Acute *Toxoplasma gondii* infection in children with reactive hyperplasia of the cervical lymph nodes

Jalal A. Bilal, MBBS, MD, *Mohamed A. Alsammani,* MBBS, MD, *Mohamed I. Ahmed,* DMB, PhD.

ABSTRACT

الأهداف: تحديد معدل انتشارمصل التوكسوبلازما الغوندية للاطفال الذين يعانون من فرط التنسج التفاعلي للعقد اللمفية الرقبية.

الطريقة: أجريت هذه الدراسة الاستطلاعية المستعرضة في مستشفى الأطفال، الخرطوم، السودان في الفترة التي أمتدت من يناير 2011م إلى أبريل 2011م. شملت الدراسة 80 طفلاً مصاباً بتضخم العقد اللمفية الرقبية. تم رشف العقد اللمفية المتضخمة لغرض السيتولوجيا كما تم سحب عينة دم من جميع المرضى لاجراء تحليل مخبري روتيني والغلوبولينٌ المناعيّ ل التوكسوبلازما الغوندية في المصل.

النتائج: من مجموع 80 طفلاً أشارت النتائج الى أن 60 منهم يعانون من فرط التنسج التفاعلي للعقد اللمفية. كان معدل انتشار مصل التوكسوبلازما الغوندية في مصل الأطفال الذين عانوا من تضخم العقد بنسبة 27.5% كما أن الدراسة قد كشفت أن المصل كان موجباً في 36.7% من الأطفال الذين يعانون من فرط التنسج التفاعلي للعقد اللمفية. لم تنبئ الخصائص السريرية ولا الاختبارات عن عدوى المصورات الزيفانية الحادة في فرط التنسج التفاعلي للعقد اللمفية.

الخاتمة: اثبتت الدراسة أن معدل اتنشار مصل المصورات الزيفانية الموجب عال (37.6%) لدى الأطفال الذين يعانون من فرط التنسج التفاعلي للعقد اللمفية. يمكن التماس الدراسات المصلية قبل الاختبارات المخبرية الغازية. الفحوصات المخبرية الروتينية لا تساعد في تشخيص عدوي التوكسوبلازما الغوندية الحادة في فرط التنسج التفاعلي للعقد اللمفية.

Objectives: To determine the seroprevalence of *Toxoplasma gondii (T. gondii)* in children with reactive hyperplasia of the cervical lymph nodes.

Methods: This cross-sectional prospective study was conducted in Khartoum Children Emergency Hospital, Khartoum, Sudan between January 2010 and April 2011. Eighty children with cervical lymphadenopathy were selected using random sampling. Their lymph nodes were aspirated for cytology, and a blood sample was collected from all patients for routine laboratory analysis and *T. gondii* IgG and IgM antibodies.

Results: Among 80 children with cervical lymphadenopathy, 60 (75%) had non-specific reactive hyperplasia. The seroprevalence of *T. gondii* among children with cervical lymphadenopathy was 27.5% (n=22), and the seropositivity of acute *T. gondii* among those with reactive hyperplasia was 36.7% (n=22/60). Lymph nodes in the *T. gondii* positive group were mobile and warm (p<0.05). The clinical features and laboratory tests were insignificant predictors of acute *T. gondii* infection with reactive hyperplasia of the cervical lymph nodes.

Conclusion: The prevalence of acute *T. gondii* infection is high among children with non-specific reactive hyperplasia of the cervical lymph nodes. Routine laboratory studies are not helpful in the diagnosis of *T. gondii* infection with reactive hyperplasia of the lymph nodes however, serological studies may be requested prior to invasive procedures.

Saudi Med J 2014; Vol. 35 (7): 699-703

From the Departments of Pediatrics (Bilal), Obstetrics and Gynecology (Alsammani), Medical Microbiology, College of Medicine, Qassim University, Buraidah, Kingdom of Saudi Arabia, and the Departments of Obstetric and Gynecology (Alsammani), and Microbiology (Ahmed), College of Medicine, University of Bahri, Khartoum, Sudan.

Received 28th December 2013. Accepted 4th May 2014.

Address correspondence and reprint request to: Dr. Jalal A. Bilal, Pediatrics Department, College of Medicine, Qassim University, Buraidah, Kingdom of Saudi Arabia. E-mail: jalalbilal2000@gmail.com

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

Lymphadenopathy is defined as lymph node enlargement beyond one cm in diameter.[1] Cervical lymph node enlargement is a common childhood problem.[2] Approximately 90% of children <8 years old have had palpable cervical lymph nodes.[3] The most common cause of cervical lymphadenopathy during childhood is infection, but occasionally, it might be due to a serious disorder such as malignancy. The enlargement of the lymph node may be due to the proliferation of benign cells intrinsic to the node, or infiltration with malignant cells.[4] However, reactive hyperplasia is the most common cause of cervical lymphadenitis in children. It occurred in 59.7% of children with cervical lymphadenopathy,[5] and in 25.4% in another study.[6] Fine needle aspiration cytology (FNAC) is a simple, safe, reliable, rapid, and inexpensive method of establishing the diagnosis of various causes of cervical lymphadenopathy in children, with an estimated 90.9% overall diagnostic sensitivity.[7] Reactive hyperplasia is a benign and self-limiting condition, and thus, no further management is usually recommended. In clinical practice, a pathology report of "benign reactive hyperplasia" will usually satisfy clinicians, and make them reluctant to search for further causes. The aim of this study was to estimate the seroprevalence of acute *Toxoplasma gondii* (*T. gondii*) infection in children with reactive hyperplasia of the cervical lymph nodes.

Methods. This cross-sectional hospital-based study was conducted in Khartoum Children Emergency Hospital, Khartoum, Sudan between January 2010 and April 2011. Eighty patients with cervical lymphadenopathy aged 1-13 years from the outpatient department were included in this study. The Development and Ethics Committee of the hospital approved the study. After explaining the study objectives to the parents/caregivers of the children included in the study, guardians signed the written consent.

The sample size was calculated using a prior pilot study where 250 patients were assigned following systemic proportional stratified random sampling. A sample size of 80 patients (51 males and 29 females) was then calculated, constituting approximately 30% of the assigned population. Inclusion criteria were the presence of one or more lymph nodes in the cervical region measuring more than one centimeter in both dimensions. Patients with malaria or with a known diagnosis of tuberculosis or malignancy were excluded from the study. Careful medical history included fever, sore throat, malaise, headache, cough, loss of weight, loss of appetite, rash, and contact with animals, while physical examination included characteristics of the lymph node (site, size, consistency, warmness, mobility and tenderness), and laboratory studies investigated the erythrocyte sedimentation rate (ESR), hemoglobin level, and the total and differential white blood cell count, which were recorded with routine laboratory tests for all patients. Using a thin needle, the largest palpable cervical lymph node was aspirated, and the obtained deposit was spread onto 2 slides for staining using Giemsa stain for one slide and hematoxylin and eosin stain for the other. Patients' sera were tested for both immunoglobulin G and M of *T. gondii* by enzyme-linked immunosorbent assay (ELISA) technology (in vitro test) using special kits provided by Alpha Diagnostic International (ADI, San Antonio, Texas, USA).

The Statistical Package for Social Sciences for Windows version 15 (SPSS Inc., Chicago, IL, USA) was used to record and analyze the data. The descriptive analyses used included the mean, standard deviation, and frequency distribution. A multivariate logistic regression was conducted to test whether the demographic characteristics, clinical findings, and routine laboratory tests are associated with *T. gondii* seropositive reactive hyperplasia of the lymph nodes. The results of the analysis were expressed as odds ratios (ORs) and 95% confidence intervals (95% CI). A p-value of <0.05 was considered significant.

Results. Of the 80 patients, 60 (75%) were found to have non-specific reactive hyperplasia, 8 (10%) had tuberculosis, 8 (10%) had acute bacterial infection, and 4 (5%) had lymphoma. The number of patients with reactive hyperplasia that had both IgG and IgM positive sera for *T. gondii* was 22 (36.7%), signifying an acquired acute toxoplasma infection; all of them were treated by pyrimethamine, sulfadoxine, and folinic acid for 2 weeks. The patients' mean age was 5.84±3.2 years with a range of 1-13 years, and there was no significant gender difference (p>0.05). The most frequent complaints are shown in **Table 1**. The anterior cervical lymph nodes were the most commonly affected group, followed by the posterior cervical group, whereas the post-auricular lymph nodes were the least affected. Most of these lymph nodes were firm and freely mobile, and most were not tender. Splenomegaly was detected in 18.3% of cases and only 5% had hepatomegaly (**Table 1**).

Comparison of *T. gondii* seropositive cases with those with seronegative hyperplasia indicated that their lymph nodes tended to be tender (31.8% versus 5.3%) had less mobility (72.7% versus 97.4%, p=0.016), and 40.9% versus 10.5% (p=0.006) were warm. There was no significant difference between the 2 groups with regard to age, gender, clinical findings, weight, and

Table 1 - Symptoms and relevant clinical findings of 60 children with reactive hyperplasia of cervical lymph nodes.

History and clinical examination findings	n	(%)
Fever	48	(80.0)
Cough	34	(56.7)
Loss of weight	31	(51.7)
Sore throat	27	(45.0)
Loss of appetite	23	(38.3)
History of animal contact	23	(38.3)
Headache	16	(26.7)
Malaise	10	(16.7)
Groin mass	7	(11.7)
Anterior cervical lymph nodes	56	(93.3)
Posterior cervical lymph nodes	31	(51.7)
Submandibular lymph nodes	20	(33.3)
Preauricular lymph nodes	4	(6.7)
Post-auricular lymph nodes	2	(3.4)
Firmness	58	(96.7)
Mobility	53	(88.3)
Warmness	13	(21.7)
Tenderness	9	(15.0)
Splenomegaly	11	(18.3)
Hepatomegaly	3	(5.0)

Table 2 - Demographic, clinical, and laboratory findings in association with seronegative reactive hyperplasia and acute toxoplasmosis (seropositive reactive hyperplasia) of 60 children with reactive hyperplasia of cervical lymph nodes.

Variable	Reactive hyperplasia (n=38)		Toxoplasmosis (n=22)		*P*-value
Age* (years)	5.5±2.7		5.8±3.2		0.698
Gender (M:F)	27:11		12:10		0.196
Symptoms					
Fever	28	(73.7)	20	(90.9)	0.238
Sore throat	16	(42.1)	11	(50.0)	0.554
Malaise	5	(13.2)	5	(22.7)	0.338
Headache	10	(26.3)	6	(27.3)	0.936
Cough	21	(55.3)	13	(59.1)	0.773
Loss of weight	16	(42.1)	15	(68.2)	0.051
Loss of appetite	15	(39.5)	8	(36.4)	0.811
Rash	2	(5.3)	3	(13.6)	0.258
Contact with animals	15	(39.5)	8	(36.4)	0.811
Clinical and laboratory findings					
Sub-mandibular group	25	(65.8)	13	(59.1)	0.604
Anterior cervical group	35	(92.1)	21	(95.5)	0.616
Preauricular	3	(7.9)	1	(4.5)	0.616
Posterior cervical group	19	(50.0)	12	(54.5)	0.734
Other sites	9	(23.7)	7	(31.8)	0.303
Tenderness	2	(5.3)	7	(31.8)	0.006
Mobility	37	(97.4)	16	(72.7)	0.016
Consistency	1	(2.6)	1	(4.5)	0.691
Warmth	4	(10.5)	9	(40.9)	0.006
Splenomegaly	6	(15.8)	5	(22.7)	0.503
Hepatomegaly	3	(7.9)	0		0.175
Skin rash	37	(97.4)	22	(100.0)	0.443
Weight*	15.85 ± 5.12		20.97 ± 5.12		0.712
Height*	103.86 ± 24.84		101.1955 ± 27.41		0.701
ESR*	61.31 ± 32.8		60.59 ± 30.0		0.933
Hemoglobin*	10.02 ± 1.91		9.68 ± 2.41		0.542
Total WCC*	7834.21 ± 4396.8		141.82 ± 3247.1		0.523
Neutrophils*	46.16 ± 12.85		43.32 ± 11.88		0.400
Lymphocytes*	51.86 ± 12.45		54.50 ± 11.25		0.418
Eosinophil*	2.31 ± 4.51		2.18 ± 4.60		0.913

*mean±SD, ESR - erythrocyte sedimentation rate, WCC - white cell count

Table 3 - Adjusted multivariate analysis of the association between the lymph nodes' clinical characteristics with the toxoplasma seropositive lymph node in 60 children with reactive hyperplasia of cervical lymph nodes.

Variables	OR	95% CI	*P*-value
Palpable sub-mandibular lymph nodes	1.335	0.363-4.909	0.663
Palpable anterior cervical group	0.740	0.053-10.304	0.823
Palpable posterior cervical group	0.703	0.191-2.583	0.596
Palpable lymph nodes in other sites	1.046	0.775-1.412	0.768
Lymph node number	0.898	0.610-1.322	0.586
Lymph node tenderness	0.241	0.020-2.859	0.259
Lymph node size	0.778	0.487-1.243	0.294
Lymph node warmness	0.309	0.044-2.180	0.239

There were no significant associations of either the site or the characteristics of lymph node involvement. OR - odd ratios, CI - confidence interval

height, (p>0.05) as well as for the laboratory values, as illustrated in **Table 2**.

Table 3 shows the adjusted multivariate analysis of the association between the lymph nodes' clinical characteristics with the toxoplasma seropositive lymph node. There were no significant associations of either the site or the characters of lymph node involvement.

Discussion. The seroprevalence of *T. gondii* was 27.5% (n=22) among children with cervical lymphadenopathy, along with 36.7% seropositivity of acute *T. gondii* (IgG and IGM) among those with reactive hyperplasia of cervical lymph nodes based on FNAC. The clinical features and the laboratory findings were insignificant predictors of acute *T. gondii* infection as a cause of reactive hyperplasia of the cervical lymph nodes. These results showed that there was a high prevalence of acute *T. gondii* among children with reactive hyperplasia. Literature on acute *T. gondii* infection and reactive hyperplasia of the lymph nodes is scant and has not addressed children per se. The high seroprevalence of *T. gondii* in this young group may reflect the endemicity of the disease in Sudan. A recent study[8] in the same area reported a 43.6% prevalence rate of anti IgG *T. gondii* antibodies and a 13.6% IgM seroprevalence among different healthy age groups and suspected cases of toxoplasmosis. However, their prevalence was based on positive latex agglutination tests, and the seropositivity in children was 13.6% prevalence in an apparently healthy sample that was comparable with our study. Moreover, antibody detection was performed only on samples of cord blood and did not include samples from children. Another study[9] in the same locality showed a

seroprevalence of 34.1% among women of reproductive age. In a neighboring locality, Mohamed et al[10] reported, in a cross-sectional study, a seroprevalence of 41.7%. The high seroprevalence of *T. gondii* (67%) in domestic animals in Sudan may be of public health concern, especially for those who consume raw milk.[11] Animal-human transmission is higher in children due to direct contact with cats, a habit of eating raw meat, contact with infected soil and drinking raw milk. In this study,[12] 36.4% of children reported direct contact with domestic animals. In addition, sand pits, which are used in playgrounds for children, may be a source of *T. gondii* oocytes infection. There is a wide variability in the clinical presentation of the disease. Generally, the disease is manifested clinically in immunocompromised patients.[13] A previous study[14] documented that FNAC has a high sensitivity (72.7-81.8%) and specificity (98-100%) for the diagnosis of toxoplasma lymphadenitis. However, in this study, none of the 22 specimens showed evidence of *T. gondii* infection in cytology specimens. Although FNAC was found to be comparable to open biopsy,[15] it is not yet supported by good-quality data. Demonstration of tachyzoites in lymphadenitis has been reported infrequently.[16] Therefore, the diagnosis of toxoplasmosis is primarily made by the use of serological tests. Furthermore, serologic diagnosis of *T. gondii* is complicated by the fact that antibodies may persist for years in healthy people.[17] The main presenting symptoms among the studied group were fever (90.9%), cough (59.1%), and loss of weight (68.2%). The most commonly affected lymph nodes groups were the anterior cervical (95.5%), submandibular (59.1%), and posterior cervical groups (54.5%). These lymph nodes were characteristically mobile and warm ($p<0.05$). It is known that *T. gondii* infection of the lymphatic system is commonly asymptomatic.[18] However, the symptoms may present in only 3-7% of clinically significant lymphadenopathy.[19] These symptoms include fever, malaise, night sweats, myalgia, sore throat, maculopapular rash, abdominal pain, hepatosplenomegaly, and small numbers of atypical lymphocytes.[20] The presence of symptoms in this study may be explained by recent infection in the studied patients. In addition, children are known as physiologically immune-deficient; hence, acute infection in children may be clinically subtle. Furthermore, possible confounders such as malnutrition may coexist in those children, which was not addressed in this study. In this study, the commonly affected nodes were the anterior and the posterior cervical groups. This is comparable with the results obtained by Weiss et al.[20] Spleen enlargement is a less frequent finding in toxoplasma infection; this study showed that 22.7% of cases had a palpable spleen.[21] The spleen and liver enlargement may be due to the response of the reticuloendothelial system to a recent infection, compared with past infection where spontaneous resolution is the rule. Hemoglobin concentration, ESR, and total white cell count did not differ significantly between children with reactive hyperplasia of the lymph node and those with acute toxoplasmosis. A single study[22] reported reversible neutropenia in children with congenital, but not acquired toxoplasmosis. The current study showed that the site, number, consistency, and the size of the lymph node are poor predictors of acute toxoplasmosis among children with reactive hyperplasia of the cervical lymph nodes. This may justify the request for a serologic test for *T. gondii* in children with cervical lymphadenopathy before performing invasive cytology.

The limitations of this study are the relatively small sample size and that the result of lymph node histology was based on cytology for tissue pictures rather than the gold standard, histopathologic examination. Furthermore, although we did not address the cause/effect relationship between acute *T. gondii* infection and reactive hyperplasia of the lymph nodes, the results may be an invitation for further research.

In conclusion, the prevalence of reactive hyperplasia of the cervical lymph nodes was 75%. There was a high prevalence (36.7%) of acute *T. gondii* infection among children with reactive hyperplasia of the cervical lymph nodes. Serological studies may be requested in children with cervical lymphadenopathy prior to invasive procedures. Routine laboratory studies are not helpful in the diagnosis of acute *T. gondii* infection with reactive hyperplasia of the lymph nodes.

Acknowledgment. *The authors would like to thank all children and their guardians for their contributions to this study. Great appreciation goes to Dr. Adel Hussein, Cytologist, National Laboratory, Khartoum, Sudan for reporting the cytology results.*

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