# Assessment of Knowledge, attitude and sexual behavior among youth and its association with EBV in Lebanon

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# ABSTRACT

Sexual activity constitute in the life of the adolescent, a risk-taking and possibly health-compromising behavior. Since unfortunately, knowledge about sexuality is often acquired after sexual activity has begun, sexually transmitted diseases (STDs) should be given high priority in health care and in education. Sexual education is an indispensable need, especially for adolescents, in order to protect them from irresponsible and non-conscious behavior. In the areas of adolescent sexual behavior, sexual education and knowledge, it is imperative and of paramount importance that the health-care system focus on this target group. For this reason, this project was based on two studies that were carried out with the aim of: 1) detecting the prevalence of STD, such as EBV, and its risks in a rural population of Lebanon and 2) evaluating the knowledge base for the development of sexual education in school students. To accomplish this work, data of EBV infection was collected from Becharreh Governmental Hospital to investigate its prevalence. Similarly, surveys were conducted in two schools to qualify knowledge of sexuality among students. The data collected from the studies were analyzed via SPSS 20. Results have shown that a lack of knowledge is widespread in society, as is the risk of the spread of sexually transmitted diseases and the increase in its prevalence due to unconscious and irresponsible practice. Programs and awareness should be designed in order to address the age-appropriate sexual information delivery, as well as to teach the risks associated

with STIs and proper use of prevention techniques in order to avoid the propagation of STI occurrence.

Keywords: Adolescents, Young adults, sexually transmitted diseases, sexual education, EBV

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## LIST OF ABBREVIATIONS

**BM:** basement membrane

**EBNA:** Epstein-Barr nuclear antigen

**EBV:** Epstein - Barr virus

**ELISA:** enzyme-linked immunosorbent assay

**ELFA:** Enzyme Linked Fluorescent Assay

**HIV:** Human immunodeficiency virus

**HPV:** Human papillomavirus

**HSPGs:** heparan sulfate proteoglycans

**IM:** infectious mononucleosis

**LCL:** lymphoblastoid cell line

**LMPs:** latent membrane proteins

**MHC:** major histocompatibility complex

**MS:** multiple sclerosis

**NFκB:** nuclear factor-kappa B

**PBMCs:** peripheral-blood mononuclear cells

**PCR:** polymerase chain reaction

**RA:** rheumatoid arthritis

**SLE:** systemic lupus erythematous

**STD:** sexually transmitted disease

**ST**I: sexually transmitted infection

**VCA:** viral capsid antigen

# I. Introduction

Sexual relations has long been an integral part of human behavior. As soon as the adolescents acquire a certain maturity, they begin to integrate into the sexual life, without taking into account the resulting disadvantages (Hassan and Creatsas 2000). This lack of preparation for sexual intercourse, and unprotected sexual activity leads to disastrous consequences and health illness. Sexually Transmitted Diseases (STDs) pose a real danger to public health, from treatable infection to unexpected cancer. These diseases originate from bad practices and neediness knowledge since the youngest age (Hassan and Creatsas 2000).

Adolescents learn about sexually transmitted diseases (STDs) from many sources, yet little is known about how well these educational sources are teaching them about STDs. In Lebanon, the base knowledge of sex seems to be a poor education, due to moral and ethical codes. Some people think this education is taboo and refuse to talk about it or to have their pubescents educated (Clark et al. 2002).

Sexually Transmitted Diseases (STDs) and Sexually Transmitted Infections (STIs) have been described for centuries and continue to persist all over the world; they are induced by bacterial, viral and protozoan vectors (Kent 2017). these 2 terms refer basically to the same concept and are used interchangeably. In other words, having an STI means that someone has an infection, but it has not yet developed into a disease. Usually, an STI often precedes an STD. usually, STDs have a high prevalence among adolescents and thus contribute to the increase in risks affecting psychological and physiological health (Naswa and Marfatia, 2010).

As an example of STIs. We will review both EBV and HPV in order to showcase their socio-sexual spreading potential. Epstein-Barr virus (EBV), a linear, double stranded DNA virus, belonging to the human herpes virus family, contributes to the sexually transmitted diseases

especially the mononucleosis infection. This virus usually targets adolescents. In fact, 90% of adults worldwide are estimated to have contracted EBV at some point in their life, making the prevalence of this disease predominant, and further putting adolescents at risk. The major threat of EBV infection is the fact that it begins as an asymptomatic infection via oral transmission, secreting this virus in the saliva (Rezk et al. 2018), spreading the disease to unsuspecting partners.

The aim of this study is to find out basic knowledge about sexual education and its dissemination in two subsets of the Lebanese society, especially among adolescents. Furthermore, we aim to assess preliminarily the spread of STDs in a remote population, taking as an example the Epstein bar virus from hospital patients over multiple years and in multiple age groups.

# II. Literature Review

## 1. Sexually transmitted diseases

Sexually transmitted infections (STDs) are usually caused by viruses or bacteria and are transmitted from person to person during sexual contact with the vagina, penis, anus, or even the mouth. These infections are common, diverse, and dangerous to health—extending from bacterial diseases that may be readily treatable once diagnosed to viral infections such as HIV, a life-threatening and, as yet, incurable disease (Low et al., 2017). In this section, we will be reviewing two major sexually transmitted diseases, namely EBV and HPV, due to the risks associated with the spreading in the population and the possibility of non-sexually practices possibly spreading them, especially within a risk taking adolescent population.

### 1.1. Risk factors promoting transmission in younger populations / college aged populations

Adolescence can be considered the transitional period from childhood to adulthood where the developmental tasks of independence, specifically in tough choices regarding schoolwork, social life, alcohol, sexuality, and drugs. The younger population is a vulnerable population, they make decisions and usually act in ways that put them at higher risk for STDs. Acquiring an STD is not only a function of unprotected sexual intercourse, but also reflects the prevalence of STDs in young adults' socio-sexual network, risk of sex partners (i.e., concurrency), and frequency and proficiency of correct condom use. Incidence and prevalence estimates suggest that young people aged 15–24 years in United States, acquire half of all new STDs, with one in four sexually active adolescent females having an STD, such as chlamydia or human papillomavirus infection (Unemo et al., 2017). Additionally, young college students may

have multiple sex partners, may use drugs and alcohol at high rates, and engage in high risk behaviors while under the influence of drugs and alcohol. Unfortunately, routine screening programs are not widespread, and social stigma and lack of public awareness concerning STIs often inhibits frank discussion between healthcare providers and patients about STI risk and the need for testing (DiClemente et al., 2011).

### 1.2. Risk factors associated with transmission in older populations

A great deal of research data shows that older adults are engaging in increasing sexual activity (Taylor and Gosney, 2011), mostly as risky behavior. Sexual risk behavior is defined as "sexual intercourse without condom use with a casual partner, and/or sexual intercourse without condoms with a new main partner with no prior HIV testing" (Brodbeck et al., 2010). So, sexual risk behaviors can cause negative health outcomes as social and emotional disturbances in addition to the transmission of STDs. In a study of 3,005 older adults in the United States, current sexual activity was reported for 73% of those aged 57 to 64 years, 53% of those aged 65 to 74 years, and 26% of those aged 75 to 84 years While older adults are engaging in riskier sexual behaviors, population needs further education regarding routine assessments of sexuality, methods of prevention, and risks of STDs.

## 2. Epstein - Barr virus – EBV infection

Epstein-Barr virus (EBV), known as human herpes 4, is a gamma-herpes virus that infects the bulk of the world's population. Initial infection with EBV infection is usually asymptomatic but also it can manifest as infectious mononucleosis. In 1964, Anthony Epstein and Yvonne Barr isolated viral particles in lymphoblasts from a patient with Burkitt lymphoma as reported in the literature (Shuichi Fujita et al., 2004; Sugden, 2014), thus keying the term Epstein-

Barr virus. Viral infections contribute to an estimated 15% to 20% of all human cancers (Esau, 2017). Many viral infections have been found to be related with an increased risk of lymphomas. However, one of the foremost unsupportive findings concerning the association of EBV with rare cancers is that EBV prevalence within the population is very high, reaching over ninetieth of the adult population worldwide (Shuichi Fujita et al., 2004; Sugden, 2014) .

## 2.1. Mechanism of EBV infection

EBV infection begins in the oral mucosa and genital secretions (Ambinder and Cesarman, 2007). Replication in epithelial cells is typically lytic and this latent infection of epithelial cells can result in gastric cancer or nasopharyngeal carcinoma, and it is also associated with HIV and many autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and dermatomycosis. EBV infects epithelial cells and B cells of the immune system and EBV latency persists in the B cells of the individual for a lifetime. Early studies demonstrated EBV ability to transform resting human B cells into lymphoblastoid cell lines (LCLs), further supporting the oncogenic potential of this virus (Stanfield and Luftig, 2017).The replication cycle of EBV is divided to 4 parts: entry of the cell, lytic replication, latency and reactivation. After replication in the epithelia, virus is primed for entry into B cells, where a transient growth program is thought to mimic a germinal center reaction, ultimately promoting maturation of the infected cell into the peripheral memory B-cell compartment (Stanfield and Luftig, 2017). In adolescence or adulthood, EBV infection may cause the clinical symptoms of infectious mononucleosis (IM). Then EBV persists in latent state in the host within long-life memory B cells. B-cell entry requires five viral glycoproteins: gp350/220 allows for attachment by binding to CD21; gp42 binds to major histocompatibility complex (MHC) class II to initiate entry; and the core herpes-virus fusion machinery consisting of gB and the heterodimer gH/gL (Stanfield and Luftig, 2017). When EBV enters the cells, the viral

capsid is dissolved, and the viral genome is transported to the cell nucleus. The lytic cycle results in the production of infectious virions.

The lytic cycle includes three stages: immediate-early stage, early stage and late stage. Some of the gene products, which act as trans activators, of the first stage are: $BZLF_1$ associated with its product gene ZEBRA and $BRLF_1$ associated with its product gene RTA. Following this stage, the Epstein-Barr nuclear antigen (EBNA) latency promoter, Wp, is active and promote the expression of *EBNA-LP* and *EBNA2*. This period lasting approximately the first 2 weeks following resting B-cell infection is termed latency IIb (Stanfield and Luftig, 2017). At this time, the virus expresses all of the EBNA proteins and minimally expresses latent membrane proteins (LMPs) 1, 2A, and 2B (Stanfield and Luftig, 2017). LMP1 is expressed as early as 2 days post infection; however, during this period, inhibition of early nuclear factor-kappa B (NFκB) activation does not affect transformation, supporting the distinction of this latency phase from the LCL state, which requires LMP1-mediated NFκB activity for survival (Stanfield and Luftig, 2017). Cells then transition from a period of rapid proliferation and high Myc activity to the steady-state proliferation (about 24 hours per cycle) observed in LCLs with lower Myc and high NFκB activity (Stanfield and Luftig, 2017).

## 2.2. Epidemiology and symptoms of EBV

EBV infection has become ubiquitous in all human populations. The paradigmatic disease associated with EBV is infectious mononucleosis. The diagnosis of mononucleosis begins with a detailed physical examination. Mainly, primary infection is asymptomatic, and the virus is leftover as a benign latent infection for the lifetime of the host. But in young adulthood this infection can cause infectious mononucleosis, a benign self-limiting disease and may cause fever, fatigue,

swollen lymph nodes in the neck, enlarged spleen or rash (Niedobitek et al., 2000; Becker and Smith, 2014) (Niedobitek et al., 2000; Ascherio and Munger, 2007; Becker and Smith, 2014). Children in developing countries acquire the infection in the first few years of life, and universal seroconversion is often seen by ages 3–4 years, whereas infection in developed countries often is delayed until adolescence (de-The et al., 1975). In developed countries, it has been described as a bimodal infection rate with peaks in children under 5 years and again after 10 years of age. The explanation of this bimodality is the oral EBV secretions between infants and parents, and adolescence's intimate partners and early adulthood. Even though adults don't handle Epstein-Barr virus as well as kids, by the age of twenty five years most of them are already seropositive and are not liable to reinfection (Stephen E. Straus et al., 1993). In general, the incidence of infectious mononucleosis increases from the age of 2–4 years to have a maximum range in adolescence and early adulthood, and then decreases, becoming very rare after the age of 40. Mononucleosis-like infections may occur more than once, but such episodes are generally not caused by a resurgence of viral activity (Straus et al., 1993). Reactivation disease appears exclusively in transplant recipients and similarly immuno-impaired persons (Ho et al., 1988). In healthy persons, a symptomatic reactivation of EBV disease has never been shown. The main symptoms to Infectious Mononucleosis are neck swelling and sore throat; also, there are non-specific symptoms such as headaches, vague discomfort, fever, and chills. Some of the main serious symptoms to suggest infectious mononucleosis are spleen tenderness and rashes. Most mononucleosis-like infections are caused by chronological infections with many pathogens. Salivary tissues are the recognized repositories of EBV, and periodic shedding from such tissue is a necessary feature of this virus's biology (Straus et al., 1993).Shedding is sustained for months after infection and then falls gradually; in 15% to 20% of all attempts, the virus can be recovered from saliva (Golden et al.,

1973). Immune compromised transplant recipients and AIDS patients show higher rates of shedding (average range = 50% to 80%) (Sumaya et al., 1986). Sexual transmission of EBV has not been proven, but this virus has been present in cervical epithelium and semen. Studies of natural or experimental transmission documented an incubation period of 3 to 7 weeks (Svedmyr et al., 1984). Epstein-Barr virus replicates in epithelial and B cells and the sore throat is typically caused by lysis of the oropharyngeal epithelial cells. The swelling of the neck is due to the enlargement of lymph nodes as the infected B cells replicate to normal cells. EBV is a stimulator of other more serious diseases and threatening cancers. In addition to systemic lupus erythematous (SLE) and rheumatoid arthritis (RA), multiple sclerosis (MS) is an additional EBV associated chronic inflammatory disease with rhythmic damages of myelin sheet in the central nervous system. As suggested by familial cases, these 2 diseases SLE and RA are complex disorders with a genetic background. Patients of both diseases have higher titers of anti-EBV antibodies, impaired T cell responses to EBV antigens and higher viral load in PBMCs (James et al., 2001; Poole et al., 2006). A proposed disease mechanism is the interplay between genetic predisposition and EBV infection leads to the development of cross reacting autoantibodies, and later the non-cross-reactive antibodies against auto epitopes (Toussirot and Roudier, 2008). As well, multiple sclerosis is a disease that has been linked to environmental and genetic factors. The most promising data suggesting EBV as the viral factor is that the risk of MS is 20 times higher among people who have contracted IM, as compared with seronegative individuals (Ascherio and Munger, 2007).However, research studies are needed in order to determine specific pathways that could link EBV to MS.

## 2.3. Treatment of EBV

Similar to other viral infections, EBV infection cannot be treated with antibiotics and there is no vaccine available to protect against infectious mononucleosis. Restricting intimate contact during acute mononucleosis can reduce the transmission of EBV but, will needlessly hamper contact with many persons who are already seropositive and, in cases of susceptible children, may even delay virus acquisition to an age when symptomatic mononucleosis is more likely (Straus et al., 1993) . Symptoms of infectious mononucleosis can be reduced by drinking fluids to stay hydrated, getting plenty of rest and taking over-the-counter medications for pain and fever. Prevention can be difficult because EBV is widespread in populations and can be transmitted even when an infected person has no symptoms. Individuals can reduce their risk of infection by not using utensils, toys, or other objects used by infected individuals and by practicing good hand-washing techniques. Avoiding contact with any body fluids, mainly saliva, will reduce the chance of infection, avoiding kissing or having sex with an infected person is also a good way for prevention.

## 2.4. Analysis techniques of EBV detection and their reliability

Doctors look for the main symptoms known as fever, inflamed throat, swollen lymph nodes in the neck, and an enlarged spleen. In infected people, the number of normal B lymphocytes in blood usually increases and the cells may look "atypical" under the microscope (Odumade et al., 2011). Also, most people develop anemia caused by the destruction of the red blood cells, although mild elevations in liver enzymes in blood are observed. In addition, IgM antibodies occur early, are transient, and indicate new or "acute" infection. In contrast, IgG antibodies to the viral capsid antigen, develop immediately and persist for life. Moreover, antibodies to the nuclear antigen (EBNA) develop three to four weeks into the illness and persist for life. Several antibody tests are

available to determine if a person has had a past infection or a recent infection with EBV. PCR tests that detect EBV DNA may be available in many laboratories. Using only three parameters [viral capsid antigen (VCA) IgG, VCA IgM and EBV nuclear antigen (EBNA)-1 IgG],it is normally possible to distinguish acute from past infection: the presence of VCA IgM and VCA IgG without EBNA-1 IgG indicates acute infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection (De Paschale and Clerici, 2012). Serology builds upon detection of EBV-specific antibodies, which is preferably done with a single acute-phase serum sample.

## 3. Human papillomavirus (HPV) infection

HPV is a group that contains more than 150 related viruses. Each HPV virus in this large group is given a number which is called its HPV type. HPV is a DNA tumor virus that causes epithelial proliferation at mucosal and cutaneous surfaces. HPV includes about 30 to 40 strains that infect the human genital tract. Of these, there are oncogenic or high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, and 58) that are associated with cervical, vulvar, vaginal, and anal cancers, and non-oncogenic or low-risk types (6, 11, 40, 42, 43, 44, and 54) that are associated with genital warts (Munoz et al., 2003). HPV 16 is the most oncogenic, accounting for almost half of all cervical cancers, and HPV 16 and 18 together account for approximately 70% of cervical cancers (Clifford et al., 2003). The most HPV types associated with genital warts, are HPV-6, and HPV-11. Research in the last decade conclusively demonstrated that sexually-transmitted infection with carcinogenic types of HPV, often referred to as high-risk types of HPV, is required for the subsequent development of virtually all cervical cancers (Anhang et al., 2004). The recently approved quadrivalent (types 6, 11, 16, and 18) HPV vaccine targets the HPV strains responsible for approximately 70% of cervical cancers and 90% of genital warts (Braaten and Laufer, 2008).

The vaccine is optional for all girls aged 11 to 12, with catch-up vaccination for women aged 26 and up.

## 3.1. Mechanism of HPV infection

Human papillomaviruses have a unique mechanism of infection that has evolved to limit infection to the basal cells of stratified epithelium, which is the only tissue in which they replicate. New studies in a mouse cervicovaginal challenge model indicate that the virus cannot firstly bind to keratinocytes in vivo. Rather it must first bind via its L1 major capsid protein to heparan sulfate proteoglycans (HSPGs) on segments of the basement membrane (BM) exposed after epithelial trauma and undergo a conformational change that exposes the N-terminus of L2 minor capsid protein to furin cleavage (Schiller et al., 2010). L2 proteolysis exposes a previously occluded surface of L1 that binds an as yet undetermined cell surface receptor on keratinocytes that have migrated over the BM to close the wound (Schiller et al., 2010) .The entry of HPV *in vitro* is initiated by binding to a cell surface receptor in contrast to the *in vivo* situation where the basement membrane has recently been identified as the primary site of virus binding (Horvath et al., 2010). HPV binds and triggers conformational changes and affect both capsid proteins L1 and L2; such changes are a requirement for interaction with the elusive uptake receptor. Most HPV types enter the cell via a clathrin-dependent endocytic mechanism. Furthermore, the productive entry of HPV is a process that occurs slowly and asynchronously and it is characterized by an unusually extended residence on the cell surface (Horvath et al., 2010).

## 3.2. Epidemiology and symptoms of HPV infection

There are 510,000 women diagnosed with invasive cervical cancer per year worldwide and 288,000 deaths, with approximately 80% of these cases in the developing world (Braaten and

Laufer, 2008). Further the neck and head area especially the oropharynx, mainly the tonsils and tongue base are the common sites associated with HPV.

The HPV virus is transmitted through direct skin-to-skin contact. Although infection is most often spread through penetrative vaginal or anal intercourse, other types of sexual contact can transmit HPV, and infection has been reported in self-reported "virgins" (Winer et al., 2003). Most HPV infections are acquired within the first years of sexual activity, as demonstrated by a study of 603 college students, in which it was found that approximately 40% of HPV infections are acquired within 2 years of the first sexual experience (Winer et al., 2003). In most cases and studies, the risk of infection is proportionately related to number of sexual partners. This may occur through skin-to-skin transmission via the epidermis due to direct contact of a plantar wart virus with broken skin, sexually during intercourse, or orally during sexual activity or kissing (Dixit et al., 2011). Condoms can decrease the risk of this transmission; on the other hand, it should be noted that condoms are not 100% protective.

## 3.3. Treatment of HPV infection

Most HPV infections go away on their own, but some last for a long time and can cause cancer or other health problems. The vaccines should provide protection from 5 to 10 years. In 2006 the Food and Drug Administration approved a HPV vaccine. The Gardasil vaccine, a series of 3 shots given over 6 months, helps to prevent against 4 types of HPV: 6, 11, 16, and 18. The vaccine is given for females aged between 9 and 26. HPV vaccine is very effective to prevent cancer and other health problems caused by this virus. Efficacy of HPV vaccination is greatest when it is given to HPV-naïve women. The ideal time to give the vaccine is prior to initiation of

sexual activity. Most vaccines protect against at least HPV types 18 and 16 that cause the highest risk of cervical cancer.

## 3.4. Analysis techniques of HPV detection and their reliability

Studies on the natural history of HPV infections have shown that in young women, most human papillomavirus infections are transient. A survey of female students at a US university reported that while one-third of participants knew that a woman under age 18 should have her first Pap test soon after having sexual intercourse for the first time, less than one-third were aware that a Pap test might detect changes indicative of HPV infection (Anhang et al., 2004). An American Social Health Association study of 489 HPV-positive men and women, 60% with visible genital warts, reported that initial reactions to HPV diagnosis include anger, depression, isolation, fear of rejection, shame, and guilt (Clarke et al., 1996). In 2006, the American Society for Colposcopy and Cervical Pathology (ASCCP) published guidelines for the management of women with abnormal cervical or cervical neoplasia cancer screening tests. These recommendations were largely based on the results of the National Cancer Institute ASCUS/LSIL Triage Study (ALTS), a large, multisite clinical trial designed to evaluate 3 methods screening: immediate colposcopy (cervical exam), cytologic follow-up, and triage by HPV DNA testing (Schiffman and Solomon, 2003).

In addition, self-sampling is a method to collect cervical specimen by using a special designated device to collect cervical cells at squamous columnar junction by the user without assistance of medical personnel (Othman and Mohamad Zaki, 2014). Simpler and lower-cost devices have been tested to self-collect cervico-vaginal cells with general good results, such as cotton swabs, vaginal tampons and lavages (Surriabre et al., 2017). Tampon is a cylindrical mass

of absorbent material, primarily used as a feminine hygiene product. At present, tampons are designed to be easily inserted into the vagina during menstruation and absorb the user's menstrual flow (Othman and Mohamad Zaki, 2014). Sizable cellular pellet can be collected by tampons that can be used for PCR purposes. Cervico-vaginal lavage is a type of device that releases liquid into the vagina and re-collects the fluid (Othman and Mohamad Zaki, 2014). This method is usually used in reproductive health studies. Cervico-vaginal lavage may have the advantage of increased sampling surface area and collection of a large sample volume, which can be fractionated for various analyses (Lorenzato et al., 2002; Othman and Mohamad Zaki, 2014).

# 4. Relation between EBV and HPV infection

## 4.1. Physiological aspects of EBV and HPV co-infection

Both Epstein-Barr virus and human papillomavirus are human tumor viruses that can cause neck and head cancers. As described previously, EBV is an enveloped double-stranded DNA virus with tropism for epithelial cells and resting B lymphocytes, and was the first virus to be associated with human cancers. This virus is related to several lymphoid and epithelial cancers including Hodgkin disease, Burkitt's lymphoma, nasopharyngeal carcinoma, and gastric cancers. Further, HPVs are non-enveloped double-stranded DNA viruses with a tropism for epithelial cells. Most HPVs are associated with 5% of human cancers including cervical and other cancers, and all HPV's are epithelial in origin. The productive phases of both viruses are attached to stratified epithelia highlighting the risk that these viruses may affect their respective lifecycles. Makieslski et al. (2016) have established an in-vitro model system to test the effects of HPV and EBV co-infection in stratified squamous oral epithelial cells. The results indicate that HPV increases maintenance of the EBV genome in the co-infected cells and promotes lytic reactivation of EBV in the upper layers of stratified epithelium (Makielski et al., 2016). Expression of the HPV

oncogenes E6 and E7 were found to be necessary and sufficient to account for HPV-mediated lytic reactivation of EBV (Makielski et al., 2016).These findings show that HPV increases the ability of epithelial cells to support the EBV lifecycle, which in turn could increase EBV-mediated pathogenesis in the oral cavity. Studies which look at the prevalence of EBV and HPV and their relation with the expression of p53 and PCNA in people with oral carcinoma, found out that HPV and EBV infection with the expression of p53 and PCNA were more observed in advanced stages of the disease. Others revealed that the co-infection of EBV and HPV plays an important role in the initiation of a neoplastic transformation of carcinogenesis (Shi et al., 2016). Interestingly, there is an interaction between EBV and HPV in vivo as well as an interaction between HPV and EBV oncoproteins [e.g., latent membrane protein 1 (LMP1) reduces apoptosis in vitro] (Ammatuna et al., 2000). The co-infection of HPV and EBV is usually found in nasopharyngeal carcinoma (NPC) patients from endemic regions (Shi et al., 2016). In one study, NPCs in Iranian patients were detected by in situ hybridization, and a low percentage (15%) of EBV-positive NPC patients had HPV sequences (HPV type 6/11 or HPV type 16/18) (Mirzamani et al., 2006). The co-infection of EBV and HPV was found in 47.7% of 88 Chinese NPC patients, and the “high-risk” types, including HPV types 16 and 18, accounted for 66.7% of 45 HPV-positive samples (Tung et al., 1999). On the other hand, some studies showed that co-infection of HPV and EBV was less frequent in NPC patients from some regions in the United States compared with that from South China. HPV might be the etiologic factor in some EBV-negative, non-keratinizing NPC patients, including Caucasian North American patients (Rassekh et al., 1998). A recent study in a population with a low incidence of NPC reported that both HPV-positive and EBV-positive NPC patients had similar overall survival (OS), whereas the HPV-positive, EBV-negative NPC group had shorter OS (Dogan et al., 2014). This study can support the etiologic role of HPV in NPC (Odumade et

al., 2011). Risk factors for acquisition of primary EBV infection, the proportion that result in infectious mononucleosis, and the distribution of their severity can only be determined by prospective studies (Grimm et al., 2016). The burden of primary EBV infection can be defined by these investigations, and it will give information about the appropriate use of a prophylactic vaccine like the gp350-based EBV vaccine reported to avoid infectious mononucleosis. Grimm et al., 2016, conducted a study among University of Minnesota undergraduate students who were seen every 2 weeks throughout their freshman year. Behavioral and clinical data, venous blood and oral washes were obtained. EBV antibodies were quantified by viral loads and enzyme immunoassay by PCR. For 8 months of observation, 24 cases/100 person experienced primary EBV infections. Eleven subjects had infectious mononucleosis with a median duration of 21 days, two subjects were hospitalized. Infections were initially identified in 12 subjects by finding EBV DNA in oral cells before onset of symptoms and in 2 subjects by symptom reporting, and EBV DNA and viral capsid antigen (VCA) IgM and gp350 IgG antibodies were present in the blood before onset of illness (Grimm et al., 2016). They observe that deep kissing is the only significant risk factor for acquisition of EBV infection. Most significantly, elevated amounts of gp350 antibody is linked to a lower severity of infectious mononucleosis ($P<0.0001$), which strengthens the rationale for a gp350-based prophylactic EBV vaccine.

The reported rates for Infectious Mononucleosis (IM) within schools and universities and their settings differ extensively from study to study. Williams-Harmon et al. (2016) examined earlier reported literature on the incidence rate, of infectious mononucleosis, in universities and military settings taking into account these possible factors: misdiagnosis, ambiguity in the reported sample populations, and number of students who visited and were diagnosed at their campus's health service centers. Some studies reported that IM was the second most common cause of

college student's admission into infirmaries. The annual incidence rate among these populations can be as high as 11 to 48 cases per 1000 (Williams-Harmon et al., 2016).

## 4.2. Social aspects and risks associated with EBV and HPV infection

Mononucleosis is a major repercussion of EBV infection (Scanlon, 1955; Evans, 1957; Bender, 1959; Evans and Espiritu-Campos, 1971; Evans et al., 1971; Sawyer et al., 1971; Brodsky and Heath, 1972; Finlay, 1976; Carter et al., 1977; Chang et al., 1979; Chang et al., 1988; Dan and Chang, 1990; Crawford et al., 2002; Macsween et al., 2010; Balfour et al., 2013; Williams-Harmon et al., 2016). The incidence of mononucleosis at the universities of California and Hawaii from 1971 through 1977 showed that students attending University of Hawaii at Manoa (UHM) was 37 cases per 100,000 per academic school year, much lower compared to the IM incidence rate at University of California at Davis (UCD), where 1,212 cases per 100,000 students per academic school year were observed(Chang et al., 1979). Similar results were observed in the military schools in the US more recently (Williams-Harmon et al., 2016). So, as a conclusion the variance in the incidence rates can be due to the differences in the populations studied, true epidemiologic or geographic variation.

In developing countries, most children over the age of 2 years are infected with EBV. In contrast, in developed counties where standards of living are high, childhood infection is usually escaped, but these individuals are then vulnerable to primary EBV infection later in life when infectious mononucleosis may occur, usually between 15–25 years in high socioeconomic groups. Higgins et al. (2007) surveyed 12000 university entrants, collecting questionnaires and serum samples to identify sexual and nonsexual risk factors for EBV seroconversion overall and, for the first time, separately by EBV type. Students were asked to complete a questionnaire that asked

about demographic, medical, behavioral, and sexual characteristics. Students were also asked to provide a blood sample to test IgG antibodies in plasma and EBV type, by polymerase chain reaction (PCR) of peripheral-blood mononuclear cells (PBMCs). The prevalence of EBV seropositivity was significantly higher among females, older students, those who had lived in tropical countries, those with siblings, and those who were sexually active, particularly if they had had numerous sex partners (Higgins et al., 2007). In contrast, risk was lower among participants who used condom all the time than among those who had sexual intercourse without the use of condom. Risk factors for type 1 EBV infection were similar to those for EBV overall, but no associations were found between nonsexual risk factors and type 2 infection (Higgins et al., 2007).The risk of type 2 infections is increased by sexual activity; however the increase in risk with number of sex partners was less consistent than for type 1 infections. Hence, EBV may be sexually transmitted and risk factors may differ for type 1 and type 2 infections.

As previously stated, human papillomavirus (HPV) is a sexually transmitted infection associated with high cancer risks. Prior studies have identified barriers to HPV vaccination, but they have focused on parental concerns, as the initial recommendation is for vaccination in the adolescent years when parents make health care decisions for children (Kester et al., 2013). Barnard et al. (2017) examined human papillomavirus (HPV) vaccine attitudes and knowledge in college students, factors associated with vaccination status utilizing the Precaution Adoption Process Model (PAPM). Their sample included 383 undergraduates' participants (freshmen through seniors) from a public university who participated in February and March 2015, with a mean age of 21.01 years. Students were emailed an unnamed online survey assessing attitudes, knowledge, and perceptions related to HPV, in addition to their stage in the PAPM about vaccination completion. Significantly more females (47.3%) than males (15.8%) were vaccinated.

While most students had basic knowledge of HPV, they had low perceptions of their susceptibility to contract HPV (Barnard et al., 2017).The unvaccinated students did not have enough information related to vaccination. In conclusion, this study indicated that students need help from providers, in addition to education and information regarding vulnerability to HPV.

## 4.3. Detection of oncogeneicity of EBV and HPV

The existence of human papillomavirus (HPV) infection in the major cases of cervical neoplasia has been considered a sign of an etiological role of HPV in cervical cancer (Kraus et al., 2006). The persistent infection by high-risk human papillomavirus (HR-HPV), such as genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 is essential for the development of cervical lesions (zur Hausen, 1991), and half of cases are caused by HPV16 (Munoz et al., 2003). HPV positive cervical carcinoma lines are dependent on E6/E7 expression and cellular regulatory pathways that are targeted by E6 and E7 are rendered latent but remain essentially functional (Goodwin et al., 2000; Mesri et al., 2014).While EBV encodes a viral oncogene, LMP1 (BNLF1) which is expressed in EBV-associated lymphoma and is responsible for B-cell transformation and for disruption of cellular signal transduction (Zheng, 2010). Oncogenic E6 genes contain an intron, whereas non-oncogenic E6 genes do not (Zheng, 2010).

Polymerase Chain reaction (PCR) with consensus primers can potentially detect most mucosal HPV types (Gravitt and Jamshidi, 2005). There are several consensus primers described: GP5/6 and their improved GP5+/6+ (Jacobs et al., 1999; Romero-Pastrana, 2012); My09/11 and their improved PGMY09/11 (Gravitt et al., 2000; Gravitt and Jamshidi, 2005); L1C1 with L1C2 and L1C2M (Yoshikawa et al., 1991); and pU-1M/pU-2R and their enhanced pU-1M-L and pU-2R-N (Gravitt and Jamshidi, 2005; Romero-Pastrana, 2012). There are different techniques for

the amplification fragments such as, hybridization to specific probes [25], direct DNA sequencing (Speich et al., 2004), or restriction fragment length polymorphisms (Lungu et al., 1992; Romero-Pastrana, 2012). To amplify the HPV oncogene modified, the amplification of papilloma virus oncogene transcripts (APOT) assay was used and was based on nested PCR reactions (Klaes et al., 1999; Lu et al., 2014).

## 5. Survey data for the analysis of sexual activity

Many countries conducted formative research to better understand the attitudes, awareness and behaviors of youth (15-29 years) relating to sexual health. In October 2005, online interviews conducted by Ipsos Canada with 1171 Canadian teenagers, found out that 27 % of teens were sexually active at a mean age of 15 years, with an average of 2.5 lifetime partners, had been in their current relationship for longer than eight months, and 76% used a condom the last time that they had sex (Frappier et al., 2008). They found that teens lacked knowledge about sexually transmitted infections and their consequences. So governments, parents and public health authorities should have an essential role to play to make sexual health information accurate, accessible, inclusive and salient to the reality of Canadian adolescents (Frappier et al., 2008). In the United States, Leigh et al. (1993) collected data on sexual behavior such as frequency of intercourse, number of sexual partners, and condom use. As a result, nearly all respondents were sexually experienced, a significant proportion of individuals were found to have intercourse with multiple partners without using condoms, and the minority of these respondents acknowledged that their behavior may place them at risk for HIV transmission (Leigh et al., 1993).

## 6. Objective and importance of this study

Little is known about sexuality among older persons in Lebanon, despite the aging of the population. Sexuality encompasses partnership, activity, behavior, attitudes, and function. Sexual activity is associated with health, and illness may considerably interfere with sexual health. A massive and growing market for drugs and devices to treat sexual problems targets older adults. Driven in part by the availability of drugs to treat erectile dysfunction, the demand for medical attention and services relating to sexual health is increasing. In addition, there is limited information on sexual behavior among older adults and how sexual activities change with aging and illness. Yet, sexual problems may be a warning sign or consequence of a serious underlying illness such as diabetes, an infection, urogenital tract conditions, or cancer. In Lebanon, there is no comprehensive, nationally representative, population-based data to inform physicians' understanding of the sexual norms and problems of older adults. Human papillomavirus (HPV) and Epstein Bar virus (EBV) infections are specific types of sexually transmitted diseases, and adults should have knowledge about their transmission and the way of prevention. So, the aim of this study is to explore on the sexual activity, behaviors, and problems of older adults and university students. Evaluate the knowledge-base for the development of sexual education and encourage the implementation of programs by schools in order to reduce risky sexual behaviors.

# III. Materials and Methods

## Study 1: Prevalence of EBV infection in Lebanese youth in the Bechareh Kaza, a risk analysis study

The main aim of this study was to a) detect the prevalence of EBV infection in a rural community in Lebanon; b) determine whether or not adolescents are at a higher risk of EBV infections than

other age groups, and c) whether or not this infection has changed across the last 5 years (2015-2019).

## 1. Data collection

Patients suspected of being infected with EBV are routinely tested at the Becharre Governmental hospital. Following study approval by the ethical and personal data security authorities of the hospital, records of 169 patients of various ages were reviewed retrospectively from 2015 to 2019 and laboratory records reviewed in order to assure a consistent diagnosis of the patients with clinical files. VCA Igg was analyzed using VIDAS EBV EBNA IgG an automated test for use on the VIDAS instruments, for the qualitative detection of anti-EBNA IgG in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay). Detection of these specific antibodies is an aid in diagnosing infectious mononucleosis (IM). Human serum (glass or plastic dry tube with coagulation activator and tube with separation gel) was used after centrifugation, then **100 µl** of sample were pipetted into the kit well. The SPRs and strips were inserted into the instrument. The color labels with the assay code on the SPRs and the Reagent Strips were checked for matching. The results was obtained within approximately 40 minutes. EBV infection was confirmed by positivity of IgG anti VCA. Information about age, marital status, level of IgG and other infections was collected and securely kept with the researcher.

## 2. Statistical analysis of study 1

Data from study 1 were analyzed using IBM SPSS 20 three different ways. First, the population was considered as one data set and gender and marital status compared using the independent t-test with respect to EBV values. Age Class and year were compared using One-Way

Anova with respect to EBV values. Second, data were split with respect to year and gender and marital status were compared using the independent t-test with respect to EBV values. Age Class were compared using One-Way ANOVA with respect to EBV values. Third, data were split with respect to age class and gender and marital status were compared using the independent t-test with respect to EBV values. Year was compared using One-Way Anova with respect to EBV values.

## Study 2: Analysis of sexual activity and risk in school students of various age

## School selection

Two private catholic-run schools across Lebanon were visited with a lesson plan to deliver customized, age-appropriate sexual education lectures to students. Before start of the sessions, anonymous surveys were administered to students to assess the level of basic knowledge, and questions and clarifications that arose during the filling out of the questionnaires were consequently addressed during the session.

### 1. Survey design and Participants

Students aged 12-13 (pre-pubescent) and 16-17 (pubescent) years from two private schools were asked to anonymously answer a survey designed to assess their knowledge concerning the physiology, anatomy and function of their body (questions 1,2), the risks associated with sexually transmitted infections (questions 4,5), and the sources of these acquired informations (question 2). Additionally, they were asked if they discuss sex (question 5) or have been involved in any sexual activities (questions 9, 10). Students were instructed to answer as truthfully as possible, and assured that no school administrator, teacher or parent will have access to their answers.

### 2. Statistical analysis of study 2

Data from study 2 were analyzed using IBM SPSS 20.0 using descriptive statistics to calculate the Knowledge Base of the students and the distribution of the answers. Further ANOVA was used to compare the various age groups. Significance was considered at $P<0.05$.

## IV. Results

## Study 1: Prevalence of EBV in Lebanese youth in the Bechareh Kaza, a risk analysis study

### a. Percent EBV prevalence in the population of Becharre Kaza

By studying the level of EBV infection shown in figure 3.a, results show that out of the total sample (n=169), 60% were men and 40% were women. Also, when we searched the marital status, we found that 75% of cases were single people and 25% were married people.

As for the analysis of EBV Infection variation with age groups as shown in figure 3.b, of the 169 cases, the majority of the infected patients (22.5%) were aged between 19-24 years. Pediatric cases (0-12 years) and those aged between 30-35 years contributed the least (11.2%). Patients aged 13-18 years and 25-29 years comprised 15.4% and 18.3% of the total cases, respectively. Those aged above 36 years showed an ascending percentage after decreasing (21.3%).

### b. IgG levels of EBV infection in Becharre

The IgG level in patients was measured, which reflects the EBV-positivity in the blood and presented in figure 4. As expected, the level of these antibodies increases through years depending on the age group. Pediatric patients, over the years of the study, showed an almost stable IgG level

with a mean around 1.5. Young population aged 13-18 show a significant increase of IgG over the years from an average of 1 in 2015 to 3 in 2019. After the age of 19 years, the level of IgG antibodies in blood in patients is described as high from 2015 to 2019. It is important to note that EBV infection expressed as IgG levels increased significantly throughout the study years.

### c. Evolution of EBV infection in the past 5 years in Becharre Kaza

Between 2015 and 2019, the evolution of IgG values was analyzed and values were compared among married and single patients as presented in figure 5. Mean IgG levels were higher among married subjects compared to single ones. Mean IgG levels change through years to get at 2019 the same average of infection approximately. Infection (or mean IgG levels) increased from an average of 1.5 in 2015 to 2 in 2019. Also for singles, EBV infection appears in high means since 2015 till 2019 from 2 to 2.5.

In fact, between 2015 and 2019, there was a slight increase in EBV infection among both married and single patients. The level and prevalence of EBV indicate a diffused infection in the entire population independently of marital status. This is due to the excessive sexual activities spread in the population, out of range of one partner relation and good practices to be safe.

## Study 2: Analysis of sexual activity and risk in high school students of various age

In this study, we assessed students of different sexes from two age groups (pre-pubescent and pubescent), in confidentiality about their knowledge about the physiology, anatomy and function of their body (figure 6), risks associated with sexually transmitted infections (figure 7), and source of such information (Figure 8). Further, we inquired whether they discuss sex (Figure

9) or have been involved in sexual activity (Figure 10). Results are reported as highest percentages for qualitative answers ranging from least to well.

Initially, students were asked basic knowledge about body and sex (Figure 6). The pre-pubescent group showed that males retain a moderate knowledge about their own body (43%), the female body (43%), and sex (58%), while 67% of females had well knowledge about both their own bodies and sex, but only a mild knowledge (34%) about the opposite sex. The pubescent group showed that males have well knowledge (63%) about their own bodies and sex, with a moderate (54%) familiarity about the opposite sex. Females showed a consistent moderate knowledge (75%) about all three aspects.

Students were asked about STIs and their dangers (Figure 7). The pre-pubescent group showed that males have a least to moderate knowledge (48%) about STIs, and a mild awareness (30%) of their dangers, while females showed least to moderate (32%) familiarity with STIs. The pubescent group showed more knowledge as males scored well (44%), while 53% of females, reported moderate level of knowledge about STIs and their dangers.

When asked about their understanding concerning protection from STIs (condom use), we found that males know more about it (Figure 8). The majority of pre-pubescent and pubescent females (61%) have a more advanced idea about the menarche than males in a moderate way (42%). However, 58% of the pre-pubescent group (males and females) showed least familiarity about condom and contraceptive pill use. The pubescent group demonstrated that males retain greater well knowledge (42%) about condoms and pills, but females still showed weak understanding (47%) of their use.

Students were then asked about the source of information about their body and sex (figure 9), in addition to who they turn to talk or inquire about their bodies and sex. Most pre-pubescent males gained knowledge about their body from other sources (34%) (Internet, movies, social media), but they seem mostly comfortable talking about their bodies (26.5%) and sex (28%) with their parents. On the other hand, pre-pubescent females gather body knowledge mainly from friends (27%), and prefer to talk about their bodies with their parents (38%), but turn to others for conversations about sex (28%). The pubescent males gained this information from parents and other sources (29%), but prefer to talk about their bodies (35%) and sex (35%) with friends and others. Females opt to receive information from parents (38%) and friends (27%), but rely mainly on friends and others when it comes to talking about their bodies and sex.

However, about 45% of pre-pubescent males and 42% of pre-pubescent females consider talking about their body and sex "taboo", with another majority of females never even talks about it. Whereas, 58% of pubescent males consider talking about body and sex as normal, while majority of pubescent females answered "yes" when asked if they talk about their body (39%), but "taboo" when asked if they talk about sex (44%) (Figure 10).

Teenagers were asked if they have experimented with their body, and answered with variable answers depending on age and sex. About 30% of the pre-pubescent males mostly answered with "mild", while 31% of pre-pubescent females answered by "moderate". Meanwhile, 72% of pubescent males answered "well" when asked about experience with their body; however 58% of pubescent females answered by "mild".

While 28% of pre-pubescent males and females had minimal experimentation with a partner, 63% of pubescents had moderately experimented with a partner (Figure 11).

# V. Discussion

Examination of a series of patients from Becharreh Governmental Hospital revealed that EBV infection has increased from 2015 to 2019 irrespective of age or sex of patients suspected to have EBV, though the surprising infection was in children. The pediatric cases showed the smallest percentage, indicating a non-sexually related behavioral transmission, such as the virus spreading by family members' practices of kissing on the mouth, or unsanitary salivary exchange via contaminated utensils. Before the age of 10 years, EBV infection is usually asymptomatic, though not the case in our analyses. As for all other ages, the EBV infection is widespread and is anticipated via engagement in bodily fluid exchange including kissing or sexual activity. The teenagers being infected is quite puzzling if attributed to sexual activity, since youth between 13 and 18 are not yet engaged in bodily relations as shown in our second study. Thus teenagers more likely are infected via non-sanitary practices.

In a study where a series of 55 pediatric cases from three geographical locations of various cultural backgrounds, namely the United Kingdom, Brazil, and Saudi Arabia and the relationship between country, age, sex, histological subtype, EBV was increased in pedriatric patients (Armstrong et al., 1993). Further, in Argentina, children were infected rather early in their life and nearly 70% of children were seropositive by the age of 2 years (Chabay and Preciado, 2013). With the infection with EBV early in life increasing the risk of developing EBV-associated lymphoma (Chabay and Preciado, 2016), it is essential to develop guidelines for prevention of such early infections.

Furthermore, patients between 19-24 years have the highest rate of infection, most likely due to sexually active behavior, in addition to the lack of knowledge about sexual transmitted disease.

During this age, adolescents discover their bodies, with little information about diseases transmitted via kissing or urogenital contact during sexual intercourse. In addition, youth present a more risk-associated behavior, such as drugs, alcohol and multiple partners. With partner stability at the age of 25 to 35 years, and more exposure to information, the percentage of infection with EBV decreases. The majority of this age group is married, so the infection may be the result of transmission between the partners themselves. Infection after 36 years shows an ascending trend, assuming that unmarried people have sex independent of a single partner, further supported by an increased level of infidelity as seen by an increase in divorce rate. In the United States, Epstein-Barr virus seroprevalence of EBV was valued to be nearly 66.5% among children ages 6–19 (58.5% for children 6–12 and 73.4% for those 12–19) from 2003 to 2010 (Dowd et al., 2013).

The EBV test is also known as "EBV antibodies", identifies EBV infection in the blood by detecting the presence of antibodies to EBV antigens. An abnormal result means that the test has detected EBV antibodies. This indicates that the patient is currently infected with EBV or have been infected with the virus in the past. The level of antibody detected in the blood, called the titer, does not have any impact on how long you have had the disease or how severe the disease is. The presence of VCA IgG antibodies indicates that an EBV infection has occurred at some time recently or in the past. The results from studies of the different types of cancer regularly conflict, estimate antibodies against a limited number of EBV proteins, or fail to explore multiple antibody types (e.g., IgG and IgA), thus limiting the ability to draw absolute conclusions about the role of the humoral immune response to EBV in expecting disease risk (Coghill and Hildesheim, 2014). As demonstrated before, the pediatrics groups show abnormal positivity in their case, and this positivity is also due to the absence of knowledge. Infected parents and siblings may be a way of transmission to children through saliva: oral secretions introduce a source through familiar intact

or sharing food and utensils. The most impressive change was observed among young populations, in which EBV-positivity increased. This widespread phenomenon is associated with an attitude of non-responsible that leads to undesirable problems affecting their health. Parents, school, and non-developed society are also factors that explain the frequent presence of this virus. In developing countries worldwide, more than 90% of the population is infected with EBV in early childhood (Henle and Henle, 1976; Takeuchi et al., 2006; Dowd et al., 2013). In Western developed countries, 45% of 5–9 year olds in England, (Morris et al., 2002; Dowd et al., 2013) and 74% for 3–17 year olds in Germany, (Dowd et al., 2013) are infected with EBV. In our study, between 2015 and 2019, there was a slight increase in EBV infection among both married and single patients. The level and prevalence of EBV indicate a diffused infection in the entire population independently of marital status. This is due to the excessive sexual activities spread in the population, out of range of one partner relation and good practices to be safe.

A moderate, low level of knowledge about sexually transmitted diseases is evident among schools students from this study. In 2009 a survey conducted in seven private and public universities in Lebanon showed that 73% of male and 22% of female unmarried university students reported having a sexual relationship (Barbour and Salameh 2009). These results may undervalue the right prevalence of young people's sexual activity. Therefore, we need to promote further discourse and awareness in the topics of sex and affiliated dangers by removing the stigma from sexual education in order to make students more comfortable in seeking information and advice.

Although males seem to have more knowledge in sexual protection than females, the percentage is still relatively small and this lack of awareness can be attributed to the absence of sexual education programs at schools. In a study done in Ghana, Ghanaian youth 99% of

respondents knew of proper condom use, reporting that females and sexually inexperienced youth were the least informed (Glover et al., 2003).

Both pre-pubescent and pubescent groups, including males and females, indicated a coherent shortcoming in general body knowledge, be it anatomically or physiologically, further advocating the need for targeted biological, sexual, or body image education or workshops. Unfortunately, parental role in sex awareness is also lacking and students rely on the exchange of information, inaccurate at times, between themselves, regardless of sex, gender and age.

A great lack of comfort is evident in students speaking about their body and sex especially in males and females aged between 12 and 13 years of age. Safe spaces ought to be allocated for these students to encourage them to seek advice and information, especially during this impressionable stage of development. .

There is a wide gap between 12-13 and 16-17 year-olds in terms of their experience with their body and with a partner: pre-pubescent appears not to be involved in sexual activities. A significant number of students from the pubescent group have experimented with their body and with a partner, which can lead to unwanted consequences. For this reason, awareness-raising conferences must be intensified in schools to reduce the risk of unprotected sexual practices.

## VI. Conclusion

The unavailability of cultural awareness and educational programs to prevent these health risks including sexually transmitted diseases or infections (STDs or STIs) and unwanted pregnancies, alongside psychological strain produced from these situations including depression, anxiety, suicide ideation, and even child abuse. STIs are a severe health concern for young people.

Hence the necessity of education, awareness, and prevention of negative outcomes. These programs can help youth adopt lifelong attitudes and behaviors that support overall health and well-being that can eventually reduce the risk for STD transmission. With sexually transmitted disease prevention closely linked to a proper sexual education curriculum worldwide, and with EBV-positivity in Lebanon increasing from 2015 to 2019, awareness on the consequences of the sociological interactions, and on proper sexual education is essential to curb the infection in EBV specifically, but also to help manage the spread of all STIs. Students lack the knowledge base on STIs but also on more broad information as it relates to protection and the proper functioning of the reproductive system, a great concern in Lebanon. There is an urgent need for public health and school administrators, as well as government entities, to seriously investigate STI-associated risks in Lebanese public, private, and higher education systems.

# VII. BIBLIOGRAPHY


Ambinder, R. F., and E. Cesarman. 2007. Clinical and pathological aspects of EBV and KSHV infection. In: A. Arvin et al. (eds.) Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis, Cambridge.

Ammatuna, P. et al. 2000. Presence of human papillomavirus and Epstein-Barr virus in the cervix of women infected with the human immunodeficiency virus. J Med Virol 62: 410-415.

Anhang, R., A. Goodman, and S. J. Goldie. 2004. HPV communication: review of existing research and recommendations for patient education. CA Cancer J Clin 54: 248-259.

Armstrong, A. A. et al. 1993. Association of Epstein-Barr virus with pediatric Hodgkin's disease. The American journal of pathology 142: 1683-1688.

Ascherio, A., and K. L. Munger. 2007. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol 61: 288-299.

Balfour, H. H., Jr. et al. 2013. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. J Infect Dis 207: 80-88.

Barnard, M., P. George, M. L. Perryman, and L. A. Wolff. 2017. Human papillomavirus (HPV) vaccine knowledge, attitudes, and uptake in college students: Implications from the Precaution Adoption Process Model. PLoS One 12: e0182266.

Becker, J. A., and J. A. Smith. 2014. Return to play after infectious mononucleosis. Sports health 6: 232-238.

Bender, C. E. 1959. Clinical epidemiology of mononucleosis at a state university. Northwest medicine 58: 697-700.

Braaten, K. P., and M. R. Laufer. 2008. Human Papillomavirus (HPV), HPV-Related Disease, and the HPV Vaccine. Rev Obstet Gynecol 1: 2-10.

Brodbeck, J., U. L. Vilen, M. Bachmann, H. Znoj, and F. D. Alsaker. 2010. Sexual risk behavior in emerging adults: gender-specific effects of hedonism, psychosocial distress, and sociocognitive variables in a 5-year longitudinal study. AIDS education and prevention : official publication of the International Society for AIDS Education 22: 148-159.

Brodsky, A. L., and C. W. Heath, Jr. 1972. Infectious mononucleosis: epidemiologic patterns at United States colleges and universities. Am J Epidemiol 96: 87-93.

Carter, C. D., T. M. Brown, Jr., J. T. Herbert, and C. W. Heath, Jr. 1977. Cancer incidence following infectious mononucleosis. Am J Epidemiol 105: 30-36.

Chabay, P., and M. V. Preciado. 2016. Epidemiology of Epstein-Barr virus-associated pediatric lymphomas from Argentina. Boletin medico del Hospital Infantil de Mexico 73: 47-54.

Chabay, P. A., and M. V. Preciado. 2013. EBV primary infection in childhood and its relation to B-cell lymphoma development: a mini-review from a developing region. International journal of cancer 133: 1286-1292.

Chang, R. Dan, and R. C. Chan. 1988. Epstein-Barr virus infections among university students in a tropical country. Journal of American college health : J of ACH 37: 115-118.

Chang, R. S., D. F. Char, J. H. Jones, and S. B. Halstead. 1979. Incidence of infectious mononucleosis at the Universities of California and Hawaii. J Infect Dis 140: 479-486.

Clarke, P., C. Ebel, D. N. Catotti, and S. Stewart. 1996. The psychosocial impact of human papillomavirus infection: implications for health care providers. Int J STD AIDS 7: 197-200.

Clifford, G. M., J. S. Smith, T. Aguado, and S. Franceschi. 2003. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 89: 101-105.

Coghill, A. E., and A. Hildesheim. 2014. Epstein-Barr virus antibodies and the risk of associated malignancies: review of the literature. Am J Epidemiol 180: 687-695.

Crawford, D. H. et al. 2002. Sexual history and Epstein-Barr virus infection. J Infect Dis 186: 731-736.
Dan, R., and R. S. Chang. 1990. A prospective study of primary Epstein-Barr virus infections among university students in Hong Kong. The American journal of tropical medicine and hygiene 42: 380-385.
de-The, G. et al. 1975. Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study - a review. IARC Sci Publ: 3-16.
De Paschale, M., and P. Clerici. 2012. Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. World J Virol 1: 31-43.
DiClemente, R. J., J. M. Sales, F. Danner, and R. A. Crosby. 2011. Association between sexually transmitted diseases and young adults' self-reported abstinence. Pediatrics 127: 208-213.
Dixit, R., C. Bhavsar, and Y. S. Marfatia. 2011. Laboratory diagnosis of human papillomavirus virus infection in female genital tract. Indian J Sex Transm Dis 32: 50-52.
Dogan, S. et al. 2014. Human papillomavirus and Epstein-Barr virus in nasopharyngeal carcinoma in a low-incidence population. Head Neck 36: 511-516.
Dowd, J. B., T. Palermo, J. Brite, T. W. McDade, and A. Aiello. 2013. Seroprevalence of Epstein-Barr virus infection in U.S. children ages 6-19, 2003-2010. PLoS One 8: e64921.
Esau, D. 2017. Viral Causes of Lymphoma: The History of Epstein-Barr Virus and Human T-Lymphotropic Virus 1. Virology (Auckl) 8: 1178122X17731772.
Evans, A. S. 1957. Acute respiratory disease in University of Wisconsin students. The New England journal of medicine 256: 377-384.
Evans, A. S., and L. Espiritu-Campos. 1971. Acute respiraotry diseases in students at the University of the Philippines, 1964-69. Bulletin of the World Health Organization 45: 103-112.
Evans, A. S., J. C. Niederman, and R. N. Sawyer. 1971. Prospective studies of a group of Yale University freshmen. II. Occurrence of acute respiratory infections and rubella. J Infect Dis 123: 271-278.
Finlay, S. E. 1976. Physical diseases in university students. British medical journal 2: 1312-1314.
Frappier, J. Y. et al. 2008. Sex and sexual health: A survey of Canadian youth and mothers. Paediatrics & child health 13: 25-30.
Glover, E. K. et al. 2003. Sexual health experiences of adolescents in three Ghanaian towns. International family planning perspectives 29: 32-40.
Golden, H. D., R. S. Chang, W. Prescott, E. Simpson, and T. Y. Cooper. 1973. Leukocyte-transforming agent: prolonged excretion by patients with mononucleosis and excretion by normal individuals. J Infect Dis 127: 471-473.
Goodwin, E. C. et al. 2000. Rapid induction of senescence in human cervical carcinoma cells. Proceedings of the National Academy of Sciences of the United States of America 97: 10978-10983.
Gravitt, P. E., and R. Jamshidi. 2005. Diagnosis and management of oncogenic cervical human papillomavirus infection. Infectious disease clinics of North America 19: 439-458.
Gravitt, P. E. et al. 2000. Improved amplification of genital human papillomaviruses. Journal of clinical microbiology 38: 357-361.
Grimm, J. M. et al. 2016. Prospective studies of infectious mononucleosis in university students. Clin Transl Immunology 5: e94.
Henle, W., and G. Henle. 1976. The sero-epidemiology of Epstein-Barr virus. Advances in pathobiology: 5-17.
Hess, R. D. 2004. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. Journal of clinical microbiology 42: 3381-3387.
Higgins, C. D. et al. 2007. A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. J Infect Dis 195: 474-482.

Ho, M. et al. 1988. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. Transplantation 45: 719-727.
Horvath, C. A., G. A. Boulet, V. M. Renoux, P. O. Delvenne, and J. P. Bogers. 2010. Mechanisms of cell entry by human papillomaviruses: an overview. Virol J 7: 11.
Jacobs, M. V. et al. 1999. Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison. Journal of clinical pathology 52: 498-503.
Jurjus A. , K. J. 2004. Knowledge, attitude, beliefs, and practice of the lebanese population concerning aids. KABP-Lebanon.
Kester, L. M., G. D. Zimet, J. D. Fortenberry, J. A. Kahn, and M. L. Shew. 2013. A national study of HPV vaccination of adolescent girls: rates, predictors, and reasons for non-vaccination. Matern Child Health J 17: 879-885.
Klaes, R. et al. 1999. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer research 59: 6132-6136.
Kraus, I. et al. 2006. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. Journal of clinical microbiology 44: 1310-1317.
Leigh, B. C., M. T. Temple, and K. F. Trocki. 1993. The sexual behavior of US adults: results from a national survey. American journal of public health 83: 1400-1408.
Lorenzato, F. R. et al. 2002. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. American journal of obstetrics and gynecology 186: 962-968.
Low, N., N. Broutet, and R. Turner. 2017. A Collection on the prevention, diagnosis, and treatment of sexually transmitted infections: Call for research papers. PLoS medicine 14: e1002333.
Lu, X. et al. 2014. Multiple-integrations of HPV16 genome and altered transcription of viral oncogenes and cellular genes are associated with the development of cervical cancer. PLoS One 9: e97588.
Lungu, O., T. C. Wright, Jr., and S. Silverstein. 1992. Typing of human papillomaviruses by polymerase chain reaction amplification with L1 consensus primers and RFLP analysis. Molecular and cellular probes 6: 145-152.
Macsween, K. F. et al. 2010. Infectious mononucleosis in university students in the United kingdom: evaluation of the clinical features and consequences of the disease. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 50: 699-706.
Makielski, K. R. et al. 2016. Human papillomavirus promotes Epstein-Barr virus maintenance and lytic reactivation in immortalized oral keratinocytes. Virology 495: 52-62.
Mesri, E. A., M. A. Feitelson, and K. Munger. 2014. Human viral oncogenesis: a cancer hallmarks analysis. Cell host & microbe 15: 266-282.
Mirzamani, N., P. Salehian, M. Farhadi, and E. A. Tehran. 2006. Detection of EBV and HPV in nasopharyngeal carcinoma by in situ hybridization. Exp Mol Pathol 81: 231-234.
Morris, M. C. et al. 2002. Sero-epidemiological patterns of Epstein-Barr and herpes simplex (HSV-1 and HSV-2) viruses in England and Wales. J Med Virol 67: 522-527.
Munoz, N. et al. 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England journal of medicine 348: 518-527.
Naswa, S., and Y. S. Marfatia. 2010. Adolescent HIV/AIDS: Issues and challenges. Indian journal of sexually transmitted diseases and AIDS 31: 1-10.
Niedobitek, G., A. Agathanggelou, N. Steven, and L. S. Young. 2000. Epstein-Barr virus (EBV) in infectious mononucleosis: detection of the virus in tonsillar B lymphocytes but not in desquamated oropharyngeal epithelial cells. Molecular pathology : MP 53: 37-42.

Odumade, O. A., K. A. Hogquist, and H. H. Balfour, Jr. 2011. Progress and problems in understanding and managing primary Epstein-Barr virus infections. Clin Microbiol Rev 24: 193-209.

Othman, N. H., and F. H. Mohamad Zaki. 2014. Self-collection tools for routine cervical cancer screening: a review. Asian Pacific journal of cancer prevention : APJCP 15: 8563-8569.

Rassekh, C. H. et al. 1998. Combined Epstein-Barr virus and human papillomavirus infection in nasopharyngeal carcinoma. Laryngoscope 108: 362-367.

Romero-Pastrana, F. 2012. Detection and typing of human papilloma virus by multiplex PCR with type-specific primers. ISRN microbiology 2012: 186915.

Sawyer, R. N., A. S. Evans, J. C. Niederman, and R. W. McCollum. 1971. Prospective studies of a group of Yale University freshmen. I. Occurrence of infectious mononucleosis. J Infect Dis 123: 263-270.

Scanlon, J. H., Jr. 1955. Report of an epidemic of infectious mononucleosis in a small college town. The Journal of the Kansas Medical Society 56: 284-290.

Schiffman, M., and D. Solomon. 2003. Findings to date from the ASCUS-LSIL Triage Study (ALTS). Arch Pathol Lab Med 127: 946-949.

Schiller, J. T., P. M. Day, and R. C. Kines. 2010. Current understanding of the mechanism of HPV infection. Gynecol Oncol 118: S12-17.

Shi, Y. et al. 2016. Co-infection of Epstein-Barr virus and human papillomavirus in human tumorigenesis. Chin J Cancer 35: 16.

Shuichi Fujita, Nathan Buziba, Atsushi Kumatori, A. Y. Masachika Senba, and K. Toriyama. 2004. Early Stage of Epstein-Barr Virus Lytic Infection Leading to the “Starry Sky” Pattern Formation in Endemic Burkitt Lymphoma. Archives of Pathology & Laboratory Medicine 128: 549-552.

Speich, N., C. Schmitt, R. Bollmann, and M. Bollmann. 2004. Human papillomavirus (HPV) study of 2916 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. Journal of medical microbiology 53: 125-128.

Stanfield, B. A., and M. A. Luftig. 2017. Recent advances in understanding Epstein-Barr virus. F1000Res 6: 386.

Stephen E. Straus, Jeffrey I. Cohen, Giovanna Tosato, and J. Meier. 1993. Epstein-Barr Virus Infections: Biology, Pathogenesis, and Management Annals of Internal Medicine 118: 45-58.

Straus, S. E., J. I. Cohen, G. Tosato, and J. Meier. 1993. NIH conference. Epstein-Barr virus infections: biology, pathogenesis, and management. Ann Intern Med 118: 45-58.

Sugden, B. 2014. Epstein-Barr virus: the path from association to causality for a ubiquitous human pathogen. PLoS biology 12: e1001939.

Sumaya, C. V. et al. 1986. Enhanced serological and virological findings of Epstein-Barr virus in patients with AIDS and AIDS-related complex. J Infect Dis 154: 864-870.

Surriabre, P. et al. 2017. Self-sampling for human papillomavirus DNA detection: a preliminary study of compliance and feasibility in BOLIVIA. BMC women's health 17: 135.

Svedmyr, E. et al. 1984. Virologic, immunologic, and clinical observations on a patient during the incubation, acute, and convalescent phases of infectious mononucleosis. Clin Immunol Immunopathol 30: 437-450.

Takeuchi, K. et al. 2006. Prevalence of Epstein-Barr virus in Japan: trends and future prediction. Pathology international 56: 112-116.

Taylor, A., and M. A. Gosney. 2011. Sexuality in older age: essential considerations for healthcare professionals. Age and ageing 40: 538-543.

Toussirot, E., and J. Roudier. 2008. Epstein–Barr virus in autoimmune diseases. Best Pract. Res. Clin. Rheumatol. 22: 883-896.

Tung, Y. C., K. H. Lin, P. Y. Chu, C. C. Hsu, and W. R. Kuo. 1999. Detection of human papilloma virus and Epstein-Barr virus DNA in nasopharyngeal carcinoma by polymerase chain reaction. Kaohsiung J Med Sci 15: 256-262.

Unemo, M. et al. 2017. Sexually transmitted infections: challenges ahead. The Lancet. Infectious diseases 17: e235-e279.

Williams-Harmon, Y. J., L. A. Jason, and B. Z. Katz. 2016. Incidence of Infectious Mononucleosis in Universities and U.S. Military Settings. J Diagn Tech Biomed Anal 5.

Winer, R. L. et al. 2003. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 157: 218-226.

Yoshikawa, H. et al. 1991. Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. Japanese journal of cancer research : Gann 82: 524-531.

Zheng, Z. M. 2010. Viral oncogenes, noncoding RNAs, and RNA splicing in human tumor viruses. International journal of biological sciences 6: 730-755.

zur Hausen, H. 1991. Human papillomaviruses in the pathogenesis of anogenital cancer. Virology 184: 9-13.

## TABLES AND FIGURES

***Table 1**: Comparative analysis of methods for detecting of EBV*

| Methods (serology) | Reliability | Reference |
|---|---|---|
| Gold standard or IFA | Classical method; highly specific; staging of EBV infections is possible; single serum sample is used. | (Hess, 2004) |
| Elisa | Rapid method; suitable for automation; single serum sample is used. | (Hess, 2004) |
| Complement fixation reaction | Less specific and less sensitive; not widely used. | (Hess, 2004) |
| IgG avidity determination | It is a special method; used for confirmation of indeterminate results. | (Hess, 2004) |
| Blot techniques | Highly specific; confirmatory method; staging of EBV infections is possible; single serum sample is used. | (Hess, 2004) |
| Nucleic acid detection | Used only in specific laboratories; long lasting test from 4 to 8 weeks. | (Hess, 2004) |

***Table 2**: Summary of oncogenes-associated with HPV and EBV and their detection methods*

| Gene region (oncogenesis) | Cancer Risk | Virus type | Technique of detection | reference |
|---|---|---|---|---|
| None | Low<br>– benign grade cervical cell changes<br>– rarely associated with invasive cancer | HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, 73, 81 | PCR | (Jurjus A. , 2004). |
| E6, E7 | Intermediate<br>– 2% to 4 % of cancers | 31, 33, 35, 51, 52 | PCR | |
| E6, E7, E2 | High<br>– Associated with invasive cancers of the vulva, penis, anus, or cervix | 18, 45 | PCR | |
| E6, E7, E5 | – | 16 | | |
| | – APOT | E6, E7 oncogene | APOT | (Klaes et al., 1999; Lu et al., 2014). |
| EBNA, LMP-1, LMP-2A | – | EBV type | PCR | (Zheng, 2010). |

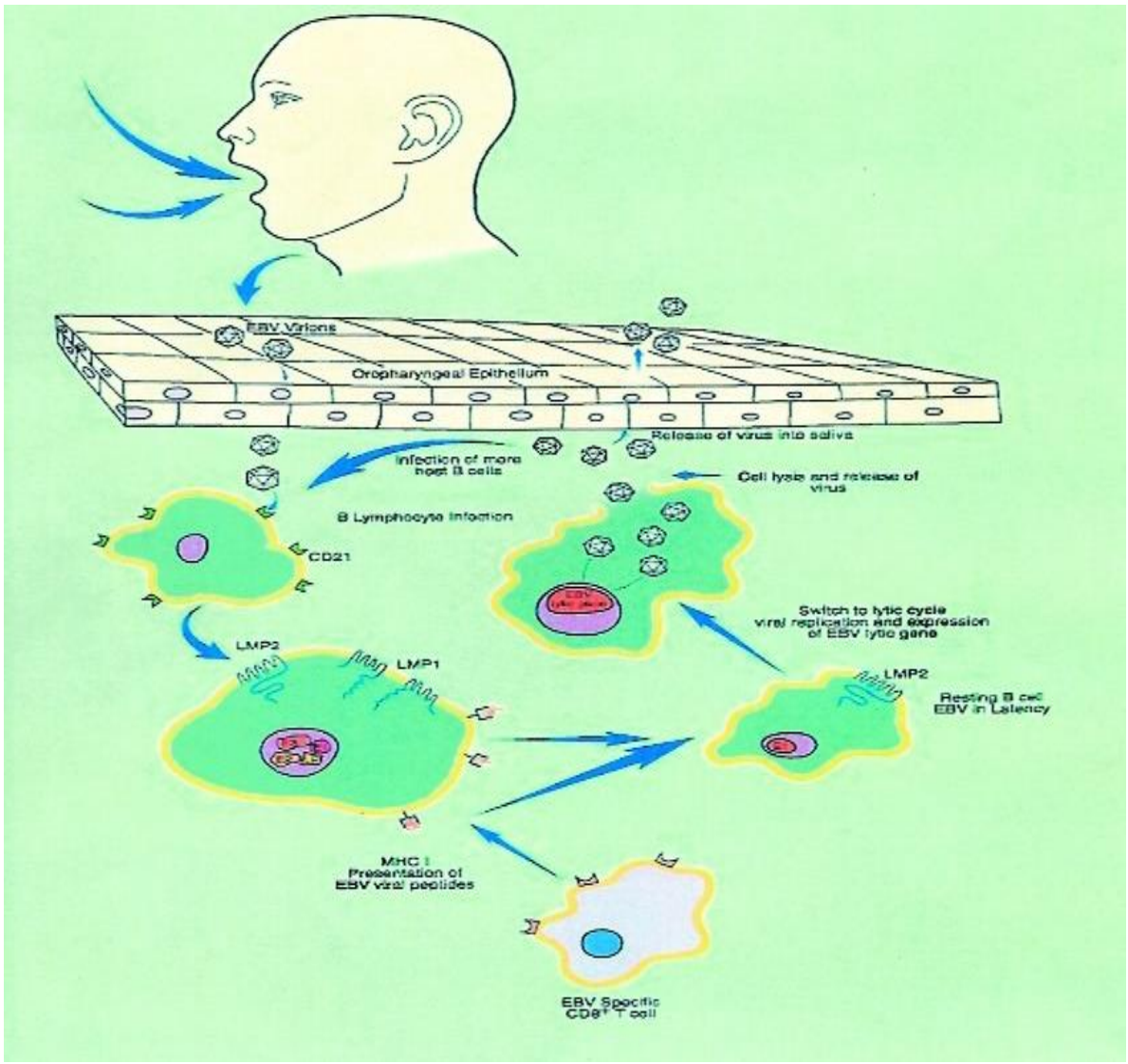


**Figure 1** : The EBV life cycle. (Hess, 2004).

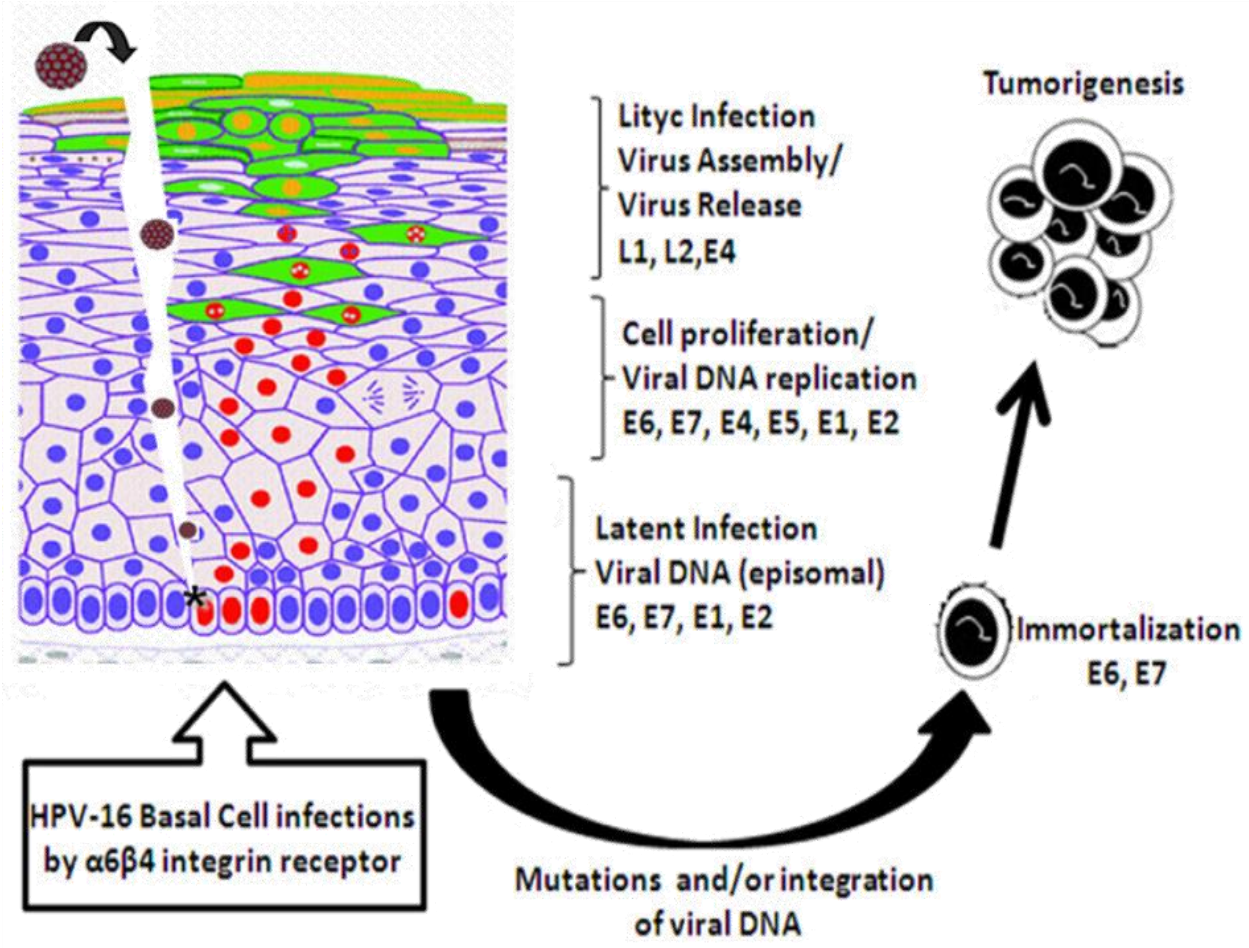


**Figure 2** : HPV mechanism (Anantharaman et al., 2013)

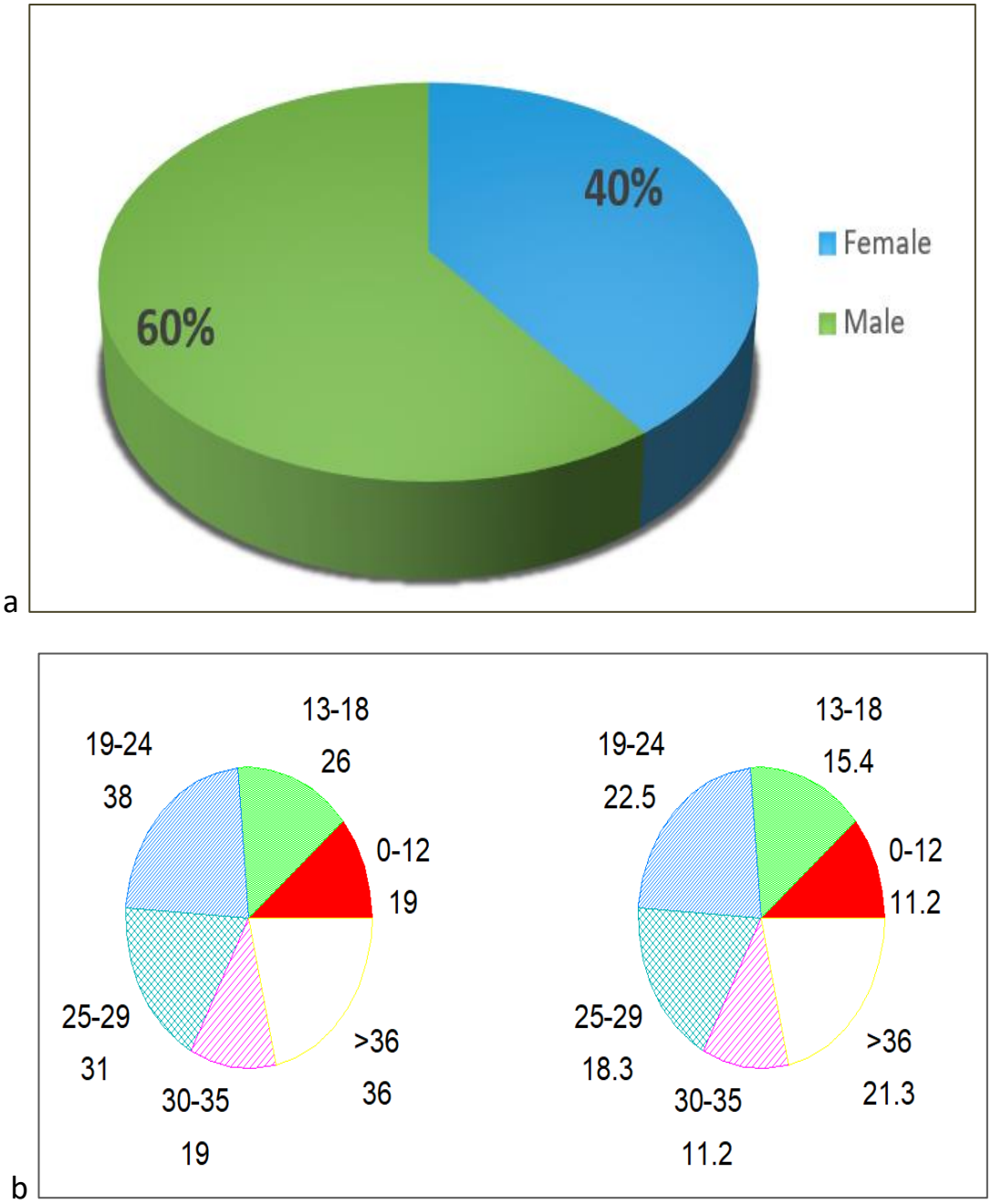


**Figure 3**: Percentage of EBV prevalence in the population of Becharreh Kaza.

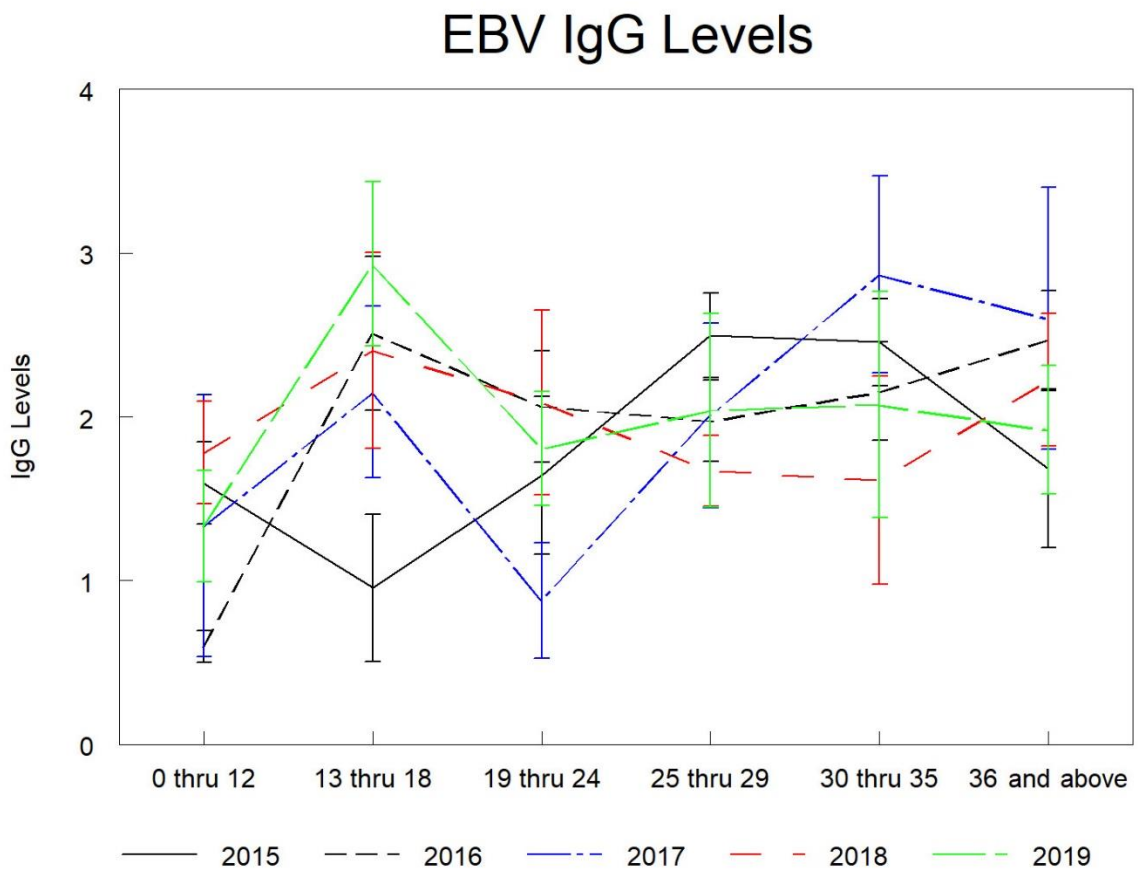


**Figure 4:** Evolution of IgG levels of EBV infection in the past 5 years in Becharreh Kaza.

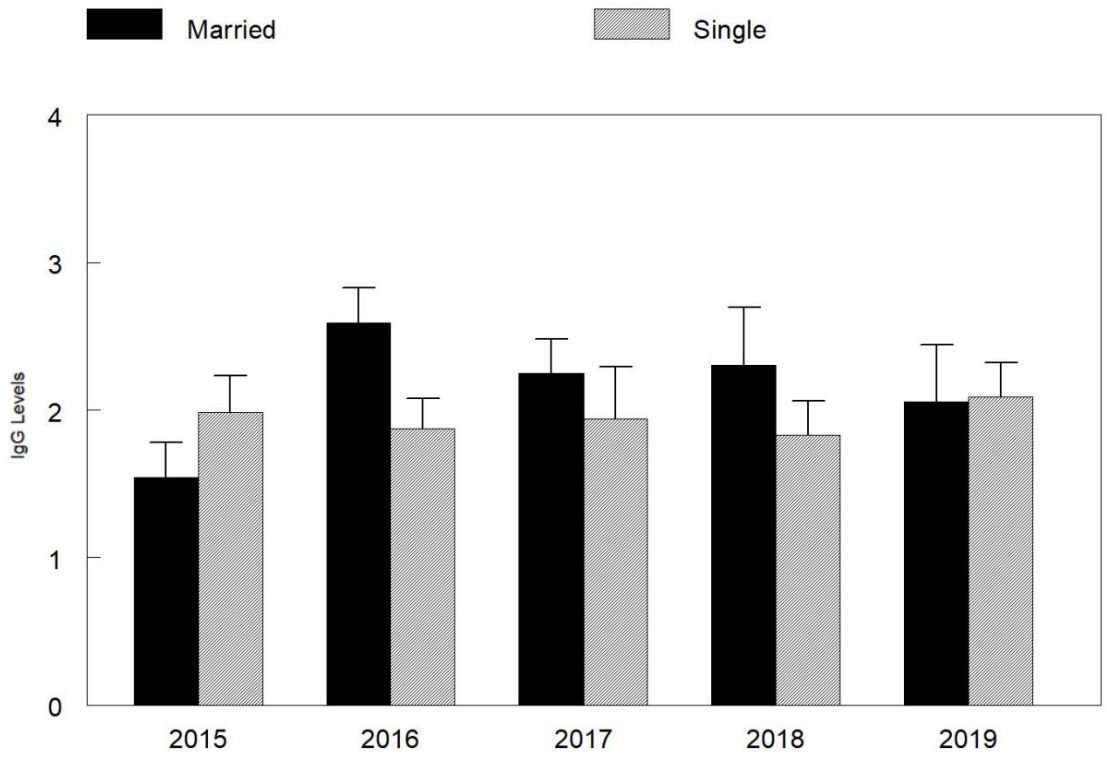


**Figure 5**: evolution of EBV infection from 2015 to 2019 with marital status.

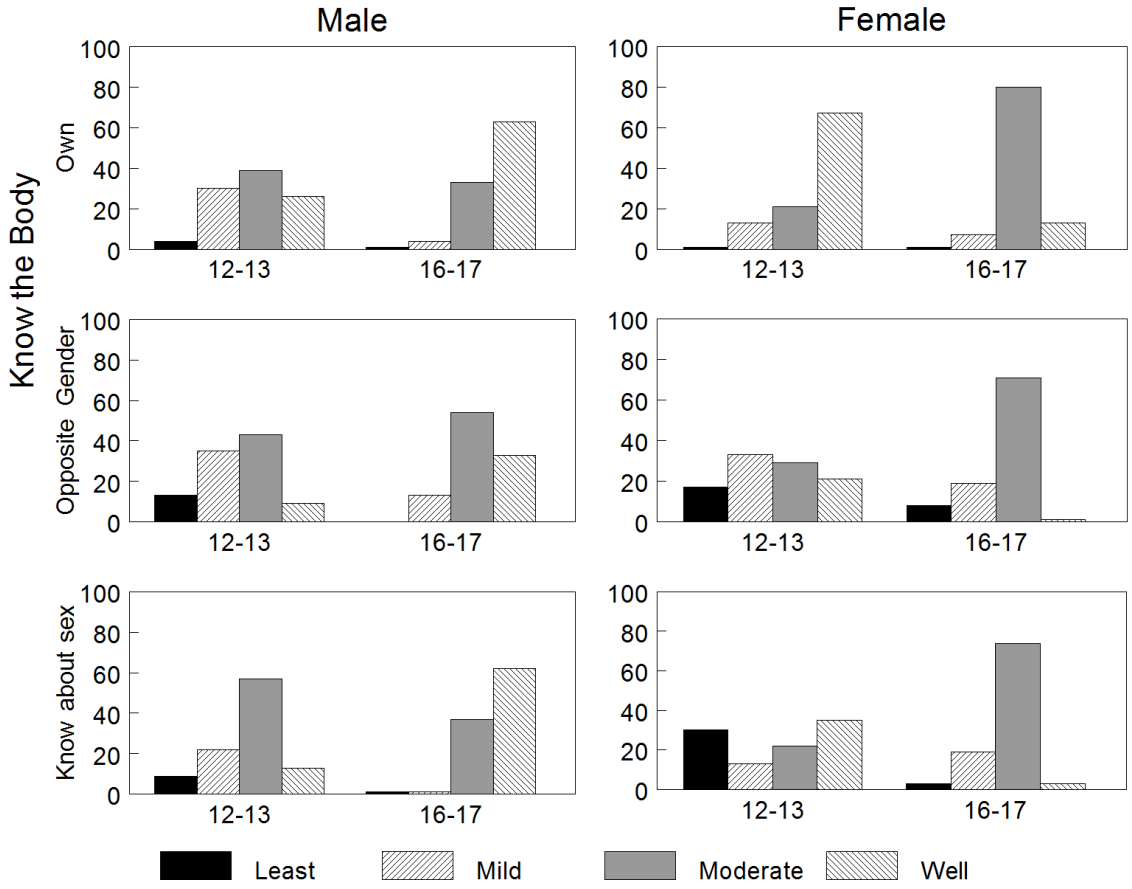


**Figure 6** : Degree of the knowledge about body and sex in Lebanese school students.

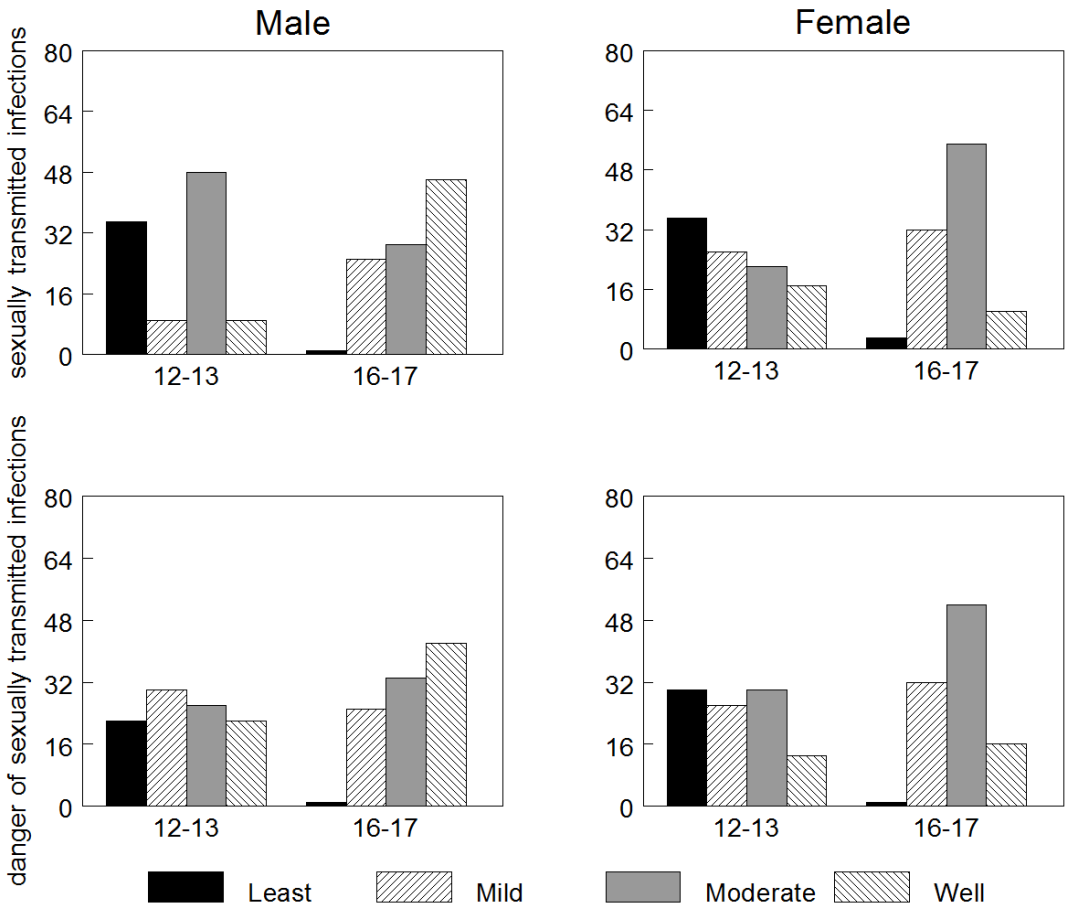


**Figure 7**: degree of knowledge about STDs in Lebanese school students.

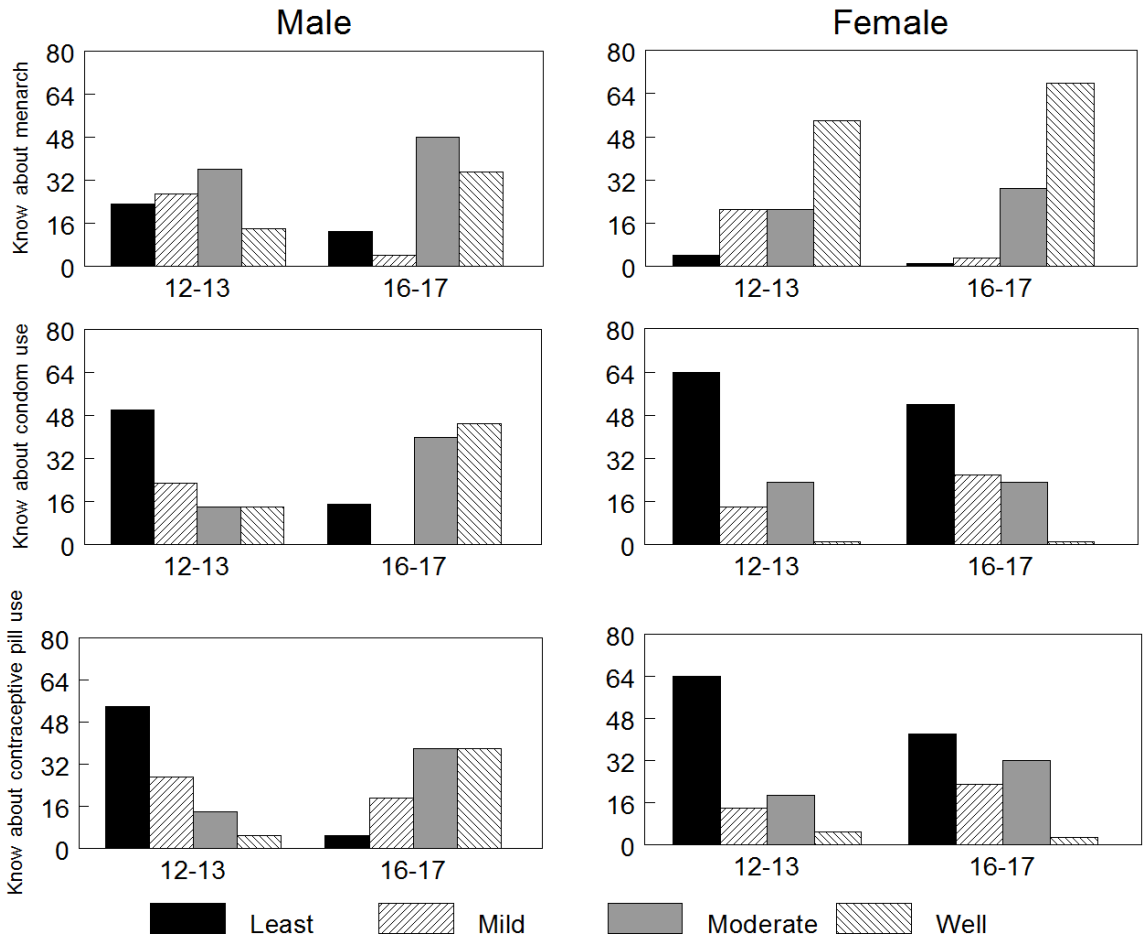


**Figure 8:** degree of knowledge about protection ways in Lebanese school students.

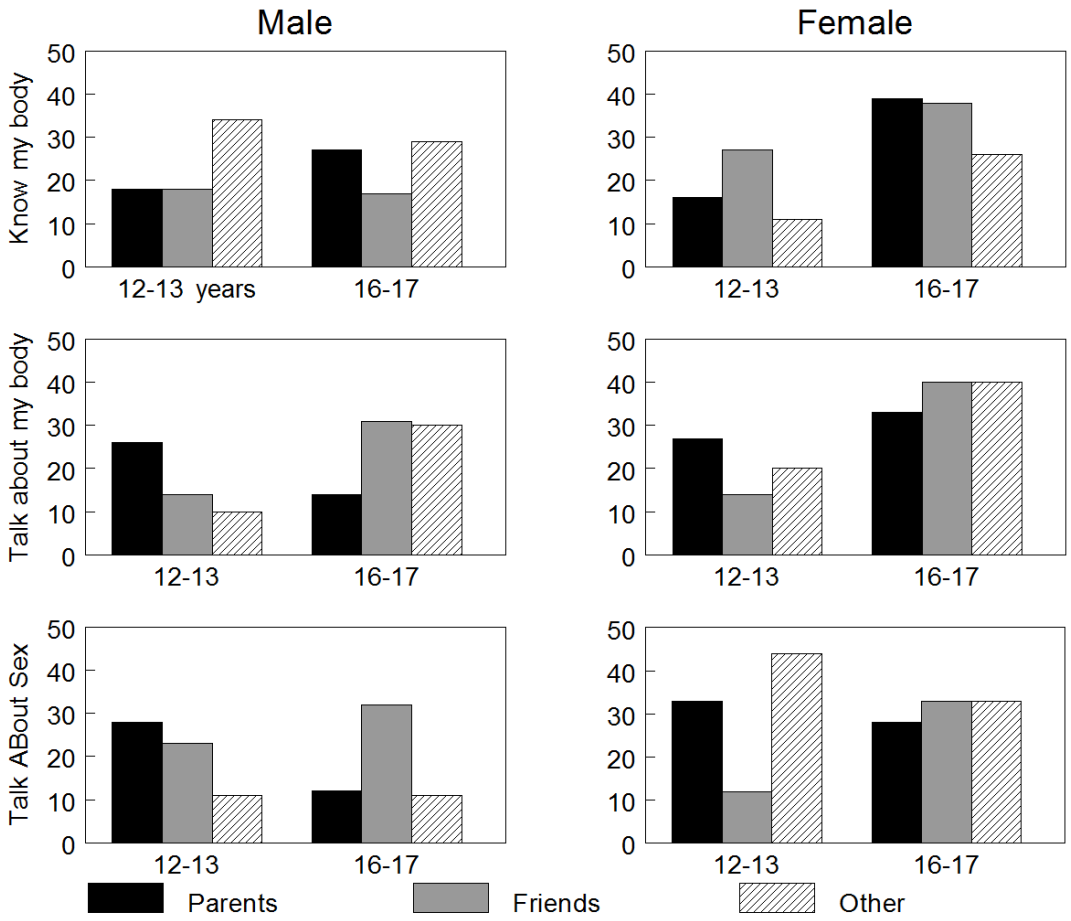


**Figure 9**: The source of student's knowledge about body and sex.

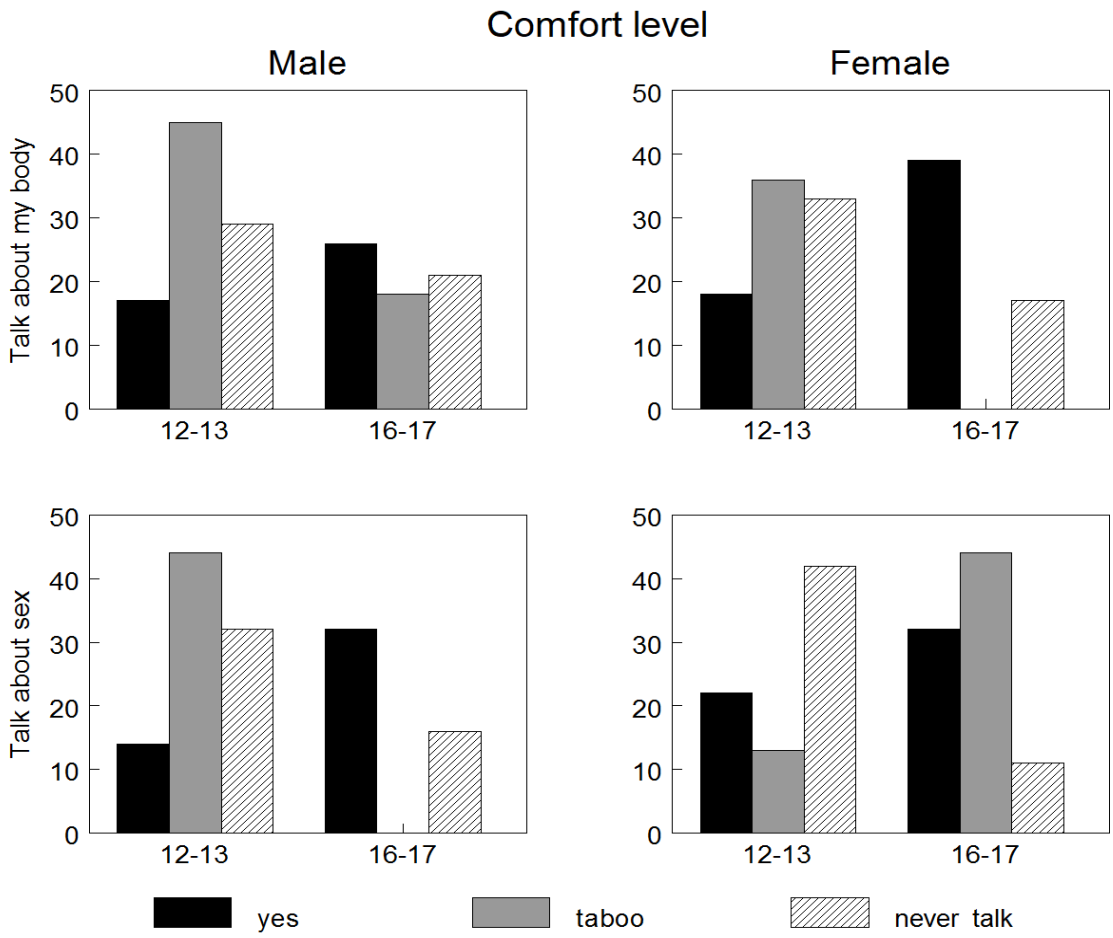


**Figure 10**: The comfort level of the students talking about body and sex.

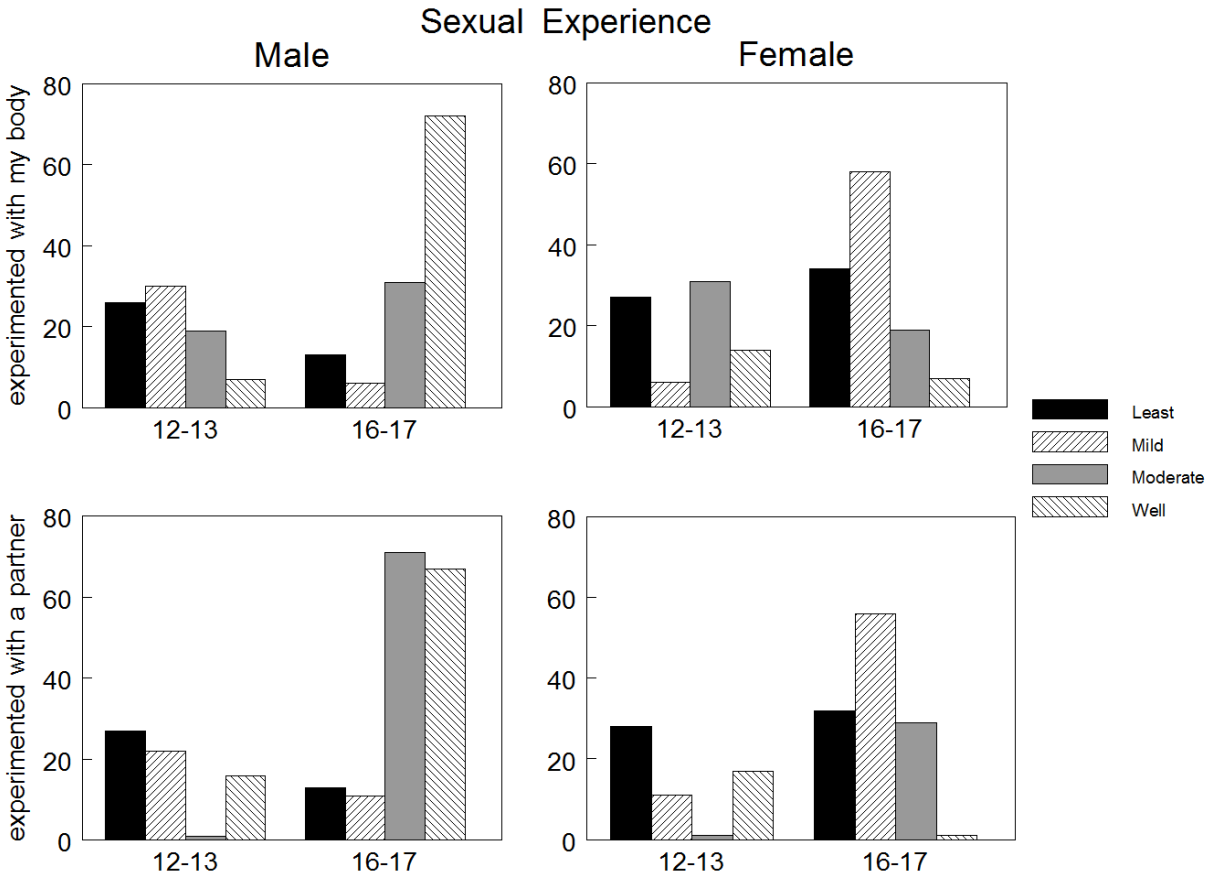


**Figure 11**: Degree of sexual activity in Lebanese school students.