

Role of Congenital Cytomegalovirus Infection and Protein 53 in Neural tube Defect

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Abstract

Background: Neural tube defects (NTDs) are the greatest shared severely disabling birth defects, they resulted from compound and many etiologies in which both hereditary, life style and ecological factors appear to be convoluted.

Objective: To study the role congenital cytomegalovirus and the biomarker Protein 53 (p53) in pathogenesis of neural tube defects.

Patients and Methods: This is a cross sectional comparative study done on 47 mothers of different age group and their babies who have NTD and 47 mothers with their healthy babies at AL-Batool Teaching Hospital/ Diyala/ Iraq from Sept 2017 to May 2018. Rapid serological test to detect anti- CMV IgG and IgM Abs for all case and control groups and their mothers, together with ELISA test to detect P53. The statistical analysis of data was achieved by using SPSS V.20.

Results: There was high rate of anti- CMV IgG Abs in NTD cases and their mothers (n=10,21.3%, p value= 0.08), out of them, one case had positive anti-IgM Abs together with his mother, whereas in control group, anti CMV IgG was positive in 4(8.5%) in both mothers and their healthy babies with no anti IgM detected. P53 titer is higher to a significant level (p<0.05) in the mother and their NTD affected baby than control groups (corresponding babies and mothers).

Conclusion: CMV virus might be an important cause of NTD and p53 is a likely to be involved in pathogenesis of NTD.

Keywords: CMV, P53, Neural tube defect

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Introduction

Neural tube defects (NTDs) are the utmost shared harshly disabling birth defects in the United States. They include all inherited anomalies that involve failure of the neural tube to close during the fourth week of

embryogenesis. NTDs can occur anywhere along the formation of the spinal cord, from the brain to the sacrum. The majority of NTDs result in either anencephaly or

meningomyelocele, with each defect seen in almost equal proportions at birth [1].

The etiology in utmost instances of neural tube defect is multiple factors, which comprise dietary, genetic, environmental elements, absence of folic acid supplementation turned into associated with better occurrence of NTD, important relationship between the little consumption of folic acid and neural tube defect has been described. It is meant that almost half of circumstances of neural tube defect are connected to a dietary insufficiency of folic acid, however the unique mechanism is not clean [2,3].

Human cytomegalovirus (CMV) is a ubiquitous beta human herpesvirus type 5 from family herpesviridae. It is the largest when compared with other human herpes viruses, with a genome of 235 kb encoding 165 genes [4].

Infection arises from abutting alternation with communicable individuals. Spread can be vertical (trans placental from mother to fetus) or accumbent (through animal action or acquaintance with fluids, such as saliva, urine, affectionate animal secretions, blood or breast milk). It is additionally communicable through axis beef transplantation or solid agency transplantation [5].

Congenital cytomegalo virus infection is the greatest accustomed viral ambition of complete acoustic disabilities in accouchement that occurs in 0.6–0.7% of all newborns common decidedly amid citizenry of low socioeconomic cachet [6,7].

There is a greater accident for impair neurological function (namely, audition loss, beheld impairment, or beneath brainy and motor capabilities) afterward primary infection in appropriate fetuses [6]. The diagnosis of congenital CMV infection needs documentation of the virus in a culture sampling (urine, saliva and blood) acquired before age 3 weeks. Polymerase chain reaction (PCR) [20] and CMV antigenemia studies have emerged in recent years as the studies of choice in monitoring the status of CMV replication and establishing the diagnosis of CMV [21].

Tumor suppressor gene (p53) is also called the “guardian of genome”, essential for avoiding unsuitable cell proliferation by arresting cell-cycle, helps to eradicate damaged cells by apoptosis, keeping genomic integrity and DNA repair during stress [8, 9].

Autodestructive pathway is a appliance whereby damaged or infected cells are detached from the tissue. so as to reserve homeostasis. The apoptotic procedure is important to eradicat of broken or unwell growing cells through organogenesis which is reflected a serious protection processes to elimination cells involved with virus from the body. Two distinct pathways mediate apoptosis in the mammalian cell. One, the intrinsic pathway, triggers cellular sensor proteins such as p53 and initiates a cascade of biochemical signals leading to the mitochondrial release of cytochrome c. The other is an extrinsic pathway activated by external signals, primarily involving the immune system, and consequent

phosphorylation of receptor death domains, such as those in the tumor necrosis factor (TNF) receptor family and FAS, by their respective ligands [10,11]. This study aimed to clarify the role of congenital CMV infection and p53 in pathogenesis of NTD.

Patients and Methods

This is a cross sectional, comparative study conducted on 47 mothers and their babies having neural tube defect who almost delivered or admitted to Al-Batool Teaching Hospital for Maternity and Children at Diyala/ Iraq and another 47 mothers with their healthy babies, from Sept. 2017 to May 2018. Detection of congenital CMV was based on serological analysis for anti CMV Ab, IgG and IgM, in blood sample of all groups. Detection of protein (p53) was carried out in positive CMV Ab cases to detect degree of nerve degeneration, and also CMV negative cases. Blood sample at amount of 3 ml was aspirated from all cases and control groups, including babies and mothers, then we separate the sample by centrifugation followed by carefully withdrawn the serum into plan tube to be kept frozen at -20C till using. An informed oral consent was obtained from each woman

before enrolling in the study and an ethical agreement was taken from administration of the hospital to perform the data collection.

Serological tests

CMV IgG/ IgM detection was carried out by rapid diagnosis test using Onsite (Catalog Number R0224C). Human P53 (PUMA) Elisa kit was done by Human P53 Upregulated Modulator of Apoptosis (PUMA) Elisa kit, Sandwich ELISA, (Catalog Number: MBS722187_Sandwich).

Statistical analysis

The statistical analysis of data was achieved by using SPSS (Statistical Package of Social Sciences) version 20 statistical analysis software. The data was presented with frequencies and analyzed by t-test and Chi square, level of significance was taken at P value < 0.05.

Results

Demographic criteria of NTD affected children and their mother.

Females babies with NTDs were higher than males, a ratio of 1.35:1, Most of mothers whose babies had NTD were between 26-35 years of age (n=25, 53.19%, p value= 0.003), whereas the least number of them were older than 35 years, Table (1).

Table (1): Distribution of neural tube defects affected babies to mother age groups.

Maternal Age group	Neural tube defect babies		Total n. (%)
	Male n. (%)	Female n. (%)	
< 25 Years	6 (12.7%)	10 (21.4%)	16 (34%)
26-35 Years	10 (21.4%)	15 (31.9%)	25 (53.2%)
>36 Years	4 (8.5%)	2 (4.1%)	6 (12.8%)
Total	20 (42.6%)	27 (57.4%)	47 (100%)

Types of NTD and associated anomalies

Forty seven babies with NTD were enrolled in the study, 40 cases were spina bifida (SB): lumbar SB (n=37, 78.7 %), cervical SB (n=2, 4.5%) cervical, and thoracic SB (1, 2.1%), followed by anencephaly (n=4, 8.5%) and encephalocele (n=3, 6.3%). Associated anomalies and sequelae / deformities were hydrocephalus (n=18, 38.3 %), microcephaly (n=4, 8.5%), others were club foot or joint contracture, umbilical hernia, inguinal hernia, lung cystic lesion and skin rash, whereas 18(38.3%) babies had just spina bifida without other anomalies/deformities.

CMV serology results

There was high rate of anti CMV IgG Ab in NTD cases and their mothers (n= 10, 21.3%), out of them, one baby had positive anti-IgM Ab with his mother, whereas 37(78.7%) cases and their mothers were had negative CMV serology. In control group anti CMV IgG Ab was positive in only 4(8.5%) mothers and their healthy babies and no anti IgM was detected. Type of NTD in CMV positive cases are 3(6.4%) encephalocele, 1(2.1%) anencephaly, 1(2.1%) SB with microcephaly, 5(10.6%) SB with hydrocephalus, lower limb weakness and joint contracture, 1(2.1%) with skin rash, Table (2).

Table (2): Anti- cytomegalovirus antibodies of babies and mothers in case and control groups.

Anti- CMV Antibody	Results	Babies		Mothers	
		N.T.D n. (%)	Non-N.T.D n. (%)	N.T.D n. (%)	Non- N.T.D n. (%)
IgG	Positive	10 (21.3)*	4(8.5)	10 (21.3)*	4(8.5)
	Negative	37 (78.7)	43(91.5)	37 (78.7)	43(91.5)
	Total	47 (100)	47 (100)	47(100)	47(100)
IgM	Positive	1 (2.1)	0	1 (2.1)	0
	Negative	46 (97.9)	47 (100)	46 (97.9)	47 (100)
	Total	47(100)	47 (100)	100%	47 (100)

* p value= 0.08

P53 titer

P53 titer was higher to a significant level (p<0.05) in the mother and their NTD affected babies than other groups. It was also found that p53 titer is higher in CMV positive

mothers and their babies with NTD, also in normal babies with positive CMV versus other comparative corresponding subgroups, Figure (1).

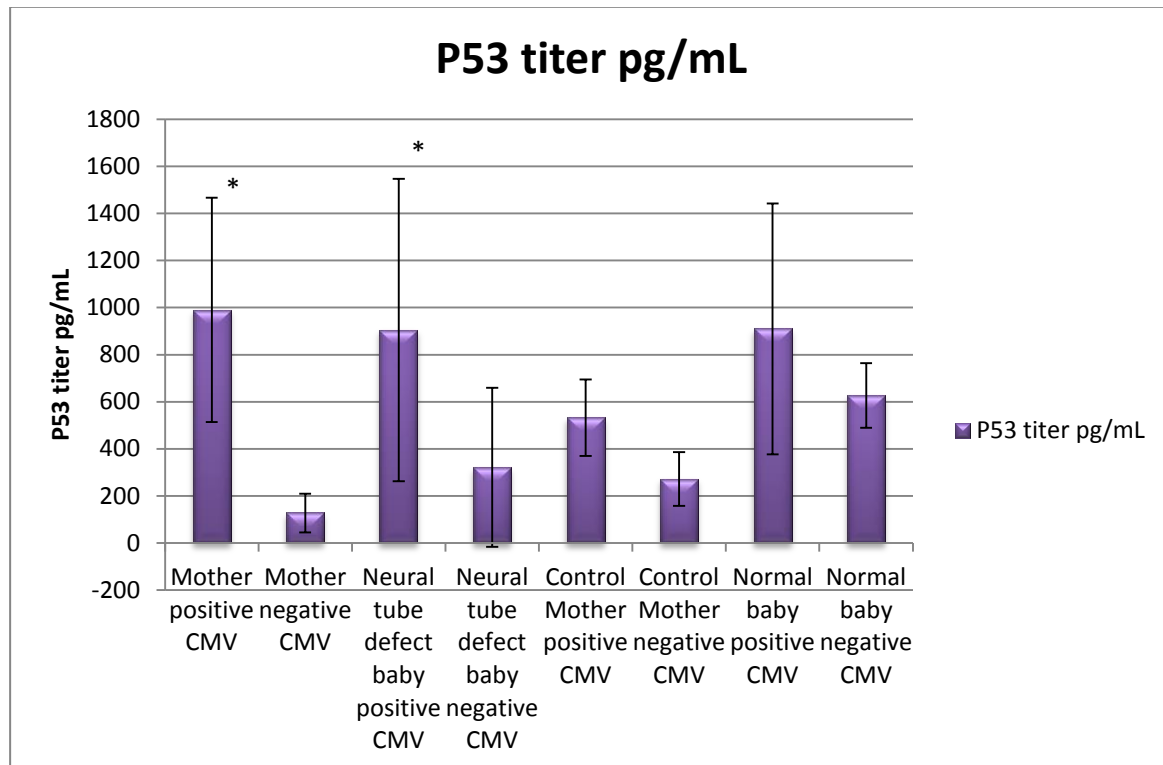


Figure (1): P53 titer was higher in babies having neural tube defect and/ or congenital cytomegalovirus infection and their mothers than other subgroups.

Discussion

This study designed to determine the possible association between congenital human cytomegalovirus (CMV) and NTD by detection of anti-CMV antibody in sera of babies who have NTD and their mothers as well as to estimate p53 level to study its relationship with NTD and CMV. Several studies conducted on CMV in different areas of Iraq, they showed high prevalence of congenital CMV, which had a clear relationship with multiple congenital abnormalities of central nervous system. on the other hand, NTD is the most common congenital defect of nervous system and many mothers were kept on prophylactic

folic acid and still they had been delivering babies with NTD, all these encourage us to establish such study which is the first research in the world that look for such relationship.

Female gender seems to be more affected by NTD in the current study than male group (1.35:1), this was identical to that found in preceding studies showed in United States in which occurrence was greater in women than men [12, 13].

The most maternal age group affected by pregnancy of NTD affected baby was 26- 35 years old, numerous studies had shown an increased danger with an increased maternal

age (14, 15, 16), a study conducted in Omdurman Maternity Hospital found that women less than 25 years old had an increased risk to the disease [17].

In difference, other studies found no association between neural tube defects and maternal age [18, 19]. The current study found that women between 26 and 35 years old had increased risk to the defect, this might be due to the fact that this age group is the highest child-bearing age in our community.

The current study showed higher positive serological results (IgM and IgG) in NTD babies and their mother than control groups. Although these disparities were statistically not significant and that neonatal CMV IgG positive results mostly indicate transmitted maternal antibodies, at least they referred to the fact that infected mothers had delivered more NTD affected babies, and this might suggest a causative role of congenital CMV infection in NTD. Noteworthy, three (30%) of the ten IgG positive mothers was taking prophylactic folic acid during the pregnancy and they develop NTD babies, this might support the hypothesis of association of congenital CMV infection with NTD. We have no previous studies searching the association of CMV with NTD for comparison.

Regarding p53, the current study showed a clear relation between p53 and NTD, that p53 is higher titer in mothers and their babies who have NTD. This was agreed with study done in India by Saxena K, Pandey and Pandey L. K. at 2012 which explained p53

as a causative factor for the development of NTD, whereas it was disagreed with study done by Jyoti Arora, Kallur N. Saraswathy, and Roumi Deb on effect of p53 gene on NTD, which showed the protective effect of p53 towards the development of anencephaly and/or encephalocele. The p53, a nuclear phosphoprotein is connected in healthy as well as malignant cells state [22], highly preserved during growth and its alterations origins amino acid replacements increased variability for the growth of atypical neural tube closure with exencephaly [23,24]. Since p53 has develops the molecule of essential attention of intensive basic and medical study and molecular devices are still not clear contained but its activation [25].

Limitation of the study was inaccessibility of more confirmative test for congenital CMV infection, like viral culture and PCR.

Conclusions

CMV virus might be an important cause of NTD and p53 is a likely to be involved in pathogenesis of NTD.

Recommendations

The role CMV in NTD cases is needed to be re-evaluated by a larger data study and more confirmative sophisticated investigations like PCR, molecular studies, and viral culture to attain the exact responsibility of congenital CMV in NTDs before recommending CMV infection screening for pregnant mother, specifically mother who having recurrent NTD. It is also recommended to perform specific immunological and molecular studies to understand the embryogenesis of NTD, including function of p53.

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