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Evaluating the diagnostic potential of saliva in respect of periodontal disease as well as changes occurring within the endothelium

Ocena potencjału diagnostycznego śliny – w aspekcie chorób przyzębia oraz zmian w obrębie śródbłonka naczyniowego

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Abstract

Technological advancement has been observed over the last few decades within many branches of medicine. This advancement has had an impressive impact on diagnostic developments and new methods of treatment that aid in improving treatment quality and the survival rate of patients. Dentistry is a field in which advancement is progressing at a rapid pace, which encourages dental practitioners to carry out interdisciplinary treatment planing and expanding their field of interest beyond the oral cavity. Saliva, once considered as an insignificant bodily secretion can, according to the latest research, be seen as a reflection of the human organism. Utilized for the evaluation of hormone levels, pro-inflammatory cytokines, medications and antibodies it constitutes an easily accessible and extremely valuable diagnostic material. With a simple as well as non-invasive method of obtainment saliva is an ideal medium which could be potentially used as an alternative method for standard laboratory tests. The aim of the following study is to present potential methods of saliva utilization in diagnostic procedures for periodontal diseases, as well as changes within the endothelium as a diagnostic factor for cardiovascular diseases.

Keywords: saliva, endothelium, periodontal disease.

Streszczenie

Obserwowany od kilku dekad postęp technologiczny znajduje swoje odbicie we wszystkich gałęziach medycyny. Wywierając ogromny wpływ na rozwój diagnostyki oraz nowych metod leczenia, znacząco przyczynia się do poprawy jakości oraz długości życia pacjentów. Stomatologia jest dziedziną, w której zmiany te są szczególnie widoczne, co skłania lekarzy dentystów do interdyscyplinarnego podejścia do pacjenta oraz wysunięcia obszaru zainteresowania poza jamę ustną. Coraz częściej podkreśla się rolę dentysty jako lekarza pierwszego kontaktu przy wykrywaniu objawów chorób ogólnoustrojowych w jamie ustnej. Ślina, niegdyś uznawana za małowartościową wydzielinę, w świetle najnowszych badań może posłużyć jako nośnik informacji o stanie całego organizmu. Wykorzystywana między innymi do oceny poziomu hormonów, cytokin prozapalnych, leków oraz przeciwciał, stanowi obecnie łatwo dostępny i niezwykle wartościowy materiał diagnostyczny. Prosty oraz nieinwazyjny sposób pozyskiwania sprawia, że ocena składu śliny może stać się alternatywną metodą dla standardowych badań laboratoryjnych. Celem niniejszej pracy jest przedstawienie w oparciu o najnowsze piśmiennictwo potencjalnych możliwości wykorzystania śliny w diagnostyce chorób przyzębia oraz zmian w obrębie śródbłonka naczyniowego jako czynnika diagnostycznego chorób układu sercowo-naczyniowego.

Słowa kluczowe: ślina, śródbłonek naczyniowy, choroby przyzębia.

Introduction

Saliva is a secretory product of the salivary glands producing stability in the oral cavity [1]. It is a mixture derived from three glands (parotid, submandibular and sublingual) as well as minor salivary glands located throughout the vestibular mucosa, buccal mucosa, palate as well as the floor of the mouth. Saliva also contains minor constituents in respect to its volume that significantly affect its composition; crevicular fluid, oral lavage, serum, red blood cells as well as bacteria and their metabolites: viruses and fungi, deepithelized cells of the squamous epithelium as well as remnants of food particles [2].

Saliva maintains the proper environment of the oral cavity. A proper amount of saliva ensures the integrity of the oral mucosa and periodontal tissues. Mucins are included in the salivary products and play a major role in protecting the hard tissues of dentition from mechanical degradation.

The continuous flow of saliva plays a significant role in terms of the debridement of food remnants and the products of bacterial metabolism, as well as the supply of calcium, phosphorus and fluoride ions, enabling enamel remineralization. Furthermore, saliva plays a buffering and protective function through specific and nonspecific immunity factors.

Non-stimulated mixed saliva is currently utilized due to its simple method of attainment and the ease of its acquisition without the need for specialized equipment [3]. Furthermore, it shows a greater diagnostic value when compared to stimulated saliva; the composition of which as well as the pH value vary, depending on the type of stimulation. The current methods for obtaining saliva are drainage, expectoration, suction and absorption [4]. In the majority of cases, patient convenience is increased through the employment of expectoration methods, which include a sialometer with an appropriate funnel.

Currently, research employing saliva is not limited to diseases of the oral cavity. Dentists play an instrumental role in the early recognition of systemic diseases due to their initial contact with a given patient. Research utilizing salivary markers could act as a diagnostic tool and enable disease monitoring. The idea is based upon the presence of various immunological elements contained within saliva [5].

The aim of this paper focuses on research development and the employment of saliva in establishing cardiovascular disease progression in the endothelium as well as the diagnosis of periodontal diseases.

The markers of periodontal disease in saliva

Periodontitis is a multifactorial disease of the suspensory apparatus of the tooth, mainly caused by a biofilm [6]. It is characterized by a chronic inflammatory destruction of the connective tissue and alveolar bone. The development as well as the progression of the disease is dependent upon the virulence factors of microorganisms, but also dependent on the host's immune system. Due to it being widespread and the risk of local and general consequences, periodontitis is currently considered a social disease [7]. Recently, a close connection between periodontal disease and diseases such as arteriosclerosis, ischemic heart disease, and diabetes has been established [8, 9]. Research carried out at the end of the 20th century has labelled periodontitis as a potentially independent factor and cause of premature birth as well as the low birth weight of infants [10]. Proper diagnosis as well as the effective treatment of periodontitis plays a crucial role not only from an oral health perspective, but also in terms of overall health. The employment of saliva as an information carrier of periodontal status as well as a periodontal disease risk predictor, could lead to a breakthrough and improve the ease of diagnosis. The non-invasive nature of this test could render it widely available in the diagnostic field.

Currently, amongst the factors discovered in the saliva are pro-inflammatory cytokines, which cause the destruction of connective tissue as well as the loss and remodelling of the alveolar process.

Inflammatory markers

Generally, periodontal disease is a final product of the progression of untreated gingivitis. Bacterial biofilm found on the surface of a tooth structure leads to the progression of an inflammatory state as well as its establishment in the deeper suspensory apparatus of the tooth. Throughout this process a cascade of cytokines is established which involves numerous elements responsible for destructive as well as reparative processes.

IL-1 is one of the main cytokines playing a major role throughout the development of periodontitis. Between the two major forms (IL-1beta and IL-1alfa) IL-1 beta, has a greater bone resorption potential and is also the most common form. In periodontal tissues, the main sources of this cytokine are predominantly monocytes/macrophages, neutrophils, fibroblasts and fat cells. These cells synthesize IL-1β as a result of their activation by the LPS, the main constituent of the gram negative bacterial cell wall as well part of the complement system C5a [11]. Miller et al., 2006 has reported significantly higher levels of IL-1 beta in patients affected by periodontitis when compared with a control group. Positive correlation has been established between cytokine levels and clinical attachment loss (CAL), bleeding on probing (BOP) and periodontal pocket depth greater than 4 mm. Furthermore, an increased level of salivary IL-1 beta was connected with a higher risk of developing periodontitis. [13].

Similarly, interleukin-6 could also be produced by a variety of cells such as lymphocyte T and B, macrophages, endothelial and epithelial cells as well as fibroblasts [14]. An increased liberation of IL-6 could be stimulated by the action of activated of IL-1 beta and TNF-alfa. One of the main functions of IL-6 is the stimulation of lymphocyte B maturation and the production and activation of osteoclasts. It is generally believed this plays an instrumental role in the bone remodelling process throughout the course of periodontitis. Research data indicates a correlation between an accelerated bone loss in chronic periodontal disease and elevated levels of this particular cytokine [15].

TNF-alfa is a pro-inflammatory cytokine, produced mainly by macrophages and monocytes. It plays a key role in the recruitment of inflammatory cells and in unison with IL-1 induces differentiation of osteoclasts, increases the activity of resorptive cells, halts the production of collagen and simulates the production of collagenase; in effect ac-

celerating the resorptive process of bone and the destruction of periodontal tissues. *In vitro* analysis of TNF-alfa revealed a stimulatory effect on fibroblasts, resulting in the production of collagenase and the degradation of collagen.

Numerous studies have focused on TNF-alfa analysis, providing various results; some of which report difficulties in establishing TNF-alpha in saliva [16], and other reports showing no statistical difference in TNF-alpha levels in patients diagnosed with periodontitis and the control group [17]. Fridge et al., however, [18] showed not only TNF-alpha levels to be significantly increased in patients with periodontal disease compared to the control group, but also a correlation of this cytokine level with the number of sites with bleeding on probing (BOP), loss of attachment and the number of periodontal pockets.

B-glucuronidase is a lysosomal enzyme responsible for degradading basic substances and proteoglycans. It is an indicator of an influx of polymorphonuclear granulocytes in the crevicular fluid. The study shows an increased concentration of this substituent in patients' with an acute periodontal disease compared to a benign form. An increased level of B-glucuronidase in saliva correlates with the amount of periodontal pockets with a probing depth greater than 5 mm [19]. Prabhahar et al. [20], also showed an increase in CAL along with a spike in the concentration of B-glucuronidase, which makes this element another potential biomarker responsible for the destruction of the periodontium.

Endothelial biomarkers

The blood vessels, lymphatic tissues and heart chambers are lined by endothelial cells. They form a single layer of cells, separating blood from the wall of the vessel and account for a surface area of 1–7m2. For many decades endothelium has been perceived only as a barrier separating the blood from tissues; nowadays, however, it is believed to take part in various physiological functions. Anggard et al., has named it the largest gland of internal excretion [21].

Its properties control the processes of hemostatis, regulate blood flow dynamics and platelet activation, and most importantly, participate in the modulation of immunological and inflammatory processes.

Endothelial dysfunction may lead to the development of various life threatening diseases such as hypertension, atherosclerosis, or tumor progression. Markers for the endothelial functions involved with the above mentioned diseases are a main topic of current research. Discovering these substances in saliva through the utilization of relatively simple methods may be an alternative or enhancement of current standard procedures.

sICAM (intracellular Adhesion Molecule 1) is an adhesive molecule participating in the regulation of an immune response; more precisely, enabling the transmigration of leukocytes through endothelial cells to the extracellular matrix. Diapedesis of leukocytes occurs only in the inflamed area entailing a greater adhesin expression (including ICAM-1) on the endothelial surface. NF papa B (molecular transcription factor papa B) is a transcription factor enhancing the expression of ICAM-1, which in turn is activated through pro-inflammatory cytokines, LPS (Lipopolysaccharide) or free radicals.

ICAM-1 is one of the most commonly utilized markers for the analysis of endothelial cell damage in people with acute coronary syndrome. An increased level of sICAM in saliva has also been observed in people with primary Sjoegren`s syndrome [22]. Elevated ICAM expression has also been observed throughout the course of periodontal disease, acting as risk factor for the development of ischemic heart disease. The concentration of salivary sICAM in periodontally involved patients was shown to be statistically significant compared with the control group [23]. The interpretation of these results could be used as a positive prognostic factor of ICAM-1 levels in inflammatory processes taking place in other parts of the body.

The main enzyme of fibrinolysis is plasmin, made from an inactive pro-enzyme, plasminogen. The activator of this reaction is t-PA (Tissue-Plasminogen); the inhibitor is PAI-1 (plasminogen activator inhibitor). The balance between these two factors is crucial for the maintenance of hematostasis. An imbalance of fibrinolytic cascade factors, mainly t-PA and PAI-1 could lead to an acute myocardial infarction caused by a sudden obstruction of a coronal artery by a thrombus formed previously on atherosclerotic plaques [24]. It is believed that a high concentration of PAI-1 is an independent risk factor in the case of ischemic heart disease as well as during myocardial infarction. Analysis of salivary PAI-1 could be utilized as a prognostic factor of ischemic heart disease as well as acute coronary syndrome. According to Xi Zang [25], the presence of stable salivary PAI-1 levels throughout a 24 hour period, however, did not correlate with PAI-1 levels obtained from serum, rendering it unsatisfactory and necessitating further analysis.

VEGF (vasculo endothelial growth factor) is a stimulatory molecule which plays a major role in the process of angiogenesis. Angiogenesis is a process of new blood vessel formation from pre-

existing networks of capillaries. Physiologically, this process is observed during the process of wound healing and the menstrual cycle. Throughout the course of many pathological states; such as inflammation, tumor progression, diabetic retinopathy or rheumatoid arthritis; uncontrollable angiogenesis occurs, comprised of significantly higher levels of VEGF. Increased levels of VEGF play a key role in the etiopathogenesis of periodontal diseases. Numerous studies have confirmed high levels of VEGF in inflamed areas of those affected by periodontal disease [26]. Salivary VEGF marking is currently employed in various fields of medicine ranging from risk assessment of recurrent aphtous stomatitis [27], to tumor analysis [28, 29].

Conclusion

All the factors presented represent only a small part of the potential diagnostic capabilities of the salivary medium. Carrying out highly sensitive and exact tests enables detailed information regarding a particular organism to be attained. Extremely advantageous is the ease of obtaining saliva along with, most importantly, its non-invasive nature. Unfortunately, the high cost of reagents as well as the need for expensive laboratory apparatus limits its utilization. We certainly hope further research will enable the gathering of other promising results that will permit for the further development of this novel technology.

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References

[1] Pink R, Simek J, Vondrakova J. Saliva as a diagnostic medium. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 2009;153:103–110.
[2] Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000. 2009;50:52–64.
[3] Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Christodoulides N, Floriano PN, Simmons G, Bhagwandin B, Jacobson JW. Current developments in salivary diagnostics. Biomark Med. 2010;4:171–189.
[4] Jach M, Gońda M, Lisiecka K, Bober J, Mokrzycka M, Kuczak M. Wykorzystanie wybranych badań fizykochemicznych śliny w diagnostyce stomatologicznej – na podstawie piśmiennictw. Czas Stomatol. 2008;61:353–358.
[5] Król K, Grocholewicz K. Wybrane białka śliny jako biomarkery miejscowych i ogólnych procesów chorobowych. Przegląd piśmiennictwa. Annales AMS – Roczniki PAM. 2007;53:78–82.
[6] Wolf HF, Rateitschak EM, Rateitschak KH. Periodontologia. 3rd ed. Lublin: Czelej; 2012. p. 95–95.
[7] Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366:1809–1820.
[8] Kapłon–Wożakowska B, Filipiak K, Opolski G, Górska R. Związek chorób przyzębia z cukrzycą i nefropatią cukrzycową. Diabetol Prakt. 2009;10:2.
[9] Buhlin K, Gustafssan A, Pockley AG. Risk factors for cardiovascular disease in patients with periodontitis. Eur Heart J. 2003;24:2099–2107.
[10] Paradowska-Stolarz A. Periodontitis and risk of preterm birth and low birthweight a metaanalysis. Ginekol Pol. 2012;83:446–453.
[11] Konopka Ł, Brzezińska-Błaszczyk E. Cytokiny w płynie kieszonki dziąsłowej jako potencjalne markery diagnostyczne i prognostyczne zapalenia przyzębia Dent Med Probl. 2010;47:206–213.
[12] Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc. 2006;137:322–329.
[13] Tobon-Arroyave SI, Jaramillo-Gonzalez PE, Isaza-Guzman DM. Correlation between salivary IL-1β levels and periodontal clinical status. Arch Oral Biol. 2008;53:346–352.
[14] Okada H, Murakami S. Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med. 1998;9:248–266.
[15] Ng PY, Donley M, Hausmann E, Hutson AD, Rossomando EF, Scannapieco FA. Candidate salivary biomarkers associated with alveolar bone loss: cross-sectional and in vitro studies. FEMS Immunol Med Microbiol. 2007;49:252–260.
[16] Aurer A, Jorgic-Srdjak K, Plancak D, Stavljenic-Rukavina A, Aurer-Kozelj J. Proinflammatory factors in saliva as possible markers for periodontal disease. Coll Antropol. 2005;29:435–439.
[17] Hojatollah Y, Robati M, Jahangirnezhad M, Ghafourian BM, Taghipour MJ. Evaluation of salivary tumor necrosis factor-alpha in patients with the chronic periodontitis: A case-control study. Indian Soc Periodontol. 2013;17;737–740.
[18] Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol. 2008;79:1913–1919.
[19] Lamster IB, Kaufman E, Grbic JT, Winston LJ, Singer RE. β-glucuronidase activity in saliva: relationship to clinical periodontal parameters. J Periodontol. 2003;74:353–359.
[20] Prabhahar CS, Niazi KM, Prakash R, Yuvaraj A, Goud S, Ravishekar P. Estimation of salivary β-glucuronidase activity as a marker of periodontal disease: a case control study. J Int Soc Prevent Communit Dent. 2014;4(Suppl. S3):193–198.
[21] Anggard EE. The endothelium – the body's largest endocrine gland? J Endocrinol. 1990;127:371–375.
[22] Cuida M, Halse AK, Johannessen AC, Tynning T, Jonsson R. Indicators of salivary gland inflammation in primary Sjogren's syndrome. Eur J Oral Sci. 1997;105:228–233.
[23] Kubicka-Musiał M, Skucha-Nowak M, Hupsch-Marzec H, Wierucka-Młynarczyk B, Książek-Bąke H. Evaluation of sICAM-1 Concentration in saliva and blood serum in patients with periodontitis. Dent Med Probl. 2005;42:223–226.
[24] Bujak R, Sinkiewicz W, Błażejewski J, Budzyński J, Żekanowska E. Tkankowy aktywator plazminogenu (t-PA) i jego inhibitor typu 1 (PAI-1) u chorych z ostrym zawałem serca. Folia Cardiol. 2002;9;311–318.
[25] Zhang X, Dimeski G, Punyadeera C. Validation of an immunoassay to measure plasminogenactivator inhibitor-1 concentrations in human saliva. Biochemia Medica. 2014;24:258–265.
[26] Johnson RB, Serio FG, Dai X. Vascular endothelial growth factors and progression of periodontal diseases. J Periodontol. 1999;70:848–852.
[27] Agha-Hosseini F, Kaviani H, Bamdad K. An Investigation on the levels of Vascular Endothelial Growth Factor (VEGF) in the Unstimulated Whole Saliva of patients with

Recurrent Aphthous Stomatitis. Journal of Dentistry of Tehran University of Medical Sciences. 2005;2:96–100.
[28] Andisheh-Tadbir A, Hamzavi M, Rezvani G, Asharf MJ, Fattahi MJ, Khademi B, Kamali F. Tissue expression, serum and salivary levels of vascular endothelial growth factor in patients with HNSCC Braz. J Otorhinolaryngol. 2014;80:503–507.
[29] Upile T, Jerjes W, Kafas P, Harini S, Singh SU, Guyer M, Bentley M, Sudhoff H, Hopper C. Salivary VEGF: a no-n-invasive angiogenic and lymphangiogenic proxy in head and neck cancer prognostication. Int Arch Med. 2009;2:12.

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