genotype-dependent effects for OS (log-rank test; p=0.026).

Discussion

There have been attempts to explain the mechanisms by which patients show different response to the same drug used to treat ALL. However, there are few studies addressing the association of SNPs with response to treatment in patients with ALL. There are no studies in Mexico regarding the -317A/G and 829C/T polymorphisms of DHFR; therefore, it is important to determine their distribution in the Mexican population, its association and impact on the risk of relapse and survival in patients with ALL.

Patients with ALL predominantly showed the heterozygous A/G genotype (47.14%) of the -A317G polymorphism (Table II), a result similar to that reported by Dulucq *et al*, in a Canadian population where the A/G genotype was reported as the most frequent in individuals with ALL (48.4%) (14). However, the genotypic frequencies A/A (31.4%), A/G (48.4%) and G/G (20.2%) reported by Dulucq *et al* are statistically significant to those found in this study (p=0.040).

The genotypic frequencies of the C829T polymorphism reported in this study differ (p<0.001) from those reported by Goto *et al*, in a Japanese population with ALL, where the C/C genotype (83.8%) was the most frequently reported, followed

Table IV. Association of the -A317G and C829T polymorphisms in the DHFR gene and other clinical features with the risk of relapse of childhood acute lymphoblastic leukemia.

Model[a]	Genotype	No. (%)	OR	95% CI	p-value[b]
-A317G (rs408626)					
Co	A/A	14 (20.00)	1.00		
	A/G	33 (47.14)	4.80	1.26-18.24	0.021[c]
	G/G	23 (32.86)	8.55	1.84-39.70	0.006[c]
Do	A/A	14 (20.00)	1.00		
	A/G + G/G	56 (80.00)	7.62	2.01-28.81	0.003[c]
Re	A/A + AG	47 (67.14)	1.00		
	G/G	23 (32.86)	2.94	0.86-10.06	0.084
C829T (rs34764978)					
Co	C/C	10 (14.29)	1.00		
	C/T	53 (75.71)	6.50	1.47-28.67	0.013[c]
	T/T	7 (10.00)	14.00	1.13-172.63	0.039[c]
Do	C/C	10 (14.29)	1.00		
	C/T + T/T	60 (85.71)	7.00	1.60-30.54	0.010[c]
Re	C/C + C/T	63 (90.00)	1.00		
	T/T	7 (10.00)	3.00	0.348-26.55	0.323
Gender					
Female		25 (35.71)	1.00		
Male		45 (64.29)	1.38	0.49-3.92	0.540
Risk by age					
1-9 years (low risk)		18 (25.71)	1.00		
<1 and >9 years (high risk)		52 (74.29)	4.54	1.14-18.09	0.032[c]
Leukocytes at diagnosis					
<50,000/mm^3		18 (25.71)	1.00		
>50,000/mm^3		52 (74.29)	7.64	1.90-30.73	0.004[c]

[a]Genetic model: Co, codominant; Do, dominant; Re, recessive. [b]Obtained by logistic regression analysis; [c]Significant p<0.05; OR, odds ratio; 95% CI, 95% confidence interval.

Table V. Factors influencing the risk of relapse of ALL in a multivariate regression analysis.

	OR	95% CI	p-value[a]
-A317G (rs408626)			
A/G	4.06	0.01-0.58	0.041[b]
G/G	6.75	0.07-0.67	0.017[b]
C829T (rs34764978)			
C/T	7.49	0.08-0.71	0.015[b]
T/T	12.38	0.002-0.93	0.049[b]
Risk by age			
<1 and >9 years (high risk)	0.86	0.25-2.96	0.814
Leukocytes at diagnosis			
>50,000/mm^3	0.52	0.11-2.36	0.395

OR, odds ratio; 95% CI, 95% confidence interval. [a]Obtained by multivariate logistic regression analysis. [b]Significant p<0.05.

by the C/T genotype (10.8%) and the T/T genotype (5.4%) (13). The C829T polymorphism has been studied in other disorders that involve the metabolic pathway of folate. However, it was not identified in non-Japanese American, Caucasian or Israeli populations (21-23), suggesting that the C829T polymorphism was found more frequently in patients with ALL than in other disorders in which the activity enzymatic of DHFR is involved.

In the present study, 93.75% of the patients with relapse were carriers of the T allele (Table III); this agrees with previous experimental studies, which identified the T allele as a risk for relapse to ALL (28). This suggests that the presence of the -A317G and C829T polymorphisms, and the strong association with the risk of relapse (p<0.05) (Table IV) may be a factor that led to more than 50% of deaths in the patients with ALL included in this study.

In many studies, age has been found to be a prognostic factor in childhood ALL (24); this feature also retained its significance in our study. Patients in the age group of 1-9 years (low risk) had the best prognosis, whereas patients <1 and >9 years of age (high risk) showed the worst prognosis (OR=4.54, 95% CI 1.14-18.09) (Table IV), which agrees with other studies (25).

Regarding gender, it has been reported in various studies that females have better survival than males of the same age
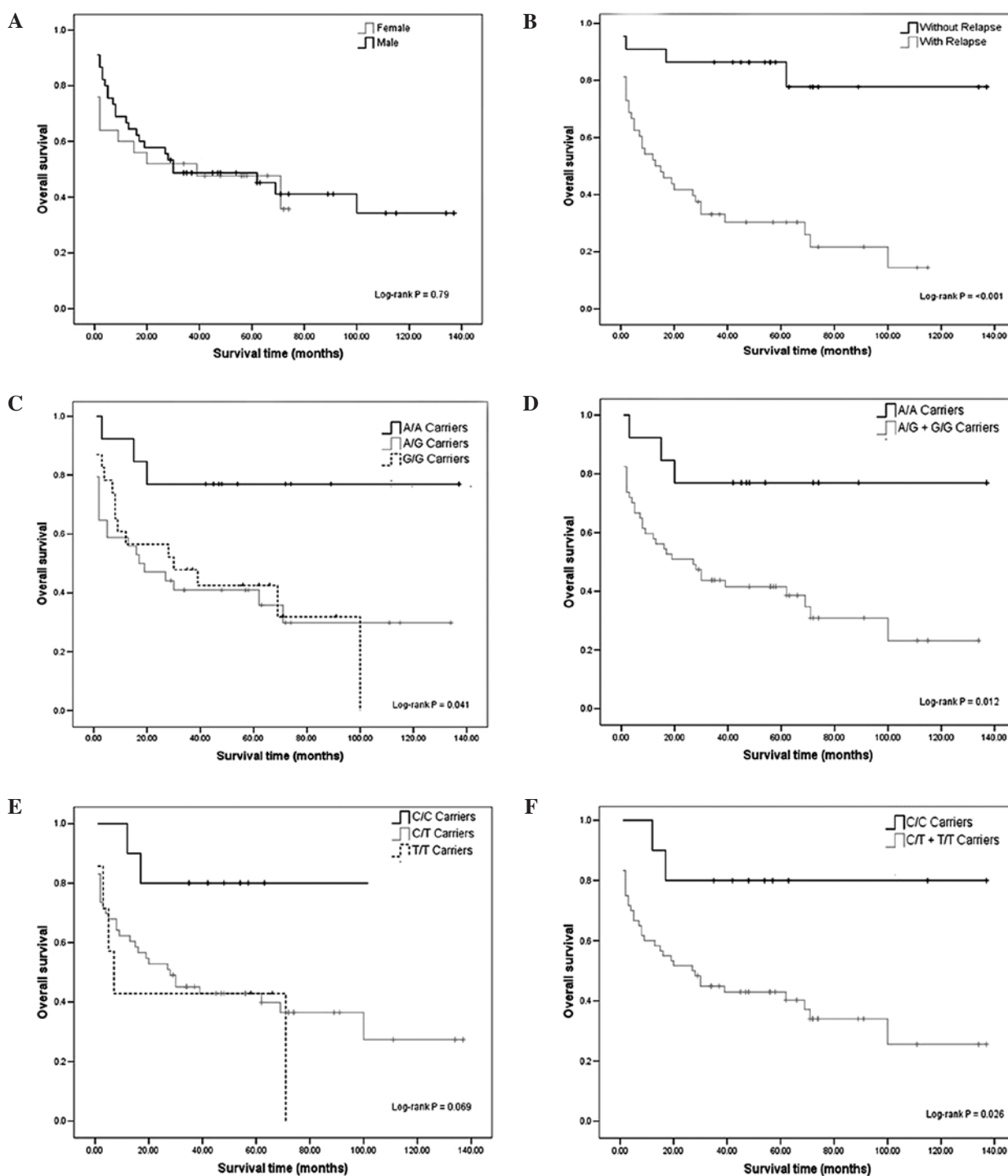
Figure 1. Kaplan-Meier curves considering the influence of gender, relapse, and dihydrofolate reductase (DHFR) -A317G and C829T polymorphisms on overall survival (OS) of acute lymphoblastic leukemia (ALL) patients. (A) OS in females and males with ALL. (B) OS between patients with and without relapse. (C) Association between OS and the DHFR -A317G polymorphism. (D) Combined genotypes A/G + G/G vs. A/A in 70 children with ALL. (E) Association between OS and the DHFR C829T polymorphism. (F) Combined genotypes C/T + T/T vs. C/C in 70 children with ALL. Ticks indicate censoring.

with ALL (24,26). However, we did not observe such a gender-based outcome (p>0.05) (Table IV), similarly to Dulucq *et al*, Kim *et al* and Dervieux *et al*, (14,27,28). This could be due to the small sample size of the female group (n=25/70) in our study. The WBC has also been reported as a prognostic factor in many studies on pediatric ALL. We observed that a WBC count more than 50×10^9 leukocytes/mm^3 at diagnosis was associated with poor outcome (OR=7.64, 95% CI 1.90-30.73)

(Table IV), similar to other studies (24,29). Immunophenotype has been considered as the most important prognostic factor impacting the therapeutic strategy. The pre-B ALL and the immature T-cell precursor are generally associated with a poorer prognosis (30). Two studies have shown that the Hispanic population has a high frequency of B-cell precursor immunophenotype (31,32). Our findings are consistent with these studies; we found that the 68.18% of cases with relapse

of leukemia were classified within this immunological lineage (data not shown).

On separate analyses of the -A317G and C829T polymorphisms in the DHFR gene, analyses of the G/G and T/T genotypes alone appear to be worse prognostic markers than leukocytes at diagnosis and age (OR=8.55, 95% CI 1.84-39.70; OR=14.00, 95% CI 1.13-172.63; OR=7.64, 95% CI 1.90-30.73; OR=4.54, 95% CI 1.14-18.09, respectively). However, in a multivariate analysis we observed that the -A317G and C829T polymorphisms were the worst independent prognostic factors. The analysis showed that G/G and T/T genotypes were independent prognostic markers, excluding leukocytes at diagnosis and age (Table V). A second goal of this study was to investigate the impact of the polymorphisms on survival. The OS rate of G and T allele carriers of the -A317G and C829T polymorphisms, respectively, was lower than that of patients carrying A and C alleles. Our results are in line with those reported by Dulucq et al (14), showing an association between the G/G variant and reduced survival in ALL patients. We also evaluated the relationship between OS and the C829T polymorphism. During follow-up, we observed a reduction in OS among T-carriers and patients with the wild-type genotype (Fig. 1).

In conclusion, the -A317G and C829T polymorphisms are strongly associated with the risk of relapse to ALL, presenting a higher risk of relapse in ALL carriers of G/G and T/T genotypes than in carriers of the A/A and C/C genotypes, respectively. Our data showed that the polymorphisms of DHFR C829T and -A317G had an impact on survival of ALL Mexican patients. These data seem to suggest a role for the DHFR polymorphisms in the relapse of ALL and overall survival. This is the first study which evaluated the effect of the C829T polymorphism on overall survival. However, this analysis was based only on 70 patients and needs to be confirmed in a larger population.

Acknowledgements

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