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Association of NLRP1 rs2670660 genotype with pediatric acute lymphoblastic leukemia.

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2024

بسِمِ اللهِ الرَّحْمنِ الرَّحِيمِ

(قالَوُا سبحَانك لا عِلْمَ لنَاَ إلِا مَا عَلمّْتنَاَ ۖ إنك أنْتَ الْعلِيمُ الْحَكِيمُ ﴿٣٢﴾)

صدق الله العلي العظيم

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الاهداء

الى نفسي اولا ثم الى من ولدتني بعد ان حملتني داخل احشائها

الى ابي الذي حملني طوال عمري

الى من كانت جداري الذي استند عليه حينما يهلكني التعب

الى اخي الذي همه امري

الى زوجته التي باتت فردا من عائلتي

الى كل الناس بعيني وروحي وعمري فهي صديقتي ورفيقة دربي

وشجاعتي وسعادتي وقلبي الى اختي التي حملت الهم مني

ثم الى كل من يحبهم القلب.

Abstract

Objectives: Meta-analysis was used to determine the association between rs2670660 polymorphism of NLRP1 gene and the risk of chronic lymphocytic leukemia (CLL).

Methods: Search for published articles about the association between the rs2670660 and CLL in PubMed, MEDINE, Web of Science, and Embase databases, with a calculated odds ratio of (OR) and 95% confidence interval (95%CI).

Results: A total of 30 cases and 30 controls in 8 studies were pooled together for evaluation of the overall association between rs2670660 and risk of CLL. Allele model (G vs C, p = 0.16, OR = 0.85, 95%CI = 0.71-1.17), homozygous model (GG vs GA, p = 0.07; OR = 0.78, 95%CI = 0.84-1.08 .

Conclusions: Our pooled data indicates that there is a correlation between the inheritance of rs 2670660 and the risk of CLL in familial.

Keywords: Chronic lymphocytic leukemia; Meta-analysis; NLRP1 rs2670660 polymorphism.

Introduction

Acute lymphoblastic leukemia (ALL) is a hematopoietic neoplasm characterized by the exacerbated proliferation of blasts in bone marrow and affects mainly children aged 2 to 15 years old. In Brazil, according to the National Cancer Institute (INCA), it is estimated that for each year of the 2020–2022 triennium, there will be 5920 new cases of leukemia (Acute and chronic) in men and 4860 in women in Brazil, which corresponds to an estimated risk of 5.67 new cases per 100 thousand men and 4.56 for each 100 thousand women.

The inflammasome complex constitutes components of innate immunity involved in inflammatory processes and has been associated with the development of autoimmune inflammatory diseases and several types of cancers[7,8]. In acute lymphoblastic leukemia, NLRP1a-induced pyroptosis in hematopoietic progenitor cells can prevent cell proliferation and differentiation, contributing to the proliferation of altered blasts that will trigger the disease. The dysregulation of the inflammasome complex can also influence the prognosis of patients, since studies report that the constitutive activation of *NLRP3* seems to cleave the glucocorticoid receptor, this being the first line of treatment for ALL, and thus increase the number of relapses[9,10].

ATERIALS And METHOD

Sampling and records collection

In the case group, samples were included from 30pediatric patients diagnosed with ALL according to the classification criteria of the World Health Organization (WHO) , and who were treated at Fundação Hospitalar de Hematologic Hemoterapia do Amazonas (HEMOAM). The patients had cryopreserved samples in the DNA library of the HLA typing laboratory of the HEMOAM, were < 18 years, of either gender or unrelated. Insufficient or lowconcentration DNA samples, and patients with a history of bone marrow transplantation were excluded from the study.

The healthy individuals (control group) consisted of 30 samples from blood donors of either gender,

DNA extraction

Blooding related in EDTA use tubes from all topics using Mini Kit (FAVORGENE) was the method used to extract DNA genes. The purity (ng/ml) of the DNA removal has been measured at 260 and 280 nm using a NanoDrop spectrophotometer (OPTIZENn POP – Korea). is able to be collected for all categories.

- *NLRP3* rs2670660 Forward 5′-ATACCCAGGTGTTCAGGAGC-3′ 328 Reverse 5′-GCCTGTGTTGTACCTTCAGC-3′ 328_{bp}

Genotyping

Using the Green master mix from Promega and the Polymerase Chain Reaction (PCR) technique, the genotyping of *NLRP3* was finished. The PCR cycling program was as follows: 94° C for 5 min; 10 cycles of denaturation at 94° C for 30 s, annealing at 62° C with 0.2° C decrements per cycle for 30 s, and extension at 72° C for 45 s; 30 cycles of 94° C for 30 s, 60° C for 30 s, and 72° C for 30 s; and a final extension

at 72° C for 5 min, followed by holding at 4° C. The amplified PCR products were performed by Sanger sequencing to identify the genotype of each individua

Analyzing

For *NLRP3* gene polymorphism, a 2.25% agarose gel was generated for gel electrophoresis. Genotypes were ascertained and gels were examined using the trans illuminator.

Statistical Analyses

Using the [chi-square] test (P value < 0.05 considered significant) and the [odd ratio] (OD) test, CI 95% to estimate the impact of this mutation with the infected group compared with the control group, the potential associations of *NLRP3*gene polymorphism with the risk of vulvovaginal candidiasis infection were analyzed. This analysis was finished using the [SPSS] program.

RESULTS AND DISCUSSION

The initial step in amplifying the target area of the *NLRP3* gene was to extract the genomic DNA shown in figure (1) from the blood samples.The [SPSS] application was used to do this study in comparison to the control group.

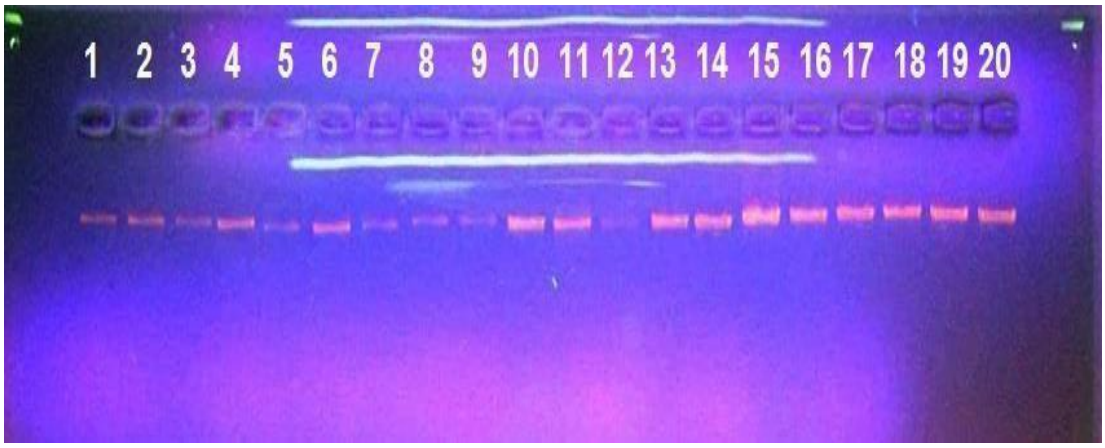

Figure 1 : Electrophoresis pattern of gnomic DNA extracted from blood samples of patients and healthy control groups. Lane 1 refers to genomic DNA control) ; Electrophoresis conditions, 1% 20from blood samples (1-10 patients & 11agarose, ethidium bromide stained 5 Ml for 15 min on high (50 volt) &60 min on low.

Association of *NLRP1* genotype with pediatric acute lymphoblastic leukemia.

The location of the *NLRP1* gene in control and patient women with pregnancy-related hypertension is displayed in Table 1. In controls, **G / G** 60%, **G / A,** 16%, and **A A** 24%; in cases, **G / G** 14%, **G / A** 66%, and **A A** 20%. Patients had a higher frequency of homozygotic mutant genotypes (TT) than controls did. The distribution of allele frequencies in patients and controls differed statistically

significantly (**G / A**: P<0.001, OR 0.3899 to , 95% CI 1.0003to 3.2665).

The *NLRP1* inflammasome is expressed in hematopoietic progenitor cells and its activation results in a process of cell death which is dependent on Caspase 1 and is called pyroptosis. Some studies report that the prolonged cytopenia, induced by the

activation of NLRP1 during infectious processes, ensures a proliferative advantage for the leukemic clone, as suggested by the Mel Greaves hypothesis on the development of ALL[32,33]. In chronic myeloid leukemia (CML), overexpression of *NLRP1* gene is associated with the promotion of proliferation and reduction of apoptosis in CML cells, in addition to inducing resistance to imatinib[34].

In this study, the *NLRP1 A/T rs12150220* genotype was associated with protection against infectious diseases. In ALL, infections are present in 49% of patients on diagnosis[35]. Studies report that susceptibility to congenital toxoplasmosis is significantly associated with SNVs and involves the locus of the *NLRP1* gene[36], which strengthens the Mel Greaves hypothesis that genetic changes in the uterus followed by the acquisition of infections by common pathogens are involved in the development of ALL[5,37].

Table (4-4): Genotype and allele distribution *NLRP1*gene polymorphism in patient and control, shown the Odd Ratio value.

Genotypes	ALL cases (N=30)		Control (N=30)	OR(95%CI)	P-value
NLRP1	**G / G [a], n(%)**	4**(14%)**	18**(60%)**		
	G / A, n(%)	20(66%)	5**(16%)**	18.0000 4.1765 to 77.5769)	0.0001*
	A / A, n(%)	6**(20%)**	7**(24%)**	3.8571 (0.8292 to 17.9411)	0.08
Allele					

Frequency	**G / , n (%)**	46 (0.46)	69 (0.69)		
	A, n (%)	53 (0.53)	31 (0.31)	0.3899 (0.2185 to 0.6960)	0.001 *

G / G -homozygous wild , G / A heterozygous, A / A / -homozygous mutant ,P<0.001: OR =(95%Cl): [a] Reference

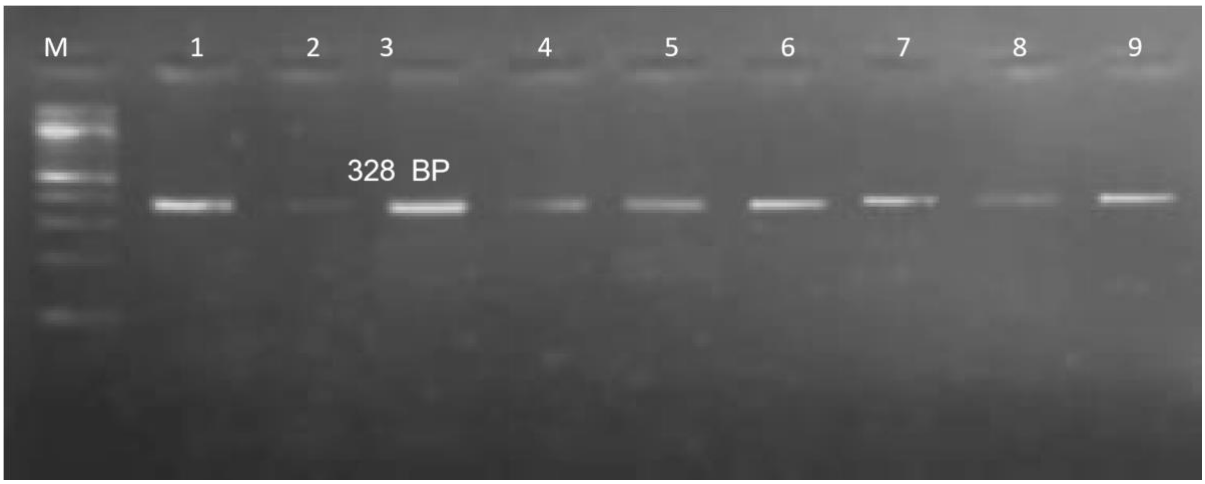

Fig.(2) Amplification of *NLRP1* **Gene of Patients and Control Groups.**

M; refers to DNA size marker (100bp) lane 1 - lane 14 refers to IL1B PCR fragments patterns.

The amplified products were one band about 304 bp in size. Electrophoresis conditions: agarose concentration 1%, power applied: 75 V, 20 mA for 1hour. Staining method; precast ethidium bromide.

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