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Detection of Epstein-Barr Virus Antibodies in Sera of a Group of Children from Diyala Province

A thesis
Submitted to the council of the College of Medicine - University of Diyala in partial fulfillment for the requirements of the degree of Master of Science in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَـٰنِ الرَّحِيمِ

(وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَى كَثِيرٍ مِنْ عِبَادِهِ الْمُؤْمِنِينَ)

صَدَقَ اللَّهُ الْعَظِيْم

سورة النمل

الآية ١٥

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Dedication

This thesis is wholeheartedly dedicated to my beloved mother, who has been my source of inspiration and gave me strength when I thought of giving up, who continually provide her moral, spiritual, emotional, and financial support.

To my beloved father, for earning an honest living for us and for supporting and encouraging me to believe in myself.

And to my dear brothers, for their help, support, and companionship.

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Declaration

I hereby declare that the thesis is my original work except for quotation and citation which have been only duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Diyala or other institutions.

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Summary

Epstein-Barr virus (EBV) is a double-stranded linear DNA human herpesvirus. EBV is transmitted primarily through saliva. Over 90% of the population is seropositive for EBV worldwide. Most children are infected by the age of 2 years and the majority of primary EBV infections are clinically silent. In older patients, it is accompanied by symptoms of infectious mononucleosis (IMN). Besides that, EBV also causes other clinical syndromes and is associated with various lymphoid and non-lymphoid malignancies.

The worldwide distribution of EBV and its important role in causing various malignancies were the main reasons behind conducting the current study to figure out the rate of EBV infection among children with clinical suspicion of IMN, and to explore the infection rate in healthy children with the assessment of effects of certain demographic factors.

Serum samples were collected from a total of 370 children (under 15 years of age); 190 of them were clinically suspected as having IMN, and 180 were normal apparently healthy children. The anti EBV VCA IgM antibodies were tested in a total of 248 serum samples (190 samples from clinically suspected children, and 58 from normal apparently healthy children). The anti EBV VCA IgG antibodies were tested in all the 370 serum samples. Statistical analyses were done using the Statistical Packages for Social Sciences-version 25 and P values equal to or less than 0.05 were considered significant.

Results showed that 24 (12.6%) suspected IMN patients were positive for anti-VCA IgM, while only 2 (3.4%) apparently healthy subjects were positive, with a statistically significant effect of clinical suspicion of IMN on IgM positivity rate ($P = 0.046$).

The IgM positive patients showed a peak in number in the age group of 1-4 years and the infection rate was slightly higher in females compared to males.

Both suspected IMN patients and control subjects had approximately similar positivity rates for anti VCA IgG (67.9%, and 70.6% respectively) with no statistically significant association (P = 0.580).

A statistically significant association was found between age and IgG positivity rate among apparently healthy subjects (P = 0.002). Gender had no statistically significant effect on IgG positivity rate. Among the suspected IMN patients, a statistically significant association was found between the IgG and IgM positivity rate (P = 0.028).

In conclusion, IMN infection rate among clinically suspected patients in Diyala province is low, and the seroprevalence of EBV among apparently healthy subjects is high.

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List of abbreviations

Abbreviation	Meaning
EBV	Epstein-Barr virus
EBNA	EBV nuclear antigen
ORFs	Open-reading frames
IR	Internal repeat
oriLyt	Lytic replication cycle origin
oriP	Origin for episomal genome replication during latency
ncRNAs	Non-coding RNAs
EBERs	EBV-encoded small RNAs
CD	Cluster of differentiation
IE	Immediate-early
E	Early
L	Late
VCA	Viral capsid antigen
ONM	Outer nuclear membrane
TGN	*Trans* Golgi network
LMP	Latent membrane protein
IMN	Infectious mononucleosis
MHC	Major histocompatibility complex
NSAIDs	Non-steroidal anti-inflammatory drugs
EAs	Early antigens
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
ISH	*In situ* hybridization
arbU/ml	Arbitrary units per milliliter
TMB	Tetramethylbenzidine
HRP	Horseradish peroxidase
SD	Standard deviation

Chapter one

Introduction

1.1. Background

Epstein-Barr virus (EBV) is a double-stranded linear DNA human herpesvirus (*Herpesviridae* family), 122-180 nm in diameter, belonging to the *Gammaherpesvirinae* subfamily and consisting of a toroid-shaped protein core that is wrapped with the viral DNA (about 172-kb molecule encoding more than 85 genes) inside an icosahedral capsid, with a tegument lining the space between the nucleocapsid and the outer envelope, into which different glycoprotein spikes are inserted. In human, two main types of EBV have been identified: EBV-1 and EBV-2 (Thompson and Kurzrock, 2004; Bouvard *et al.*, 2009; Banks *et al.*, 2012). EBV is transmitted primarily through saliva and is shed intermittently in healthy carriers (Lunn *et al.*, 2017). The EBV replication cycle can switch between lytic and latent state, and latently infected cells can sometimes be stimulated to reactivate EBV (Odumade *et al.*, 2011; Kempkes and Robertson, 2015).

Over 90% of the population is seropositive for EBV worldwide. Most children are infected by the age of 2 years in developing countries, while in developed countries EBV infection occurs more often in late childhood and adolescence (Ryan *et al.*, 2014). The majority of primary EBV infections in infants and young children are clinically silent. In older patients, it is accompanied by symptoms of infectious mononucleosis (IMN) in about 50% of cases (Jenson, 2016). IMN is a clinical syndrome characterized by pharyngitis, cervical lymphadenopathy, fever, and lymphocytosis, and is most often caused by EBV (Dockrell *et al.*, 2018). In addition to IMN, EBV also causes other clinical syndromes and is associated with various lymphoid and non-lymphoid malignancies (Carroll *et al.*, 2016).

Although heterophile tests are most commonly used to diagnose IMN, the U.S. Centers for Disease Control and Prevention (CDC) has advised against them

for "general use" because of their non-specificity and possibility of false negative results especially in young children (CDC, 2014).

More accurate diagnosis of EBV infection and disease in immunocompetent individuals was made possible by the availability of sensitive and specific EBV antibody tests. The presence of anti-VCA IgM antibodies indicates current infection. Anti-VCA IgG antibody is a marker of past infection and indicates immunity (Carroll *et al.*, 2016). Indirect immunofluorescence assay (IFA) is the gold standard test for the diagnosis of EBV infection, but its most important drawbacks are the high cost and the requirement of experienced staff. The compliance of enzyme-linked immunosorbent assays (ELISA) with IFA (as a reference method) is high, and ELISA provides advantages in terms of ease of use as it is practical and can be automated, which makes it preferable in many laboratories (Kasifoglu *et al.*, 2018).

The best test for diagnosing and monitoring EBV infections in the immunocompromised host is the blood viral load (or quantitative EBV DNAemia assay) usually performed on a polymerase chain reaction (PCR) platform (Holman *et al.*, 2012). PCR assays for EBV viral DNA can detect virus in blood, body fluids, and tissues (Carroll *et al.*, 2016). EBV is usually detected in tissue by *in situ* hybridization (ISH) using a probe for EBER, because this RNA is present at thousands of copies per cell. EBV latency proteins, including EBNA-1, EBNA-2, or LMP1, can also be detected in tissues (Longnecker *et al.*, 2013).

Chen *et al.* (2013), in a study conducted in China, among a total of 761 children (22 days to 14 years) with suspected EBV infection, found an IgM positivity rate of 14.3%. Similar studies had yielded comparable results (Balfour *et al.*, 2013 B; Fourcade *et al.*, 2017; Sohn *et al.*, 2018). Wang *et al.* (2013) found that the positivity rate among suspected IMN patients in China was (59.3%). Similarly, González Saldaña *et al.* (2012) observed a high positivity rate in Mexico (57.6%).

Chabay and Preciado (2013) reported that the anti-VCA IgG prevalence among children less than 15 years of age in Argentina was 72%. Similar results were also found in most previous studies (Huang *et al.*, 2013; Chen *et al.*, 2015; Fourcade *et al.*, 2017).

Epstein-Barr virus infection is very common in the United States, since more than 90% of adults are antibody positive by the age of 35 years. However, the prevalence among children is lower (Balfour *et al.*, 2013). EBV seroprevalence in pregnant women in Finland has remained the same in the last 20 years (Puhakka *et al.*, 2016).

Studies on the prevalence of anti-EBV antibodies in different countries had yielded different outcomes (Linton *et al.*, 2013; Devkota *et al.*, 2018).

1.2. Aims of the study

The worldwide distribution of EBV and its important role in causing various malignancies along with the lack of studies concerning this virus in Diyala province were the main reasons behind conducting the current study which came out to achieve the following goals:

1. To figure out the rate of current/reactivated or recurrent EBV infections among children who are clinically suspected as having infectious mononucleosis compared to apparently healthy controls through detection of serum anti-VCA IgM.
2. To explore the past infection rates in apparently healthy children through detection of anti-VCA IgG.
3. To assess the effects of certain demographic factors in relation with the positivity rates of anti-VCA IgM and IgG.