Pak. j. life soc. Sci. (2024), 22(1): 5465-5473 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.00403

RESEARCH ARTICLE

Detection the Level of CD_{19}, COX_2, INF gamma, and Prostaglandin (E_2) in Chronic Active Epstein Barr Virus at Acute Inflammation Patients

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ARTICLE INFO

Received: May 22, 2024

Accepted: Jul 9, 2024

Keywords

Epstein-Barr virus (EBV)

CD_{19}

COX_2

Prostaglandin (E2)

Epstein Barr Virus

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ABSTRACT

Epstein-Barr virus (EBV) always expresses latent proteins and drives B cell proliferation so that persistence lead to different acute inflammation in the patient. The aim of this study was to determent the relation of acute inflammation in the patients with EBV infection and detection the Level of CD19, COX2, INF gamma, and Prostaglandin (E2) in chronic active EBV. One hundred patients male and female Patients with acute inflammation or cancer symptoms were used in this case study. One hundred patients male and female with no inflammation or cancer symptoms were used as control. The blood sample collected from all participants to diagnosis EBV and the biological markers. The median serum level in relation EBV infection IgG with other disease and EBV patient showed high significant Statistically compare with control group, The median serum level in CD19 protocol , INFγ cytokine and Prostaglandin E2 related with infected EBV IgG compare control group data was presented high significant, While the median serum level between COX2 enzyme and EBV IgG infection Not significant , The study showed there are high significant between the patient group with different inflammation disease in addition infected with virus compare with have not disease in CD19 protocol , INFγ cytokine, COX2 and Prostaglandin E2. The conclusion: This study concluded the seriousness of chronic active Epstein-Barr virus infection to persist different types of inflammation and cause a threat to patient life.

INTRODUCTION

Epstein-Barr virus (EBV) is a common virus infected by about 90% of the population (1-7), with a 12.69% infection rate among reactive arthritis patients in Iraq (8). It belongs to the Herpesviridae family and is primarily transmitted through contact with oropharyngeal secretions. Sequencing-based research has identified two types of EBV (9,10). EBV can be transmitted through blood and blood derivative transfusions, organ and tissue transplantation, breast milk, and vaginal tract secretions, although it is a rare vertical transmission and EBV is found in vaginal tract secretions (11-14). Epstein-Barr virus (EBV) produces latent proteins that drive B-cell growth in vitro and can remain dormant in memory B cells in vivo. Studies on the expression of latent viral proteins in vivo suggest that growth-promoting latent EBV proteins are produced primarily in infected naïve B in the tonsils of healthy donors. In germinal center B cells, EBV exclusively produces latent membrane proteins 1 and 2a (LMP1 and LMP2a) and the viral epitope-associated protein, EBV nuclear Ag 1. LMP1 and LMP2 can provide necessary signals for a latently infected B cell to enter the follicle (17).

EBV infects and activates naïve B cells in the tonsil (18,19), allowing them to develop into resting memory B cells via GC using LMP1 and LMP2 signals(20-22), resulting in somatic hypermutation, class switching, survival/rescue signals, and memory cell formation(15). COX2 and PGE2 are crucial inflammatory factors in cancer, contributing to cell survival, invasion, proliferation, and immune escape, often promoting carcinogenesis and progression (23). Chronic inflammation is a common cause of cancer, as evidenced by the higher likelihood of colitis-related colon cancer in IBD patients compared to the general population (24). Tumor-associated inflammation involves intricate interactions between epithelial and mesenchymal cells, potentially leading to genetic alterations. Persistent inflammation can trigger growth factors, promoting tumor formation and causing tumours to behave like "non-healing wounds." (25).

Chronic inflammation can accelerate tumor growth by allowing tumor immune escape and creating an immunosuppressive microenvironment, both of which are cancer-related. Tumor immune escape occurs through various mechanisms, including dysfunctional antigen-presenting cells, tumor cell resistance, decreased cytotoxicity of CD8+ T cells and NK cells, and induction of immunosuppressive cells like myeloid cells, T helper cells, and macrophages (26).

COX enzymes convert arachidonic acid to PGH2, which is transformed by prostaglandin synthase to produce five prostaglandins: D2, E2, I2, F2α, and thromboxane A. COX-2 and prostaglandin E2 synthase catalyze the process. PGE2 is converted to its inactive form, 15-keto-PGE2, which is metabolized by 15-hydroxyprostaglandin dehydrogenase. High levels of PGEM increase the risk of colorectal and stomach cancers (27,28).

PGEM may be a biomolecular marker for cancer risk prediction. Prostaglandins regulate cellular functions by binding to G protein-coupled receptors on cell surfaces. PGE2 sends signals to four receptors, and the COX-2-PGE2 pathway promotes tumor immune evasion by controlling myeloid-derived suppressor cells, lymphocytes, and antigen-presenting cells. Understanding the COX-2-PGE2 pathway could provide a basis for developing innovative approaches to fight tumor immune escape (29).

MATERIALS AND METHODS

Patients and control group

One hundred patients male and female Patients with acute inflammation or cancer symptoms were used in this case study. One hundred patients male and female with no inflammation or cancer symptoms were used as control. The blood sample 5-10 mL were collected from all participants, blood serum was collected and preserved in -20o C till be used in ELISA technique to diagnosis Epstein Barr Virus and the biological markers.

The evaluation of immunological marker

Human Epstein Barr Virus early antigen antibody(IgG ELISA Kit) (Cat.No:ED0291Hu), Human Cluster of differentiation 19 ELISA(Cat.No: E3269Hu), Human Prostaglandin E2 ELISA Kit (Cat.No: E1009Hu),Human Cyclooxygenase2 (COX2) ELISA Kit (Cat.No:E0780Hu), Human Interferon Gamma (IFN-G ELISA Kit) (Cat.No: E0105Hu).The methods were performed as manufactured instruction.

Statistical analysis

Means and standard division were calculated for group. All data obtained were analyzed using student T test and probability value of $p<0.05$ were considered as significant difference.

RESULT

Relation EBV infection IgG with disease: The study showed high significant differences were found in the level P value < 0.05 when comparing infected people to uninfected people, where 26 people

with tonsillitis were found to EBV IgG positive in people with enteritis, 44 people with arthritis also had EBV IgG positivity, and 12 people with tonsillitis also had EBV IgG positivity, and this is evidence. On the outbreak and spread of the EBV virus as shown in the figure (2).

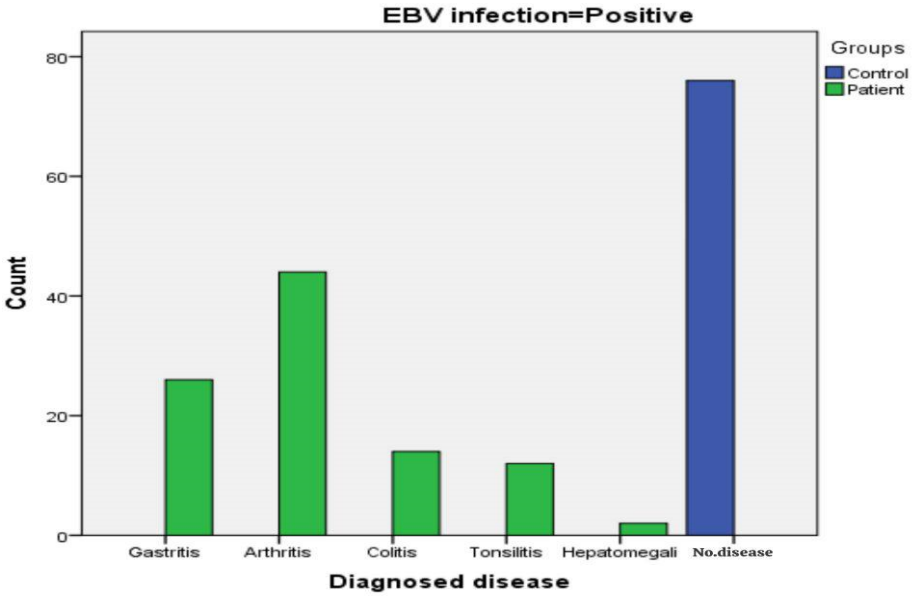

Figure 1: Show relation EBV infection IgG with other disease: P-value ≤ 0.05 Statistically significant, **Statistically high significant at level 0.000

Cluster of differentiation (CD19) protocol related with infected EBV IgG

The present study indicates a positive correlation between CD19 and EBV IgG infection at probability value P < 0.05 as shown in the table 1:

Table 1: CD_{19} related with EBV IgG infection

Groups	EBV IgG infection	CD_{19}	Number
Control	**Negative**	25.93 ± 2.902	20
	Positive	34.08 ± 15.5	76
	Total	32.38 ± 14.22	96
Patient	**Negative**	30.75 ± 7.17	6
	Positive	46.185 ± 49.72	98
	Total	45.29 ± 48.408	104
Total	**Negative**	27.044 ± 4.58	26
	Positive	40.9 ± 39.067	174
	Total	39.1 ± 36.76	200

Data was presented at (Mean ± Std.)

Cyclooxygenase 2 (COX2) enzyme related with infected EBV IgG:

In table (2) showed no relationship between COX2 and EBV IgG infection probability p-value > 0.05

Table 2: COX2 enzyme related with infected EBV IgG

Groups	EBV IgG infection	COX_2	Number
Control	Negative	40.74 ± 4.94	20
	Positive	51.57 ± 27.72	76
	Total	49.31 ± 25.12	96
Patient	Negative	34.42 ± 2.5	6
	Positive	68.67 ± 90.24	98
	Total	66.7 ± 87.94	104
Total	Negative	39.3 ± 5.21	26
	Positive	61.2 ± 70.5	174
	Total	58.35 ± 66.18	200

Data was presented at (Mean ± Std.)

Interferon gamma (INF gamma) cytokine related with infected EBV IgG.

The present study indicates a positive correlation between INFγ and EBV IgG infection at probability value P < 0.05 where shown in the table (3).

Table 3: INF gamma cytokine related with infected EBV IgG

Groups	EBV infection	INF gamma	Number
Control	**Negative**	113.51 ± 30.19	20
	Positive	161.41 ± 130.8	76
	Total	151.44 ± 118.62	96
Patient	**Negative**	86.75 ± 11.23	6
	Positive	107.4 ± 106.04	98
	Total	106.21 ± 103.05	104
Total	**Negative**	107.34 ± 29.1	26
	Positive	130.1 ± 120.18	174
	Total	127.92 ± 112.8	200

Data was presented at (Mean ± Std.),*Significant at P-Value < 0.05

Prostaglandin (E2) related with infected EBV IgG.

The result in this table showed a significant elevation at (p=0.003) in patient group compare with control group (Mean ± Std. Deviation) as shown in the table (4).

Table 4: Prostaglandin (E2) related with infected EBV IgG

Groups	EBV IgG infection	*Prostaglandin (E_2)*	Number
Control	**Negative**	42.81 ± 6.6	20
	Positive	54.89 ± 47.049	76
	Total	52.37 ± 42.19	96
Patient	**Negative**	47.51 ± 2.84	6
	Positive	70.96 ± 40.194	98
	Total	69.6 ± 39.4	104
Total	**Negative**	43.9 ± 6.23	26
	Positive	63.94 ± 43.92	174
	Total	61.33 ± 41.57	200

Data was presented at (Mean ± Std.),*Significant at P-Value < 0.05

Relation between other disease with infection EBV IgG with CD19

The present study indicates a positive correlation between inflammation other disease and infection with EBV IgG with level of CD19 in patient with Gastritis, Arthritis, Colitis, Tonsillitis, Osteonecrosis, Hepatomegalia as shown in the table (5).

Table 5: Relation between other disease with infection EBV IgG with level of CD19

Diagnosed disease	EBV infection	CD_{19}	Number
Gastritis	Negative	32.97 ± .00000	2
	Positive	60.43 ± 70.28	26
	Total	58.47 ± 68.006	28
Arthritis	Negative	37.41 ±.00000	2
	Positive	47.86 ± 47.15	44
	Total	47.4 ± 46.14	46
Colitis	Positive	34.5 ± 21.15	14
	Total	34.5 ± 21.15	14
Tonsillitis	Positive	27.3 ± 12.95	12
	Total	27.3 ± 12.95	12
Osteonecrosis	Negative	21.9 ± .00000	2
	Total	21.9 ± .00000	2
Hepatomegalia	Positive	19.49 ± .00000	2
	Total	19.49 ± .00000	2
No disease	Negative	25.93 ± 2.9	20
	Positive	34.08 ± 15.5	76
	Total	32.38 ± 14.22	96
Total	Negative	27.04 ± 4.6	26
	Positive	40.9 ± 39.07	174
	Total	39.1 ± 36.76	200

*Statistically P value < 0.05

Relation between other diseases with infection EBV IgG with COX 2

The present study indicates a positive correlation between inflammation by other disease and infection with EBV IgG with level of COX2 enzyme rate at probability value $P < 0.05$ as shown in the table (6).

Table 6: Relation between other diseases with infection EBV IgG with COX2

Diagnosed disease	EBV infection	COX_2	N
Gastritis	Negative	35.1691 ± 0.0000	2
	Positive	101.3277 ± 127.72	26
	Total	96.6021 ± 124.12	28
Arthritis	Negative	36.7695 ± 0.0000	2
	Positive	69.8398 ± 84.98	44
	Total	68.4020 ± 83.34	46
Colitis	Positive	41.9823 ± 30.89	14
	Total	41.9823 ± 30.89	14
Tonsillitis	Positive	30.7946 ± 11.96	12
	Total	30.7946 ± 11.96	12
Osteonecrosis	Negative	31.3281 ± 0.0000	2
	Total	31.3281 ± 0.0000	2
Hepatomegali	Positive	32.2883 ± 0.0000	2
	Total	32.2883 ± 0.0000	2
No disease	Negative	40.7385 ± 4.94	20

	Positive	51.5659 ± 27.9	76
	Total	49.3102 ± 25.12	96
Total	Negative	39.2809 ± 5.21	26
	Positive	61.1974± 70.5	174
	Total	58.3483 ± 66.18	200

*Statistically P value < 0.05

Relation between other disease and infection EBV IgG with level of INFγ

The present study indicates a positive correlation between inflammation by other disease and infection with EBV IgG with level of INFγ compare with no disease at probability value P< 0.05 where as shown in the table (7).

Table 7: Relation between other diseases with infection EBV IgG with level of INFγ

Diagnosed disease	EBV infection	INFγ	N
Gastritis	Negative	90.7723 ± .00000	2
	Positive	121.6178 ± 138.83524	26
	Total	119.4145 ± 133.83898	28
Arthritis	Negative	96.8057 ± .00000	2
	Positive	115.7499 ± 106.70510	44
	Total	114.9262 ± 104.38003	46
Colitis	Positive	94.2862 ± 69.13358	14
	Total	94.2862 ± 69.13358	14
Tonsillitis	Positive	57.6657 ± 37.74777	12
	Total	57.6657 ± 37.74777	12
Osteonecrosis	Negative	72.6720 ± .00000	2
	Total	72.6720 ± .00000	2
Hepatomegalia	Positive	129.2934 ± .00000	2
	Total	129.2934 ± .00000	2
No disease	Negative	113.5137 ± 30.11847	20
	Positive	161.4148 ± 130.79776	76
	Total	151.4354 ± 118.61788	96
Total	Negative	107.3374 ± 29.10140	26
	Positive	130.9952 ± 120.17872	174
	Total	127.9197 ± 112.80923	200

DISCUSSION

The results of the present study were discussed and interpreted in five key areas, each of which plays a crucial role in understanding the correlation between EBV infection and other medical conditions, Correlation between EBV illness and other medical conditions, Study and analysis of inflammatory elements, indicators, and cytokines associated with EBV infection and their connection to other autoimmune disorders and inflammatory states. All data related to the research study, which has been conducted at a significant level of p≤0.05, is of high reliability and validity.

Experiments performed in the presence of phosphonoacetic acid, an inhibitor of herpesvirus DNA polymerase, reversed the inhibition of PGE2 biosynthesis, suggesting the involvement of viral replication and newly synthesized viral proteins in this process. It was done. Therefore, inhibition of PGE2 biosynthesis in monocytes may be an additional mechanism contributing to EBV pathogenesis (30). tables 4 and 5 exhibit identical outcomes. The Cox2 and PGE2 factor levels in EBV patients significantly surpass those of healthy control subjects. Considering this aspect, our findings can be juxtaposed and deliberated upon about the outcomes presented in other scholarly publications. About the elevated expression of the bladder cytokine gene observed in our research, it can be stated that while certain studies have indicated a decrease in the level of this cytokine in the disease, other studies have contradicted this entirely. In our investigation, we observed a significant increase in the expression level of this cytokine gene in individuals with the illness compared to those who are healthy. However, it is essential to note that there are conflicting findings in this field. For instance, Morrison et al. mentioned in their 2001 study that an Epstein-Barr Virus Immediate-Early Protein inhibits IFN-γ signaling(31).

This virus predominantly affects B lymphocytes (as demonstrated by the large increase in CD19 markers among infected persons compared to healthy individuals), but it also causes the release of a variety of inflammatory cytokines, including Cox2 and PGE2. The study found that EBV patients with disease activity have considerably greater IFN-γ levels compared to control groups.These findings imply that IFN-γ may be a helpful biomarker for disease activity and contribute to developing HLH and active EBV (32). This cytokine's gene expression was also detected in the patients in our investigation.

The study discovered that IRP patients had a greater rate of EBV lytic infection than control participants, with 45.1% of IRP patients carrying EBV-DNA copies against 22.2% of control people. Furthermore, CD19 B lymphocyte EBV-DNA copy counts were considerably greater in newly diagnosed IRP patients than in remission or control participants (33). This study also found a substantial rise in the CD19 marker level on the surface of B lymphocytes compared to the control group.

CONCLUSION

The conclusion is that the EBV virus plays a significant role in various acute inflammation diseases, chronic active Epstein-Barr virus infection to persist different types of inflammation and cause a threat to patient life with elevation of the immunological markers CD19, E2 ,and INFγ that lead to increase severity and persistence of inflammation.

Conflict of interest: Authors were declared no conflict of interest

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