Original Research Article

Seropositivity for CMV, EBV, HBV, HCV, and HIV in Leukemia Patients and Its Relationship with Cytogenetic Changes

Abstract

Introduction

leukemias are involving the bone marrow and the soft tissues in the inner parts of the bones, where new blood cells are formed. This malignancy is the most common pediatric cancer, and its etiologic causes are not well understood. This multifactorial disease is believed to be linked to genetic and non-hereditary environmental factors. Cytogenic analyses of chromosomal abnormalities provide diagnostic and prognostic values in leukemia patients. Given the high prevalence of viral diseases and clinical suspicions of the relationship between certain viral infections and leukemia, it is necessary to investigate this possible relationship, especially in third-world countries. The present study recruited 65 children with leukemia (AML, CML, or ALL) who were presented to two tertiary hospitals. At first, all the patients underwent testing for HBV, HCV, CMV, EBV, and HIV. Bone marrow specimens were studied for identifying possible chromosomal abnormalities in cytogenic investigations. According to our findings, there was a relationship between the incidence of leukemia, the 12:21 chromosomal translocation, and CMV infection. Therefore, preventing CMV infection can lead to a reduced incidence of leukemia. It is expected that the findings of this study enlighten scientists to conduct more extensive research on the relationship between viral diseases and leukemia in third-world countries.

Materials and Methods

: The present study recruited 65 children with leukemia (AML, CML, or ALL) who were presented to two tertiary hospitals. At first, all the patients underwent testing for HBV, HCV, CMV, EBV, and HIV. Bone marrow specimens were studied for identifying possible chromosomal abnormalities in cytogenic investigations.

Results:

According to our findings, there was a relationship between the incidence of leukemia, the 12:21 chromosomal translocation, and CMV infection. Therefore, preventing CMV infection can lead to a reduced incidence of leukemia.

Conclusion:

In this study, we demonstrated that leukemia is relevant to the 12:21 chromosomal translocation and CMV virus infections, So the reduction in leukemia prevalence is dependent on the prevention of CMV disease. It is expected that the findings of this study enlighten scientists to conduct more extensive research on the relationship between viral diseases and leukemia in third-world countries.

Key point: Chromosomal Abnormalities, Leukemia, Viral Diseases

Introduction

Leukemia, a common hematologic malignancy in children, usually originates from the bone marrow and causes abnormal cell proliferation in the peripheral blood (1). This malignancy is classified into different categories based on the cell lines involved, including Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML), and Acute Myeloid Leukemia (AML). All leukemias result from an uncontrolled proliferation of myeloid and lymphocytic precursor cells and their accumulation in the bone marrow. There are multiple forms of leukemia occurring in children, ALL is the most common form of childhood leukemia followed by AML(2, 3). Leukemia cells also influence the proliferation of other blood cells in the bone marrow, including the erythrocytes and platelets (4). Treatment of Leukemia is complicated and depends on the patient's age and health status, as well as the type of leukemia and the extent of the disease (5, 6). Some common presentations of leukemia include fever, chills, fatigue, weakness, anorexia, nausea, weight loss, night sweats, bone, and joint pain, dyspnea, recurrent infections, and cutaneous lesions. Respiratory tract infections and pneumonia are the leading causes of death in these patients (7).

The etiology of leukemia has not yet been fully understood, and a combination of genetic and non-hereditary, environmental factors forms the major risk factors. People with exposure to smoke, ionizing radiation, chemicals like benzene, previous chemotherapy, and Down syndrome are predisposed to the disease(8). Moreover, individuals with a family history of leukemia have a higher risk of developing the disease (9). The therapeutic measures for this disease include chemotherapy, radiotherapy, targeted therapies, bone marrow transplantation, or a combination of these methods. Supportive and palliative care can be also provided(10, 11).

Similar to other cancers, leukemia is caused by DNA mutations, which activate oncogene, inhibit tumor suppressor genes, and cause malfunctioning in the normal cell cycle, cell maturation, and apoptosis (12). In addition to gene mutations, some chromosomal abnormalities can also pose an increased risk of leukemia development. For example, Down and Fanconi syndromes are risk factors for AML development(13). In general, structural and numerical abnormalities of the chromosomes can be predisposing factors for leukemia and impact the response to treatment. Therefore, several studies have emphasized the application of cytogenetic analyses of chromosomal abnormalities in diagnosing leukemia, determining its prognosis, and selecting appropriate treatments for disease management (14- 16). It is now estimated that 10% of the world's cancers are attributed to viral infections, with the vast majority (> 85%) occurring in developing countries (17).

Considering the high prevalence of viral infections and the clinical suspicions on the possible relationship between viral infections and leukemia, the importance of performing independent studies that investigate the potential relationship between these viral infections and the cytogenetic abnormalities in leukemia patients is highlighted. Therefore, the present study investigated this relationship

Methods

Participant Characteristics

This cross-sectional descriptive study recruited 65 patients with leukemia (AML, CML, or ALL) who were presented to the Amirkabir Hospital, Arak, Iran, and the Golestan Hospital, Tehran, Iran, between November 2020 and August 2021. The participants were included in the study after giving written informed consent. The bone marrow and blood samples used in the present study were primarily provided for conventional diagnostic and therapeutic purposes, and the participants did not undergo any diagnostic tests and sampling merely due to the study.

Demographic features and medical history information, including the current age, age of disease onset, family history, and medical history were initially collected.

Viral infection investigations

To diagnose HBV, HCV, CMV, EBV, and HIV, the participants were referred to the laboratories of the Amirkabir Hospital, Arak, Iran, or the Golestan Hospital, Tehran, Iran. An Enzyme-Linked Immunosorbent Assay (ELISA) technique was used for the diagnosis.

Chromosomal abnormality investigations

A volume of 3 mL of bone marrow was aspirated from the participants. Then, the bone marrow aspirates were kept in a sterile transport tube containing specific transport media with 600 µL FBS, 3 ml RPMI-1640, and 100 µl sodium heparin. On the same or the next day, 200-800 µl of each bone marrow specimen was cultured in a media containing 20% FBS, 5 ml Marrowmax medium, 100 U/ml penicillin or streptomycin, and 2 mM L-glutamine. The samples were incubated for 24 hours at a temperature of 37 ° C. Then, 50 µlcalcined(10 µg/ml) was added, followed by 20 minutes of incubation. In the following, a 7-8 ml hypotonic solution of potassium chloride was added. After 25 minutes, a fixative solution containing methanol and acetic acid (ratio: 3:1) was added to the smears of each specimen thrice. In the next step, slides were prepared from the chromosomes at metaphase in an environment with controlled humidity and temperature. The slides were incubated for 24 hours at a temperature of 65 ° C and then stained using the trypsin-Giemsa technique (19).

Statistical analysis

Data analysis was performed using descriptive and analytical statistical methods. The SPSS version 22 (IBM © Corp., Armonk, NY, USA) was used, and the results obtained were reported in absolute frequency and percentage (%). Moreover, the table and diagrams summarized the results. Finally, analyses were made to assess the frequency and the type of chromosomal abnormalities in patients seropositive for HBV, HCV, CMV, EBV, and HIV.

We evaluated 65 patients and 69.20% of them (n=45) were boys. The mean age was 6.56±2.11 and 69.23% of participants (n=45) were in 1-7 age groups.

Two patients were infected with EBV and four patients were infected with CMV and all of them had chromosomal translocation (12:21). However, according to Table 2, further analyses showed no significant relationship between HBs Ab and chromosomal abnormalities ($P>0.05$).

According to our results, no significant relationship was found between chromosomal abnormalities and viral infections with HIV, EBV, HBV, and HCV ($P>0.05$). On the other hand, our results on the prevalence of positive CMV IgG in the study participants showed that four children infected with CMV had chromosomal translocation (12:21) in their genomes. Thus, CMV infection is highly suspected to cause a chromosomal abnormality and predispose children to leukemia ($P<0.5$).

Results

We evaluated 65 patients and 69.20% of them (n=45) were boys. The mean age was 6.56±2.11 and 69.23% of participants (n=45) were in 1-7 age groups. Also, 26.15% (n=17) and 4.62% (n=3) were in the 8-15 and more than 15 years age groups respectively.

Table 1 shows the distribution of chromosomal abnormalities in the participants. Based on this table, the most of chromosomal abnormalities were related to chromosomal translocation (12:21) (n=30, 46.2%). While, inversion 16 and translocations of t(8:16), t(8:21), and t(8:14) were shown in a few cases.

None of the children were infected with HIV, HBV, and HCV. Hence, no significant relationship was found between these infections and chromosomal abnormalities in leukemia patients. Moreover, two patients were infected with EBV and four patients were infected with CMV and all of them had chromosomal translocation (12:21). However, according to Table 2, further analyses showed no significant relationship between HBs Ab and chromosomal abnormalities (P>0.05).

Table 1. Distribution of chromosomal abnormalities in the study participants

Chromosomal abnormality	Frequency (n=65)	Percentage (%)	95% CI
Chromosomal translocation			
(12:21)	30	46.2	36.57-63.42
(8:16)	1	1.5	0.04-9.23
(15:17)	4	6.2	7.34-27.42
(9:22)	4	6.2	7.34-27.42
(8:21)	1	1.5	0.04-9.23
(8:14)	1	1.5	0.04-9.23
Down mosaicism	1	1.5	0.04-9.23
Trisomy 21	3	4.6	0.4-11.90
Inversion 16	1	1.5	0.04-9.23
Without translocation/ undetermined translocation	19	29.2	18.57-63.42

Table 2. The relationship between HBs Ab and chromosomal abnormalities in the study participants			
Chromosomal abnormality	Negative N=17	Positive N=48	P-value*
t (12:21)	8 (47.05)	22 (45.83)	0.919
t (8:16)	1 (5.88)	0 (0.0)	
t (15:17)	0 (0.0)	4 (8.33)	
t (9:22)	2 (11.76)	2 (4.16)	
t (8:21)	1 (5.88)	0 (0.0)	
t (8:14)	0 (0.0)	1 (2.08)	
Down mosaicism	0 (0.0)	1 (2.08)	
Trisomy 21	0 (0.0)	3 (6.25)	
Inversion 16	1 (5.88)	0 (0.0)	
Without translocation/ undetermined translocation	4 (23.52)	15 (31.25)	
* P-value was calculated by chi-2 statistics at 95% levels of CI			

Table 3 evaluated the association between leukemia types with viral infections, including HIV, EBV, CMV, HBV, and HCV. There were no significant differences between the frequencies of these infections based on types of leukemia.

Table 3. Relationship between different types of leukemia and viral infections

Viral Infection	Type of Leukemia		P-value*
	ALL (n=59)	AML (n=6)	
HIV			
Negative	59 (100.0)	6 (100.0)	-
Positive	0 (0.0)	0 (0.0)	
HBV			
Negative	57 (96.61)	6 (100.0)	0.647
Positive	2 (3.39)	0 (0.0)	
CMV			
Negative	55 (93.22)	6 (100.0)	0.510
Positive	4 (6.78)	0 (0.0)	
HCV			
Negative	59 (100.0)	6 (100.0)	-
Positive	0 (0.0)	0 (0.0)	
HBs Ab			
Negative	15 (25.42)	2 (33.33)	0.674
Positive	44 (74.58)	4 (66.67)	
HBs Ag			
Negative	59 (100.0)	6 (100.0)	-
Positive	0 (0.0)	0 (0.0)	

* * P-value was calculated by chi-2 statistics at 95% levels of CI

Discussion

Cancer with 6.7 million deaths per year, is a devastating disease that causes serious problems for the affected people, their families, and health care systems (18). The development of treatment and prevention strategies for the management of cancer depends on our knowledge of cancer cells and the caused mechanisms. Over the past 30 years, it has become clear that several viruses play an important

role in the multistage development of human neoplasms (19, 20). Oncogenic viruses can contribute to various stages of the carcinogenic process. The association of the virus with particular cancer can be between 15-100% (20). In addition to elucidating the cause of many human cancers, the study of carcinogenic viruses has been invaluable in discovering and analyzing key cell pathways in cancer.

The present study investigated the relationship between chromosomal abnormalities in children with leukemia and with viral infections of CMV, EBV, HCV, HBV, and HIV. This study aimed to establish a better understanding of the possible relationships between leukemias and certain viral infections and thus enlighten the public healthcare system in developing countries to save people at risk of leukemia by controlling the incidence of viral diseases.

In this study, 65 children with leukemia were studied to investigate the association between viral diseases and the possibility of genetic abnormalities. Among 65 patients, 69.20% were boys and 30.80% were girls. Also, most of the children participating in the study (69.23%) were between 1-7 years old. The mean age of the children was 6.84 ± 4.40 years. The frequency of leukemia in children was found 92.3% with ALL. Also, the frequency of chromosomal displacement in children showed that most of them, 46.2%, had a 12:21 chromosomal shift in their genome.

According to our results, no significant relationship was found between chromosomal abnormalities and viral infections with HIV, EBV, HBV, and HCV ($P>0.05$). Therefore, it can be concluded that none of these viral infections can lead to chromosomal abnormalities, and thus increase the risk of leukemia. On the other hand, our results on the prevalence of positive CMV IgG in the study participants showed that four children infected with CMV had chromosomal translocation (12:21) in their genomes. Thus, CMV infection is highly suspected to cause a chromosomal abnormality and predispose children to leukemia ($P<0.5$).

The main objective of the present study was to investigate the relationship between lifelong exposure to HBV, HCV, HIV, EBV, and CMV and the possibility of gene mutations or chromosomal translocations leading to leukemia. In general, it is the CMV infection that requires greater attention compared to other viral infections. Therefore, preventive measures aiming to reduce the incidence of leukemia should be focused on controlling the cytomegalovirus infection, especially in third-world countries where viral diseases are prevalent.

Approval statement: The project was found to be following the ethical principles and the national norms and standards for conducting Medical Research in Iran.

Limitations of the study

Limitations of the study The main limitation of the current study was the decrease in births in recent years. As a result, sample collection took a lot of time.

Clinical trial registration

code: IR.AJAUMS.REC.1399.161

Evaluated by: AJA UNIVERSITY OF MEDICAL SCIENCES

Approval Date:2020-11-15

The research followed the tenets of the Declaration of Helsinki. Authors' contribution ME, NA, and VF were the main researchers of the study. SS and MN collected the samples. MN reviewed the manuscript and critically evaluated the intellectual material. All authors participated in writing the final draft of the manuscript, revised the manuscript, and critically evaluated the intellectual material. All authors read and verified the content of the manuscript and checked the accuracy and integrity of each part of the study. Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical issues

The Ethics Committee of AJA University of Medical Sciences approved this study. The institutional ethical committee at AJA University of Medical Sciences approved all study protocols(code: IR.AJAUMS.REC.1399.161). Accordingly, written informed consent was taken from all participants before any intervention. This research considered and observed all the principles of the Helsinki Declaration. Moreover, ethical issues (including plagiarism, data fabrication, and double publication) were completely observed by the authors. The neonates' parents gave their consent to publish the research. Funding/Support. This work was supported by the Deputy of Research and Technology of AJA UNIVERSITY OF MEDICAL SCIENCES). The project was found to be following the ethical principles and the national norms and standards for conducting Medical Research in Iran.

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