Quantitative Analysis of Tannerella Forsythia Level in Periodontitis Patients with or without Diabetes Mellitus

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AB did the Data collection, Data analysis and interpretation and drafting of article and critical revision of the article. Author MJ and SJ drafting of article and critical revision of the article managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Periodontitis is a systemic chronic inflammatory disease resulting from a complex polymicrobial infection causing tissue destruction as a consequence of perturbation of the homeostasis between subgingival microflora. T. forsythia has been strongly implicated in the onset of periodontitis. The level of glycemic control is the key to determining increased risk. In adults, HbA1C levels >9% have a higher prevalence rate for periodontitis than those without diabetes. In adults, random blood sugar levels >200mg/dl have higher susceptibility to periodontitis.

Materials and Methods: This study was carried out in a university setting at Saveetha dental college. Total of 8 samples were collected from November 2020 to February 2021. The subgingival plaque samples containing bacterial cells were pelleted by centrifugation at room temperature. Following centrifugation with all buffer solutions, quantitative RT-PCR was performed with stratagene MX3000P and the relative gene count was calculated using relative CT method.

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purified DNA molecules were identified by agarose gel electrophoresis and T. forsythia was identified using this method.

**Results:** The assessment of mRNA expression of *T. forsythia* assessed by RT-PCR showed that *T. forsythia* was seen with more prevalence in patients with periodontitis with diabetes mellitus (n=1,388), than in patients with periodontitis without diabetes mellitus (n=1) (P=0.0028 < 0.005). The study was statistically significant.

**Conclusion:** This study shows that *T. forsythia* is seen with more prevalence in patients with periodontitis with diabetes mellitus when compared to periodontitis without diabetes mellitus.

Keywords: Diabetes mellitus; Innovative technology; Tannerella. Forsythia; periodontitis.

1. **INTRODUCTION**

Periodontitis is a systemic chronic inflammatory disease resulting from a complex polymicrobial infection causing tissue destruction as a consequence of perturbation of the homeostasis between sub gingival microflora [1]. It can lead to root caries, eating disabilities, tooth mobility and systemic diseases in the human population [2,3]. The major microflora causing periodontitis in the human community belong to the red complex species which includes *A. actinomycetemcomitans, P. gingivalis, T. forsythia, P. nigrescens, P. intermedia, T. denticola* [4].

*T. forsythia* has been strongly implicated in the onset of periodontitis [5,6], *T. forsythia* is an anaerobic, gram negative bacteria belonging to the cytophaga-bacteroides family which was initially described as *Bacteroides forsythus* [7,8]. Diabetes mellitus increases the susceptibility to periodontitis by a threefold [9–11]. The level of glycemic control is the key to determining increased risk. In adults, HbA1C levels >9% have a higher prevalence rate for periodontitis than those without diabetes [14,15]. In adults, random blood sugar levels >200 mg/dl have higher susceptibility to periodontitis [16,17]. The present study aims to quantitatively analyse *T. forsythia* levels in periodontitis patients with or without diabetes mellitus.

2. **MATERIALS AND METHODS**

This study was carried out in a university setting at Saveetha dental college. Patients aged between 30-60 reported to the department of periodontics. Total of 8 samples were collected from November 2020 to February 2021. The samples were selected from patients diagnosed with generalized periodontitis with a history or without diabetes mellitus.

2.1 Inclusion Criteria

1. No >2 missing teeth per quadrant
2. >30% of sites with PD greater than or equal to 4mm
3. >20% of sites with CAL greater than 2mm
4. Bleed on probing greater than or equal to 30% and radiographic indication of bone loss.

2.2 Exclusion Criteria

1. If the patient had undergone and periodontal treatment in the last 6 months
2. Tobacco use and smoking
3. Alcoholism
4. Conditions that could alter healing response like periodontal abscess, acute necrotizing gingivitis, oral mucosal lesions

2.3 Isolation and Quantification of *T. forsythia* by Real time-PCR

The subgingival plaque samples containing bacterial cells were pelleted by centrifugation at room temperature. Following centrifugation with all buffer solutions, quantitative RT-PCR was performed with stratagene MX3000P and the relative gene count was calculated using relative CT method. The purified DNA molecules were identified by agarose gel electrophoresis and *T. forsythia* was identified using this method.

3. **RESULTS**

The assessment of mRNA expression of *T. forsythia* assessed by RT-PCR showed that *T. forsythia* was seen with more prevalence in patients with periodontitis with diabetes mellitus (n=1,388), than in patients with periodontitis without diabetes mellitus (n=1) (P=0.0028 < 0.005). The study was statistically significant.
Fig. 1. This graph shows the expression of mRNA of *T. forsythia* in patients with periodontitis without diabetes mellitus and patients with periodontitis without diabetes mellitus.

**Chart 1. P Value of mRNA of *T.forsythia* in patients**

| group                | Periodontitis | Periodontitis +DM | P VALUE |
|----------------------|---------------|-------------------|---------|
| *T. forsythia* mRNA  | 1 +/- 0.00    | 1.350 +/- 0.057   | 0.0067  |

4. DISCUSSION

*T. forsythia* is a Gram-negative anaerobic organism that inhabits the subgingival cavity and initiates connective tissue destruction and alveolar bone resorption in periodontal disease (PD) [18,19].

This study showed that *T. forsythia* was seen with more prevalence in patients with periodontitis with diabetes mellitus [20,21]. Investigations have demonstrated associations between periodontitis and various systemic diseases such as cardiovascular disorders, respiratory diseases, osteoporosis, immunodeficiencies and also diabetes mellitus [22,23]. Longitudinal studies have demonstrated a two-way relationship between diabetes and periodontitis [24], with more severe periodontal tissue destruction in diabetic patients and poorer glycemic control in diabetic subjects with periodontal disease [14].

Glycemic control was the most important risk factor related to severity and extent of periodontitis [25,26]. Diabetes leads to worsening of periodontal disease, and a significant association between diabetes and periodontitis has been demonstrated [27]. Periodontal disease has a higher incidence in diabetic patients, and it is more prevalent and severe if compared with a healthy population. The risk of periodontitis is 3-fold times higher among diabetic patients, being its prevalence and severity even greater in diabetic patients presenting elevated HbA1c levels [25,28]. Periodontal inflammation, as any other infections, can have an adverse effect on diabetes glycemic control, compromising diabetes management in these individuals [16]. Most evidence on this issue is derived from interventional and observational studies, indicating that periodontitis affects the glycemic control of diabetic patients [29]. HbA1c values < 7% are related with proper glycemic levels whilst > 8% values represents poorly controlled glycaemia [12]. Longitudinal studies have demonstrated that severe periodontitis is associated with poorly controlled glycaemia, higher HbA1c levels and development of diabetic systemic complications.
5. CONCLUSION

This study shows that *T. forsythia* is seen with more prevalence in patients with periodontitis with diabetes mellitus when compared to periodontitis without diabetes mellitus.

6. CONSENT

It is not applicable.

7. ETHICAL APPROVAL

As per international standard or university standard guideline ethical approval has been collected and preserved by the authors.

8. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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