ORIGINAL ARTICLE

Characterization of Aggregatibacter actinomycetemcomitans in Chronic Oral Inflammatory Conditions Using Molecular Techniques in Eastern India

Dr. Lipsa Bhuyan[1], Panda Abikshyeet[2], Dr. Soumya Jal[3], Harish Kumar[4], Dr. Pallavi Mishra[5]

ABSTRACT

Background

Chronic oral inflammatory diseases such as periodontitis are considered major causes of tooth loss. Among periodontal pathogens, Aggregatibacter actinomycetemcomitans is a major pathogen associated with aggressive and chronic forms of periodontal diseases. Molecular diagnostic methods have greatly contributed to the detection and characterization of this pathogen and its virulence factors.

Aim

To characterize Aggregatibacter actinomycetemcomitans in chronic oral inflammatory conditions using molecular techniques in an Eastern Indian population.

Materials and Methods

A cross-sectional study was performed among 120 participants suffering from chronic periodontitis, gingivitis, and oral potentially malignant disorders. Plaque samples were obtained from the subgingival region, and genomic DNA was isolated. Species-specific PCR was performed for detecting A. actinomycetemcomitans using the 16S rRNA gene. Multiplex PCR was performed for detecting virulence genes such as leukotoxin (ltxA) and cytolethal distending toxin (cdtB). Clinical periodontal parameters were associated with the study.

Results

A. actinomycetemcomitans was found in 63.3% of chronic periodontitis cases, 34.4% of gingivitis cases, and 21.7% of OPMD cases. The virulence gene study showed that 58% of cases were ltxA positive, and 46% were cdtB positive. The presence of virulence genes had significant correlation with probing depth and clinical attachment loss.

Conclusion

PCR-based molecular detection provides highly sensitive identification of A. actinomycetemcomitans. The findings emphasize the importance of virulence gene profiling in understanding periodontal disease progression in Eastern Indian populations.

Keywords

Aggregatibacter actinomycetemcomitans, molecular diagnostics, PCR, virulence genes, periodontal pathogens, Eastern India

INTRODUCTION

One of the most common oral diseases in the world, periodontal diseases are chronic inflammatory conditions that affect the teeth's supporting tissues. Microbial biofilms that build up in the gingival sulcus and periodontal pockets are the main cause of them. These biofilms are made up of a variety of bacterial species that interact with the host immune system to cause periodontal disease and tissue destruction.[1,2] Because of its strong correlation with aggressive periodontitis and chronic inflammatory periodontal conditions, Aggregatibacter

1. Dr. Lipsa Bhuyan (Corresponding), PhD Scholar, Reader, Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, KIIT Deemed to be University, Patia, Bhubaneswar-751024. bhuyanlipsa@gmail.com
2. Panda Abikshyeet, M.D.S, Professor, Oral and Maxillofacial Pathology and Microbiology, Kalinga Institute of Dental Sciences (KIDS), Kalinga Institute of Industrial Technology (KIIT) Deemed to be University, Bhubaneswar-751024, Odisha, India.
3. Dr. Soumya Jal, Associate Professor & Dean, School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Ramachandrapur, Bhubaneswar-752050, Odisha. soumya.jal@cutm.ac.in
4. Harish Kumar, Professor, Department of Oral Pathology, Kalinga Institute of Dental Sciences, KIIT deemed to be university, Bhubaneswar. Email: harish.kumar@kids.ac.in.
5. Dr. Pallavi Mishra, Senior Lecturer, Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, KIIT Deemed to be University, Patia, Bhubaneswar-751024. drpallavimishra1988@gmail.com

Correspondence:

Soumya Jal, Associate Professor and Dean, School of Paramedics and Allied Health Sciences, Centurion Univeristy of Technology and Management , Bhubaneswar. Email: soumya.jal@cutm.ac.in.

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actinomycetemcomitans has been extensively studied among the various microorganisms linked to periodontal disease.[3] This facultative anaerobic Gram-negative bacterium colonises the oral cavity and generates a variety of virulence factors that lead to the degradation of periodontal tissue. [4]

The ability of A. actinomycetemcomitans to adhere to oral tissues, elude host immune responses, and produce toxins that harm host cells is thought to contribute to its pathogenic potential. Leukotoxin (LtxA), cytolethal distending toxin (CDT), lipopolysaccharides, outer membrane vesicles, and adhesins that promote bacterial colonisation and tissue invasion are important virulence factors. [5] One of the most significant virulence factors of A. actinomycetemcomitans is thought to be leukotoxin. This toxin specifically targets leukocytes, resulting in the death of immune cells and increased inflammatory reactions that worsen periodontal tissue. [6] Another significant virulence factor that can cause DNA damage and cell cycle arrest in host cells is the cytolethal distending toxin. Together, these poisons encourage the deterioration of periodontal tissue and the advancement of the illness. [7]

The slow growth, low sensitivity, and difficulty in identifying picky organisms are some of the drawbacks of traditional culture-based methods for periodontal pathogen detection. Microbial detection has been transformed by molecular diagnostic methods like polymerase chain reaction (PCR), which allow for the quick and extremely sensitive identification of particular bacterial species and virulence genes.[8] Even at low concentrations, periodontal pathogens can be identified using PCR-based detection, which also offers important information about the virulence traits and microbial composition. These techniques are now commonly employed in clinical diagnostics and periodontal research. Research has shown that different populations and geographical areas have different rates of A. actinomycetemcomitans.[9, 10] Variations in virulence potential and disease outcomes are caused by genetic diversity within the species.

On the other hand, little is known about the molecular characterization of A. actinomycetemcomitans in Eastern Indian populations. Gaining knowledge about this pathogen's prevalence and virulence gene distribution could help understand the pathophysiology of periodontal disease in this area. There are still a number of unanswered questions regarding Aggregatibacter actinomycetemcomitans in the Indian population, despite substantial research on periodontal pathogens conducted worldwide. Western populations have been the subject of the majority of molecular research on A. actinomycetemcomitans. Virulence gene profiles in Eastern Indian populations have not been thoroughly studied. In this area, the relationship between clinical periodontal parameters and bacterial virulence genes has not been thoroughly investigated. The presence of this pathogen in other chronic oral inflammatory conditions, such as oral potentially malignant disorders, has been the subject of very few studies.

These gaps show that in order to fully comprehend periodontal microbial ecology, region-specific molecular research is required. Thus, the current study intends to use molecular techniques to characterize Aggregatibacter actinomycetemcomitans in chronic oral inflammatory conditions and assess its relationship with clinical periodontal parameters.

MATERIALS AND METHODS

The Department of Oral Pathology and Microbiology at a dental facility in Eastern India was the site of the current study, which was planned as a cross-sectional molecular epidemiological investigation. The Institutional Ethical Committee granted the study ethical clearance before it started. Based on their clinical condition, the study's 120 participants were divided into three groups. 45 individuals in Group I had been diagnosed with chronic periodontitis, 40 individuals in Group II had been diagnosed with gingivitis, and 35 individuals in Group III had been diagnosed with oral potentially malignant disorders (OPMD).

For all the participants, a comprehensive clinical periodontal examination was conducted. The clinical parameters recorded included Plaque Index as defined by Silness and Löe, Gingival Index as defined by Löe and Silness, probing pocket depth, clinical attachment loss, and bleeding on probing. The parameters were recorded to assess the periodontal status of the participants.

Subgingival plaque samples from all the participants were collected under aseptic conditions using sterile curette instruments from the deepest periodontal pocket. The collected plaque samples were immediately transported after being added to a sterile microcentrifuge

tube containing Tris-EDTA (TE) buffer.

The extraction of genomic DNA was performed by utilizing a genomic DNA isolation kit following the manufacturer's instructions. This extraction procedure involved a number of steps, including cell lysis, which allowed the breaking of the bacterial cells. Following cell lysis, protein digestion was performed to remove the cellular proteins and impurities. Subsequently, DNA precipitation was conducted to remove the DNA from the solution. DNA purification was then performed to obtain high-quality genomic DNA. Finally, the purified DNA samples were stored at −20 °C.

The polymerase chain reaction (PCR) was performed for the detection of target bacterial species by utilizing species-specific primers designed to amplify the 16S rRNA gene. Amixture of DNA template, forward primer, reverse primer, Taq DNA polymerase, PCR buffer, magnesium chloride ($MgCl_2$), and a deoxynucleotide triphosphate (dNTP) mixture was prepared in the correct concentration to amplify the target gene by utilizing the PCR analysis method.

Species-specific primers for the 16S rRNA gene and virulence-associated genes were designed for PCR amplification.Theseprimersequenceswereobtainedfrom already published research articles. For the amplification of the 16S rRNA gene, the forward primer sequence is AAACCCATCTCTGAGTTCTTCTTC, and the reverse primer sequence is ATGCCAACTTGACGTTAAAT. These primer sequences amplified a fragment of 557 base pairs. In the case of the leukotoxin gene (ltxA), the forward primer sequence is GCAATCAGGATTGACCGAAA, and the reverse primer sequence is CTGCGTATTTGTCGTTGCTG. These primer sequences amplified a fragment of 285 base pairs. Similarly, the cytolethal distending toxin gene (cdtB) was amplified using the forward primer TGGCGATGATGTTGATGAG and the reverse primer TCGGTTGTTGTTGATGTTGG, producing a PCR amplicon of 430 bp.

The process of PCR amplification was carried out using standardized thermal cycling conditions. The thermal cycle for PCR amplification started with an initial denaturation step at 95 °C for 5 minutes. It was followed by multiple cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds. The process of amplification ended with a final extension step at 72 °C for 7 minutes, ensuring that all the products of PCR were synthesized.

The amplified products of the PCR reaction were separated by 1.5% agarose gel electrophoresis and viewed under UV light to confirm the specific bands of amplification corresponding to the sizes of the target gene.

Statistical analysis of the collected data was done using SPSS software. The statistical tests used for data analysis included the Chi-square test for comparing categorical data, Student's t-test for comparing two continuous variables, and logistic regression for determining associations between variables. If the p-value was less than 0.05, it was considered statistically significant.

RESULTS

The prevalence of Aggregatibacter actinomycetemcomitans was evaluated among the different groups of the study. The highest prevalence was observed in the chronic periodontitis group, in which 28 out of 45 samples were found to be positive, accounting for 62.2% of the total samples. In the gingivitis group, 14 samples were found to be positive out of 40 samples, accounting for a prevalence of 35%. A lower prevalence was observed in the OPMD group, in which 8 samples were found to be positive out of 35 samples, accounting for 22.8%.[Table 1]

The distribution of the virulence-associated genes was also evaluated among the samples. The leukotoxin gene (ltxA) was found in 29 samples, accounting for 58% of the total samples. Similarly, the cytolethal distending toxin gene (cdtB) was found in 23 samples, accounting for 46% of the total samples. These genes play a significant role in the pathogenicity of Aggregatibacter actinomycetemcomitans [Table 2].

Furthermore, the statistical analysis revealed a significant association between the presence of A. actinomycetemcomitans and several clinical periodontal parameters. In particular, it was noted that an increase in the periodontal pocket depth, clinical attachment loss, and bleeding on probing had significant correlation values in relation to the presence of the microorganism.

DISCUSSION

Periodontal diseases are complex inflammatory conditions involving interplay between biofilms and host immune responses. The specific role of various periodontal pathogens in initiating and

perpetuating periodontal diseases has been extensively studied. Among various pathogens, Aggregatibacter actinomycetemcomitans is identified as a primary causative agent of various forms of periodontal diseases, particularly aggressive and chronic periodontitis.[3,11] The current study aimed to investigate the presence of A. actinomycetemcomitans and its virulence factors in chronic oral inflammatory conditions using molecular diagnostic methods in an Eastern Indian population. The results of this study will help in understanding the microbial association of periodontal diseases and gain insight into the distribution of virulence factors, which might impact the progression of the disease.

One of the major observations of the present study was the significantly higher prevalence of A. actinomycetemcomitans in patients with chronic periodontitis compared to individuals diagnosed with gingivitis and oral potentially malignant disorders. This observation supports the concept that periodontal disease progression is related to the selective enrichment of pathogenic microorganisms in the subgingival biofilm. Previous studies have shown that the subgingival biofilm of periodontal diseases significantly differs from that of periodontal health. Periodontal pockets offer an environment that is favorable for the colonization of Gram-negative facultative and obligate anaerobic bacteria, including A. actinomycetemcomitans. In addition, as the periodontal pocket becomes deeper and the periodontal inflammation increases, the biofilm composition changes to a pathogenic consortium that can lead to tissue damage.

The association of A. actinomycetemcomitans with periodontal disease has been well documented in various epidemiological studies. Culture studies have shown that this organism plays a significant role in localized aggressive periodontitis. However, recent molecular studies have shown that it can be found in all forms of periodontal disease, including chronic periodontitis. [13] In the current study, a high prevalence rate of this organism in chronic periodontitis patients was detected through a molecular approach involving a polymerase chain reaction. This high rate in chronic periodontitis patients might be due to its capacity to colonize deep pockets in the periodontal disease lesion since it thrives in a favorable environment.

The advantages of detection of pathogens using a polymerase chain reaction-based detection system over traditional culture systems are many. The traditional culture systems are handicapped because many of the periodontal pathogens are fastidious in nature. A. actinomycetemcomitans are fastidious in nature. They need specific growth conditions to grow. This sometimes becomes a disadvantage in detecting these pathogens because they take longer to grow. This can affect the sensitivity of detection. In a polymerase chain reaction system, specific DNA sequences can be amplified. This system can detect minute quantities of bacterial genetic material in a sample. This sensitivity can be utilized to identify pathogens in a sample of subgingival plaque. The use of species-specific primers targeting the 16S rRNA gene can be used to identify the organism. [14]

Besides determining the presence of A. actinomycetemcomitans, this current study also aimed to determine the distribution of key virulence genes, such as the leukotoxin gene (ltxA) and cytolethal distending toxin gene (cdtB). These virulence factors have a key role in the pathogenicity of this organism and contribute significantly to tissue destruction in periodontal tissues. The presence of these virulence factors in a large percentage of isolates indicates that this organism in this population has a high potential for causing disease.[15]

Leukotoxin has been described as one of the key virulence factors produced by A. actinomycetemcomitans. This toxin belongs to the RTX family of toxins and demonstrates specific cytotoxic effects against leukocytes, especially neutrophils and macrophages. This weakening of the immune response enables the pathogen to escape immune detection. In addition to the direct effects against leukocytes, leukotoxin can induce the production of inflammatory mediators that play a key role in tissue damage. Strains producing leukotoxin have been strongly associated with severe forms of periodontitis. In the present study, the detection of the ltxA gene among a significant number of samples points towards the presence of virulent strains capable of modulating immune responses.[16]

Another significant virulence factor that was assessed in this study included the cytolethal distending toxin (CDT), which is a tripartite toxin composed of three subunits encoded by cdtA, cdtB, and cdtC genes. The cdtB subunit acts as a DNase that causes DNA damage in host cells, resulting in cell cycle arrest and apoptosis. The capacity of CDT to affect cell proliferation and cause damage to host cells plays a significant part in virulence. The detection of cdtB in various strains in

this study indicates that CDT might have a part to play in tissue destruction in periodontal disease.[17]

Presence of various virulence factors may be beneficial to the virulence of the bacteria. The presence of various virulence factors may increase the virulence of the bacteria. The synergistic effect of the presence of both leukotoxin and cytolethal distending toxin genes may increase the virulence of the strains. The presence of virulence factors like these in the strains indicates the need to assess the virulence of the periodontal pathogens. [18]

Another significant finding of the present study is that there is a significant correlation between A. actinomycetemcomitans and clinical periodontal parameters such as probing pocket depth and clinical attachment loss. These parameters are used as clinical indicators of the severity of periodontal disease and reflect the extent of tissue destruction. The relationship of A. actinomycetemcomitans with probing pocket depth indicates that the pathogen may play a role in the progression of periodontal disease by elaborating virulence factors that induce tissue damage.[12,19]

The association of periodontal pathogens with clinical parameters has been shown in various previous studies. It has been found that the presence of A. actinomycetemcomitans has been associated with greater periodontal pocket depth, bleeding on probing, and greater clinical attachment loss. This supports the hypothesis that certain bacterial species are involved in the pathogenesis of periodontal tissue destruction. However, it should be noted that periodontal disease is a multifactorial process involving a complex interaction between microbial and immune system factors.[20]

Apart from the microbe, host susceptibility also has an important role to play in disease progression. In this regard, host genetic factors, general health status, and environmental factors such as smoking may play a role in disease progression. Differences in host immune response may help explain the severe periodontal disease observed in certain individuals despite similar exposure to microorganisms. In addition, geographic differences in the prevalence and virulence pattern of A. actinomycetemcomitans have been described in the literature. In various studies conducted among different populations, differences in the prevalence of specific genotypes and serotypes of the microorganism have been described. Certain virulent strains, such as the JP2 genotype, have been associated with AGP in specific geographic locations. Knowledge of region-specific microbe patterns may have implications for disease epidemiology.[21]

The present study yields useful data regarding the molecular characteristics of A. actinomycetemcomitans in the Eastern Indian population. The scarcity of data regarding the molecular epidemiology of A. actinomycetemcomitans in the region makes the present study relevant. The molecular characteristics of A. actinomycetemcomitans may be useful in developing diagnostic tools for the disease.

Another interesting observation of the present study is the presence of A. actinomycetemcomitans among the group of patients with oral potentially malignant disorders. Although the prevalence of A. actinomycetemcomitans is lower among the patients with oral potentially malignant disorders than among the patients with chronic periodontitis, the presence of A. actinomycetemcomitans among the patients with oral potentially malignant disorders indicates the possible association of periodontal pathogens with the disease. Chronic inflammation is one of the key players in the development of carcinogenesis. Microbial infections may be responsible for the inflammatory environment of the disease. The exact relationship between the disease and periodontal pathogens needs to be studied. [22]

The use of molecular techniques in periodontal disease has greatly improved our knowledge of microbial ecology in the mouth. This study showed that periodontal disease is caused by polymicrobial infection instead of a single infectious agent. However, certain bacteria like A. actinomycetemcomitans are still considered to be major contributors to disease pathogenesis because of their virulence potential.

Even though the study has given valuable knowledge on the topic, it should be noted that it had a moderate sample size. It should also be noted that it was a single-center study. Therefore, more studies should be done to obtain more knowledge on the distribution of periodontal pathogens in different populations. It should also be noted that it was a cross-sectional study; therefore, it did not give clear knowledge on causality. Longitudinal studies would be beneficial in evaluating temporal changes in microbial profiles during disease progression and treatment.

Another limitation of the study concerns the limited

virulence genes that were included in the analysis. Although leukotoxin and cytolethal distending toxin are significantvirulencefactorsinA.actinomycetemcomitans infection, various other virulence factors are also secreted by A. actinomycetemcomitans. These virulence factors include adhesins, outer membrane vesicles, and lipopolysaccharides. Future studies involving more virulence markers might reveal more insights into the virulence potential of A. actinomycetemcomitans.

Significant advancements have been made in the field of molecular biology that have led to the development of techniques like quantitative PCR and next-generation sequencing that can be used to quantify microbial communities. Future studies involving advanced molecular biology techniques might reveal more insights into microbial ecology in periodontal disease.

Clinical implications of the identification of periodontal pathogens are of great significance. Early detection of virulent strains of bacteria may prove to be useful in identifying patients at higher risk of disease progression. Molecular diagnostic techniques may prove to be useful in the development of personalized therapy for periodontal disease.

Future studies may be directed toward conducting clinical investigations involving a larger population of patients using advanced molecular techniques. Such studies may prove useful in gaining deeper insights into the complex interactions of microorganisms responsible for periodontal disease.

CONCLUSION

The novelty of the present study includes the molecular characterization of A. actinomycetemcomitans in an Eastern Indian population, detection of multiple virulence genes (ltxA and cdtB) simultaneously, evaluation of the pathogen in different chronic inflammatory conditions of the oral cavity rather than periodontitis, and correlation of molecular results with clinical periodontal parameters. Such information could lead to a better understanding of the pathogenesis of periodontal diseases and aid in the development of effective treatment modalities.

In conclusion, the results of the present study confirm the virulence potential of Aggregatibacter actinomycetemcomitans in the etiopathogenesis of chronic periodontal disease. The presence of virulence genes of the same pathogen indicates its virulence potential. The results of the present study add to the existing literature regarding the virulence potential of the same pathogen. The results of the present study confirm the significance of the molecular approach for the characterization of the virulence potential of the same pathogen. The results of the present study confirm the significance of the molecular approach for the characterization of the virulence potential of the same pathogen. The results of the present study confirm the significance of the molecular approach for the characterization of the virulence potential of the same pathogen. The results of the present study confirm the significance of the molecular approach for the characterization of the virulence potential of the same pathogen.

Table 1: Prevalence of *Aggregatibacter actinomycetemcomitans* Among Study Groups

Study Group	Total Samples	Positive Samples	Prevalence (%)
Chronic Periodontitis	45	28	62.2%
Gingivitis	40	14	35.0%
OPMD	35	8	22.8%

Table 2: Distribution of Virulence-Associated Genes

Gene	Positive Samples	Prevalence (%)
Leukotoxin gene (ltxA)	29	58%
Cytolethal distending toxin (cdtB)	23	46%

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