Materials Used for Diagnostic and Treatments in Dental Practice

DRAGOS FRATILA[1], DORIANA AGOP-FORNA[1], NORINA CONSUELA FORNA[2]*

[1] Grigore T. Popa University of Medicine and Pharmacy of Iasi, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iasi, Romania

Oral disease diagnostic research are moving toward methods whereby endodontic, periodontal or peri-implantitis risk can be identified and quantified by biomarkers. Monitoring of endodontic treatment of chronic periapical lesions can be performed using ELISA test to detect levels of MMP-8 and IL-β1 in periapical exudates. Early detection of periodontal disease and monitoring of treatment can be performed using microbiological tests, immunological tests and biochemical tests to assess levels of gingival crevicular fluid (GCF), ASAT, MMP-8, MMP-9, IL-β1. Early detection of peri-implantitis and monitoring of therapy outcome can be performed using ELISA test.

Keywords: microbiological tests, immunological tests, biochemical tests, periapical exudates, biomarkers

In dental medicine the traditional diagnostic tools, despite of their ease of use, cost-effectiveness, and noninvasive features, are limited to the assessment of disease history and not current disease status. Oral disease diagnostic research are moving toward methods whereby endodontic, periodontal or peri-implantitis risk can be identified and quantified by biomarkers [1-5].

Biomarkers of a certain disease, including dental diseases, play important roles in diagnosis, monitoring of therapy results, and drug discovery. The challenge is that use of biomarkers to allow earlier detection of disease evolution and therapy efficacy assessment. To be introduced in routine practice, it is essential to be fully understood the relation of biomarkers with the mechanism of disease progression and therapeutic intervention [6].

The aims of this paper are to present current issues biomarkers involvement in the diagnosis and monitoring of results in various dental treatments.

Biomarkers used in treatment of chronic periapical lesions

The presence of chronic periapical lesions implies the complex interactions between bacteria, their products, and the immunological host components. The immunological reaction despite their role in the prevention of infection spreading in bone tissues, also presents a destructive effect for the host tissues. Dental practice requires the diagnostic materials and techniques able to assess the results of therapy and to predict the future evolution of periapical lesions.

Application of molecular genetic methods (PCR techniques) to the analysis of the bacterial diversity in the oral cavity has revealed a still broader spectrum of extant bacteria than previously reported by cultivation approaches [7]. Diagnostic techniques at molecular level (PCR) are important for the detection of some pathogenic species that cannot be cultivated as to obtain positive results on long term. Biological molecular techniques (PCR) are considered most efficient due to speed, accuracy and specificity.

Metaloproteinases are enzymes implicated in the degradation of organic component of periapical tissues and are secreted by PMN, monocites and macrophages. The endodontic treatments that reduce levels of bacteria inside endodontic space also reduce the active forms of MMP-8 in periapical area. The assessment of Il-1β levels in periapical exudate can also be considered as a method to assess the intensity of periapical inflammatory process. High levels of IL-1β are characteristic to the fluid present inside radicular chist [8]. Ataonlu T. and colab., in 2002, found levels of Il-1β three times higher for teeth with apical secretion comparing with teeth without periapical secretion [9]. On the other way, protective factors can be assessed during therapy; for example, in an alkaline environment created by $Ca(OH)_2$, inactive TGF-β1 is transformed in active growth factor and represents a biomarker of periapical healing processes [10].

Wahlgren J. et al., in 2002 [11], using ELISA test, found a significant reduction of MMP-8 in 10 cases, in a study performed on 11 teeth with chronic periapical lesions treated with $Ca(OH)_2$. Same authors concluded that MMP-8 must be used as biomarker to indicate the therapeutic efficiency in the case of teeth with periapical lesions [1-5].

Tests used in the detection of early periodontal diseases and therapy monitoring

The aim of periodontal diagnostic procedures is to provide useful information to the clinician regarding the periodontal disease category, severity and location. These data serve as a background for treatment planning and are essential during periodontal maintenance and disease-monitoring phases of treatment. The diagnostic tests based on periodontal markers are introduced in dental practice to compensate the diagnostic conventional methods that require significant clinical changes to detect the presence of early periodontal disease. More, the complex nature of periodontal disease requires the use of combinations of host markers. The markers of periodontal disease are as follows: prostaglandines (role in the initiation of inflammatory processes), alcalin phosphatases (biomarkers of osteolytic processes), catepsine B (role in proteolisis of periodontal tissues), colagenases (MMP-8, MMP-9, MMP-13). The colagenases are considered key factors of periodontal disease due to their implication in the destruction of organic component [12]. The roles of diagnostic tests based on biological markers are as follows: the identification of therapeutic objectives, the high risk sites, the monitoring of response to the therapy.

The background of diagnostic tests is related to the microbiological and biological activity criteria of periodontal

* *email: profforna@gmail.com*

disease. The diagnostic tests used in dental practice are microbiological tests, immunological tests, biochemical tests. Microbiological tests include BANA test and ADN probes. Most studies based on immunological tests use ELISA test to detect and assess the levels of periodontal biomarkers. ELISA test is based on marked fluorescenced antibodies for the detection of the investigated antigen. Test Quantikinine (R&D System) is produced for the detection and assessment of MMP-8 collagenase and test Interleukin-β1 is used to detect and measure the IL-β1 levels. Biochemical tests include ASAT test, tests for the assessment of metabolic poducts and inflamation mediators. Biochemical tests include Periotron 8000 measuring the levels of GCF, Periogard (Colgate) assessing ASAT levels, Periocheck (AC Tech) related to colagenase levels, and Prognostik (Dentsply) using elastase levels for the detection of early periodontal disease.

Oral-fluid-based tests can detect the presence of periodontopathogens and their associated host-derived enzymes, inflammatory mediators, and tissue breakdown products, but given the complex nature of periodontal disease, it is unlikely that a sole biomarker exists for disease detection and disease prediction. Among the salivary biomarkers, IL-1β, MMP-8, MMP-9, and OPG demonstrated the highest correlation with disease status [13]. The detection of MMP-8 using ELISA test in crevicular fluid can be the background of a diagnostic instrument for the assessment of efficiency of periodontal therapy [14].The review performed by Boronat-Catala M. et al. in 2014 [15] concluded that IL-1β is higher in the saliva and/or crevicular fluid of patients with gingivitis and can be used as a diagnostic marker of the degree of inflammation in gingivitis. Yucel et al. in 2008 [16] found that the amount of IL-11 and IL-1β was higher in the gingivitis group than in patients with periodontitis. Offenbacher S. et al. in 2010 [17] found that in experimental gingivitis IL-1β and IL1α increased, while IL-8 decreased. Perozini C. et al. in 2010 [18] analyzed IL-1β and found that it was highest in the periodontitis group comparing with healthy patients, but found no differences between the gingivitis and healthy group. Becerik S. et al. in 2012 [19] found that IL-1β and IL-6 in patients with gingivitis are higher than healthy patients and then returned to initial levels following the return to normal hygiene. Ertugrul A.S. et al. in 2013 [20] found that there is a positive correlation between periodontal parameters and the interleukins IL-8, IL-1β and TNFα.

The use of these tests related to periodontal biomarkers facilitates both the introduction of individualized periodontal therapies and regulatory drugs for the inflammatory processes localized in periodontal affected areas.

ELISA test in the detection, prevention and treatment of early peri-implantitis

Biomarkers have the potential in detecting early peri-implantitis diseases [21]. IL-1β levels can be a good marker to detect peri-implant mucositis lesions before the initiation of peri-implantitis. MMP-8 levels detected in peri-implant sulcus fluid can be used for monitoring the progression of peri-implantitis. Osteoprotegerin (OPG) and receptor activator of NF B ligand (RANKL) are significantly higher in peri-implantitis areas compared with healthy implant areas. The review of Javed F. et al. in 2011[22] found literature data showing high levels of interleukin IL-1β, IL-6, IL-8, MMP-1, TNF-α in the crevicular fluid of implants affected by peri-implantitis compared to healthy sites.

Some studies showed that biomarkers can differentiate healthy peri-implant tissues from mucositis and peri-implantitis sites. Accordingly to Rakic M. et al., [23] levels of biomarkers sRANKL, RANK, OPG, are associated with peri-implant tissue destruction. The authors collected peri-implant/gingival crevicular fluid samples from patients with peri-implantitis, healthy peri-implant tissues, severe chronic periodontitis, and assessed biomarkers levels using enzyme-linked immunosorbent assays (ELISA test). When comparing peri-implantitis and periodontitis findings, RANK was significantly higher in peri-implantitis sites whereas, sRANKL were significantly higher in periodontitis sites. In implant patients pocket depths and bleeding on probing values were positively associated with high RANK levels. RANK was higher in periodontitis sites comparing with healthy peri-implant tissues. Considering these results, RANK can be considered as a pathologic determinant of peri-implantitis, and a potential parameter in assessment of peri-implant tissue inflammation. Also RANK can be considered as a potential biomarker used to design the treatment strategies. In another study, levels of RANK, sRANKL and OPG were significantly increased in patients with peri-implantitis compared with patients with healthy peri-implant tissues, while significantly increased levels of RANK in patients with mucositis comparing with patients with healthy peri-implant tissues [24].

Conclusions

The microbiological, immunological and biochemical tests can be used efficiently to identify early stages of endodontic diseases, periodontal diseases and peri-implantitis. The results of therapy can also be recorded in an objective manner using these tests at specific time intervals.

Application of molecular genetic methods to the analysis of the bacterial diversity in the oral cavity has revealed a still broader spectrum of extant bacteria than previously reported by cultivation approaches. Diagnostic techniques at molecular level are important for the detection of some pathogenic species that cannot be cultivated as to obtain positive results on long term. Also, biological molecular techniques are considered most efficient due to speed, accuracy and specificity.

Among the salivary biomarkers (IL-1β, MMP-8 and MMP-9) demonstrated the highest correlation with diseases status. The detection of MMP-8 using ELISA test in crevicular fluid can be the background of a diagnostic instrument for the assessment of efficiency of periodontal therapy.

Other biomarker is RANK who is considered as a pathologic determinant of peri-implantitis, and a potential parameter in assessment of peri-implant tissue inflammation. Also RANK can be considered as a potential biomarker used to design the treatment strategies.

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Manuscript received: 18.11. 2014