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Short Communication

Detection of EBV BNRF 1 Gene in Iraqi Schizophrenia Patients by Molecular Methods

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ABSTRACT

Epstein–Barr virus is the most common viruses that is capable of infecting the central nervous system and establishing persistent infection. EBV infects B cells of immune system and epithelial cells. A target sequence of the BNRF1 gene is a successful genetic marker for EBV. The aim of this study was to determine the relation between EBV and schizophrenia. A total number of 180 serum and plasma samples were collected from Baghdad specialized hospital for psychiatric disorder including (90 schizophrenia patients, and 90 control sample males and females. Immunological assay for evaluation of Anti-EBV IgG was accomplished by ELISA technique. Molecular analysis was achieved by real time PCR to confirm our results by detecting copy number of *BNRF1* gene to determine the infection severity. Results revealed that the distribution of EBV infection among studied group was 70 (77.7%) in patients and 8 (8.8%) in control. Investigating schizophrenia family history was also included in the study, results showed that 50 (55.5%) of patients have family history while 40 (44.4%) did not. Anti-EBV IgG was positive in 78 (86.6%) and 10 (11.1%) of schizophrenia patients and control group respectively, molecular experiments confirmed our results where it showed a high copy number of the *BNRF1* gene in patients with high Anti-EBV IgG level and low copy number in patients with low Ab serum level. In conclusion, there is a significant relation between schizophrenia and EBV infection in studied subjects, accompanied with a statistical significance in the family history factors compared with control group.

Keyword: Schizophrenia, EBV, IgG, Real-Time PCR, BNRF 1 Gene.

Introduction

Schizophrenia is one of the most serious neuropsychiatric disorders with unknown etiopathogenesis with a lifelong incidence in the USA of approximately 1 percent. Although schizophrenia has strong genetic cause. However, currently the disease risk could be explained by the influence of certain genes.[1] Environmental risk factors, including birth place, season, maternal obstetric issues, age of the parents, neonatal vitamin D levels, and prenatal infection (e.g., influenza, toxoplasmosis, and herpes simplex viruses), are believed to have a role in the etiology of Schizophrenia. Additionally low social class seems to be a cause and a consequences.[2] On the other hand, epidemiological research has shown a high clinical levels of schizophrenia in large towns, immigrants population, traumatized individuals, and cannabis users, some of them at least believed to be the product of the underlying exposures in the community.[3] Epstein–Barr virus is one of the Herpesviridae family and is known as human herpes virus 4. Epstein–Barr virus is a lymphotropic virus that induces latent disease. In addition, there are few reports of sensitivity to EBV in people with schizophrenia. Therefore, in a cohort of individuals experiencing schizophrenia, we tested antibody concentrations toward Epstein–Barr virus and identified EBV molecules then compared them against a population of control persons without any psychiatric background.[4] The virus stays alive in the host B cell and T cell, macrophages, and endothelial cells following acute infection; symptomless viral salivary discharge leads to further transmission. EBV can induce latency in several body locations, including the nervous system, in which reactivation can be connected with encephalomyelitis and specific immune responses in the brain. An immune system response to EBV infection could be regulated by measuring directed antibody levels to virus-derived antigens and specific EBV protein. Anti-Epstein–Barr virus antibodies involve: anti-early antigen that occurs early during infection, then reduces after 3 to 6 months; viral capsid antibody which also exists early during infection but remains for longer periods of time; anti-Epstein–Barr virus nuclear antigen (Epstein–Barr virus NA or EBNA) that does not occur until late during infections although persists for long periods.[5]

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Materials and Methods

Ethical consideration
This study was approved by the Ethical Committee, Department of Biology, College of Science, University of Bagdad, Baghdad, Iraq and the Iraqi Ministry of Health.

Study groups
A total number of 180 individuals (males and females) were included in the study. Ninety (90) of them were diagnosed to be positive for schizophrenia (patient group). The remaining 90 samples represented the control group with no previous history of any psychiatric disorder.

Specimen collection
Five (5) mL of blood samples were taken from each patient and control groups via vein puncture method (according to guidelines of Center of Disease Control and Prevention (CDC) 2018). These samples were then divided into two parts to obtain serum and plasma which were used for detection of anti-EBV antibodies (serum) and for viral DNA extraction (plasma) as follows:

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(i) Two (2) mL of blood were placed in an anti-coagulated tubes containing EDTA and then frozen at -20°C until used later for extraction of EBV DNA.
(ii) For separation of serum, 3 mL of blood were transferred into clot activator gel tubes and allowed to clot for ~30 minutes; these tubes were centrifuged for 3 minutes at 4000 rpm and stored at -20°C till used for detection of anti-EBV antibodies.

Detection of Anti-EBV IgG by Enzyme Linked Immunosorbent Assay (ELISA)
In the serum samples from the entire study group (90 Schizophrenia patients and 90 for controls), anti-EBV antibody (IgG) were tested via ELISA technique, the immunological part for evaluation of Anti-EBV IgG was accomplished by using ELISA technique by Manufacturing company (EUROIMMUN/Germany).[6]

Molecular Detection and EBV by Real-Time PCR
DNA was extracted using column filter G-spin total DNA extraction kit (Korea). The molecular analysis was achieved by real time PCR to confirm the results by detecting the copy number of entire *BNRF1* gene for determination of infection severity by PCR Max (qPCR Kit UK).[7] So *BNRF1* gene does not play a clear function in the replication of DNA or maturation of the virus, yet are essential for a successful infections and primary B lymphocytes transformation.[8] The program used for this reaction and its components are mentioned below (Table 1).

Table 1: PCR program for EBV genome amplification

Step	Temperature & Duration	Number
Initial denaturation	94 ˚C / 5 minutes	1 cycle
2nd denaturation	94 ˚C / 1 minute	
Annealing	53 ˚C / 1 minute	
Extension (elongation)	72 ˚C / 1 minute	35 cycles
Final extension	72 ˚C / 10 minutes	1 cycle
Final hold	4 ˚C	_____

Statistical analysis
The Statistical Analysis System- SAS (2012) program was used to analyze the effect of different factors in this study. Chi-square test was used to compare significance differences between percentages (with probability levels at 0.05 and 0.01).

Results and Discussion

Distribution of the studied schizophrenia cases according to family genetic history
The result of this research shows the family history of schizophrenia, 50(55.56%) patients with family history and 40(44.44%) patients without family history, 36(40%) out of 70 male patients and 14(15%) out of 20 females with family history (Table 2).
The statistical analysis of all findings revealed a slightly significant difference according to family history, the family history of schizophrenia is considered the strongest single predictor of the risk of individual to schizophrenia. Thus, it is important to include family history of schizophrenia as a potential risk factor in any study of risk factors for schizophrenia. The finding of a large scale Danish study which involved 9324 schizophrenia cases revealed that almost any mental disorder in first level relatives increases the individual's risk of schizophrenia, while the general medical history of the family had been shown to be a risk factor of the connection between schizophrenia and urbanization with respect to the potential influence of the birthplace.[9] Family history of mental illness also has been found to be a high risk factor for schizophrenia development with a ten-fold increase in the risk among most of the first-degree relatives. Thus many genes were detected as possible contributors to schizophrenia etiology.[10] Over the last few years, the interest in the differences between the genders (male and female) of patients has increased and this include brain morphology and neurocognitive function. Nevertheless, of the variability and methodological insufficiencies, some few questions arise from the available literature. Most interesting finding of the neuroanatomic studies showed extra enlarged ventricles and smaller frontal lobes in males than in females with schizophrenia.[11]

Detection of the immune response (IgG) to EBV by ELISA
The Enzyme Linked Immunosorbent Assay was used to test the presence of anti-EBV antibodies (IgG) in sera from the study groups (patients and controls). Seventy-eight (78) (86.6 %) of patients and 10 (11.1%) of controls were anti-EBV (IgG) positive. The statistical analysis of all these results showed a highly significant difference between each class of immunoglobulin in both groups ($P < 0.0001$). The mean IgG value in healthy control was 61.78 ± 5.04 Ru/mL, while that of the patients group was 163.10 ± 5.93 Ru/mL (Figure 1).
Consistent with the present study is the findings of a study performed at the Ministry of Health, the Russian Federation which found out that the highest number of cases indicated a positive reactions for IgG to Epstein Barr virus (96%), HSV-1 (81%) and CMV (31%) among schizophrenia patients.[12] While the study conducted by Asoode and colleagues[13] pointed out a few viruses (such as herpes viruses) in addition to neurotropic characteristics and latency as possible risk factors in many CNS disorders, including schizophrenia. The prevalence of anti-EBV IgG among patients with schizophrenia and healthy controls in their study was 100% and 89%, respectively with significant difference between the two groups with respect to the anti-EBV antibody titers.[13] Dickerson *et al* (2019)[5] found that individuals with schizophrenia have elevated levels of IgG antibodies to EBV in comparison to the control. These differences are independent of demographic factors that are known to influence EBV exposure, such as age, sex, race and socio-economic status.[5]

Detection of the prevalence of EBV infection in schizophrenia patients by Real Time-PCR
Real Time-PCR was utilized to confirm the EBV infection among the studied groups, viral nucleic acids were extracted from the serum samples of the schizophrenia patients and controls, Plasma samples was used for this purpose as EDTA anti-coagulated plasma offers some protection for EBV from nucleases present in the serum. The presence of the extracted nucleic acid was confirmed by the use of 0.5-1.5% agarose gel by electrophoresis before amplification of EBV *BNRF1* gene. The detection of EBV in all the participants of both of the studied groups (90 patients and 90 controls), which were previously tested for the presence of anti-EBV antibodies by ELISA, was achieved by amplification of *BNRF1*gene by the use of Real-time PCR. The *BNRF1* gene was detected in 70 out of 90 patients (77.7%) and 8 out of 90 controls (8.8%), the statistical analysis revealed significant differences ($P < 0.0001$) between the compared groups. As shown in Figure 2, the Mean fold induction of *BNRF1*gene in healthy controls was 2.74 ± 0.92 µg/mL, and that of the patients was 20.80 ± 1.82 µg/mL. Some infectious agents may attack the brain and interfere with its development, growth, and/or functionality.

Table 2: Distribution of sample study according to Family history

Family history	No.	Percentage (%)
Yes	50	55.56
No	40	44.44
Total	90	100%
Chi-Square ($\chi 2$)	---	4.391 *
P-value	---	0.0452
($P \leq .0.05$). *		

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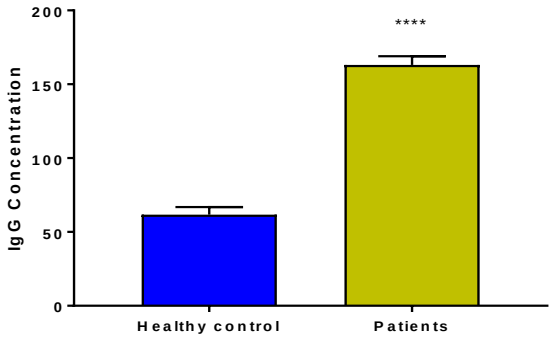

Figure 1: Detection of Serum IgG levels (Ru/mL) by ELISA test against EBV for both patients and controls

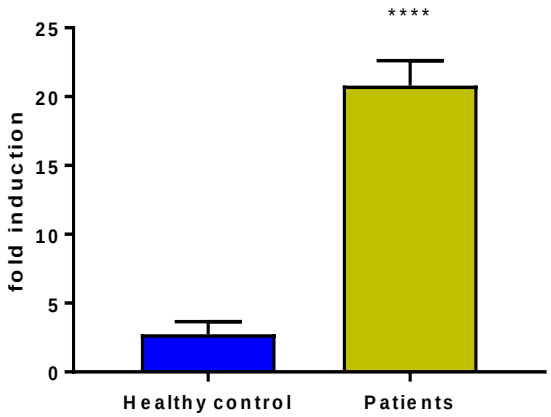

Figure 2: The prevalence of EBV infection by RT-PCR

A number of studies have shown that exposure to certain infectious agents during fetal and postnatal life can also lead to the later development of schizophrenia as well as other non-affective psychosis. This research was conducted to investigate the prevalence of EBV in Iraqi schizophrenia patients. The target sequence within the *BNRF1* gene has previously been shown to be a good genetic marker for EBV in other clinical real time PCR based studies.[14]

ELISA versus Real-time Polymerase Chain Reaction

The findings of this analysis indicated a significant difference between the number of specimens which were positive for anti-EBV IgG as detected by the ELISA technique and the number of specimens in which the EBV *BNRF1* gene was detected for each of the groups studied. The variance showed a strong significant relationship between the results of the ELISA and PCR. The ELISA positive results were 86.6% and 77.7% for the RT-PCR EBV gene in the patient's community, while the control results were 11.1% and 8.0%, respectively, as explained in Table 3.

Conclusion

From the results of our study we can conclude that the infection of EBV among the Iraqi schizophrenia patients is highly predominant with a statistical significance compared to control as shown by anti-EBV IgG immune response achieved by ELISA technique. Also, High level of Anti-body in the patients showed an observable elevation in the copy number of *BNRF1*gene as compared with group of normal controls with a high statistical significance ($P \leq 0.01$) and genetic family history showed statistical significant difference in schizophrenia patients compared to the control group.

Conflict of interest

The authors declare no conflict of interest

Table 3: Comparison between positive results of ELISA and Real-time PCR in respect to EBV infection in the studied groups

DATA DESCRIPTION			Test		TOTAL
			ELISA (POSITIVE)	RT-PCR (POSITIVE)	
GROUP	PATIENTS	COUNT%	78(86.6%)	70(77.7%)	148
		WITHIN GROUP	(52.7%)	(47.3%)	100%
	CONTROLS	COUNT%	10(11.1%)	8(8.0%)	18
		WITHIN GROUP	(55.6%)	(44.4%)	100%
	TOTAL		88	78	88
			53.0%	47.0%	53.0%

Chi-Square Tests

	value	df	Asymptotic Significance (2-sided)
CHI-SQUARE	.052	1	.819

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them..

References

1. Birnbaum R and Weinberger DR. Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci. 2017; 18(12):727-740.
2. Fuglewicz AJ, Piotrowski P, Stodolak A. Relationship between toxoplasmosis and schizophrenia: a review. Adv Clin Exp Med. 2017; 26(6):1031-1036.
3. Stilo SA and Murray RM. Non-genetic factors in schizophrenia. Curr Psych Rep. 2019; 21(10):1-10.
4. Breier A, Buchanan RW, D'Souza D, Nuechterlein K, Marder S, Dunn W, Preskorn S, Macaluso M, Wurfel B,

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Maguire G, Kakar R, Highum D, Hoffmeyer D, Coskinas B, Litman R, Vohs JL, Radnovich A, Francis MM, Dickerson FB. Herpes simplex virus 1 infection and valacyclovir treatment in schizophrenia: results from the VISTA study. Schizophrenia research. 2019; 206:291-299.

5. Dickerson F, Jones-Brando L, Ford G, Genovese G, Stallings C, Origoni A, O'Dushlaine C, Katsafanas E, Sweeney K, Khushalani S, Yolken R. Schizophrenia is Associated With an Aberrant Immune Response to Epstein–Barr Virus. Schizophr Bull. 2019; 45(5):1112-1119.
6. Pakpoor J, Giovannoni G, Ramagopalan SV. Epstein–Barr virus and multiple sclerosis: association or causation? Expert Rev Neurother. 2013; 13(3):287-297.
7. Smatti MK, Al-Sadeq DW, Ali NH, Pintus G, Abou-Saleh H, Nasrallah GK. Epstein–Barr virus epidemiology, serology, and genetic variability of LMP-1 oncogene among healthy population: an update. Front Oncol. 2018; 8:211.
8. Huang H, Deng Z, Vladimirova O, Wiedmer A, Lu F, Lieberman PM, Patel DJ. Structural basis underlying viral hijacking of a histone chaperone complex. Nat Commun. 2016; 7(1):1-10.
9. Vassos E, Agerbo E, Mors O, Pedersen CB. Urban–rural differences in incidence rates of psychiatric disorders in Denmark. Br J Psych. 2016; 208(5):435-440.
10. Käkelä J, Panula J, Oinas E, Hirvonen N, Jääskeläinen E, Miettunen J. Family history of psychosis and social, occupational and global outcome in schizophrenia: A meta-analysis. Acta Psychiatr Scand. 2014; 130(4):269-278.
11. Mendrek A and Mancini-Marïe A. Sex/gender differences in the brain and cognition in schizophrenia. Neurosci Biobehav Rev. 2016; 67:57-78.
12. Breier A. 39. Viruses and Schizophrenia: Implications for Pathophysiology and Treatment. Schizophr Bull. 2018; 44(1): S61–S62.
13. Asoode A, Yavarian J, Ahmadkhaniha H, Eshraghian M, Marashi SM, Ghaemi K. Epstein-Barr virus and herpes simplex infection assessment in schizophrenia and bipolar patients compared to healthy subjects. J Birjand Univ Med Sci. 2016; 23(2):149-157.
14. Tsai K. Tegument protein BNRF1 regulation of Epstein-Barr virus genome chromatinization during early infection. 2014.