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Original Article

Dentistry Section

The Serological Evidence of Cytomegalovirus Infection as a Potent Aetiological Factor for Cleft Lip/Palate, Mental Retardation and Deafness

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ABSTRACT

Introduction: Congenital Cytomegalovirus (CCMV) infection is estimated to occur in 0.5% to 2% of all deliveries across the world. According to the available literature about Human Cytomegalovirus (HCMV) infected children, 0.5% to 1% acquire Cytomegalovirus (CMV) in utero, 40% acquire the infection within the first decade of life, between 15% to 70% acquire CMV infection in group day care settings and continue to shed the virus for 6 to 48 months after primary infection. Although, 90% of the infected infants are clinically asymptomatic at birth, shreds of evidence show that these infants are at risk for audiological, neurological, and developmental sequelae. Despite this, HCMV still remains undetected due to silent or asymptomatic nature of the virus.

Aim: The present study was aimed to test the hypothesis that HCMV can be a potential aetiologic factor in the development of cleft lip/palate, mental retardation and deafness.

Materials and Methods: The study was carried out in a controlled setting under strict aseptic conditions. Blood samples were collected from 80 children, who were selected strictly adhering to the inclusion criteria and were divided into four groups containing 20 children each. Group 1: 20 children with cleft lip/palate, Group 2: 20 mentally retarded children, Group 3: 20 completely deaf children and Group 4: 20 normal Children (control). The samples were tested for HCMV-specific Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies by using solid phase enzyme-linked immunosorbent assay and the obtained data were analysed statistically using ANOVA and Post-Hoc Tukey's tests.

Results: In the study group (Group1, 2 and 3) children, the overall positivity for HCMV- specific IgG was 100% and 5% borderline to IgM antibodies whereas in the control group (Group 4) it was 80% negative to HCMV- specific IgG and 100% negative to IgM antibodies.

Conclusion: From the observations noted in the present study, HCMV could be suggested as the potent aetiologic factor in the development of cleft lip/palate, mental retardation and deafness.

Keywords: Birth defects, Cross-sectional studies, Infected infants

INTRODUCTION

Human cytomegalovirus typically abbreviated as HCMV or commonly but more ambiguously referred to as CMV is a species of the Cytomegalovirus genus of viruses, which is a member of Herpes viridae. It is an easily transmissible beta herpes virus which is highly prevalent [1,2] and frequently associated with the salivary glands [3]. A large double-stranded DNA genome which possesses formidable coding capacity giving rise to more than 750 translational products and also a multitude of virus-encoded micro-ribonucleic acids (miRNAs) in infected cells reflecting the exceptional power and ability of this virus to manipulate and cope with the host. HCMV also alters innate and adaptive immunity of the host and results in immune dysfunction [4].

Though HCMV infection is typically unnoticed in healthy people, it can be life-threatening for the immunocompromised like in HIV-infected persons, organ transplant recipients, or newborn infants [5]. After the primary infection, HCMV can be reactivated at any time as it remains latent within the body throughout life and eventually, it may cause mucoepidermoid carcinoma and possibly other malignancies such as prostate cancer [6].

HCMV test is rarely considered at the time of diagnosis of any disease, but is the leading cause of congenital infections worldwide and commonest non-genetic cause of childhood hearing loss in the post-rubella era and a significant cause of neurodevelopmental delay [7-10]. The worldwide neglect of this problem is underscored by the continued lack of awareness of CCMV among health care workers and the public [11].

So, the present cross-sectional seroprevalence study was aimed to evaluate the evidence of CCMV infection in children with cleft lip/palate, mental retardation and deafness.

MATERIALS AND METHODS

This cross-sectional study was conducted between the time period 6th to 18th August 2015 among 80 children between the age group of 6-14 years selected from the Parivarthan deaf school, J & J Karunodhaya manasika Vikas kendram and the outpatient Department of Pedodontics and Preventive Dentistry, St. Joseph Dental College, Eluru, West Godavari district, Andhra Pradesh, India, to evaluate the relationship between a potential risk factor (CMV) in the development of the conditions like cleft lip/palate, mental retardation and deafness. The selected sample meeting the inclusion criteria [Table/Fig-1] of respective groups were divided into 4 groups.

- Group 1: 20 cleft lip/palate children.
- Group 2: 20 complete deaf children.
- Group 3: 20 mentally retarded children.
- Group 4: 20 normal children (control).

This study was done after the approval of Ethical Committee of St. Joseph Dental College and informed written consent was taken from all the parents/caretakers of the children participating in the study. Matching was done between the study group and control groups based on age, geographic location and by obtaining a thorough case history from the parents/caretakers of all the children

in the study group to rule out other aetiologic factors which were implicated in causing the condition and in control group to eliminate any health related issues.

Study Sample Size Determination

The below formula was used to calculate the sample size for the present study.

$$N = Z^2pq/d^2$$

Where N = the desired sample size when the population is more than 10,000.

Z = the standard variation usually set at 1.96 (which corresponds to 95% confidence interval).

p = proportion in the target population estimated to have particular characteristics. The rate was estimated according to the Indian population statistics 2011; 26,183 children (between age group 6 to 14 years) were present in Eluru [12].

Out of which, 216 (between age group 6 to 14 years) were suffering from cleft lip/palate.

387 (between age group 6 to 14 years) were suffering from complete deafness.

173 (between age group 6 to 14 years) were suffering from mental retardation.

q = 1.0 – p

d = degree of accuracy required; set at 0.05.

Therefore, the minimum sample size, $n = (1.96)^2\ pq/\ (0.05)^2$

Hence, by applying the formula stated above, the minimum sample size was 12, 20 and 10 subjects for cleft lip/palate, complete deafness and mental retardation respectively. So, a minimum sample size of 20 were taken for each study group and compared with 20 control group (normal children).

Procedure

A 3 ml blood was drawn from all 80 children transferred to vials and sent to Thyrocare® lab immediately for viral titre estimation using antibody testing (serology). Blood samples sent to the lab were clotted and centrifuged for serum separation prior to testing. All the sera were stored at -20°C. The serum samples were tested for CMV- IgG and IgM antibodies using commercially available µ-capture enzyme immunoassay method (ELISA kits -RADIM) for qualitative detection. In this test system, "Biotin-Streptavidin Complex" has been used to increase the sensitivity of the procedure. The specificity of the procedure used was also 100% with no nonspecific binding due to rheumatoid or other herpatic viruses.

Interpretation of the results was based on the controls provided with the kit. The test sample was said to be positive for IgG or IgM antibodies when its absorbance value was higher than the absorbance value of the cut-off control [Table/Fig-2].

STATISTICAL ANALYSIS

Descriptive statistics, ANOVA and Post-hoc Tukey's tests were performed with the obtained data using the Statistical Package for Social Sciences (SPSS) software Version 20.0 (Chicago, IL, USA) and presented as percentages, frequencies and mean values.

Groups	Inclusion Criteria	Exclusion Criteria
Group 1	Children suffering from cleft lip/ palate.	Syndromes, environmental risk factors during pregnancy like lack of folic acid, smoking, alcohol consumption, obesity, medications such as anti-seizure/ anticonvulsant drugs, acne drugs containing accutane, and a drug methotrexate which is commonly used for treating cancer, arthritis, and psoriasis
Group 2	Children suffering from complete deafness.	Genetic conditions – syndromes, late pregnancy, diseases in mother such as diabetes, heart and kidney disease.
Group 3	Children suffering from mental retardation.	Hereditary disorders: if any of the family members has a similar problem, genetic disorders- syndromes, and hearing loss due to trauma or loud noise
Group 4	Children without any underlying medical conditions.	Children with any underlying medical conditions

[Table/Fig-1]: The selection criteria for the study groups and control group.

Anti-HCMV Antibodies	Reference Range		
	Negative	Borderline	Positive
IgG	< 0.8	0.8-1.2	>1.2
IgM	< 0.9	0.9-1.1	>1.1

[Table/Fig-2]: The reference range values for anti-HCMV antibodies [13].

RESULTS

Results of the present study have been presented in [Table/Fig-3-5].

DISCUSSION

In developing countries and in communities with lower socioeconomic status, HCMV infection is more widespread and it represents the most significant viral cause of birth defects in industrialized countries [14]. In India, serological surveys in different parts have shown the prevalence of 80-90% seropositivity [15-18] of CMV antibodies (IgG) in women of childbearing age, but there is limited literature reporting the occurrence of birth defects due to CMV infection in India [19].

The estimated risk of permanent sequelae in infants surviving with symptomatic CMV disease ranges from 17% to 90%, with either intellect or hearing handicap occurring in 90% of patients [20]. According to the available literature congenital HCMV is the most significant infectious cause of deafness, learning disabilities, and intellectual disability in children [21,22]. However, there are no studies from India reporting its role in the development of deafness and mental retardation in children. Hence, in the present study 20 children with complete deafness and 20 children with mental retardation were included strictly adhering to the inclusion and exclusion criteria. The analysed data from the serological survey yielded a striking difference between the anti-HCMV IgG values of the complete deaf and mentally retarded groups when compared with the control group, which strongly supports the presence of latent infection. In case of anti-HCMV IgM values there was no

Variables		Group-1		Group-2		Group-3		Group-4	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
HCMV IgG	Positive	20	100	20	100	20	100	0	0
	Negative	0	0	0	0	0	0	16	80
	Borderline	0	0	0	0	0	0	4	20
HCMV IgM	Positive	0	0	0	0	0	0	0	0
	Negative	19	95	20	100	20	100	20	100
	Borderline	1	5	0	0	0	0	0	0

[Table/Fig-3]: Distribution of anti-HCMV IgG and IgM antibodies in all the four groups.

Mean optical density (OD ratio)	Group-1 cleft lip/palate	Group-2 complete deafness	Group-3 mental retardation	Group-4 normal children	ANOVA test
HCMV IgG	2.45 OD ratio	3.75 OD ratio	3.05 OD ratio	0.40 OD ratio	p-value < 0.001
HCMV IgM	0.30 OD ratio	0.10 OD ratio	0.10 OD ratio	0.10 OD ratio	0.05

[Table/Fig-4]: Mean Optical Density (OD) values of the anti-HCMV IgG and IgM antibodies in the study groups (1, 2 and 3) and Group 4 (control).

Post Hoc Tukey's test		Mean Diff.	p-value and significance
IgG	Group-4 vs Group-1	2.05	< 0.001, Highly Significant
	Group-4 vs Group-2	3.35	< 0.001, Highly Significant
	Group-4 vs Group-3	2.65	< 0.001, Highly Significant
IgM	Group-4 vs Group-1	0.20	< 0.001, Highly Significant
	Group-4 vs Group-2	0	1, Not Significant
	Group-4 vs Group-3	0	1, Not Significant

[Table/Fig-5]: Comparison between means and standard deviation of optical density of the study groups (1, 2 and 3) with the Group 4 (control) using Post-hoc Tukey's test.

difference which infers that there was no recent reactivation of the virus in these children. Serological evaluation was chosen in the present study as it is the most commonly used test to detect the presence of anti-HCMV IgG and IgM antibodies [23].

No cross-sectional studies till date reported the evidence that HCMV infection which could be the probable aetiological factor for the development of cleft lip/palate in children. So, the present study aimed to test this hypothesis, 20 cleft lip/palate children were evaluated serologically to detect the evidence of HCMV in their blood. The serological survey has yielded a striking difference between the anti-HCMV IgG values of cleft lip/palate group when compared with the control, which strongly supports the presence of latent infection. Marked difference was also noted between the anti-HCMV IgM values of the cleft lip/palate children and the normal children indicating a recent reactivation of HCMV infection that may have resulted in the increased antibody titres.

The results obtained in the present study further support the evidence provided by Weichert A et al., in their case report that micrognathia and cleft lip were potential effects of early CMV infection in developing foetus as the oral-facial organogenesis is very vulnerable to HCMV infection, because it is highly dependent on mesenchymal integrity and epithelial-mesenchymal interactions where the virus mostly inhabits [24].

Seroprevalence is age-dependent, 58.9% of individuals aged six years and 90.8% of individuals aged 80 years and older were positive for HCMV [25]. Ross SA et al., stated that serological tests were useful for determining whether a patient had HCMV infection in the past, by the presence or absence of antibodies IgG, IgM against HCMV [26]. So, in the present study, serology testing has been chosen to evaluate HCMV titres to estimate the previous exposure of the study groups to HCMV infection. The reported serological findings yielded 100% anti-HCMV IgG positivity in all the three study groups.

IgM antibodies are present in most individuals within a week or two after the initial exposure to CMV infection, they are the first line of defense by the host against HCMV and their production rises for a short time period and then declines. After several months, the level of CMV IgM antibody usually diminishes below detectable levels and additional IgM antibodies are produced when latent CMV is reactivated [27-30].

During the period of active CMV infection, the levels of anti-HCMV IgG accelerate and then stabilize. Once a person has been exposed to CMV, he will have some measurable amount of CMV IgG antibody in his blood for the rest of the life. Therefore, anti-HCMV IgG antibody testing can be used to confirm the exposure to a previous CMV infection [22].

The following factors can explain the low profile of CMV. First, most maternal and newborn infections are asymptomatic and therefore are not recognized at birth [31,32]. Second, the sequelae of CMV infection are frequently delayed in onset, at which point a retrospective diagnosis is challenging [33]. Third, the dogma that congenitally infected children who are born to women with pre-existing antibodies have normal outcomes has led to inattention to CMV in developing countries [34].

Our study unequivocally provides evidence of the role of CMV in the development of these conditions, thereby quoting the need for early diagnosis as maternal CMV infection can cause a considerable burden on society in terms of an increased number of babies with birth defects. The importance may primarily be given to the introduction of antenatal screening for CMV infection in the developing countries like India has been implemented against HIV. Data generated in our study might help the health authorities of India to make policy decisions against CMV infection prevailing in the country.

LIMITATION

The present cross-sectional study limitations were the invasive procedure followed for sample collection and also the failure to ascertain the maternal HCMV serostatus of the reviewed subjects which could be a possibility for HCMV transmission. But, the current study adds up to the existing information that previous exposure to HCMV infection in the early childhood could result in the development of birth defects like cleft lip/palate, mental retardation and progressive deafness.

CONCLUSION

By the facts presented in the present study, we can conclude that detection of anti-HCMV IgG antibodies in the blood of the cleft lip/palate, mentally retarded and deaf children suggests the past exposure or the latent CMV infection which may have led to the development of the aforementioned conditions. Good hygiene, antenatal screening, antiviral therapies, development and introduction of vaccine may achieve the goal of controlling HCMV related congenital defects in the newborns.

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