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ASSOCIATION OF *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* AND *FRETIBACTERIUM FASTIDIOSUM* WITH AGGRESSIVE PERIODONTITIS – A CLINICO-MICROBIOLOGICAL STUDY

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ABSTRACT Studies show that a group of specific bacteria is responsible for the initiation and progression of periodontal disease.[1] *Aggregatibacter actinomycetemcomitans (A.a)* is specifically associated with destructive forms of periodontitis.[2] *Fretibacterium fastidiosum (F.f)* is a commensal bacterium of the oral cavity but fewer studies are there to find out the role of *F.f* with aggressive periodontitis (AgP).[3] The present study was designed to find out the association of *A.a.* and *F.f.* with aggressive periodontitis (AgP). This study involved randomly selected patients with Aggressive Periodontitis (AgP) from North East India. Genomic analysis using PCR was employed to detect *Aggregatibacter actinomycetemcomitans (A.a)* and *Fretibacterium fastidiosum* (*F.f*) and assess their association with AgP. Findings suggest a strong link between *F.f.* and *A.a.* in AgP cases in this region. While *F.f.* is typically a commensal oral bacterium, it may become pathogenic under disrupted oral conditions.[3] The results indicate that *F.f.,* alongside *A.a.,* may contribute to the onset and progression of AgP.

KEYWORDS : aggressive periodontitis, polymerase chain reaction, fretibacterium fastidiosum, aggregatibacter actinomycetemcomitans, north east india

INTRODUCTION

Aggressive periodontal disease (AAP,1999) comprises a group of specific pathological conditions where rapid destruction of the periodontal tissues occurs especially in the early decades of life.[4-5]

Studies indicate that *A. actinomycetemcomitans (A.a)* is strongly associated with aggressive periodontal diseases. *A.a.* produces virulent factors like leukotoxin, which disrupts the host's innate immune system, facilitating the invasion of pathogenic organisms into tissues. Leukotoxin also induces apoptotic cell death in lymphocytes, impairing immune function.[5] *Fretibacterium fastidiosum (F. f)* is a gram-negative, mesophilic, anaerobic, motile bacterium found in the oral cavity. Research suggests that *F.f.* may play an active role in the development of periodontal disease, particularly in hosts with compromised immunity or altered oral conditions.[3,6] Scanty literature is available on the association of *A.a.* along with *F.f.* in the initiation and progression of an aggressive form of periodontal disease. The present study was designed to find out the association of these two bacteria with AgP in a group of research participants from the northeastern region of India.

MATERIAL AND METHODS

The study received prior approval from the institutional ethics committee. Forty-five patients diagnosed with Aggressive Periodontitis were randomly selected from the Periodontics outpatient department at Regional Dental College, Guwahati. All participants gave written informed consent after being briefed on the procedure and its benefits. Individuals with systemic conditions, pregnancy, lactation, tobacco use, recent antibiotic use, or a history of periodontal treatment were excluded. Baseline clinical parameters were recorded during the initial examination.

Sample collection: Plaque samples were collected from the deepest pocket of each arch with a sterile area-specific Gracey curette after proper isolation, placed in separate Eppendorf tubes with 400µL phosphate buffer solution (PBS), and transported in a biosafety box. Samples were stored at (-80°C) in the freezer. **Lab analysis of the plaque samples:** Commercial DNA extraction kit (Qiagen) - was used for DNA isolation from the plaque samples following the steps mentioned below 1) **Initial Preparation**: Samples were combined with molecular-grade ethanol (99.9%) and a customized buffer solution. 2) **Homogenization and Incubation**: A 400 µL aliquot of the sample, mixed with phosphate-buffered saline (PBS), was vortexed (Fig. 1) and centrifuged at 8000 rpm for 1 minute, followed by incubation at 53°C for 15 minutes. 3) **Lysis and Separation**: The resulting solution from Step 2 was treated with lysis buffer. Two aliquots (300 µL [A] and 600 µL [B]) were prepared: Aliquot A was centrifuged at 8000 rpm for 1 minute. Aliquot B was centrifuged at 14,000 rpm for 3 minutes. The supernatants were discarded after each spin. 4) **Washing**: Both pellets (A and B) were resuspended in 500 µL of wash buffer and centrifuged at 8000 rpm for 1 minute which was repeated twice followed by a final dry spin. 5) **Elution**: DNA was eluted via centrifugation at 8000 rpm for 1 minute. The extracted DNA was assessed for quality and stored at (-20°C) for downstream PCR analysis.

Table 1.1. Showing Program for DNA amplification *Fretibacterium fastidiosum (F.f.)* and *Aggregatibacter actinomycetemcomitans (A.a.)*:

Temperature	Time	
95^{0}C	5 min	
95^{0}C	30 sec	35 cycles
53^{0}C	30 sec	
72^{0}C	60 sec	
72^{0}C	10 min	
4^{0}C	Hold	

Table 1.2. Showing Primers used for identification of ***Fretibacterium fastidiosum (F.f)*** **and** ***Aggregatibacter actinomycetemcomitans (A.a)*:**

Aggregatibacter actinomycetemcomitans (A. a.)

Forward	ATTGGGGTTTAGCCCTGGT
Reverse	GGCACAAACCCATCTCTGA

Fretibacterium fastidiosum (F. f)

Forward	TGGTAACACGGAATGGCATAC
Reverse	ACCAACATCTCTGCTCGC

Disposal of the Sample:

At the end of the study, lab samples were disposed of, as per standard biomedical waste disposal protocol.

RESULTS

Statistical Analysis - Data were analyzed using GraphPad Prism

(version 8.0.1), with Pearson correlation used to evaluate associations between categorical variables. A p-value of ≤ 0.05 was considered statistically significant. As shown in Tables 2.1 and 2.2, both *A. actinomycetemcomitans (A.a)* and *F. fastidiosum (F.f.)* demonstrated significant positive correlations with probing depth and clinical attachment loss. *A.a*. was also significantly linked to both localized and generalized bone loss, while *F.f* showed a significant association only with generalized bone loss (p = 0.18 for localized loss, not significant).

Table 2.1 – Showing Mean And Standard Deviation Of All Variables

Clinical Parameters	Mean ± SD
Age(years)	22.49 ± 4.5
Gender, n (%)	
Male	36.17%
Female	59.6%
PPD (mm)	
CAL (mm)	7.9 ± 1.3
Organisms	9± 2.0
A. actinomycetemcomitans	66.6 %
F. fastidiosum	86.6 %
Bone loss	
Generalized	44.44%
Localized	86.66%

Table 2.2 Showing Different Variables Of Correlation Coefficient, Confidence Interval, P-value

	A. actinomycetemcomitans	*F. fastidiosum*
Clinical Attachment Level		
Correlation Coefficient (r)	0.50	0.49
Confidence Interval	95% (0.2416 to 0.6917)	95% (0.2244 to 0.6822)
P Value	(.0005)	(0.0007)
Mean Pocket Depth		
Correlation Coefficient (r)	0.49	0.48
Confidence Interval	95% (0.2320 to 0.6864)	95% (0.2201 to 0.6797).
P Value	(.0006)	(0.0008)
Generalized Bone Destruction		
Correlation Coefficient (r)	0.44	0.44
Confidence Interval	95% (0.172 to 0.65)	95% (0.17 to 0.65)
P Value	(0.002)	(0.002)
Localized Bone Destruction		
Correlation Coefficient (r)	0.46	0.2
Confidence Interval	95% (0.192 to 0.66)	95% (0.09 to 0.47)
P Value	(.0016)	(0.18)

DISCUSSION

Over the decades, various theories have been advocated on the etiology and pathogenesis of periodontal disease. H. Loe et al in their classic study on human models highlighted the non-specific bacterial theory which was later reviewed based on evidence-based studies of specific plaque hypothesis where a group of specific pathogenic bacteria was believed to be responsible for the etiopathogenesis of periodontal disease.[7-8] Aggressive periodontitis (AgP) is linked to specific bacterial species in susceptible individuals. Lafaurie et al. observed that the presence of pathogens such as *P. gingivalis*, *E. corrodens*, *T. forsythia*, and *A. actinomycetemcomitans* varies across geographic and ethnic groups, showing that colonization patterns are influenced by regional, cultural, and genetic factors.[9]

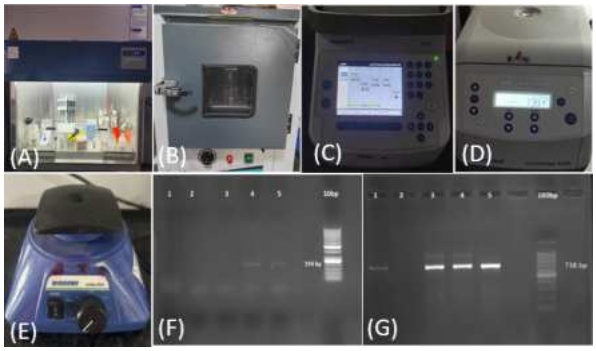

Fig 1 (A) showing DNA extraction workstation (B) showing Incubator (C) showing Centrifuge machine (D)showing PCR Thermocycler (E) showing Vortexer (F) Gel image showing amplification of Aggregatibacter actinomycetemcomitans product size 194bp. (G) Gel image showing amplification of Fretibacterium fastidiosum product size 738bp

This study shows a significant association between *Fretibacterium fastidiosum (F.f.)* and *Aggregatibacter actinomycetemcomitans (A.a.)* in patients with Aggressive Periodontitis (AgP), indicating a possible synergistic role as pathobionts. Both *A. actinomycetemcomitans (A.a)* and *Fretibacterium fastidiosum (F.f)* showed significant associations with AgP. *A.a* was strongly correlated with mean pocket depth (p = 0.0006), clinical attachment loss (p = 0.0005), localized bone loss (p = 0.0016), and generalized bone loss (p = 0.002). *F.f.* also showed significant correlations with mean pocket depth (p = 0.0008), clinical attachment loss (p = 0.0007), and generalized bone loss (p = 0.002), but not with localized bone loss (p = 0.18).

CONCLUSION

The development of precise qualitative and quantitative techniques like polymerase chain reaction for identification of periodontal microbiota leads to the evolution of the theories of the existing literature on the etiopathogenesis of periodontal disease.[10] Studies indicate that periodontal biofilm dysbiosis contributes to the initiation and progression of periodontal disease.[11] Aggressive periodontitis is characterized by rapid destruction of supporting tissues.[12,4] Research shows that the colonization of periodontopathic bacteria varies across different populations with AgP.[13] As no studies have explored the association of *A. actinomycetemcomitans (A.a)* and *Fretibacterium fastidiosum (F.f)* with AgP in northeastern India, this study was aimed to investigate their association through genomic analysis using PCR.

F.f. is considered to be a commensal bacterium of the oral cavity and could be a pathobiont if the ecology of the oral environment is altered.[3] The result of the present study indicates that both *A. actinomycetemcomitans (A.a.)* and *F. fastidiosum (F.f.)* are linked to Aggressive Periodontitis (AgP), with *F.f.* detected more frequently than *A.a.* in participants. Further research in the northeastern Indian population may uncover genetic and metabolic risk factors. The development of low-cost, AI-based chairside diagnostic tools could enable early identification and prevention of AgP before significant periodontal damage occurs.

Conflict Of Interest: None

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