Review Article

Salivary Biomarkers of Chronic Periodontitis

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Abstract:

Periodontitis is chronic inflammatory conditions of the tissues that surround and support the teeth and is initiated by inappropriate and excessive immune responses to bacteria in subgingival dental plaque leading to loss of the integrity of the periodontium, compromised tooth function, and eventually tooth loss. Traditional clinical measurements such as probing pocket depth, bleeding on probing, clinical attachment loss; plaque index and radiographs used for periodontal diagnosis are often of limited usefulness as they are indicators of previous periodontal destruction rather than predict patient susceptibility, disease activity, and response to treatment. Studies of the immunopathogenesis of periodontitis and analysis of mediators in saliva have allowed the identification of many potentially useful biomarkers. Based on the evidence, it can be concluded that several sensitive salivary indicators of periodontitis are available to detect the presence, severity and response to treatment. Further studies are warranted to analyze the sensitivity and reliability of these indicators that might help in developing non-invasive tests that could help in the diagnosis of periodontal disease in chair-side or as home-test.

Key Words: Biomarkers, diagnosis, periodontal disease, saliva.

Introduction

Periodontitis is a chronic infectious disease of multifactorial nature characterized by the irreversible destruction of collagen fibres and other matrix constituents of the gingiva, periodontal ligament and alveolar bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium (1). Consequently, periodontitis often results in loose teeth, pain, and impaired mastication and is a common cause of tooth loss. Furthermore, periodontitis is time-consuming and expensive to treat and, therefore, prevention, early detection, and management of extent of the disease are critical issues. Also, periodontitis patients have significantly poorer physical, psychological, and social oral-health-related quality of life measures as compared to periodontal healthy individuals (2).

As the number of implant placements is rapidly increasing, the potential occurrence of implant failure as a result of peri-implantitis is consequently also expected to increase. Long-term maintenance of osseointegration depends on the

preservation of healthy soft and hard tissues surrounding oral implants (3, 4, 5).

Periodontitis is initiated by dental plaque biofilms triggering an altered host response leading to soft tissue inflammation and alveolar bone loss (6). Etiologic bacteria of periodontal diseases typically include gram-negative anaerobic bacteria: among those, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis (formerly Bacteroides forsythus), and Treponema denticola are strictly anaerobic and Aggregatibacter (formerly Actinobacillus) actinomycetemcomitans and Campylobacter rectus are facultative /microaerobic (7). The presence of these bacteria has been shown to be a useful indicator of active disease and of increased risk of gingival attachment loss (8). It is recognized that the severity of periodontal disease is dependent on a dynamic equilibrium of interactions between the microbial challenge and host immuno-inflammatory responses (1). Oral bacteria induce inflammation by activating host cells to produce pro-inflammatory mediators, which in turn promote connective tissue and alveolar bone destruction. Components of microbial plaque, especially lipopolysaccharide and other soluble products, stimulate lymphocytes, macrophages and neutrophils to secrete a wide range of proinflammatory components, for example interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF-α), prostaglandin E2 (PGE2), and IL-6 (9).

A periodontal diagnostic tool provides relevant information for differential diagnosis, localization of disease, and severity of infection. These diagnostics, in turn, serve as a basis for planning treatment and provide a means for assessing the effectiveness of periodontal therapy. They include probing pocket depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs that quantify alveolar bone levels (10). These measures provide information primarily about disease severity, and are not useful measures of disease activity. It has long been realized that a rapid and simple diagnostic test that can provide a reliable evaluation of periodontal disease and identify patients at risk for active disease would be of value to both clinicians and patients (11). Saliva is an optimal biological fluid to serve as the diagnostic tool for periodontitis (12).

Advantages of saliva over GCF sampling

The saliva collection is simple, non-invasive and painless, so it is considered to be useful for the screening tests for periodontitis, and a means of monitoring the response to treatment. The majority of research reports have used gingival crevicular fluid (GCF) as sampling method. However, sampling of GCF is time consuming and only reflects periodontal inflammation at each specific site sampled (1, 12,13).

Saliva: secretion, components, composition and functions

Saliva is a clinically informative, biological fluid (biofluid) that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases (14). Saliva'' is a generic term. The fluid sample can be considered as whole saliva, it originates mainly from three salivary glands: parotid, submandibular and sublingual. Minor salivary glands (bucal, labial, palatal, palate glossal, lingual) located in the oral cavity, gingival crevicular fluid with bacteria, epithelial cells, erythrocytes, leukocytes and food debris can contribute in small volume to the formation of what is designated as "oral fluid" or "whole saliva". In this review we will adopt the term "saliva" to designate the whole oral fluid present in the mouth (Fig.1). Each salivary gland secretes a characteristic type of saliva, with different ionic and protein characteristics. The glandular secretory

contribution varies depending on its stimulatory status i.e., unstimulated: submandibular ~ 65%, parotid gland ~ 20%, sublingual ~ 5% and minor glands ~ 10%; artificially stimulated: parotid gland > 50%, submandibular ~ 35%, sublingual and the minor mucous glands ~ 7-8% each. In resting conditions, an individual secretes approximately 0.1 to 0.3 mL/min, reaching a maximum of 7 mL/min when artificially stimulated (4,15).

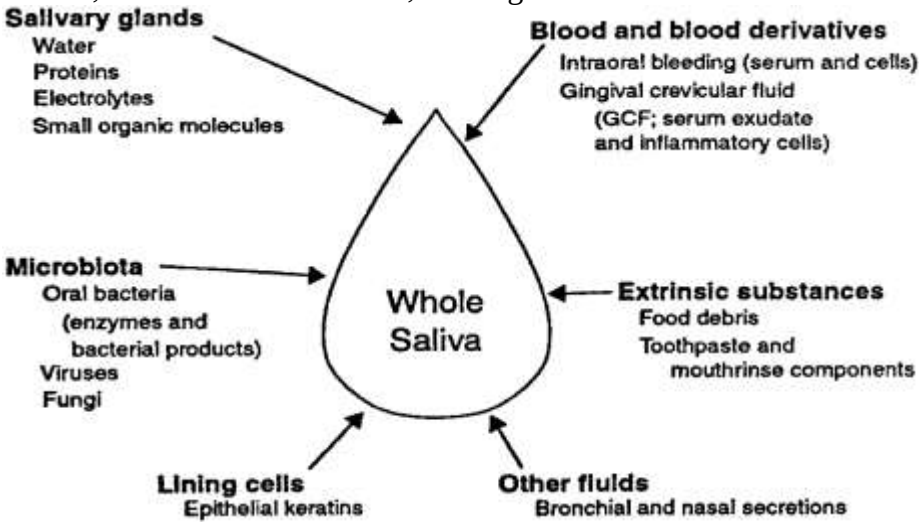

Fig.1 Components of whole saliva (11).

Saliva is mainly composed of water (98%), and other compounds (2%) are electrolytes, glycoproteins, antibacterial compounds, and various enzymes. This unique biological fluid has multiple functions, such as rinsing, solubilisation of food substances, food and bacterial clearance, lubrication of soft tissues, bolus formation, dilution of detritus, swallowing, speech and facilitation of mastication, all of which are related to its fluid characteristics and specific components. In addition, saliva components contribute to mucosal coating, digestion and antibacterial defence (Table 1) (16).

Table 1. The major functions of saliva

Functions	Salivary components involved
1. ***Protective functions***	
Lubrication	mucins, proline-rich glycoproteins, water
Antimicrobial	salivary proteins: lysozyme, lactoferin, lactoperoxidase, mucins, cystatins, histatins, secretory IgA; proline-rich glycoproteins
Mucosal integrity	mucins, electrolytes, water
Lavage/cleansing	water
Buffering	bicarbonate, phosphate ions, proteins
Remineralisation	calcium, phosphate, statherin, anionic proline-rich proteins
2. Food and speech related functions	
Food preparation	water, mucins

Digestion	amylases, lipase, ribonuclease, proteases, water, mucins
Taste	water, gustin
Speech	water, mucins

Adapted from FDI working group 10, Core (1992), and Fox (1989)(11).

Collection of Saliva

Whole saliva is most frequently studied because its collection is easy, non-invasive and rapid to obtain without the need for specialized equipment. It can also be collected with or without stimulation. Unstimulated whole saliva is commonly collected by the `draining' method where the subject's head is tilted forward so that saliva moves towards the anterior region of the mouth and the pooled saliva is drooled into a wide-bore sterile vessel (Fig. 2). Stimulated whole saliva is generally obtained by masticatory action (i.e., from a subject chewing on paraffin) or by gustatory stimulation (i.e., use of citric acid or sour candy drops on the subjects tongue) and is expectorated into a tube. Stimulated whole saliva is less suitable for diagnostic applications because the foreign substances used to stimulate saliva tend to modulate the fluid pH and generally stimulate the water phase of saliva secretion, resulting in a dilution in the concentration of proteins of interest (17).

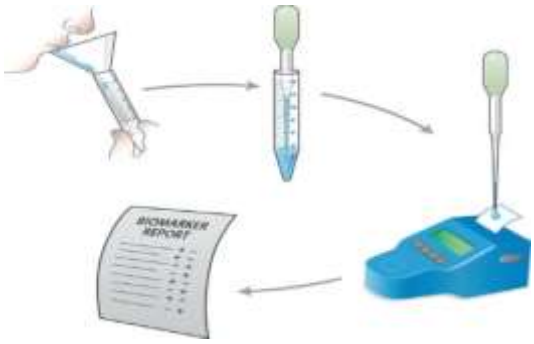

Fig. 2. Strategy for oral fluid sampling and analysis with a rapid point-of-care or lab-on-a-chip device for the generation of a periodontal disease biomarker report (18).

Salivary Biomarkers

Biomarkers have been defined as "cellular, biochemical, molecular, or genetic alterations by which a normal, abnormal, or simply biologic process can be recognized or monitored" . Biomarkers must be indicative of physiological health, pathological processes, and/or response to therapy. Also, biomarkers must be discriminatory, robust, and validated in clinical studies. However, although a number of potential biomarkers are under investigation for diagnosis of oral and systemic diseases, a suitable marker for the detailed investigation of periodontal disease remains to be fully characterised (2, 19) .

Biomarkers of disease in succession play an important role in life sciences and have begun to as a greater role in diagnosis, monitoring and therapy outcomes and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements (20).

For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting breast cancer (21), oral cancer (22), caries risk (23), salivary gland diseases (24), periodontitis (25), and systemic disorders such as hepatitis C and the presence of human immunodeficiency virus (HIV) (26). It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. Various mediators of chronic inflammation and tissue destruction have been detected

in whole saliva of patient with oral diseases (27). Also saliva is a preferred source to assess the levels of cotinine as it correlates well with that of serum (28). The salivary test has been used for a wide variety of forensic studies. It used to detect blood-group substances or salivary genetic proteins (14).

Salivary Biomarkers of Periodontal diseases

Whilst clinical and radiographic evaluation of periodontal disease remains the basis for patient's evaluation, analysis of saliva, a fluid that contains local and systemically-derived markers of periodontal disease, may offer the basis for a patient-specific diagnostic test for periodontitis / peri-implantitis (1). A panel of optimal biomarkers must be carefully selected based on the pathogenesis of periodontitis (Fig. 3) (12).

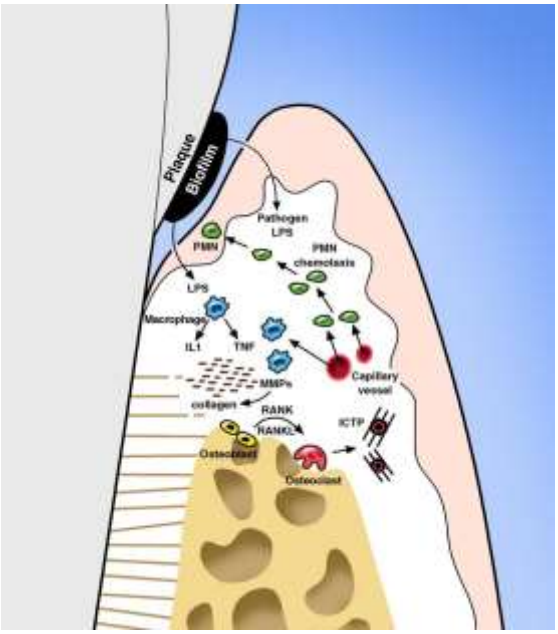

Fig. 3. Schematic overview of the pathogenic processes in periodontal disease. Initial events are triggered by lipopolysaccharide (LPS) from gram-negative bacteria on the tooth root surfaces. As a first line of defense, polymorphonuclear leukocytes (PMNs) are recruited to the site. Monocytes and activated macrophages respond to endotoxin by releasing cytokines [tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β)] which stimulate further tissue destruction. Matrix metalloproteinases (MMPs), powerful collagen destroying enzymes, are produced by fibroblasts and PMNs. TNF-α, IL-1β and receptor activator of NF-kB ligand (RANKL) are elevated in active sites and mediate osteoclastogenesis and bone breakdown. Bone-specific markers such as pyridinoline crosslinked carboxyterminal telopeptide of type I collagen (ICTP) are released into the surrounding area and transported by way of gingival crevice fluid (GCF) into the pocket and serve as potential biomarkers for periodontal disease detection (29, 18).

Salivary markers that have been studied for periodontal diagnosis include proteins of host origin (i.e., enzymes and immunoglobulins), phenotypic markers, host cells, hormones, bacteria and bacterial products, ions and volatile compounds(13). Since major periodontal bacteria are commonly found in adults, a combination of pathogenic bacteria in saliva may represent a marker for disease (30). New strategies that combine microbial identification with host response or tissue breakdown factors using discriminant analysis may better improve the ability of microbial analysis to predict future periodontal disease around teeth and dental implants (3). Ideal biomarkers of periodontitis must be able to: (1) diagnose the presence of periodontal disease, (2) reflect the severity of the disease (3) monitor the response of the disease to treatment, and (4) predict the prognosis/progress of the disease. A number of biomarkers that satisfy at least one of the four requirements have been identified in saliva (Tables2–5). Salivary biomarkers of periodontal disease can originate from both bacteria and the host (12).

Possible Salivary Biomarker (Tables2–5)

TABLE 2 | Bacteria-derived salivary biomarkers (12)

Salivary biomarkers		References
DNA	*Porphyromonasgingivalis*	(31-36)
	Prevotella intermedia	(31-36)
	Tannerella forsythia	(32-35)
	Treponema denticola	(34, 35)
	Campylobacter rectus	(31, 34,35)
	pseudomonasaeruginosa + *Acinetobacter* spp.	(37)
	Peptostreptococcus micros	(31)
	Fusobacterium nucleatum	(33)
	Aggregatibacter Actinomycetecommitans	(31, 33)
Proteins	Dipeptidyl peptidase	(38)

TABLE 3| Host-derived salivary biomarkers associated with inflammation.

Salivary biomarkers		References
Proteins	IL-1β	(39-50)
	MIP-1α	(44, 50)
	Arginase	(35, 52,53)
	soluble CD14	(54, 55)
	IFN-γ and IFN-γ/IL-22ratio	(56)
	Lactoferrin	(57-59)
	Dipeptidyl peptidase	(38)
	Chemerin	(60)
	Procalcitonin	(61)
	Calprotectin	(34)
	Myeloperoxidase	(62)
	IL-18	(63)
	TLR4	(63)
	β-glucuronidase	(47,64)
	CRP	(41, 65, 66)
	IL-6	(40, 55, 67)
	IL-8	(50)
	TNFα	(68)
Metabolite	Nitric oxide	(69-73)
	8-hydroxydeoxyguanosine	(74-79)
	Platelet activating factor	(80)
	Neopterin	(81)
	ω-3(docosapentaenoate) and ω-6(linoleate and arachidonate) fattyacids	(82)

TABLE 4 | Host-derived salivary biomarkers associated with soft tissue destruction.

Salivary biomarkers		References
Protein	MMP-8	(34, 39, 41, 46, 48, 67, 83-86)
	HGF	(87-90)
	Aspartate aminotransferase	(63, 91-93)
	Lactate dehydrogenase	(91, 93-95)
	MMP-9	(34, 85, 96)
	TIMP-2	(62)
	Alanine aminotransferase	(63, 91)
	TIMP-1	(84, 96)
Metabolites	Purine degradation metabolites (e.g., guanosine and inosine)	(82)
	Dipeptide, amino acid, carbohydrate, lipids, and nucleotide metabolites	(97)

TABLE 5| Host-derived salivary biomarkers associated with hard tissue destruction.

Salivary biomarkers		References
Protein	Alkaline phosphatase	(92, 95, 98)
	Osteonectin	(88, 99)

	RANKL	(100)
	Osteoprotegerin	(34, 101)
Metabolites	Calcium	(102)
	Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen	(84)

Also, there are substantial confirmatory studies for only a limited number of these candidate biomarkers; therefore few such biomarkers can be described as "robust." Salivary mediators analysed in these cross-sectional studies and an assessment of the possible role of these proteins as biomarkers for periodontitis are presented in Figure 4&5 (2).

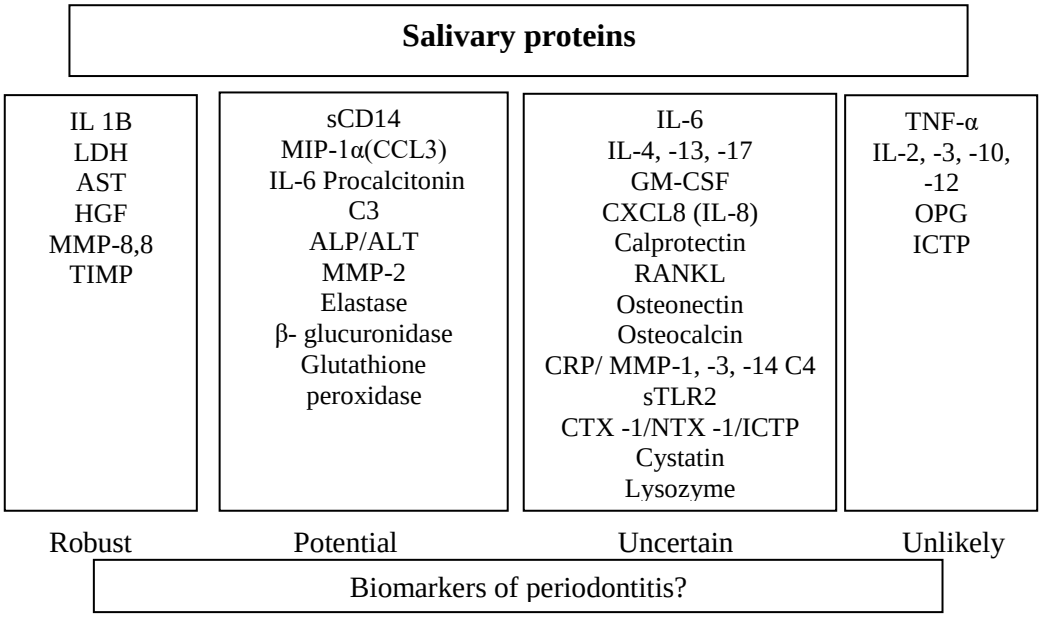

Figure 4: The possible role of salivary proteins as biomarkers of periodontitis. "Robust" biomarkers are defined as those salivary proteins which have been shown to discriminate between periodontitis and oral health in at least 3 cross-sectional studies (with comparatively little or no published evidence to the contrary) and for which there may be supporting evidence from longitudinal studies investigating the natural course of periodontitis and/or the effects of treatment on biomarker levels. "Potential" biomarkers are identified using identical criteria to "robust" biomarkers with the exception that there are 2 replicated cross-sectional studies showing disease discrimination in addition to possible supporting evidence from longitudinal studies but for which there may be limited contradictory studies. It is accepted that the entries in the "robust" and "potential" categories may be interchangeable depending on the existence of further studies which remain unpublished for commercial reasons. "Uncertain" biomarkers are proteins for which there are only single studies showing discrimination of periodontitis or for which there are several studies from which the evidence is contradictory. "Unlikely" biomarkers are those proteins for which there are 3 or more studies which fail to provide evidence for an association with periodontitis in the absence of any evidence to the contrary (2).

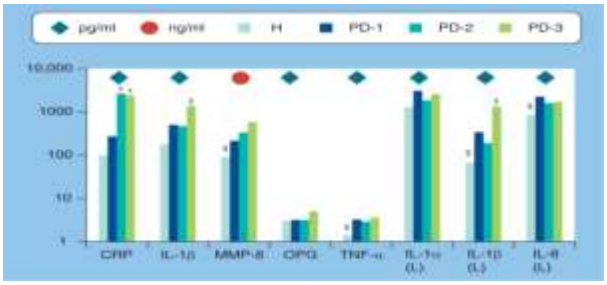

Figure 5. Mediator levels of six putative biomarkers of periodontal disease in whole expectorated saliva analyzed by enzyme immunosorbent assays and Luminex© technology

Bars denote mean levels in 35 healthy and 18 periodontitis patients. Severity of periodontitis was categorized based on increasing clinical disease severity (i.e., H < PD-1 < PD-2 < PD-3) using frequency of sites with bleeding on probing, pocket depths of at least 5 mm and clinical attachment levels of at least 2 mm.

*Significantly greater than H and PD-1.
‡Significantly greater than other categories at least at $p < 0.01$.
§Significantly less than other categories at least at $p < 0.01$
using Kruskal–Wallis ANOVA on ranks with *post-hoc* Dunn's test for pairwise comparisons.
CRP: C-reactive protein; H: Healthy; L: Luminex; MMP: Matrix metalloproteinase; OPG: Osteoprotegerin; PD: Periodontitis (17).

The future of the diagnosis of periodontal diseases:-

Common aims in periodontal research on salivary diagnostics are to find markers that could be used, preferably as chair-side tests, for example, to determine the activity of periodontitis or the results of periodontal treatment, or to a lesser extent to detect periodontitis in field studies (104). Saliva is an optimal biological fluid to serve as a near-patient diagnostic tool for periodontitis. Recent developments in point-of-care (POC) testing indicate that a diagnostic test for periodontitis using saliva is now technically feasible (12). A self-administered home test that serves as a screening tool for periodontal diseases could play an important role in making individuals aware of the fact that a pathological process is occurring in their oral cavities and that a visit to a provider of dental services should be prioritized (Figure 6). An analogy to such screening test for periodontal diseases would be home pregnancy tests, where females who show positive results are encouraged to visit their physicians to confirm their pregnant statuses and receive appropriate care thereafter. In the case of periodontal diseases, saliva serves as an attractive vehicle on which a screening test could be conducted. Saliva is in close proximity with sites that are present with gingival and periodontal inflammation; therefore, it contains biological markers associated with these diseases. Moreover, saliva is an abundant fluid that is easy to collect and store, making it a convenient medium for conducting a high sensitivity screening test for periodontal diseases (105). Based upon the potential value of saliva as a non-invasive screening tool for oral disease(s), this study focused on the quantification of a group of analytes that may act as biomarkers for periodontitis and aid in the development of personalized approaches for periodontal risk assessment. Movement toward an era of personalized medicine and individualized clinical decisions in periodontology requires significant improvement in our ability to define risk and predict disease progression (106).

Figure 6. Lab Now analyzer and Nano-Biochip
(A) Analyzer, by contrast to the actual production configuration, is shown here with transparent outer covering to allow inner features to be viewed. **(B)** A representation of the multiple biochip functions performed within the credit-card-sized Nano-Biochip, which serve to eliminate constraints imposed by traditional laboratory-confined methods (17).

Conclusion:

1- Analysis of saliva may offer a cost-effective approach to assessment of periodontal disease in large populations.

2- Detection of analytes in saliva shows great promise for enhancing the ability to diagnose periodontal disease at the chair/side.

3- Use of saliva as a quick, easy and reliable method of assessing and monitoring periodontal disease will provide important diagnostic information that improves and speeds treatment decisions and moves the field closer to individualized point-of-care diagnostics.

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