Detection of Factor V Leiden among Sudanese Acute Lymphoblastic Leukemia Patients at Khartoum state, 2023

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Abstract:

Background: Factor V Leiden is an inherited disorder in which hemostatic balance tends towards thrombophilia blood clotting. Factor V Leiden is the name of a specific gene mutation that results in thrombophilia, which is an increased tendency to form abnormal blood clots that can block blood vessels, which represents an increase in rates of thrombophilic morbidity and, when found in acute lymphoblastic leukemia patients, increases the risk of thrombotic events. The study was designed to detect Factor V Leiden in ALL Sudanese patients. ***Material and method:*** This was a descriptive case-control study. A total of one hundred (100) participants were included in this study after their approval had been acquired. 50 ALL patients were enrolled as case groups, and 50 as control groups. The detection of Factor V Leiden was done using conventional PCR. This study was conducted from January to March in Khartoum State. ***Results***: The investigation revealed that out of 50 patients, none of them had the Factor V Leiden mutation, and the control group also had no mutation. ***Conclusion***: According to the results of this study, the factor V Leiden mutation was not detected in ALL Sudanese patients. No association was observed between factor V and ALL.

Keywords: Factor V Leiden, Detection, ALL, leukemia.

Introduction

Leukemia is a group of blood cancers that usually begin in the bone marrow and result in high numbers of abnormal blood cells **[1].** These blood cells, often known as blasts or leukemia cells, are not fully matured. Symptoms may include bleeding and bruising, bone pain, fatigue, fever, and an increased risk of blood tests or bone marrow biopsy, infections, these symptoms occur due to a lack of normal blood cells. Diagnosis is typically made by blood tests or bone marrow biopsy **[1].** The exact cause of leukemia is unknown, A combination of genetic factors and environmental (non-inherited) factors are believed to play a role **[2].** Risk factors include smoking, ionizing radiation, petrochemicals (such as benzene), prior chemotherapy, and Down syndrome **[2].** People with a family history of leukemia are also at higher risk **[2].** There are four main types of leukemia—acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) as well as several less common types **[3].** In young children, acute lymphoblastic leukemia (ALL) is the most prevalent kind of leukemia. Adults are affected as well, particularly those 65 and older. Standard treatment involves chemotherapy and radiotherapy. Acute bi-phenotypic leukemia, Burkitt's leukemia, precursor B acute lymphoblastic leukemia, and precursor T acute lymphoblastic leukemia are among the subtypes. While most cases of ALL occur in children, 80% of deaths from ALL occur in adults **[4].** Around 6,540 new cases of ALL (3,660 males and 2,880 females) and 1,390 ALL-related fatalities (700 men and 690 females) are expected worldwide in 2022. Less than half of one percent of cancer cases in the US are associated with ALL, indicating that it is not a prevalent malignancy. The average person's lifetime risk of getting ALL is about 1 in 1,000. The risk is slightly higher in males than in females, and higher in Whites than in African Americans **[4].** In African countries, although data is somewhat fairly scant. It seems that the occurrence of leukemia is not as far-off lower as it arises in European countries **[5].** Cancer registries in Africa suffered from a lack of good medical supply and incompetent methods for detection such as diagnosing by blood smears or by applying cytology procedures **[5].** Moreover, in the practice of pediatric knacks in the tropics, diagnosis of leukemias might have been unexploited since the common clinical presentation of childhood lymphadenopathy, leukemia fever, and anemia is easier to get confusing with extra reciprocal circumstances **[6]**, In Sudan acute ALL accounts for (19.8%) among all childhood malignancies **[7].** The cause of ALL is unknown in general, ALL is most likely to affect children and older adults. The following factors may raise a person's risk of developing ALL: children younger than 15 and adults older than 50 are more likely to develop ALL **[2].** White people are somewhat more likely than black people to develop ALL for reasons that are not understood **[2].** Genetic conditions: people with some genetic conditions have a higher risk of ALL than the general population, these

conditions include the following syndromes (Down syndrome, Fanconi anemia, etc.) **[2].** High dose of radiation: people who have been exposed to high levels of radiation may be more likely to develop ALL **[9].** Viruses: ALL can be associated with previous viral infection eg (EBV, or infectious mononucleosis **[10].** Acute lymphoblastic leukemia (ALL) and acute lymphocytic leukemia (ALL) originate from distinct lymphocyte subtypes. Almost 85% of cases of ALL begin from B cells (also called B lymphocytes) and 15% begin from T cells (also called T lymphocytes) **[11].** Factor V Leiden is an autosomal dominant disorder discovered in 1993, and was found in 20% to 60% of patients with recurrent thrombosis with no previously recognized inherited thrombotic disorder. The majority of cases (92%) are inherited and caused by mutation of factor V, Arg506Gln, referred to as factor V Leiden. Factor V Leiden is the most common inherited cause of thrombosis in the white population of northern and western Europe. In the United States, factor V Leiden is seen in 6% of whites. The homozygous form of factor V Leiden has an 80-fold increased risk of thrombosis, while heterozygous carriers have a 2- to 10-fold increase in thrombosis. Recall that the protein C–protein S combination inactivates factors V and VIII. Overproduction of clots is caused by mutated factor V, also known as factor V Leiden, which is not inactivated. If additional inherited or acquired risk factors present, the likelihood of thrombosis is significantly elevated. The thrombotic complications associated with factor V Leiden are venous thromboembolism (VTE) **[12].**

Materials and methods

Study Design

The study will be a descriptive case-control hospital-based study.

Study Area

This study was carried out in Khartoum State; samples were collected from the Radiation and Isotope Centre in Khartoum. A laboratory investigation was done in the Exon Laboratory for Molecular Biology in Khartoum State.

Study Period

This study was conducted during the period from January to March 2023.

Study Population:

Sudanese patients diagnosed by ALL according to the radioisotope center protocol for leukemia diagnosis identification in Khartoum who attend Radiation and ISO Tops Centre Khartoum.

Sample Size

A total of one hundred (100) samples, fifty (50) Sudanese acute lymphoblastic leukemia-diagnosed patients as cases, and fifty (50) healthy Sudanese non-acute lymphoblastic leukemia-diagnosed individuals as controls.

Inclusion criteria

Sudanese patients diagnosed with ALL were enrolled as the case group in Khartoum state, and healthy Sudanese individuals for the control group were enrolled in this study.

Exclusion criteria

Patient professionally diagnosed ALL who were critically ill or with other chronic.

Sample collection

Under full septic techniques, 3 ml of venous blood samples will be collected from all participants in the ethylene diamine tetraacetic acid (EDTA) anticoagulant for DNA extraction and the detection of the Factor V Leiden mutation using conventional PCR (Sensquest-Lab-Cyclone)

Genomic DNA Extraction

Instruction 300 ml whole blood were added to 1.5 ml in tube containing 900 ml RBC lysis solution, then mixed thoughts by vortexing and incubated for 5 minutes at room temperature centrifugation at 10000xg for 1 min then removed supernatant, then 300 ml cell lysis solution were added to the resuspend cells and did pipettes up and down to lyse the cell , and chilled samples to room temperature and then 100 ml PPT buffer were added to the cell lysate and vortexing vigorously at high speed for 20 seconds , then Centrifuged at 13000 – 16000xg for 3-5 minutes , then 300 ml of the supernatant that containing DNA

was taken into 1.5 ml tube, 300 ml 100% Isoprpanol were added and the samples were Mixed by inverting gently for several times to wash the DNA pallet , then Centrifuged at 13000 - 16000 xg for 1 min , and inverted the tube on a clean absorbent paper and allowed to air dry for 10 - 15 minutes , 150 ml DNA rehydration buffer was added dehydrated DNA by incubating at 65C for 30 min, for long term storge collected DNA is stored at 20c . Then the DNA purity O.D. 260/280 ratio and detected by electrophoresis to make sure it was free of any protein contamination.

Polymerase Chain Reaction (PCR)

Principle of PCR: The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer-mediated enzymes. DNA polymerase synthesizes strands of DNA complementary to the template DNA. The DNA polymerase can add an anucleotide to the pre-existing 3-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3rd end of the DNA polymerase.

Gel electrophoresis

Is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. The molecules travel through the pores in the gel at a speed that is inversely related to their lengths. This means that a small DNA molecule will travel a greater distance through the gel than a larger DNA molecule. When charged molecules are placed in an electric field, they migrate to either the positive or negative pole according to their charge.

Preparation of primers

For 100 pmol/µ from each primer was dissolved in DW. For 10 pmol/ µL, 10 µl of each primer was dissolved in 90 µl of DW.

Master maxime PCR PreMix

PCR premix (Intron Biotechnology Korea) which contains (Tag polymerase 5U/µl,dNTPs, reaction buffer (10x), and gel loading buffer) was premixed, ready to use a solution containing all reagents required for PCR (except template DNA and primers) and additional compound needed for direct loading onto an agarose gel and two tracking dyes (blue and yellow)that allow monitoring progress during the electrophoresis.

Protocol used for amplification

The protocol of amplification with Factor V developed by Exon laboratory, Multiplex PCR was used of a Thermocycler. The PCR mixture was subjected to an initial denaturation step at 94˚Cfor 10 min, followed by 40 cycles of denaturation at 94˚C for 1 min,55˚C for 1min, 72˚C for 1min and final extension at 72˚C for 10 min.

Preparation of 1X TBE buffer:

Ten ml of 10X was added to 90 ml of deionized water and heated until completely mixed.

Preparation of ethidium bromide

Five milligrams of ethidium bromide were dissolved in 500 µl DW and kept in a brown bottle.

Preparation of agarose gel

The amount of 2 g of agarose powder was dissolved in 100ml 1X TBE buffer and heated in a microwave for 1 minute. Then the mixture was cooled to 55 ˚C in the water bath. Then 2.0 µl of (20mg/ml) ethidium bromide was added, mixed well, and poured into a casting tray that was taped up appropriately and equipped with a suitable comb to form well in place. Any bubbles were removed and the gel was allowed to set at room temperature. After solidification, the comb was gently removed and the spacer from the opened slides was removed.

Visualization of PCR products

The gel tray was covered with TBE buffer up to the surface, and then 5µl of each PCR product was loaded into each well. Then to the first well of the casting tray 5µl of DNA ladder (100bp) was injected for each run. The electrophoresis apparatus was connected to the power supply (primer, 125v, 500mA, UK). The electrophoresis was done at 140v for 45min, after that, the gel was removed by gel holder and visualized by U.V transilluminator (Uvite-UK). The results were photographed using a gel documentation system.

Ethical considerations

This study was approved by the Ethical Committee of the Faculty of Graduate Studies and Scientific Research of the National University and the Ministry of Health, Khartoum State.

Data Analysis

The data obtained through the study were entered and analyzed by computer using Microsoft Office Excel and the SPSS statistical program to calculate the mean, standard deviation, and *P-value* by t-test. $P<0.05$ was considered statistically significant.

Results

This was an analytical Hospital-based study conducted to study the association of factor V Leiden mutation gene in acute lymphoblastic leukemia among Sudanese patients in Khartoum state. The investigation revealed that out of 50 cases, zero of the patients had factor V Leiden gene mutation, and the study also showed that the control group had no mutation (Table 1). Considering the case group; 74% of the population was male and 26% was female, and According to the control group, 64% of the population was male and 36% was female (Table 4). In the population with a chronic disease, in the case and control groups, 100% of the participants had no history of chronic disease (Table 5).

Table 1. Result of factor V Leiden gene by conventional PCR among case and control

Study Population	Result PCR	Frequency	Percent %
Case	Positive	00	00
	Negative	50	100
	Total	**50**	**100**
Control	Positive	00	00
	Negative	50	100
	Total	**50**	**100**

Table 2. Correlation between Factor V Leiden and other factors among case groups.

		Age	Gender	Chronic disease	History of ALL
FVL mutation	*P value*	0	0	0	0
	No	50	50	50	50

Table 3. Frequency of age group among case and control groups.

Study Population	Age group	Frequency	Percent %
Case	3 – 6	15	30
	7 – 11	23	46
	12 – 15	9	18
	16 – 20	3	6
	Total	**50**	**100**
Control	3 – 6	17	34
	7 – 11	16	32
	12 – 15	11	22
	16 – 20	6	12
	Total	**50**	**100**

Table 4. Frequency of gender among case and control group

Study population	Gender	Frequency	Percent %
Case	Male	37	74
	Female	13	25
	Total	**50**	**100**
Control	Male	32	64
	Female	18	36
	Total	**50**	**100**

Table 5. Frequency of chronic disease among case and control group

Study Population	Chronic disease	Frequency	Percent %
Case	Yes	00	00
	No	50	100
	Total	**50**	**100**
Control	Yes	00	00
	No	50	100
	Total	**50**	**100**

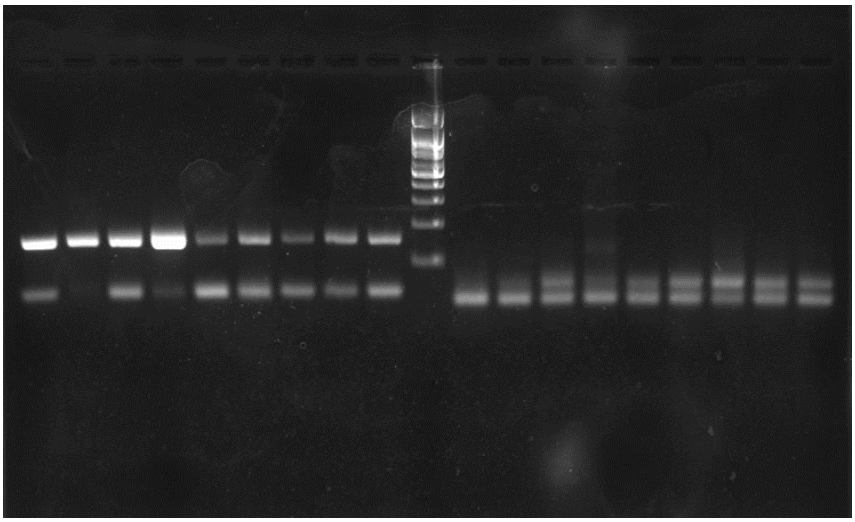

Figure 1. Result of Factor V Leiden by U.V transilluminator (Uvite-U)

Discussion

Factor V Leiden mutation is an established one of the risk factors for venous thromboembolic disorder, FVL mutation was reported with high prevalence in Caucasians (1–15%) but was absent in non-Caucasians like Africans and Asians. This is the first study to identify the association between FVL mutation and acute lymphoblastic leukemia in Sudan. Several studies have reported that the mutation of Factor V Leiden is a risk factor for increasing thrombotic events in acute lymphoblastic leukemia. This study detected the mutation of factor V Leiden susceptibility to acute lymphoblastic leukemia in the Sudanese population, other studies have been performed to detect the association between factor V Leiden mutation and increased thrombotic events in acute lymphoblastic leukemia but results were different. The current study shows no detection of Factor V Leiden among the case and control groups, this agrees with a study done by M.M Jadaon in Sub-Saharan Africa where there is no found of Factor V Leiden in this population **[13]**, Similarly de Stefano et al reported in their study not found Factor V Leiden among African American and Australia **[14].** Another study done by L.G.Mitchell in North America, her result did not show any relation between the presence of Factor V Leiden and thrombotic event, this disagrees with a study done by a Catholic University in Roma-Italy where their result showed the mutation of Factor V Leiden increased risk to venous thromboembolism in acute lymphoblastic leukemia patients, which disagree with the current study. The variance between the two studies' results may be due to the difference in ethnicity and geographical distribution.

Conclusion

According to the results from this study, the mutation of the factor V Leiden gene was completely absent in this study and didn't show any significant correlation, which indicates that there's no association of factor V Leiden mutation with ALL among Sudanese patients.

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Conflict of Interest

The author has affirmed that there are no conflicting interests.

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