

Serological and molecular detection of parvovirus b19 in children with acute lymphoblastic leukemia

Iqbal M. Abdullah* BSc, MSc
 Shatha F. Abdullah* FICM Path
 Mazin F. Al-Jadiry ** MD

Summary:

Background: Human parvovirus B19 (B19V) is the only human pathogenic parvovirus, It is highly erythrotropic and preferentially replicates in erythroid progenitor cells (EPCs). Recently the effects of B19 infection have been well studied in children with acute lymphoblastic leukemia (ALL).

Objective: To detect parvovirus B19 among children with ALL.

Methods: A cross sectional study involved forty five patients with ALL (21 patients were newly diagnosed ALL and 24 children who underwent chemotherapy) who were attending department of oncology in Children's Welfare Teaching Hospital in Baghdad Medical City between December 2012 and April 2013, compared to forty five of apparently healthy children as a control group, age and sex were matched. A serological and molecular detection of parvovirus B19 were done using enzyme linked immunosorbant assay (ELISA) and Real-time PCR.

Results: B19-IgM, IgG and DNA were detected in study cases as (15.6%, 40% and 13.3%) compared to control group (4.4%, 13 % and 0%) respectively. B19-IgG and B19-IgM were detected in newly diagnosed cases in (41.7% and 19.05%) as well as in (38.1% and 12. 5%) of cases on chemotherapy respectively, all cases with B19-DNA signals were underwent chemotherapy (25%) with mean viral load of (36x104 copies/ml).

Conclusion: children who suffering from ALL are at increased risk of B19 infection. Notable percentage of persistent B19 infection was recorded among children with ALL who are receiving ongoing treatment.

Key words: parvovirus B19, acute lymphoblastic leukemia (ALL), real time PCR.

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Introduction:

B19V belong to Parvoviridae family which includes small non-enveloped single stranded DNA viruses, that infect a wide range of animals. (1). As a result of increased awareness of and screening for B19, a number of novel genotypes have been identified (2,3). The virus is spread by respiratory droplets (4) ,blood and blood products (5). Nosocomial infection has been also described (6), B19V is highly erythrotropic and preferentially replicates in erythroid progenitor cells (7). The pattern of clinical disease caused by B19 varies and is influenced by both the hematological and the immunological status of the infected individual in immunocompetant host. B19V can cause self limit infection including fifth disease (erythema infectiosum) in children and pure red cell aplasia in immunocompromised patients (8,9),it may cause chronic anemia in immunocompromised patients especially in patients who suffering from hematological malignancies.(10,11).

Patients and methods:

A cross sectional study involved 45 patients with ALL were currently attending department of oncology in Children's

Welfare Teaching Hospital in Medical City of Baghdad between December 2012 and April 2013. Twenty one patient who newly diagnosed with ALL and 24 who underwent chemotherapy. Their age ranged from 8 months to 15 years with mean age \pm SD equal to 6.54 \pm 4.2 years, compared to 45 of apparently healthy children who were already under pre operative screening tests as a control group, age and sex were matched. The study was approved by the ethics committees of Ministry of Health and all patients' parents gave informed consent. Three ml of blood samples were harvested and divided as following: One and half ml centrifuged for 10 min. X 3000 rpm, then the serum were aliquots in Eppendorf tubes and 1.5 ml collected in tubes with anti-coagulant substances (K3-EDTA) centrifuged for 15 min. X 1500 rpm, the sera and the plasma were immediately frozen at -20°C.

Detection of B19 IgM & IgG antibodies using Immunoassay kit for the semi-quantitative determination of human parvovirus B19 antibody (IgM) concentrations in serum by ELISA for in vitro diagnostic use (Nova Tec, NovaLiza, Germany, Nova Tec, NovaLiza catalog, Germany). Recombinant purified B19 antigens are coated to a microwell plate. The final measurement is carried out at 450 nm on a spectrophotometer. Samples were considered positive when the absorbance value was higher than 10% over the cut-off.

Viral DNA extraction and amplification were done

*Dept. of Microbiology College of Medicine Baghdad University

**corresponding Author, Dept. of Pediatric Heamatology, Children's Welfare Teaching Hospital, shthabdullah@yahoo.com

using [Parvovirus B19 Real-TM Quant kits (Sacace BIOTECHNOLOGIES, Italy)]. The Parvovirus B19 Real-TM is a Real-Time test for the quantitative detection of Parvovirus B19 in the biological materials. DNA is extracted from samples, amplified using real time amplification with fluorescent reporter dye probes specific for Parvovirus B19 and Internal Control (IC). Test contains an IC which served as an amplification control for each individually processed specimen and to identify possible reaction inhibition. Rounds of amplifications were done in a DNA thermal cycler (Smart Cycler) as follows: First round where initial denaturation for 15 min at 94°C. Followed by the second round of 38 cycles of denaturation at 94 °C for 5 sec, annealing at 60 °C for 20sec and extension at 72 °C for 15 sec then 60 °C and 72 °C for 15 sec. The results of amplification step was appeared on screen that linked to the thermal cycler (Smart Cycler). The Internal Control (IC) was detected on the FAM channel and Parvovirus B19 on the Joe/HEX/Cy3 channel. For each control and patient specimen, the concentration of Parvovirus B19 was calculated by using the following formula: Parvovirus B19 DNA copies/specimen (Joe/HEX/Cy3 channel) IC DNA copies/specimen (FAM channel) x coefficient* = copies DNA B19/ml. Statistical Analysis: data were analyzed by using statistical package for social sciences (SPSS 17). The quantitative data were expressed as mean ± standard deviation. The chi-square and t- test was used to compare between two groups. The ANOVA test was used to compare more than two variables. Significant P value was < 0.05. Odd ratio was used as a measure of relative risk.

Results:

Table -1 demonstrates B19 - serological markers among study groups, B19-IgM/IgG and B19-DNA were detected in (15.6%, 40% and 13.3%) of cases compared to (4.4%, 13 % and 0%) respectively in apparently healthy children as control group, statistical significant difference was clearly noticed in B19-IgG/DNA detection between cases and control groups (P-value < 0.05) and the risk of parvovirusB19 infection in children with ALL was 3.96 times (odds ratio).

Table -1: Parvovirus B19 IgM / IgG detections among study groups.

Study groups	(ve+)IgM No. (%)	IgG(+ve) No. (%)	B19-DNA (+ve) No. (%)
Cases (45)	7(15.6)	18 (40)	6(13.3)
Control (45)	2(4.4)	6(13)	0

odds ratio=3.96 p<0.05(IgG and DNA among study groups) Four out of 21(19.05%) children whom newly diagnosed with ALL had acute parvovirus B19 infection compared to 3 out of 24 (12.5%) children on chemotherapy who gave positive B19-IgM. B19 IgG antibodies were detected in 8 out of 21(38.1%) of newly diagnosed children with ALL compared to 10 out of 24 (41.7%) children on chemotherapy and all cases with

positive B19 -DNA signals were underwent chemotherapy which represented in 6 out of 24(25%)(p<0.05) as shown in table-2.

Table-2: Parvovirus B19 IgM / IgG /DNA detection among study cases.

ALL cases	IgM(+ve) No.(%)	IgG(+ve) No.(%)	B19-DNA (+ve) No. (%)
Newly diagnosed (21)	4(19.05)	8(38.1)	0
after receiving chemotherapy (24)	3(12.5)	10(41.7)	6(25)

P<0.05

The viral load was ranged from (65x10³-10⁶ copies/ml) with mean of (36x10⁴ copies/ml). Figure -1.

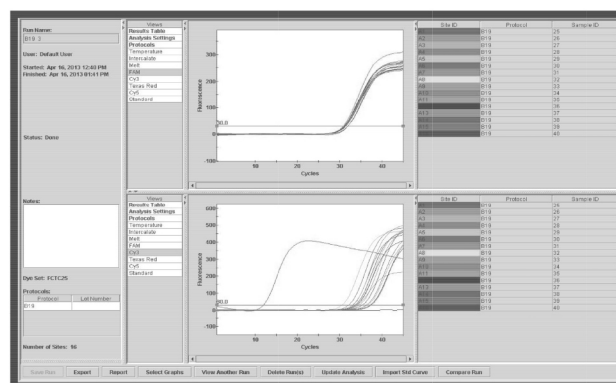


Figure-1: Real-time polymerase chain reaction curves for DNA detection of parvovirus B19.

In newly diagnosed ALL cases, only one case (4.7%) had IgM/IgG antibodies and 3 out of 21 cases (14.3%) gave IgM positive antibodies. While children on chemotherapy, a combined IgM/IgG were detected in 3 out of 24(12.5%), 3 cases with both IgG and B19 DNA signal detection and only two (8.3%) children on chemotherapy gave positive B19 DNA signal (P-value < 0.05).Table-3.

Table -3: The profile of B19 markers detected in recently infected patients.

B19 markers Total No. (%)	ALL cases	
	Newly diagnosed (21)	after receiving chemotherapy (24)
IgM	(14.3)3	0
IgM/IgG	(4.7)1	(12.5) 3
IgM/B19 -DNA	0	0
IgG/ B19- DNA	0	(12.5) 3
B19 DNA	0	(8.3) 2
B19-DNA/IgM/IgG	0	(4.2) 1

P-value < 0.05

Discussion:

Acute lymphoblastic leukemia is the most common cancer in childhood. Children younger than 5 years were at a higher risk of developing ALL (12). Multiple risk factors contribute to infection-related morbidity, chief among them the immunosuppressive effects of leukemia itself and of cytotoxic chemotherapy (13). In this study, PVB19- IgM was detected in 15.6% of cases, and children with ALL were at risk of getting parvovirus B19-infection as about 4times, however, this difference was not statistically significant compared to 4.4% among control group, this probably due to limited number of our cases. In comparison to studies done in Egypt among acute leukemic children, they suggested 37% of parvoB19- seropositivity (14), another recent research reported a prevalence of acute B19 infection in 30% of acute leukemic children (15) and in an Indian study was 27.7% (16). In contrast, low parvovirus IgM detected in only 5.1% (17) and 4.4% (18). Not surprisingly that in immunocompromised patients unable to mount a neutralizing antibody response due to a persistent bone marrow insufficiency (19). These figures are nearly similar in the comparison to results of other studies done recently which registered B19-IgG percentage (36%, 40%, 41.6% and 47.5%) by (14,15,18,20) respectively. The presence of anti B19V-IgG seems to indicate previous infection by B19V (21). In this study, the rate of IgG positivity against B19 infection in newly diagnosed ALL children was the highest 38% ,as well cases on chemotherapy, IgG was found in 41.7%. The higher prevalence of B19-IgG antibodies may be due to multiple blood transfusion which play a cornerstone in the transmission of the virus (22). On the other hand other studies included children on chemotherapy rated the positivity of B19-IgM as 18% up to 26.7 % respectively by (23,24). Higher rates of IgM seropositivity were reported ranged from 35% of leukemic children at time of first presentation and 25% of those during chemotherapy, while the frequency of IgG among recently diagnosed ALL was reported as 30% and was highest as 50% in those on chemotherapy (15). High frequencies of IgM and IgG were reported among recently diagnosed cases followed by those on maintenance chemotherapy (14). Furthermore; IgG and IgM rates of 34.3% and 27.8% were recorded in recently diagnosed children with ALL (16). These differences in rates may be attributed to different socioeconomic standards, frequency of blood transfusions and possibility of nosocomial exposure (15). Persistence of B19 infection was reported in such patients in serum and bone marrow samples by (24) that led to the conclusion that there may be an aberrant immune response to B19 in patients with acute leukemia as the presence of neutralizing antibodies to B19 didn't clear the virus and in this case high levels of antibodies are associated with the persistence and not to the clearance of the virus (15). In this study, B19-DNA targeted towards VP1 region was measured by real time-PCR technology. A close finding was given by (15) while lower B19 DNA Positivity was shown in 5.7% by (16). Compared to a relatively higher rates of Parvovirus B19 DNA in 27.1% by (17) to 37.14%

of patients (25) up to 45% (Zaki and Ashray, 2010). On the contrary The B19V NS1 was detected recently in 16% of bone marrow samples of acute lymphoblastic leukaemia (26). These results suggest that B19 infection may be a relatively common findings in patients suffering from haematological malignancies. The limit of detection in our PCR was found to be from 800 -10.000.000 genome equivalents/ml of plasma . Thus our PCR positive cases had viral load higher than this value. Hence actual numbers of B19 infected children with ALL may be more than were actually detected (16). It's well known that ALL Patients with viremia at diagnosis is lesser than patient on chemotherapy; thus might be attributed to chemotherapy which induced immune suppression with activation of persistent infection or from repeated blood transfusions (25). The actual number of children infected with B19V may be more than observed in the present study due to compromised status of these children which causing failure to amount detectable IgM and brief DNAemia (16, 25). Combined B19-DNA/IgG detection in the patients on chemotherapy may indicate either persistent infection after IgM/ IgG switch or reactivation of infection due to immune suppression. Accordingly, it is impossible to exclude the passive acquisition of antibodies by blood transfusion. On the other hand, B19-IgG may indicate serum clearance of viral nucleic acid. In only one case on maintenance chemotherapy there was combination of the three markers; the suitable interpretation is reactivation of viral infection with no sufficient clearance by B19-IgM due to immune suppressed bone marrow. We conclude that leukemic children are at increased risk of B19 infection and persistent B19 infection was recorded among children with ALL who are receiving ongoing treatment. It is advisable, if IgM turns out to be negative, to continue the search for possible parvovirus B19 infection by employing PCR method as a second line of diagnostics assay (25).

Author Contributions:-

Iqbal M. Abdullah: acquisition of data analysis, interpretation of data.

Shatha F. Abdullah: drafting of manuscript, design, interpretation of data and critical revision

Mazin F. Al-Jadiry: study conception, design, and interpretation of data.

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