Research Article

Detection of prothrombin mutation G20210A among acute lymphoblastic leukemia Sudanese patients at Khartoum state

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Abstract:

Background: Cancer is one of the most common causes of disease-related mortality in children in many countries. The most prevalent kind of leukemia in children is acute lymphoblastic leukemia (ALL). Is a hematological malignancy that originates from the abnormal proliferation of B-type or T-lymphocytes in the bone marrow. The prothrombin mutation gene (G20210A) increases the risk of thrombosis, and it is the second most common hereditable thrombophilia defect found in patients with venous thromboembolic disorder (VTE). ***Objectives***: This study aimed to find out how to detect the prothrombin mutation gene (G20210A) in children with acute lymphoblastic leukemia. DNA was extracted from whole blood by using iNtRON biotechnology (G-DEX TM IIb), and the mutation analysis for prothrombin (G20210A) was used by conventional thermal cycle PCR (sensoquest) and agarose gel to amplify the PCR product, which was visualized under UV light by the gel documentation system ***Methods***: This is a case-control study conducted in Khartoum state at the Radiation Isotope Centre Khartoum, which included one hundred, 50 children with acute lymphoblastic leukemia (case group), male and female, aged 3–20 years, and 50 healthy children of matched age and gender. From each participant, 3 ml of venous blood was collected in Ethylene Diamine Tetra Acetic (EDTA) vacutainers. ***Results***: The study result revealed that out of 50 participants, no prothrombin mutation (G20210A) was detected among ALL patients. The study also showed no mutation among the control group. Conclusion: The study concluded that there is no association between the prothrombin mutation (G20210A) gene and Sudanese acute lymphoblastic leukemia patients.

Keywords: *Prothrombin, Mutation, G20210A, ALL, leukemia*.

Introduction:

Leukemias are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood **[1].** Acute lymphoblastic leukemia (ALL) is caused by an accumulation of lymphoblasts in the bone marrow. It is the most common malignant disease of childhood; 75% of cases occur before the age of 6 years. Eighty-five percent of cases are of B-cell lineage, with the rest being of T-cell lineage **[2].** Acute lymphoblastic leukemia (ALL) is a heterogeneous group that results from the monoclonal proliferation and expansion of immature lymphoid cells in the bone marrow, blood, and other organs **[3].** Every year, about 6,000 new cases of acute lymphoblastic leukemia (ALL) are identified in the US. ALL is the most common pediatric cancer (representing approximately 25% of cancer diagnoses), and approximately 60% of all cases occur in children and adolescents younger than 20 years, with an annual incidence of 36.2 per 1 million persons and a peak age of incidence of two to five years (at which there are >90 cases per 1 million persons). ALL is diagnosed more frequently in boys than in girls, with a ratio of approximately 1.3:1 **[4].** Several genetic factors (most prominently Down's syndrome) are associated with an increased risk of ALL, but most patients have no recognized inherited factor **[5].** Epidemiologic studies of acute leukemia in children have examined several possible risk factors (e.g., environmental, genetic, or infectious) to determine the etiology of the disease. Only one environmental risk factor (ionizing radiation) has been significantly linked with ALL **[6].** The French American British (FAB) morphological criteria, which separated ALL into three subtypes (L1, L2, and L3) based on cell size, cytoplasm, nucleoli, vacuolation, and basophilia, was the first attempt at defining the disease. In 1997, the World Health Organization (WHO) proposed a composite classification in an attempt to account for the morphology and cytogenetic profile of the leukemic blasts and identified three types of ALL: B lymphoblastic, T lymphoblastic, and Burkitt-cell leukemia. In 2016, two new provisional entities were added to the list of recurrent genetic abnormalities, and the hypodiploid was classified as either low hypodiploid or hypodiploid with TP53 mutations. **[7].** Acute lymphocyte leukemia is divided into subtypes by immunological criteria based on the presence of a specific receptor or antigen on the cell surface of leukemic blast cells **[8].** Clinical features of ALL are a result of bone marrow failure; they include anemia. Neutropenia and Thrombocytopenia). Organ infiltration causes tender bones, lymphadenopathy, moderate splenomegaly, hepatomegaly, and meningeal syndrome (headache, blurring of vision, and diplopia) **[9].** Some symptoms of ALL can be vague. They include fatigue, fever, loss of appetite, and weight loss. Night sweats and tiny red spots just under your skin. Many of these symptoms happen because your body is reacting to a lack of healthy blood cells **[10].** ALL is diagnosed when the bone marrow aspiration and biopsy contain 20% or more immature cells or blasts determined to be lymphoid in nature. One important test is immunophenotyping, which determines whether the cells are lymphoid rather than myeloid **[11].** Patients with high-risk features usually receive induction therapy with agents including vincristine, prednisone, and L-asparaginase gaunorubicin). intensive consolidation therapy. Usually employing multiple agents and frequently repeating induction therapy and maintenance therapy that often includes late intensification or periodic pulses of intensified therapy **[12].** Many genetic and acquired risk factors are known to cause venous thromboembolic disorders (VTE); one of these is the

prothrombin gene mutation (G20210A), which was discovered in 1996. Prothrombin is a protein in the blood that is required for the blood to clot; it is also called factor II **[13].** The prothrombin gene mutation (G20210A) is a specific change in the genetic code that causes the body to produce too much of the prothrombin protein. An excess of prothrombin increases the risk of blood clotting. A prothrombin gene mutation is also called a factor II mutation or prothrombin variant **[14].** It's also defined as a single missense mutation; guanine is substituted by an adenine base pair in the nucleotide position G20210A of the 3'-untranslated region of the prothrombin gene, resulting in abnormal thrombin production predisposing to both arterial and venous thrombosis **[15].** The factor II mutation gene (G20210A) increases plasma prothrombin activity and increases the risk of venous thromboembolism **[16].** And also associated with deep vein thrombosis (DVT) and pulmonary embolism (PE) **[17].** Prothrombin gene mutation (G20210A): it is associated with various obstetric complications such as venous thromboembolism, recurrent pregnancy loss, preeclampsia, and abruption of the placenta **[18].** The Prothrombin variant is the second most common hereditable thrombophilic defect found in patients with venous thromboembolic disorder (VTE) **[19].** It's inherited as an autosomal dominant pattern with variable penetrance **[20].** The homozygous or double heterozygous prothrombin mutation (G20210A) is a rare inherited thrombophilic trait. Individuals with this genetic background have an increased risk of recurrent vascular thrombosis **[21].** The prothrombin gene mutation (G20210A) presents with an incidence of approximately 5% in a heterogeneous population and 45% to 63% in the thrombophilic population **[22].** Heterozygous prothrombin gene mutation (G20210A) is the most common inherited thrombotic disorder in the Caucasian population **[23].** And the prevalence of prothrombin gene mutation (G20210A) in Africans living in Africa, with Asians and Native Americans having the lowest rate of zero in western and southern African countries and 2.4%–3.9% in North African countries **[24].**

Materials and methods:

Study design

The study was a case-control hospital-based study.

Study area:

This study was performed at the Radiation and Isotopes Centre in Khartoum, and laboratory processing was performed at Exon Lab (Sudan-Khartoum-Elgamhuriaya Street).

Study population:

Sudanese patients diagnosed by ALL according to radiation and isotope center protocol for leukemia diagnosis identification in Khartoum who attend Radiation and Isotope Centre Khartoum.

Inclusion criteria:

Patients are professionally diagnosed with ALL according to radiation and the isotope center protocol for leukemia diagnosis and identification.

Exclusion criteria:

Patients were professionally diagnosed with all the other chronic diseases.

Sample Size:

100 samples were collected in this study, and 50 patients were in the case group. 50 were healthy individuals in the control group.

Sample collection:

Under full septic techniques, 3 ml of venous blood samples were collected from all

participants in the ethylene diamine tetra acetic acid (EDTA) anticoagulant for the DNA extraction.

Molecular method:

The mutation analysis for the prothrombin gene (G20210A) was done by polymerase chain reaction (PCR). Polymerase chain reaction (PCR) is an extremely important tool for molecular diagnosis as it can specifically amplify nucleic acid templates for sensitive detection, which can be performed in a capillary tube pseudo-isothermally within a significantly short time. Thermal cycling is implemented by including thermal convection inside the capillary tube, which stratifies the reaction into spatially separated and stable melting. Annealing and extension zones are created by temperature gradients.

DNA Extraction:

The DNA was extracted from a blood sample using G-DEX TM IIB Genomic DNA Extraction Kids (iNtRON Biotechnology, Korea).

Polymerase chain reaction:

The PCR were carried out to confirm the presence of prothrombin mutation allele (G20210A) the amplification were carried out by (conventional thermo cycler PCR-sensoquest) and using primer which is highly conserved region in prothrombin gene (G20210A) forward (5'-GCACTGGAGCATTGAGG ATC-3') and reverse (5'-TCTAGAAACAGTTGCCTGGCAG-3')

PCR assay 20ul final reaction mixture which consisted 4ul green master mix (intron biotechnology 'Korea) which consisted (Taq polymerase reaction buffer and DNTps and 1ul forward and 1ul reverse primer specific for amplification of prothrombin mutant allele (G20210A) and 5ul from DNA template and 9ul from D W to complete the volume to 20ul final reaction mixture The reaction tube incubated in the thermal cycler programme was performed with an initial denaturation for 10 minutes at 95°C, then 10 cycles of denaturation for 30 seconds at °C, annealing f for 30 seconds at 60°C, and extension for 1 minute at 72°C, and 25 cycles of denaturation for 30 seconds at 94°C, 'annealing for 30 seconds at 55°C, and final extension for 1 minute at 72°C.

Gel Electrophoresis:

2% (20 g) agarose gel was dissolved in 1X Tris Borate EDTA (TBE) buffer, and then put in a microwave (until it cleared). Wait for 2–3 minutes to cool down, then add 2 µl of ethidium bromide (iNtRON Biotechnology, 'Korea). Put the combs inside the gel casting try and to pour the gel onto the casting. When the gel is cooled and solidified, pull the combs out of the gel (the teeth of the comb leave impressions in the gel called wells where the DNA will be loaded). The gel is then placed in the electrophoresis chamber (a tank full of TBE). Then, add 3 µl from the ladder as control into wells on both the right and left sides of the gel, and add 5 µl from each DNA sample into the wells. The electrodes are connected to the power supply.

The amplified products were electrophoresed at 140 volts for 45 minutes and visualized under UV light by the gel documentation system. All these steps are applied in the case and control groups.

Data analysis:

Data was entered and tabulated using Excel, then analyzed by the statistical package for social science (SPSS) software version 23.

Ethical consideration:

This study was approved by the National University-Sudan ethical committee board. Informed consent will be obtained from every participant in this study before the samples are taken.

Results:

The study was conducted in a case-control hospital in the Radiation and Isotope Centre Khartoum from January to March with a total of 100 samples, divided equally into the case (patients with ALL) and control (healthy individuals). To detect the prothrombin (G20210A) mutation gene in all patients. According to the case group, 74% of the population was male and 26% was female. And in the control group, 36% of the respondents were female and 64% were male **(Table 1).** The age group of the cases ranged from 3 to 20 years. The age of the study population was categorized into two groups. Group (1): 3–11 years represents 67%. Group (2): 12–20 years represents 24% of the population. The age group of control was categorized into 2 groups: Group 1 (3–11 years) represents 66%, and Group 2 (12–20 years) represents 34% of the population **(Table 2).** Among the case group, for a total of 50 samples with ALL, the prothrombin mutation was not detected (as in the control group) **(Table 3).**

Table 1: Frequency of patient and control group according to the gender

Study population	Gender	Frequency	Percent (%)
Case	Male	37	74.0
	Female	13	25.0
	Total	50	100.0
Control	Male	32	64.0
	Female	18	36.0
	Total	50	100.0

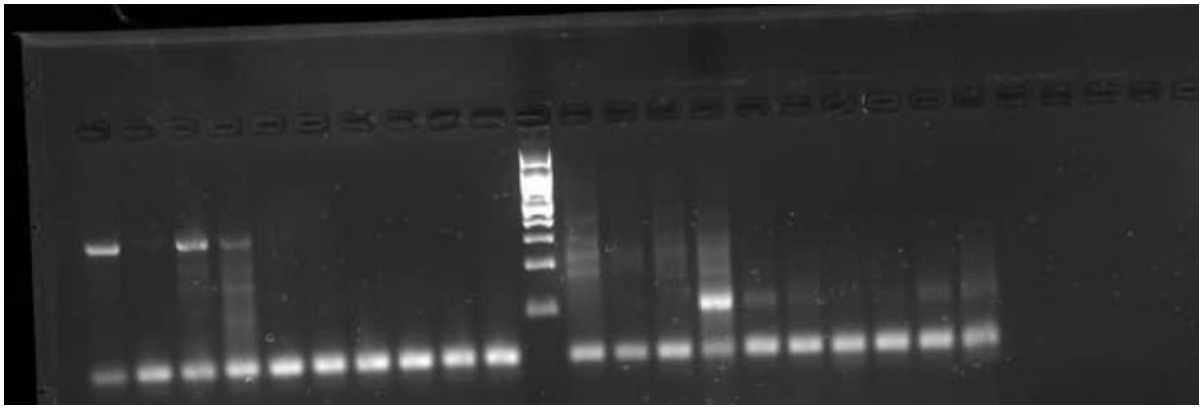
Table 2: Frequency of patients and control group according to age

Study population	Age group	Frequency	Percent (%)
Case	(3-11) years	38	76.0
	(12-20) years	12	24.0
	Total	50	100.0
Control	(3-11) years	33	66.0
	(12-20) years	17	34.0
	Total	50	100.0

Table 3: Result of prothrombin mutation by PCR among case and control groups.

Study population	Result of PCR	Frequency	Percent (%)
Case	Positive	00	00.0
	Negative	50	100.0
	Total	50	100.0
Control	Positive	00	00.0
	Negative	50	100.0
	Total	50	100.0

Figure 1. Results of PCR



Discussion:

The study was conducted in a case-control hospital in the Radiation and Isotope Centre Khartoum from January to March, with a total of 100 samples divided equally into case (patients with ALL) and control (healthy individuals). The study reported a lack of prothrombin mutation among ALL patients; this finding was consistent with the study of Dalara Falma and his colleagues, 2011, who reported a lack of prothrombin mutation among the Turkish ALL population **[25].** Zohreh Rahimi et al. (2013), reported a lack of prothrombin mutation in the Iranian ALL population **[26].** And Azza Hamdy Elsissy et al. (2014), reported a lack of prothrombin mutation among the Egyptian ALL population **[27].** The result was unmatched by the studies of Nicola Santoro, M.B. et al. (2006), who reported the presence of prothrombin mutations among the Italian ALL population **[28].** , and Destefano V. et al. (2003), who reported the presence of prothrombin mutations among the Italian ALL population **[29].**

Conclusion:

According to the result of this study, the mutation of the prothrombin G20210A gene was completely absent and did not show any different significance. It was concluded that there is no association of the prothrombin G20210A mutation with Sudanese acute lymphoblastic leukemia among Sudanese patients.

Recommendation:

Further thrombophilic molecular screening targeted the ALL population. Other studies should be done with a larger population.

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Conflict of Interest:

The author has affirmed that there are no conflicting interests.

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