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Herpes Simplex Virus Seroprevalence in Children with Autism Spectrum Disorder: A Cross-Sectional Study

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Abstract

Background: exposure to these viruses, HSV-1 and HSV-2, has been linked to neurological and behavioral problems in children that exhibit symptoms similar to those of schizophrenia and autism, these Viruses can cause neuronal cell death and trigger a widespread inflammatory response, which may impact the development of autism. Several case reports suggest a link between herpes simplex virus (HSV) infection and the onset of autism spectrum disorder (ASD). **Objectives:** to investigate the prevalence of herpes in autistic children. **Material and Methods:** Collected 400 blood samples from autistic children, with 258 males and 142 females. The age range was 2 to 16 years, with a mean age of 5.87 ± 2.544 years. ELISA technology identified the presence of a herpes infection. The samples were centrifuged, separated, and stored at 4°C until the detection tests for HSV1 and HSV2 were conducted. **Results:** Individuals diagnosed with autism spectrum disorder (ASD) had a discernible inclination towards elevated levels of antibodies against the Herpes Simplex Virus HSV-1 (60%), and HSV-2(25.5%).

Conclusions: Although the outcomes are positive, there remains a possibility that HSV contributes to ASD for two specific reasons. Firstly, the presence of elevated antibody levels and the diversity in age, sex, ASD levels, or severity necessitate additional research. Furthermore, the virus can operate as a catalyst or underlying cause for specific infants who have a genetic or environmental inclination towards these problems**.**

Keywords: HSV-1, HSV-2, Autism spectrum, viral infection

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social communication difficulties, restricted interests, and repetitive behaviors [1].

It usually appears in early childhood and is thought to be caused by a combination of genetic, environmental, and neurological factors [2].

Autism spectrum disorder (ASD) is becoming more common, affecting one out of every 88 infants in the United States, with a clear male predominance. Only 10% of ASD individuals have a recognized cause, while the other 90% are categorized as idiopathic. In the absence of a clearly defined causal agent, preventative interventions become unfeasible [3].

Although significant research has been conducted on genetic tendencies, there has been a recent shift toward environmental causes, such as viral infections. Herpes viruses, a type of DNA virus containing double-stranded genetic material, have

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been proposed as probable causes of ASD. This is because they can infect the central nervous system (CNS) for a long period and influence the body's immunological responses. [4].

Infections, the measles vaccine, vitamin D deficiency, and oxidative stress have all been investigated as possible causes of autism spectrum disorder. According to a recent theory, autism spectrum disorder (ASD) is caused by abnormal immune system reactions triggered by viral infections. This eventually leads to the malfunctioning of specific parts of the central nervous system. Several case studies show a link between herpes simplex virus (HSV) infection and the onset of autism spectrum disorder (ASD) [5, 6].

Viruses can cause neuronal cell death and a systemic inflammatory response, which may influence the development of autism. Exposure to these viruses, specifically HSV-1 and HSV-2, has been related to neurological and behavioral abnormalities in children that mimic symptoms of schizophrenia and autism. [7].

HSV-2 infection during early pregnancy can double the risk of developing autism in male fetuses, according to a Norwegian study. The study found that increasing HSV-2 IgG levels in maternal mid-pregnancy plasma doubles the risk of autism in male fetuses. [7].

In recent years, there has been increased attention given to the potential involvement of infectious agents, specifically viruses, in the development of ASD. Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are of particular interest among these viruses because they tend to infect the nervous system and can remain dormant there for a person's entire life. HSV-1 mostly causes oral lesions, commonly known as cold sores, but can also result in genital herpes through sexual transmission. On the other hand, HSV-2 is mainly linked to genital herpes [8, 9].

Multiple studies have examined the frequency of HSV-1 and HSV-2 infections in persons with ASD, with the hypothesis that viral infections might play a role in the onset or worsening of ASD symptoms. It is essential to understand the frequency of herpes simplex virus antibodies in individuals with ASD to clarify the possible link between viral infections and ASD. This knowledge is also important for guiding therapeutic management approaches and preventive measures [10].

This study aims to investigate the frequency of HSV-1 and HSV-2. By investigating the connection between herpes simplex virus infections and ASD, we can gain vital knowledge about the intricate interaction between viral variables and neurodevelopmental outcomes. This can potentially lead to innovative strategies for preventing and treating ASD.

2. Material and Method

The study has been approved by the College of Medical Al-Iraqia University; the sample was collected from each child after obtaining consent from the parents. Between September 2023 and March 2024, at the National Autism Center/Teaching Hospital for Children/Medical City in Baghdad, 400 blood samples. The age distribution of the patients ranged from 2 to 16 years. ELISA technology identified the presence of a herpes infection.

2.1. Patients and Sampling:

2.2. ELISA Protocol:

For each case, we extracted a volume of 5 mL of blood from each patient using a new syringe. We placed the specimen in gel tubes and centrifuged it at 3000 rpm for 10 min to separate the different components of the blood. We then transferred the serum to Eppendorf tubes. We kept the sample tubes for the serological assay at a temperature of 4 °C until we used commercially available ELISA

kits (Bioactiva; Germany) (4 kits with 96 wells) for the detection test for HSV1 (IgG) and HSV2 (IgG).

2.3 Statical Analysis:

Data were entered, checked, and analyzed using computer software programs of Statistical Package of Social Science (SPSS) version 26.

Results

Immunoglobulin tests were conducted on samples to detect Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2). The complete sample was tested for both viruses separately. The average concentration of IgG for HSV-1 was 21.58266 ± 16.961378, while for HSV-2 it was 8.68502 ± 5.717347 (Figure 1). According to the cutoff value, most patients tested for HSV-1 were found to have a positive test result (60.5%), while approximately three-quarters of patients tested for HSV-2 were negative (74.5%) (Figure 1).

When we compared the age of the study's sample with the severity of the disease, no significant differences were found between study comparative groups among both types of herpes virus infection -1 and -2 respectively (P> 0.05) as in (table 1).

However, significant differences were observed solely among patients' age groups of herpes virus infection type 1 (HSV-1), as higher proportions of three forms of the severity of mild, moderate, and severe were observed among the age group of 3-6 years old (66.1%, 77.9% and 41.9%) respectively in comparison to other age groups (Likelihood Ratio: 19.566, df: 8, P = 0.012). Similarly, among herpes virus -2 infection, the three forms of ASD disease were predominately observed among the age group of 3-6 years old but unfortunately such differences were not statistically significant (P > 0.05) as in (table 2).

Regarding patients' sex and their autism spectrum disorder (ASD) severity among herpes infection type 1, moderate and severe infections were significantly higher among male than female patients as compared to mild infection that was female dominant (75.2% and 71% vs. 51.8%) respectively ($x2$: 12.613, df: 2, P = 0.002). Nevertheless, no significant differences were observed regarding patients' sex and their ASD severity among herpes infection type 2 (P > 0.05) (Table 3).

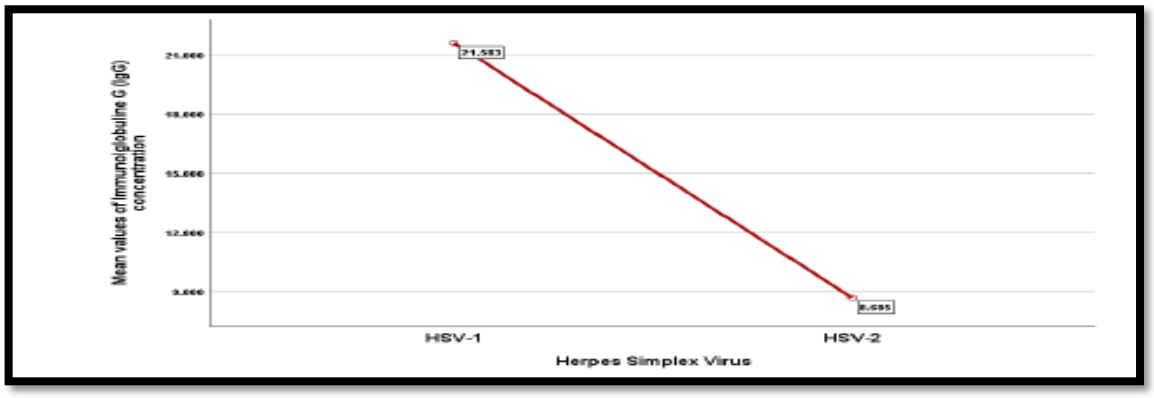

Figure 1. The mean value of IgG concentration among the study's sample test of herpes simplex virus (each n=200)

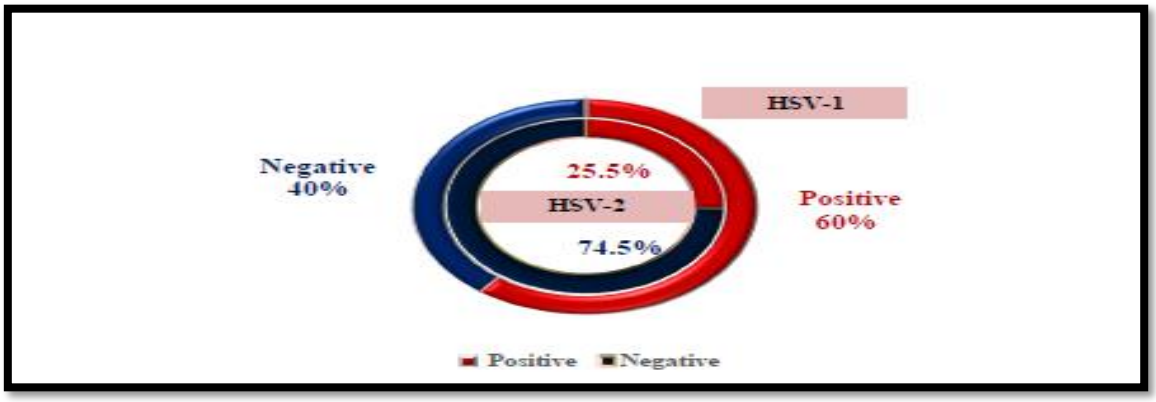

Figure 2 Positivity test of herpes simplex virus type test among the study's sample (each n=200)

Table 1 Comparison of the study's sample mean of age according to ASD disease severity among herpes simplex virus groups (n= 400)

ASD Severity	Hereps simplex virus groups					
	HSV-1[a] (n=200)			HSV-2[b] (n=200)		
	n	Mean ± SD	Mean difference [c]	n	Mean ± SD	Mean difference [c]
Mild	56	5.96 ± 2.896		47	5.83 ± 2.593	
Moderate	113	5.49 ± 2.215	-0.47	131	5.63 ± 2.132	-0.2
Severe	31	8.16 ± 3.289	2.2	22	5.80 ± 2.758	-0.03

[a]: F= 1.343, df: (44, 155), P= 0.098, [b]: F= 0.770, df: (30, 169), P= 0.799, [c]: Mean differenc from Mild disease group.

Table 2 Distribution of patients by their severity of ASD disease and their age groups among herpes virus groups (n=400)

Patients' age- groups (Years)	HSV-1[a]						HSV-2[b]					
	ASD Severity						ASD Severity					
	Mild (56)		Moderate (113)		Severe (31)		Mild (47)		Moderate (131)		Severe (22)	
	n	%	n	%	n	%	n	%	n	%	n	%
< 3	2	3.6	2	1.8	-	-	1	2.1	-	-	-	-
3-6	37	66.1	88	77.9	13	41.9	35	74.5	92	70.2	15	68.2
7-10	13	23.2	17	15	10	32.3	7	14.9	35	26.7	5	22.7
11-13	3	5.4	5	4.4	6	19.4	4	8.5	4	3.1	2	9.1
≥ 14	1	1.8	1	0.9	2	6.5	-	-	-	-	-	-

[a]: *Likelihood Ratio*: 19.566, df: 8, P = 0.012, [b]: *Likelihood Ratio*: 7.975, df: 6, P = 0.240

Table 3 Distribution of patients by their severity of ASD disease and their sex among herpes virus groups (n=400)

Patients' sex	HSV-1[a]						HSV-2[b]					
	ASD Severity						ASD Severity					
	Mild (56)		Moderate (113)		Severe (31)		Mild (47)		Moderate (131)		Severe (22)	
	n	%	n	%	n	%	n	%	n	%	n	%
Female	29	51.8	28	24.8	9	29	18	38.3	49	37.4	9	40.9
Male	27	48.2	85	75.2	22	71	29	61.7	82	62.6	13	59.1

[a]: x^2: 12.613, df: 2, $P = 0002$, [b]: x^2: 0.101, df: 2, $P = 0.951$

Discussion

Researchers have hypothesized that a virus may be a cause of autism spectrum disorder (ASD), and this virus is thought to be associated with the Herpesviridae family [7].

These viruses exhibit a preference for infecting the central nervous system and engage in intricate interactions with the host immune system, which may potentially lead to the development of various immunological illnesses [11].

This is our finding on immunoglobulin tests on samples to detect HSV-1 and HSV-2. Key findings include high average IgG concentrations for HSV-1 (21.58266), suggesting a significant level of chronic infection or widespread transmission in the population studied [13]. The lower IgG concentration for HSV-2 (8.68502) is in line with previous studies showing lower transmission rates and more recent infections. The 60.5% positivity rate for HSV-1 is consistent with global trends, especially in older children and adults. The 25.5% positivity rate for HSV-2 is within the expected range, reflecting transmission dynamics and lower overall seroprevalence [14].

Significant differences in HSV-1 infections among different age groups, particularly higher severity in children aged 3-6 years, might indicate that younger children are more susceptible to more severe presentations of HSV-1.[4]

Gender differences in HSV-1 severity, with males having more moderate and severe forms while females predominantly have mild infections, could reflect underlying biological or social factors influencing disease presentation and severity. The lack of significant differences in HSV-2 infections regarding sex and ASD severity is consistent with some prior research indicating that HSV-2's impact might be more uniform across different demographic groups [15].

However, the association between age groups and ASD severity in HSV-2 infection was not statistically significant. The study also found that male patients had higher rates of moderate and severe infections compared to females, while mild infections were more prevalent in females [12].

For herpes infection type 1, the chi-square test revealed a significant difference in ASD severity levels by sex, but it found no significant differences between patients' sex and ASD severity for herpes

infection type 2. Despite the positive results, it is still possible that HSV plays a role in ASD for two reasons. Firstly, the high levels of antibodies and the variations in age, sex, ASD levels, or severity indicate the need for further investigation. Second, the virus could potentially serve as a trigger or causative factor for certain children with a genetic or environmental predisposition to these disorders.

Conclusion

Based on evidence illustrated from the study, the study concluded herpes infection was prevalent among autistic child with variation among their sex and their type and severity of infection.

Conflicts of interest

"There are no conflicts to declare".

Funding

"None"

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