Advances in Environmental and Life Sciences 4(1) (2023) 31-42

Contents lists available at Egyptian Knowledge Bank

Advances in Environmental and Life Sciences

journal homepage: https://aels.journals.ekb.eg

Screening for human parvovirus B19 genome in patients with acute leukemias in Suez Canal University hospital by Real-Time PCR technique

Rabab H A Hasanain[a,*], Hanaa H Gomaa [a], Fadia M Attia [b]

[a] *Department of Botany and Microbiology, Faculty of Science, Suez Canal University, 41522, Ismailia, Egypt*

[b] *Department of clinical pathology, Faculty of medicine, Suez Canal university, 41522, Ismailia, Egypt*

Abstract

Introduction Human parvovirus B19 belongs to family Parvoviridae. The replication of human parvovirus B19 is autonomous replication. When acute leukemia patients acquire an infection with human parvovirus B19, the disease progression of acute leukemia will be complicated causing cytopenia, leading to reduced treatment intensity and to complications during treatment also causing myalgia, bone pain, bone marrow aplasia and sinusoidal obstruction syndrome at the end of the induction treatment. Infection with human parvovirus B19 constitutes a serious risk in certain group of patients such as immunodeficient patients, organ transplant recipients, pregnant women and patients with acute leukemias. The genome of the virus can persist in patients with acute leukemia. So, it's highly recommended to screen patients with acute leukaemias for an infection with human parvovirus B19 genome by using Real-Time PCR technique to avoid any possible complication that might occur if these patients were infected with human parvovirus B19 during their treatment.

Objective: Screening for the genome of human parvovirus B19 in acute leukemias patients in Suez Canal University Hospital by Real-Time PCR technique in order to obtain a clear image about the prevalence of the virus among acute leukemia patients.

Methods Seventy-five blood samples of patients with acute leukemias and seventy-five blood samples of healthy individuals (used as control group) were screened for parvovirus B19 genome by real time PCR technique in Suez Canal University Hospital.

Results The percentages of acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) patients within the 75 patients of acute leukemia represented within the study population were as the following; 41.3% of the patients who participated in this study were AML patients, and 58.7% were ALL patients. The genome of the virus was detected in 17 patients of the 75 leukemia patients within the study group (22.7%). The number of leukemia patients in the study group who previously received blood or platelet transfusions was (50) leukemia patient. The blast cells count ranged from (20-70) in leukemia patients and there was absence of blast cells in the control group of healthy individuals, the blast cell count rises in case of leukemia patients. The study detected the genome of human parvovirus B19 in 17 of the leukemia patients within the study group (22.7%), these patients also have a high leukocytic count (20-64), a decreased hemoglobin level (5-7), decreased platelet count (8-25) and high blast cells count (35-65). Also, 66.7% of these leukemia patients (50 leukemia patients) have received blood or platelet transfusions during their treatment.

Conclusion Patients with acute leukemia are susceptible to acquiring an infection with human parvovirus B19 which is considered a serious risk for this group of patients. So, screening patients with acute leukemias for human parvovirus B19 genome is highly recommended.

*Corresponding author.

Email address : r_hasanain@yahoo.com (Rabab H A Hasanain)

doi 10.21608/aels.2023.203374.1030

Received: 11 April 2023, Revised : 25 April 2023
Accepted: 27 April 2023, Published: 27 April 2023

1. Introduction

Human Parvovirus B19 is a DNA virus, it measures 20-25 nm in diameter, contains 5.6kb genome, non-enveloped, single stranded DNA virus, an icosahedral shaped virus. DNA replication process and protein products of human parvovirus B19 seems to be similar to the way of replication of other animal parvoviruses, yet, human parvovirus B19 has many particular replication features which make it is distinguished from other animal parvoviruses. These features comprise initiation of transcription at p6 (strong left side promoter), the lack of functional internal promoter and the exceptional transcription map of the virus. Human parvovirus B19 transcription map indicates overlapping transcripts, similar initiation and transcription and including different-sized introns **[1].**

Despite the fact that these agents have a morphology and buoyant density that strongly suggest a parvovirus, at that stage there was no knowledge available about the nature of these viruses' genetic material, hence, when papers on the diseases concerning these viruses came out, authors avoided any conclusive classification of the virus and used the terms parvovirus-like agent (PVLA) and serum parvo-virus like agent (SPVLA), yet recent papers gave more specific virus classification, it belongs to the family parvoviridae, subfamily parvovirinae, genus eryhthrovirus **.** human parvovirus B19 has a single-stranded DNA genome of 5.6 kb, which codes for three proteins: a nonstructural protein (NS1) and two viral capsid proteins VP1 and VP2, the major viral capsid protein (VP2) makes out 96%of the total capsid proteins, the minor viral capsid protein (VP1) makes out the remaining 4% of the total capsid proteins, it has an additional 227 amino acids at the amino terminus of VP1 (called VP1U or the VP1 unique region) the VP1U shows high sequence variability which causes non conservative amino acid alterations, which result in mutations these mutations causes modified surface of the virus which result in development of persistent infection, Vp1U is the main antigenic target for the neutralizing antibodies, responsible for initiating infection, also responsible for induction of both chronic inflammation and autoimmune responses by unknown mechanisms, NS1 is the most abundant non-structural protein its'molecular mass is 77 KDa, NS1 plays many roles ,including site specific DNA binding, DNA nicking, regulation of gene transcription, helicase function, controlling replication by trans regulating viral P6promoter and some cellular promoters, induction of apoptosis by interaction with caspase 3, NS1 cytolytic activity is responsible for most of the clinical manifestations of B19 by being responsible for damaging the erythroid precursors, NS1 is encoded by genes om the left side of the genome, VP1 and VP2 capsid proteins are encoded by genes on the right side of the genome, VP1 is encoded by the sequence from nucleotides 2444 to 4486 and has a molecular mass of 84 KDa, VP2 is encoded by the sequence from nucleotides 3125 to 4786 and has a molecular mass of 58 KDa **[2].**

Human parvovirus B19 can be transmitted through respiratory secretions, contaminated blood products or vertically. The most accurate and reliable method to diagnose human parvovirus B19 infection is to detect the genome of the virus in peripheral blood, bone marrow or tissues by using PCR techniques. Over the years, accurate molecular diagnostic assays have been developed to be more rapid **[3], [4].** The PCR protocols have been improving and evolving in order to guarantee the validity, preciseness and efficiency of the diagnostic assays. This development was achieved through the added enhancements over the years and in particular by comprising the internal controls and competitor within these advanced PCR protocols **[5], [6], [7].**

At the present time, quantitative PCR techniques including both Real-Time PCR and PCR assays implementing internal control. These PCR assays are the most reliable yet typical and well-established analytical procedure to detect the genome of human parvovirus B19 **[8], [9], [10], [11].**

It's expected that the ongoing technical progress will definitely give rise to new analytical and molecular detection assays which will enhance the execution procedures and also will reduce time needed and expenses. At last, it should be mentioned that there are specific assays considered

beneficial as complementary assays besides the implemented PCR assay for screening and detecting an infection with human parvovirus B19 in bioptic specimens. These complementary assays include in situ hybridization techniques (which detect the genome of the virus) and immunohistochemical assay which detects the specific proteins of the virus. These complementary assays are very valuable as it helps to identify the host cells infected with virus and subsequently differentiate between types of parvovirus B19 infection **[12], [13], [14], [15], [16], [17], [18].**

The DNA amplification assays (and PCR assays in particular) have a sensitivity estimated to be nearly about 102 genome copies per milliliter, whereas direct assays for DNA hybridization have a sensitivity estimated to be nearly about 106 genome copies per milliliter **[19], [20].**

According to French-American-British (FAB) group, acute leukemias are classified based on morphology and cytochemistry in addition to immunophenotyping, several different leukemia types became classified according to FAB by the morphological features and cytochemical studies, particularly myeloperoxidase (or Sudan black B) and non-specific esterase staining. FAB provided very clear guidelines for classification but failed to discriminate various immunophenotypic groups of acute lymphoblastic leukemias (ALL) and resulted in some subcategories of little clinical significance. Also, it did not recognize the significance of myelodysplastic changes in acute myeloid leukemias (AML) or cytogenetic abnormalities in either leukemia type, AML are classified into eight subtypes (M0 to M7) and ALL are classified into three subtypes (L1 to L3) according to the original classification scheme of the (FAB) Cooperative Group

Leukemia takes place in hematopoietic cells. The development of this type of malignancies results in the dispersed replacement of both bone marrow and peripheral blood cells with the malignant cells. There are two types of leukemia according to the disease progress pathway. These two types include acute leukemia and chronic leukemia. Since 1990 to 2018, collected data and statistics indicated that the incidence of leukemia around the globe has risen uniquely in this time span **[21].**

Leukemia risk factors could be genetic or environmental, including being exposed to ionizing radiation, certain chemicals and being subjected to specific infection **[22], [23], [24].**

The role of the immune system is highly important and fundamental in order to prevent and also treat leukemia. Its role in treating leukemia could be described by the development of both recombinant T-cells and the immune checkpoint inhibitors along with the antigen chimeric receptors **[25].**

The term leukemia refers to the type of cancer that commence in cells that usually evolve into various kinds of blood cells. Usually, the onset of leukemia takes place in white blood cells specifically, in the early stages of white blood cells, however, there are types of leukemias which take place in other kinds of blood cells such as the megakaryocytic leukemia or erythroleukemia. According to the pattern of the development of leukemia, there are several types of leukemia which are classified into acute which develops quickly and chronic which develops slowly. Another classification of the types of leukemia is also performed according to the specific cells where its onset has taken place as the leukemia could begin in the myeloid cells or in lymphoid cells **[26], [27].**

Many studies including patients with different hematological conditions have shown a rise in the numbers of cases infected with human parvovirus B19 within this category of patients. In 1994, Frickhofen and coworkers carried out a retrospective study which included fifty-seven patients suffering from acquired chronic pure red cell aplasia. The percentage of B19 infection among these patients was 14% as the genome of the virus was disclosed in the serum of eight (8 patients of the total 57 patients included within this study) **[28], [29].**

In 2007, Tercan and coworkers carried out a prospective study which included seventy-nine immuno-compromised patients with chronic anemia suffering from severe hematological disorders. The purpose of the study was investigating the percentage of infections with human parvovirus B19 among this specific group of patients. The percentage of infection with human parvovirus B19

among these patients was 29.1% as the genome of the virus was disclosed in the serum of twenty-three patients of the total seventy-nine chronic anemic immuno-compromised patients included within this study.

Human parvovirus B19 is considered one of the viruses that causes leukemia (viral triggers), there is a link between human parvovirus B19 and nuclear factor kappa B, tumor necrosis factor alpha, and interleukin 6 levels. And plays an important role in tumorigenesis and thyroid cancer development through the virus inflammatory mechanisms. **[30], [31], [32] Human pa**rvovirus B19 infects the bone marrow erythroblasts, as well as different other cell types, when patients with shortened red cell survival acquire an infection with human parvovirus B19 it causes aplastic crisis which is a prodrome of ALL in 2% of patients **[33], [34] Human p**arvovirus B19 also causes aplastic anemia, which occurs several months before onset of acute leukemia **[35], [36].**

There are many viruses which cause human cancer (oncoviruses), such as Epstein–Barr virus, hepatitis B virus, hepatitis C virus, human papillomaviruses, Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) and Merkel cell polyomavirus, human T-cell lymphotropic virus. Several distinct aspects of the life cycle of human parvovirus B19 resemble that of oncoviruses. Some parvoviruses are human oncolytic viruses including Rat parvovirus H-1. **[37], [38], [39], [40] , [41]. Rat parv**ovirus H-1(known as ParvOryx) is now a subject of clinical trials in order to use it as a treatment for human glioblastoma multiforme **[42], [43], [44], [45].**

Human parvovirus B19 is responsible for complicating acute leukemia clinical course, either by an opportunistic infection or by causing reactivation of a latent B19 virus, also acute human parvovirus B19 infection takes place before diagnosing acute leukemia by 180 days and in certain cases human parvovirus B19 is responsible for causing aplastic crisis which is known to proceed onset of acute leukemia in a few numbers of patients **[46], [47].**

Human Parvovirus B19 infects red blood cell precursors in the bone marrow and results in stopping erythrocyte production. This can complicate anemia and worsen the general condition of patients, and causes life-threatening complications in severe anemia patients including cerebrovascular attack, circulatory collapse, congestive heart failure, or acute splenic sequestration **[48]. (A**cute B19 infection is linked to low circulating IL-10 consistent with a vigorous immune response. Deficient production of IL-10 was found to be associated with the following development of acute leukemia **[49].**

In 2015, a study conducted by Wolfromm and coworkers showed that ALL patients with hereditary hemolytic anemia included within the study were positive for human parvovirus B19 DNA.

Inherited hemolytic anemias, showed high-titer replication, which results in suppression of bone marrow, causing a life-threatening drop of hemoglobin values resulting in profound anemia, or aplastic crisis. A human parvovirus B19 screening program is not applied till the present day either for blood transfusion used in the hemotherapy or for high-risk group of patients including leukemia patients, which is a cause of a great concern especially for this group of patients**, [50]**

Sekiguchi and coworkers in 2014 reported a persistent parvovirus B19 infection (genome of the virus remained positive). This persistent infection with the virus comprises a risk of recurrence and also bone marrow failure.

Aim of the study: Detection of human parvovirus B19 in blood of patients with acute leukemias at Suez Canal university hospital.

2. Methods

2.1. Sample collection:

This study is a case control study that has been implemented at Suez Canal University Hospital, Ismailia, Egypt. This study included seventy-five patients with acute leukemias and another seventy-five healthy negative control individuals at Suez Canal University Hospital.

Seventy-five blood samples were withdrawn from patients with acute leukemias and another seventy-five blood samples were withdrawn from healthy negative control individuals, age group (4-55). Both genders (males and females for both

patients with acute leukemias and the healthy individuals as well) were included in this study.

The 75 blood samples for patients with acute leukemias and the other 75 blood samples for the healthy control individuals have been subjected to main 4 screening tests and proved to be negative to these tests. The core tests are Antibody to HIV, Hepatitis B Surface Antigen, Antibody to Hepatitis C, usually subtypes 1 and 2 and finally Serologic test for Syphilis. Exclusion criteria was Acute leukemias Patients' refusal or refusal of the healthy control individuals. Data were collected from each patient with acute leukemia as well as each healthy individual. The individuals were asked about the following data; Name, Age, Sex, Mobile phone number and Address.

Sampling process was conducted and included one hundred and fifty (150) blood samples. The blood samples were collected into 3ml plain tube with EDTA, (75) blood samples from healthy control individuals and another (75) blood samples from patients with acute leukemias, at Suez Canal University Hospital. The serum samples from each blood sample were separated and stored at (–20°C) till further use for real-time PCR.

2.2. Real-time polymerase chain reaction:

Human parvovirus B19 genome was detected in 150 serum samples using RealStar® Parvovirus B19 PCR Kit 1.0.

2.3. Sample preparation:

Extracted DNA was the starting material for the RealStar® Parvovirus B19 PCR Kit 1.0.

Type of gene detected was human parvovirus B19 gene.

DNA extraction from EDTA blood samples and we assessed purity of extracted DNA by nanodrop then amplification of specific sequence of parvovirus B19 was used for detection of acute infection by Real-time PCR.

2.4. Setup of the master mix:

All reagents and samples were thawed completely, mixed and centrifuged briefly before use.

The RealStar® Parvovirus B19 PCR Kit 1.0 contained a heterologous Internal Control (IC), the IC was used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix was set up according to the following pipetting scheme:

Number of Reactions (rxns)	**1**	**12**
Master A	**5 µl**	**60 µl**
Master B	**15 µl**	**180 µl**
Internal Control	**1 µl**	**12 µl**
Volume Master Mix	**21 µl**	**252 µl**

The IC was used as a control for the sample preparation procedure and as a PCR inhibition control, the IC must be added during the nucleic acid extraction procedure.

2.5. Setup of the reaction

Reaction Setup	
Master Mix	**20 µl**
Sample or Control	**10 µl**
Total Volume	**30 µl**

Twenty (20) µl of the Master Mix was pipetted into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube, Ten (10) µl of the sample was added (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control), One Positive (QS) and one Negative Control were used per run, The samples and controls were thoroughly mixed with the Master Mix by pipetting up and down, The 96-well reaction plate was closed with appropriate lids or optical adhesive film and the reaction tubes were closed with appropriate lids, The 96-well reaction plate was centrifuged in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

2.6. Programming the Real-Time PCR Instrument

- Settings

Settings	
Reaction Volume	**30 μl**
Ramp Rate	**Default**
Passive Reference	**ROX^{TM}**

Target	Detector Name	Reporter	Quencher
Parvovirus B19 specific DNA	**Parvovirus B19**	**FAM^{TM}**	**(None)**
Internal Control (IC)	**IC**	**JOE^{TM}**	**(None)**

2.7. -Fluorescence Detectors (Dyes)

The fluorescence detectors (dyes) were defined:

2.8. Temperature Profile and Dye Acquisition

-The temperature profile and dye acquisition were defined:

2.9. -qualitative analysis

2.10. Data Statistical analysis

IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) was used for the analysis. The normality of distribution of variables was verified using the Kolmogorov- Smirnov; comparison between groups for categorical variables was done using Chi-square test

Area about 100% is the best performance for the test. area more than 50% gives acceptable performance and Significance of the obtained results was judged at the 5% level.

Student t-test was used to compare two groups for normally distributed quantitative variables while Mann Whitney test was used to compare between two groups for not normally distributed quantitative variables.

3. Results

The percentages of acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) patients within the 75 patients of acute leukemia represented within the study population were as the following; 41.3% of the patients who participated in this study were AML patients and 58.7% of them were ALL patients.

The table shows the prevalence of the genome of human Parvovirus B19 within the study group and the control group. The genome of the virus was detected in (17) patients of the (75) leukemia patients representing a percentage of 22.7% of prevalence of human parvovirus B19 within the study group. The table shows also the history of blood transfusion in both the control group and the study group. The number of leukemia patients in the study groups who previously received blood transfusion in this study was (50) leukemia patients.

The table shows the elevated presence of blast cells ranging from (20-70) in leukemia patients in the study group and the absence of these blast cells in the control group of healthy individuals.

The table shows that the genome of human parvovirus B19 was detected in 17 leukemia patients of the 75 leukemia patients within the study group. These patients also have high leukocytic count (20-64), decreased hemoglobin level (5-7), decreased platelet count (8-25) and high presence of blast cells (35-65). Also 66.7 % of these leukemia patients have received blood or platelet transfusions during their treatment.

4. Discussion

Screening patients with acute leukemias for human parvovirus B19 genome is not yet implemented in Egypt despite the fact that the virus is compromising this group of patients due to its ability to present in high titers, causing infection and causing serious complications in this group of patients. Human parvovirus B19 is also capable of resisting heat inactivation procedures, so there is a great need to investigate the prevalence of the virus genome in patients with acute leukemias of our country.

This study detected the genome of the virus within the study group of leukemia patients and control group of healthy individuals. 22.7% of the leukemia patients have the DNA of human parvovirus B19 in the serum samples collected

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	**Hold**	**1**	**-**	**95**	**10:00**
Amplification	**Cycling**	**45**	**-**	**95**	**00:15**
			yes	**58**	**01:00**

Detection Channel		Result Interpretation
FAM™	**JOE™**	
+	**+***	**Parvovirus B19 specific DNA detected.**
-	**+**	**No parvovirus B19 specific DNA detected. Sampledoes not contain detectable amounts of parvovirus B19 specific DNA.**
-	**-**	**PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.**

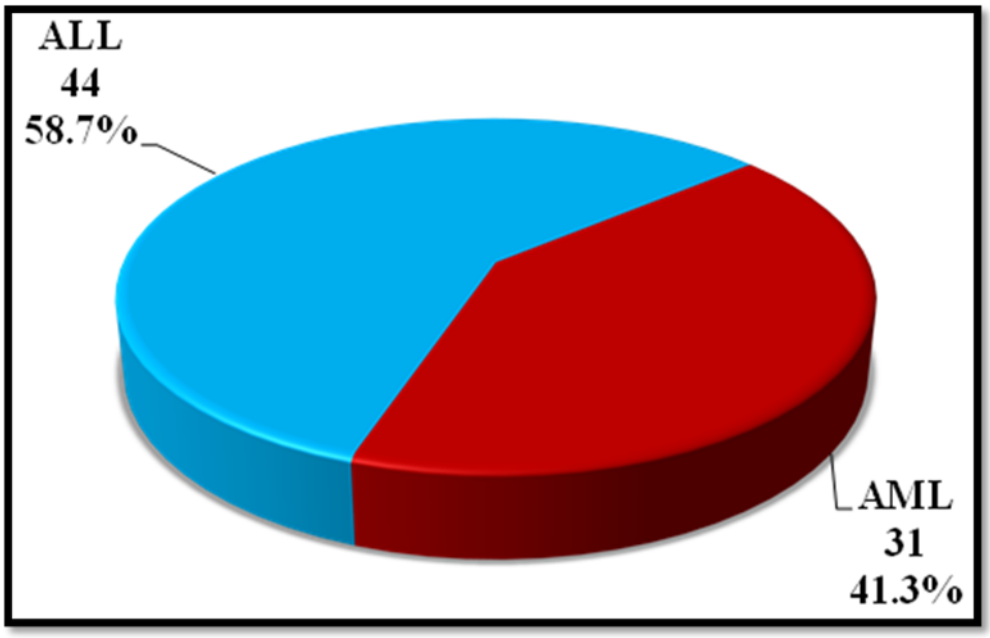

Figure 1: Distribution of the studied sample

from these patients, while the genome of the virus wasn't detected in the control group within this study. This result leads to the conclusion that the leukemia patients are more susceptible to infection with human parvovirus B19 than normal individuals.

The percentage of leukemia patients who had previously received blood transfusions in this study was 66.7%. The study shows a drop in both platelet count and the hemoglobin level in the study group of leukemia patients combined with an elevated leukocytic count.

This study included children, females and males in both the control group and the study group. The study group included 36.0% males, 50.7% females, 13.3% children, while the control group in

Table 1: **Comparison between the two studied groups according to different parameters**

	Cases(n = 75)	**Control(n = 75)**	**Test of Sig.**	**p**
		Sex		
Male	27 (36.0%)	30 (40.0%)		
Female	38 (50.7%)	35 (46.7%)	c^2=0.281	0.869
Child	10 (13.3%)	10 (13.3%)		
		Age (years)		
Min. – Max.	4.0 – 55.0	4.0 – 55.0		
Mean ± SD	31.51 ± 12.78	33.85 ± 11.85	U=2535.00	0.296
Median (IQR)	33.0 (25.0–41.0)	37.0 (32.50– 40.0)		
		WBCs (x1000/μl)		
Min. – Max.	20.0 – 70.0	4.0 – 9.0		
Mean ± SD	38.81 ± 12.73	6.84 ± 1.32	t=21.635*	<0.001*
Median (IQR)	40.0 (30.0–47.0)	7.0 (6.0–8.0)		
		Hb (gm/dl)		
Min. – Max.	5.0 – 7.0	11.0 – 15.0		
Mean ± SD	5.99 ± 0.68	12.68 ± 1.20	t=42.027*	<0.001*
Median (IQR)	6.0 (5.60 – 6.30)	12.50 (12.0 – 13.50)		
		Platelets (x1000/μl)		
Min. – Max.	5.0 – 30.0	170.0 – 320.0		
Mean ± SD	15.45 ± 4.78	232.52 ± 36.29	t=51.360*	<0.001*
Median (IQR)	15.0 (12.0 – 20.0)	220.0 (200.0– 250.0)		
		Blast cells		
Min. – Max.	20.0 – 70.0	–		
Mean ± SD	45.27 ± 9.57	–	–	–
Median (IQR)	45.0 (40.0–50.0)	–		
		B19 PCR		
Negative	58 (77.3%)	75 (100.0%)	c^2=19.173*	<0.001*
Positive	17 (22.7%)	0 (0%)		
		History of blood transfusion		
No	25 (33.3%)	75 (100.0%)	c^2=75.00*	<0.001*
Yes	50 (66.7%)	0 (0%)		

* p value is significant

this study included 40.0% males, 46.7% females, 13.3 % children. The age group in this study for both the control group and the study group was (4 -55 years) in order to assess the prevalence of the genome of human parvovirus B19.

The results of our study showed that the genome of human parvovirus B19 was detected in 17 of the leukemia patients within the study group. Those patients also showed an elevated leukocytic count, decreased hemoglobin and platelet counts with high presence of the blast cells in the blood samples of these patients. The detected human Parvovirus B19 genome in these patients refers to the presence of a current viremia stage within these leukemia patients. These results are in consonance with the study conducted in 1998 by Broliden and coworkers, where during their study 59 bone marrow samples of children suffering from various malignancies including ALL were screened for human parvovirus B19 DNA implementing PCR, and the results showed that seven samples out of the 59 samples were positive for human par-

Table 2: **Relation between Parvovirus B19 infection with different parameters in cases group (n= 75)**

	PV B19 PCR	
	Negative(n = 58)	**Positive(n = 17)**
WBCs (x1000/μl)		
Mean ± SD	39.19 ± 12.82	37.53 ± 12.72
Median	40 (20 – 70)	35 (20 – 64)
t (p)	0.470 (0.640)	
HB (gm/dl)		
Mean ± SD	5.97 ± 0.67	6.06 ± 0.71
Median	6 (5 – 7)	6 (5 – 7)
t (p)	0.450 (0.654)	
Platelets (x1000/ul)		
Mean ± SD	15.50 ± 4.87	15.29 ± 4.61
Median	15 (5 – 30)	15 (8 – 25)
t (p)	0.155 (0.877)	
Blast cells		
Mean ± SD	4.90 ± 9.82	46.53 ± 8.83
Median	45.0 (20.0 – 7.0)	44.0 (35.0–65.0)
t (p)	0,616 (0,540)	
History of blood transfusion		
No	19 (32.8%)	6 (35.3%)
Yes	39 (67.2%)	11 (64.7%)
c^2 (p)	0.038 (0845)	

vovirus B19 genome. The seven samples consisted of three samples of patients with ALL receiving chemotherapy, one patient with ALL at early stage, two patients suffering from non-Hodgkin's lymphoma and one patient suffering from Ewing's sarcoma **[51], [52].**

The prevalence of the genome of PB19 virus in the current study is in consonance with the study conducted by Zaki and Ashray in 2010, where in their study included 85 pediatric patients between (2007-2008), in the Children Hospital Mansoura University. The study included two groups of patients, the first group included 45 patients with acute leukemia on chemotherapy; 30 patients with ALL on maintenance chemotherapy and 15 patients with AML on consolidation therapy, while the second group included 40 patients with acute leukemia; 24 with ALL and 16 with AML, Prevalence of viral DNA was 22.2% of the Group I patients and 45% of the patients in Group II. The prevalence of human Parvovirus B19 IgM antibodies was 31.1% in the Group I patients and 50% of Group II patients The prevalence of human Parvovirus B19 IgG was 40% of ALL patients **[53], [54].**

The prevalence of human parvovirus B19 genome in the current investigation is higher than previous study conducted by Lindblom and coworkers in 2008 who tested 86 bone marrow samples for detection of human parvovirus B19 genome and found that B19 DNA was detected in 6 out 86 (7%) of ALL patients at diagnosis (4 patients have anemia and 5 patients have thrombocytopenia) and in 12 patients out of 31 (39%) ALL patients during or between chemotherapy **[55]. T**he prevalence of genome of the virus in our study is much higher than previous studies, in 2009, Soliman and coworkers screened 59 patients for human parvovirus B19 infection, (39 patients with ALL and 20 patients with solid tumors). All patients were receiving chemotherapy and their study included control group of 30 individuals. The genome of human parvovirus B19 was detected in 11/39, 5/20 and 0/30 respectively. Anti-human parvovirus B19 IgG antibodies were detected in 26/39, 10/20, 18/30 respectively. Anti-human parvovirus B19 IgM antibodies were detected in 0/39, 3/20, and 0/30 respectively. The patients who are positive for B19 genome showed higher rates of anemia. They have received cell transfusions previously, and their hospital time is longer than other patients who are negative to human parvovirus B19 genome **[56].**

The prevalence of B19 genome in our study is higher than previous study conducted in 2013 by Da Costa and coworkers, they screened 78 ALL patients, 155 AML, and 16 CML patients for the detection of genome of B19 in bone marrow. The prevalence of the B19 genome in their study was 12 of 78 (15.4%) ALL, 25 of 155 (16.1%) AML and 3 of 16 (18.7%) CML Genotype 1a was detected in 35 of the 40 patients (62.5%) and genotype 3b was detected in 50 patients (37.5%); co-infection with

genotypes 1a and 3b was detected in 12 of 40 (30%) of the participants, one patient showed multiple genotypes, a new human parvovirus B19 virus intergenotypic recombinant (1a/3b) and NS1 non-recombinant genotype 1a. **[57].**

The prevalence of the genome of the virus in our study is much higher than a previous study conducted in 2002 by Heegaard and coworkers, which included 65 children with ALL in a study to detect genome of human parvovirus B19 in their serum. The results showed no genome of the virus in any child of the 65 children screened within the study but only one child had the genome of the virus 5 months before being diagnosed with ALL. The child was suffering from pancytopenia episode.

The prevalence of the genome of the virus in this current investigation is in consonance with a previous study conducted in 2002 by Heegaard and coworkers, they screened 75 patients with ALL; 48 of these patients were seronegative for human parvovirus B19 genome and 27 of these patients were seropositive for human parvovirus B19 genome. Four ALL patients of the 48 patients negative for the genome of the virus seroconverted and became positive for the genome of the virus and showed evidence of viremia**. [58] The** prevalence of the genome of the virus in our current study is in consonance with a previous study conducted in 2010, Zaki and Ashray conducted a case control study to detect B19 genome as well as IgG and IgM against the virus within the control group and the two study groups of patients, Human parvovirus B19 genome was detected in 10 of the 45 ALL patients (22%), These patients were receiving chemotherapy, The DNA of the virus was also detected in 18 of the 40 ALL who were diagnosed with leukemia and also DNA of the virus wasn't detected in any of the 20 healthy individuals within the control group. IgM antibodies against the virus were detected in 14 of 45 (31%) ALL patients on chemotherapy, in 20 of the 40 (50%) ALL patients recently diagnosed, and wasn't detected in any of the healthy control individuals. IgG antibodies against the virus were detected in 18 out of the 45 (40%) ALL patients during chemotherapy and 16 out of the 40 (40%) ALL patients recently diagnosed and wasn't detected in any of the healthy control individuals **[59].**

5. Conclusion

Human parvovirus B19 genome can persist in patients with acute leukemias, for long periods. The prevalence of human parvovirus B19 genome is high within the population of patients with acute leukemias in Ismailia, Egypt and this is clearly an evident that there is an active human parvovirus B19 transmission in this study group. Therefore, screening for human parvovirus B19 for the acute leukemia patients is highly recommended and can be considered as an essential procedure to be undertaken all through their treatment routine in the future to ensure that these patients will not encounter serious life-threatening complications that might worsen their state or delay their recovery process.

The seroprevalence of human parvovirus B19 within acute leukemia patients in our study is high; hence there is a serious risk for this group of patients especially if they were subjected to blood transfusion many times during their treatment as if the blood is contaminated with human parvovirus B19 became transfused to such high-risk group of patients.

Acquiring an infection with B19 constitutes a high risk for patients with acute leukemias, but till the present days there are no strict regulatory procedures to screen this group of patients for human parvovirus B19 to control and exclude any chances of human parvovirus B19 transmission to these patients.

References

[1] J. & Kuhn, Radoshitzky, & Sheli, S. & Bavari, P. Jahrling (2013).
[2] E. D. Heegaard, K. E. Brown, Human parvovirus B19, Clin Microbiol Rev 15 (3) (2002) 485–505.
[3] L. Kaikkonen, M. Söderlund-Venermo, J. Brunstein, O. Schou, P. Jensen, I. Rousseau, S. Caul, E. O. Cohen, B. Valle, M. Hedman, L. Hedman, K, Diagnosis of human parvovirus B19 infections by detection of epitope-type-specific VP2 IgG, J Med Virol 64 (3) (2001) 360–365.
[4] C. Ducloux, B. You, A. Langelé, O. Goupille, E. Payen, S. Chrétien, Z. Kadri (2020).
[5] L. Kaikkonen, H. Lankinen, I. Harjunpaa, Acute phase specific heptapeptide epitope for diagnosis of parvovirus B19 infection, J. Clin. Microbiol 37 (1999) 3952–3956.

[6] E. Manaresi, G. Gallinella, S. Venturoli, M. Zerbini, M. Musiani, Detection of parvovirus B19 IgG: choice of antigens and serological tests, J. Clin. Virol 29 (2004) 51–53.
[7] M. Enders, G. Schalasta, C. Baisch, Human parvovirus B19 infection during pregnancy value of modern molecular and serological diagnostics, J. Clin. Virol 35 (2006) 400–406.
[8] M. Zerbini, D. Gibellini, M. Musiani, S. Venturoli, G. Gallinella, G. Gentilomi, Automated detection of digoxigenin labelled B19 parvovirus amplicons by a capture hybridization assay, J. Virol. Methods 55 (1995) 1–9.
[9] A. Ishikawa, Y. Yoto, T. Tsugawa, H. Tsutsumi, Quantitation of human parvovirus B19 DNA in erythema infectiosum and aplastic crisis, J Med Virol 86 (12) (2014) 24962467–24962467.
[10] D. Peterlana, A. Puccetti, R. Corrocher, C. Lunardi, Serologic and molecular detection of human parvovirus B19 infection, Clin. Chim. Acta 372 (2006) 14–23.
[11] M. Musiani, G. Gallinella, S. Venturoli, M. Zerbini, Competitive PCR ELISA protocols for the quantitative and the standardized detection of viral genomes, Nat. Protoc 2 (2007) 2511–2519.
[12] S. A. Baylis, N. Shah, P. D. Minor, Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma, J Virol Methods 121 (1) (2004) 7–16.
[13] L. D. Rogo, T. Mokhtari-Azad, M. H. Kabir, F. Rezaei, Human parvovirus B19: a review, Acta Virol 58 (3) (2014) 199–213.
[14] D. Peterlana, A. Puccetti, R. Corrocher, C. Lunardi, Serologic and molecular detection of human Parvovirus B19 infection, Clinica Chimica Acta 372 (2006) 14–23.
[15] A. Lundqvist, A. Isa, T. Tolfvenstam, G. Kvist, K. Broliden, High frequency of parvovirus B19 DNA in bone marrow samples from rheumatic patients, J Clin Virol 33 (1) (2005) 71–75.
[16] F. Gruber, F. G. Falkner, F. Dorner, T. Hammerle, Quantitation of viral DNA by real-time PCR applying duplex amplification, internal standardization, and two-color fluorescence detection, Appl. Environ. Microbiol 67 (2001) 2837–2839.
[17] E. Manaresi, G. Gallinella, E. Zuffi, F. Bonvicini, M. Zerbini, M. Musiani, Diagnosis and quantitative evaluation of parvovirus B19 infections by real-time PCR in the clinical laboratory, J. Med. Virol 67 (2002) 275–281.
[18] S. A. Baylis, L. Ma, P. D. J, H. A, Y. M, Collaborative study to establish a World Health Organization International genotype panel for parvovirus B19 DNA nucleic acid amplification technology (NAT)-based assays, Vox Sang 102 (2012) 204–211.
[19] E. D. Heegaard, K. E. Brown, Human parvovirus B19, Clin Microbiol Rev 15 (3) (2002) 485–505.
[20] F. Bonvicini, C. Filippone, E. Manaresi, Peptide nucleic acid-based in situ hybridization assay for detection of parvovirus B19 nucleic acids, Clin. Chem 52 (2006) 973–978.
[21] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, T. L, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin 68 (2018) 394–424.
[22] R. G. Verhaak, B. J. Wouters, C. A. Erpelinck, S. Abbas, H. B. Beverloo, S. Lugthart, B. Lowenberg, P. Valk, Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling, Haematologica 94 (2009) 131–134.
[23] A. Sayad, A. Hajifathali, A. A. Hamidieh, F. Esfandi, M. Taheri, Fas-antisense long noncoding RNA and acute myeloid leukemia: is there any relation?, Asian Pacific Journal of Cancer Prevention (APJCP) 19 (2018) 45–45.
[24] A. Sayad, A. Hajifathali, M. D. Omrani, A.-J. S. Hamidieh, A. Taheri, M, Expression of TNF-and HNRNPL-related immunoregulatory long non-coding RNA (THRIL) in acute myeloid leukemia: is there any correlation?, Iranian Journal of Allergy, Asthma and Immunology 17 (2018) 274–280.
[25] K. Kuželova, B. Brodska, J. Schetelig, C. Rollig, Z. Račil, J. S. Walz, G. Helbig, O. Fuchs, M. Vrana, P. Pecherkova, Association of HLA class I type with prevalence and outcome of patients with acute myeloid leukemia and mutated nucleophosmin, PLoS One 13 (2018) 204290–204290.
[26] J. E. Kolitz (2018).
[27] Adult Acute Myeloid Leukemia Treatment, National Cancer Institute. Physician Data Query (PDQ) (2018).
[28] N. Young, Acute and chronic parvovirus infection, in: D. Schlossberg (Ed.), Clinical Infectious Disease, Cambridge University Press, 2015, pp. 1216–1219.
[29] A. Wolfromm, C. Rodriguez, M. Michel, A. Habibi, V. Audard, E. Benayoun, O. Rogier, D. Challine, O. Chosidow, J. D. Lelièvre, C. X, L. Bras, F. Pautas, C. Imbert, M. Pawlotsky, J. M. Wagner-Ballon, O, Spectrum of adult Parvovirus B19 infection according to the underlying predisposing condition and proposals for clinical practice, Br J Haematol 170 (2) (2015) 25920561–25920561.
[30] E. D. Heegaard, K. Schmiegelow, Serologic study on parvovirus b19 infection in childhood acute lymphoblastic leukemia during chemotherapy: clinical and hematologic implications, J Pediatr Hematol Oncol 24 (5) (2002) 368–73.
[31] Z. Mel, S. Hassan, S. A. Seleim, T. Lateef, R. A, Parvovirus B19 infection in children with a variety of hematological disorders, Hematology 11 (4) (2006) 261–266.
[32] M. Zaki, R. E. Ashray, Clinical and hematological study for parvovirus B19 infection in children with acute leukemia, International Journal of Laboratory Hematology 32 (2) (2010) 159–166.
[33] L. Villarreal-Martínez, J. C. Jaime-Pérez, M. Rodríguez-Martínez, O. González-Llano, D. Gómez-Almaguer (2012).
[34] Y. Liang, L. Ding, X. Li, W. Wang, X. Zhang, Transient pancytopenia preceding adult acute lymphoblas-

tic leukemia with chromosomal abnormalities including the Philadelphia chromosome: A case report and review of the literature, Oncol Lett 10 (6) (2015) 4665684–4665684.

[35] G. Sitar, C. L. Balduini, L. Manenti, A. Castello, D. Balanzin, E. Ascari, Possible evolution of human parvovirus B19 infection into erythroleukemia, Haematologica 84 (10) (1999) 957–959.

[36] T. Fisgin, N. Yarali, D. F, K. A, Parvovirus-B19 infection preceding acute myeloid leukemia with orbital granulocytic sarcoma, Leukemia and Lymphoma 43 (10) (2002) 2059–2061.

[37] J. Rommelaere, K. Geletnek, A. L. Angelova, Oncolytic parvoviruses as cancer therapeutics, Cytokine Growth Factor Reviews 21 (2-3) (2010) 185–195.

[38] L. Olivier-Gougenheim, F. Dijoud, A. Traverse-Glehen, S. Benezech, Y. Bertrand, S. Latour, E. Frobert, C. Domenech, Aggressive large B-cell lymphoma triggered by a parvovirus B19 infection in a previously healthy child, Hematological Oncology 37 (2019) 483–486.

[39] T. A. Zykova, E. A. Shevyakova, Parvovirus B19: Features Of Prevalence In Oncological Patients, Medical Journal of the Russian Federation 24 (2019) 206–212.

[40] A. M. B. Fard, S. Atashi, S. Amiri, M. Kaviani, A. Gholampour, M. Ajami, Parvovirus B19 affects thrombopoietin and IL-11 gene expression in human bone marrow mesenchymal stem cells, Future Virology 16 (8) (2021) 519–526.

[41] G. Gallinella (2022).

[42] K. Geletneky, J. Huesing, J. Rommelaere, Phase I/IIa study of intratumoral/intracerebral or intravenous/intracerebral administration of Parvovirus H-1 (ParvOryx) in patients with progressive primary or recurrent glioblastoma multiforme: ParvOryx01 protocol, BioMed Central Cancer 12 (2012) 99–99.

[43] J. Hwee, C. Tait, L. Sung, C. Jeffrey, R. Kwong, J. D. Sutradhar, A. Pole, Systematic review and meta-analysis of the association between childhood infections and the risk of childhood acute lymphoblastic leukaemia, British Journal of Cancer 118 (2017) 127–137.

[44] R. Eren, M. H. Doğu, Ş. Altindal, O. Yokuş, E. Suyani, The seasonality in the diagnosis of acute leukemia: A single center data from Turkey, Marmara Medical Journal (2018) 112–115.

[45] S. Ramanathan, G. Narula, M. Prasad, T. Vora, G. Chinnaswamy, S. Banavali, Parvoviral disease in childhood cancer: Clinical outcomes and impact on therapy at a tertiary cancer center in India 65 (2018) 11–11.

[46] J. R. Kerr, A review of blood diseases and cytopenias associated with human parvovirus B19 infection, Reviews in Medical Virology 25 (2015) 224–240.

[47] M. Thvilum, F. Brandt, T. H. Brix, L. Hegedüs, Month of birth is associated with the subsequent diagnosis of autoimmune hypothyroidism. A nationwide Danish register-based study, Clinical Endocrinology 87 (2017) 832–837.

[48] E. Heegaard, K. E. Brown, Human parvovirus B19, Clin Microbiol Rev 15 (2002) 485–505.

[49] J. R. Kerr, D. L. Mattey, The role of parvovirus B19 and the immune response in the pathogenesis of acute leukemia, Rev Med Virol 25 (2015) 133–55.

[50] S. N. Slavov, S. Kashima, A. Pinto, C. D, Human parvovirus B19: general considerations and impact on patients with sickle-cell disease and thalassemia and on blood transfusions, FEMS Immunol Med Microbiol 62 (2011) 247–62.

[51] S. Fattet, P. Cassinotti, M. B. Popovic, Persistent human parvovirus B19 infection in children under maintenance chemotherapy for acute lymphocytic leukemia, J Pediatr Hematol Oncol 26 (8) (2004) 497–503.

[52] E. D. Heegaard, K. E. Brown, Human Parvovirus B19, Clinical Microbiology Reviews 15 (2002) 485–505.

[53] M. E. S. Zaki, R. E. Ashray (2010).

[54] A. Yalcin, M. S. Serin, G. Emekdas, N. Tiftik, G. Aslan, G. Eskandari, S. Tezcan, Promoter methylation of P15 (INK4B) gene is possibly associated with parvovirus B19V infection in adult acute leukemias, International Journal of Laboratory Hematology 31 (2009) 407–419.

[55] A. Lindblom, M. Heyman, I. Gustafsson, O. Norbeck, T. Kaldensjö, A. Vernby, J. I. Henter, T. Tolfvenstam, K. Broliden, Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy, Clin Infect Dis 46 (4) (2008) 528–564.

[56] Parvovirus B19 Infection in Pediatric Oncology Patients: Diagnostic Value of Clinical and Serologic Parameters Compared With Nested PCR, Journal of Pediatric Hematology/Oncology (31) (2009) 173–176.

[57] D. Costa, A. C. Bendit, I. D. Olivera, A. C. S. Kallas, E. G. Sabino, E. Sanabani, S. S, Investigation of human parvovirus B19 occurrence and genetic variability in different leukemia entities, Clin. Microbiol. Infec 19 (1) (2013) 31–43.

[58] E. D. Heegaard, K. Schmiegelow, Serologic study on parvovirus B19 infection in childhood acute lymphoblastic leukemia during chemotherapy: clinical and hematologic implications, Journal of Pediatric Hematology/Oncology 24 (5) (2002) 368–373.

[59] M. E. Zaki, R. E. Ashray, Clinical and hematological study for parvovirus B19 infection in children with acute leukemia, International Journal of Laboratory Hematology 32 (2) (2010) 159–166.