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Original Research Article

Determination of serotype distribution of aggregatibacter actinomycetemcomitans and its relationship to Herpes virus in patients with aggressive periodontitis

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ABSTRACT

Background: Aggregatibacter actinomycetemcomitans (A. a) and its serotypes were commonly associated with Aggressive Periodontitis (AgP). Herpes virus along with A.a in sites of destruction, suggested that their co-occurence reduces the ability of macrophages to respond to bacteria and inhibits their phagocytic activity. JP2 clone is a highly leukotoxic strain of A.a, associated with Keywincreased progression of periodontal disease.
Aim: The aim of the study was to determine the serotype distribution of A.a and its relationship to the presence of Herpes virus [Epstein Barr virus (EBV) and Cytomegalovirus (CMV)] in patients with AgP.
Materials and Methods: The study included 20 patients with AgP and 20 periodontally healthy subjects. Subgingival plaque samples were collected from deepest pockets using Gracey curettes and transferred to transport media. The cDNA was extracted and analysed by PCR.
Results: Serotype d and JP2 were found to be significantly more prevalent among the participants and showed a positive correlation with increased PD and CAL, suggesting an association with the severity of AgP. Though CMV and EBV were also found to be associated with AgP, the results were insignificant. Females showed more association with disease severity than males, but the results were insignificant.
Conclusion: There was a positive correlation between serotype d and JP2 with the periodontal status. CMV and EBV were also associated with the severity of AgP, but the results were insignificant. Further quantitative studies should prove the exact role of co- occurrence of serotypes with CMV and EBV and their effect on AgP.

1. Introduction

Aggressive Periodontitis (AgP) is an uncommon type of periodontitis that has a familial pattern of occurrence and a rapid rate of disease progression, affecting people who are otherwise healthy. AgP occurs in localized and generalized forms that usually affects people under 30 years of age.

Aggregatibacter actinomycetemcomitans (A. a) is a Gram negative, facultative anaerobic, non- motile, rod shaped, opportunistic pathogen that is found to be most commonly associated with AgP. A.a has five serotype based on structurally and antigenically distinct O polysaccharide (O-PS) components of their respective lipopolysaccharide molecules, which determines the virulence of the organism.[1] They are serotype a, b, c, d and e. Later a new untypable serotype, named serotype f was detected.[2] Much later in 2010, serotype g was also identified.[3]

Herpesviruses especially Epstein-Barr virus (EBV) and Cytomegalovirus (CMV), have been detected at destructive periodontitis sites in combination with A.a and was found to be related to the etiology of AgP by inhibiting the phagocytic activity and reducing the ability of macrophages to respond to bacteria or by increasing the risk of

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bacterial infection by providing new sites for attachment of periodontopathogens.[4]

The present study determines the types of serotypes of A.a present in the subgingival plaque samples of patients with AgP and examines its relationship with the presence of Herpes virus.

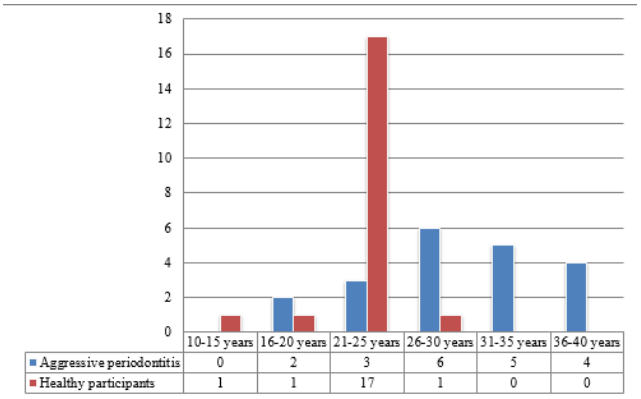
2. Materials and Methods

Subjects were recruited from the patients reporting to the Department of Periodontics. After clinical examination and selection of the subjects, a signed consent was obtained. The study was approved by the Institutional Ethical Committee. Study included two groups, 20 participants in AgP group (test group) and 20 participants in Healthy group (control group). Patients with a history of diabetes, HIV infections, medical conditions requiring prophylactic antibiotic coverage, pregnant women, patients under current orthodontic treatment, patients who have undergone professional dental cleaning or antibiotic therapy in the past 3 months, smokers and patients with less than 20 teeth were excluded from the study.

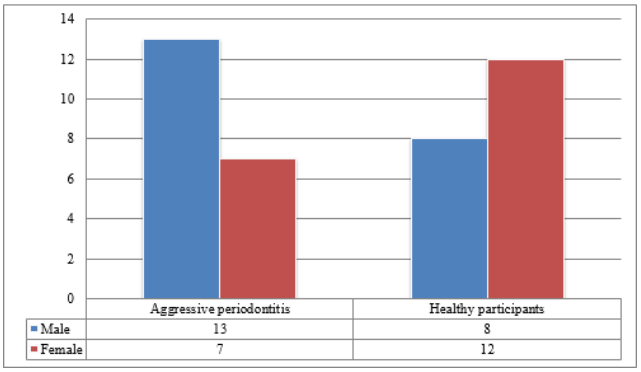
Plaque Index (PlI), Gingival Index (GI), Probing Depth (PD) and Clinical Attachment Level (CAL) were measured in six periodontal sites per tooth using a manual periodontal probe. Subgingival plaque samples were collected from site with deepest periodontal pocket using a Gracey curette after isolating the site with cotton pellets. The sample is then transferred to a plastic vial containing transport media (Phosphate buffered saline- PBS) and is taken for extraction of viral and bacterial DNA.[5]

DNA extraction was done using a modified proteinase K method and A.a serotypes and Herpes virus was detected using Polymerase Chain Reaction.A. a serotypes, JP2, CMV and EBV were detected using “sure cycler 8800” PCR unit (Agilent technologies). The primers used for DNA amplification were designed as per the primer guidelines (**Table 1**). The PCR products were analyzed by electrophoresis on a 2% agarose gel and photographed under UV light by the Gel doc system.[5]

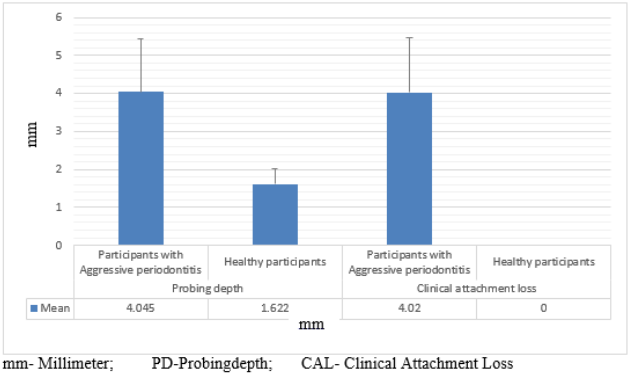
The comparison of mean Plaque Index (PlI) and Gingival Index (GI) between test and control groups were done using independent sample t test. The intergroup comparison between the presence or absence of serotypes of A.a and Herpes Virus was done using Chi-square test. Association of serotypes of A.a, Herpesvirus (CMV and EBV) and JP2 with the presence or absence of AgP was done using Binary logistic regression analysis. Association of serotypes of A.a, Herpesvirus (CMV and EBV), JP2 and gender with PD and CAL was done using Linear regression analysis.



Graph 1: Age distribution among study participants



Graph 2: Gender distribution among study participants



mm- Millimeter; PD-Probingdepth; CAL- Clinical Attachment Loss

p value < 0.05- statistically significant; p value > 0.05- statistically insignificant

Graph 3: Comparison of mean PD and CAL between test and control group

Table 1: Primer sequences for DNA amplification:

S.No	Serotypes	Forward Primer Reverse primer
1	Serotype a	FORWARD 5' -GCAATGATGTATTGTCTTCTTTTGGA-3' REVERSE 5'CTTCAGTTGAATGGGGATTGACTAAAAC-3'
2	Serotype b	FORWARD 5'-CGGAAATGGAATGCTTGC-3' REVERSE 5'-CTGAGGAAGCCTAGCAAT-3'
3	Serotype c	FORWARD 5'-AATGACTGCTGTCGGAGT-3' REVERSE 5' -CGCTGAAGGTAATGTCAG-3'
4	Serotype d	FORWARD 5'-TTACCAGGTGTCTAGTCGGA-3' REVERSE 5'-GGCTCCTGACAACATTGGAT-3'
5	Serotype e	FORWARD 5'-CGTAAGCAGAAGAATAGTAAACGT-3' REVERSE 5'-AATAACGATGGCACATCAGACTTT-3'
6	JP2 clone	FORWARD 5'- CAGATCAAAACCTGATAACAGTATT- 3' REVERSE 5'- TTTCTCCATATTCCCTCCTTCTGT-3'
7	CMV	FORWARD 5'-TCCACGCCGTTCAAGAGA-3' REVERSE 5'-GACTGACGCTGAAACGGA-3'
8	EBV	FORWARD 5'-TATTGCCGCCTCGTGTTT- 3' REVERSE 5'-TCTCCTTCTGTACGCTAGTA- 3'

Table 2: Age distribution among study participants

Age groups	Aggressive periodontitis n(%)	Healthy participants n(%)
10-15 years	0(0)	1(5)
16-20 years	2(10)	1(5)
21-25 years	3(15)	17(85)
26-30 years	6(30)	1(5)
31-35 years	5(25)	0(0)
36-40 years	4(20)	0(0)
Mean age	29.25 years	22.6 years

Table 3: Gender distribution among study participants

Gender	Aggressive periodontitis n(%)	Healthy n(%)
Male	13(65)	8(40)
Female	7(35)	12(60)

n- Number of participants

Table 4: Comparison of mean PD and CAL between test and control group

	Groups	Mean (mm)	Std. Deviation (mm)	t value	p value	Mean difference	95% confidence interval of difference	
							Lower	Upper
PD	Participants with Aggressive periodontitis	4.045	1.3797	7.534	<0.001**	2.423	1.7565	3.0895
	Healthy participants	1.622	0.4065					
CAL	Participants with Aggressive periodontitis	4.020	1.4598	12.315	<0.001**	4.020	3.3368	4.7032
	Healthy participants	0.000	0.000					

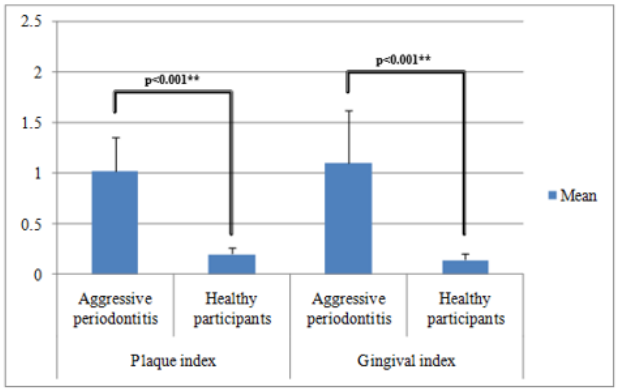
PD- Probing depth; CAL- Clinical Attachment Level; mm- millimeter

Value < 0 05- statistically significant; p value > 0 05- statistically insignificant
**-Highly significant

Table 5: Comparison of mean plaque and gingival index between test and control group - Independent samples t test

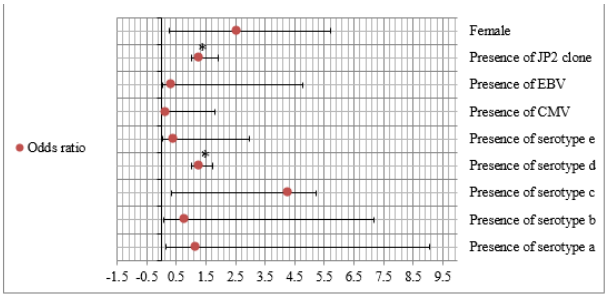
Groups		Mean	Std. Deviation	t value	p value	Mean difference	95% confidence interval of difference	
							Lower bound	Upper bound
Plaque index	Aggressive periodontitis	1.0200	.33995	10.566	<0.001*	0.819	0.662	0.976
	Healthy participants	0.2010	.06782					
Gingival index	Aggressive periodontitis	1.1035	.52529	8.113	<0.001*	0.959	0.719	1.198
	Healthy participants	0.1446	.05871					

Value < 0 05- statistically significant; p value > 0 05- statistically insignificant



p value < 0.05- statistically significant; p value > 0.05- statistically insignificant

Graph 4: Comparison of mean plaque and gingival index between test and control groups

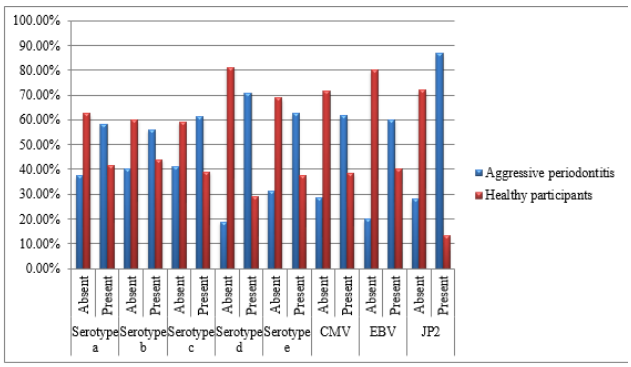


Dependent variable= Presence or absence of Aggressive periodontitis

CMV- Cytomegalovirus; EBV- Epstein Barr virus

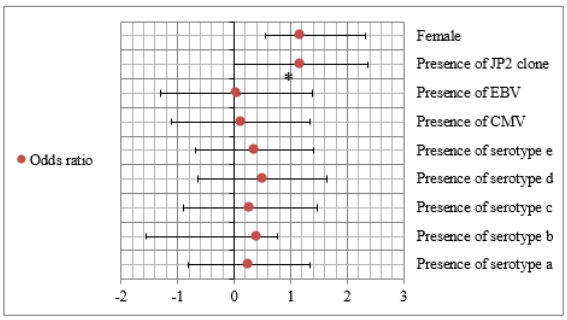
p value < 0.05- statistically significant; p value > 0.05- statistically insignificant

Graph 6: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpes virus (CMV and EBV), JP2 and gender individually with the presence or absence of Aggressive Periodontitis



CMV- Cytomegalovirus; EBV- Epstein Barr virus

Graph 5: Distribution of different serotypes of Aggregatibacter actinomycetemcomitans,



Dependent variable: Probing depth

PD- Probing depth; CMV- Cytomegalovirus; EBV- Epstein Barr virus

p value < 0.05- statistically significant; p value > 0.05- statistically insignificant

Graph 7: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpes virus (CMV and EBV), JP2 and gender individually with PD

Table 6: Distribution of different serotypes of Aggregatibacter actinomycetemcomitans, CMV, EBV and JP2 clone among test and control group

Serotypes			Groups		Total	Chi square value	p value
			Aggressive periodontitis	Healthy			
Serotype a	Absent	n	6	10	16	1.667	0.197
		%	37.5%	62.5%	40.0%		
	Present	n	14	10	24		
		%	58.3%	41.7%	60.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
Serotype b	Absent	n	6	9	15	0.960	0.327
		%	40.0%	60.0%	37.5%		
	Present	n	14	11	25		
		%	56.0%	44.0%	62.5%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
Serotype c	Absent	n	9	13	22	1.616	0.204
		%	40.9%	59.1%	55.0%		
	Present	n	11	7	18		
		%	61.1%	38.9%	45.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
Serotype d	Absent	n	3	13	16	10.417	0.001*
		%	18.8%	81.2%	40.0%		
	Present	n	17	7	24		
		%	70.8%	29.2%	60.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
Serotype e	Absent	n	5	11	16	3.750	0.053
		%	31.2%	68.8%	40.0%		
	Present	n	15	9	24		
		%	62.5%	37.5%	60.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
CMV	Absent	n	4	10	14	3.965	0.047*
		%	28.6%	71.4%	35.0%		
	Present	n	16	10	26		
		%	61.5%	38.5%	65.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
EBV	Absent	n	2	8	10	4.800	0.028*
		%	20.0%	80.0%	25.0%		
	Present	n	18	12	30		
		%	60.0%	40.0%	75.0%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		
JP2	Absent	n	7	18	25	12.907	<0.001**
		%	28.0%	72.0%	62.5%		
	Present	n	13	2	15		
		%	86.7%	13.3%	37.5%		
	Total	n	20	20	40		
		%	50.0%	50.0%	100.0%		

CMV- Cytomegalovirus; EBV- Epstein Barr virus
Value < 0 05- statistically significant; p value > 0 05- statistically insignificant

Table 7: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpes virus (CMV and EBV), JP2 and gender individually with the presence or absence of Aggressive Periodontitis- Binary logistic regression analysis

Independent variables		Odds ratio	Standard Error	p value	95% C.I. for odds ratio Lower	Upper
Serotype a	Absent	1.000(Ref)	1.054	0.897	0.145	9.045
	Present	1.146				
Serotype b	Absent	1.000(Ref)	1.131	0.829	0.085	7.186
	Present	0.783				
Serotype c	Absent	1.000(Ref)	1.277	0.255	0.350	5.215
	Present	4.278				
Serotype d	Absent	1.000(Ref)	1.306	0.029*	0.004	0.744
	Present	1.258				
Serotype e	Absent	1.000(Ref)	1.002	0.381	0.058	2.963
	Present	0.416				
CMV	Absent	1.000(Ref)	1.235	0.140	0.014	1.815
	Present	0.161				
EBV	Absent	1.000(Ref)	1.358	0.417	0.023	4.751
	Present	0.332				
JP2	Absent	1.000(Ref)	1.129	0.040*	0.011	0.901
	Present	1.299				
Gender	Male	1.000(Ref)	1.181	0.430	0.251	5.705
	Female	2.539				

Dependent variable= Presence or absence of Aggressive periodontitis
CMV- Cytomegalovirus; EBV- Epstein Barr virus
Value < 0 05- statistically significant; p value > 0 05- statistically insignificant

Table 8: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpesvirus (CMV and EBV), JP2 and gender individually with PD – Linear regression analysis

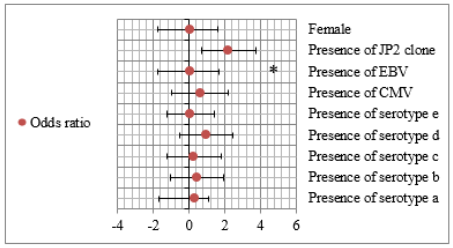
			Coefficients		**p value**	**95% Confidence Interval for B**	
			B	Std. Error		Lower Bound	Upper Bound
Independent variables	Serotype a	Absent			Ref		
		Present	0.263	.531	.623	-.819	1.346
	Serotype b	Absent			Ref		
		Present	0.388	.569	.501	-1.548	.772
	Serotype c	Absent			Ref		
		Present	0.287	.576	.622	-.887	1.460
	Serotype d	Absent			Ref		
		Present	0.504	.561	.376	-.640	1.648
	Serotype e	Absent			Ref		
		Present	0.363	.514	.486	-.686	1.411
	CMV	Absent			Ref		
		Present	0.121	.601	.842	-1.105	1.346
	EBV	Absent			Ref		
		Present	0.044	.655	.947	-1.292	1.381
	JP2	Absent			Ref		
		Present	1.179	.576	.049*	.004	2.355
	Gender	Male			Ref		
		Female	1.172	.576	0.833	0.561	2.320

B- Odds ratio; PD- Probing depth;
Dependent variable: Probing depth
CMV- Cytomegalovirus; EBV- Epstein Barr virus
Value < 0 05- statistically significant; p value > 0 05- statistically insignificant

Table 9: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpesvirus (CMV and EBV), JP2 and gender individually with CAL – Linear regression analysis

			Coefficients		p value	95% Confidence Interval for B	
			B	Std. Error		Lower Bound	Upper Bound
Independent variables	Serotype a	Absent			Ref		
		Present	.307	.681	.656	-1.696	1.083
	Serotype b	Absent			Ref		
		Present	.471	.730	.524	-1.018	1.961
	Serotype c	Absent			Ref		
		Present	.283	.739	.704	-1.224	1.791
	Serotype d	Absent			Ref		
		Present	.976	.720	.185	-.494	2.445
	Serotype e	Absent			Ref		
		Present	.081	.660	.904	-1.265	1.426
	CMV	Absent			Ref		
		Present	.619	.772	.428	-.955	2.193
	EBV	Absent			Ref		
		Present	.032	.842	.970	-1.748	1.685
	JP2	Absent			Ref		
		Present	2.223	.740	.005*	.713	3.732
	Gender	Male			Ref		
		Female	0.074	0.832	0.930	-1.771	1.623

B- Odds ratio; CAL- Clinical Attachment Level
Dependent variable: Clinical Attachment Level
CMV- Cytomegalovirus; EBV- Epstein Barr virus
Value < 0 05- statistically significant; p value > 0 05- statistically insignificant



Dependent variable: Clinical attachment loss

CAL- Clinical attachment loss; CMV- Cytomegalovirus; EBV- Epstein Barr virus

p value < 0.05- statistically significant; p value > 0.05- statistically insignificant

Graph 8: Association of serotypes of Aggregatibacter actinomycetemcomitans, Herpes virus (CMV and EBV), JP2 and gender individually with CAL

3. Results

The age distribution in the test group showed 10% (n= 2) of the subjects aged between 16 and 20 years, 15% (n= 3) were between 21-25 years, 30% (n= 6) were between 26 and 30 years, 25% (n= 3) of subjects were between 31- 35 years and 20% (n= 4) of the patients were aged between 36 and 40 years with a mean age of 29.25 years. In healthy group, 5% (n= 1) of the subjects were aged from 10-15 years, 5% (n= 1) aged between 16 and 20 years, 85% (n= 17) were aged between 21 and 25 years and 5% (n= 1) of the subjects were aged from 26 to 30 years with a mean age of 22.6 years. (Table 2Graph 1)

The gender distribution was 13 males (65%) and 7 females (35%) in AgP group (test group) and 8 males (40%) and 12 females (60%) in healthy group (control group). (Table 3Graph 2) AgP group included 19 GAgP and 1 LAgP patients.

The mean PD of AgP group was 4. 045±1.3797 mm and in healthy subjects it was found to be 1.622±0.4065 mm. The difference between the groups was found to be highly statistically significant($p<0.05$) with a mean difference of 2.423 mm. (Table 4Graph 3) The mean CAL in subjects with AgP was 4.020±1.4598 mm. The mean was observed to be 0 mm in healthy subjects. On comparison, the result was statistically significant ($p<0.05$) with a mean difference of 4.020 mm. (Table 4Graph 3)

The mean Plaque Index (PlI) for AgP group was 1.02 and for healthy group was 0.201. The result was statistically significant ($p<0.05$). (Table 5Graph 4) The mean Gingival Index (GI) for AgP group was 1.1035 and for healthy group was 0.1446. The result was also statistically significant ($p<0.05$). (Table 5; Graph 4)

All patients with AgP showed various levels of serotypes, CMV and EBV distribution. Serotype a and b was observed in 70% (n= 14) of participants, serotype c in 55% (n= 11), serotype d in 85% (n= 17) and serotype e in 75% (n= 15) of participants. Presence of CMV and EBV was detected in 80% (n= 16) and 90% (n= 18) of participants respectively

while JP2 was observed in 65% (n= 13) of patients with AgP. The control group patients also exhibited all types of serotypes, CMV and EBV distribution. Serotype a was detected in 50% (n= 10) of subjects, serotype b in 55% (n= 11), serotype c in 35% (n= 7), serotype d in 35% (n= 7) and serotype e in 45% (n= 9) of subjects. While CMV and EBV was observed in 50% (n= 10) and 60% (n= 12) of subjects respectively, JP2 was noticed in 10% (n= 2) of healthy participants. The difference in the presence of serotype d, CMV, EBV and JP2 clone in test and control groups were found to be statistically significant. ($p<0.05$) (Table 6; Graph 5)

Table 7; Graph 6 shows the association of serotypes of A.a, CMV and EBV with the presence or absence of AgP. The result was statistically significant for two independent variables i.e., Serotype d and JP2 of A.a. It was observed that the presence of serotype d in patients, statistically significantly increased the severity of AgP ($p<0.05$) as compared to the absence of the serotype. Though patients who harbored CMV and EBV showed increased association with AgP compared to subjects who showed absence of CMV and EBV, the results were not statistically significant ($p>0.05$). Patients with the presence of JP2 serotype had statistically significant increased association with AgP ($p>0.05$). Though females showed more association with AgP than males, the result was not statistically significant ($p> 0.05$).

Table 8; Graph 7 shows the association of serotypes of A.a, Herpesvirus (CMV and EBV) and gender with PD. Amongst all the serotypes, serotype d was most highly associated with increased PD though the value was not statistically significant ($p>0.05$). Though patients who harbored CMV and EBV showed increased PD when compared to subjects who showed absence of CMV and EBV, the results were not statistically significant($p>0.05$). The result was statistically significant only in relation to one independent variable i.e., JP2($p<0.05$). Participants with the presence of JP2 serotype had 1.179 times deeper PD than participants exhibiting absence of JP2. Though females had deeper PD than males, the result was not statistically significant. ($p>0.05$)

Amongst all the serotypes, serotype d was most highly associated with increased CAL (0.976 times) though the value was not statistically significant($p>0.05$). Though patients who harbored CMV and EBV showed increased CAL when compared to subjects who showed absence of CMV and EBV, the results were not statistically significant($p>0.05$). The result was significant for JP2 variable alone with its presence increasing the risk of CAL by 2.223 times($p<0.05$). Though females had a higher chance of developing increased CAL than males (0.074 times) the result was not statistically significant($p>0.05$). (Table 9; Graph 8)

4. Discussion

The study consisted of 13 males and 7 females with AgP and 8 males and 12 females who were periodontally healthy. Results of the present study suggested that females had a higher risk of having increased PD and CAL than males. Also females exhibited more association with AgP when compared to males. Though the findings were statistically insignificant, it was in agreement with the study by *Melvin WL et al., (1991)*[6] who found higher prevalence of AgP among Caucasian females than males.

Studies have investigated the presence of A. a and its serotypes in AgP. A study by *Joshi et al., (2017)*[7], investigated the presence of serotypes and evaluated its effect on Chronic periodontitis with the co- occurrence of Herpes virus. To the best of our knowledge, this is the first study to investigate and compare the co- occurrence of serotypes of A. a and Herpes virus and its effect on patients with AgP.

A. a seems to be the most common microorganism associated with AgP as studied by *Slots et al., (1982),*[8] *Hillman et al., (1982),*[9] Zambon et al, (1983),[10] *Saarela et al., (1992),*[11] *Jensen AB et al., (2020).*[12] The relation between A. a and periodontal disease risk had been previously determined by *Fine et al., (2007).*[13]

Most of the participants in the present study harbored more than one serotype. This finding correlates with the study by *Teixeira et al., (2006),*[14] *Sakellari et al., (2011),*[15] *Cortelli JR et al., (2012),*[16] *C. Akrivopoulou et al., (2017),*[17] *Suprith SS et al., (2018)*[18] who found combinations of more than one serotype among patients with AgP.

The prevalence of serotypes among AgP group is more than those among healthy participants. In the present study, serotype a and b was observed in 70% of participants, serotype c in 55%, serotype d in 85% and serotype e in 75% of participants with AgP. Whereas, in healthy participants, serotype a was detected in 50% of subjects, serotype b in 55%, serotype c and d in 35% and serotype e in 45% of subjects which was lesser when compared to those with AgP, suggesting that increased prevalence of various types of serotypes has increased risk of developing AgP. This finding was in agreement with the study by *Yang et al., (2005)*[19] who found significantly higher prevalence of serotypes in AgP when compared to healthy participants.

In the present study, the most prevalent serotype among the patients with AgP was serotype d. The presence of serotype d in patients significantly increased the severity of AgP ($p<0.05$) as compared to the absence of the serotype. However, this finding was contradictory to the findings by *Cortelli JR et al., (2012)*[16] who found low prevalence of serotype d.

CMV and EBV were found to influence the host response by reducing the ability of macrophages to kill periodontopathogens. Thereby, they inhibit the phagocytic activity of macrophages and promotes the progression of

periodontitis. The present study detected the presence of CMV and EBV among both AgP and healthy patients as found in the studies by *Parra and Slots., (1996)*[4] and *Contreras A et al., (1999).*[20]

The prevalence of CMV among AgP patients were found to be 80% and in healthy participants it was found in 50% of subjects. EBV was detected in 90% of AgP patients and 60% of healthy participants. This shows nearly equal distribution of EBV and CMV among patients with AgP. This report is in accordance with the studies done by Saygun et al., (2004),[21] *Kubar et al., (2005)*[22] and *Imbronito et al., (2008)*[23] who found nearly equal distribution of CMV and EBV in patients with AgP.

The presence of CMV in AgP patients led to an increased the severity in PD and CAL, 0.121 and 0.619 times respectively. Similarly, the presence of EBV in AgP led to increased PD and CAL, 0.044 and 0.032 times respectively. These values were however statistically insignificant ($p>0.05$). Similar results were obtained in study by *Blankson PK et al., (2019)*[24] who found an increased association of HSV with AgP, though the values were not significant.

The prevalence of JP2 clone of A. a was higher in AgP group when compared to healthy participants. The prevalence of JP2 clone was 65% in AgP group, whereas healthy participants showed only 10% prevalence of JP2 clone, suggesting a positive association of JP2 clone with AgP. Results of this study shows that, the PD among patients who were positive for JP2 clone was found to be 1.179 times greater than those patients who lack JP2. And similarly, CAL among patients who were positive for JP2 clone was found to be 2.223 times greater than those patients who show absence of JP2. Both the values were statistically significant ($p < 0.05$). These results were similar to the finding obtained by *Haubek et al., (2004)*[25] who found that there was increased progression of PD and CAL in patients who were detected positive for JP2 than in patients who lack JP2 clone.

5. Conclusion

This study was conducted to assess the prevalence of serotypes of A.a, Herpes virus and JP2 clone. It was established that serotype d and JP2 are associated with increased PD and CAL. The qualitative PCR results could not analyze the exact role of HSV and the influence of it's mere presence on the periodontal status of the patients since all the participants in the study showed the presence of any one of the viruses. Thus, there is no possible way to ascertain the compounding effect of HSV over serotype of A.a on the periodontal status of the patients. Further studies should be carried out at a greater scale with a larger sample size using quantitative PCR to detect the load of HSV and justify its influence on the periodontal status.

6. Source of Funding

None.

7. Conflict of Interest

None.

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