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Evaluation of Expression Profiles of Salivary Ghrelin in Periodontitis Patients

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ABSTRACT:

Background-Ghrelin, a peptide hormone known for its influence on the immune system, was the focus of this study. The primary objective was to check the levels of ghrelin in the plasma of individuals with chronic periodontitis and explore potential associations between ghrelin and periodontal parameters.
Methodology- This study comprised 20 individuals with chronic periodontitis (CP) and a control group of periodontally healthy individuals (C). Various periodontal parameters were documented, and saliva samples were collected to analyze ghrelin levels.
Results- A significant improvement was observed in both the groups ($p<0.05$). However, we did not identify a direct correlation between the levels of ghrelin and periodontal parameters. Our findings suggest an elevation in ghrelin levels among individuals with chronic periodontitis.
Conclusion- Moreover, investigations involving larger cohorts, encompassing the analysis of ghrelin levels in oral tissues and fluids are necessary to validate the potential involvement of ghrelin in periodontitis

Keywords: Ghrelin, Biomarker, salivary biomarker, Periodontitis, anti-inflammatory

1. Introduction

Ghrelin is a hormone (peptide) released mainly by the stomach, and some othe cells and tissues including glands (salivary and pituitary), odontoblasts, osteoblasts and defense cells [1]. Ghrelin exists in two main forms, namely des-acylated and acylated. They play crucial physiological roles in the release of growth hormone, regulation of appetite and metabolism [2]. Its acylated form is essential to bind to the GHS-R1a and stimulate the expression of

peptide (growth hormone). Some investigators have revealed a correlation between ghrelin's anti-inflammatory effect and the degree of acylation [3].

The impact of ghrelin on endocrine function has been a focal point of extensive scientific investigation. Recent research suggests that ghrelin may influence the immunity and bone homeostasis [4]. It demonstrates potent anti-inflammatory properties, inhibiting the release of pro-inflammatory cytokines like interleukin IL-1b and tumor necrosis factor (TNF)-a, induced by lipopolysaccharide (LPS) [5]. Ghrelin not only possesses anti-inflammatory properties but also promotes the maturation of osteoblastic cells, contributing to enhanced osteogenesis [6]. Studies indicates that ghrelin escalates the release of markers associated with osteoblast differentiation, such as alkaline phosphatase (ALP), osteocalcin (OSC), and collagen type 1. Moreover, there is no evidence suggesting relation between ghrelin levels and the soluble receptor activator of nuclear factor kappa-B (sRANKL), however a positive correlation with osteoprotegerin (OPG) levels exists [7]. Chronic illness such as ankylosing spondylitis, Crohn's disease, and inflammatory bowel disease have been associated with elevated ghrelin levels [8]. Conversely, lower levels of ghrelin are observed in conditions like type 2 diabetes mellitus, obesity, and metabolic syndrome [9]. While the precise mechanisms regulating ghrelin production are still not fully understood, studies like these suggest that systemic inflammation plays a role in influencing circulating ghrelin levels. In periodontitis, a chronic inflammatory disease characterized by bacterial accumulation, there is a destruction of both hard and soft tissues [10]. Despite microbes being the primary cause of inflammation, the destruction of periodontal tissues is facilitated by chemical mediators of inflammation. Studies have demonstrated significantly high levels of pro-inflammatory cytokines, in the serum and tissues of individuals with periodontitis [11]. The elevated levels of these may lead to the induction of RANKL expression and the suppression of OPG expression, resulting in alveolar bone loss [12]. Additionally, recent research indicates that periodontal disease affects the surrounding structures of teeth as well as has an impact on a patient's overall health [13]. Given the systemic consequences of the disease, we hypothesized that periodontitis might influence salivary expression of ghrelin, similar to other chronic inflammatory conditions.
The primary objective of the current study was to assess the expression profile of ghrelin in the salivary samples of chronic periodontitis patients.

2. Materials and Methods

Study Population

This research enlisted 20 participants, aged between 29 and 42, with 10 individuals diagnosed with chronic periodontitis (CP) and another 10 without the condition (C). Subjects were selected from individuals receiving dental care at the department of periodontology of Saveetha Dental College and Hospitals. Exclusion criteria comprised major systemic diseases, current or past smokers, pregnancy, ongoing lactation, use of oral contraceptive drugs. Ethical Approval was obtained from the regional ethics committee. All eligible participants were thoroughly briefed about the study and furnished with a written consent form.

Periodontal Parameters

During the periodontal examination, the same clinician assessed various clinical factors, including the plaque and gingival indices (PI and GI), percentage of bleeding on probing sites (BOP%), probing depth (PD), and clinical attachment levels (CAL).

Laboratory Analysis

Commercially available ELISA kits (Millipore Human Ghrelin Active/Total ELISA kit, USA) were employed to assess the ghrelin levels in saliva. The Human Ghrelin (GHRL) ELISA Kit utilized a two-site sandwich ELISA method to quantify ghrelin in samples. In this process, a microplate was pre-coated with an anti-human ghrelin antibody. Standard and sample liquids were introduced into the wells, which allowed the immobilized antibody to bind to any present ghrelin. Following the removal of any unbound compounds, HRP-conjugated ghrelin detection antibody was poured. Subsequently, a Chromogen solution was introduced after a wash to eliminate any unbound HRP reagent, and colour development occurred in proportion to the amount of ghrelin bound in the initial phase. The development was then halted, and the colour intensity was measured.

Statistical Analysis

All statistical analyses were performed using statistical software (SPSS 21.0 Chicago, IL, USA). Statistical significance was attributed to p-values below 0.05. An independent t-test was employed to assess significance and the Pearson correlation test was utilized to analyze the relationship between periodontal parameters and ghrelin levels.

3. Results

This investigation involved a total of 20 participants, comprising 10 individuals with chronic periodontitis and 10 controls. All participants completed the examinations conducted in this study. The periodontal param of both the groups are detailed (Table 1) revealing that the C group significantly outperformed the CP group in all clinical metrics ($p < 0.05$). Table 2 presents the levels of ghrelin and optical density in saliva for both the study groups and the control group. Optical density results were lower in the periodontally healthy group (C group) and higher in the chronic periodontitis group (CP group). Table 3 outlines the correlation between ghrelin levels and outcome measures, indicating a positive correlation.

Table 1. Clinical periodontal parameters of study groups (mean ± SD)

Periodontal Parameters	C (n=10)	CP (n=10)	p Value
GI	0.15 ± 0.07	1.32 ± 0.33	0.000
PI	0.22 ± 0.08	2.11 ± 0.64	0.000
BOP	6.90 ± 5.76	93.74 ± 11.03	0.000
PD	1.67 ± 0.14	4.12 ± 0.43	0.000
CAL	1.69 ±0.16	4.91 ±0.86	0.000

Table 3. Optical density (OD) and ghrelin levels in study groups (mean ± SD)

Group	OD	levels (in ng/ml
group C (n=10	0.313 ± 0.05	0.701 ± 0.42
group CP (n=10)	0.741 ± 0.08	1.616 ± 0.33

Table 3. Correlation between ghrelin levels and periodontal parameters in group CP

Group	variables	r value	p
Group CP	ghrelin levels and GI	-0.008	0.972
Group CP	ghrelin levels and PI	0.115	0.625
Group CP	ghrelin levels and BOP	0.142	0.547
Group CP	ghrelin levels and PD	0.562**	0.010
Group CP	ghrelin levels and CAL	0.562**	0.010

4. Discussion

The present study examines the salivary concentrations of ghrelin in individuals diagnosed with chronic periodontitis. Association of bone turnover and inflammation, and the levels of ghrelin have already been explored [14]. Since the identification of various effects of ghrelin beyond its well-known role in hunger regulation, most studies have focused on its involvement in inflammation. The majority of investigations have demonstrated that ghrelin hinders the proliferation of inflammatory cells. Similar studies have been done to check the level of salivary biomarkers and its correlation with periodontitis [15-18]. Kodama et al. [19] revealed that the introduction of ghrelin leads to a suppression of inflammation. Delivery of ghrelin externally has been demonstrated to enhance IL-10 formation while reducing the release of IL-1b and TNF-a. Additionally, it was observed that T-cells stimulated by ghrelin increases IL-13 and IL-4. Shimizu et al found that ghrelin treatment improved endothelial dysfunction in rat endothelial cells [20]. Furthermore, according to Chow et al. ghrelin exhibits an anti-inflammatory effect against lipopolysaccharide. [21] Another study revealed that ghrelin is produced by the epithelial fibroblasts, and individuals without periodontitis exhibited higher levels of ghrelin in their gingival crevicular fluid (GCF) compared to their salivary levels. Furthermore, the study reported that ghrelin suppresses pro-inflammatiory cytokines. [22]
This study represents the first attempt to assess salivary ghrelin levels in chronic periodontitis patients. Our findings indicated that participants affected with periodontal disease exhibited elevated levels of ghrelin. According to Hataya et al., the initial effects of LPS administration resulted in a decline in ghrelin levels, while subsequent LPS administration led to an increase [23]. Furthermore, heightened levels of ghrelin have been associated with inflammatory conditions such as celiac disease, inflammatory bowel disease, and ankylosing spondylitis. Poykko et al. reported a positive correlation between plasma ghrelin levels and the early stages of atherosclerosis development. One theory posits that the release of pro-inflammatory

cytokines during an inflammatory response stimulates ghrelin production [24]. Mafra et al. observed an association between the plasma total ghrelin concentration and inflammation [25]. Similarly, an investigation noted that systemic illness like diabetes impacted circulating ghrelin levels [26].

All these investigations have indicated that cytokine production and inflammation may impact blood ghrelin levels, though the specific influence of cytokine production and/or the stage of disease progression on ghrelin levels remains unclear. It is important to note that ghrelin must be acylated to exert its anti-inflammatory effects [27].

A positive correlation was identified between PD and CAL when comparing the ghrelin levels of the chronic periodontitis group with their periodontal measurements. These findings suggest a connection between ghrelin levels and the inflammatory processes associated with periodontitis. To ascertain whether ghrelin plays a role in the pathophysiology of periodontitis, further research involving larger populations, including trials with exclusively male or female participants, is warranted. Conducting a study that investigates ghrelin levels in plasma, gingival tissue, and/or GCF would be valuable in clarifying the association between ghrelin levels and chronic periodontitis.

5. Conclusion

In summary, individuals with chronic periodontitis exhibited elevated ghrelin levels. Additionally, for a more comprehensive understanding of ghrelin activity, comparing plasma ghrelin levels with levels in GCF, saliva, and gingival tissue among individuals with varying types and severities of periodontitis would be beneficial. Exploring the impact of management of periodontitis on ghrelin levels and its isoforms is also a potential avenue for further investigation.

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Conflict of Interest: Both the authors declare no conflict of interest for the study.

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