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Molecular study of HumanHerpes Virus 6- A and- B, in Patients with Autism Spectrum Disorders in Basra/ south Iraq

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ABSTRACT

Background: Autism Spectrum Disorders (ASDs) are complex, pervasive neurodevelopmental conditions with a largely unknown etiology. HHV6 is a common childhood viral infection. The latency of the virus in the CNS might act with other co-factors to disrupt the neurons of the risky children and predisposed them to get ASD so the aim of the present study was to evaluate the prevalence of HHV6 A and B in children with ASD compared to controls.**Patients and methods:** This is a case – control study, in which 94 Autistic children and 100 apparently healthy control individual were enrolled. The prevalence of HH6V genotypes A and B were detected by **q**PCR .**Results:**There was no significance variation in the percentage of HH6A positive among autistic and the control groups (P >0.05) while HH6V B were detected in 33 (35.1%) out of 94 ASD patients versus 2 (2%) out of 100 control children and statistically the difference was significant (P value < 0.038).**Conclusion:** our study shows that the rate of HHV-6 exposure is dissimilar between the studied groups of children
Keywords: Autism Spectrum Disorders , HHV-6

Introduction

Autism Spectrum Disorders (ASDs) are complex, pervasive neurodevelopmental conditions with a largely unknown etiology. ASDs are behaviorally defined and characterized by deficits in social communication and interaction, and the presence of restricted, repetitive patterns of behavior, interests, or activities[1].The etiology and pathogenesis of autism are poorly understood, there is evidence that a multifactorial agents might included like environmental factors, such as infectious diseases and teratogen exposure in utero, can cause autism and that in some cases there must be an interaction between genetic and environmental factors [2].There are multiple genetic and environmental risk factors including a variety of mutated and variant genes, advanced paternal age, exposure to toxins and medications in early development, prematurity, and birth complications [3].Viral agents,due to their potential latency and neurotropism are considered as possible agents in the pathogenesis of many central nervous system (CNS) disorders [4]. Human herpesvirus 6 (HHV-6) is a member of *Herpesviridae*family, isolated, at first, from patients with AIDS andlymphoproliferative disease [5]. Herpes viruses have been supposed as the key pathogenic factors in mental diseases [6]. Two variants of HHV-6 have been identified on the basis of differences in epidemiology, in vitro growth properties, reactivity with monoclonal antibodies, restriction endonuclease mapping, and nucleotide sequence, leading to adoption of the nomenclature HHV-6A (variant A) and HHV-6B (variant B).HHV-6 may play a pathogenic role in neurologic disorders such as epilepsy, multiple sclerosis as well as skin diseases [7].The aim of the present study was to evaluate the prevalence of HHV6 A and B in children with ASD compared to controls.

Material and Methods

This is a case – control study carried out fromDesember 2018 to October 2020 in which 94 autistic children (patients group) 78 male and 16 female who had been diagnosed by specialist psychatrician, and 100 apparently healthy control individual (control group): 80 male and 20 female, who were matched by age and sex with the patients group were enrolled from the same population living in Basra providence in Iraq.

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Ethical aspects

This study was approved by the Primary Health Care Centers manager and family of both patients and control kids. Its procedures and purpose were clarified to all studied population, and Every individual member of the family has obtained written informed consent.

Collection of Blood Samples:

Five mL ofvenous bloodwas drawn from each Participants ,were collected in tube contain EDTA (EthylineDiamine Tetra Acetic acid) as anticoagulant .

sample was centrifuged at 3000 rpm for 10 mints and got 3 layers

- ✓ First layer was plasma
- ✓ Second layer was buffy coat which collected in microcentrifuge to use to extraction viral DNA
- ✓ Third layer included the erythrocytes which was discarded.

Viral DNA Extraction:

Viral DNA was extracted from buffy coat of each samples by using DNA Blood Mini Kit (Favorgen - Taiwan Cat. No: HB10.03.10 UK) depending on the instruction supplied by the kit. The extracted DNA was stored at -20 until use.

Determination of Viral DNA Yield

A Nano-drop Spectrophotometer (Thermofisher, Germany), an absorbance based method, was used to measure the DNA yield at 260nm,which was the wavelength of maximum absorption for DNA.

Detection of HH6VA and HH6VB by qPCR:

HHV6 variant A &B was detected in samples by using Rotor-Gene Q 6000 Thermal Cycler (Germany) from the previously extracted DNA samples using genesig® Advanced Kit(UK) cat. no.HB10.03.10 PCR setup and conditions were described as in the manufacturer's instructions. The assay included a heterologous amplification system (internal control) to identify possible PCR inhibition and Endogenous controlto confirm extraction of a valid biological template. During PCR amplification, forward and reverse primers hybridize to the HHV6 DNA. A fluorogenic probe was included in the same reaction mixture which consists of a DNA probe labeled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe was cleaved and the reporter dye and quencher were separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Statistical analysis:

The Statistical Package of Social Science (SPSS, version 20) was used to analyze and processed data. Fisher Exact test and $\chi 2$ test were used. A *p*-value of <0.05 two-sided test was considered statistically significant.

Result

The distribution of HHV-6 genotyping A and B in both autistic and control groups were clarified in table 1. From the table below we demonstrate that the frequency of positive cases with HH6VB virus was (18%) which was more than HH6A (1.5%) in the study population. The overall distribution of HH6 A virus in case and control groups were summarized in table 2 which show that by using the Fisher Exact test there was no significance variation in the percentage of HH6A positive among autistic and the control groups (P >0.05).As shown in table 3,HH6V B were detected in 33 (35.1%) out of 94 ASD patients versus 2 (2%) out of 100 control children and statistically the difference was significant (P value < 0.038).

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Table 1. Frequency of HH6V A and B in the study population

Result of Qpcr	HHV6ANo. (%)	HHV 6BNo.(%)
Positive	3(1.5)	35(18)
Negative	191(98.5)	159(82)
Total	194(100)	194(100)

Table 2. The distribution of HH6 A virus in the study population

Study groups	HH6A		TotalNo.(%)
	PositiveNo. (%)	NegativeNo.(%)	
Autistic group	2(2.1)	92(97.9)	94
Control group	1(1)	99(99)	100
Total	3	191	194
Fisher Exact test= 0.612 df= 2 OR=0.465 P>0.05			

Table 3. Distribution of HH6V B infection in patients and Autistic groups

Study groups	HHV6 B		Total No.(%)
	Positive No. (%)	Negative No.(%)	
Autistic group	33(35.1)	61(64.9)	94 100
Control group	2(2)	98(98)	100 100
Total	35	159	194
X^2= 35.916 df=2 OR= 0.038			P<0.05

Table 4. The quantitative standards; *Ct value and the number of viral copies

No.	Color	Name	Type	*Ct	Given Conc (copies/reaction)	CalcConc (copies/reaction)

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No.	Color	Name	Type	*Ct	Given Conc (copies/reaction)	CalcConc (copies/reaction)
1		S1	Standard	21.86	200000	151436
2		S2	Standard	24.10	20000	28017
3		S3	Standard	27.51	2000	2135
4		S4	Standard	30.71	200	191
5		S5	Standard	33.79	20	19
6		S6	Standard	36.78	2	2
7		16	Patient 16			
8		17	Patient 17			
9		65	Patient 65	27.02		3099
10		14	Patient 14			
11		68	Patient 68	26.81		3612
13		19	Patient 19			
14		29	Patient 29	34.96		8
18		61	Patient	29.23		583
20		5	Patient			
21		8	Unknown	28.70		871
22		NC	Negative Control			

*CT: cycle threshold defined as the number of cycles required for the fluorescent signal to cross the threshold.

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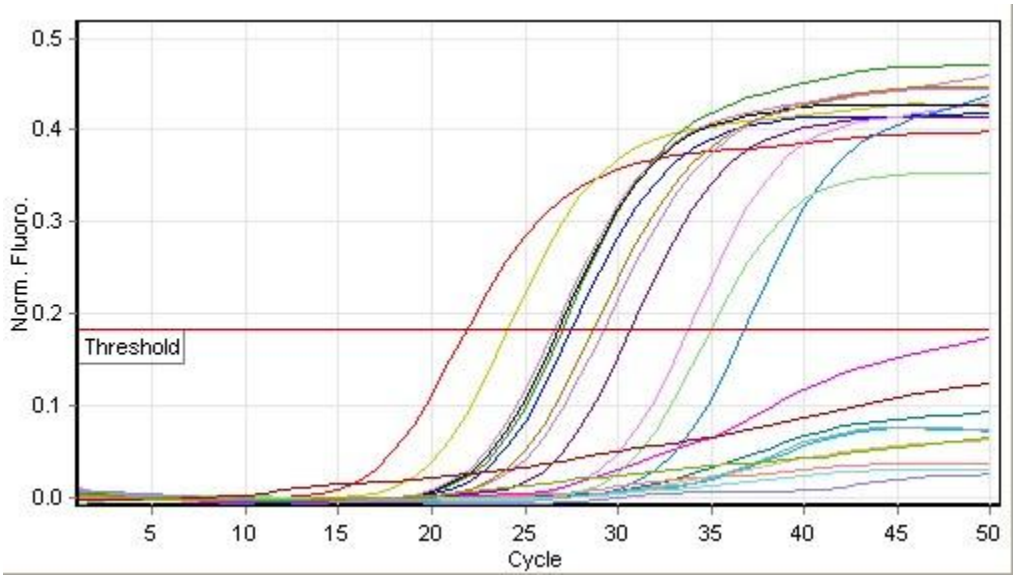

Figure 1. The Amplification Plot; the concentration of the amplified product in fluorescence channel; fluorescent signal appear when above the background noise (i.e. Cycle threshold);the first four curves (red, yellow, blue and purple) represents the 1st-4th quantitation standards. The last three curves represent patients samples. All other samples (case and control) were tested below the threshold line that considered as negative for HHV-6.

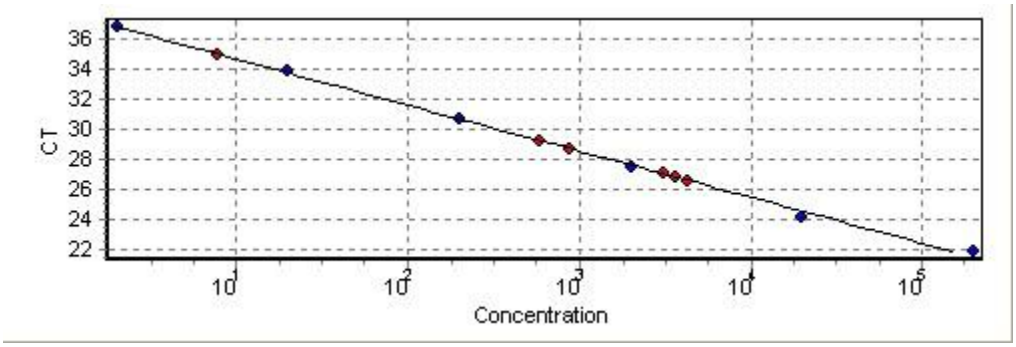

Figure 2. The standards curve of the real time-PCR virus assay. Serial logarithmic dilutions of the standards of known concentrations were analyzed by using standard amplification conditions. The Ct (y axis) of each of the dilutions is plotted against the log concentration (x axis). The number of molecules of the target sequence was doubled during each replication cycle, indicates amplification efficiency.

Discussion:

The etiology of ASD remains controversial. Although some viral infections had been linked with the ASD like Herpes Simplex Virus HSV1, Epstein-Barr virus, Rubella virus, Measles virus, Cytomegalovirus, and othersbut still the role of other viruses like HHV6 have not been implicated well[8].The Herpes Viruses family appeared to be the more sensible to treatment with anti-viral formula, this family may be the most frequently implied in autism. HHV6 is a common childhood viral infection. It has the ability to reside in the central nervous system (CNS) (generally where nerves of the body or theface entered spinal cord or the brain). The latency of the virus in the CNS might act with other co-factors to disrupt the neurons of the risky children and predisposed them to get ASD[9].The brain development is a very dynamic place, with synapses connecting and

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disconnecting rapidly and continuously, on a time scale of milliseconds to seconds. The synaptic relation just become constant by becoming myelinated, and myelination continues on an hour to day time scale.The oligodendrocytes cells were generated the six proteins which necessary to myelinate and stabilize the newly formed neural CNS connections, many of this cellsif becameinfected with HHV6 so the processes of myelination will be affected and the efficacy of the axon to conduct the electrical signals from one neuron to another will be interrupted[10]. In this study we found that there was no significance differencein the prevalence of HHV-6Aamong control and patients group this in agreement with Gentile *etal* 2013[8], who found that levels and seropositivity percentage of HHV-6 and HHV-8 antibodies had no changes in autistic children and non -autistic groups. Regarding HH6V B infection : in this study we found their was adifference in the percentage of HHV-6 B positive sample in the case and control group ($p < 0.05$) these result in harmony of a publication reported that infection, particularly from HHV-6, may implicated in some patients of ASD, by using site-specific primers of PCR in acase-control design withDNA of HHV-6B was most frequently detected virus [11].Also Nicolson *etal* 2007[12]reported that a greater numbers of autistic individuals reveals evidence of infection with HHV-6. Singh etal 1998[12] indicated that an enormous number of autistic cases who had HHV-6 antibody in sera was also had autoantibody AMBP for brain:it supported the hypothesis that an autoimmune response which induced by virus may implicate in ASD.However many autistic immune panels showedsymptoms of atypical infections and abnormal cell counts. In conjunction withtrait-related cerebral hypometabolism and hypo perfusion, these findings suggestedhypothesis: Some autistic subgroups derived from intra-monocyte pathogens such as measles virus, CMV, HHV-6, and Yersinia enterocolitica[14]. In addition, their effects manifested as diminished hematopoiesis, impaired peripheral immunity, and altered function of blood-brain barriers often accompanied by demyelination, with much interchild variation.One or more of these pathogens persisted in some such children as a chronic-active, seemingly subclinical infection that is etiologically significant to the autistic traits of the child. [14].

References:

[1] American Psychological Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed). Washington, DC: American Psychiatric Association.

[2] Gillberg, C., & Coleman, M. (2000). The biology of the autistic syndromes. Cambridge: Cambridge University Press.

[3] Coleman, M., &Gillberg, C. (2012). The autisms (4th ed). Oxford, UK: Oxford University Press, Oxford, p. 286–302.

[4] Niebuhr, D. W., Millikan, A. M., Yolken, R., Li, Y., & Weber, N. S. (2008). Results from a hypothesis generating case–control study: Herpes family viruses and schizophrenia among military personnel. Schizophrenia Bulletin, 34(6), 1182–1188. doi:10.1093/schbul/sbm139

[5] Salahuddin, S. Z. (1992). Human herpesvirus 6: Epidemiology, molecular biology, and clinical pathology, 4 (pp. 3–8). Amsterdam, The Netherlands: Elsevier Biomedical Press.

[6] Hannachi, N., El Kissi, Y., Samoud, S., Nakhli, J., Letaief, L., Gaabout, S., . . . Boukadida, J. (2014). High prevalence of Human herpesvirus 8 in schizophrenic patients. Psychiatry Research, 216(2), 192–197. doi:10.1016/j.psychres.2013.12.035

[7] Reynaud, J. M., &Horvat, B. (2013). Human herpesvirus 6 and neuroinflammation. ISRN Virology, 2013, 1–11, article ID 834890. doi:10.5402/2013/834890

[8] Gentile, I., Zappulo, E., Coppola, N., Bonavolta, R., Portella, G., Cernia, D. S., . . . Bravaccio, C. (2013). Prevalence of HHV-6 and HHV-8 antibodies in patients with autism spectrum disorders. In Vivo, 27(6), 843–849.

[9] Pellett, P. E., Ablashi, D. V., Ambros, P. F., Agut, H., Caserta, M. T., Descamps, V., . . . Razonable, R. R. (2012). Chromosomally integrated human herpesvirus 6: Questions and answers. Reviews in Medical Virology, 22(3), 144–155. doi:10.1002/rmv.715

[10] Blumberg, B. M. (2017). The secret lives of human herpes virus 6 (HHV-6). Journal of Neurological Disorders, 05(3), 352. doi:10.4172/2329-6895.1000352

[11] Sweeten, T. L., Croen, L. A., Windham, G. C., Odell, J. D., Stubbs, E. G., & Torres, A. R. (2019). Brief report: Low rates of herpesvirus detection in blood of individuals with autism spectrum disorder and controls. Journal of Autism and Developmental Disorders, 49(1), 410–414. doi:10.1007/s10803-018-3691-x

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11379 - 11385
Received 05 March 2021; Accepted 01 April 2021.

[12] Nicolson, G. L., Gan, R., Nicolson, N. L., & Haier, J.. (2007). 2007Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. Journal of Neuroscience Research, 85(5), 1143–1148. doi:10.1002/jnr.21203

[13] Singh, V. K., Lin, S. X., & Yang, V. C. (1998). Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism. Clinical Immunology and Immunopathology, 89(1), 105–108. doi:10.1006/clin.1998.4588

[14] Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. Medical Hypotheses, 56(4), 523–531. doi:10.1054/mehy.2000.1247