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Prevalence of Epstein Barr Virus in children of leukemia and lymphoma

[*1] Kumar Shambhu Nath, [2] Bhatia BD, [3] Vineeta Gupta

[1] Associate Professor, Katihar Medical College, Katihar, Bihar, India
[2] Professor, Banaras Hindu University, Varanasi, Uttar Pradesh, India.
[3] Banaras Hindu University, Varanasi, Uttar Pradesh, India.

Abstract
Objective: Study was to evaluate the clinicohematological profile in childhood leukemia and lymphoma, and prevalence of Epstein Barr Virus of these leukemia and lymphoma cases.
Methods: Children were examined and various investigations were performed, and patients with ALL were treated with modified UK MRC X protocol.
Results: statistical analysis was done by using X^2-test, and P value was taken less than 0.05 for significant differences.
Conclusions: Patients with ALL were poor nutritional status and more high risk features. Majority of patients with Hodgkin's lymphoma were advanced disease and systemic symptoms. And prevalence of *EBV* was more in cases of ALL and Hodgkin's disease.

Keywords: Epstein Barr Virus, leukemia, lymphoma

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and represents around 23% of cancer diagnosis below 15 years of age, amounting to and annual incidence of approximately 30-40 per million cases (Ries LA *et al.*, 1999) [1]. There has been a gradual increase in incidence of ALL in the past 25 years with peak incidence in children aged 2 to 3 years.

Among the various risk factors for all the most accepted non-genetic risk factor is exposure to radiation/X-rays in both prenatal as well as post-natal life (Ross JA *et al.*, 1994) [2]. increased incidence of ALL is also associated with certain genetic conditions like - Down syndrome, Bloom syndrome, Schwachman syndrome, neurofibromatosis and ataxia telangiectasia. Viral etiology has also been implicated in ti re pathogenesis of ALL But there are few reports in the literature regarding this. No more studies have been conducted in this part of the country on clinicohematological profile and association of EBV of ALL

The lymphoma constitutes a heterogeneous group of neoplasm of lymphoid system that includes distinct clinicohematological entities representing clonal expansion of a normal precursor cell. These can further be divided into Hodgkin's and non-Hodgkin's lymphoma (NHL) based on the presence of Reed-Sternberg cell (present in Hodgkin's lymphoma). The risk of having the disease is increased by having weakened immune system (such as chemotherapeutic drugs and irradiation). The Genetic factors play a lesser role, but the viral infections like EBV and Human Immunodeficiency Virus (HIV) etc. are implicated in the etiology of lymphoma. The EBV has been more commonly associated with Hodgkin's lymphoma rather than non-Hodgkin's lymphoma.

Epstein Barr Virus (EBV) or Human Herpes Virus 4 infects most of the individual by adulthood as evidenced by the presence of antibodies to EBV in >90% adults. This is *to* bring to your kind notice that the first *virus* associated with human malignancy like lymphoproliferative disease, nasopharyngeal carcinoma. Burkitt's lymphoma, Hodgkin's disease and B-cell lymphoma. There are various methods used for detection of Epstein Barr Virus (EBV) in patients with malignancy. Katalin k, *et al* [3]. studied 109 cases of Hodgkin's disease and found 43% of cases were positive for LMP-1 of EBV. According to A. Arnstromy *et al* [4], 69% of Hodkgkin's disease were positive for EBV by RNA in situ hybridization, southern blot or immunohistochemical analysis. Study done by Lu *et al* [5]. 26. 9% cases of ALL were positive for EBV DNA in their peripheral blood mononuclear cells. There have been few studies for detection of EBV genome by PCR but no more studies have been conducted in this region.

Objective of our study was to find the clinicohematological profile in childhood leukemia and lymphoma. And to study the prevalence of Epstein Barr Virus of these leukemia and lymphoma cases.

Methods & materials

The study was conducted in the Department of Pediatrics and Microbiology, Sir Sunder Lal Hospital, Institute of Medical sciences, Banaras Hindu University, Varanasi, India.

Study Population: Sixty five children (Leukemia 32, Lymphoma 13, controls 20) attending Pediatric OPD and admitted in the pediatric ward of Sir Sunder Lal Hospital between December 2006 and June 2008 were included in the study. Informed consent was taken from the parents/ guardians. Attendants/parents/guardians of entire subject signed an informed consent, approved by the institutional ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India was sought.

Study Protocol: Children were examined clinically and anthropometry. The various investigations were performed using standard methods, like, total blood count, hemoglobin, platelet count on automated hematology analyzer (manufactured by Wipro), liver profile (Bil, SGOT/SGPT, TP :Alb), renal profile (Urea, Creatinine), serum uric acid on Flexor XL (netherland) and synchron CX5 (US), peripheral blood smear examination for blast cell, bone marrow examination with special staining, immunophenotyping wherever possible, FNAC/ Biopsy from lymph node in lymphoma cases, USG computed tomography of the chest and abdomen as required in the cases of Lymphoma for diagnostic specimen and clinical staging and PCR for Epstein Barr Virus.

Patients with ALL were treated with modified UK MRC X protocol. Those with Hodgkin's disease were treated with either COPP (cyclophosphamide, oncovin, procarbazine, prednisolone) or alternating COPP and ABVD (adriamycin, bleomycin, vinblastine, dacarbazine). 6 to 8 cycles were given depending on the stage of the disease. Patients with NHL received CHOP (cyclophosphamide, adriamycin, oncovin, prednisolone).

Sample collection: 10 mL of venous blood was collected from peripheral vein. 5ml serum was separated and stored at -40°C for DNA isolation by phenol alcohol extraction method. From rest sample other hematological profile were done.

Extraction of DNA from Blood Samples

Serum sample (3ml)
↓
Incubate with l mg (100µ1) of lysozyme at 37°C for 60 min.
↓
1 ml 0.1 % Triton-X 100 and 5µ1 Proteinase-K added and incubated at 65°C for 120 min.
↓
To this equal volume of chloroform: Isa-Amyl Alcohol (IAA) 24: 1 dilution was added and mixed by vortexing for 15 seconds.
↓
Centrifuge at 10,000 rpm for 10 mins.
↓
The aqueous phase was collected
↓
140µ1 of phenol: cholorophorm: IAA (25:24:1)/ml of aqueous was added, vortexed for 15 seconds.
↓
Centrifuge at 10,000 rpm for 10 minutes
↓
The aqueous phase was collected
↓
Equal volume of phenol: chlorophorm: lAA (25:24:1) was added and vortexed for 15 sec.
↓
Centrifuge at 10,000 rpm for 10 minutes
↓
Aqueous phase was collected
↓
2 µl of RNase (30µ1 *I* ml final concentration) was added
↓
Incubated for 30 min at 37°C
↓
Equal volume of isopropanol was added
↓
The solution was kept at room temperature for 5 minutes.
↓
The above was centrifuged at 10,000 rpm for 10 minutes and supernatant decanted, the pellet (colorless, usually not evident visually) washed by 200µ1, 70% ethanol
↓
The above solution was Centrifuge at 10,000 rpm for 10 minutes.
↓
Pellets were dried at 37°C (in inverted condition), for 30 min
↓
The pellet was re-dissolved in 20 µl TE (pH8)

PCR of Extracted DNA

Amplification of conserved sequences of Epstein-Barr virus BNRFl gene for pl40 by using specific primers:

Primers Design from BNRFl gene

Forward and reverse oligo-nucleotide primers derived from the conserved region of the Barr virus BNRFl gene for pl40 were designed and synthesized (Gene Bank: Accession no. NC_ gi |59165 I emb| X67777.1).

BNR Fl 5' AAGCTGAATGAGTGGGTGTTTG 3' (G+C=l0/21, 48%), BNR Rl 5'ATAGTGCTGGCAGCCTGCCATATC 3' (G+C=13/24, 54%)

Internal primers derived from the region in between the external primer sequences and were synthesized for use in nested PCR amplification. BNR F2 5' ACACGAACCGCTCATAGTTTGG 3' (G+C=11/22, 50%) BNR R2 5' AAGTAGCTGCTGACAAACTG 3', (G+C=9/20, 45%)

Table 1: Master Mix (25 µI) for first round PCR

S.N.	Constituents	Volume(µI)	Final Cone.
1	Autoclaved ultra- filtered water (PH 7.0)	17.17	-
2	1 Ox PCR buffer*	2.5	lx
3	dNTPmix	2	200mM each nucleotide
4	Primer F(BNR Fl)	1	10 p mole
5	Primer R (BNR Rl)	1	10 p mole
6	Taq DNA polymerase	0.33	1 unit
7	Genomic DNA template	1	lOOng/25 µl

*lOxPCR buffer contains: 500mM; lOOmMTris-HCl; 15mm MCl2

using thermal cycler (Biometra, Germany), the reaction mixture was subjected *to* 40 cycles of PCR.

Running conditions for first round PCR

Table 2: Lid Temp> 105°C

Step no	Step	Temp.	Duration
1	Initial Denaturation	95°C	7min
2	Denaturation	94°C	45 sec
3	Annealing	55°C	45 sec
4	Extension	72°C	45 sec
5	Go To 2, 39 cycle		
6	Final extension	72°C	10 min
7	Stop	4°C	Pause

Table 3: Master Mix for nested PCR

S. N.	Constituents	Volume(µl)	Final Cone
1	Autoclaved ultra-filtered (pH7.0)	17.17	
2	1 Ox PCR buffer*	2.5	lx
3	dNTPmix	2	200mM each nucleotide
4	Primer F(BNR F2)	1	10 p mole
5	Primer F(BNR R2)	1	lOp mole
6	Taq DNA polymerase	0.33	1 unit
7	DNA template (l" PCR product)	1	-

Using thermal cycler (Biometra, Germany), the reaction mixture was subjected *to* 40 cycles of PCR.

Running conditions for second round PCR

Table 4: Lid Temp> 105°C

Step no.	Step	Temp.	Duration
1	Initial Denaturation	95°C	7 min
2	Denaturation	94°C	45sec
3	Annealing	55°C	45 sec
4	Extension	72°C	45sec
5	Go To 2, 39 cycle		
6	Final extension	72°C	lOmin
7	Stop	4°C	Pause

10µ1 of PCR products were electrophoresed on 1.5% agarose gel stained with ethidium bromide (4µ1 *I* 100 ml) agarose gel solution Working conc. 0.5 µg/ml) (Stock Conc. 10 mg/ml) and the bands (638 bp} were visualized by UV transillumination. Negative and positive reaction controls using water and DNA from the reference strain of Epstein Barr Virus respectively, as templates were performed in each PCR experiment.

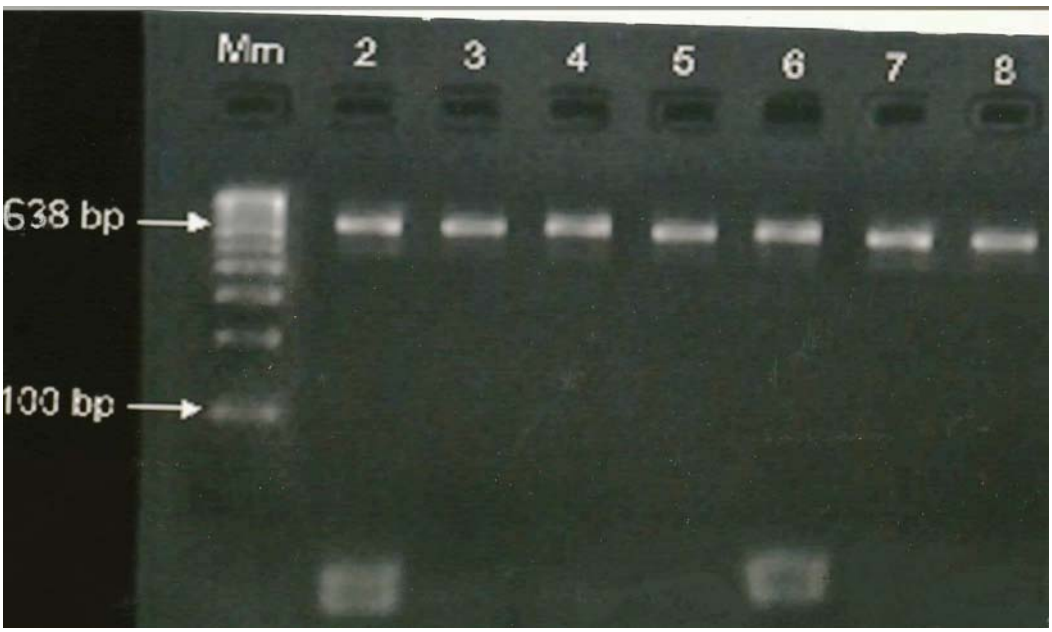

Fig 1: Results of PCR from subjects with childhood leukemia and lymphoma

Statistical Analysis

Data was analyzed by using statistical methods, and taken X^2 test for variables. P value was taken less than 0.05, for significant differences.

Observations

In our study, total 65 subjects were taken. In total of 65 subjects, 45 were in experimental group and 20 were in controlled group. In experimental group, 32 subjects (27 males and 5 females) with ALL and 13 with lymphoma (10 Hodgkin's and 3 non-Hodgkin). In the lymphoma group, all patients were male.

The nutritional status of the study subjects with ALL was found, that weight for age, height for age and BMI were <3rd percentile in 18 (56.3%), 10 (31.3%) and 16 (50%) respectively. Only 11 (34.4%}, 21 (46.9%) and 10 (31.3%) of the patients were having >10th percentile of weight for age, height for age and BMI respectively.

Out of 13 patients of lymphoma 10 were of Hodgkin's disease (7 were stage III, 2 were stage II and 1 was stage IV) and 3 were non-Hodgkin's disease. In the patients with Hodgkin's lymphoma 8 were having B symptoms in the form of either fever or significant weight loss. Nutritional status. It was found that weight for age was <3rd percentile in 10 (76.9%)

cases. Only 2 (15.4%) patients were weight for age of >10th percentile. The height for age and BMI was <3rd percentile in 6 (46.2%) patients. Height for age was between 3rd - 10th percentile in 4 patients and >10th percentile in 3 patients. BMI was between 3rd to 10th percentile in 3 patients and >10th percentile in 4 patients. The nutritional parameters of 45 control subjects were found that wt. for age was <3rd percentile in 6 patients (13.3%) 3rd_10th percentile (24.4%) and >10th percentile in (62.2%). Height for age was <3rd percentile in 5 (11.1 %) and >10th percentile in 26. (57.8%). BMI was <3rd percentile in 10 (22.2%) between 3rd – 10th, (22.2%) and 25 (55.6%) subjects had BMI >10th percentile.

As it was observed that a significant number of patients were falling below 3rd percentile for weight for age, height for age and BMI. It was further compared to the control population of the study and the difference was found to be significant statistically ($p<0.05$). The similar interpretation was seen in lymphoma patients. Significant population was falling below 3rd percentile for weight, for age, height for age and BMI. Weight for age and height for age was statistically significant in comparison to control population.

The age distribution of the patients with ALL and lymphoma, was found that 46.9% (15/32) patients. This patients were <5 years of age and most of the patients (84.4%) were below 10 year of age. In the lymphoma group, majority of the patients (69.2%) were between 5-10 year of age. 3 patients (23.1 %) and 1 patient (7. 7%) were below 5 and above 10 year of age respectively. There was no significant difference in the age distribution of the patients between leukemia and lymphoma group.

The polymerase chain reaction for detecting the EBV genome was done in the serum sample of all the cases and (20) control. It was found that none of the control was positive for EBV but 4 of the ALL (12.5%) were positive for EBV. Similarly 4 patients of Hodgkin's Lymphoma (40%) were positive for EBV whereas none of the patients of NHL were PCR positive. The hemoglobin, total leukocyte count, platelet count in patients with ALL were performed. It was observed that 28 (87.5%) of the patients were Hb of <8 gm% of which 13 (40.6%) were Hb of <5gm%. The total leukocyte count was <10,000/mm3 in (12.5%) cases. 16 cases (50%) were TLC of >50000/mm3 and 12 cases (37.5%) were TLC of 10,000-50,000/mm3. Platelet count was $<20Xl0^9$/L in one subject. $20Xl0^9$-$50Xl0^9$/L in 22subjects and $50Xl0^9$-$100Xl0^9$/L in 7 subjects. Only 2 patients had platelet count > $100Xl0^9$/L.

The hemoglobin of subjects were divided in to 4 groups <5gm%, 5-8gm%, 8-11 gm% and >11 gm% and it was compared with PCR positivity. And found that out of 28 patients who were PCR negative (16 patients) were having Hb of <8gm% whereas those who were PCR positive (4 patients) were equally distributed in all four groups. The distribution of Hb in the two groups did not differ significantly.

The total leukocyte count and PCR positivity was compared, and found that out of 4 patient who were PCR positive 2 patients were TLC 10000 to 50000 /mm3 and 2 patients were TLC between 50000 to 1 lac/mm3. None were TLC of either < 10000 or > 1 lac/mm3 whereas out of 28 PCR negative patients, 10 patients each was TLC of 10,000 to 50000/mm3 or 50000 to 1 lac/mm3. Only 4 patient were TLC of <10000or >1 lac. The distribution of TLC in the two PCR groups did not differ significantly.

The absolute platelet count and PCR positivity was compared, and found that all the cases who were PCR positive were having platelet count between $20Xl0^9$ - $50X10^9$/L. Out of 28 patients who were PCR negative 18 patients were platelet count were $20X10^9$- $50X\ 10^9$/L. Platelet count $<20X10^9$/L was seen in one patient, $50Xl0^9$- $100Xl0^9$/L in 7 and $>100Xl0^9$/L in 2 patients respectively. There was no significant difference in two PCR groups when they were compared for platelet count.

The Hb, TLC and platelet count was done and observed that 4 patients each were having Hb of <5 gm% and 5-8 gm% respectively. 5 patients with lymphoma were Hb of 8-11 gm%. The TLC was < 10000/mm3 in 8 patients (61.5%) and the platelet count was > 100 Xl09/L l in 12 patient (92.3%) only one patient was platelet count of< 100 Xl09/L (7.7%).

Table 5: Comparison of hemoglobin and PCR positivity in subjects of Lymphoma.

Hemoglobin (gm %)	PCR			
	Positive		Negative	
	No.	%	No.	%
<5gm/dl	0	0	4	44.4
5-8 gm/dl	1	25.0	3	33.3
8-11 gm/dl	3	75.0	2	22.2
>11 gm/dl	0	0	0	0
Total	4	100	9	100

x^2 =3.846; d.f.=2; P=0.146

The Hb of the patient with lymphoma was compared with PCR positivity and it was found that 3 out of 4 patients (75%) who were PCR positive was Hb, 8-11 gm% and 1 patient was Hb of 5-8gm %. Out of 9 PCR negative lymphoma 4 patients were Hb <5gm%, 3 were Hb of 5-8gm% and 2 were Hb of 8-1 l gm%. The distribution Hb in two PCR groups did not differ significantly (p=0.146).

Table 6: Comparison of total leukocyte count and PCR positivity in subjects of Lymphoma.

Total leukocyte count	PCR			
	Positive		Negative	
	No.	%	No.	%
<10,000/mm3	3	75.0	5	55.6
10,000 - 20,000/mm3	1	25.0	4	44.4
Total	4	100	9	100

x^2 = 0.442; df. = 1; P=0.506

The TLC and PCR positivity was compared, and it was observed that 3 out of 4 patients who PCR positive were TLC < 10000/mm3. Among 9 PCR negative 5 patients were having TLC of <10000/mm3 and 4 were having TLC of 10000-20000/mm3. These two group did not differ significantly (p=0.506).

Table 7: Comparison of Platelet count and PCR positivity in subjects of Lymphoma.

Platelet count	PCR			
	Positive		Negative	
	No.	%	No.	%
50 X 10^9/L - 150 X 10^9/L	1	25.0	0	0
>150 X 10^9/L	3	75.0	9	100
Total	4	100	9	100

x^2 =2.438; d.f.=1; P=0.118

The platelet count and PCR positivity was compared, and it was found that 3 cases (75%) out of 4 patients with PCR positive were platelet count > 150 $Xl0^9$/L, whereas all the PCR negative cases were platelet count > 150 $Xl0^9$/L. It was not significant differences.

Discussion

Leukemias are most common malignant neoplasm in childhood accounting for about 41 % of all malignancies. Similarly lymphoma is the 3rd most common cancer among children. In most of the cases of ALL etiology of ALL is unknown, although several genetic and environmental factors are associated with childhood leukemia. Apart from certain chromosomal abnormalities such as Down syndrome, Bloom syndrome, Ataxia telangiectasia and fanconi syndrome, there has been rising concern that environmental factors like EBV, HHV-6 and CMV are related with an increase in incidence of acute leukemia and lymphoma in children. If we look at the age distribution of ALL patients, 84.4% of the cases were below 10 years of age, majority of whom were below 5 years of age. This is in accordance with the usual presentation of ALL where peak incidence occurs between 2 to 5 years of age. In case of Hodgkin's disease, typical bimodal age distribution is seen in developed countries. The early peak occurs between 20 to 25 years and a second peak after the age of 50 years. In contrast, three distinct forms of Hodgkin's dlsease are seen in developing countries, a childhood form {age 14 years or younger), a young adult form (15 to 35 years) and an older form between 55 to 75 years (Hudson MM *et al*, 1997) [6]. In the present study also, more number of patients (69.2%) were between 5 to 10 years of age, indicating an early childhood peak. None of the patients were below 5 years of age.

The nutritional status of subjects with acute lymphoblastic leukemia showed that 56.3% of the patients were having acute malnutrition with <3rd percentile of weight for age. Similar finding was seen in BMI where 50% of the patients were falling below 3rd percentile, but the height for age which is a marker of chronic malnutrition was not affected much as only 31.3% of case were falling below 3rd percentile. Similar finding was seen in cases of lymphoma where 76. 9% and 46.2% of the patients had <3rd percentile, for weight for age and BMI respectively. When this was compared to control group, it clearly indicates that cases of ALL and lymphoma had poor nutritional status. It is known that nutritional status is a significant prognostic factor for outcome in patients with leukemia and lymphoma. In this study also, patients were significant malnutrition. Malnutrition in Indian children has been implicated in the poor treatment outcome of acute lymphoblastic leukemia. Such children have less tolerance for chemotherapy and receive suboptimal doses of chemotherapeutic drugs.

Most of the patients of ALL (87.5%) were severe anemia at the time of presentation. Total leukocyte count- of the patients with ALL was >50,000/mm3 in 16 patients (50%), satisfying the high risk criteria. The other prognostic criteria that is age < l year and > 10 year was seen is 1 and 5 patients respectively. Out of these 6 cases, 4 had TLC >50,0000 *I* mm3 who were classified in high risk category. If we consider the NCI criteria of high risk patients: age <1 year or > 9 years and initial WBC count of >50,000/mm3, 18 patients, (56.2%) in the present study satisfied the high risk criteria. This is at variance with Western figures where more patients fall in the standard risk group. Chessel JM, *et al.*(2000) [7] studied and said that standard risk factor is 65 % in age 1-9 years of children with leukocyte count < 50000cumm^3, higher risk factor is 25 % in age > 10 years with leukocyte count >50000cumm^3, highest risk factor is 8-9 % in infant bellow 1 year with hypodiploidy, and special risk is 1-2 % with B-ALL.

In the lymphoma group also, apart from the earlier age of presentation, more number of patients were advanced disease (state III/IV). Of the 10 patients only 2 had stage II disease. 80% of the patients were B symptoms. This picture is consistent with the presentation of HD in Indian children where advanced disease with B symptoms is more common. Rajlaxmi *et al.* 2006 [8], has reported an Indian study on Hodgkin's lymphoma and Epstein Barr virus by immunohistochemistry and found that EBV was positive in 55% of cases. Similar finding was found in the study done by Dinand *et al* [9]. where EBV was detected in 91.1% of 146 children with Hodgkin's lymphoma by LMP-1 detection with immunohistochemistry. Many studies have shown that EBV association in Hodgkin's lymphoma is more common in <10 year of age, in males, in less developed regions and mixed cellular subtype (Glassar *et al*, and Katalin k *et al*) [9, 3]. In present study 4 out of 10 cases of Hodgkin's Lymphoma were positive for EBV and none of the cases of nonHodgkin's lymphoma were positive for EBV by PCR. Of these four cases, 2 were mixed cellularity and 2 were of nodular sclerosis type. Naresh *et al* [11] and Dinand *et al* 9 reported association of EBV in 78% and 91.1 % of patients with Hodgkin's lymphoma respectively.

In present study of 32 cases of ALL 4 cases were positive of EBV by PCR (12.5%). The findings are similar to Lu *et al.* who studied EBV DNA in peripheral blood mononuclear cell in 26 cases of ALL and detected DNA in 7 (26.92%) cases. Sazawal *et al* [12] studied sera of 102 cases of acute lymphoblastic leukemia and compared it with 142 healthy subject for antibody to EBV antigen by enzyme immunoassay. The positivity was significantly higher in comparison to healthy controls (67% vs 51 %; P < 0.05). Sakajiru *et al* [13] reported a case of T-cell ALL, whose leukemic cells had EBV, confirmed by southern blotting and in situ hybridization.

Loutfy SA *et al*, 2006 [14] has also studied antibody for EBV in children with leukemia. He observed that EBV was positive in 83% of leukemic children and 95% of the control subjects were also positive for EBV thus excluding any causative association. Researchers, (Bogdanovic G. *et al.*) [15] have also tried to find association between perinatal exposure to EBV and development of acute leukemia in later life. 54 patients of ALL and 47 healthy controls matched for age and birth place were tested negative for EBV DNA in their cord blood sample by PCR.

Summary and conclusion

Our study was to evalatuated the clinicohematological profile and the prevalence of EBV in childhood leukemia and lymphoma. A total of 65 cases (45 patients and 20 controls) were included in the study. In total of 45 patients, 32 patients with acute lymphoblastic leukemia and 13 patients with lymphoma (10 Hodgkin's and 3 non-Hodgkin) were included. Detailed clinical and hematological profile was noted in all

the patients. EBV genome was identified by PCR using specific primers.

The following findings were obtained

i) Majority of the patients 27 (84.4%) with ALL were below 10 years of age of which 15 (46.9%) were below 5 years.
ii) In the lymphoma group, majority 9 (69.2%) were between 5-10 years of age.
iii) Out of 32 patients of ALL 18 (56.3%) were below 3rd percentile of weight for age, and 16 (50%) had a BMI below 3rd percentile. The difference was statistically significant when compared with controls ($p<0.001$ for weight for age and <0.05 for BMI).
iv) In the patients with lymphoma, 10 cases (76.9%) were below 3rd percentile for weight for age and 6 (46.2%) had BMI below 3rd percentile. Again on comparison with controls, the differences were statistically significant.
v) Out of 32 patients of ALL 18 (56.2%) were satisfied the high risk criteria of the disease.
vi) Majority of the patients (80%) had advanced disease (state III/IV) in the Hodgkin's disease group. B symptoms were present in 8 cases (80%).
vii) *EBV* was found to be positive in 4 out of 32 cases (12.5%) of ALL there was no significant association of *EBV* positivity with Hb, TLC or platelet count.
viii) Out of 13 patients of lymphoma 4 (30.8%) cases were found to be positive for *EBV*. All the cases were in the Hodgkin's lymphoma group (4/10). There was no significant difference between *EBV* positive and negative groups in relation to Hb, TLC and platelet count.

Thus, our study concluded that patients with ALL had poor nutritional status and more high risk features. Majority of patients with Hodgkin's lymphoma were advanced disease and systemic symptoms. Prevalence of *EBV* was more in cases of ALL and Hodgkin's disease in comparison to control subjects.

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