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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF SERUM TITRES OF HUMAN CYTOMEGALOVIRUS [HCMV] IN GINGIVITIS, AGGRESSIVE AND CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE I THERAPY - A RANDOMIZED CONTROLLED CLINICAL TRAIL

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ABSTRACT

Background: The role of virus in the etiology of periodontitis has long been understood. Human cytomegalovirus has been postulated to be associated with periodontitis. The role of HCMV in initiation and progression of periodontitis has been demonstrated by Conterars and Slots. Immunoglobulin G [IgG], Immunoglobulin M [IgM] are two antibodies produced by human body against HCMV. IgG is formed and elevated during active infection, whereas IgM is elevated during their initial infection stage. In this study we compared and evaluated the serum titres of IgG, IgM against HCMV in Gingivitis, Chronic periodontitis, Aggressive periodontitis before and after scaling and root planing.

Aim: To evaluate the serum titres of antibodies viz; Immunoglobulin G [IgG], Immunoglobulin M [IgM] against HCMV in three Groups of Gingivitis, Chronic periodontitis and Aggressive periodontitis patients before and after Scaling and Root planing [SRP].

Materials & Methods: Thirty subjects were assigned in to three groups, Group A [Gingivitis], Group B [Chronic Periodontitis], Group C [Aggressive periodontitis] with ten subjects in each group. All clinical parameters like Gingival Index [GI], Plaque Index [PI], Probing Pocket Depth, Clinical Attachment Level & biochemical parameters like serum titres of IgG, IgM antibodies levels were evaluated at baseline and three months after SRP.

Results: The present study showed a significant reduction in mean values of IgG with a greater significance in Group C. There is no significant reduction in IgM values of Group A than Group B & C.

Conclusion: Within limitations of the study, there is a significant relation of viral titres and periodontal diseases. An increase in IgG values before SRP and their value reduction after SRP showed the positive correlation of viral titres and periodontitis.

INTRODUCTION

Periodontitis is a multifactorial, polymicrobial disease wherein connective tissue destruction was brought about by inducing an alteration in host immune responses. The role of virus as an etiological agent in the pathogenesis of periodontitis has been proposed by various studies.[1,2,3]

Existing body of literature states that Herpes viruses viz; Herpes simplex virus 1, HCMV, Epstein barr virus were found to be associated with periodontitis.[1,2,3] The role of HCMV was first reported by Morgan in 1983 in a case of necrotising periodontitis of HIV infected person. The role of HCMV in the initiation and progression of periodontitis has been demonstrated by Conterars and Slots in 1998.[4] HCMV belongs to the family Herptoviridae commonly known as Human Herpes Virus 5.

IgG, IgM are two antibodies produced by human body against HCMV. IgG is formed and elevated during active infection, whereas IgM is elevated during the initial infection stage. HCMV triggers thx release of proinflammatory cytokines that have the potential to activate osteoclasts and Matrix metalloproteinases and to impair antibacterial immune mechanisms, causing an up-growth of periodontopathic bacteria.[1] It targets endothelial, ductal epithelial cells ,gingival monocytes/ macrophages, T-lymphocytes by inducing abnormalities in adherence, chemotaxis, phagocytic, oxidative, secretory, and bactericidal activities of polymorphonuclear neutrophils.[5]

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Various studies regarding detection of HCMV using various types of Polymerase Chain Reaction [PCR],[6,7] DNA-DNA hybridisation[8] has been done. So, far very few studies are done in correlating serum antibody titres against HCMV. So, the present study was performed to evaluate and compare the serum titres of antibodies [IgG & IgM] against HCMV in Gingivitis, Chronic periodontitis and Aggressive periodontitis groups before and after SRP.

MATERIALS AND METHODS

Thirty subjects aged between 20- 60 years of age were selected from the outpatient segment from the Department of Periodontics, St Joseph Dental College, India .All 30 subjects fulfilled the Inclusion criteria for the study, which includes the age limit and otherwise systemically healthy patients who were eligible to participate in this randomized clinical trial. The patients were assigned in to three groups of 10 each Group A [Gingivitis], Group B [Chronic Periodontitis], Group C [Aggressive Periodontitis] based on criteria of American academy of periodontology 1999[G.C Armitage].

The patients with a history of periodontal treatment within the past 6 months, under any medication, patients who have smoking habit, pregnant and lactating women and with history of any viral infection in past 6 months were excluded from the study .Subjects fulfilling the selection criteria were chosen successively and ethical clearance was obtained from the institutional review board. Admissible information regarding the study protocol was elucidated to each patient, and written informed consent was obtained from all participants. Initially the deepest pocket is measured using an acrylic stent.

The following parameters were recorded: Plaque index [PI] (Silness and Loe, 1964), Gingival index [GI] (Loe and Silness, 1963), Probing Pocket Depth [PPD] (measured with Williams periodontal probe), and Clinical Attachment Level [CAL] (measured from a fixed reference point i.e Cemento enamel junction).

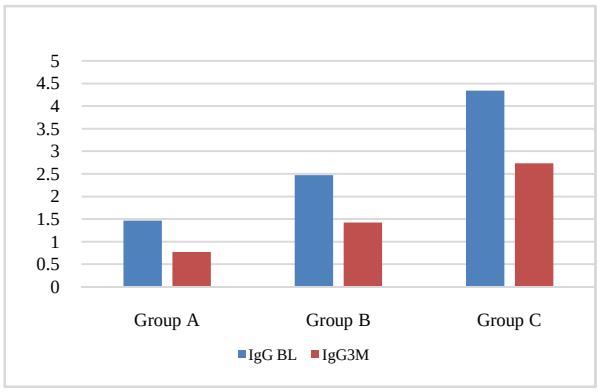
5 ml of blood samples are collected from anti cubital vein of all the three groups (Group A, Group B, Group C) at baseline and recalled after 3 months for assessment of parameters. Serum is separated by centrifugation and stored at -4 degree centigrade and it is transferred to Thyrocare® labs for further evaluation of antibody titres {IgG and IgM} of HCMV which is done through solid phase immune assay ELISA.

Statastical Analysis

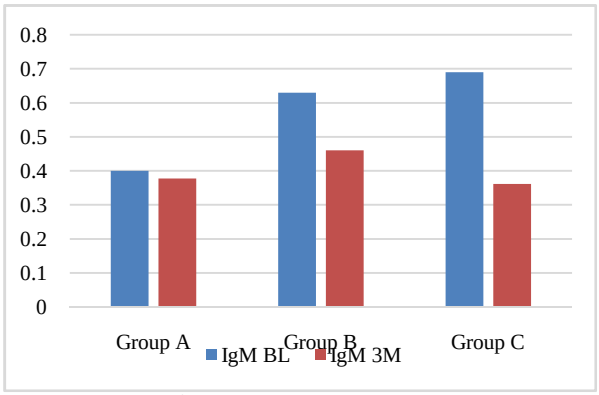
Paired t test is used for intra group comparison and One Way ANOVA test is used for inter group comparisons using graph pad prism version 6.0.

RESULTS

The mean values of Plaque Index, Gingival Index, Clinical Attachment Level, Probing Pocket Depth showed significance in all the three groups. The mean values of serum titres of IgG values showed significance in all the three groups A,B,C with P values [0.003,<0.0001,<0.0001] respectively. The mean values of serum titres of IgM in all the three groups A,B,C with P values[0.479,0.0019,0.0009] respectively with no significance in Group A when compared to Group B&C.



Graph1 Comparison of IgG values in all three groups in Baseline [BL] & 3months [3M]



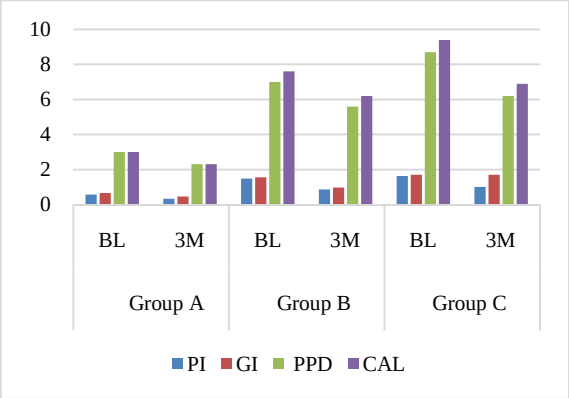
Graph2 Comparison of IgM values in all three groups in Baseline {BL} & 3months {3M}

Table1 Comparison of P values of PI, GI, PPD, CAL, IgG, IgM in all the three groups

parameters	Group A		P value	Group B		P value	Group C		P value
	Baseline Mean ± SD	3months Mean ± SD		Baseline Mean ± SD	3months Mean ± SD		Baseline Mean± SD	3months Mean ± SD	
PI	0.575±0.248	0.328 ±0.170	0.003	1.48±0.27	0.865±0.25	<0.0001	1.631±0.46	1.007±0.25	<0.0001
GI	0.654±0.262	0.464 ±0.239	0.006	1.55±0.201	0.96±0.163	<0.0001	1.704±0.21	1.097±0.13	<0.0001
PPD	3±0	2.3±0.483	0.001	7±1.49	5.6±1.505	0.0067	8.7±1.33	6.2±1.31	<0.0001
CAL	3±0	2.3±0.48	0.001	7.6±1.34	6.2±1.686	0.0067	9.4±2.5	6.9±1.969	<0.0001
IgG	1.463±1.01	0.775±0.182	0.003	2.47±1.04	1.426±0.36	<0.0001	4.34±0.799	2.734±0.58	<0.0001
IgM	0.409±0.197	0.377±0.185	0.479	0.63±0.149	0.465±0.120	0.0019	0.69±0.194	0.361±0.12	0.0009

Table 2 Comparative evaluation of IgG, IgM levels at baseline and three months in all the three groups

HCMV	IgG		IgM	
	Baseline	3Months	Baseline	3Months
Group A	1.463±1.01	0.77±0.182	0.4±0.197	0.377±0.185
Group B	2.472±1.04	1.42±0.36	0.63±0.149	0.69±0.1203
Group C	4.34±0.799	2.734±0.58	0.46±0.194	0.361±0.125



Graph 3 Comparison of PI, GI, PPD, CAL in all the three groups at Baseline [BL], 3Months [3M]

DISCUSSION

Recently, some studies suggested the role of HCMV in periodontitis.[9,10,11] Studies regarding saliva, Gingival crevicular fluid antibody titres[12] have been done. So, far very few studies are done evaluating the serum titres of antibodies against HCMV. Serum titres are preferred in the study as it is usually used for the detection of active phases of periodontal disease and to identify individuals at higher risk for future disease occurrence. With the arrival of highly sensitive techniques, traces of markers can be accurately established in Serum. It has no contamination during colonisation, translocation and migration, it can predictably used as a biomarker.

HCMV exhibits marked tropism for cells of the immune system, it modulates antigen presentation in the Major histo compatibility class I and II pathways,[13] it also shows direct cytopathic effect on inflammatory cells stimulating the release of cytokines and chemokines which impairs the periodontal immune defense. So, it helps in enhancing the virulence of already resident bacteria.[14]

There is a significant reduction in GI & PI values in 3 groups from baseline to 3 months which could be attributed to constant reinforcement of oral hygiene instruction and Hawthorne effect as given by Knowles *et al*., in 1979 and Ramfjord *et al*.,1987.[15] There was significant reduction in the PPD and gain in CAL in Group B&C compared to Group A which was in accordance to the study done by Shibata Y 1989 where in there was decrease in the probing depth and gain in attachment level after SRP.[16]

At the baseline, viral titres of serum IgG are increased [normal values0.8-1.20 odds ratio] in all the three groups, with Group C showing a more inflated value. The increased serum IgG values indicated the active infection of cytomegalovirus in all 3 groups.

At the baseline, viral titres of serum IgM are within normal limits {0.9-1.10 odds ratio} in all the three groups. This could be attributed to the fact that IgM antibodies are the first to be produced by the body in response to a HCMV infection.

There was a significant reduction in serum titres of IgG, values in all the three groups after SRP with a more significant reduction in Group C. The decreased serum IgG values could be contributed to SRP, which was effective in reducing the amount of viral load. The above results are in accordance to the study done by Grenier *et al* 2009[16] who found reduction in the scores of HCMV, EBV and HSV after SRP.

There is no significant reduction in IgM values of Group A when compared to Group B & C this could be attributed to no latent infection of this virus which was in accordance with Contreras *et al*., 1999. Furthermore, Li Jane Ling *et al* reported higher percentage of HCMV than EBV and HSV by using nested PCR method.[18]

The present study showed a significant reduction in mean values of IgG with a greater more significance in Group C. However, IgM values showed no significance in Group A which was in accordance with the studies done by Vahid Esfahanian *et al*, 2013.[12]

CONCLUSION

Within limitations of the study, there is a significant relation of viral titres and periodontal diseases. An increase in IgG values before SRP and their value reduction after SRP showed the positive correlation of viral titres and periodontitis. Further studies with large samples are needed regarding serum tires of IgG, IgM for the findings to be generalised.

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