DETERMINATION OF EPSTEIN-BARR VIRUS IN GASTROINTESTINAL LYMPHOMAS (WITH SPECIAL REFERENCE TO NON-HODGKIN'S LYMPHOMA OF SMALL INTESTINE): STUDY ON MALAYSIAN PATIENTS.

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MAY 2005

Dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science (Medical Research).
This work was carried out in the Advanced Medical and Dental Institute (AMDI), University Sains Malaysia, Penang under the direct supervision of Associate Professor Dr. Mohamed J.E.M.F Mabruk

DECLARATION

Here, I declare that this research has sent to University Sains Malaysia for degree of Master of Science. It is also not be send to any other universities. With that, this research might be use for the consultation and will be photocopy as references.

ASMAH MD YUNOS

MAY 2005

DEDICATION

For Mother and Father

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
BCIP	5-bromo-4-chloro-3-indolylphosphate
BL	Burkitt's lymphoma
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
EBER	Epstein-Barr virus encoded RNA
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
GI	Gastrointestinal
GIT	Gastrointestinal tract
H_2O	Water
HD	Hodgkin's disease
HIV	Human Immunodeficiency Virus
HL	Hodgkin's lymphoma
ICH	Immunohistochemistry
IM	Infectious mononucleosis
ISH	*In situ* hybridization
LMP	Latent membrane protein
MALT	Mucosa-Associated Lymphoid Tissue
NBT	Nitroblue tetrazolium
NHL	Non-Hodgkin's lymphoma
NPC	Nasopharyngeal carcinoma

OHL	Oral hairy leukoplakia
PCR	Polymerase chain reaction
PK	Proteinase K
PNA	Peptide nucleic acid
RNA	Ribonucleic acid
RS	Reed-Sternberg
TBS	Tris-Buffered Saline
Min	Minute
µm	Micrometer
e.g.	example guide
%	Percentage
Sec	Second
N/A	Not available

ABSTRACT

Non-Hodgkin's lymphoma (NHL) is ranked twelfth among all cancers world-wide, in which it is more prevalent in males compared to females.

The aim of the present study was to determine the presence and distribution of Epstein-Barr virus (EBV) in non-Hodgkin's lymphomas (NHL) of the gastrointestinal tract (GIT) tissue samples obtained from 25 Malaysian patients diagnosed clinically and histopathologically. The gastrointestinal lymphomas analysed in the present study was divided into NHL of the ileocecal (9 cases), NHL of the small intestine (7 cases), NHL of the stomach (5 cases), NHL of the abdomen (1 case), NHL of the wedge ulcer (1 case), NHL of rectum fotic (1 case) and finally NHL of the hemicolon (1 case). The presence of EBV in all of the above mentioned tissue samples, after formalin fixation and paraffin embedding was carried out by using conventional *in situ* hybridization technology.

Two out of 25 cases of NHL of the GIT analysed in the present study, demonstrated positive signals for EBV/EBER. In the first positive case, EBV/EBER signals were located in transformed lymphocytes in the serosa layer of the small intestine (14.3%). In the second EBV positive

case, EBV/EBER signals were located in diffuse B-cell lymphomas of the ileocecal (11.1%).

In conclusion, these finding demonstrate a rare association between EBV and lower gastrointestinal lymphomas (excluding stomach).

ABSTRAK

Non-Hodgkin's lymphoma (NHL) disenaraikan sebagai kanser ke-12 di antara semua kanser di seluruh dunia, di mana ianya lebih cenderung dihidapi oleh lelaki berbanding perempuan.

Matlamat kajian ini adalah bagi mengenalpasti kehadiran dan penyebaran Epstein-Barr virus (EBV) di dalam non-Hodgkin's lymphomas melalui sampel tisu saluran laluan gastro (GIT), yang telah diambil daripada 25 orang pesakit Malaysia, yang telah di diagnosis secara klinikal dan histopathologikal. Analisis saluran laluan gastro lymphomas dalam kajian ini telah dibahagikan kepada NHL di ileocecal (9 kes), NHL di usus kecil (7 kes), NHL di perut (5 kes), NHL di abdomen (1 kes), NHL di cebisan ulser (1 kes), NHL di rektum fotik (1 kes) dan akhir sekali NHL di hemikolon (1 kes). Semua sampel tisu di atas di diletakkan dalam formalin dan dibekukan dengan paraffin. Bagi mengesan kehadiran EBV, kaedah teknologi konvensional *in situ* hybridization digunakan.

Dua daripada 25 kes NHL di laluan saluran gastro yang telah di analisis dalam kajian ini menunjukkan tanda positif untuk EBV/EBER. Dalam kes positif yang pertama, tanda EBV/EBER dikesan semasa pemindahan lymphocytes di lapisan serosa, dalam usus kecil (14.3%).

Dalam kes EBV positif yang kedua, tanda EBV/EBER dikesan dalam penyerapan sel B lymphomas di ileocecal
(11.1%).

Kesimpulannya, penyelidikan ini menunjukkan bahawa hubungan EBV dan saluran laluan gastro lymphomas (kecuali perut) adalah jarang-jarang berlaku.

CHAPTER 1

INTRODUCTION

This study was initiated to determine the prevalence of Epstein-Barr virus (EBV) in gastrointestinal (GI) lymphomas with special reference to non-Hodgkin's lymphoma (NHL) of small intestine. EBV was discovered 40 years ago during examination of electron micrographs of cells cultured from Burkitt's lymphoma sample (Young and Rickinson, 2004). This finding became the first of an unexpectedly wide range of associations between EBV and malignancies (Rickinson and Kieff, 1996).

Lymphoma in general is defined as a primary malignant tumour of the lymphoid cells (either nodal or extra-nodal). There are two types of lymphoma; Hodgkin's disease (HL) and non-Hodgkin's lymphoma (NHL). Lymphomas, ranked twelfth among all cancers world-wide, are more prevalent in males compared to females (Peh, 2003; Muller *et. al,* 2005).

From the literature, it has been shown in many studies that EBV is associated with several lymphoid and epithelial human malignancies (Fahraeus *et. al,* 1988; Lee *et. al,* 1997; Wong *et. al,* 2003). To date, there were no studies conducted to determine the prevalence of EBV in NHL of lower gastrointestinal tract in Malaysian populations. In addition and to our knowledge, worldwide, there are only few studies that looked at the

prevalence of EBV (EBERs) in NHL of the stomach (Ogata *et. al*, 2004; Royer *et. al*, 1997; Tossing, 1996), and there is only one case study that looked at the prevalence of EBV in NHL of the ileocecal (Sadahira *et.* al, 2001) and only few case repot studies that looked at the prevalence of EBV in NHL of the small intestine (Borisch *et. al*, 1992; Yang *et. al*, 1998; Kersten *et. al*, 1999).

The experimental approach that has been employed in the present study for the detection of EBV in NHL of lower GI tract was *in situ* hybridization (ISH). The technology of ISH is based on the detection of specific DNA or RNA sequences in tissue sections or cell preparations using a labeled complementary nuclei acid sequence or probe (Mabruk, 2004). The probe used in the present study was the EBER/EBV probe. EBER ISH is considered as the gold standard technique for detecting and localizing latent EBV in tissue samples (Gulley, 2001).

The present study is a retrospective analysis conducted to determine the prevalence of EBV in formalin fixed paraffin embedded tissue samples obtained from twenty-five Malaysian patients diagnosed with GI lymphomas. The GI lymphomas tissue samples used in the present study were divided into the following: NHL of the stomach (5 cases), NHL of the small intestine (7), NHL of the ileocecal (9), NHL of the abdomen (1), NHL of the rectum fotic (1), NHL of the wedge ulcer (1), and

NHL of the hemicolon (1). All of these samples were collected from the year 1992 to 2004, at the Pathology Department School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan.

The positive control for this study consisted of nasopharyngeal carcinoma (NPC) and Hodgkin's lymphoma (HL) known to be positive for EBV (EBERs). The negative control consisted of a serial section of the same test samples exposed to a negative control probe.

The detection of EBV in NHL of lower gastrointestinal tract may help in better patient management and treatment by administration of anti-herpesviruses drugs in combination with the conventional therapy (e.g.; radiotherapy and chemotherapy).

CHAPTER 2

OBJECTIVE

To determine the presence and distribution of EBV (EBERs) in non-Hodgkin's lymphoma of gastrointestinal tract tissue samples.

CHAPTER 3

LITERATURE REVIEW

3.1 Hodgkin's lymphoma (HL)

Hodgkin's disease (HD) was identified for the first time by Thomas Hodgkin in 1832 (Devita *et. al*, 1985). Hodgkin's disease is divided into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphomas (NHL). Non-Hodgkin's lymphomas (NHL) comprise a large and heterogenous group of lymphomas (Manch and Armitage, 2000; Nocini *et. al,* 2000).

The differences between Hodgkin's lymphoma (HL) and NHL is based on the presence of Reed-Sternberg cell (RS cell) in HL tissue samples at the histopathological level. These RS cells are found in most cases of HL (Tsuchiya, 2002). Kato *et.* al, 2000, reported that 62.5% (10 out of 16) RS cells were stained positive for EBV/EBER. These RS cells are not usually found in other lymphomas. For this reason, lymphomas that do not contain RS cells are called NHL. This may not seem a very big difference, but it is important because the treatment for Hodgkin's and NHL can be very different (Gulley *et. al,* 2002).

The presence of EBV in Hodgkin lymphoma and particularly in RS cells has been investigated previously by many workers using a

combination of EBER *in situ* hybridization and immunostaining for the detection of EBV-encoded latent membrane protein (LMP) (Khan *et. al*, 1993; Aguilera *et. al*, 1996; Kato *et.* al, 2000; Abrahamsen, 2000; Guidoboni *et. al*, 2005).

3.2 Non-Hodgkin's lymphoma (NHL)

Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of malignancies of the lymphoid system (Coffey *et. al,* 2003). The lymphoid system includes the lymph nodes as well as extranodal sites, such as bone marrow, the spleen, the tonsils, adenoids, the thymus gland, and Payer's patches in the small intestine (Waldman, 2003). The exact etiology for most lymphomas has not been determined (Mladenova and Pellicano, 2003). The first evidence which linked EBV to lymphomas was found in the 1960s, by the detection EBV in an explanted Burkitt's lymphoma (Pagano, 2002).

3.2.1 Clinical presentation of NHL

In NHL, tumours develop from white blood cells (lymphocytes) (Kato *et. al,* 2000). These tumours can occur at different locations in the body (Waldman, 2003). The cancer can spread to almost any organ or tissue in the body, including liver, bone marrow, spleen and nose (Guarner

et. al, 1991; Ott *et. al,* 1997; Zucca *et. al*, 2003). There are more than thirty types of NHL (Waldman, 2003). The most common subtypes are lymphoblastic, Burkitt's (Klumb *et. al,* 2004) and anaplastic large cell lymphomas (Noorali *et. al,* 2004).

According to the data from the World Health Organization International Agency for Research on Cancer (IARC), the NHL rate is increasing world wide and it is higher in developed countries than in Africa and Asia (Ferlay *et. al,* 2000). In England and Wales, NHL mortality has increased in older adults and decreased in young age probably due to improvements in methods of treatment (Swerdlow, 2003). Recent data indicated that a higher risk of developing lymphoma is associated with children of certain ethic origins (Cho *et. al,* 2001). The pattern of malignant lymphoma is known to vary in different populations (Magrath, 1992; Anderson *et. al*, 1998). There are several risk factors that have been postulated to be associated with NHL, including: exposure to chemicals, viral infection, organ transplantation and blood transfusion, family history, and lifestyle factors (Zinzani, 2005).

3.2.2 Prevalence and site of occurrence

Extranodal NHL of the GI tract accounts for about one third of all extranodal NHL (Zucca *et.* al, 2000; Koh *et.* al, 2001; He *et. al,* 2005).

Commonly involved extranodal sites include the stomach, tonsil, adenoid, skin and small intestine (Oikawa, 1995). These organs account for 50% or more of all extranodal involvement sites (Lee *et. al,* 2001). Primary GI lymphomas are the most common type of extranodal lymphomas and they have different biologic behaviors compared to nodal lymphomas (Isaacson and Spencer, 1987; van Krieken *et. al,* 1990).

The majority of primary GI lymphomas are B-cell, NHL type (Chan, 1996). They arise from Mucosa-Associated Lymphoid Tissue (MALT). The gastric low-grade B-cell lymphomas of MALT (Isaacson, 1994), as well as enteropathy-associated intestinal T-cell lymphomas are known to have precursor lesions (Isaacson and Wright, 1978; Isaacson *et. al,* 1985; Parsonnet *et. al,* 1994).

3.3 Treatment of NHL

The conventional method for NHL treatment is surgery, radiotherapy, and chemotherapy. Now a days, intensive treatments have been developed, however it is unknown whether this intensive treatment is more effective than conventional methods (Escalon *et. al*, 2005). Jazirehi and Bonavida, 2005 reported that clinical application of rituximab, alone and/or combined with chemotherapy has significantly ameliorated the treatment outcome (Jazirehi and Bonavida, 2005).

In addition, it has been shown, that treatment with anti-herpesviruses in combination to conventional therapy may produce good clinical results in terms of tumour resolving and patients recovery (Feng *et. al,* 2002; Battegay *et. al,* 2004; Feng *et. al,* 2004; Copur *et. al,* 2005; Lewington, 2005; Marcus, 2005; Zinzani, 2005).

3.4 Epstein-Barr virus

The Epstein-Barr virus (EBV) is a member of the gamma subfamily of the human herpesviruses family (Oikawa, 1995; Okano, 2000). It is the causative agent of infectious mononucleosis (IM) (Tsuruta *et. al*, 2003). EBV is a ubiquitous pathogen that is strongly associated with certain types of lymphoid disorders, such as endemic Burkitt's lymphoma (Gan *et. al*, 2002), Hodgkin's disease (Glaser *et. al,* 2005; Weiss *et. al*, 1987; Weiss *et. al*, 1989), non-Hodgkin's lymphoma (Libra *et. al*, 2005; Lui *et. al,* 2005; Mitarnun *et. al*, 2004), as well as B-cell lymphomas arising in immunocompromised individuals (MacMahon *et. al,* 1991). EBV has been linked to geographic, ethnic, hygienic status, and socioeconomic factors, with lower rate in developed countries (Sumaya *et.* al, 1975; Tsuchiya, 2002; Cordova Perez *et. al*, 2003).

3.4.1 EBV structure and genomic organization

The Epstein-Barr virus is composed of double stranded DNA surrounded by protein coat called capsid. The viral nucleic acids and capsid is termed the nucleocapsid. The entire virus is cloaked in an envelope with external glycoprotein spikes. There are two sub-types of EBV (type A and type B). These sub-types are distinguished by their distinct DNA sequence and protein antigenic variation for the EBV nuclear antigen (EBNA) (Peh *et. al*, 1995, Peh *et. al*, 2002). The locations of the EBV latent genes are shown in **Figure 3.1**.

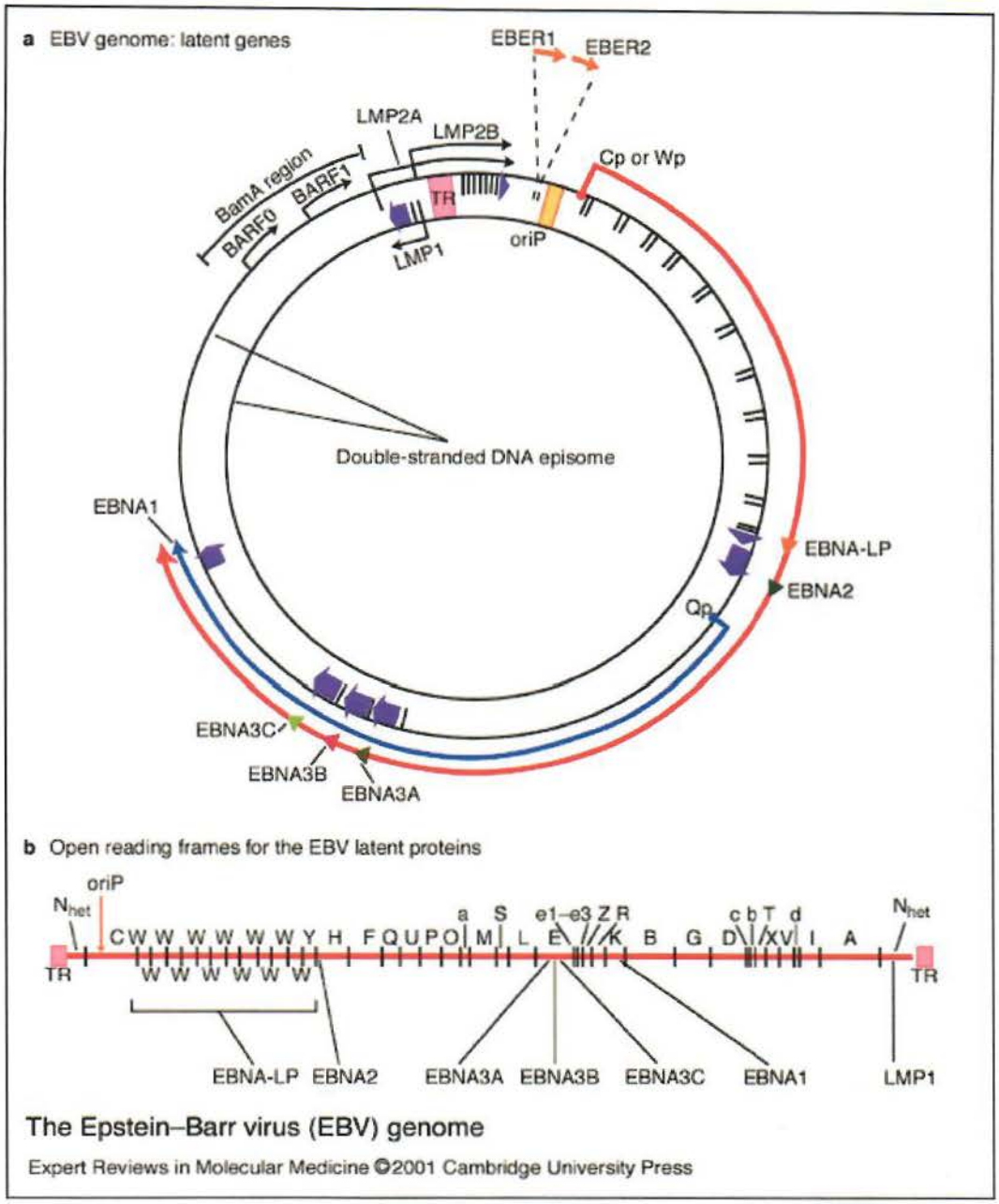

Figure 3.1 The Epstein-Barr virus (EBV) genome. (a) Diagram showing the location and transcription of the EBV latent genes on the double-stranded viral DNA episome. (b) Diagram showing the location of open reading frames for the EBV latent proteins (Adapted from http://www-ermm.cbcu.cam.ac.uk/01003854h.htm).

3.4.2 Receptors of EBV

3.4.2.1 Latent Infection

Analysis of lymphoblastoid cell lines infected with EBV led to the identification of two groups of genes and correspondent proteins: the first group is latent genes and the second group is lytic genes (Rickinson *et. al,* 1984; Kieff, 1996).

EBV persists in its hosts through its ability to establish a latent infection that periodically reactivates (Brooks *et. al,* 1993). EBV has shown to have three transcriptionally distinct forms of latency. These are known as latency I, II, and III (Rowe *et. al*, 1992; Brooks *et. al*, 1993). The latent proteins, comprise of six EBV-specified nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C, and leader protein, EBNA-LP) three latent membrane proteins (LMPs 1, 2A and 2B), and EBV encoded RNA (EBERs) (Tsuchiya, 2002).

It is well established, that two EBV encodes RNAs, EBER1 (166 bases) and EBER2 (172 bases) are expressed in all forms of latency (Gilligan *et. al*, 1990; Ambinder and Mann, 1994; Harris *et. al*, 1994). Because the EBER1 and EBER2 RNAs are expressed at high levels in latently infected cells (approximately 10^7 copies per cell), they are useful targets and markers for detection of latent EBV (Leong *et.* al, 2001; Peh *et. al*, 2004).

The exact function of EBERs is still not known, although studies suggest that the EBERs may work at the level of replication, transcription, or RNA processing (Gilligan *et. al*, 1990; Harris *et. al*, 1994).

3.4.2.2 Lytic Activation

Infection of B-lymphocytes is predominantly latent and results in lymphoproliferation (Knecht *et. al,* 2001; Ooka, 1985). Infection of epithelial cells can be latent, as in nasopharyngeal carcinoma, but usually leads to complete viral replication resulting in cytolysis (Sista *et. al*, 1995). The EBV encoded protein, Z, plays a dominant role in the switch of EBV from latent cycle to productive infection, and is transcribed from the immediate early gene, BZLF1 (Flemington and Speck, 1990).

3.4.3 EBV infection and life cycle

EBV is transmitted mainly through saliva and initially infects oropharyngeal squamous epithelial cells (Sixbey *et. al*, 1984). If the virus replicates and released from the epithelial cells, B lymphocytes in the nearby lymphoid tissue become infected (Leong *et.al*, 2001). If the host immune system is unable to counteract this growth, the potential for malignancy arises (Young, 1999; Takada, 2000) (**Figure 3.2**).

EBV carries a set of latent genes that, when expressed in resting B cells, induce cell proliferation and thereby increase the chances of successful virus colonization of the B-cell system during primary infection and the establishment of persistence (Crawford, 2001). This virus persistence might lead to uncontrolled cellular proliferation as a result of genetic alteration within infected cells. This uncontrolled cellular proliferation may lead to malignancy (**Figure 3.3**).

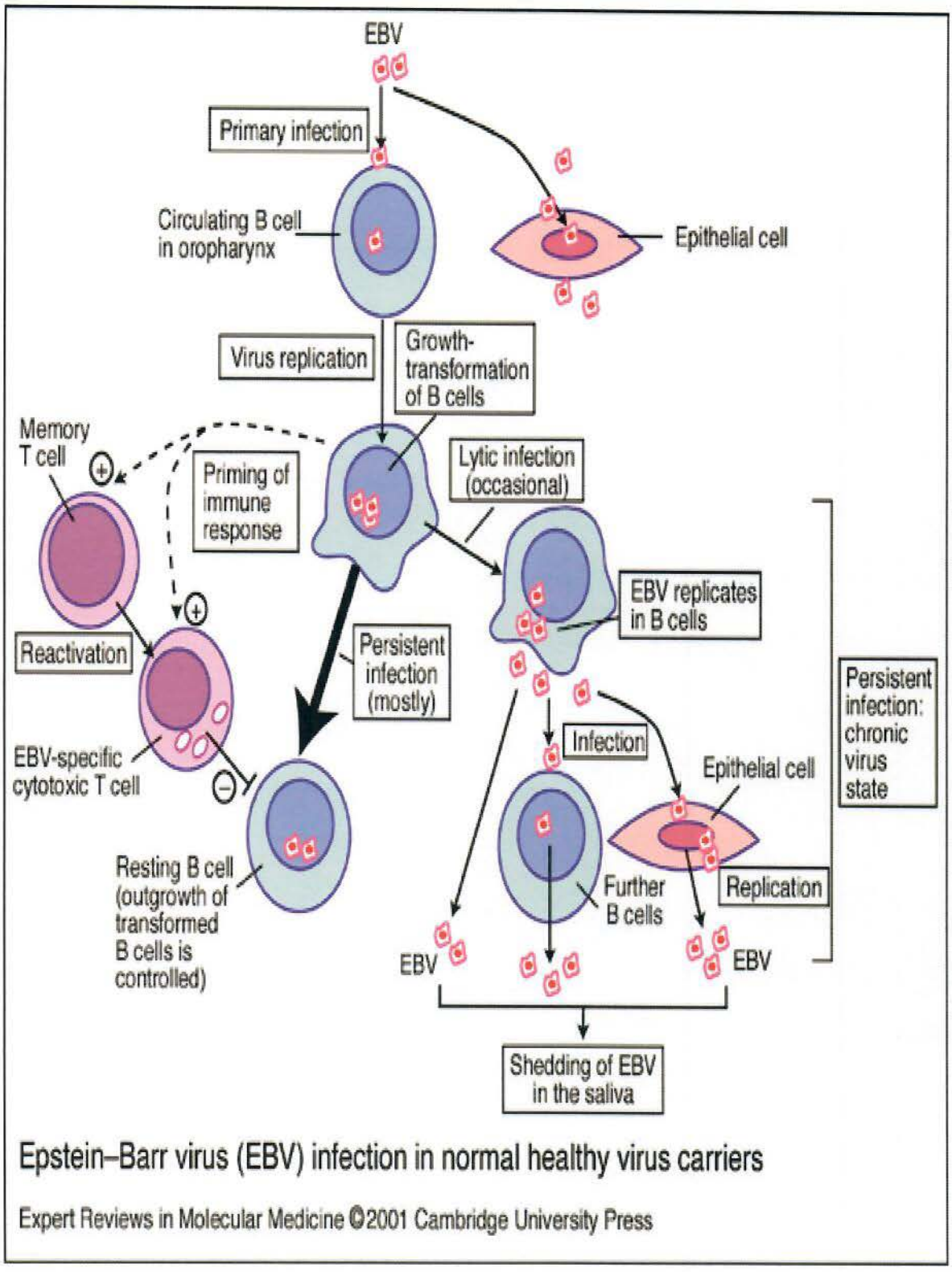

Figure 3.2 EBV infections in normal healthy virus carriers.

(Adapted from http://www-ermm.cbcu.cam.ac.uk/nfig002lyb.gif).

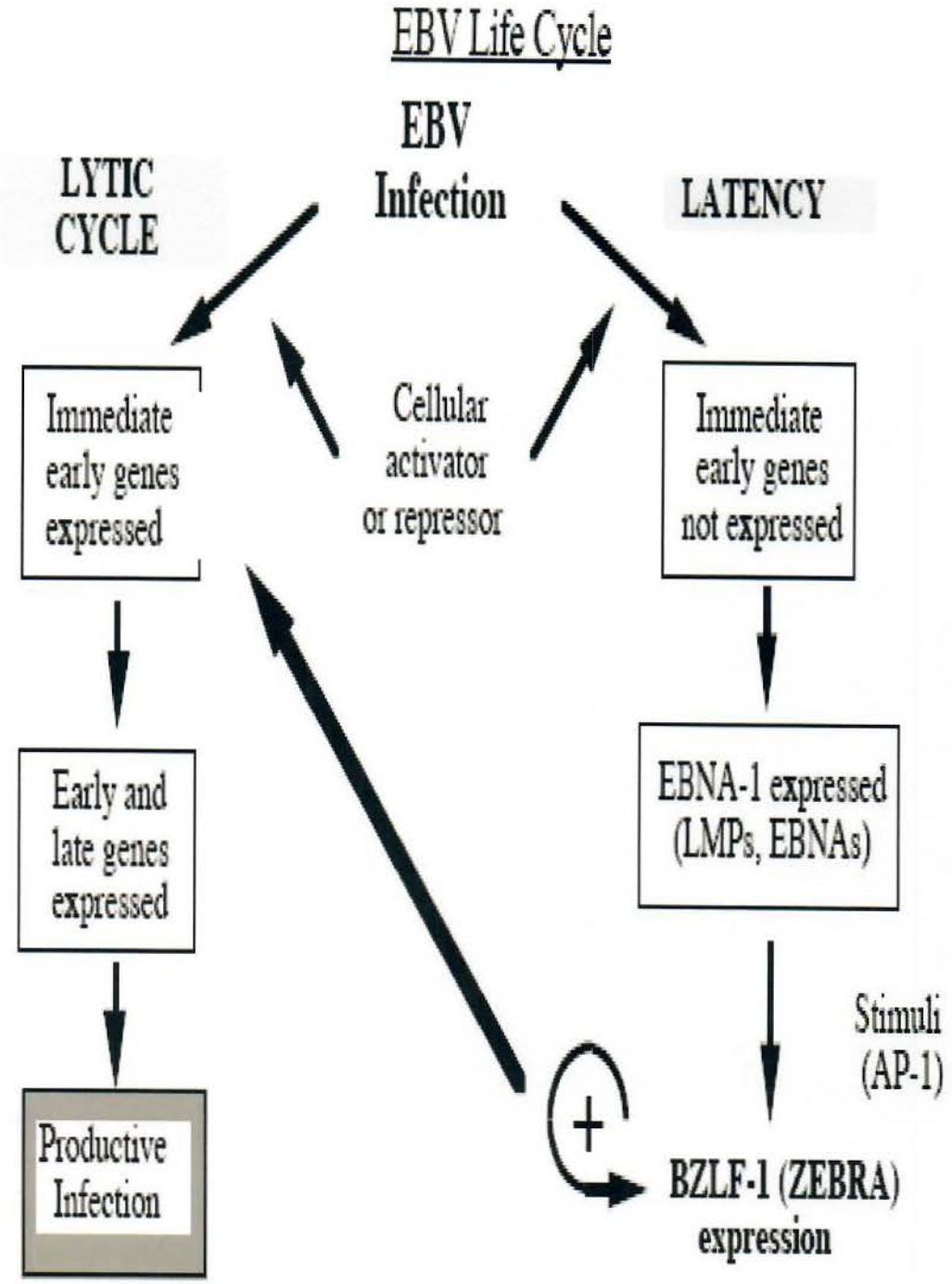

Figure 3.3 EBV life cycle. (Adapted from Fields Virology, 4th Edition).

3.4.4 Molecular events in EBV neoplasia

Burkitt's lymphoma (BL) is invariably associated with a chromosomal translocation that dysregulates the expression of *c—myc* gene (Dalla-Favera *et. al*, 1982; Taub *et. al*, 1982).

According to Hecht and Aster, 2000, *c-myc* normally plays a central role in the transcriptional regulation of an emerging set of downstream genes that control diverse cellular processes, including cell cycle progression and programmed cell death [apoptosis].

Over expression of *c-myc* as a result of insertion between EBV and cellular chromosome, may result in increased expression of the oncogenes and uncontrolled proliferation of lymphocytes and ultimately to malignancy (Purtilo *et. al,* 1985; Van Krieken and Kluin, 1992; Drotar *et. al,* 2003).

Another aspect describing molecular events in EBV neoplasia indicated that EBV/LMP1 and EBV/LMP2, might contributed to the escape cells from apoptosis which ultimately may lead to lymphomagenesis (Vockerodt *et. al,* 2002).

3.4.5 EBV associated diseases

3.4.5.1 Infectious mononucleosis (IM)

Infectious mononucleosis (IM) is an acute lymphoproliferation disorder caused by EBV (Gowing, 1975). IM is commonly manifested by fever, sore throat, lymphadenopathy, and splenomegaly (Lowinski *et. al,* 2001). A high proportion IM patients demonstrating biochemical hepatic involvement (Gowing *et. al,* 1975; Yao *et. al,* 1985). The characterization of IM is high grade fever, lymphadenopathy, hepatosplenomegaly, and pharyngitis or tonsillitis (Sumaya and Ench, 1985; Yao *et. al*, 1985).

3.4.5.2 Oral hairy leukoplakia (OHL)

Oral hairy leukoplakia (OHL) is a white corrugated lesion, that occurs on the lateral border or the ventral surface of the tongue, mainly in acquired immunodeficient (e.g. HIV and AIDS) patients and post-transplantation immunodeficient patients (Cohen, 2000). OHL is associated with lytic EBV infection (Mabruk *et. al,* 1995; Mabruk *et. al,* 2000). Previously, Mabruk *et. al*, 2000 reported that 100% of OHL biopsies obtained from HIV-seropositive were positive for EBV-DNA by PCR and ISH.

3.4.6 EBV associated malignancy

EBV has been classically associated with a number of malignancies, for examples Burkitt's lymphoma (Crawford, 2001; Purtilo *et. al,* 1985), nasopharyngeal carcinoma (Hsu and Glaser, 2000), Hodgkin's disease (Guidoboni *et. al,* 2005), and non-Hodgkin's lymphoma (Meijer *et.* al, 1996; Quintanilla-Martinez *et.* al, 1998; Wang *et. al*, 2004).

3.4.6.1 Burkitt's lymphoma (BL)

Burkitt's lymphoma (BL) is a malignant form of tumour that associated with EBV (Epstein *et. al*, 1964; MacMahon *et. al*, 1991; Knowles *et. al*, 1995; Leong *et. al*, 2001). The association between EBV and BL has long been established (MacMahon *et. al*, 1991; Leong *et. al*, 2001; Gottschalk *et. al*, 2005).

The histological appearance of BL, is classified into three types, endemic, sporadic and AIDS-associated (Purtilo *et. al,* 1985; Razzouk *et. al*, 1996). The typical anatomic site of the endemic type is the jaw, whereas sporadic and AIDS-related cases generally involve the abdomen (Tsuchiya, 2002). **Figure 3.4** depicts Burkitt's lymphoma in African child.

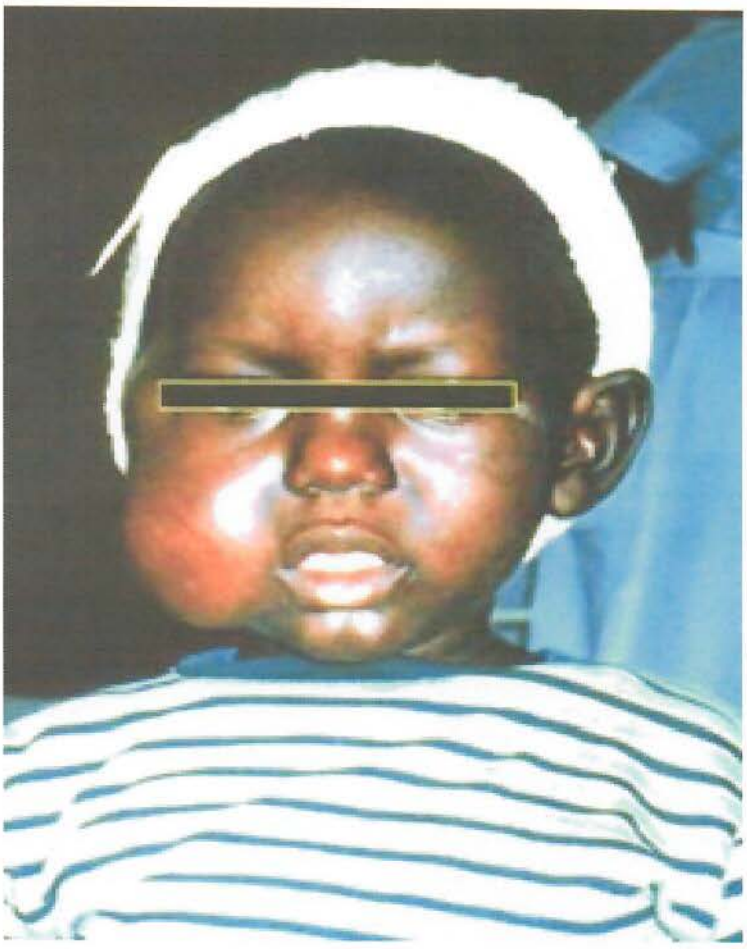

Figure 3.4 Burkitt's lymphoma in African child.

(Adapted from http://www.kochi-ms.ac.jp/~ff_mcrbi/BL-web.jpg).

3.4.6.2 Nasopharyngeal carcinoma (NPC)

Nasopharyngeal carcinoma (NPC) is another carcinoma that has been found to be associated with reactivation of latent EBV (Okano, 2000; Wang *et. al,* 2004). This disease is a malignancy of the epithelium of the upper respiratory tract and usually the epithelial cells of nasopharynx contain EBV DNA. Previously, Peh *et. al*, 1995 reported that 41% of NPC

were EBV/EBER positive. There was also another report, showed that 94% of NPC were EBV/EBER positive (Fahraeus *et. al*, 1988). The reason of disperancy in the frequency of EBV/EBER positivity could be contributed socioeconomic condition, geographical differences, ethnic differences, which affect EBV infection rates (Yang *et. al,* 1998).

3.4.6.3 Hodgkin's lymphoma (HL)

Hodgkin's disease (HD) differs in prevalence, morphologic findings, and association with EBV in various parts of the world (Paulino *et. al*, 1996). The aetiology of HD remains only partially understood (Jarrett, 1992; Swerdlow, 2003).

Epstein-Barr virus (EBV) is detected in HL in variable frequencies: It has been reported that the prevalence rates of EBV in HL were 40% in the United States (Glaser *et. al*, 1997), 43% in the Philippines (Paulino *et. al*, 1996), 65% in Hong Kong (Chan *et. al*, 1995), and 52% in Malaysians (Peh *et. al*, 1997). Macak *et. al*, 2000 and Trimeche *et. al*, 2005 reported that 83-84% of HL cases were EBV-positive in the Czech and Tunisie. However, Glaser *et. al*, 2005 reported a lower frequency of 23% of HL were EBV-positive in the United States.

3.4.6.4 Non-Hodgkin's lymphoma (NHL)

NHL has increased in incidence in many countries, particularly in the West (Swerdlow, 2003). Several aetiological factors may contribute the development for NHL (Seow *et. al*, 1996).

Epstein-Barr virus (EBV) is known to be involved in the genesis of many human tumours, including NHL (Liu *et. al*, 2005). Noorali *et. al*, 2004 reported that 16.7% of NHL cases were positive for EBV/EBER. A higher frequency of 66.6% positivity for EBV/EBER in anaplastic large cell lymphoma has been previously reported by He *et. al,* 2003. In addition, a case report study detected EBV/EBER positive signals in primary NHL of the vagina tissue sample (Domingo *et. al*, 2004). It has been reported that the prevalence rates of EBV in NHL was 30.8% in China (Chan *et. al*, 2001), 26% in Berlin, Germany (Hummel, 1995), and 41.4% in Malaysians (Peh *et. al*, 1995).

3.4.6.5 NHL of GI lymphomas

There are only 5 reports on the association between EBV and lower GI tract lymphoma (Borisch *et. al,* 1992; Yang *et. al*, 1998; Kersten *et. al,* 1999; Royer *et. al*, 1997; Wong *et. al*, 2003). Most of these reports were carried out as a case report study on 1-2 cases.

Although the GI tract is the most common site of extranodal NHL, primary small intestine lymphomas remain relatively rare, especially localized low- grade follicular B-cell lymphomas (Poggi *et. al,* 2002). Worldwide, there have been few studies that looked at EBV association with NHL of small intestine (Borisch *et. al,* 1992; Yang *et. al*, 1998; Kersten *et. al,* 1999). EBV/EBER has been detected in NHL of small intestine tissue sample obtained from a kidney donor patient (Kersten *et. al*, 1999). In a second case report study, enteropathy-associated T-cell lymphoma obtained from renal transplant patient was EBV/EBER positive (Borisch *et. al*, 1992). Both Borisch *et. al,* 1992 and Kersten *et. al,* 1998 concluded that NHL of small intestine may be EBV-related. In addition, there was one case study carried by Yang *et. al,* 1998, showed EBV positivity in NHL of small intestine.

To date, there was also only one case study demonstrated positive signal for EBV/EBER in lymphoma of colorectal tissue sample (Wong *et. al*, 2003).

In regard to the prevalence of EBV in NHL of the ileocecal, a literature search showed only one case report study that looked at the presence of EBV in NHL of the ileocecal. The result from this study; showed positive signals for EBV/EBER in lymphoma cells and hyperplastic follicular germinal centre cells (Sadahira *et. al,* 2001).

All published reports on the prevalence of EBV in NHL of lower GI tract are summarized in **Table 3.1**.

3.4.6.6 Prevalence of EBV in NHL of gastrointestinal lymphomas in Malaysian patients.

To date there have been no studies conducted to determine the prevalence of EBV in lower GI lymphomas in Malaysian patients. There is only one report on the prevalence of EBV in upper GI by published by Peh *et. al*, 2001, this report showed that 12 out of 29 of upper-aerodigestive tract lymphomas were EBV/EBER positive.

As mentioned earlier, no studies have been carried out to date to determine the prevalence of EBV in NHL of lower digestive tract in Malaysian populations and that only few case studies were carried out worldwide. For this reason this preliminary study was initiated to provide data on the prevalence of EBV (EBERs) in lower GI lymphomas tissue samples (in respect to stomach). The future aim for this study is to provide new tool in additional to conventional therapy regarding the possibility of using anti-herpesviruses drugs for treatment of NHL patients.