

Inhibition Of Human Herpes Virus Type 6&8 Associated With Non-Genetic Autism Spectrum Disorders

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Abstract

Samples were collected on 3-15-2017 until 8-20-2019 from children with autism spectrum, as well as samples were taken from the mother for each child during the study. All samples were diagnosed through RT-PCR by designing primers for each virus as well as for the disease depending on the location of NCBI. The infected children were divided into three groups starting from a year and a half until age 11.5 years and the first group was (23 case) higher than the rest of the groups, while the ages of mothers were divided into three groups, starting from 25 upto 42 years, noting that the second group had a higher (25 case) of the virus than the first and third groups (11 and 18 cases), respectively. And with a significant difference at $p < 0.05$. Then both the diagnosed viruses were implanted on the tissue culture cells infective dosage (TCID₅₀/0.1 ml) was ($10^{7.6}$) of virus. and then the viruses were inactivated through the use of a dose-controlled gamma ray (6.864 mSv/h.) and then the cellular changes were observed on the tissue culture cells between 24-48 hours and the study continued for three years. In addition of diagnosis two groups of cytokines, including pro and anti-inflammatory (TNF- α and IL-37) of human infected.

The first study in Iraq is in terms of diagnosing non-inherited autism spectrum disease and due to the associated herpes virus type 6 and 8.

Introduction

Disorders recognize neuropsychiatric considered through knowingly abnormal, communication capabilities and dialectal and considerable cramped arrangement of actions and benefits called autism spectrum [1]. As the symptoms begin to appear on children clearly after the third year, in addition to the presence of some methods that show abnormal perception, sensory treating, focus and slow learning [2].

In recent years, the incidence of infection increased from 1 in 88 newborns, and this led to interest in disorders that spread during the previous years due to the association with comorbidities, which include mental retardation, aggression, epilepsy and self-harm [3,4]. A number of studies have shown that viruses are related to autism, including mainly herpesviridae [herpes simplex (HSV), cytomegalovirus (CMV) [5], varicella zoster (VZV) [6, 7 and 8], mumps [9], influenza [10], lymphocytic choriomeningitis [11] and polyomaviruses [12, 13]. Four types of autism disturbances were diagnosed until 2013 including; [autistic, Asperger's syndrome, childhood disintegrative and pervasive developmental disorder].

The properties virus Herpes viruses a linear double stranded DNA which comprises an source of replication, two 8–10 kb ,Both directions are left and right duplication termini, and a unique fragment that is 143–145kb have a central viral core [14]. This DNA was a hoop shape, typified by a hole during the mid and the DNA was fixed in a proteinaceous spinning wheel [15]. The capsid two fold symmetry has sixteen surfaces (icosahedral) and its diameter from a hundred up to one hundred and twenty of partly depends on the thickness of the surfaces owns one hundred sixty-two capsomeres. The capsid composition of HHV-8 was determined through an electron microscope that has three dimensions consisting of twelve pentons, one hundred and fifty-hexons, three hundred and twenty-three triple order of twenty faces [16].

TNF- α was principally secreted by macrophages and was generally convoluted in the innate immune response during the TNF receptor and activates NF- κ B to organize downstream genetic material. TNF- α , in addition to IL-1's and IL-6, can totally irritate for the peripheral blood until the brain to immediately impact brain duty through their receptors [17]. Interleukin -37 belongs from IL-1 family and considered anti-inflammatory and a role in the treatment of autism spectrum [18,19].

2 .Material and Methods

2.1 Real Time PCR Technique: This technique was relied on for the diagnosis of viral infection, and an extraction was used from (Canvax HigherPurity™ Viral DNA/RNA Extraction Kit AN0605.UK.). The primers designed according to NCBI by us for the purpose of diagnosing disease and serotype virus including HHV type 6&8 also TNF-alpha and IL-37 are shown in the table (1).

Table (1): Primes design of HHV type 6&8 as well autism spectrum disorders depending on the NCBI

Type/subtype	Name	Sequences	Base s	PCR product size
Autism spectrum disorders	Primer F	GTGTCCGTGGAGTTTGGAGT	20bp	248 bp
	Primer R	GCTGCAAGAGGAATCAGACC	20bp	
HHV type 6	Primer F	CTCGGGACAACCTGGCTAAAC	20bp	155 bp
	Primer R	CTCGAGACCCGCAATGTTAT	20bp	
HHV type 8	Primer F	CACAGTGTACGGCTCGAGAA	20bp	178 bp
	Primer R	GGTGATCCTCCTCACTGGAA	20bp	
TNF alpha	Primer F	CATTCTCTTCTCCCAACA	20bp	191 bp
	Primer R	TTTCACCTCCATCCATCCTC	20bp	

IL-37	Primer F	CCCCACCATGAATTTTGTC	20bp	184 bp
	Primer R	GGATTGCTTCCACAAAGGA	20bp	

2.2. Preparation of cell culture :

The virus isolation method was based on (Carloset al ., 2014), but chicken embryo fibroblast cells were prepared in the laboratory from chicken embryonated eggs locally.

3.Results :

3.1.Diagnosis viruses and cytokines:

Samples were collected from children with autismspectrum and according to clinical signs from the specialized centers for patients, as well as taking samples from mothers, where the total number of samples was 86 samples and the samples that appeared positive 54 which were associated with the virus ,human infected including 33 cases of HHV type 6 while 21 cases of HHV type 8in figure (1)and the diagnosis was made through examination real time - PCR.In addition, it was divided into three groups according to age for infected children and mothers, where the results showed that group (1.5-4.5) years for children and group (31-36) years for mothers had the highest rate of viral infection, which was(23) and (25)respectively compared with other groups in figure (2 and 3).

While of diagnosis TNF-alpha appear after 20-24 cycle according one stepe of acute,but IL-37 show from 28-34 cycle of chronic infected patientsand it is considered within the second and third stage according to the concentrationin figure (4).

After 48 hours, the cellular effects were observed as shown in the figure (5) for the infected cells with the virus, where the cells were enlarged in size and shape compared to non infected cells with the virus,as well as with the infected cells, and then they were inhibited by gamma rays including cobalt (Co-60) and cesium (Cs) elements.

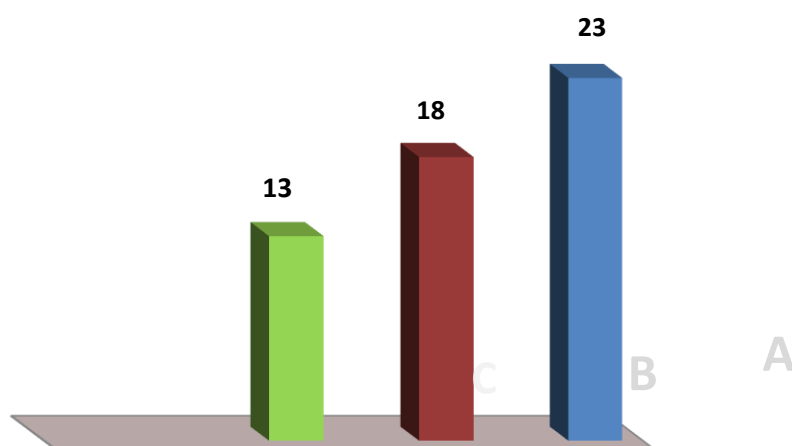


Figure (1): The infected children number with autism spectrum disorders associated human Herpes virus type 6&8 dependent different age including three groups : A (1.5-4.5) year ,B(5.5-8.5) year and C(9.5-11.5) year .

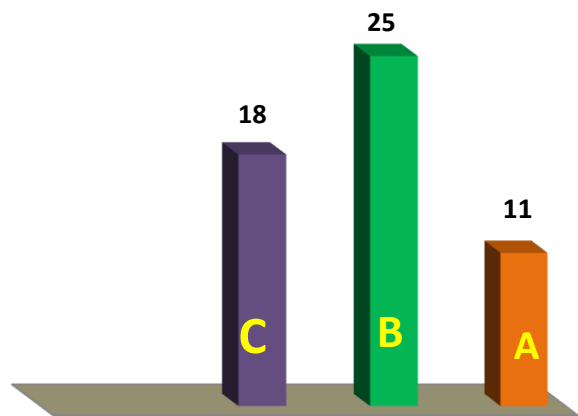


Figure (2): It shows carrying of virus mothers number during pregnancy according to age groups including three groups :A(25-30) year, B(31-36) year and C(37-42) year.

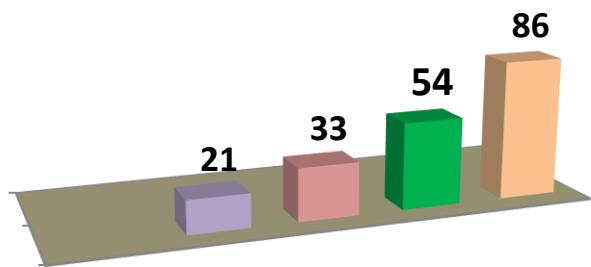


Figure (3): Total cases of collection samples was 86 cases but 54 case human infected including 33 case of HHV type 6 while 21 case of HHV type 8

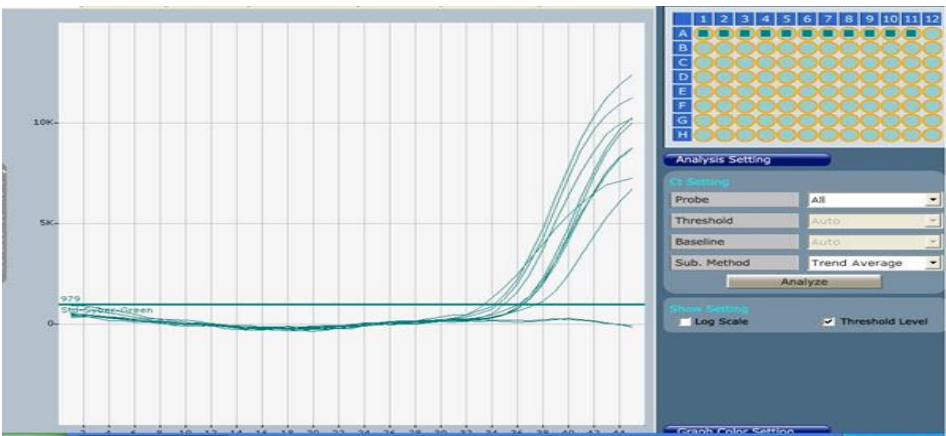


Figure (4) Diagnosis of Autism spectrum disorders with HHV type 6 & 8 also TNF-alpha and IL-37 by RT-PCR.

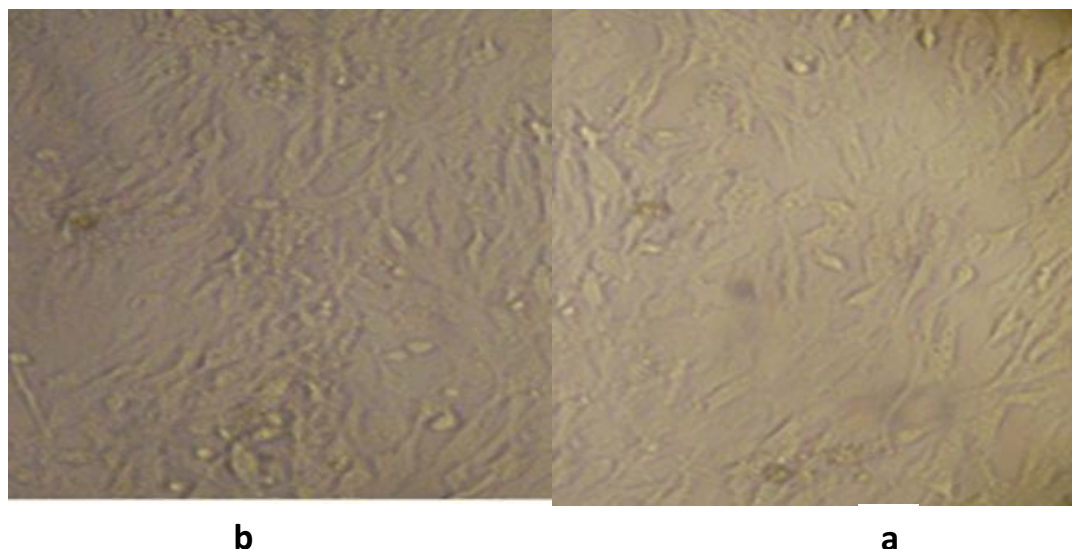


Figure (5) Cytopathic effect of human infected cells in (b) after 48 h inoculated of human Herpes virus type 6&8, non-infected (a)

Arithmetical examination:

The “Statistical package for social science”: (SPSS) blueprint (version-19) was used in statistical .

Discussion:

Whereas in Catherine et al ., 2006 a study, the diagnosis of infection from the age of 9 was higher than age 2, but less severe in symptoms compared to age 2 ,While in my study the group (1.5-4.5) years was higher compared to the other groups .

Through our research, we found that the cause of non-genetic autism spectrum is due to the presence of a latent viral infection in the mother’s body, which previously caused due to many abortions repeated the virus, and cases that did not cause abortions resulted in a child with autism spectrum.

In the second group of mothers , the concentration of the virus was high compared to the other groups, because the mothers had undergone continuous abortions before the birth of the affected child, and thus the virus was transmitted to the children and caused them to have autism spectrum disorders that is not hereditary compared of cause genetic.

The types of autism spectrum are different and some of the types only affect females. In addition to that, the onset of the disease varies according to the type, so it is necessary to search for the onset of the disease in relation to whether the cause is not hereditary or the cause is a virus, so it is necessary to know the onset of symptoms in children and compare it with the genetic type, age, gender, as well as the rest babies who are born after an affected child is likely to develop autism spectrum or not.

The diagnosing of both viruses, the study by the real time PCR method, and the virus was also diagnosed by many studies from 2000 up to 2020 for each of the (Francis ,2000; David

and Newell ,2012 and Niesters, 2020) .In addition to many other studies that have not been addressed, but the design of the primers was different, as the primers were designed depending on the location of (NCBI) and the type of disease.

The CPEobserved of the infected cells as described in the results, and these changes were also observed in the study of (Nerurkar,1983; Samiksha et al ., 2011 and Joshua et al ., 2018) but the cells culture of cell line and disease were differential.While in cytokines that includes (TNF-alpha and IL-37)) there are no studies in terms of diagnosis through real time PCR .

There are no previous studies on the use of gamma rays to inhibit the human Herpes virus type 6 and 8 , but in our study, gamma rays were used for cobalt (Co-60) and caesium (Cs) elements, and both viruses were successfully inhibited with a dose (6.864) mSv/h.) .And then it was confirmed by diagnosing the inhibited samples through realtime PCR, as well as through tissue culture, where no cellular changes were observed on the cells that were inhibited after 4th passage.

References

- 1.Charles A. Dinarello and Richard E. 2019. Contributed by Susan E. Leeman.
2. Autism spectrum disorder - Symptoms and causes. 2019.Mayo Clinic.
- 3.Ruggieri V .2020. Autismo, depresión y riesgo de suicidio [Autism, depression and risk of suicide]. Medicina (in Spanish). 80 Suppl 2: 12–16.
4. Richa S. Fahed M. Khoury Eand Mishara B .2014. Suicide in autism spectrum disorders. Archives of Suicide Research. 18 (4): 327–39.
- 5.Sanchack K and Thomas C. 2016. Autism Spectrum Disorder: Primary Care Principles. American Family Physician. 94 (12): 972–979.
- 6.Oswald Dand Sonenklar N. 2007. Medication use among children with autism spectrum disorders. Journal of Child and Adolescent Psychopharmacology. 17 (3): 348–355.
7. Samsam M.Ahangari Rand Naser.2014. Pathophysiology of autism spectrum disorders: revisiting gastrointestinal involvement and immune imbalance. World J Gastroenterol . 20 (29): 9942–9951.
- 8.Geschwind D. 2008. Autism: many genes, common pathways. Cell. 135 (3): 391–395
- 9.F84. 2013. Pervasive developmental disorders. ICD-10: International Statistical Classification of Diseases and Related Health Problems: Tenth Revision. World Health Organization. 2007.
- 10.Pinel J .2011. Biopsychology (8th ed.). Boston, Massachusetts: Pearson. p. 235.

11. Rogers S. 2009. What are infant siblings teaching us about autism in infancy. *Autism Res.* 2 (3): 125–137.
12. Rapin I and Tuchman R. 2008. Autism: definition, neurobiology, screening, diagnosis. *Pediatric Clinics of North America.* 55 (5): 1129–1146.
13. Filipek P, Accardo P, Baranek G, Cook E, Dawson G, Gordon B, Gravel J, Johnson C, Kallen R, Levy S, Minshew N, Ozonoff S, Prizant B, Rapin I, Rogers S, Stone W, Teplin S, Tuchman R and Volkmar F. 1999. The screening and diagnosis of autistic spectrum disorders. *J Autism Dev Disord.* 29 (6): 439–484.
14. Tang H, Kawabata A, Yoshida M, Oyaizu H, Maeki T, Yamanishi K and Mori Y. 2010. Human herpesvirus 6 encoded glycoprotein Q1 gene is essential for virus growth. *Virology.* 407 (2): 360–7.
15. Autism Spectrum Disorder, 299.00 (F84.0). 2013 In: American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.* American Psychiatric Publishing.
16. Chaste P and Leboyer M. 2012. Autism risk factors: genes, environment, and gene-environment interactions. *Dialogues in Clinical Neuroscience.* 14 (3): 281–292.
17. Banks W. 2015 The blood-brain barrier in neuroimmunology: tales of separation and assimilation. *Brain Behav Immun.* 44:1–8.
18. Amaral D. 2017. The effects of neonatal amygdala or hippocampus lesions on adult social behavior. *Behav Brain Res* 322(Pt A):123-137.
19. Irene T, Arti B, Patel H, Sabina B, Pio C, Profile Susan E, Leeman, and Theoharis C. Theoharides. 2019. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. *PNAS.* 116 (43) 21659-21665.
20. Carlos N, Yumi H, Carla F, Galhardi N, Lopes W, Andrade B, Korki d, Cândido E, Marcos M, Azevedo A, dos S, Gon. 2014. Elisa Carvalho Linhares. *An. bras. dermatol.* 89 (3) .
21. Catherine L, Susan R, Pamela S, DiLavore, Cory S, Audrey T and Andrew P. 2006. Autism From 2 to 9 Years of Age. *Arch gen psychiatry.* Vol. 63.
22. Francis L, Nathalie D, Willy R, Jean-Claude N. and Vincent M. 2000. Quantitative Analysis of Human Herpesvirus 8 Viral Load Using a Real-Time PCR Assay. *Journal Of Clinical Microbiology,* p. 1404–1408 Vol. 38, No. 4.
23. David J and Newell W. 2012. Detection of human herpes virus 8 by quantitative polymerase chain reaction: development and standardization of methods. *Speicher and Johnson BMC Infectious Diseases.* 12:210.

24.Niesters H.Doornum J. Guldemeester G. and Osterhaus A.2020.Diagnosing Herpesvirus Infections by Real-Time Amplification and Rapid culture. Journal of Clinical Microbiology. Vol. 41, No. 2.

25.Nerurkar L. Jacob A. Madden D and SeverJ.1983.Detection of genital herpes simplex infections by a tissue culture-fluorescent-antibody technique with biotin-avidin. Journal ListJ Clin Microbiol.17(1).

26.Samiksha C. Maribel M. Frances A. Frank S. Yuanxiang Zand Vaibhav T .2011.Herpes Simplex Virus Type-1 (HSV-1) Entry into Human Mesenchymal Stem Cells Is Heavily Dependent on Heparan Sulfate. BioMed Research International .

27. Joshua G. Eric E.Maya Tand Ursula A.2018. Analyses of Tissue Culture Adaptation of Human Herpesvirus-6A by Whole Genome Deep Sequencing Redefines the Reference Sequence and Identifies Virus Entry Complex Changes. Viruses. 10(1): 16.