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Research Article

Genotype Analysis of Single Nucleotide Variant in *NCF4* and *CBR3* Genes Associated with Reduced Systolic Fractional Shortening in Pediatric Patients with Acute Lymphoblastic Leukemia Treated with Doxorubicin

Jesús Alonso Gándara-Mireles[1,2], Ismael Lares-Asseff[1,2]*, Elio Aarón Reyes Espinoza[3], Javier G Blanco[5], Antonio Emilio González Font[4], Lourdes Patricia Córdova Hurtado[3], Verónica Loera-Castañeda[1,2], Ignacio Villanueva Fierro[1,2], Isaías Chairez Hernández[1], Hugo Payan-Gándara[3], Leslie Patrón Romero[6] and Horacio Reyes-Almanza[6]

[1]*Academia de Genómica, Instituto Politécnico Nacional, CIIDIR-Unidad Durango, México*

[2]*Red Latinoamericana de Implementación y Validación de Guías Clínicas Farmacogenómicas (RELIVAF-CYTED)*

[3]*Servicio de Hemato-Oncología Pediátrica, Centro Estatal de Cancerología, CECAN Durango, México*

[4]*Servicio de Cardiología Pediátrica, Hospital Materno Infantil, Servicios de Salud de Durango, México; México*

[5]*School of Pharmacy and Pharmaceutical Sciences, University of Buffalo, The State University of New York*

[6]*Facultad de Medicina y Psicología de la Universidad Autónoma de Baja California, Tijuana, México*

***Corresponding Author:** Ismael Lares-Asseff, Academia de Genómica, Instituto Politécnico Nacional, CIIDIR-Unidad Durango, México.

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Abstract

Cardiotoxicity (CT) is a frequent complication due to the use of anthracyclines (AC) in chemotherapy in various types of cancer. The reduction in the systolic shortening fraction (SF) is an echocardiographic parameter that, together with other biochemical values, is used to assess the presence of CT. Studies suggest that some genetic variants may be involved in the risk of cardiotoxicity due to the use of anthracyclines. Although it has been reported that SF alone cannot be taken as a measurement to definitely assess CT due to the use of AC, reports documenting the impact of single nucleotide variant (SNV) on CT risk considering SF as a marker in pediatric patients from Latin American countries with cancer, are scarce. This study aimed to evaluate the association between the genotypic status of *NCF4* rs1883112 and *CBR3* rs1056892 SNV with the SF indicative of CT by AC in a group of Mexican children with acute lymphoblastic leukemia (ALL). Sixty-nine children (6 to 17 years of age) with ALL were treated at the Centro Estatal de Cancerología (CECAN) in Durango-Mexico. The *NCF4* and *CBR3* genotypes were examined by real-time PCR. Fractional shortening was evaluated as a marker of systolic CT due to the use of AC. The homozygous AA genotype of the *NCF4* rs1883112 SNV was significantly associated with reduced SF by the use of AC (OR = 8.87, 95% CI = 1.8066 to 147.6678, p = 0.01). There was a significant association between the dominant homozygous GG genotype of the *CBR3* rs1056892 SNV and the reduced SF by the use of AC (OR = 5.33, 95% CI = 1.4008 to 20.3060, p = 0.01). This pilot study suggests that selected SNV may affect the risk of CT by AC, SF may be a value that, together with other parameters such as left ventricular ejection fraction (LVEF) and diastolic filling volume may give a broader picture of CT risk in Mexican pediatric patients treated with AC.

Keywords: *Fractional shortening; Cardiotoxicity; Anthracyclines; Leukemia*

Abbreviations

AC: Anthracyclines; ALL: Acute Lymphoblastic Leukemia; CHF: Congestive Heart Failure; CT: Cardiotoxicity; Dox: Doxorubicin; DRZ: Dexrazoxane; ECG:Echocardiographic; HWE: Hardy-Weinberg Equilibrium; LVEF: Left Ventricular Ejection Fraction; qPCR: Real-Time Polymerase Chain Reaction; ROS: Reactive Oxygen Species; SF: Shortening Fraction; SNV: Single Nucleotide Variant

Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common pediatric cancer in the world; it is a malignant disease of the white blood cells with a multifactorial etiology that involves an interaction of several variables including genetic ones.[1] It is estimated that more than 60% of patients diagnosed with ALL are children under 15 years of age, with a higher incidence between 2 - 5 years of age. In Mexico, the incidence of pediatric ALL is 44.9/million inhabitants [2,3].

With the treatment regimen currently in use, the cure rate for this disease is 90% [4,5]. The strategy of implementing risk-stratified therapy has had very encouraging results, as survival rates for ALL have improved significantly [6-8].

Treatment of pediatric ALL consists of combination chemotherapy regimens that include AC; and doxorubicin (Dox) is one of the most widely used AC in cancer treatment [9]. Treatment with Dox is associated with the development of CT in some patients.[10] It is well known that anthracyclines such as Dox can cause congestive heart failure (CHF) during or after treatment of childhood ALL; the risk of CT is increased with a total cumulative dose and is modified by individual factors, including cancer diagnosis at a younger age, female sex, and concomitant radiation to the heart [11,12].

One of the strategies to reduce the risk of cardiotoxicity is the use of cardioprotective drugs. Dexrazoxane (DRZ) has been shown to successfully prevent or reduce anthracycline-related cardiotoxicity in children with cancer by acting as a scavenger of metal ions in cardiomyocytes, preventing the iron-anthracycline complex from entering the oxidoreduction cycle and forming reactive free radicals [13]. Enalapril is another drug that provides temporary cardioprotection against the development of cardiotoxicity when using Dox therapy [14,15]. With more childhood cancer survivors now reaching adulthood, it is vital to understand the adverse effects of cancer treatment on the cardiovascular system and their long-term consequences to identify and establish optimal prevention and management strategies that balance oncologic efficacy with long-term safety; therefore in addition to the use of cardio protectants, one strategy that has aided the treatment and clinical assessment of the adverse effects of Dox has been an echocardiographic evaluation [16,17]. Van Dalen E., *et al* 2006 [18] wrote one of the most comprehensive review article regarding the echocardiographic assessment of cardiotoxicity in which they evaluated twelve protocols that included AC therapy in regards to the minimally required diagnostic tests to assess CT, the parameters and definitions of Anthracycline-induced cardiotoxicity. In most of these 12 protocols they used the left ventricular shortening fraction (LSF) to evaluate cardiac function. In this sense, several authors agree that a reduction in the value of SF < 29% will be considered a significant functional deterioration [19,20].

As mentioned above, several factors may be involved in the development of CT. It has been shown that some genetic SNV of genes that participate significantly in the metabolic pathway of Dox, may have a potentiation effect or decrease the probability of presenting CT in patients using this antineoplastic agent.

The *NCF4* gene is part of a subunit of NAD(P)H oxidase, directly involved in the formation of reactive oxygen species (ROS) in the mitochondria, and it has been reported that the rs1883112 SNV is associated with the development of CT [21]. On the other hand, the *CBR3* gene encodes for the protein carbonyl reductase, it has an important role in the metabolic pathway of Dox since in cardiomyocytes, it participates in the synthesis of the metabolite doxorubicinol which is known to be cardiotoxic. The rs1056892 SNV of the *CBR3* gene has been reported as an enhancer of CT [22].

Although the assessment of SF to evaluate CT by AC relies heavily on the accuracy of the sonographer, several studies have considered that it can be a good support that together with other echocardiographic (ECG) [18] values, it can help monitor cardiotoxic effects during and after the use of AC. This work aimed to identify the association of the genetic SNV *NCF4* rs1883112 and *CBR3* rs1056892 with SF reduction in pediatric patients with acute lymphoblastic leukemia treated with Dox.

Material and Methods

Population

This was a longitudinal case-control study where sixty-nine children diagnosed with childhood ALL treated with Dox, from the Pediatric Hemato-Oncology Service of Centro Estatal de Cancerología (CECAN) of the Secretary of Health of Durango-Mexico were included. Of the 69 children included, 12 presented a reduced SF after the application of Dox, these children were assigned to the cases group. A reduction in SF ≤ 28% was considered a significant decrease after Dox dosing. Of the 69 children included in this study, fifty six children did not present a reduced SF, these children were assigned to the control group.

All patients were diagnosed with ALL according to the criteria of the French-American-British Hematology Association [23]. This research was approved by the Research Ethics Committees of the General Hospital of Durango, Mexico, with registration number 516/019, in accordance with the Helsinki declaration and the Mexican General Health Law. Each patient was undergoing chemotherapy treatment according to the St Jude TOTAL XV protocol [24]. Patients under observation, that is, children who had already completed their drug treatment, were also included. All patients' parents were asked to give written informed consent; in addition, children older than 9 years were also asked to give written informed assent.

Cardiotoxicity assessment criteria

All 69 patients diagnosed with ALL underwent an echocardiogram (ECG) to evaluate cardiotoxicity, which was found to be normal (SF > 28%) [25] before starting Dox treatment. One month after the first dose of Dox, a second ECG was performed. A third ECG was performed four months later, a fourth study eight months later, and a fifth ECG a year and a half after receiving Dox. Subsequently, after receiving Dox, a decrease in SF ≤ 28% was considered cardiotoxic due to a reduced SF.

Evaluation of cardiovascular risk factors in patients

Evaluation of cardiovascular risk factors at the Centro Estatal de Cancerología (CECAN) of Durango, México is currently carried out considering arterial hypertension, high cholesterol, overweight and obesity, smoking, physical inactivity, male patients, and age. None of the patients considered in this study presented any of the cardiovascular risk factors.

Genotyping

DNA was obtained from whole blood using the "DTAB-CTAB" extraction procedure [26]. Its integrity and purity were determined by horizontal 1% agarose gel electrophoresis, staining was performed with Texas Red, and quantification by spectrophotometry on a Nanodrop® (Thermo Scientific, USA). *NCF4* rs1883112 (C__11521119_1_) and *CBR3* rs1056892 (C___9483603_10) SNV were determined by real-time polymerase chain reaction (qPCR) using TaqMan technology on a dedicated thermal cycler, Bios® Step One®.

Statistical analysis

Data is presented as means ± standard deviation or proportions. Differences between numerical variables were established with Student's t-test for independent samples (Fisher's exact test) for categorical variables. Multivariate analysis was performed with a correspondence test, using TIBCO Statistica 13.3 software [27]. Genotype frequencies were obtained by direct counting and Hardy-Weinberg equilibrium (HWE) was calculated by X2 goodness-of-fit statistics; both analyses were performed using SNP stats software (http://bioinfo.iconcologia.net/SNPstats) [28]. The association between the SNV and fractional shortening reduction was assessed with Odds ratio analysis. Statistical significance was set at $p < 0.05$ with a 95% confidence interval (CI). PASW Statistics 18.0.0 software was used for statistical analysis.

Results

Table 1 shows the demographic data of children with (cases) and without (controls) SF reduction, statistically significant differences were found between children in height (p = 0.04) and BMI (0.04). table 2 shows the genotypic and allelic frequencies of the SNV'S; in the case of the *NCF4* rs1883112 SNV, there is a statistically significant difference in the WT genotype (p = 0.04), also, there is a difference between the G alleles (p = 0.01). In the case of the *CBR3* rs1056892 SNV, there is a statistically significant difference in the HM genotype (p = 0.02), and HT genotype (p =0.03), additionally, there is a difference between the A alleles (p = 0.01).

Variables	Cases (*N* = 12)	Controls (*N* = 57)	P
Age (years)	11.69 ± 4.3	12.22 ± 4.6	0.4
Sex(F/M)	04/08	22/35	
Weight (kg)	34.4 ± 8.8	36.7 ± 7.6	0.3
Height (cm)	144.7 ± 24.7	126.8 ± 22.3	0.04
BMI (kg/m^2)	17.1 ± 3.3	20.4 ± 2.2	0.04

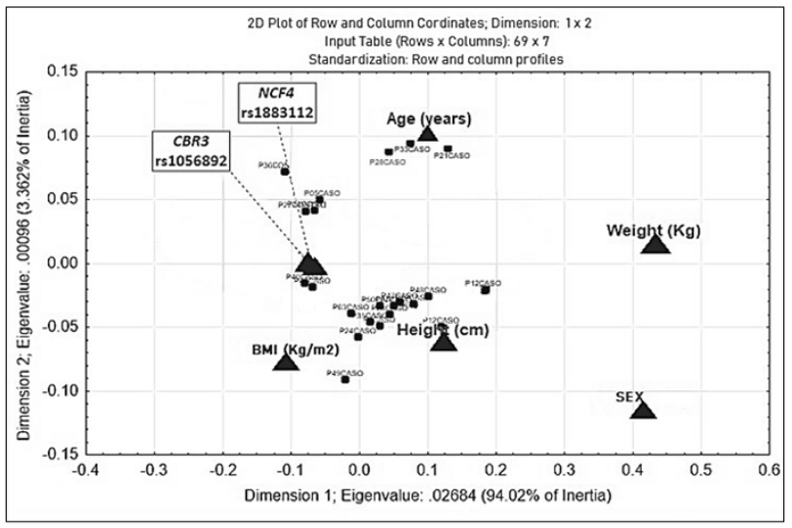
Table 1: *Comparison of demographics of children with acute lymphoblastic leukemia with and without systolic fractional shortening. T Student t-test.*

	Patients with ALL (n=69)			
	Genotype	**Cases n (%)**	**Controls n (%)**	**p***
NCF4 rs1883112	WT (GG)	01 (8.33%)	28 (49.12%)	0.04
	HT (GA)	04 (33.33%)	17 (29.81%)	0.07
	HM(AA)	07 (58.31%)	12 (21.04%)	0.5
	Alleles			
	Major allele (G)	06 (25.00%)	73 (64.03%)	0.01
	Minor allele (A)	18 (75.00%)	41 (35.97%)	0.08
CBR3 rs1056892	**Genotype**			
	WT (GG)	06 (50.00%)	09 (15.78%)	0.1
	HT (AG)	03 (25.00%)	23 (40.34%)	0.03
	HM(AA)	03 (25.00%)	25 (43.85%)	0.02
	Alleles			
	Major allele (G)	15 (62.50%)	41 (35.97%)	0.07
	Minor allele (A)	09 (37.50%)	73 (64.03%)	0.01

Table 2: *Genotypic and allelic frequencies of NCF4 rs1883112 and CBR3 rs1056892 SNV'S in patients with and without systolic shortening fractional.*

**Fisher´s exact test.*

Graph 1 shows the association between the variables of age, sex, weight, height, BMI, and genetic SNV with the patients who presented SF reduction using Dox by a multivariate analysis, in which a significant association was obtained (x2 = 328.189, p = 0.0001). The association between the variables and the patients who had a reduction in SF is shown, it can be observed that the variables that had the greatest association were: Height (Relative Inertia: 0.19844), *NCF4* rs1883112 SNV (Relative Inertia: 0.18984), BMI (Relative Inertia: 0.10541) and *CBR3* rs1056892 SNV (Relative Inertia: 0.09547).



Graph 1: *Two-dimensional graph showing the association between cases (patients with fractional shortening reduction). (●), as well as the 7 variables (▲).*

Regarding Hardy Weinberg Equilibrium (HWE), we found that the *NCF4* rs1883112 SNV is in disequilibrium, while the *CBR3* rs1056892 SNV are in HWE.

Table 3 shows the crude analysis of allelic status by inheritance models, which shows that the rs1883112 SNV of the *NCF4* gene was significantly associated with reduced SF, shown through a homozygous AA in the codominant model (OR 8.87; 95% CI 1.8066 to 147.6678, p = 0.0171).

	Inheritance models of the ALL patients with and without systolic fractional shortening (n = 69).					
	Codominant Model	**Yes(n=12)**	**No(n=57)**	**OR**	**IC**	**P**
NCF4 rs1883112	WT (GG)	01	28	1		
	HT (GA)	04	17	6.2882	0.6788 to 63.9427	0.1040
	HM (AA)	07	12	8.8700	1.8066 to 147.6678	0.0171
	Dominant Model					
	WT (GG)	01	28	0.0942	0.0114 to 0.7782	0.0283
	HT (GA) +HM(AA)	11	29			

	Additive Model					
	WT (GG)+HM(AA)	08	40	0.8500	0.2253 to 3.2063	0.8104
	HT (GA)	04	17			
	Major allele (G)	06	73	0.1872	0.0689 to 0.5089	0.0010
	Minor allele (A)	18	41			
	Minor allele (A)	18	41	3.7330	1.6982 to 8.2059	0.0010
	Major allele (G)	06	73			
	Recessive Model					
	HM(AA)	07	12	5.2500	1.4130 to 19.5059	0.0171
	WT(GG) + HT (GA)	05	45			
	Codominant Model					
	WT (GG)	06	09	1		
	HT (AG)	03	23	0.1957	0.0401 to 0.9554	0.0438
	HM(AA)	03	25	0.1800	0.0370 to 0.8752	0.0333
CBR3 rs1056892	**Dominant Model**					
	WT (GG)	06	09	5.3333	1.4008 to 20.3060	0.0141
	HT (AG) + HM (AA)	06	48			
	Additive Model					
	WT (GG) + HT (AG)	09	32	2.3438	0.5736 to 9.5760	0.2356
	HM (AA)	03	25			
	Major allele (G)	15	41	2.9675	1.1937 to 7.3769	0.0192
	Minor allele (A)	09	73			
	Minor allele (A)	09	73	0.3370	0.1356 to 0.8377	0.0192
	Major allele (G)	15	41			
	Recessive Model					
	HM (AA)	03	25	0.4267	0.1044 to 1.7433	0.2356
	WT(GG) + HT (AG)	09	32			

Table 3: *Association by inheritance models of NCF4 rs1883112, CBR3 rs1056892 SNV'S in children with ALL with systolic fractional shortening.*

**Odds ratio test.*

The dominant model shows us that homozygous GG has a protective effect of the SF reduction (OR 0.0942; 95% CI 0.0114 to 0.7782, p = 0.02). The major allele (G) was associated with the protection against SF reduction (OR 0.1872; 95% CI 0.0689 to 0.5089, p = 0.001), while the minor allele (A) was associated with the risk of SF reduction (OR 3.73; 95% CI 1.6982 to 8.2059, p = 0.001). Finally, the recessive model shows the risk effect of reduced SF through the homozygous AA (OR 5.25; 95% CI 1.4130 to 19.5059, p = 0.01).

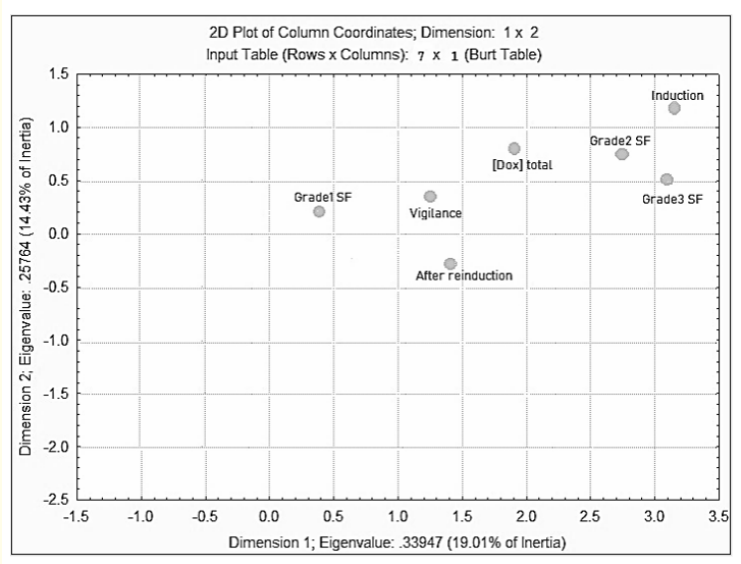
For *CBR3* rs1056892 SNV, the genotype WT codominant model (OR 5.33; 95% CI 1.4008 to 20.3060, p = 0.01) was associated with the risk of SF reduction by Dox use. The minor allele (A) was associated with protection against the risk of reduced SF (OR 0.3370; 95% CI 0.1356 to 0.8377, p = 0.01), while the major allele (G) was associated with the risk of reduced SF by Dox use (OR 2.9675; 95% CI 1.1937 to 7.3769, p = 0.01).

Table 4 shows the degree of SF reduction of the patients who presented reduced SF, the temporal distribution of the SF findings, as well as the cumulative and total doses of Dox. We can observe that the "Vigilance" stage was where the highest number of cases of patients presented a SF reduction occurred (7 patients). Similarly, SF grade 1 was the one with the highest number of patients, since 10 of the 12 who presented SF reduction were grade 1.

Number of patients with systolic fractional shortening / Sex	**Classification of systolic fractional shortening according to the severity**			**Temporal Distribution**			**[Dox] final accumulated**	**Body Surface m^2**	**[Dox] total**
	Grade 1 toxicity Shortening fraction: 24% ≤ SF <30%	Grade 2 toxicity (Moderate to severe cardiotoxicity) Shortening fraction: 15% ≤ SF <24%	Grade 3 toxicity (Symptomatic congestive heart failure) Shortening fraction: < 15%	Induction	After reinduction	Vigilance			
1 /F			X			X	230 mg/m^2	1.15	264mg
2 /M	X				X		230 mg/m^2	0.91	209mg
9 /M	X				X		230 mg/m^2	0.99	228mg
10/M	X				X		230mg/m^2	1.49	343mg
11/F	X				X		150 mg/m^2	1.32	198mg
13/F	X				X		150 mg/m^2	1.46	219mg
15/M	X					X	230 mg/m^2	1.27	292mg
21/M	X					X	230 mg/m^2	0.91	209mg
22/M		X				X	230 mg/m^2	1.13	260mg
38/M	X					X	230 mg/m^2	1.31	301mg
46/F	X					X	230 mg/m^2	0.96	221mg
57/M	X					X	230 mg/m^2	1.21	278mg

Table 4: *Temporal distribution of systolic fractional shortening findings and Dox dose in patients with ALL.*

A multivariate analysis was performed using "TIBCO Statistica 13.3.0"28 software using a correspondence model (Graph 2). The results show that among the variables taken into account in the temporal distribution of the findings in patients with SF reduction, those with the greatest association were: Grade 1 toxicity fractional shortening (relative inertia: 0.1265), Vigilance (relative inertia: 0.069444), and total [Dox] (≥ 220mg) (relative inertia: 0.055556).



Graph 2: *Temporal distribution of findings in patients with reduced fractional shortening.*

Finally, we found a significant association between patients with reduced FS and the *CBR3* rs1056892 SNV at the vigilance stage (OR = 6.133 CI: 1.3353 to 65.3291 p = 0.03). In addition, we found a significant association between total [Dox] ((≥ 220 mg) and the AA genotype of the *NCF4* rs1883112 SNV (OR: 4.448, CI: 1.3353 to 35.3291, p = 0.04).

Discussion

The use of AC such as Dox is limited due to the development of cardiotoxicity, which depends on various factors such as dose, age, height, sex, and genetic SNV´S. In 2006 van Dalen, E., *et al* [18] published an extensive review of guidelines for monitoring CT during AC treatment in children and the monitoring recommendations used in European pediatric oncology trials. Six of the 12 guidelines reviewed mark SF (≤ 28%) as a parameter for monitoring CT. On the other hand, Steinherz., *et al*. [20] recommended that a drop in SF by an absolute value of ≥ 10 percentile units or SF < 29% after the use of AC will be considered a significant deterioration of cardiac systolic function. In Mexico, SF has been reported as an indicative marker of cardiotoxicity due to the use of AC [29].

In the meta-analysis performed by Leong SL, Chaiyakunapruk N and Lee SW [30] the association between genetic markers and cardiotoxicity by AC considering the reduction of SF as one of the parameters to evaluate it, through a literature search until May 2016 they examined 84 genes and 147 SNV, they reported that three variants significantly increased the risk of CT by AC, these were; *ABCC2* rs8187710, *CYBA* rs4673, and *RAC2* rs13058338. One of the genes studied by this group was included in our study, thus, we found that

the homozygous GG in the dominant model of the rs1056892 SNV of the *CBR3* gene was associated with reduced SF due to the use of Dox. It is important to note that the minor allele (A) of this gene was associated with protection to SF reduction while the major allele (G) was associated with risk of SF reduction, for this, it is necessary to have two copies of the mutated allele of this gene to possess a true risk to have SF reduction by Dox use. Although the *NCF4* gene is not included in the study, we have studied the contribution of the rs1883112 SNV of this gene, due to its importance in the metabolic pathway of Dox, this gene showed a significant association as a risk factor in the reduction of SF by Dox in the homozygous AA of the codominant model, However, the heterozygous G/A genotype also shows a risk effect to the reduction of SF by the use of Dox, this is because the minor allele (A) of this gene alone has a risk effect, in such a way that a single copy of this allele confers a risk to have a reduction of SF by the use of Dox.

As we have mentioned, several factors can influence Dox metabolism that can end up causing CT. In our study, we found that of the different variables studied (SNV'S, age, height, sex, weight, and BMI), height and BMI are among the first three with the greatest association with the reduction of SF. This is interesting since in the work reported by Hanley, M., and collaborators in 2010 [31] they mention that anthropometric measures of people significantly modify the pharmacokinetics of drugs, in our work we have found significant differences between the height of cases and controls, however, although our study patients do not fall into malnutrition evaluated by BMI (probably because they are on treatment with steroids [32,33] we did find that cases (patients with reduced SF after receiving Dox) had lower BMI than controls with a statistically significant difference. This lower BMI in cases may contribute to reduced SF as mentioned by Batte, A., *et al.* in 2017 [34] in whose study found that underweight, thinness, delayed growth and development, and malnutrition were more frequent among children with cardiovascular disease.

Corremans R., *et al.* [38] emphasizes that a high cumulative dose of anthracycline is a well-recognized risk factor for cardiac damage, mentioning that other risk factors include an extreme range of age (> 65 or < 4 years), diabetes, previous mediastinal radiotherapy, hypertension, concomitant treatments, and the presence of heart disease, continuous rather than dichotomous variables, which makes it difficult to quantify the risk of any patient. In our study, we found that the variables of height, *NCF4* rs1883112 SNV, BMI, and *CBR3* rs1056892 SNV are associated with patients who developed reduced SF due to Dox use.

On the other hand, in our work, we have explored the temporal distribution of the findings of systolic fractional shortening and Dox dose in patients with ALL. For this reason, Kocabaş A., *et al.* [35] evaluated early-onset chronic progressive cardiotoxicity in the left and right ventricles with increasing cumulative doses of anthracycline; they analyzed diastolic and systolic parameters and compared them with the controls, although the myocardial performance index increased significantly in both segments, abnormalities in right ventricular diastolic function and left ventricular systolic function were observed even with a cumulative dose of anthracycline < 120 mg/m^2. Bansal N, Amdani SM., *et al*. [37] revealed in their work that there is no "safe" dose of anthracyclines. This is consistent with what we found when performing multivariate analysis since we observed that the variable of total dose concentration ≥ 220 mg is one of the three that has the greatest association with the reduction in systolic fractional shortening.

In contrast to a study reported by Wojonowsky., *et al.* in 2005[36] in which they examined acute and chronic Dox toxicity for three years, in our study ECG follow-up was performed for a year and a half and we observed that of the 12 patients who had reduced SF, 7 were in the observation stage, and 5 had reduced SF after reinduction. This is interesting because this result shows the importance of cardiological follow-up of patients who have completed treatment with AC.

Conclusion

In the clinical practice, we can point out that the results obtained show, through inheritance models, the impact of the *NCF4* rs1883112 and *CBR3* rs1056892 SNV'S in the reduction of FS due to the use of Dox. In the case of the *NCF4* gene SNV, a single copy of the A allele confers a risk of having a reduction in FS from Dox use.

It is worth highlighting the importance of inheritance models, which evaluate the effect of gene variation on the risk or protection of the FS reduction caused by the use of anthracyclines.

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Disclosure of Interest

The authors report no conflict of interest.

Author Contributions

All authors were involved in the study. Lares-Assef, Gándara-Mireles, Blanco and Reyes-Espinoza mainly contributed to study conception and design. Gándara-Mireles, Lares-Assef, Loera Castañeda, González Font, Córdova Hurtado, Chairez Hernández and Payan-Gándara were involved in acquisition, analysis, and interpretation of data. The first draft of the manuscript was written by Gándara-Mireles, Patrón Romero, Almanza Reyes and Lares Assef. All authors participated in critical revision of the manuscript, contributed comments, and approved the final version.

Declaration of Data Availability

Data supporting the findings of this study are available upon request from the corresponding author.

The variables that presented the greatest association are: Height (Relative inertia: 0.19844), *NCF4* rs1883112 SNV (Relative inertia: 0.18984), BMI (Relative inertia: 0.10541) and *CBR3* rs1056892 SNV (Relative inertia: 0.09547).

A multivariate analysis using a correspondence model is shown, where the importance of each variable with the association of the systolic shortening fraction is evident, the first 3 variables in order of importance were; Toxicity grade 1 Fractional shortening (relative inertia: 0.1265), Vigilance (Relative inertia: 0.069444) and total [Dox] (≥ 220mg) (Relative inertia: 0.055556).

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Volume 10 Issue 8 August 2022