IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

# Prevalence of Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) Subclinical Infection in Patients with Acute Immune Thrombocytopenic Purpura (ITP)

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Received: 24, Dec, 2019
Accepted: 28, Jun, 2020

**ABSTRACT**

**Background**: Immune thrombocytopenic purpura (ITP) is defined as a bleeding disorder in which the number and production of platelets are reduced by the immune system; however, the destruction of peripheral blood platelets also occurs. Although its exact etiology and pathogenesis have not already known, several studies have shown that Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are known as possible causative agents of ITP. This investigation aims to evaluate the presence of CMV and EBV in two groups of case and control by polymerase chain reaction (PCR).

**Materials and Methods**: we considered the presence of CMV and EBV in 48 acute ITP patients and 48 healthy people. Study participants were recruited from Ahvaz Shafa Hospital between 2017 and 2018 and the presence of two viruses was investigated by (PCR).

**Results:** Out of 48 acute ITP patients, the CMV DNA was detected from the blood of 12 (25%) patients and the EBV DNA from the blood of 2 (4.2%) other patients. Also, only one patient was (2.1%) co-infected with CMV and EBV. In contrast, in 48 healthy subjects, 3 (6.6%) had CMV and none of the control group was infected with EBV.

**Conclusion:** Due to the presence of both EBV and CMV in the acute ITP patients in Ahvaz, they can be considered as factors in the progression of this disease. Therefore, consideration of the methods of elimination and treatment of these two viruses in these patients may be used as a treatment strategy in ITP patients in the future.

**Keywords:** Immune thrombocytopenic purpura (ITP); Cytomegalovirus (CMV); Epstein-barr virus (EBV)

## INTRODUCTION

Immune Thrombocytopenic purpura (ITP) is a type of autoimmune disorder which is characterized by the destruction of peripheral blood platelets or inhibition of megakaryopoiesis in bone marrow by the produced autoantibodies against the surface antigens of platelets and megakaryocytes [1]. The most important antigen on platelets and megakaryocytes is glycoprotein IIb-IIIa which autoantibodies are produced against it[2]. Consequently, the number of platelet has dropped to below $100 \times 10^9$/L and is accompanied by complications such as GI bleeding, petechiae, and purpura[3]. Also, the results of previous studies have shown that the expression of GPIIb / IIIa on the surface of megakaryocytes and the production of autoantibodies against them can stimulate cytotoxic T cells and lead to the destruction and suppression of maturation of Megakaryocytes[4]. ITP can be divided into three categories: acute, subchronic, and persistent[5]. Acute thrombocytopenic purpura is more commonly reported in children following viral infections[6]. This type of disease eventually responds to treatment, but the subchronic and persistent forms that occur in adults do not respond well to treatment and last more than 12 months[7-9]. Several studies have mentioned various causes of the disease that bacteria and viruses were formed about 60 to 80 percent of these factors [10, 11]. Various viruses including HIV, CMV, EBV, varicella, herpes simplex, rubella, measles, parvovirus, influenza A and hepatitis C in association with this disease have been reported[12]. Viral infections play a significant role in causing the destruction and reduction through the reticuloendothelial system because of constant stimulus of the production of autoantibodies against platelet-derived surface antigens in infected patients[13]. On the other hand, megakaryocytes as progenitor cells of platelets which are originated from the bone marrow can be targeted for viral infections and produced defective platelets that have a role in stimulating the production of autoantibody in case of removing and facilitating them more quickly[13]. EBV and CMV viruses that belong to the herpesviridae family, due to their presence in the blood and targeting blood cells such as B cell, T cell, and platelets play a major role in blood diseases such as mononucleosis, lymphoproliferative and ITP[13,14]. Due to the presence of these two viruses in ITP patients and their effect on megakaryocytes and platelets, this study aimed to investigate their prevalence in ITP patients in Ahvaz. If there is a significant relationship between these two viruses and ITP, future studies must apply antiviral therapies as a treatment strategy for these patients.

## Material and methods

### Samples collection

This case-control study was performed on 96 samples: 48 patients with acute ITP vs 48 healthy individuals. Both groups were recruited from Ahvaz Shafa Hospital between 2017 and 2018. The diagnosis of ITP in patients was made according to the American Society of Hematology (ASH) criteria. On the other hand, patients with the history of thrombocytopenia with malignant disease or other viral agents such as HIV and HCV were excluded from this study. Also, 48 healthy individuals selected randomly did not have any family history of malignant diseases or other conditions leading to thrombocytopenia. According to the American Hematology Association, the cytomegalovirus test is not necessary to rule out secondary causes of the ITP; however, in some references, such as Williams' hematology it is necessary to perform these tests. In this study, blood samples (3cc) from each individual were taken and transferred to tubes containing EDTA and then stored at -20 ° C after obtaining personal consent.

### DNA extraction

The CMV and EBV genome were extracted by the High Pure Viral Nucleic Acid (Roche-GmbH-Germany) kit according to the *manufacturer's instructions*. Then, the concentration of DNAs was measured using a NanoDrop.

### The CMV and EBV DNA detection by PCR

All samples were subjected to PCR for detection of CMV and EBV DNA using the IGF (IGF-Denmark) kit according to the manufacturer's instruction. There is a master mix inside the kit, including dNTPs, MgCL2, reaction buffers and primers from the manufacturer. The Taq DNA Polymerase enzyme is also separately

available in the kit. PCR amplification was conducted using 25µl reaction volumes containing 5 µl of DNA template, 0.3 µl of a taq and 20 µl of the PCR master mix shown in Table 1. Then, PC products were detected on 1.5% agarose gel electrophoresis followed by staining with safe stain.

**Table 1.** Three step PCR cycles' temperature and time

| | Step | Temp | Time | Number of cycles |
|---|---|---|---|---|
| Step 1 | Initial denaturation | 94 ℃ | 3 min | 1 |
| Step 2 | Denaturation | 94 ℃ | 30 sec | 40 |
| | Annealing | 66 ℃ | 30 sec | |
| | Extension | 72 ℃ | 30 sec | |
| Step 3 | Final extension | 72 ℃ | 7 min | 1 |

**Statistical analysis**

The collected data were analyzed using T-test, Chi-squared test and SPSS version 22. P <0.05 was considered statistically significant.

**RESULTS**

Out of 96 subjects who were examined in this study, 30 were male, and 66 were female. Analysis of data showed that the rate of infections with CMV and EBV was not associated with gender. (P: 0.35). Participants' age ranged between 2 months and 28 years, and the mean age in the group of CMV (+) was 15.35 ± 13.35, EBV (+) 4.24 ± 4.00, and non-infected group was 15.47 ± 12.12 SD years, respectively. However, it was determined that there was no significant relationship between the mean age of participants with CMV and EBV and People who were not infected with these viruses (P: 0.76) (Table2). The prevalence of EBV and CMV in the case and control groups was as follows: 12 (25%) out of 48 patients were positive for CMV genome, and 2 (2.4%) patients had EBV (Figures 1, 2). Moreover, one patient (2.1%) was co-infected with CMV and EBV. Three (6.3%) out of 48 subjects in the control group had CMV, whereas no subject from the control group showed signs of an EBV infection. After statistical analysis, it was found that the difference between the prevalence of CMV and EBV infections in ITP patients and control was significant (P-Value: 0.002) (Table2). Additionally, the mean platelet count (MPC) before and after treatment (one month later) was reported 29.5 ± 19.2 and 181.91±113.22, respectively. In the patients with CMV, EBV, and non-infected patients the MPC was 204.00 ± 127.27, 185.27±99.19, respectively (Table2). However, these results showed no significant correlation between mean platelet count after treatment in patients with CMV, EBV and non-infected subjects. (P: 0.84).

Table 2: Correlation of viral infections with clinical parameters in patients

| Parameter | CMV (+) | EBV (+) | Non-infected | P |
|---|---|---|---|---|
| Gender Male/female | 5/7 | 0/2 | 9/24 | 0.35* |
| Age SD± | 15.35±13.25 | 4.24±4.00 | 15.47±12.12 | 0.76** |
| Mean platelet count SD± | 181.91±113.22 | 204.00±127.27 | 185.27±99.19 | 0.84*** |

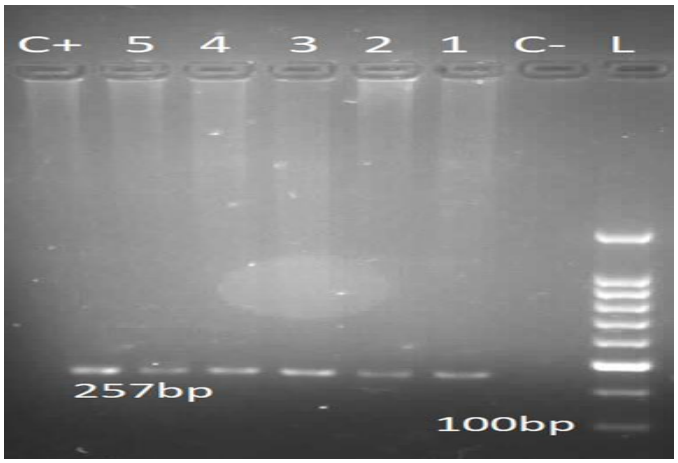

Figure 1: PCR analysis of CMV DNA samples extracted from ITP patient

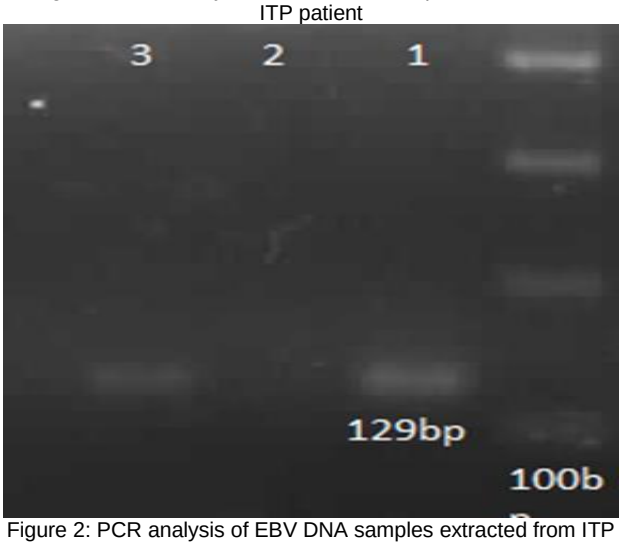

Figure 2: PCR analysis of EBV DNA samples extracted from ITP patient

**DISCUSSION**

In general, ITP is one of the hematological disorder in which platelets and megakaryocytes are targeted and destroyed by autoantibodies[12]. Although various studies have mentioned several factors to stimulate autoantibody production and platelet destruction in this disease, the exact pathogenesis of this disease has not been elucidated[15]. However, in various studies, viruses such as HIV, CMV, EBV, varicella, herpes simplex, rubella, measles, parvovirus, influenza A and hepatitis C associated with this disease and its progressive factors have been reported[12]. CMV and EBV viruses are viral agents that their roles have been considered in the pathogenesis of ITP disease in various studies, as in the study of Wu et al, the role of 21.4% cmv and 19% EBV virus has been reported[13]. In this study, among 48 patients with ITP, 12 (25%) were infected with CMV compared to the control group in which 3 (6.3%) individuals were infected with this virus; a significant correlation was observed (P: 0.002) .Various studies have mentioned several factors such as an endemic area for CMV, the association of the virus with organ transplantation, involvement in neonatal infections, and immunosuppressive patients as a reason for the relatively high prevalence of the virus in ITP [12, 16-18]. EBV was another virus with a 4.2% role in 48 ITP patients in this study and since no subject of the control group was positive for EBV, we can conclude that there is a relationship between the virus and the disease (P: 0.002). In the studies conducted by Maysaa, Tsai and Hsiao various factors such as the virus being endemic in Taiwan, chewing food as one of the intimate relationships in Chinese family culture, and overcrowding and intimate contacts between family members in Egypt were reported as the causes of high prevalence of EBV virus [19,20]. In the case of co-infection of these two viruses, it has been found that due to their synergistic influence on allergen-specific B-cell responses and NK-cell cytokine production they can lead to a more pathological consequence, so co-infection of two viruses is more common in children and immunosuppression patients[21]. In addition, in diseases such as mononucleosis and ITP in which the immune system is impaired the co-infection of the two viruses have also been reported[10, 22]. In the current study, only one co-infected patient was found. In this study, the relationship between two viruses and other clinical parameters such as platelet count after treatment, gender and age were also investigated. After data analysis, mean post-treatment platelet count was as follows: (one month later) in patients with CMV (181.91 ±113.22), EBV (204.00 ±127.27) and in patients who did not have viral infections (185.27 ±99.19). These results showed that although the mean in the CMV group was slightly lower, but in the EBV group was higher, the difference between the mean platelet count after treatment in all three groups was not statistically significant. But, previous studies have shown that platelet count and treatment can be aided by antiviral therapy in these patients. According to studies by Bessy and Dina, prescription

of Cyclovir or Val Gun Cyclovir antiviral therapy along with other treatments such as injectable immunoglobulins and steroids and also reduction of CMV load can increase platelet count in these patients[18,23]. Another study reported that if IVIG is selected as a treatment in EBV-related ITP patients, it can lead to a reduction in EBV DNA copy number, an increase in platelet count and a decrease in bleeding[24]. Age and gender were other parameters that were evaluated in various studies for their association with these viruses and no significant relationship was found. This study, like previous ones, showed no significant relationship between viruses and age (P: 0.76) as well as viruses and gender (P) [10,13]. Of course, other factors such as the ability of the CMV virus to infect megakaryocytes and prevent their proliferation, the ability of the Gb protein to encourage the production of autoantibodies, and the continued viral DNA replication have made ITP disease significant [16, 17]. In the case of EBV, important factors such as disruption of the differentiation and proliferation of megakaryocytes and mononucleosis infection can make the conditions for ITP more prepared [24].

## CONCLUSION

CMV and EBV viruses can be considered as risk factors in patients with ITP. Diagnosis of these viral infectious agents and consideration of eliminating or reducing methods can be good strategies to improve and prevent ITP and increase platelet count in ITP patients. Hence, the use of antiviral drugs, evaluating viral antibodies before and after transplantation, preventing the transmission of viruses may be adjunctive therapies that may be used in the future therapeutic strategies.

## CONFLICTS OF INTEREST

The authors declared that they had no conflict of interest in this manuscript.

## ACKNOWLEDGEMENTS

This work was financially supported by grant OG-6124 from vice chancellor for research affairs of Ahvaz Jundishapur University of Medical Sciences. This paper is taken from the thesis authored by Farshad Abbasi.

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