The Role of Congenital Cytomegalovirus (CMV) In Autism Children

***Gaber Ajeel , S1 , Salman Hashim, S2,**

1.Sattar Gaber Ajeel , department for microbiology , medicine collage from Ibn Sina University of medicine and pharmaceutical science , Iraq – Baghdad .
2. Saad Salman Hashim , department of medicines , medicine collage from Ibn Sina University of Medical and pharmaceutical science , Iraq – Baghdad .
Author: drstaargaber@gmail.com
sattar.gaber.ajeel@ibnsina.edu.iq

Conclusions

Cytomegalovirus (CMV) an important virus that play a critical rule in autism , which was higher rate in children with autism , with viral infected those mother .

Key Words : RT-PCR , autism , Cytomegalovirus .

Introduction :-

The Cytomegalovirus (CMV) is important member for herpes viruses that is known pathogenic to peoples especially (mother and child) (1). Congenital cytomegaloviruses has many manifestation of infection that is varies between the effect of immunologic and hematological status for the body (2).

The role of Cytomegalovirus (CMV) that related with with pregnant woman, that serological test is positive ,which represent the genome of the virus in the blood (3). Infection is occasionally, especially in before and through pregnant (4) Cytomegalovirus (CMV) , the important of healthy (5) . Persistent Cytomegalovirus (CMV) infection is manifested as autism in children (5).

Viral infection is the most prevalent comorbidity in pregnant (6) The Cytomegalovirus (CMV) replication autonomously and in the sometime the machinery cells support the cycle of through the natural selection (7). The reasons for Cytomegalovirus (CMV) may be an important pathogen in autism children . One of this reasons, The Cytomegalovirus (CMV) have transmogrify strategies that coopt the host for organism that imortans site for their evaluation . the important roles are directed to the cellular machinery and include complication and the ingenious solution that optimization the replication ,assembly and the shedding happened to virus (8,9). Cytomegalovirus (CMV) directly effect to the immune system and causes the reduction in product and survival of erythrocyte (10) most common complications of Cytomegalovirus (CMV) that infect in the autism child patients (11). Cytomegalovirus (CMV) target the bone marrow especially the progenitors of erythrocyte by the glycosphingolipid globoids building (Gb4) The link between Cytomegalovirus (CMV) (12).

Autism the disorder that affects brain development called autism spectrum disorder (ASD), the diagnostic of autism

and studied the psychological world after 1910 (13) .

Methods and Subjects :-

The study history and taken samples was performed on 100 mother from autism child and 100 autism children , and 100 apparently healthy children ,that used as control group (50 males and 50 females), the age of all patients and control group ranged between (2 – 4) years . And 100 women as control group (healthy) , All children were attended from centers care for autism child during the period from February 2022 till December 2022 .

Sample collection: -

We taken (5) ML from blood that aspirated from children , mothers from patients and heathy group included in this study . the samples from blood taken which divided in the two tubes , the first from the blood that collected in the EDTA the second that centrifuged at 3000RMP for 15 minute after clot . Serum kept in deep frozen,

The procedure of DNA extraction

Cytomegalovirus (CMV) the DNA that extracted for serum according to the manufacturers instruction .

1.then lysis the solution and wash solution (1and 2) were brought to the (60-66C) until not appearance of ice crystals .

2. 300 ml of solution that lysis and (100) ml from the sample was added to appropriate tube .

3 . the tubes wrere incubate to (5min) at (65 c) after that time centrifuged (8 sec)at 15000g.

4 .then the supernatants transferred to appropriate new tube .

5 . 20 ml of sorbent that added to all the tubes and then put in vortex for (6 Sec) and then incubate from 3 min at room temperature .

6.centrifuge the tubes to(30 sec), in 5000 g , after that use micropipette to remove the supernatants for all the tubes .

7 .300 ml washing solution , one that added to all tubes and then used vortex after that centrifuged to 30 sec in 8000 g then remove supernatant

8 .500 ml of washing two that added to all tubes then use vortex , centrifuged to (35 sec, in 8000 g) after that remove supernatant

(Repeated step 8 again) .

9 . the tubes incubated for 5b min in 66c .(the cap remind open)

10 . 15 ml from DNA added and then incubate from (5min – in 66 c)

11 . all tubes were centrifuge in (2 min in 12000 g) .

12 . the supernatant for tubes contain DNA transferred in to the new tube and stored in deep freeze .

Measure the extracted DNA concentration :-

The viral DNA extraction for all samples (mothers & children), that measure the concentration from DNA by using nucleic acid measuring instrument , analytic a gene for (USA) nano drop that put 5ml from extract DNA the cuvette which name (chip cuvette). Extracts with purity in between of ≈ 1.9 were included in the study, the extraction of DNA of the all samples which repeated

Diagnostic of cytomegalovirus CMV by (q RT-PCR)

Principle :-

By real time (PCR) production that made by reaction of a molecular fluorescent which were report in DNA that increased and appropriation in signal fluorescents . the florescent chemistry

employed that included the binding dyes for DNA specific sequence of the probe that florescent . the thermal cycler that equipment with fluorescent amplifications accrues in the end of thermal protocol , RT-PCR instrument software automatically that calculate by baseline cycler and thresholds instrument software automatically that calculating via baseline cycle and threshold , the standard curve that plot by use the data for defined standard , (Y) Axis the CT for cycle of threshold and (X) Axis the DNA for copy number of the viral . .

Results :-

Result of this study show that present of CMV Ab in serum of autism child from (18) in all all autism children (100) in compared with control group of children (100) in the centers of care child who taken for diagnosis the causes of the autism This mean that children have infection that perhaps transport from yours mothers found that table (1) .also table (2) show that the percentage of males (88.00%)infected with autism more than females (12.00%) , in table (3) show the arrangement (number) of autism child on his family we seen that the high number in the 3rd child(44.00%) and then in the 1stchild(30.00%) rather than the 3rd (20.00%) and other arrangement child ,in Table (4) show that number of male (88.00%) more than female (12.00%) autism children in the study groups, present of CMV antibody in serum of mother autism children till now (22.00%)) mothers from all mothers that taken for study (100) in compered with non-present (78.00%) and compered with control group (100) show in table (5) also , table (6) show that infection mother for autism child with CMV and taken vaccine in the 1st trimester was very low(4.00%) and after 1st trimester (14.00%) and through 3rd trimester (41.00%) respectively in compered with other trimester group and control groups , the number of mother that taken vaccine before pregnancy ,Table (7) show high infection of mother autism child with CMV through the 1st pregnant (42.00%) then (27 .00%)in the 2nd pregnant and(19 .00%) in the 3rd pregnant rather than other group and controls group , in table (8) show lower mother that taken vaccine before they decided to become pregnancy (31.00%) and the mother of autism children that non taken vaccine were (69.00%) in compered with control group that taken vaccine were(93.00%) , Table (9) Show that mother that exposure to abortion of first child (30.00%) more than other group and control group , Table (10) infection of mother autism child with CMV and taken vaccine before pregnancy was(30.00%) and after pregnancy (44.00%) in compered with control group before pregnancy, (90.00%) and (3.00%) after pregnancy, a significant showed difference (p>0.001) in all different tables .

Table (1) present of CMV antibody in serum of autism child and control

Abject	Study groups	
	Control	Autism children
Positive CMV	0	18
%	0.00%	18.00%
Negative CMV	100	82
%	100.00%	82.00%

p value		>0.001

Table (2) number of male and female autism child contain CMV antibody in serum

	Study groups		Study groups		
Gender	Control	Autism children	Control	present CMV Ab	Non-present CMV Ab
Female	50	12	50	4	8
%	50.00%	12.00%	50.00%	4.00%	8.00%
Male	50	88	50	14	74
%	50.00%	88.00%	50.00%	14.00%	74.00%
Total	100	100	100	18	82
%	100.00%	100.00%	100.00%	100.00%	100.00%
p. value.	>0.001		>0.001		

Table (3) the arrangement (number) of autism child on his family

Subject	groups			
	No .Control	%	No .Autism	%
1st child	30	(30.00%)	52	(52.00%)
2nd child	20	(20.00%)	22	(22.00%)
3rd child	44	(44.00%)	18	(18.00%)
Other	6	(6.00%)	8	(8.00%)
Total	100	(100.00%)	100	(100.00%)
p. value			>0.001	

Table (4) number of male and female autism child contain in the study groups

Subject	Control	%	Autism	%
Male	50	(50.00%)	88	(88.00%)
Female .	50	(50.00%)	12	(12.00%)
Total	100	(100.00%)	100	(100.00%)
P.Value			>0.001	

Table (5) present of CMV antibody in serum of mother autism child till now .

Subject	Control	%	Autism mother	%
Present	6	(6.00%)	22	(22.00%)
Non	94	(94.00%)	78	(78.00%)
Total	100	(100.00%)	100	(100.00%)
P. value			>0.001	

Table (6) infection mother for autism child with CMV and taken vaccine

Subject	Control	%	Autism mother	%
Through1st trimester	62	(62.00%)	4	(4.00%)
After 1st trimester	20	(20.00%)	14	(14.00%)
Through2ndtrimester	8	(8.00%)	19	(19.00%)
Through3rdtrimester	6	(6.00%)	41	(14.00%)
Non taken	4	(4.00%)	22	(22.00%)
Total	100	(100.00%)	100	(100.00%)
p. value .			>0.001	

Table (7) infection of mother autism child with CMV through pregnancy

Subject	Control	%	Infected	%
1st pregnancy	4	(4.00%)	42	(45.00%)
2nd pregnancy	2	(2.00%)	27	(27.00%)
3rd pregnancy	1	(1.00%)	19	(19 .00%)
4th pregnancy	0	(0.00%)	8	(8.00%)

Non infected	93	(93.00%)	4	(5.00%)
Total .	100	(100.00%)	100	(100.00%)
p. value			>0.001	

Table (8) mother of autism child who taken CMV vaccine before decided pregnancy

Subject	Control	%	Mother	%
Taken	93	(93.00%)	31	(31.00%)
Non	7	(7.00%)	69	(69.00%)
Total	100	(100.00%)	100	(100.00%)
p. value			>0.001	

Table (9) infection of mother autism child with CMV and causes abortion

Subject	Control	%	Infected	%
1st pregnancy	9	(9.00%)	30	(30.00%)
2nd pregnancy	2	(2.00%)	15	(15.00%)
3rd pregnancy	0	(0.00%)	9	(9.00%)
1st and 2nd pregnancy	3	(3.00%)	12	(12.00%)
1stand3rdpregnancy	0	(0.00%)	4	(4.00%)
2nd and 3rd pregnancy	0	(0.00%)	2	(2.00%)
Non abortion	86	(86.00%)	28	(28.00%)
Total	100	(100.00%)	100	(100.00%)
P. value			>0.001	

Table (10) infection of mother autism child with CMV and taken vaccine

Subject	Control	%	Infected	%
Before pregnancy	90	(90.00%)	30	(30.00%)
After pregnancy	3	(3.00%)	44	(44.00%)
Non taken	7	(7.00%)	26	(26.00%)
Total	100	(93.00%)	100	(100.00%)
p. value			>0.001	

Statistical analysis:- the data for this study was expressed , mean +_ SD . that used to analysis the average of samples, one away of analysis for varation (ANOVA) used to study the variations between . ratio compartment by test chi –squre . also the parametares analysis by the correlation Pearson.($p<0.001$) that accept as difference of statistically. groups all the data was expressed as mean +_SD. Students test was used

Conclusions :-

The congenital cytomegalovirus (Ccmv) lead to infection of fetal and causes neurodevelopment through infect the encephalon from fetus , also stimulation for responses of neuroimmune that damage the nerve cells and the effect of prodection of gene that inhibition of neuro progenitor cells by reduce or prevention of differentiation and proliferation ,which causes the inhibit of migration and the formation of the synapse , or triggering of information's the placenta and thus disrups supply Oxygen from the fetus that causes the mental retardation , abnormalities of neurodevelopment , cerebral neoplasm , and complications of ophthalmic , Autism infantile , additionally CCMV that infection during developing of hearing system which lead to auditory impairment . The damage in CNS is irreversible , the prognosis of congenital cytomegalovirus infection that improve , the prenatal diagnosis and the diagnostic from acquired perinatal should be also improved . The importance age for autism children in this study between 3-12 years , but some of children before two years old the result of my study agreement with (13)That he found that high prevalence of infection in children between 3-6) years , also the prevalence of autism is (0.8-1.0%) , and prevalence in male more than four time in female this result agreement with (14) . the variation in genetic traits , intrauterine environmental abnormalities and neurodevelopment mechanism that increase the percentage of infection with autism , the abnormalities of environmental which the one of the most factors for autism developing (15) . congenital cytomegalovirus infected fetus during the third trimester from gestation the result of this study agreement with (16) . incidence of cytomegalovirus in children with autism was detected DNA from CMV in DBS that show the main causes of autism is CMV , table (6) showing the mother that infected with CMV and taken vaccine in the first

trimester was very low percentage of infection in competence with the other trimester groups , this study agreement with (17) . The number of mothers that taken vaccine before becoming pregnancy

Table (7) show the high prevalence of infected mothers with cytomegalovirus in the first month of pregnancy more than other months . table (8) seen the lower of percentage from the mothers of autism children those taken vaccine before decided from become pregnant. table (9) explain the mother those exposure to abortion with the first child (the first pregnancy of mother) than the other pregnancy of other mothers from autism children . Table (10) progressive the mothers for autism children that infected with cytomegalovirus taken vaccine Before pregnancy (30.00%)while after pregnancy (44.00%) and non taken vaccine (26.00%)this result agreements with (18) .

References

1. Luck SE, Wieringa JW, Blázquez-Gamero D, et al. Congenital cytomegalovirus: a European expert consensus statement on diagnosis and management. Pediatr Infect Dis J 2017
2. Turner KM, Lee HC, Boppana SB, *et al* Incidence and impact of CMV infection in very low birth weigh infants. Pediatrics 2014
3. Schleiss MR. Congenital cytomegalovirus: impact on child health. Contemp Pediatr 2018 .
4. Chien-Heng Lin, I.-Ching Chou, Inn-Chi Lee, Syuan-Yu Hong, Cytomegalovirus Infection in Infancy May Increase the Risk of Subsequent Epilepsy and Autism Spectrum Disorder in Childhood, Children, 10.3390/children8111040, 8, 11, (1040), (2021)
5. Fernańdez-Alcántara M, García-Caro MP, Pérez-Marfil MN, et al. Feelings of loss and grief in parents of children diagnosed with autism spectrum disorder (ASD). Res Dev Disabil 2016.
6. 48. Melnick M, Sedghizadeh PP, Allen CM, Jaskoll T. Human cytomegalovirus and mucoepidermoid carcinoma of salivary glands: cell-specific localization of active viral and oncogenic signaling proteins is confirmatory of a causal relationship. Exp Mol Pathol 2012.
7. 49. Baryawno N, Rahbar A, Wolmer-Solberg N, Taher C, Odeberg J, Darabi A, et al. Detection of human cytomegalovirus in medulloblastomas reveals a potential therapeutic target. J Clin Invest 2011.
8. Tidake, Vishal & Mazumdar, Nilanjan & Kumar, A. & Rao, B. & Fatma, Dr Gulnaz & Raj, I.. (2023). Sentiment Analysis of Movie Review using Hybrid Optimization with Convolutional Neural Network in English Language. 1668-1673. 10.1109/ICAIS56108.2023.10073750.
9. Kandavalli, S. R., Wang, Q., Ebrahimi, M., Gode, C., Djavanroodi, F., Attarilar, S., & Liu, S. (2021). A brief review on the evolution of metallic dental implants: history, design, and application. *Frontiers in Materials*, 140.

10. M. Lourens, A. Tamizhselvi, B. Goswami, J. Alanya-Beltran, M. Aarif and D. Gangodkar, "Database Management Difficulties in the Internet of Things," 2022 5th International Conference on Contemporary Computing and Informatics (IC3I), Uttar Pradesh, India, 2022, pp. 322-326, doi: 10.1109/IC3I56241.2022.10072614.
11. Prabha, C., Arunkumar, S. P., Sharon, H., Vijay, R., Niyas, A. M., Stanley, P., & Ratna, K. S. (2020, March). Performance and combustion analysis of diesel engine fueled by blends of diesel+ pyrolytic oil from Polyalthia longifolia seeds. In *AIP Conference Proceedings* (Vol. 2225, No. 1, p. 030002). AIP Publishing LLC.
12. Abd Algani, Y. M., Caro, O. J. M., Bravo, L. M. R., Kaur, C., Al Ansari, M. S., & Bala, B. K. (2023). Leaf disease identification and classification using optimized deep learning. *Measurement: Sensors*, *25*, 100643.
13. Ratna, K. S., Daniel, C., Ram, A., Yadav, B. S. K., & Hemalatha, G. (2021). Analytical investigation of MR damper for vibration control: a review. *Journal of Applied Engineering Sciences*, *11*(1), 49-52.
14. Abd Algani, Y. M., Ritonga, M., Kiran Bala, B., Al Ansari, M. S., Badr, M., & Taloba, A. I. (2022). Machine learning in health condition check-up: An approach using Breiman's random forest algorithm. *Measurement: Sensors*, *23*, 100406. https://doi.org/10.1016/j.measen.2022.100406
15. Mourad, H. M., Kaur, D., & Aarif, M. (2020). Challenges Faced by Big Data and Its Orientation in the Field of Business Marketing. *International Journal of Mechanical and Production Engineering Research and Development (IJMPERD)*, *10*(3), 8091-8102.
16. Ruban, S. R., Jayaseelan, P., Suresh, M., & RatnaKandavalli, S. (2020, December). Effect of textures on machining of carbon steel under dry cutting condition. In *IOP Conference Series: Materials Science and Engineering* (Vol. 993, No. 1, p. 012143). IOP Publishing.
17. Naidu, K. B., Prasad, B. R., Hassen, S. M., Kaur, C., Al Ansari, M. S., Vinod, R., ... & Bala, B. K. (2022). Analysis of Hadoop log file in an environment for dynamic detection of threats using machine learning. *Measurement: Sensors*, *24*, 100545.
18. Suman, P., Bannaravuri, P. K., Baburao, G., Kandavalli, S. R., Alam, S., ShanthiRaju, M., & Pulisheru, K. S. (2021). Integrity on properties of Cu-based composites with the addition of reinforcement: A review. *Materials Today: Proceedings*, *47*, 6609-6613.
19. Kandavalli, S. R., Rao, G. B., Bannaravuri, P. K., Rajam, M. M. K., Kandavalli, S. R., & Ruban, S. R. (2021). Surface strengthening of aluminium alloys/composites by laser applications: A comprehensive review. *Materials Today: Proceedings*, *47*, 6919-6925.

20. Priel E, Wohl A, Teperberg M, Nass D, Cohen ZR. Human cytomegalovirus viral load in tumor and peripheral blood samples of patients with malignant gliomas. *J Clin Neurosci* 2015.
21. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, et al. Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* 2002; 62:3347–3350. doi: Published June 2002
22. Daniela Elgueta, Paola Murgas, Erick Riquelme, Guang Yang, Gonzalo I. Cancino, Consequences of Viral Infection and Cytokine Production During Pregnancy on Brain Development in Offspring, Frontiers in Immunology, 10.3389/fimmu.2022.816619, 13, (2022).
23. Roberto Coccurello, Maria Cristina Marrone, Mauro Maccarrone, The Endocannabinoids-Microbiota Partnership in Gut-Brain Axis Homeostasis: Implications for Autism Spectrum Disorders, Frontiers in Pharmacology, 10.3389/fphar.2022.
24. Dziurzynski K, Wei J, Qiao W, Hatiboglu MA, Kong LY, Wu A, et al. Glioma-associated cytomegalovirus mediates subversion of the monocyte lineage to a tumor propagating phenotype. *Clin Cancer Res* 2011.
25. Landa RJ. Diagnosis of autism spectrum disorders in the first 3 years of life. *Nat Clin Pract Neurol* 2008.
26. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcin C, et al. Global prevalence of autism and other pervasive developmental disorders. *Autism Res* 2012.
27. Soderberg-Naucler C, Johnsen JI. Cytomegalovirus infection in brain tumors: a potential new target for therapy? *Oncoimmunology* 2012.
28. Samhita Jain, Rebecca J. Baer, Charles E. McCulloch, Elizabeth Rogers, Larry Rand, Laura Jelliffe-Pawlowski, Xianhua Piao, Association of Maternal Immune Activation during Pregnancy and Neurologic Outcomes in Offspring, The Journal of Pediatrics, 10.1016/j.jpeds.2021.04.069, 238, (87-93.e3), (2021).
29. Cinatl JJ, Vogel JU, Kotchetkov R, Wilhelm DH. Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. *FEMS Microbiol Rev* 2004.
30. Maussang D, Langemeijer E, Fitzsimons CP, Stigter-van WM, Dijkman R, Borg MK, et al. The human cytomegalovirus-encoded chemokine receptor US28 promotes angiogenesis and tumor formation via cyclooxygenase-2. *Cancer Res* 2009.