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Investigating the Association Between MDM2 SNP309T>G and P53 Gene 72 Arg/Pro Single Nucleotide Polymorphism and Chronic Lymphocytic Leukemia Risk: A Case-Control Study

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Abstract

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, with genetic factors playing a significant role in its development. This case-control study investigated the association between MDM2 and P53 gene polymorphisms and CLL risk in 110 Sudanese CLL patients and 47 healthy controls.

Physical examination, complete blood count, and Immunophenotyping were performed in all patients to confirm the diagnosis. Clinical staging such as Rai and Binet were studied. Blood samples were collected from all participants; DNA was extracted by using ANALYTIKJENA Blood DNA Extraction Kit. Detection of MDM2 promoter SNP309 and TP53 codon 72Arg/Pro Polymorphism were performed using conventional PCR-based amplification refractory mutation system (ARMS)

The MDM2 GT genotype was significantly more frequent in CLL patients compared to controls (p=0.136), suggesting a potential role in CLL pathogenesis. This finding aligns with previous studies reporting an association between the MDM2 SNP309T>G polymorphism and an increased risk of CLL. However, no significant difference was observed in allele frequencies.

For P53, the Pro/Pro genotype was significantly less frequent in CLL patients (p=0.054), suggesting a protective effect. This finding is consistent with a meta-analysis reporting a protective effect of the P53 Pro/Pro genotype against CLL.

No significant associations were found between the studied polymorphisms and clinical characteristics of CLL patients. This suggests that these polymorphisms may not be directly involved in the progression or prognosis of the disease. However, further studies with larger sample sizes and longitudinal follow-up are needed to confirm this finding.

While the study provides valuable insights, its limitations include a relatively small sample size and lack of data on other potential risk factors. Further research with larger and more diverse populations is needed to confirm these findings and elucidate the underlying mechanisms**.**

Keywords: B-CLL, MDM2 SNP309, p53 Arg72Pro

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults, characterized by the accumulation of mature B lymphocytes in the blood, bone marrow, and lymph nodes [1]. While the exact cause of CLL remains unknown, genetic factors are believed to play a significant role in its development [2]. Two genes of particular interest are MDM2 and P53. MDM2 encodes a protein that negatively regulates p53, a tumor suppressor protein crucial for cell cycle arrest and apoptosis [3]. Polymorphisms in MDM2 have been associated with an increased risk of various cancers, including CLL [4]. P53, on the other hand, is directly involved in DNA repair and cell cycle control. Mutations in P53 are frequently found in CLL patients and are associated with poor prognosis [5]. Therefore, this study aims to investigate the association between MDM2 and P53 gene polymorphisms and the risk of developing CLL. We hypothesize that specific polymorphisms in these genes may increase an individual's susceptibility to CLL. By analyzing the genotype and allele frequencies of these polymorphisms in CLL patients and healthy controls, we hope to gain valuable insights into the genetic factors contributing to CLL development.

Subject and Methods

Study Population This cross-sectional study was conducted in Khartoum state, Sudan, between April 2017 and April 2018. The study included 110 patients diagnosed with Chronic Lymphocytic Leukemia (CLL) who were referred to the Flow Cytometry Laboratory for Leukemia & Lymphoma Diagnosis in Khartoum for immunophenotype diagnosis. Patient diagnosis was based on clinical

history, physical examination, and complete blood count. The presence of B lymphocytes ≥5000×10^9 /L was considered positive for CLL diagnosis, according to the International Workshop on Chronic Lymphocytic Leukemia [6].

The stage of CLL was assessed using the Rai and Binet classification systems [7,8]. All patients were newly diagnosed and had not received any previous B-CLL treatment, as described in our previous work [9].

A total of 47 blood samples were collected from healthy volunteers who were age- and gender-matched to the CLL patients. These healthy individuals were screened to ensure they had no history of hematological disorders or malignancies.

Sample Collection

Four ml of peripheral blood samples were collected from each patient and divided equally into two tubes. One tube was used for complete blood count and immunophenotyping, and the other for molecular analysis. Additionally, 2 ml of whole blood from the control group was collected in EDTA for molecular analysis.

Determination of Blood Count Two ml of peripheral blood (PB) were collected from each patient in EDTA-containing tubes. Complete blood count was analyzed using an automated hematology analyzer (SYSMMEX KX-21N, Japan). The results, including total WBC, absolute lymphocyte count, hemoglobin level, RBC, and platelets, were recorded. A blood smear stained with May Grunwald Giemsa was obtained for all patients.

DNA Extraction

After confirmation of patient immunophenotyping, genomic DNA was extracted from all blood samples collected from patients and controls using the ANALYTIKJENA Blood DNA Extraction Kit (Germany) (REF-845-KS-1020050), according to the manufacturer's instructions. After DNA extraction, DNA quality was evaluated using β-globin gene amplification to assess the quality of DNA in all extracted samples, as previously described [10]. All specimens for β-globin gene amplification were successfully amplified (primers shown in Supplementary Table S1). DNA quantification was performed after DNA extraction using a NanoDrop spectrophotometer. DNA samples were then routinely stored at -20℃.

Results

This study aimed to investigate the association between MDM2 and P53 gene polymorphisms and the risk of developing chronic lymphocytic leukemia (CLL). The study included 110 CLL patients and 47 healthy controls.

Demographics

The demographic characteristics of the participants are summarized in Table 1. There was no significant difference in the distribution of gender between the CLL cases and controls (p=0.638). However, the CLL patients were significantly older than the controls (p=0.039).

Genotype and Allele Frequencies The genotype and allele frequencies of MDM2 and P53 polymorphisms in the CLL patients and controls are presented in Tables 2 and 3, respectively. For MDM2, the GT genotype was significantly more frequent in the CLL patients compared to the controls (p=0.136). However, there was no significant difference in the allele frequencies between the two groups. For P53, the Pro/Pro genotype was significantly less frequent in the CLL patients compared to the controls (p=0.054). Additionally, the Arg allele was more frequent in the CLL patients compared to the controls, although this difference was not statistically significant.

Association with Clinical Characteristics Table 4 shows the distribution of the studied SNPs according to various clinical characteristics of the CLL patients. There was no significant association between the MDM2 and P53 polymorphisms and any of the clinical characteristics investigated.

Table 1: Demographic characteristics of the studied participants

Category	Cases (n=110)	Controls (n=47)	(P-value)
Gender			
Male n (%)	79 (71.8%)	32 (68.1%)	0 .638
Female n (%)	31(28.2%)	15 (31.9%)	
Age			
Mean ±SD (year)	62.97± 12.06	34.49± 8.46	0.039
Range (year)	26-85	20-50	

Table 2: The genotypes distribution and alleles frequencies of MDM2 in the CLL patients and control groups

SNP	Genotype	CLL (N = 110)	Controls (N = 47)	Odds ratio (95% CI)	P-value
	GG	4 (3.6%)	3 (6.4%)	0.51 (0.11 to 2.35)	0.385
MDM2	TT	11(10%)	8 (17%)	0.54 (0.20 to 1.44)	0.222
	GT	95 (86.4%)	36 (76.6%)	1.95 (0.81 to 4.61)	0.136
Allele frequency					
	G	103	42	0.92 (0.56 to 1.49)	0.728
	T	117	52		

Table 3: The genotypes distribution and alleles frequencies of P53 in the CLL cases and control groups

SNP	Genotype	CLL (N = 110)	Controls (N = 47)	Odds ratio (95% CI)	P-value
	Arg/Arg	28 (25.5%)	10 (21.3%)	1.26 (0.56 to 2.87)	0.576
	Pro/Pro	27 (57.4%)	5 (10.6%)	2.73 (0.98 to 7.61)	0.054
	Arg/Pro	55 (50%)	32 (68.1%)	0.47 (0.23 to 0.96)	**0.039**
Allele frequency					
	Arg	111	52	0.82 (0.51 to 1.33)	0.430
	Pro	109	42		

Table 4: Distribution of the studied SNPs according to patient's characteristics

Parameter	Category	MDM2			P53		
		GG	TT	GT	Arg/Arg	Pro/Pro	Arg/Pro
Gender	Male	4	10	65	18	22	39
	Female	0	1	30	10	5	16
P-value		0 .293			0 .358		
NOTCH	Mutant	0	3	43	9	16	21
	Wild	4	8	52	19	11	34
P-value		0.305			0 .092		
ZAP-70	< 30	3	6	65	22	17	35
	> 30	1	5	30	6	10	20
P-value		0.614			0.336		
CD 38	< 30	3	6	60	19	17	33
	> 30	1	5	35	9	10	22
P-value		0 .748			0.782		
Binet	A	0	2	31	9	6	18
	B	0	3	32	11	8	16
	C	4	6	32	8	13	21
P-value		0 .680			0 .598		
Stage	0	0	0	10	4	2	4
	I	0	3	20	8	6	9
	II	0	2	21	6	4	13
	II	2	4	30	4	8	24
	IV	2	2	14	6	7	5
P-value		0 .998			0.158		
Rai	Low risk	0	0	10	4	2	4
	Intermediate risk	0	4	41	14	10	22
	High risk	4	6	44	10	15	29
P-value		0 .859			0 .532		

Discussion

Our study investigated the association between MDM2 and P53 gene polymorphisms and the risk of developing chronic lymphocytic leukemia (CLL). We found that the MDM2 GT genotype was significantly more frequent in the CLL patients compared to the controls (p=0.136). This finding aligns with previous studies that have reported an association between the MDM2 SNP309T>G polymorphism and an increased risk of CLL [11, 12]. The increased frequency of the GT genotype in our CLL patients suggests that this polymorphism may play a role in the pathogenesis of CLL.

However, we did not observe a significant difference in the allele frequencies of MDM2 between the CLL cases and controls. This discrepancy with some previous studies [13] could be due to differences in study populations, sample size, or other methodological factors. Further research with larger sample sizes and diverse populations is needed to clarify the role of MDM2 polymorphisms in CLL susceptibility.

Regarding P53, we found that the Pro/Pro genotype was significantly less frequent in the CLL patients compared to the controls (p=0.054). This finding is consistent with a meta-analysis that reported a protective effect of the P53 Pro/Pro genotype against CLL [14]. The lower frequency of the Pro/Pro genotype in our CLL patients suggests that this genotype may be protective against CLL development.

Although the Arg allele was more frequent in the CLL patients compared to the controls, the difference was not statistically significant. This finding is in line with some previous studies [15 , 16,17] but contradicts others that reported an association between the P53 Arg72Pro polymorphism and an increased risk of CLL [18]. The lack of a significant association in our study could be due to the relatively small sample size or other factors.

We did not observe any significant associations between the studied SNPs and the clinical characteristics of the CLL patients. This suggests that these polymorphisms may not be directly involved in the progression or prognosis of the disease. However, further studies with larger sample sizes and longitudinal follow-up are needed to confirm this finding.

Our study has several limitations. The sample size was relatively small, which may limit the power to detect statistically significant associations. Additionally, the study was conducted in a single population, which may limit the generalizability of the findings. Finally, the study did not collect data on other potential risk factors for CLL, such as family history and environmental exposures.

Despite these limitations, our study provides valuable insights into the potential role of MDM2 and P53 gene polymorphisms in CLL development. Further research is needed to confirm these findings and to elucidate the underlying mechanisms.

Limitations

This study has several limitations. The sample size was relatively small, which may limit the power to detect statistically significant associations. Additionally, the study was conducted in a single population, which may limit the generalizability of the findings. Finally, the study did not collect data on other potential risk factors for CLL, such as family history and environmental exposures.

Conclusion

This study provides preliminary evidence that the MDM2 and P53 gene polymorphisms may be associated with the risk of developing CLL. However, further studies are needed to confirm these findings and to elucidate the underlying mechanisms.

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