Original Article

Gingival crevicular fluid and salivary levels of neuregulin-4 and its receptor ErbB4 in patients with stage III and IV periodontitis with and without type 2 diabetes: An Observational study

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Abstract

Aim: Periodontitis and diabetes mellitus are common diseases that affect a large population worldwide. While researching the possible mechanisms linking periodontitis and diabetes, adipokines emerged as a promising common biological mediator between both diseases. The purpose of this study is to assess the levels of Neuregulin-4, and its receptor ErbB4 in the GCF and saliva of stage III and IV periodontitis patients with and without type II diabetes.

Subjects and methods: Saliva and GCF samples were collected from 42 participants, 14 healthy participants, 14 stage III or IV periodontitis patients with diabetes mellitus and 14 non-diabetic stage III or IV periodontitis patients. Samples were analyzed using ELISA and results were correlated with clinical periodontal parameters, glycosylated hemoglobin, and fasting plasma glucose.

Results: Elevated levels of Nrg4 and its receptor ErbB4 were found in the periodontitis and diabetes mellitus groups, with a statistically significant difference between the periodontitis and non-diabetic groups, as well as the periodontally healthy group. There was no significant correlation between periodontal clinical parameters, glycosylated hemoglobin, or fasting plasma glucose levels and Nrg4 and its receptor levels.

Conclusion: We found significant differences in the levels of Nrg4 and ErbB4 in gingival crevicular fluid (GCF) and saliva between healthy individuals, periodontitis patients with diabetes, periodontitis patients without diabetes, and periodontitis patients with and without diabetes. These differences indicate that Nrg4 and ErbB4 are involved in the pathophysiology of periodontitis, and their levels are affected by the presence of diabetes.

Keywords: Neuregualin-4; ErbB4; DM; periodontitis; gingival crevicular fluid, saliva.

Introduction

Periodontal disease is a chronic inflammatory disorder that has a detrimental effect on both oral health and the periodontium. It is a common disease that causes irreversible destruction of periodontal hard and soft tissues.

Severe periodontitis is rated as the sixth most prevalent human disease [1]. It is defined as a multifactorial inflammatory disease associated with dysbiotic dental biofilm characterized mainly by clinical attachment loss, radiographic bone loss, and gingival recession[2]. Recent data showed that diabetes mellitus and periodontitis

are linked in a bidirectional manner. Patients with diabetes may experience impaired immune function, including reduced neutrophil chemotaxis and phagocytosis, which can compromise the body's ability to fight infections such as periodontitis. Diabetes can lead to increased levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which can exacerbate the inflammatory response in periodontitis. Diabetes can impair the normal healing process, leading to delayed healing of the periodontal tissues and making the periodontium more susceptible to infection and damage. High blood glucose levels in diabetes can provide an optimal environment for the growth of bacteria associated with periodontitis, as they can utilize glucose as a source of energy, leading to increased bacterial activity and plaque formation. Periodontal infection and inflammation can lead to the release of pro-inflammatory cytokines into the bloodstream, which can contribute to insulin resistance and elevated blood glucose levels in individuals with diabetes. [3–6] .

Adipokines play a crucial role in the regulation of metabolism, inflammation, and insulin sensitivity and are emerging as indicators of the functional status of adipose tissue. Evidence suggests a link between adipokines and multifactorial chronic diseases such as type 2 diabetes mellitus and periodontitis. It has been demonstrated that adipokines play a coordinated role in the health and inflammation of periodontal tissues [5].

Neuregulin-4 (Nrg4) is a novel adipokine and a member of the epidermal growth factors family that acts as an extracellular ligand. It binds specifically to the receptor tyrosine kinase ErbB4 which is one of the ErbB/HER protein-tyrosine kinase receptors family[7,8]. Several studies suggest that Nrg4 and ErbB4 are involved in the pathophysiology of numerous chronic inflammatory diseases[9], particularly metabolic disorders such as diabetes mellitus, non-alcoholic fatty liver disease[10], obesity [11]and cardiovascular diseases [12–15] .

Nrg4 and ErbB4 levels in the gingival crevicular fluid (GCF) were positively correlated with the severity of various periodontal clinical parameters, suggesting that they play a significant role in the pathogenesis of periodontitis[9]. A resolving mechanism to eliminate the prolonged inflammation seen in many diseases, is to eradicate the proinflammatory macrophage phenotype (M1) from the tissues. Recent research indicates that the Nrg4-ErbB4 signaling pathway anti-inflammatory mechanism involves encouraging the apoptosis of proinflammatory macrophages[16].

Consequently, targeted inhibition of inflammatory cell subsets may be a therapeutic approach for chronic inflammatory diseases such as periodontitis based on the activation of the ErbB4- Nrg4 signaling pathway. Hence, the current study aims to assess the levels of Nrg4 and ErbB4 in periodontitis stage III and stage IV patients with and without type 2 diabetes mellitus.

Subjects and Methods

This analytical observational cross-sectional study was registered in the U.S. National Institutes of Health Clinical Trials Registry , ClinicalTrials.gov Identifier: ID: NCT04930588, and the study protocol was approved by the Ethics Committee of Scientific Research, Faculty of Dentistry, and Cairo University (1-3-21). The complete steps were thoroughly presented to all participants, and patients who decided to engage in this study gave a written informed consent.

The present study was conducted on forty-two participants recruited from the outpatient clinic of the department of Oral Medicine and Periodontology, Faculty of Dentistry-Cairo University from June 2021 till December 2023. Screening of patients continued until the target sample was achieved. Identifying and recruiting potential subjects was achieved through patient's examination and periodontal charting.

Forty two participants were divided equally into the three groups:

Group (1): patients with periodontitis and type 2 DM with no other systemic disease.

Group (2): stage III or IV periodontitis and systemically healthy patients

Group (3): periodontally and systemically healthy individuals
Eligibility criteria

For stage III and IV periodontitis patients in group 1 and 2 [17–19] should have bleeding on probing (BOP) ≥ 30%, having at least four non-adjacent teeth sites with CAL ≥ 5 mm and PD ≥ 6 mm in one or more sites, tooth loss due to periodontitis ≥ 4 teeth and have a minimum of 15 natural remaining teeth. For patients with type 2 diabetes mellitus, they were diagnosed as having type 2 diabetes mellitus ≥ 5 years before the study and treated with stable doses of oral hypoglycemic agents and/or insulin under the supervision of an endocrinologist with their glycated hemoglobin level (HBA1c) > 6%. Regarding non-diabetic participants in the group 2 and 3, they were selected as having fasting plasma glucose (FPG) <126 mg/dl. Patients were excluded if they had any systemic disease other than type 2 diabetes, had received systemic antibiotics and anti-inflammatory drugs within the past 3 months, or were former or current smokers.

Periodontal examination

All subjects had a full-mouth periodontal examination. In periodontitis patients, clinical parameters including plaque index (PI), bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) were assessed. A manual UNC periodontal probe was used to measure the pocket depth from the gingival margin to the bottom of the gingival sulcus. It was also used to measure the clinical attachment level from the CEJ to the bottom of the gingival sulcus. For each tooth, these readings were taken at six different locations (mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, distolingual, and mid-lingual). The same investigator used a manual UNC periodontal probe to capture clinical information.

Gingival crevicular fluid (GCF) samples collection

To avoid mixing GCF with blood when probing inflamed sites, samples for all groups were collected the day after the patients had received clinical assessment. Teeth were isolated with cotton rolls before sample, supragingival plaque was eliminated with a sterile curette, and the surfaces were gently air-dried. The deepest site was sampled for GCF. GCF was extracted by introducing absorbent sterile paper strips for 30 seconds into the gingival sulcus or periodontal pocket. Blood or saliva contaminated paper strips were rejected (Meraci et al., 2020).

Two strips were taken from each study's participant, placed in the same coded Eppendorf tube, merged to make a single sample, and rapidly kept at -80C until they could be examined.

Saliva samples collection

All individuals in this investigation provided unstimulated entire expectorated saliva samples. Each individual provided a saliva sample of 5 mL. The patients were instructed to rinse their mouths with tape water. Then, saliva sample was taken by expectorating the patient into an Eppendorf tube while set upright. The supernatant phase was transferred to Eppendorf tubes and kept at 80°C until analysis (Navazesh, 1993).

Quantitation of Human neuregulin-4

Saliva and GCF from each patient were prepared for detection of Human neuregulin-4 level by using ELISA kit provided by Bioassay Technology Laboratory, China with Cat. No E3931Hu. This ELISA kit is sandwich kit for the quantitative detection of Human neuregulin-4 (also known as NRG4) in serum and other biological fluids.

Quantitation of Human Receptor tyrosine-protein kinase ErbB-4

Saliva and GCF were produced from each patient for erbB-4 detection using an ELISA kit donated by Bioassay Technology Laboratory, China. This sandwich kit is used to detect Human Receptor Tyrosine-Protein Kinase ErbB-4 in serum and other biological fluid.

Chi- square test was used to compare between the groups. Correlation between various clinical parameters and GCF and saliva of neuregulin-4 and its receptor ErbB4 levels were determined using Spearman's correlation coefficient. The significance level was set at P ≤ 0.05. Data were analyzed using IBM SPSS advanced statistics.

Results

The current study comprised 34 persons, 13 men and 21 women, and there was no statistically significant difference in gender among groups (P = 0.2409). The participants' mean age was 44.47 10.7 years, with a statistically significant difference in age across groups (P = 0.001). The entire sample size was 42, however the data of six healthy group members were lost, hence the number was reduced to 34.

Regarding the clinical periodontal parameters, there was a statistically significant difference when comparing all groups (P < 0.001). Further pairwise comparisons indicated a statistically significant difference between healthy individuals and both diabetic patients with periodontitis, as well as non-diabetic patients with periodontitis. However, there was no statistically significant difference between diabetic patients with periodontitis and non-diabetic patients with periodontitis.

In terms of Nrg4 and ErBb4 levels in the gingival crevicular fluid (GCF), the intergroup comparison among all groups showed a statistically significant difference (P < 0.001), as shown in Table 1. Additionally, pairwise comparisons revealed a statistically significant difference between the diabetic and non-diabetic groups with periodontitis compared to healthy controls. There was also a statistically significant difference between the diabetic and non-diabetic groups with periodontitis.

Table (1): Mean and standard deviation of Neuregulin-4 and ErbB4 in the GCF/saliva (μL) for all groups

	Healthy Mean ±SD	Periodontitis + Diabetes Mean ±SD	Periodontitis + non-diabetes Mean ±SD	*P*
Neuregulin-4 in the GCF (μL)	7.06a ± 1.2	25.65c ± 1.65	12.86b ± 1.6	*P* < 0.001*
ErbB4 in the GCF (ng/ml)	6.86a ± 0.39	24.62c ± 1.03	14.36b ± 0.57	*P* < 0.001*
Neuregulin-4 in the saliva (μL)	3.81a ± 1.23	7.06b ± 2.07	5.7ab ± 1.09	*P* = 0.045*
ErbB4 in the saliva (ng/ml)	3.38a ± 0.69	7.06c ± 1.58	5.5b ± 1.12	*P* < 0.001*

Statistically significant differences were observed in the intergroup comparison of Nrg4 and ErbB4 levels in saliva. When comparing healthy individuals to diabetic patients with periodontitis, there was a statistically significant difference. However, no significant differences were found between healthy individuals and non-diabetic patients with periodontitis, or between diabetic patients with periodontitis and non-diabetic patients with periodontitis in pairwise comparisons.

The data obtained from the study does not indicate any correlation between the levels of Nrg4 and its receptor in the gingival crevicular fluid and saliva, and the clinical periodontal parameters in any of the groups.

Discussion

Nrg4, an adipokine, has been found to possess anti-inflammatory properties by inhibiting the pro-inflammatory M1 macrophage phenotype and promoting the anti-inflammatory M2 macrophage genotype, as indicated by the CD163 marker. This suggests that Nrg4 may play a role in regulating the inflammatory response in conditions such as diabetes and periodontitis. These findings highlight the potential of Nrg4 and adipokines as targets for future therapeutic interventions and as markers for assessing the progression of these diseases.

The serological increase in Nrg4 levels may be a compensatory reaction to counteract the reduced expression of Nrg4 in adipose tissue or the impaired Nrg4/ErbB4 pathway due to receptor resistance, known as "Nrg4 resistance." In Nrg4 resistance, the activation of Nrg4 is impaired as a result of diabetes mellitus[10]. On the other hand, it was reported decreased serum Nrg4 levels in association with increased type II diabetes mellitus risk. The authors claimed that there were two reasons why their results differed from those of the studies mentioned later[13]. The first feature is that Nrg4 is secreted not only from brown adipose tissue but also from other tissues such as the liver, stomach, and muscles, all of which contribute to the level of Nrg4 in the blood [20] . The second feature is that the expression of Nrg4 in adipose tissue varies depending on the temperature [21].

These contradictory results could be explained by the subsequent reasons. The ethnic differences between the subjects enrolled in the studies that resulted in heterogenicity of their clinical features, the fact that all of these studies were observational with small sample sizes and a lack of representativity, making it impossible to establish a causal relationship between Nrg4 and diabetes mellitus, and finally the differences in methods of detecting serum Nrg4. Numerous association studies have been conducted to investigate the role of Nrg4 in diabetes mellitus. However, the role of Nrg4 in periodontitis has not been widely researched.

Meraci et al. [9] were the first to assess Nrg4 and ErbB4 levels in serum and GCF in periodontal health and disease. The GCF total amounts of Nrg4 and ErbB4 were found to be elevated at baseline and were positively linked with periodontal clinical parameters. Due to a lack of available data in the literature, these findings were compared to previous biochemical findings that assessed Nrg4 levels in other chronic diseases that share common pathogenesis with periodontitis.

Our findings are partially consistent with that of Meraci et al. [9]. This may be explained by the fact that the present study's correlation results were done with the concentration levels of Nrg4 and ErbB4 in GCF, whereas Meraci et al (2020) correlated with total volume levels. Total volume levels, on the other hand, reflect general indications of inflammation and better reflect the level of biomarkers in the GCF. The overexpression of the ErbB4 receptor in response to proinflammatory macrophage activation may lead to binding with Nrg4, enhancing the mitochondrial apoptotic pathways of proinflammatory macrophages and reducing inflammation. During acute inflammation phases, the excessive proinflammatory macrophages may deplete endogenous Nrg4 levels, but its expression increases during recovery phases.

Small sample size and a cross-sectional study design are two of the study's limitations. Strong evidence can be obtained from well-designed studies with larger sample sizes and matching results after a longer follow-up period following periodontal treatment.

Conclusion

Nrg4 and its receptor ErbB4 suggested to have a role in periodontitis and periodontitis with diabetes mutual relationship.

Conflict of Interest:

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university

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