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Research Article

Relation between Epstein-Barr virus (EBV) with some biochemical variables in high-risk aborted women in Mosul city, Iraq

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Abstract
Epstein-Barr Virus (EBV), or Kissing Virus, is a member of the Herpes virus that can be a contributory factor for compromised pregnant, high-risk aborted women worldwide. The present study aimed to detect high-risk EBV by the Monospot test for pregnant, high-risk aborted women, to detect immunoglobulin IgM and IgG for EBV using Enzyme-linked immunosorbent assay (ELISA) technique, to distinguish the infections as acute, chronic, or reactivated, and to determination of Enzymes as Aspartate Transaminase (AST), Alkaline phosphatase (ALP), and Alanine Transaminase (ALT).A cohort of 91 serum samples were collected from high-risk aborted women (ages 15-45 years) who attended Al-Medina Private Laboratory from February to December 2022.Sera were tested for heterophile antibodies(HA) associated with Infectious Mononucleosis (IM) caused by EBV by Latex Agglutination slide test (IM Quick test) and were tested for IgM and IgG antibodies against EBV-CA in serum using the ELISA kit.Sera from the patients and healthy controls were analyzed for Glutamate-Pyruvate Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT), Lactate Dehydrogenase (LDH), and Alkaline phosphatase. Compared to healthy controls, the data showed that the late phase with loss and reactivated infection was responsible for 25% of cases and that the acute and late infection cases had a high of 64%. There were significant differences in the level of these hormones;aborted women showed increased levels of serum ALP (70.83) while having a reverse effect with serum ALT (11.7) and AST (25.43). EBV activation was higher in the aborted women. The study would help to determine the role of EBV in the pathogenesis of abortion.

Keywords: Epstein-Barr virus (EBV), Enzyme-Linked Immunosorbent Assay (ELISA), Alkaline phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST)

INTRODUCTION

Epstein-Barr Virus (EBV), or Kissing Virus, is a member of the Herpes virus that infect humans and animal and can be considered a contributory factor for compromised pregnant, high-risk aborted women worldwide.

Herpesviridae is a family of DNA viruses that infect humans and other animals. Its name is derived from the Greek herpes “to creep,” referring to the latent, recurring infections typical of this group of viruses (Weidner-Glunde *et al*., 2020).EBV is one of Herpes viruses that belongs to the family Herpesviridae. It is classified as a species of Lymphocryptovirus genera in the subfamily Gammaherpesvirinae, also known as Human Gamma Herpesvirus 4 (Frappier *et al*., 2021 and Hosomi *et al*., 2021). This family is divided into three subfamilies: α - Herpesviruses: Herpes simplex virus types 1 and 2, and Varicella-Zoster virus have a broad host range; β - Herpesviruses: Cytomegalovirus, and Human Herpesviruses 6 and 7, with restricted host range; γ- Herpesviruses: Epstein-Barr virus and human Herpesvirus 8, with a very restricted host range.

Epstein-Barr virus (EBV), or Human Herpesvirus 4 (HHV4), possesses a linear double-stranded DNA molecule of 172 kb flanked by Terminal Repeats (TR), packaged into an icosahedral capsid, which is surrounded by a proteinaceous structure called the tegument, and an envelope composed of Several viral glycoproteins embedded in a lipid bilayer (Zanella *et al*.,2021;Smatti *et al*.,2018). There are two strains of

EBV, with Type 1 being the more frequent of the two outside of equatorial Africa and New Guinea, where Type 2 is at least as common as Type 1. Slight differences in the genes encoding EBV nuclear proteins account for the distinction between the two kinds(Chua *et al*., 2022).

EBV has two major target tissues *in vivo*, B lymphocytes and squamous pharyngeal epithelium (Smatti *et al*.,2018).EBV-infected autoreactive B-cells likely seed the thyroid gland, generate autoantibodies and communicate with autoreactive T-cells in genetically vulnerable patients (Slough and Randolph, 2021). More than 90% of persons in the United States will have been exposed to EBV by age 20, with at least 25% of those people getting mononucleosis. Multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, celiac disease, Type 1 diabetes, juvenile idiopathic arthritis, and lupus are only some autoimmune illnesses linked to it in recent studies (Naughton *et al*., 2021). DNA from the Epstein-Barr virus was isolated in 71.9% of the samples, and it was also discovered that the presence of EBV was linked to high-risk aborted women (Banko *et al*.,2022; Schechter and Lamps 2018;Smatti *et al*.,2018).In the analytical cycle of EBV proliferation, the biosynthetic precursors to the seven constitutional glycoproteins and proteins that make upViral capsid antigens (VCA) are biosynthesized. Recombinant VCAs, including the full-length N-terminus of p23 and the carboxylhemisphere of p18, are used in the serological diagnosis of VCA. In 2000, an endogenous gene fusion linked these proteins in vitro, laying the groundwork for developing novel EBV ELISAs (Fahriye*et al*., 2022; Färber *et al*., 2001). Anti-VCA Abs from both the IgG and IgM families are described. In most cases, the timing of determining humoral reaction to VCA precedes the appearance of clinical symptoms. Student instances of Epstein-Barr virus (EBV) were studied, and VCA-IgM was detected by enzyme immunoassay 7 days before symptoms emerged (Rostgaard*et al*., 2019). VCA-IgM is transiently brought out and used as a late major infection signal. VCA-IgM is no longer revealed following the recovery period and usually does not occur again in life (Hess, 2004). Although VCA-IgM came out soon and aids in diagnosing severe EBV contagion. For instance, children besides adults may have non-definitive VCA-IgM trendy major severe contagion, cross-reactivity of EBV-IgM with other antigen-related infections, particularly CMV (Guerrero-Ramos *et al*., 2014). VCA-IgG has been found in severe, convalescent, or previous infections, as it begins to come into sight at the exact time as VCA-IgM. EBV nuclear Ag (EBNA) consists of six proteins. Pained cells express the EBNA-1 protein, and IgG against this protein is a late indicator of Epstein-Barr virus infection (Rostgaard *et al*.,2019). EBNA_ 1Abs come into sight late, 4 to 5 months following the period of illness, then decrease but persist to exist at a considerable scale for life. Since EBNA-IgG has never evolved in about 6-11% of healthy individuals with EBV, and this proportion is substantially more in under-impaired patients, VCA-IgG detects past infection more precisely than EBNA-IgG. (Rostgaard*et al*., 2019; Hess, 2004). When looked at, the IgM class of EBNA-1 was found to be significantly higher than what is typically detected, it signals the presence of a latent major contagion; however, it may continue for several months following the initial infection, and can be seen again in the steps of reactivation (De Paschale and Clerici, 2012).

Cross-reactivity between EBNA-1 IgM and other viruses including CMV and Parvovirus B19 might lead to false non-definitive results. During the lytic cycle, cells damaged by the Epstein-Barr virus express a complex of non-structural proteins known as EA. An EA consists of a diffuse EA-D and a constrained EA-R (Crowley *et al*., 2012). IgG Abs contra to EA is transiently revealed up to 4 months or longer during mononucleosis. Normally, EA Abs are seen in the severe stage and then decrease to non-observed levels. However, investigations have shown that only 65-90% of patients with severe infection have positive EA outcomes and that 25-35% of strong persons with previous EBV contagion take observable heights of EA Abs. Since of the above reasons, the recognition rate of those Abs remains controversial (Rostgaard*et al*., 2019;Hiss *et al*.,2004). The phrase TORCH composite infection or TORCHs infection indicates the inherited infection of Toxoplasmosis, others (Epstein–Barr viruses EBV, Hepatitis or other), Rubella, Cytomegalovirus (CMV), and Herpes simplex. A collective of parasites and viruses sources them (Jaan and Rajnik, 2022;Al-Taie*et al*., 2019). Intrauterine transference of this infection to the embryo results in diverse signs once the baby is born. Maternal danger determinants comprise periods of immunizations and Sexually Transmitted Diseases(STDs). The scrupulousness of maternal contagion if it is a major epidemiological factor due to fetal harm, generally relies on the gestational age. Excluding the Herpes simplex virus, infection during the first trimester has the worst outcome (Jaan and Rajnik, 2022;Al Taie*et al*., 2019).

The TORCH sera panel is overwhelmingly requested during pregnancy due to the relation of fetal infection with a range of ultrasound anomalies. It stays an ordinary action nowadays, despite the worry about superfluous testing and extra expenses for patients and healthcare systems, as well as the consequences of false positive results (Fitzpatrick *et al*., 2022).

Laboratory liver tests are widely defined as tests useful in evaluating and treating patients with impaired liver function where the liver metabolizes carbohydrates, proteins, and fats. Important biochemical markers for liver dysfunction include bilirubin, alanine aminotransferase (GOT), aspartate aminotransferase (GPT), alka-

line phosphatase, and gamma-glutamyl transferase (Nayagam *et al*. 2022).The severity of hepatic complications in the case of IM varies with age, which is estimated at 10% in young people and 30% in the elderly people. It is known that 90-80% of patients with IM have a moderate increase in aminotransferase levels, usually a less than fivefold increase compared to a normal level (Čalkić*et al*., 2019). There is liver damage, although it is usually mild and resolves independently or can be managed with supportive care alone. In extremely rare situations, patients with strong immunocompetence have reported severe or deadly hepatitis due to EBV infection. Liver failure contributes to greater mortality rates in patients with chronic active Epstein-Barr virus infection, which occurs when an infection persists for more than 6 months (Schechter and Lamps, 2018). Previous studies showed that more than 80% of patients with mononucleosis had abnormal levels of transferase, especially ALT, but jaundice was uncommon (Chen*et al*., 2023).Thepresent study aimed to i) Detect Monospot test for patients, ii) Detect immunoglobulin IgM and IgG for EBV using Enzyme Linked Immuno Assay(ELISA) technique, iii) Distinguish the infections as acute, chronic, or reactivated,iv) Determine Enzymes as Aspartate Transaminase (AST), Alkaline phosphatase (ALP), and Alanine Transaminase (ALT).

MATERIALS AND METHODS

Study area

The serum of one hundred women who had abortions (more than 2-3 abortions) while at high risk and thirty healthy women served as controls,who have attended Al-Medina Private Laboratory and their ages ranging between (15-45) years old and no infection with one of TORCH test except EBV infection during the period from February to December 2022

Methodology

The treating physicians diagnosed the women and confirmed the diagnosis bya specific testintendedthe IM Quick test (Haemagglutination test for detection of HA associated with IM)was completed according to the manufacturer's instruction (Demeditec, Germany). The test was considered negative when no difference in agglutination was observed between the specimen and negative control;positive control and positive sera must show distinct agglutination within 2 minutes. Using quantitative commercial ELISA kits, EBV antibodies were detected in the serum of all investigated patients. The following antibodies against viral capsid antigen (VAC) were evaluated: antibodies against (lgM and IgG,). lgM antibodies are specific to EBV early antigen (EA) and EBNA-1, the virus' nuclear antigen. The analytical sensitivity of the test that was carried out, on average, was 1.3 IU/ml (Feyzioğlu *et al*.,2009). An absence of all antibodies (VAC IgM, VAC IgG, EBNA-1-IgG), and EA IgG indicated that EBV infection was absent. A diagnosis of acute infection might be made if both VAC IgM and VAC IgG were present despite the absence of EBNA-1-IgG. Indicating a previous infection was the presence of VAC IgG and EBNA-1-IgG in the blood without the presence of VAC IgM in the blood. A reactivation was indicated by the presence of VAC IgG, EBNA-1-IgG, and EA IgG simultaneously (Feyzioğlu*et al*.,2009).

Blood was collected aseptically by venipuncture and placed in a sterile SST tube without anticoagulant. It should then be allowed to clot at room temperature. During the Ist reaction step, diluted patient samples (1/5) were incubated for 30 minutes in wells. After that, they were washed three times using a working wash buffer and emptiedthe wells. In the case of positive samples, specific lgM antibodies will bind to antigens P3H3 extracted from EBV of human Burkitt in wells of kit.To detect the bound antibodies, 2nd incubation was carried out after adding a conjugate enzyme (Peroxidase-labeled anti-human lgM) into each well, after washing 100 µl of the substrate was added into microplate wells and incubated in the dark for 15 minutes at room temperature then finally stopped the reaction and read the absorbance at 620nm within 30 minutes by using Micro ElISA (washer and reader) Semi-quantitative evaluation was performed by comparing the extinction value of the control or patient sample to that of the calibrator using the following formula. The results are presented as a numerical value along with interpretation.

A negative expected value indicated that the subject was not exposed to EBV. Neither IgG nor lgM antibodies could be detected. Serum samples were tested for a liver enzyme for alkaline phosphatase (ALP) using ELISA kits (BioMerieux, Lyon, France) according to the guidelines provided by the manufacturer (A450(cases)/ MPCx100).

Geometric analysis

The results were analyzed by Spss version 21 after translating data into codes. A suitable statistical method was used to analyze and assess the data. The mean and standard deviations wereconsidered significant if the $p \leq 0.05$ and highly substantial if the $p \leq 0.001$.

Ethical approval

The study was conducted according to the ethical principles originating in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number 4189 (in 1/2/2022) to get this

approval.

RESULTS AND DISCUSSION

The Heterophiles antibody (HA) test/Monospot Test is one of the standard tests used in the diagnosis of EBV virus, which depends on the detection of HA resulting from the activation of B cells produced by the immune system in response to EBV infection. It depends on the ability of the patient's serum to agglutinate or adhere to red blood cells resulting from an animal source such as a horse or goat (Hess, 2004).The results of the Monospot test showed that 37 out of 100 cases were positive for IgM (9 IgM+ and 22 IgM-) with a sensitivity of 32.14 % and specificity of 65.07%, but 31 out of 100 were positive for IgG (IgG+ 29 and IgG- 2) with a sensitivity of 32.18% and specificity of 50%.

This result of the ELISA test gave (28/91) positive cases for IgM with a percentage of 30.7%, while it gave (87/91) positive cases for IgG at the rate of 95.6%.The statistical analysis revealed no significant differences(P value 0.59) between the two tests when utilized in the diagnosis. The difference between the results of the Monospot test and ELISA test is that the first test detects heterotrophs, which peak in the first weeks of the initial infection, before the rise of IgG, and IgM antibodies.The similarity between the ELISA test for the detection of IgG and IgM immunoglobulins and the HA test is the fact that, in some cases, the HA persistence was observed for a few months after the initial infection.Using an HA test during the first weeks of infection was recommended to detect EBV infection. It is not preferable to use it in the general diagnosis of infection. Therefore, it was replaced by the ELISA test for the detection of immune antibodies, and the rise in the antibodies recorded in the ELISA test for IgG antibodies can be caused by a previous infection, which the HA test cannot diagnose. Regardless of this test's false negative results, it has other equals, including it is a nonspecific test and can give false positives for infections other than EBV, including viral hepatitis, German measles, and autoimmune diseases. The statistical analysis showed no significant difference when comparing the heterologous antibody test with the ELISA assay for detecting IgG and IgM immunoglobulins.As a ratio of sensitivity and specificity of the above.The appearance of positive cases for both tests (heteroantibodies and IgM) indicates an acute primary infection. Still, in the case of the presence of seropositivity for anti-IgG only and seronegative for anti-IgM and heterogeneous antibodies, this indicates the previous infection. The recovery period from infection or the period of reactivation of the virus from the latent stage to the lytic stage was in people who had previous infections. The percentage of previous infections was 64 (6%) cases and among all cases, 9.8 % involved a reactivation of the virus, and 30.7% were late primary infections. Results from the EBV VCA-IgG, VCA-IgM, and EBNA-IgG antibody tests used to paint a complete picture of EBV infection are given in Table1.

Table 1. A comprehensive picture of EBV infection According to Type of anti-EBV antibodies using ELISA

Condition	VCA IgG	VCA IgM	EBNA-1 IgG
EBV seronegative	-	-	-
Acute infection	+	+	-
Past infection	+	-	+

+=Presence of antibody;- = absence of antibody

Table 2. Showing numbers and percentages of the Pregnancy group based on their Anti-EBV serological Profile

Anti-EBV Serological Profile	Pregnancy Group		P
EBV VacA IgM (IU/ml)	**No.**	**%**	
Negative (< 0.8-0)	0	0.0	
Equivocal (0.8-1.2)	0	0.0	0.490
Positive (>1.2)	28/91	30.7	
EBV VacA IgG (IU/ml)	No.	%	P
Negative (< 0.8-0)	0	0.0	
Equivocal (0.8-1.2)	0	0.0	0.003
Positive (>1.2)	87/91	95.6	
EBNA-1 IgG (IU/ml)	No.	%	P
Negative (< 0.8-0)	0	0.0	
Equivocal (0.8-1.2)	0	0.0	0.323
Positive (>1.2)	80/91	72.8	
EBV EA IgG (IU/ml)	No.	%	P
Negative (< 0.8-0)	60/91	66.7	
Equivocal (0.8-1.2)	0	0.0	0.001
Positive (>1.2)	31/91	34.3	

Table 2 shows that among the pregnant cases(80) and all control groups, all tested antibodies were positive except for EA IgG, which was positive among the pregnant cases (20) compared to only the four cases (P=0.004) in the control group. In addition, the pregnant women group had significantly higher mean serum levels of EBV VAC IgG and EBV EA IgG than the control group (P= 0.323 and P= 0.001, respectively). While the presence of most tested antibodies in both cases and controls is not surprising given the endemic nature of EBV in Iraq, the presence of EA IgG antibodies in cases but not controls should raise awareness of the potential role of virus reaction in disease pathogenesis

Table 3. Number of infected cases according to type of immunoglobulins in EBV infection under study

Number of Infected Cases	Total Number of Cases(100)
Previous Infection	60 have IgM 30 have IgM and HA for both
Early Primarily Infection	31 have IgG and HA for both
Reactivation of Virus	12 have IgM, IgG, and HA

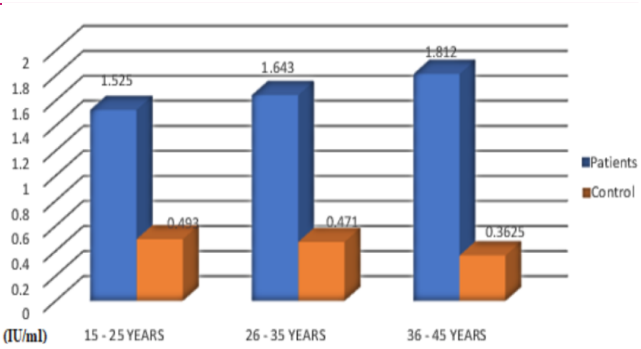

Fig.1.*Percentage of Seropositive Epstein Barr Virus (EBV)*

and/or progression. However, the present study found no significant difference in EBV serological profile between high-risk aborted women groups, suggesting that virus reactivation may play a role in disease pathogenesis rather than progression (Rutkowska and Pokorska,2023). A viruscan be considered an etiological factor inthese conditions (Taxirovich.,2023). Since EBV persists in the body throughout life, it plays a role in the chronic course of autoimmune diseases (Houen andTrier ., 2021), frequently accompanied by symptom exacerbations (Kronzer*et al*., 2021; Pyzik*et al*., 2019). Three female cases of infectious mononucleosis caused by a newly diagnosed and acute primary EBV infection were studied by Akahori *et al*. (2010). As shown by Jane Ova*et al*. (2015), elevated titers of antibodies against various EBV antigens are present in various autoimmune disorders. Titers of VCA IgG were more often found in patients than in the control group. The positivity of antibodies against early antigen (IgG–EA) was also significantly more often found in patients than in the control group. The appearance of positive cases for both tests (hetero-antibodies and IgG) in 30 indicates previous infection. The period of recovery from infection or the period of virus reactivation from the latent stage to the lytic stage in people who had previous infections was only in 12 cases, as shown in Table 3and Fig.1.

The results of the liver function test showed that30 personsout of a total of 100 showed a significant increase in AST enzyme according to the results of the statistical analysis when comparing the level of AST enzyme with the results of the ELISA test. It was noted that all positive cases of ELISA assay for IgM^+ antibody had an increase in the level of AST enzyme (p = 0.051) as in Table4 and this explains that EBV virus affected the liver only in the initial acute infection and that the previous infections did not show a significant increase in liver enzymes.Losavio and Te (2007) recorded an increase in the level of AST enzyme in their study groups, and the peak of the increase in the level of the enzyme lay in the first and second week of infection, and it returned to its normal position after 90 days. Patients infected with the EBV virus had an increase in the enzyme AST (85%) of EBV patients suffering from liver dysfunction to varying degrees, and 6% of all liver infections are caused by the EBV virus, which raises the level of transaminase in the body, which in turn leads to impaired liver function (Ali*et al*., 2023). A patient infected with EBV viruscontains a large number of lymphocytes, and the enzymes enter the plasma through the liver membrane when its tissues are injured and therefore, the level of liver function enzymes rises in the affected people compared to the uninfected people and this matched the results of the present study with the difference in terms of moral differences, as indicated by the researcher) Chen *et al*., 2023). Although ALP and ALT enzyme rates were much higher in the positive instances of the IgM antibody ELISA test, no significant difference was found between the positive and negative cases. The appearance of significant differences between the infected and healthy patients on the level of the AST enzyme and the absence of significant differences in the enzyme levels ALP,ALT (P>0.05)) agrees with what was mentioned by Chen*et al*. (2023). More than 80% of people infected with the virus over the age of 16 years showed abnormal results for aminotransferase in different age groups (Linthorst *et al*., 2023; Kronzer *et al*., 2021;Griffin*et al*., 2013). The results of the present study converged with those (Chen*et al*., 2023;Theodory*et al*., 2023; Čanović*et al*., 2015), who indicated that the AST enzyme was elevated in all groups ofstudy that EBV infection does not

Table 4. Liver function enzymes and IgM ELISA EBV

Groups of Study	ALT (U/L)		ALP (U/L)		AST(U/L)	
100	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
IgM+(30)	11.7	6.7	70.83	18.43	25.43	19.83
P – value	0.519		0.191		0.0018	
IgM-(70)	9.93	5.57	68.26	17.07	18.03	5.45

have a direct effect on hepatocytes, and that this defect in liver function during infection with EBV is caused by type II hypersensitivity reactions also (Ali *et al*., 2023). The effect of EBV on liver function varies according to the severity, frequency of the disease, and if they are in constant exposure to the virus due to the nature of their work (Yamada *et al*., 2023). Rather, the effect is the immune response to viral antigens, which in turn pressures the hepatocytes, causing their damage and the release of enzymes into the blood serum, as EBV infects and activates CD8, T-cell, and thus leads to their accumulation in the liver, causing enlargement of the liver, spleen and lymph nodes (Pebdeni *et al*.,2023; Banko *et al*.,2022).

Conclusion

The EBV affects pregnant women and high-risk abortion patients and relates to some of their biochemical variables. Twenty-five per cent of patients were attributable to the late phase with loss and reactivated infectionwith EBV virus, and a large percentage of 64 patients were attributable to the acute and late infection phases. The levels of these hormones varied significantly; aborted women had elevated serum ALP levels on average of 70.83, but ALT and AST levels were in the opposite direction of 11.7% and 25.43, respectively. Further, it is suggested to compare the serological parameters with the molecular study for EBV.

Conflict of Interest

The authors declare that they have no conflict of interest.

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