

Prevalence and Clinical Characteristics of Primary Epstein–Barr Virus Infection Among Children Presented with Cervical Lymphadenopathy

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ABSTRACT

Introduction: Epstein-Barr virus is a known cause of childhood cervical lymphadenopathy. In practice this infection is not sought as a cause in low-resources settings. The virus among children with cervical lymphadenopathy was not studied before in this country. The aims of this study were to estimate the prevalence of primary EBV infection and to describe clinical characteristics of children presenting with cervical lymphadenopathy.

Materials and Methods: A cross-sectional study was conducted among children presented with cervical lymphadenopathy to Khartoum Children Emergency Hospital during the period from February 2009 to January 2010. Eighty-two children were randomly selected. Demographic and clinical data were recorded. Routine laboratory values were determined and children's sera were analysed for anti-viral capsid antigens IgM Epstein–Barr virus ELISA tests. Mean and SD were calculated. Regression analysis was to identify association between demographic, clinical and laboratory variables and IgM seropositivity of EBV.

Results: Epstein-Barr virus infection was diagnosed in 13 (15.9%) children with cervical adenopathy (n=82). The average age of these children was 7.5 (SD±3.3) years. The male to female ratio was 1.6. The most frequent symptoms were fever, loss of appetite and cough. The anterior cervical group of lymph nodes was most commonly implicated. All affected patients had mobile lymph nodes and the majority, 12 (92.3%), of patient had firm nodes. The lymph node number ranged from 2-6 nodes; with a mean of 3.5 nodes and average size of 3.6 cm. Splenomegaly and pharyngo-tonsillitis were found in 30.8% and 23.1% of patients respectively and leukocytosis was noted in 3 (23.1%). The odds of IgM seropositivity decreased with age ≤ 9 years (OR 0.15, 95% CI 0.03-0.78, $p=0.023$).

Conclusion: EBV infection is not an uncommon cause of childhood cervical lymphadenopathy. The commonest symptoms are fever, loss of appetite and cough. Anterior cervical nodes are the most affected. Younger children (age ≤ 9 years) are less likely to be sero-positive.

Keywords: Lymph nodes, Child, Neck

INTRODUCTION

Epstein–Barr virus (EBV) is a double-stranded DNA virus, a member of the Herpes viridae family, is a known cause of infectious mononucleosis (IM) in children [1]. The virus usually becomes latent subsequent to the primary infection and more than 90% of adults are known to be seropositive and hence associated EBV malignancies are likely [2]. In low resources settings transmission of EBV generally occurs during infancy and early childhood and virtually all children are infected by the age of three years, resulting in asymptomatic infection resembling many febrile infections that occur during childhood [2]. Cervical lymphadenopathy (CL) is common in children with a range estimate of 38% to 90% of otherwise healthy children [3]. The commonest causes of childhood CL in similar settings as ours are reactive hyperplasia, specific infection, and malignancy [4]. Although it is not a serious disease, IM is known to present with CL [5]. In clinical practice many febrile children can present with IM-like syndrome and hence IM is not sought as a cause in children with lymphadenopathy particularly in low-resources settings as ours [2]. Furthermore, the prevalence of EBV infection among children with CL was not studied before in Sudan.

The aims of this study were to estimate the prevalence of primary EBV infection and to describe clinical characteristics of children presenting with cervical lymphadenopathy at an outpatient clinic of tertiary level Children's hospital.

MATERIALS AND METHODS

This cross-sectional study was conducted among children who presented for the first time with cervical lymph nodes enlargement

(lymph node of ≥ 10 millimeters) to the outpatient clinic of Khartoum Children Emergency Hospital (KCEH), Khartoum, Sudan during the period from February 2009 to January 2010. KCEH is the main referral hospital for children in Sudan with the capability of 240 beds and serving approximately five million people, the population of the capital, Khartoum [6].

Since the prevalence of cervical lymphadenopathy among Sudanese children was not known, a pilot study was conducted to determine the sample size. A pilot survey was first conducted among 60 patients attending the outpatient clinic. Twenty of them were selected by simple random sample and were found to have cervical adenopathy. Among those 20, the group aged 1-5 constitute 45% of the sample with sex ratio 5/4, and age group 6-10 constitutes 50% with even sex ratio while only 5% was found in the age group 11-15 year. According to the result of this survey and since the age is one of the important determinant factors; a population of 250 patients was assigned following a systemic proportional stratified random sampling. A sample size of 82 patients (52 males & 30 females) was chosen constituting about 30% of the assigned population. A child was included if he/she had single or multiple lymph nodes in the cervical region measuring ≥ 1 centimeter in both dimensions. Children who were known to have tuberculosis or malignancy were excluded from the study.

A preformed data sheet was designed to record demographic, history and clinical examination data. Five milliliters of venous blood were obtained for estimation of haemoglobin concentration, ESR, total and differential white blood cell count and centrifuged for sera. Sera were then analysed for anti viral capsid antigens

(VCA)-IgM Epstein-Barr virus (Ortho Diagnostic Systems) ELISA test. The analysis was performed according to the standards of the manufacturer's guidelines [7]. The diagnosis of primary EBV infection was based on the presence of IgM antibodies to VCA in the children's sera [1]. Children who were seropositive and symptomatic were treated by appropriate doses of acetaminophen for pain and fever and encouraged to take adequate fluids and rest and then followed up until resolution of the disease [8]. Seronegative children were subjected for further investigations for other causes of lymphadenopathy and were accordingly managed.

Parents/caregivers of the children were informed about the study and each had signed a written consent. KCEH Development and Ethical Committee had approved this study.

STATISTICAL ANALYSIS

Data were double entered and analysed into SPSS statistical software for windows version 20 (SPSS Inc., Chicago, IL, U.S.A.). Parametric tests were calculated for numerical variables where appropriate and frequency tabulation was done. Fisher Exact test was used to compare the difference between categorical data. Univariate and multivariate regressions were conducted to detect correlation between demographic, clinical and laboratory variables as independent variables and IgM seropositivity of EBV as a dependent outcome. Results were expressed as odds ratio (OR) within 95% CI limit. The level of significance was set at $p \leq 0.05$.

RESULTS

Eighty-two children with CLA were included in this study. Primary Epstein-Barr virus infection was serologically diagnosed in 13 (15.9%) children with CLA. The average age of these children was 7.5 (SD±3.3) years. Eight (61.5%) of them were males, while 5 (38.5%) were females with a male to female ratio of 1.6. However, there was no significant gender difference among children of different age groups ($p=0.188$).

The most frequent symptoms in children with primary IM and lymphadenopathy were fever, loss of appetite, cough and headache.

Clinical variable	Frequency	%
Symptoms		
Fever	9	69.2
Loss of appetite	7	53.8
Cough	6	46.2
Headache	4	30.8
Malaise	2	15.4
Arthralgia	1	7.7
Lymph nodes examination Findings Lymph node site		
Anterior cervical	10	76.9
Submandibular	7	53.8
Posterior cervical	7	53.8
Preauricular	2	15.4
Generalized lymphadenopathy	5	38.5
Lymph nodes characters		
Mobility	13	100
Firmness	12	92.3
Tenderness	1	7.7
Other examination findings		
Pharyngo-tonsillitis	3	23.1
Mouth ulcers	1	7.7
Splenomegaly	4	30.8
Hepatomegaly	1	7.7

[Table/Fig-1]: Clinical findings of acute Epstein-Barr virus infection in patients with cervical lymphadenopathy

The most affected group of lymph nodes were the anterior cervical, and the least were the preauricular. Generalized lymphadenopathy was reported in 5 children (38.5%). All patients had mobile lymph nodes and 12 (92.3%) of these children had firm lymph nodes, whereas only one (7.7%) had tender lymphadenopathy. The other frequent physical signs were splenomegaly (30.8%) and pharyngo-tonsillitis (23.1%). These are further illustrated in [Table/Fig-1]. The number of lymph nodes varied from 2-6 nodes, with a mean of 3.5 nodes and their average size was 3.6 cm (range 2-7 cm). Chest examination was normal in all children.

[Table/Fig-2] illustrates the laboratory values of CL patients with primary EBV infection. The mean white blood cell count was $7.43 \times 10^9/L$ and three (23%) children had leukocytosis ($WBC > 10 \times 10^9/L$).

Age was the only significant association with seropositivity of IgM antibody of EBV (OR 0.81, 95% CI 0.67-0.99, $p=0.041$) [Table/Fig-1,2]. The odds of being seropositive decreased when the child with CL was ≤ 9 years of age (OR 0.18, 95% 0.04-0.78 CI $p=0.022$). In a multivariate regression model, age retained association with EBV

Laboratory variable	Mean±SD*
Haemoglobin (g/dL)	9.9±1.96
ESR (mm/1 st hour)	59±23.05
White blood cell count ($10^9/L$)	7.24±3.6

[Table/Fig-2]: Laboratory values in patients with acute Epstein-Barr virus infection in children with cervical lymphadenopathy
* SD refers for standard deviation

Variables	OR*	95% CI †	p-value
ESR/1 st hour	1.01	0.99–1.03	0.565
Haemoglobin concentration g/L	0.99	0.72–1.38	0.984
White cell count $10^9/L$	1.01	0.92–1.00	0.805

[Table/Fig-3]: Association of laboratory values with acute Epstein-Barr virus infection in children with cervical lymphadenopathy using univariate regression
* OR refers to odds ratio
† CI refers to confidence interval

seropositivity (OR 0.15, 95% CI 0.03-0.78, $p=0.023$) in children with CL [Table/Fig-3].

DISCUSSION

This is the first report on the prevalence of EBV infection among children with cervical lymphadenopathy in Sudan, and is an addition to the available literature on prevalence studies of EBV infection in children.

Comparison of prevalence of EBV in children in literature

Despite the relatively small sample size, this prevalence (15.9%) was approximately similar to the figure reported by Abdel-Aziz et al., from Egypt [5]. Higher prevalence of up to 40% has been reported from Ugandan patients of different age groups, including adults. The higher rates of concomitant HIV infection in their sample might be a factor of the high prevalence. Furthermore, the majority of children in central Africa is usually asymptotically infected with or has mild EBV by the age of 3 years [2].

Comparison of Clinical features of EBV in literature

Our study results are significant for evidence of prior EBV infection has been reported in 36.3% of paediatric patients with leukemia in Sudan [9]. Prior EBV infection has also been demonstrated in 37% biopsies of adult Sudanese oral squamous cell carcinoma patients [10].

In this study the mean age of children with primary EBV infection (7.5 years) was higher compared to the more common younger age of preschool children observed by other authors from India and Egypt [5,11].

The most frequent associated symptom in children with cervical lymphadenopathy and primary EBV infection in our study was fever, reported in approximately 70% of patients. Abdel-Aziz et al., have also documented similar rates, however, Gao et al., reported a higher figure (92.3%) from Northern China [5,12]. Though all patients in this study had CL, pharyngitis was seen in less than a third of patients, a lower than reported from Egypt and China but comparable to a study from India [5,11,12].

The anterior cervical group in contradiction to Abdel-Aziz et al., were commonly enlarged in this series, followed by submandibular, posterior groups and then generalized lymphadenopathy [5]. There was no significant variation of the characteristics of the nodes in different studies [5,8,11-14]. Splenomegaly and hepatomegaly were detected among approximately 30% and 8% of patients respectively in this study, while Gao et al., recorded 47.4% for splenomegaly and 58.1% for hepatomegaly among their cohort [12]. Thompson et al., noticed hepatosplenomegaly among 27.1% of their cases while Abdel-Aziz et al., reported splenomegaly in 58.3% and hepatomegaly in 25% of their cases [5,15,16].

Leukocytosis was detected in 23% of patients in this study, lower than the 45.8% and 67.5% leukocytosis reported by other authors. Children with varied age groups including infants were recruited our, which may provide an explanation for some these differences.

Age younger than 9 year was the only correlated factor with seropositivity of EBV among all demographic, clinical and laboratory factors. The odds of contracting infection in an earlier age are a well-known trend in developing countries [1,17,18].

LIMITATIONS OF THE STUDY

This study has several limitations. The relatively small sample limits the generalizability of the results. Detection of IgG antibodies in patients' sera would have added further information on the nature of primary versus secondary infections. Follow-up complications were not sought in this cohort because of its cross-sectional nature. A larger scale longitudinal survey in this country will provide further information on this topic.

CONCLUSION

Epstein-Barr virus primary infection is not an uncommon cause of cervical lymphadenopathy among Sudanese children. The commonest presenting symptoms are fever, loss of appetite and cough. The anterior cervical nodes were the most affected and they are characteristically mobile but infrequently tender. Splenomegaly is not an infrequent finding. The odds of EBV-IgM seropositivity decreased with an age ≤ 9 years.

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REFERENCES

- [1] Haque T, Crawford DH. Epstein-Barr virus. In: Zuckerman JA, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, editors. Principles and Practice of Clinical Virology, 6th ed. John Wiley & Sons Ltd. Hoboken, NJ, (USA); 2009; pp. 199-221.
- [2] Jensen HB. Epstein-Barr virus. *Paediatr Rev.* 2011;32(9):375-83.
- [3] Rosenberg TL, Nolder AR. Paediatric Cervical Lymphadenopathy. *Otolaryngol Clin North Am.* 2014;47(5):721-31.
- [4] Moore SW, Schneider JW, Schaaf HS. Diagnostic aspects of cervical lymphadenopathy in children in the developing world: a study of 1877 surgical specimens. *Paediatr Surg Int.* 2003;19:240-44.
- [5] Abdel-Aziz M, El-Hoshy H, Rashed M, Qotb M, Awad S, Naguib N. Epstein-Barr virus infection as a cause of cervical lymphadenopathy in children. *Int J Paediatr Otorhinolaryngol.* 2011;75(4):564-67.
- [6] The fifth population census data: Sudan Central Bureau of statistics. 2008. Available from: <http://www.cbs.gov.sd/en/Accessed> 15/2/2015.
- [7] Ortho Diagnostic Systems. Ortho EBV ELISA: VCA IgM, VCA IgG, EBNA IgG: recueil fiches techniques. Roissy en France: Ortho Diagnostic Systems, 2001.
- [8] Luzuriaga K, Sullivan JL. Infectious mononucleosis. *N Engl J Med.* 2010;362(21):1993-2000.
- [9] Ahmed HG, Osman SI, Ashankyti IM. Incidence of Epstein-Barr Virus in Paediatric Leukemia in the Sudan. *Clin Lymphoma Myeloma Leuk.* 2012;12(2):127-31.
- [10] Jalouli J, Ibrahim SO, Sapkota D, Jalouli MM, Vasstrand EN, Hirsch JM, et al. Presence of human papilloma virus, herpes simplex virus and Epstein-Barr virus DNA in oral biopsies from Sudanese patients with regard to toombak use. *J Oral Pathol Med.* 2010;39(8):599-604.
- [11] Balasubramanian S, Ganesh R, Kumar JR. Profile of EBV associated infectious mononucleosis. *Indian Paediatr.* 2012;49(10):837-38.
- [12] Gao LW, Xie ZD, Liu YY, Wang Y, Shen KL. Epidemiologic and clinical characteristics of infectious mononucleosis associated with Epstein-Barr virus infection in children in Beijing, China. *World J Paediatr.* 2011;7(1):45-49.
- [13] Balfour HH Jr, Holman CJ, Hokanson KM, Lelonek MM, Giesbrecht JE, White DR, et al. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. *J Infect Dis.* 2005;192:1505-12.
- [14] Williams H, Crawford DH. Epstein-Barr virus: the impact of scientific advances on clinical practice. *Blood.* 2006;107:862-64.
- [15] Bowers JW, Zhang L. Reactive lymphadenopathy with diffuses paracortical pattern-infectious etiology. In: Cuaing HD, Bhargava P, Sandin RL, editors. Non-Neoplastic Hematopathology and Infections. 1st ed. John Wiley & Sons, Inc; 2012. pp. 323-46.
- [16] Thompson SK, Doerr TD, Hengerer AS. Infectious mononucleosis and corticosteroids: management practices and outcomes. *Arch. Otolaryngol. Head Neck Surg.* 2005;131:900-4.
- [17] Ebell MH. Epstein-Barr virus infectious mononucleosis. *Am Fam Physician.* 2004;70(7):1279-87.
- [18] Xiong G, Zhang B, Huang MY, Zho H, Chen LZ, Feng QS, et al. Epstein-Barr virus (EBV) infection in Chinese children: a retrospective study of age-specific prevalence. *PLoS One.* 2014;10(9):e99857.

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