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## Immunodiagnostic investigations for children with lymphadenopathy at Tanta University hospital

Saida M. Amer<sup>1</sup>, Nanis G. Alam<sup>1</sup>, Mohamed R. El-Shanshory <sup>2</sup>, Fatma A. Ibrahim<sup>3</sup>,  
Ahmed S. El-shafey<sup>1\*</sup>

<sup>1</sup> Department of Botany and Microbiology, Faculty of Science, Tanta University, Egypt.

<sup>2</sup> Department of Pediatrics, Faculty of Medicine, Tanta University, Egypt.

<sup>3</sup> Department of Biochemistry, National Research Center, Egypt.



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### ABSTRACT

The lymphatic system is composed of lymphatic vessels and lymphoid organs such as the thymus, tonsils, lymph nodes, and spleen. Lymph nodes are small glands that are responsible for filtering fluid from the lymphatic system. Lymphadenopathy refers to the enlargement of lymph nodes that can occur due to autoimmune disease, malignancy or microbial infections. Immunological investigations were done from 2018 to 2023 to children with lymphadenopathy to help in diagnosis the causes of lymphadenopathy. Viruses as Epstein-Barr virus and cytomegalovirus were detected in blood samples by immunoassays. The obtained results indicated presence of Epstein-Barr virus and (45.5%), cytomegalovirus (18.2%) in children with lymphadenopathy. The results showed no significant differences in levels of serum Anti-Nuclear Antibody (ANA) for children with lymphadenopathy compared with their levels in healthy control children. Only one case (2.9%) out of 35 children with lymphadenopathy was diagnosed with Kawasaki disease and had positive ANA test. There was no significant difference in immunoglobulin A (IgA) in children with lymphadenopathy compared to their levels in healthy control children.

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### Introduction

Lymph nodes are found at the convergence of major blood vessels, and an adult will have approximately 800 nodes commonly sited in the neck, axilla, thorax, abdomen, and groin. These filter incoming lymph and play a role in infection as well as in malignancy (Bujoreanu & Gupta 2023). Lymphadenopathy is a common finding in clinical practice. The cause of enlarged nodes on clinical examination alone is challenging and there may be multiple reasons for this enlargement. It may become enlarged due to stimulation by infectious agents or the involvement of metastasis or malignant diseases, such as lymphoma (Vesnic et al. 2024). Although lymphadenopathy in the presence of fever usually

represents an infection or lymphoma, the differential diagnosis is actually quite broad. The list of differential diagnoses includes infectious mononucleosis (Epstein Barr virus), cytomegalovirus infection, toxoplasmosis, syphilis, subacute bacterial endocarditis, histoplasmosis, sarcoidosis, salmonellosis, tuberculosis, acquired immunodeficiency syndrome, Hodgkin disease, non-Hodgkin lymphoma, angioimmunoblastic lymphadenopathy, mixed essential cryoglobulinemia, systemic mastocytosis, chronic lymphocytic leukemia, agnogenic myeloid metaplasia, Waldenström macroglobulinemia, multiple myeloma, systemic lupus erythematosus, rheumatoid arthritis, Kawasaki disease, Whipple disease, serum sickness, and Kaposi sarcoma (Habermann & Steensma, 2000).

Corresponding author Email address: [ahmedsamymmd@gmail.com](mailto:ahmedsamymmd@gmail.com) (Ahmed Samy El-shafey)



Epstein Barr virus is a herpes virus with double-stranded DNA enclosed by proteins. The envelope of the virus has glycoproteins, which are important for attachment and entry into the host cells (B cells and epithelial cells). Epstein Barr virus targets B cells by utilizing their molecular machinery to replicate the viral genome. The virus causes B cells to differentiate into memory B cells, which then can move into the circulatory system, or become latent until a trigger causes reactivation (Plosa et al. 2012). Bilal (2015) reported that Eighty-two children with Cervical lymphadenopathy were included in his study. Primary Epstein-Barr virus infection was serologically diagnosed in 13 (15.9%) children with Cervical lymphadenopathy. 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 ( $p=0.188$ ). Epstein-Barr virus (EBV) and cytomegalovirus (CMV) cause infectious mononucleosis (IM), characterized by pharyngitis and subacute (development over weeks) bilateral anterior cervical lymphadenopathy or generalized lymphadenopathy (Berce et al. 2023).

Cytomegalovirus is a double-stranded DNA virus and is a member of the herpesviruses. Like other herpesviruses, after recovery of the initial infection, CMV remains dormant within the host. Cytomegalovirus (CMV) has been identified as an important viral pathogen in humans for more than a century. CMV infects multiple human cell types, including salivary gland epithelial cells, hence the original name of the virus: salivary gland virus. In 1960, Weller designated the virus as CMV based on the appearance of the swollen virus-infected cells labeled as cytomegalic. CMV infects nearly 1% of all newborns,  $\approx$  40,000 infants per year, in the United States. Infection with CMV is the most common cause of nonhereditary sensorineural hearing loss (SNHL). In addition to congenital and perinatal infection, CMV causes significant morbidity in immunocompromised patients, including chorioretinitis, pneumonia, colitis, and neuropathy (Odumade et al. 2011).

Among primary antibody deficiencies, the most significant prevalence of lymphadenopathy is reported in patients with common variable immunodeficiency (CVID), while it is described with lower frequency in patients with other disorders, including X-linked agammaglobulinemia (XLA), hyper-immunoglobulin (Ig)M syndrome (HIGM) and selective IgA deficiency (Costagliola & Consolini 2021).

Autoimmune disease-associated lymphadenopathy shows marked histopathological and clinical diversity. The patients had multicentric lymphadenopathy in association with clinical and laboratory findings suggestive of an

autoimmune disease as systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome and Sjogren's syndrome (SS), rheumatoid arthritis (RA) and chronic thyroiditis (Kojima et al. 2001).

Cervical lymphadenopathy is commonly encountered in children. Although infection is the most frequent cause, physicians should be aware of the differential diagnosis, including Kawasaki disease (KD), cat-scratch disease, Kikuchi-Fujimoto disease, and lymphoma. Kawasaki disease is one of the most important causes of cervical lymphadenopathy because 20% of untreated patients will develop coronary artery abnormalities (Otani et al. 2021).

## Materials and Methods

### Sampling

Blood samples were taken from all children with lymphadenopathy (group I; 35 children) and healthy control children (group II; 20 children) at the Hematology unit, Tanta University Hospital. Blood samples were collected in test tubes (Wassermann tubes) without anticoagulant substances to obtain serum for detection of Cytomegalovirus IgM (CMV IgM), Epstein Barr virus IgM (EBV IgM), and measuring serum level of Immunoglobulin A (IgA) and Antinuclear Antibody (ANA). This study was done according to guidelines of Ethics Committee of Scientific Research for medical research at Tanta university with code number 32017/02/18.

### *Determination the serum level of Epstein - Barr virus IgM*

Serum samples were collected for in vitro analysis by using IMMULITE 2000 Systems Analyzers for the qualitative detection of IgM antibodies to viral capsid antigen of Epstein-Barr virus in human serum according to Pochedly (1987).

### *Determination the serum level of Cytomegalovirus Virus IgM*

The electrochemiluminescence immunoassay "ECLIA" is determined by the cobas e 801 immunoassay analyzer for the qualitative determination of IgM antibodies to cytomegalovirus in serum samples according to Genser et al. (2001).

### *Determination of serum level of immunoglobulin A (IgA)*

A quantitative turbidimetric assay was used for measurement of IgA in serum samples. Anti-human IgA antibodies form insoluble complexes when mixed with samples containing IgA. The scattering light of the immunocomplexes depends on the IgA concentration in the patient sample, and can be quantified by comparison from

a calibrator of known IgA concentration (Friedman & Young 1997).

#### **Determination of Serum Anti-Nuclear Antibody (ANA)**

ELISA-based test system was used for the qualitative measurement of IgG class autoantibodies against SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, centromere B, Jo-1 in serum samples (Meroni & Schur 2010).

#### **Results**

Analysis of serum samples collected from children exhibiting lymphadenopathy as well as from healthy children indicate a notable rise in the blood level of Epstein-Barr Virus IgM in children with lymphadenopathy when compared to healthy control children. This difference

was statistically significant, with a P value of 0.001 (Table 1).

Table 2 revealed that there was no significant difference in Cytomegalovirus IgM serum levels between children with lymphadenopathy and healthy control children (P value = 0.164).

The results in table 3 showed no significant difference in blood immunoglobulin A (IgA) levels between children with lymphadenopathy and healthy control children (P value = 0.112).

According to table 4 there was no significant difference in blood ANA levels between children with lymphadenopathy and healthy control children (P value = 0.788).

**Table 1:** Comparison between children with lymphadenopathy and healthy control children.

Groups	Serum level of Epstein-Barr Virus IgM			T test	
	Range (mg/dl)	Mean	± SD	T-test	P-value
With lymphadenopathy	0.30 - 43	11.149	± 10.4666	2.563	0.001*
Healthy control	0.6 - 9.0	5.020	± 2.65		

**Table 2:** Comparison between children with lymphadenopathy and healthy control children as regards serum level of Cytomegalovirus IgM

Groups	Serum level of Cytomegalovirus IgM			T test	
	Range (mg/dl)	Mean	± SD	T-test	P-value
With lymphadenopathy	0.20 - 3.0	0.5331	± 0.61242	1.068	0.164
Healthy control	0.21 - 0.72	0.3835	± 0.159		

**Table 3:** Comparison between children with lymphadenopathy and healthy control children as regards serum IgA level

Groups	Serum immunoglobulin A (IgA)			T test	
	Range (mg/dl)	Mean	± SD	T-test	P-value
With lymphadenopathy	26 - 278	116.60	± 54.11	1.614	0.112
Healthy control	29 - 215	94.54	± 37.99		

**Table 4:** Comparison between children with lymphadenopathy and healthy control children as regards serum ANA level

Groups	Serum Anti -Nuclear Antibody (ANA)			T test	
	Range (mg/dl)	Mean	± SD	T-test	P-value
With lymphadenopathy	0.21 - 1.46	0.6877	± 0.21624	2.023	0.788
Healthy control	0.24 - 1.0	0.569	± 0.19647		

## Discussion

Lymphadenopathy is an irregularity in the size and texture of the lymph nodes, which is quite common in childhood. When the enlargement of lymph nodes is caused by inflammatory and infectious processes, it is called lymphadenitis (Pecora et al., 2021).

Infectious agents that can cause Lymphadenopathy are many as Epstein-Barr virus, cytomegalovirus, herpes simplex virus, human immunodeficiency virus, hepatitis B virus, mumps virus, measles virus, rubella virus, dengue virus (dengue fever), *Staphylococcus aureus*, *Streptococcus pyogenes*, anaerobes (periodontal disease), *Bartonella henselae* (cat-scratch disease), *Francisella tularensis* (tularemia), *Yersinia pestis* (bubonic plague), *Corynebacterium diphtheriae* (diphtheria), *Haemophilus ducreyi* (chancroid), *Brucella sp* (Brucellosis), *Leptospira sp*, (leptospirosis), *Chlamydia sp* (lymphogranuloma venereum), *Salmonella sp* (typhoid fever), *Mycobacterium tuberculosis* (Tuberculosis), *Coccidioides sp* (Coccidiomycosis), *Cryptococcus sp* (cryptococcosis), *Histoplasma sp* (histoplasmosis), *Toxoplasma sp* (Toxoplasmosis), *leishmania sp* (leishmaniasis), *Borrelia burgdorferi* (Lyme disease) and *Treponema pallidum* (syphilis) (Friedmann, 2008).

The obtained results in present study indicated that Epstein-Barr virus was detected in 45.5% of children with lymphadenopathy but cytomegalovirus was detected in 18.25%. This is agreement with the previously mentioned results that reported presence of Epstein-Barr virus as a cause of cervical lymphadenopathy in children, Abdel-Aziz et al., 2011 mention that EBV infection is not a rare cause of cervical lymphadenopathy in children while Twenty-four cases (15%) showed positivity to EBV serology, all of them had posterior cervical lymph nodes enlargement, 70.8% had fever, 66.6% had tonsillopharyngitis, 58.3% had splenomegaly, 25% had hepatomegaly, 41.6% had generalized lymphadenopathy, while skin rash was detected in 12.5%, and both palatal petechiae and palpebral edema were detected in 8.3%. The obtained results are with the same line with Bilal, 2015 who showed that Epstein-Barr virus infection was diagnosed in 13 (15.9%) children with cervical adenopathy (n=82). EBV infection is a common cause of childhood cervical lymphadenopathy. The commonest symptoms are fever, loss of appetite and cough. Evaluation of Peripheral Lymphadenopathies in children could be explained by Işık et al., 2024 who reported that the most common infectious causative agent for lymphadenopathy in children was Epstein Barr Virus (EBV). In the same context Yu et al., 2021, reported clinicopathological features of CMV-positive cases were studied while those cases suffer from lymphadenopathy.

In the present work, the obtained results showed that there were no significance differences in the serum level of Immunoglobulin A (IgA) between children with lymphadenopathy compared to healthy control children. This was because all children with lymphadenopathy have normal level of serum IgA according to their age. In same context Costagliola and Consolini, 2021 studied the relation between lymphadenopathy and immunodeficiency. They showed that lymphadenopathies can be part of the clinical spectrum of several primary immunodeficiencies, including diseases with immune dysregulation and auto inflammatory disorders. Additionally, the finding of lymphadenopathy in a patient with diagnosed immunodeficiency raises the question of the differential diagnosis between benign lymphoproliferation and malignancies.

Also present results showed no significance differences in levels of serum Anti-Nuclear Antibody (ANA) for children with lymphadenopathy compared with their levels in healthy control children and this may due to presence of autoimmune diseases (Kawasaki disease) in one case (2.9%) from children with lymphadenopathy. In agreement with the previously mentioned results of Gru and O'Malley, 2018 who studied autoimmune that induced lymphadenopathies and reported that some common autoimmune disorders that could affect the lymph nodes Some of these disorders are more characteristic of individuals in the pediatric age group (autoimmune lymphoproliferative syndrome, Kawasaki disease), while others present in older individuals (rheumatoid arthritis, lupus erythematosus, sarcoidosis). In the same way, Autoantibody profile in children with Kawasaki disease (KD) was studied by Basha et al., 2018 that reported there were 36 children with Kawasaki disease have lymphadenopathy while ANA was detected in three patients.

## Conclusion

Epstein-Barr virus was the most common cause for lymphadenopathy in children for this study. There was no significant difference in immunoglobulin A (IgA) in children with lymphadenopathy compared to their levels in healthy control children. Results showed only one case (2.9%) that diagnosed with autoimmune disease (Kawasaki disease).

## Conflict of interest

The authors declare that they have no conflict of interest.

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