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P14 Gene Polymorphism in Iraqi Acute Myeloid Leukemia Patients Infectious with Human Herpes Virus-6-B

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ABSTRACT

Background: There has been a lot of interest in the pathogenic functions of human herpes virus-6 (HHV-6) in acute leukemia. There have been conflicting findings in attempts to relate HHV-6 activation to the development of acute leukemia. Through a functional network, cMYC, P14ARF, MDM2 and P53 are essential for cell viability

Objective: This study was designed to determine the percentage of Herpes virus-6-B(HHV-6B) and P14 gene polymorphism in Patients Suffering from acute myeloid leukemia of a group of Iraqi patients.

Patients and methods: 100 freshly whole blood were obtained from patients with acute myeloid leukemia enrolled in this study; while control groups in current study included 100 freshly whole blood. Viral and total DNA genomic extraction were done to detect the HHV-6-B by PCR technique and P14 gene polymorphism by sequencing, respectively.

Results: The positive of viral genome extraction was found 42% (42 out of 100) of the specimens have viral genome , wwhile 58% (58\100) specimens not contain viral genome. The rate of human herpes virus -6 B infection according to the PCR was 28.6% (12 out of 42) while the negative result was 71.4% (26 out of 42). the findings demonstrated that the GA, CT, AT, and AG genotypes P14 rs3731249 polymorphism was present in 53.1% (17 out of 32 cases), 21.9% (7 out of 32 cases), 15.6% (5 out of 45 cases), and 9.3% (3 out of 32 cases) of AML patient group, respectively 60% (3 out of 5) and 40% (2 out of 5), respectively, in control group, and 60% (3 out of 5) and 40% (2 out of 5), respectively, in the experimental group.

Conclusion: In view of the relatively small numbers included in our study, the present results indicate the possibility that HHV-6 B as well as P14 rs3731249 may play a role in the tumor biology of the examined subset of acute myeloid leukemia and may contributed to their development.

INTRODUCTION

When aberrant myeloblasts build up, usually in the bone marrow, they cause bone marrow failure and mortality, which is the cause of acute myeloid leukemia (AML). Peripheral blood involvement is common, although organ infiltration particularly worrisomely the brain and, or lungis uncommon and more frequently observed in high blood blast patients counts (e.g., >50 000/L). AML that appears to be restricted to locations other than bone marrow or blood is known as granulocytic sarcoma (GS). Because GS typically advances to marrow involvement within a year, it should be handled similarly to AML with marrow involvement[1]

HHV6 infection typically proceeds according to the standard herpesvirus replication cycle, whih includes the release of contagious virions and cell lysis. For unknown causes, HHV6 can potentially integrate into the host DNA,

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resulting in a "unclassl" type of latency. According to research in vitro infection by Arbuckle et al,can result in HHV6 chromoso gration (ciHHV6) with a chance of reactivation and the production of infectious HHV-6.[2]

Early to mid-1990s saw the first reports of ciHHV-6 in vivo when Luppi et al. discovered a potentially and partial full-length integrated of HHV-6 genome in newly isolated a peripheral blood mononuclear cells (PBMC) DNA.[3,4]

1 copy of the integrated virus is found in the cell in ciHHV-6-positive people, which is suggestive of hereditary transmission[5]

The cause of roseola infantum, often known as the sixth childhood ailment , is the widely ubiquitous virus known as HHV-6B.[6] In hematopoietic transplant recipients, HHV-6B is a worry as well because of the connection between viral reactivations and a number of illnesses that can range in severity from minor to fatal. While nearly everyone on the planet has HHV-6B, HHV-6A seems less common in North America, Japan, and Europe. Interestingly, in sub-Saharan Africa, HHV-6A is the most common variant linked to viremic newborn infections.[7]

early antigen p14 for HHV6 was discovered in megakaryocytes and 54% of the 36 patients with chronic myelogenous leukemia had blasts in their bone marrow, according to a single study that looked into the antigen's expression in leukemia.[8]

tumor suppressor called P14ARF is activated by mitogenic arousal, where in enhanced MYS activity is mediated by RAS activates P14ARF, which in turn prevents MDM2 from performing its role. Additionally, a P53/MDM2 feedback loop exists in which P53 can activate the MDM2 protein, while MDM2 can also deactivate P53[9]

The essential p16INK4A and p14ARF which control the cell cycle are a protein that the CDKN2A encodes; 9p21.3 (cyclin-dependent kinase inhibitor 2A) gene, and they also play a crucial part in programmed by the tumor-suppressive mechanisms,cell die functions. Therefore, p16INK4A and p14ARF tumor suppressor expression and activity are diminished with deletion, mutation, or genetic polymorphisms of CDKN2A, which results in a dysregulation of cell proliferation and the emergence of cancer[10]

The current study was designed to unravel the rates of HHV-6B infection and P14 rs3731249 gene polymorphism in patients with Acute Myeloid Leukemia.

MATERIAL AND METHODS

This study is designed as case-control study.

Study groups

Blood samples from each patient in the research with AML should be enrolled, that sort according to.

- Group of blood samples from Patients with AML.
- Blood from ostensibly healthy people serves as the control group.

Sample Collection

The samples will be taken from patients treated with Patients with AML and apparently healthy persons as control group from the general hospitals and numerous smaller clinics in the center Euphrates -Iraq.

Extraction of Viral Genome

Viral genomic DNA from both patients and control groups' blood was extracted using akit for extracting viral DNA (PATHOGENE- INTRON\ KOREA). After then was detection of HHV-6 by PCR technique.

Detection of SNP of P14 rs3731249 by Sequencing

Total genomic DNA from both patients and control groups' blood was extracted using DNA extraction kit (G -SPIN-INTRON\ KOREA). After then was the detection of determine of P14 rs3731249 SNP by Sequencing.

Statistical Analysis

To detect the significance between the studied variables in this study, Chi-square test was applied, where all these statistical analyses were done using the Version– 23 SPSS program where the $p < 0.05$ value was considered significant.

RESULTS

Age distribution of study groups

The studied tissues s were related to patients with acute myeloid leukemia whose age ranged from 2 to 80 years (mean = 51.6 + 11.5 years), while their control counterparts have a mean of 49.5 + 12.4 years. However, on comparing the age of these two groups, no significant variations were detected ($p > 0.05$) (Table 1).

- Detection Rates of HHV-6B by Using PCR Technique
- Extraction of Viral Genome

It is found that 42% (42 out of 100 cases) of the acute myeloid leukemia specimens were having DNA viral genome. While, the control group, 5 out of the 100 (5%) blood specimens were having DNA viral genome (Table 2). A statistical significant difference between the results of study groups ($p = 0.01$).

- Detection of HHV-6B Genome by Conventional PCR:

Thirty –three percent (14 out of 42 cases) have HHV-6B genome in patients with AML .While ,no positive HHV-6B genome in AHC specimens (Figure and Table). The statistical analysis of the differences between these two groups were significant ($p = 0.02$).

- The HHV-6B Results in Acute Myeloid Leukemia specimens according to the Age Groups

The frequency percent of HHV-6B according to age groups (1-20 years), (21-40 years), (41-60 years) and (61-80 years) were 12.2; 8.1; 4.2 and 4.1%, respectively. Significant differences ($p<0.05$) were found according to age groups (Table 4).

- The PCR HHV-6B- DNA Positive in Patients with AML According to the Gender.

Table 1: The age of patients with acute myeloid leukemia

Range(years)		*S. E*	*S. D*	*Mean of age (years)*	*No.*	*Study groups*
Maximum	*Minimum*					
80	6	1.97	11.5	51.6	100	AML
66	8	2.43	12.4	49.5	100	AHC
P-value = 0.3 No. sign. (P<0.05)					200	Total

Table 2: Viral genome detection in blood specimens

Viral Genome		*AML group*	*AHC group*⁺	*Pearson Chi-Square (p-value)*
Positive	N	42	5	P=0.01 Hig.sign. (P>0.05)
	%	42%	5%	
Negative	N	58	95	
	%	58%	95%	
Total	N	100	100	
	%	100%	100%	

⁺AHC means apparently health control

The highly percentage of gender of patients with AML that have positive HHV-6 B-DNA PCR results was males 66.7% (8 out of 12 cases) followed by females 33.3% (4 out of 12 cases). Statistical analysis revealed significant differences in gender whom are positive for HHV-6B-DNA PCR (P<0.05).

- Genotyping of P14 rs3731249 Among study groups

The findings revealed that in the GE patient group, the DNA polymorphism distributions were 53.1% (17 out of 32 cases), 21.9% (7 out of 32 cases), 15.6% (5 out of 45 cases), and 9.3% (3 out of 32 cases) according to GA, CT, AT, and AG genotypes of P14 rs3731249 polymorphism.However, the P14 rs3731249 polymorphism distributions for the GA and AG genotypes were 60% (3 out of 5 cases) and 40% (2 out of 5 cases), respectively

DISCUSSION

The most prevalent viruses that causes cancer in human r were previously reviewed;[11] they are likely inducing immunosuppression, triggering oncoproteins to modify host cells, and altering the expression of a protein in the host cells. Brain tumors, as heterogeneous, complex, multifactorial, and non-communicable neurological diseases, arise in the brain parenchyma and affect both adults and children.[12]

It was shown that HHV-6 has infected over 90% of children before their 3 years of age, where it established viral latency is present in lymphocytes and is highly potent to trigger chronic inflammation and /or immunosuppression pathways.[13,14] It was reported that the natural HHV-6 infection among those infected healthy adults is clinically either asymptomatic or has presented with non-specific symptoms.[15]

A small significant relationship between elevated HHV-6seropositivity and with AML, 95% CI 1.07-1.33) was seen in the biggest case control using a serology study undertaken on individuals with haematologic malignancies who had not yet received immunosuppressive treatment. The findings provided here are consistent with case control research, in which AML

Table 3: Results of HHV-6 B- DNA in AML specimens among study groups

	AML N/ %	*AHC* N/ %*
Positive	12 (28.6%)	0\00
Negative	32 (71.4%)	5(100%)
Total	42 (100%)	5 (100%)

AHC* means apparently health control

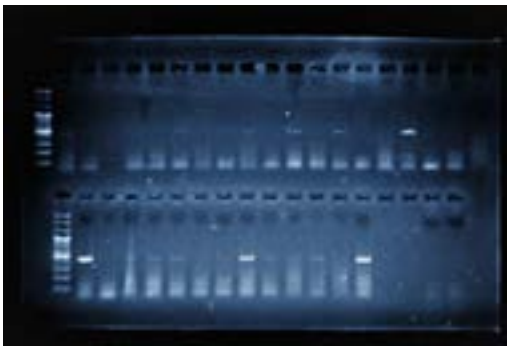

Figure 1: Detection of HHV-6 B from patients with acute myeloid leukemia, Lanes 1.5% agarose gel electrophoresis, TBX 1X , at voltage 85 Volt for 1h , lanse(480bp) were positive

Table 4: Frequency of HHV-6B-DNA positive among according to their age groups patients with acute myeloid leukemia.

Patient	*HHV6-B*	*p-value*
Age (1-20)	12.2%	Anova test P=0.04 (P<0.05)
Age (21-40)	8.1%	
Age (41-60)	4.2%	
Age (61-80)	4.1%	

Table 5: PCR results of HHV-6B- DNA in AML based on the gender of patients

Gender of Patients	*HHV-6- Infected AML*	
	No.	*%*
Men	8	66.7
Women	4	33.3
The analysis Statistical	(P<0.05)= 0.04	

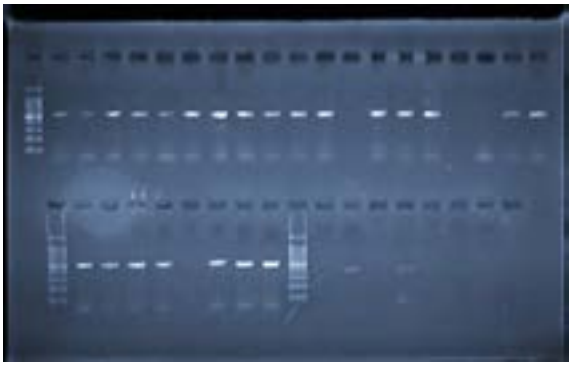

Figure 2: P14 rs3731249 from patients with acute myeloid leukemia, Lanes 1.5% agarose gel electrophoresis, TBX 1X , at voltage 85 Volt for 1h , lanse (525 bp) were positive

Table 6: the Comparison between patient with AML and AHC on percentages of P14 rs3731249 expressed gene polymorphism

Polymorphism of P14 rs3731249	*Type of Mutation*	*Study group*		*OR [Patients]*	*OR [Control]*	*p-value*	*95% C.I for OR [Patients]*	
		HC NO.(50)	*GE NO.(150)*				lower	Upper
G\A	Transtion	60%	53.2%	0.8	1.7	*0.001*	0.83	0.99
C\T	Transversion	0.0%	21.8%	0.7	1.4	*0.008*	0.77	0.97
A\T	Transversion	0.0%	15.5%	0.6	1.3	*0.006*	0.80	0.96
A\G	Transtion	40%	9.4%	0.8	1.1	*0.003*	0.88	0.95

patients had a greater prevalence of HHV-6 antibodies than the control group (95% CI 1.90-3.74)[16]

In addition, malignant cells in two cases of monoblastic leukemia were shown to have HHV-6 DNA, immature viral particles,and antigens,[17]

whereas ten AML patients in another research had undetectable HHV-6 DNA upon blot hybridization.[18] According to several investigations done on healthy persons,the prevalence of HHV6 might be affected by age, sex, region and ethnicity, with titres being higher in children than in adults, in females than in men, and in Ghanaians compared to Asians and White Americans.[19-21] In our investigation, neither age nor sex-based differences in seropositivity were found

The (CDKN2A/B) genes' inactivation is a major factor in the etiology and medication resistance of ALL and can result in the malignant proliferation of tumor cells(.CDKN2A/B) inactivation was found to increase pre-B cells' capacity for self-renewal, ensure the development of their full leukemic potential, and amplify the resistance pcr ABL1 positive ALL mouse models treated with tyrosine kinase inhibitors (TKIs), according to in vitro and in vivo studies.[22,23] In both childhood and adult ALL, deletions of CDKN2A/B occur commonly (30–50%). Numerous research have looked at the prognostic significance of CDKN2A/B deletions, although the findings are still debatable.[24]

Previous studies have linked two SNPs at the CDKN2A gene to ALL risk. first SNP, rs3731249 C>T, is found in exon 2 of the(CDKN2A0 gene and causes Ala148Tr (Alanine to Treonine) alteration that affects the function of the p16INK4A and p14ARF proteins as well as the reduction in gene expression[25,26] This study discovered a significant correlation between ALL and the genotype and allele frequency of the rs3731249 gene. Similar results were found in a research by Gutierrez-Camino et al. that showed rs3731249 increased incidence of B-ALL in Spanish population.[27] Another study found that this SNP tripled ALL incidence in children from European countries.[28]

Additionally, according to Vijayakrishnan et al.,[29] the link between rs3731249 and ALL was not limited to a particular subgroup of B-cell ALL.

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