Minireview

Detection of inflammatory biomarkers in saliva and urine: Potential in diagnosis, prevention, and treatment for chronic diseases

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Abstract

Inflammation is a part of the complex biological response of inflammatory cells to harmful stimuli, such as pathogens, irritants, or damaged cells. This inflammation has been linked to several chronic diseases including cancer, atherosclerosis, rheumatoid arthritis, and multiple sclerosis. Major biomarkers of inflammation include tumor necrosis factor, interleukins (IL)-1, IL-6, IL-8, chemokines, cyclooxygenase, 5-lipooxygenase, and C-reactive protein, all of which are regulated by the transcription factor nuclear factor-kappaB. Although examining inflammatory biomarkers in blood is a standard practice, its identification in saliva and/or urine is more convenient and non-invasive. In this review, we aim to (1) discuss the detection of these inflammatory biomarkers in urine and saliva; (2) advantages of using salivary and urinary inflammatory biomarkers over blood, while also weighing on the challenges and/or limitations of their use; (3) examine their role(s) in connection with diagnosis, prevention, treatment, and drug development for several chronic diseases with inflammatory consequences, including cancer; and (4) explore the use of innovative salivary and urine based biosensor strategies that may permit the testing of biomarkers quickly, reliably, and cost-effectively, in a decentralized setting.

Keywords: Inflammation, chronic diseases, biomarkers, urine, saliva, cancer

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Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, irritants, or damaged cells. The word inflammation was derived from the Latin word *inflammare,* which meant, 'to set on fire.' The classic signs of inflammation as first described by a Roman encyclopedist, Cornelius Celsus (25 BC–50 AD), were *calor* (heat), *dolor* (pain), *rubor* (redness), and *tumor* (swelling). Much later, in the 1800s, German pathologist Rudolf Virchow, added *functio laesa* (loss of function) to the definition of inflammation.[1]

Inflammation occurs as a stereotyped response, and therefore is considered a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Therefore, inflammation is not a synonym for infection, even in cases when inflammation is caused by an infection. In current medicine, inflammation is classified to be acute (an initial, immediate, and short-term response of the body to harmful stimuli) or chronic (a long-term response leading to a progressive shift in the types of cells present at the site of inflammation, characterized by simultaneous destruction and healing of the tissue from the inflammatory process).[2] Some of the known potential sources of inflammation may include environmental/industrial pollutants (e.g. cigarette smoke, carbon dioxide emissions, smog, sulfur dioxide/nitrogen dioxide emissions); ultraviolet radiation; dietary factors (e.g. consumption of a Westernized dietary pattern comprising of refined carbohydrates and/or fried foods or red/processed meats and cooking methods such as grilling/charbroiling); other lifestyle factors (e.g. smoking, alcohol consumption); obesity; bacteria (e.g. *Helicobacter pylori, Salmonella typhi, Streptococcus bovis, Escherichia coli, Chlamydia, pneumonia*); viruses (e.g. herpes simplex virus B, hepatitis viruses, human papilloma virus, human immunodeficiency virus [HIV], Epstein-Barr virus); as well as stress factors (e.g. pH, hypoxia, chemotherapy, heavy metals).[3]

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Link between inflammation and chronic diseases

Most chronic diseases are caused by chronic inflammation. However, acute inflammation exerts therapeutic potential. The association between inflammation and chronic diseases is supported by epidemiological, pharmacological, and genetic studies.[4] In 1850, Rudolf Virchow, a German scientist from Wurzburg, was the first to link inflammation with various chronic diseases, which include cancer, atherosclerosis, arthritis, diabetes, asthma, multiple sclerosis, and Alzheimer's disease.[5] To date, over 200 different types of diseases caused by inflammation have been described. Usually inflammatory diseases are represented by suffix 'itis.' For example, arthritis, bronchitis, sinusitis, gastritis, esophagitis, pancreatitis, meningitis, rhinitis, and gingivitis are, respectively, inflammation of the joints, bronchi, sinuses, stomach, esophagus, pancreas, brain, nose, and gums. Today, inflammation is considered a risk factor for most cancers (e.g. 11–24% of patients with bronchitis progress to develop lung cancer; 15% of patients with esophagitis progress to develop esophageal cancer; 10–15% of patients with asbestosis progress to develop mesothelioma; ≤10% of patients with pancreatitis progress to develop pancreatic cancer; and 1% of patients with inflammatory bowel disease progress to develop colorectal cancer).[6]

How chronic inflammation resulted in the development of chronic diseases is well documented by numerous studies. Various reports revealed that free radicals are generated during chronic inflammation, which activates the process of damage and deterioration in target cells and organs and leads to further chronic disease. Mechanistically, these free radicals are known to induce the activation of signaling molecules and transcription factors associated with several chronic diseases.[4,7,8] The master inflammatory transcription factors are nuclear factor-kappaB (NF-κB) and STAT3. Activation of these transcription factors have been implicated in a wide variety of diseases, including cancers, diabetes mellitus, cardiovascular diseases, autoimmune diseases, viral replication, septic shock, neurodegenerative disorders, ataxia telangiectasia, arthritis, asthma, inflammatory bowel disease, and several other inflammatory conditions.[9]

Besides these transcription factors, several other biomarkers of inflammation are implicated in chronic diseases. Common factors include inflammatory cytokines and chemokines (tumor necrosis factor [TNF]-α, interleukin-1 [IL-1], IL-6, IL-8, and monocyte chemotactic protein 1), proinflammatory enzymes (e.g. cyclooxygenase [COX]-2, 5-lipoxygenase [LOX], 12-LOX, and matrix metalloproteinases [MMPs]), prostate-specific antigen [PSA], C-reactive protein, adhesion molecules, vascular endothelial growth factor [VEGF], and TWIST.[10] Thus, it is clear that most chronic diseases are preceded by a chronic low level of inflammation.

Biomarkers for detection of diseases

Diagnosis, stratification, prognosis, and therapy for inflammation include the measurement of biological markers (i.e. biomarkers) in biological specimens. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathogenic, or pharmacological responses to a therapeutic intervention. Numerous biomarkers of inflammation have been discovered in the last two decades (e.g. cytokines [TNF, IL-1, -6, etc], chemokines, COX-2, 5-LOX, MMPs, VEGF, TWIST, cell surface adhesion molecules).[11] Since NF-κB is an up-regulator of all these biomarkers, its activation is considered a major mediator of inflammation in most chronic diseases, including cancer. Biomarkers may be measured in several biological specimens (e.g. blood, urine, sputum/saliva, hair, feces, cerebrospinal fluid, body tissues). It is evident that examining inflammatory biomarkers in blood is a standard practice when it comes to clinical diagnosis; however, recent trends suggest that measuring diagnostic biomarkers in novel biological specimens (e.g. saliva and urine) is becoming increasingly popular due to its non-invasive nature.[12]

The diagnostic use of salivary and urinary biomarkers poses several advantages over the traditional measurements of inflammatory biomarkers in blood. For example, (i) they not only spare the patients of the stress from venipuncture, but also eliminate the need of specialized technicians for specimen collection, thereby reducing the healthcare costs; (ii) they do not clot, and therefore eliminate the need of anticoagulation treatment after collection; (iii) they are less likely to transmit diseases than blood through contact or needle-stick injury and therefore are safer; (iv) they are 'quiescent,' meaning that their molecular activity doesn't change much after sampling; (v) they are a relatively clean medium and contain few interfering proteins; and (vi) the simplicity of specimen collection opens an avenue for self-collection of specimens at home by the patients.

In this review, we aim to (1) further discuss the advantages of using novel biological specimens (i.e. saliva and urine) for detecting inflammatory biomarkers over those in blood, while also weighing on the challenges and limitations of their use; (2) examine their role(s) in connection with diagnosis, prevention, treatment, and drug development for several chronic diseases with inflammatory consequences, including cancer; and (3) explore the use of innovative salivary and urine based biosensor strategies that may permit the testing of biomarkers quickly, reliably, and cost-effectively, in a decentralized setting.

Importance of saliva and urine for detection of biomarkers

Saliva and urine are important physiologic fluids containing a highly complex mixture of substances including proteins and genetic molecules. Saliva provides non-invasiveness and stress-free sample collection, easy and multiple sampling opportunities, reduced need for sample pre-processing, and minimal risk of contracting infectious organisms, and it is also an ideal biofluid for collecting specimens from patients in developing countries.[13] Urine is also one of the most interesting and useful biofluids for

routine testing and provides an excellent resource for the discovery of novel biomarkers, with the advantage over tissue biopsy samples due to the ease and less invasive nature of collection.[14] Because of these, saliva and urine could be used in clinical practice for monitoring and diagnosis.

Saliva is used diagnostically for numerous diseases including neurological diseases, Cushing's syndrome,[15] HIV,[16] and cancer.[17,18] The possibility to identify and measure biomarkers in saliva also opens the avenue for screening at-risk populations and monitoring disease progression and compliance with treatment modalities. As an example, cetuximab (Erbitux) is an epidermal growth factor receptor (EGFR) inhibitor currently used for the treatment of metastatic head and neck squamous cell carcinoma (HNSCC). Saliva contains soluble EGFRs, and by analyzing the levels in saliva over time it is possible to determine the patient's response to cetuximab treatment.[19] In urine, approximately 40% of the pyridinoline, biomarker of osteoclastogenesis, crosslinks are eliminated as free components, and the rest as peptide-bound forms.[20,21] A number of biomarkers in urine including PSA for urologic cancers such as prostate cancer are determined.[22] Considering these facts, saliva and urine could be used as an important source of biomarkers for disease diagnosis.

Saliva in diagnosis, prevention, and treatment of diseases

There are several methods, criteria and procedures are used for disease diagnosis. Saliva and urine screening are being used for the diagnosis, prevention, and treatment of numerous diseases. A large number of medically valuable analytes in saliva are gradually unveiled, and some of them represent biomarkers (Figure 1) for diseases including cancer, autoimmune diseases, chronic kidney diseases, diabetes, oral diseases, and cardiovascular diseases[23,24] (see Table 1).

Cancer

Evidence from various studies revealed that a number of salivary proteins are altered in oral cancer patients, including Cyfra 21-1, TPS and CA12, various oxidative stress-related salivary parameters, and biochemical and immunological parameters. In a study, concentrations of four potential salivary biomarkers—choline, betaine, pipecolinic acid, and l-carnitine—were present in significantly different concentrations between oral squamous cell carcinoma (OSCC) patients at stages I and II and healthy individuals.[42] DNA promoter hypermethylation in saliva of oral cancer patient was also found to be useful for the early diagnosis

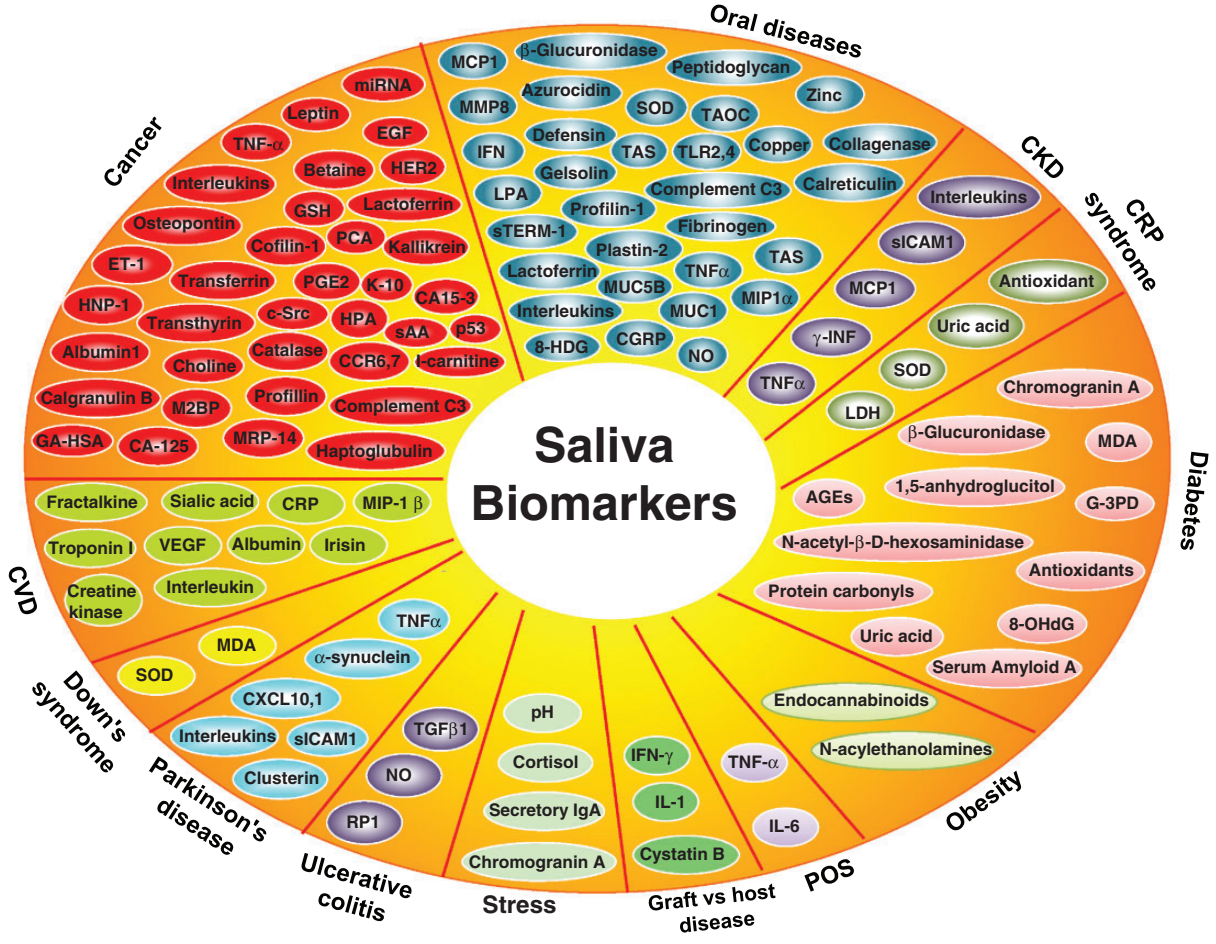

Figure 1 Salivary biomarkers linked with diagnosis, prevention, and treatment of various diseases. (A color version of this figure is available in the online journal.)

Table 1 Detection of inflammatory biomarkers in saliva of patients with various chronic diseases

Biomarkers	References
Cancer	
Oral Squamous Cell Carcinoma	
TNF-α, IL-1α, IL-6, IL-8	(25)
IL-1β, Osteopontin	(26)
ET-1	(27)
HNP-1	(28)
Albumin	(29)
CA-125	(30)
Glutathione	(31)
Ig heavy constant, Cofilin-1, Region gamma	(32)
Transferrin, Transthyretin, Calgranulin B	(32)
M2BP, MRP-14, Profillin, Catalase	(33)
p53 antibodies	(34)
Haptoglobin, Complement C3	(35)
PGE2	(36)
CCR6, CCR7, c-Src	(37)
Sialyl lewis-x	(38)
Total antioxidant	(39)
IL-10	(40)
HPA, sAA, K-10, GA-HSA	(41)
Choline, Betaine, Pipecolinic acid, l-carnitine	(42)
Head and neck squamous cell carcinoma	
miR-9, miR-134, miR-191	(43)
Breast Cancer	
EGF	(44)
EGF Receptor, HER2	(18)
CA15-3, EGFR	(45)
Solid Tumors	
Kallikrein	(46)
Parotid Tumors	
Leptin	(47)
Lung Cancer	
Lactoferrin	(48)
Ovarian Cancer	
CA 125, Glycoprotein complex	(45)
Oral Diseases	
Periodontal Disease	
MIP-1α	(49, 50)
MCP-1	(51)
MMP-8	(52)
NO	(53)
Lysophosphatidic acid	(54)
sTREM-1	(55)
β-Glucuronidase	(56)
IL-1β, -6, -8, MMP-8, IFN-α	(57)
Lactoferrin	(58)
8-Hydroxydeoxyguanosine	(59)
Azurocidin	(60)
Collagenase, Profilin-1, Calreticulin, Peptidoglycan	(61)
Lactotransferrin, Fibrinogen Alpha Chain, Plastin-2	(61)
Alpha-2- Macroglobulin, Alpha-2-HS-Glycoprotein	(61)
Complement C-3, MMP-9, Gelsolin, Defensin	(61)
SOD, TAS, IL-1β	(62)
TOS, TAOC	(63)
Oral Lichen Planus	
IL-6, -8, -18, TNF-α	(64)
IL-1α, -6, -8	(25)
IFN-γ, IL-4	(65)
TLR-2, TLR-4	(66)
IFN-γ, IL-4	(67)
Dental Caries	
TNF-α, IL-6, -8	(68)
sTLR-2	(69)
SOD, Copper, Zinc levels	(70)
MUC1, MUC5B	(71)
Burning Mouth Syndrome	
CGRP	(72)
Autoimmune Diseases	
Sjorgen's Syndrome	
CXCL10	(73)
CXCL13	(74)
sICAM-1	(75)
IL-17, -22, -23	(76)
IL-4, IL-5, Clusterin	(77)
Crohn's Disease	
IL-1β, -6, TNF-α	(78)
Atherosclerosis	
TNF-α, IL-6, -10	(79)
Parkinson's Disease	
α-synuclein	(80)
Chronic Kidney Disease	
IL-1β, -6, -8, TNF-α, γ-INF, MCP-1, sICAM-1	(81)
Complex Regional Pain Syndrome	
SOD, Uric acid, Antioxidant, Albumin, LDH	(82)
Ulcerative Colitis	
TGF-β1, NO, RP-1	(83)
Diabetes	
β-Glucuronidase	(56)
G-3PD, Serum amyloid A	(84)
1,5-anhydroglucitol	(85)
N-acetyl-β-D-hexosaminidase	(86)
8-OHdG, Protein carbonyls	(87)
MDA, Uric acid	(88)
Chromogranin A	(89)
Antioxidant enzymes	(90)
Advanced glycation endproducts	(91)
Obesity	
Endocannabinoids, N-acylethanolamines	(92)
Polycystic Ovary Syndrome	
TNF-α, IL-6	(93)

Table 1 Detection of inflammatory biomarkers in saliva of patients with various chronic diseases

Biomarkers	References
Graft vs. Host Disease	
IFN-γ	(94)
IL-1, Cystatin B	(95)
Sub-acute Thyroiditis	
CRP	(96)
Down's Syndrome	
SOD, Malondialdehyde	(97)
Stress	
pH	(98)
Chromogranin A	(99)
Cortisol	(100)
Secretory IgA	(101)
Cardiovascular Diseases	
Myocardial Infarction	
Irisin	(102)
Fractalkine, IL-17, IL-6, IL-9,	(103)
MIP-1 β, CRP, VEGF, IL-5	(103)
Oxidative stress	(104)
Ischemia-modified albumin	(105)
Creatine kinase MB, Creatine phosphokinase	(106)
Free sialic acid	(107)
Heart Failure	
CRP, Troponin I	(108)

EGF, Epidermal Growth Factor; CRP, C Reactive Protein; TNF, Tumor Necrosis Factor α: Alpha; IL, Interleukin; ET, Endothelin; HNP, Human alpha-Defensin; CA, Cancer Antigen; Ig: Immunoglobulin; M2BP, Mac-2 Binding Protein; MRP, Myeloid-Related Protein; PGE2, Prostaglandin E2; CCR, Chemokine Receptor; IFN, Interferon; TLR, TolI-Like Receptor; TGF, Transforming Growth Factor; NO, Nitric Oxide; MIP, Macrophage Inflammatory Protein; MCP, Monocyte Chemoattractant Protein; MMP, Matrix Metalloproteinases; s-TREM, soluble-Triggering Receptor Expressed on Myeloid Cells; CXCL: Chemokine Ligand; sICAM, soluble Intercellular Adhesion Molecule; SOD, Superoxide Dismutase; LDH, Lactate Dehydrogenase; RP-1, Recognition Protein-1; G-3PD, Glyceraldehyde-3 P Dehydrogenase; CGRP, Calcitonin gene-related peptide.

of oral cancer and potentially can be applied in a clinical setting.[109] Salivary biomarker MMP-1 with 100% specificity was also detected in saliva rinse that could be potentially useful for non-invasive diagnosis of HNSCC of the oral cavity or oropharynx.[110] Collectively these data are predictive in using saliva for diagnosing some types of cancer.

Autoimmune diseases

In autoimmune diseases such as Sjögren's syndrome, saliva ferning patterns were used as a diagnostic test for dry mouth in the primary or secondary stage of diseases. Patients with Sjögren's syndrome showed abnormal salivary smears, usually with a combination of reindeer antler ferning, thick branching ferning, and mucosal squamous.[111] Hu et al.[112] identified 24 antibodies that can differentiate healthy patients from patients with primary Sjögren's syndrome and patients with systemic lupus erythematosus. They also evaluated four other antibodies—anti-transglutaminase, anti-histone, anti-Sjögren's-syndrome-related antigen A (anti-SSA), and anti-Sjögren's syndrome B—in the saliva of patients, which exhibit as biomarkers. Recently, BP180 and BP230 biomarkers have shown diagnostic tool for the detection of Bullous pemphigoid subepidermal blistering disease.[113] Besides these, there is an also increasing interest in using microRNAs (miRNA) as biomarkers in autoimmune diseases.

Infectious diseases

Infectious diseases can be also diagnosed by salivary biomarkers. In fact, saliva is considered the best diagnostic tools for periodontitis. The presence and number of bacterial taxa and other protein markers differentiate the diseases.[114] In the diseases caused by flavivirus, nonstructural protein 1 has been clinically accepted as an alternative biomarker and detection of this protein in the saliva, allowing early diagnosis of the disease.[115] Detection of *Leishmania siamensis* DNA in the saliva of an asymptomatic patient by polymerase chain reaction also could be used as a marker to diagnose the disease.[116] Besides these, in a cohort study among secondary school students in Tanzania, the presence of HIV antibodies in saliva has been investigated. One percent and 5.5% students at the rural and urban schools, respectively, tested positive for HIV antibodies in saliva.[117] Thus, providing HIV knowledge based on this salivary antibody test, the incidence and progression of AIDS can be prevented.

In addition, saliva exhibits antimicrobial and antifungal properties, which manifest its efficacy in the treatment of infectious disease. Saliva was found to be useful in the treatment of the sexually transmitted disease gonorrhea, since it is a powerful and specific inhibitor of *Neisseria gonorrhoeae*. The alpha-amylase present in saliva was found to be responsible for inhibition of this bacterial growth.[118]

Oral disease

Within the past few years, the use of saliva as a diagnostic tool has gained considerable attention. Salivary biomarkers are used as early predictors for clinical disease, contribute to the effective prevention and treatment of diseases, and enhance the assessment of potential health risks. In case of dental caries, saliva also plays an important role in the prevention and development of enamel caries. The components present in saliva protect the enamel surface taken by dental biofilm.[119] In another study with caries-susceptible and caries-resistant female subjects (20 to 21 years old), it was found that the lipid concentrations in parotid saliva in caries-susceptible subjects were higher than those from caries-resistant ones, and differences in fatty acid composition also were found. Thus, the variations in the lipid levels and fatty acid composition may be associated with those in caries development.[120] In addition, saliva contains

several types of antimicrobial peptides that play a role in innate immunity. Salivary peptides such as alpha/beta-defensins and human neutrophil defensins or human neutrophil peptide 1 (HNP)-1, -2, and -3 exhibit antimicrobial activity. Thus, these peptides could be useful for the treatment of periodontal disease and for the prevention of caries and periodontitis.[121] Another component of saliva, fluoride, can also prevent dental caries by acting very early on remineralization and demineralization processes in enamel surfaces.[122]

Saliva has been linked with the treatment condition of diseases. In human subjects, salivary chitotriosidase activity was found to be decreased after non-surgical periodontal treatment in patients having periodontitis with or without coronary atherosclerosis. Thus, salivary chitotriosidase can be used as a marker for the evaluation of periodontal treatment and host response.[123] Other studies have shown an increase in the levels of salivary antioxidants after non-surgical periodontal treatment. In chronic periodontitis patients, an increase of uric acid, albumin, glutathione peroxidase, and total antioxidative status was detected with a decrease of superoxide dismutase activity after treatment.[124] Guentsch et al.[125] also showed that non-surgical periodontal treatment leads to a reduction of salivary malondialdehyde, glutathione peroxidase to levels comparable to healthy controls. Salivary proteins including tissue inhibitor of metalloproteinases 1 (TIMP1) also modulated by the treatment of doxycycline in patients of chronic periodontitis. Doxycycline resulted in significant improvement in clinical parameters compared with the conventional periodontal treatment. Although doxycycline did not produce significant reductions in MMP-8 and MMP-9 levels, increases in the TIMP-1 concentration and the MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios in saliva were observed.[126] Besides these, the salivary defense factors lactoferrin, chitinase, and eta-*N*-acetylhexosaminidase are also found to be suitable for monitoring periodontal treatment results. It has been shown that concentrations of these factors decrease after periodontal treatment.[127]

Other

The role of saliva in the diagnosis, prevention, and treatment of other diseases including peptic ulcer has also been emphasized. Salivary mucus swallowed with food reduces the alkalinity of bile and therefore prevents damage of mucous cells.[128] Saliva is also helpful in the diagnosis and prevention of occupational diseases. For example, exposure to the environmental contaminant phthalate results in several diseases. Detection of phthalate metabolites in human saliva indicate incidence of phalate-associated diseases.[129] Therefore, by taking necessary preventive measures, disease occurrence can be prevented. Furthermore, in cardiovascular disease, determinations of total serum amylase and salivary amylase activity can be also used as a biomarker.[130] Conclusively, such assessments are in the initial stages of development and require considerable further research to determine their clinical utility.

Urine in diagnosis, prevention, and treatment of diseases

Numerous experimental and clinical studies revealed that urine could be used as tool for the diagnosis, prevention, and treatment of various diseases, e.g. cancer, kidney diseases, infectious diseases, autoimmune diseases, tuberculosis, and cardiovascular diseases (Table 2). Urine contains several molecules that act as biomarkers for the diagnosis, prevention, and treatment of diseases (Figure 2).

Cancer

Cancer can also be diagnosed with urine biomarkers. In a study with transitional cell carcinoma (TCC) in dogs, a total of 379 proteins were identified and 96 were unique to the TCC animals. This indicates that these biomarkers can be used for diagnosing cancer such as TCC.[207] Methylation of several tumor suppressor genes is also correlated with clinicopathological variables and diagnosis of diseases. The presence of the methylated tumor suppressor genes CCND2, SCGB3A1, BNIP3, ID4, and RUNX3 in the urine of bladder cancer patients represents the usefulness of urine not only for diagnostic assessment but also as independent bladder cancer prognosticators.[119] VEGF and endostatin were also found higher in urine of patients with bladder cancer than those in patients with urological disorders and healthy controls. Thus, urine levels of VEGF and endostatin could be a clinically useful aid in the diagnosis of bladder cancer.[208]

Urine biomarkers are useful as indicators in cancer prevention. The DNA damage caused by *N*-nitroso compound in red meat-consuming people has been detected in urine. Taking this DNA damage in account, some types of cancer such as colorectal cancer can be prevented by dietary manipulation.[209] In high-grade prostatic intraepithelial neoplasia or prostate cancer patients, urinary F2-isoprostane provides a biomarker for the role for oxidative stress in prostate carcinogenesis. Thus, F2-isoprostanes may also serve to target oxidative stress mechanisms in prostate cancer prevention or treatment.[210] Besides these, antioxidant status such as glutathione *S*-transferase,[211] urine pH,[212] and other biomarkers can play a role in preventing diseases including some types of cancer. Additionally, the presence of tea polyphenol metabolites in tea-consuming individual indicates its significance in cancer prevention, since tea polyphenols are a well-known cancer preventive agent.[213]

Accumulated evidence suggests that urine biomarkers have important applications in the monitoring of disease treatment including cancer. In a study with cancer patients, a high level of cyclic guanosine 3′,5′ monophosphate (cyclic GMP) urine biomarker has been found. However, successful cancer treatment was found to be accompanied by a dramatic fall in the urine cyclic GMP concentrations, whereas if the treatment was unsuccessful the level did not change.[214] Similarly, in bladder cancer patients, the presence of IL-2 in the urine was demonstrated. IL-2 production was found to be directly related to the Bacillus Calmette–Guérin (BCG; a vaccine) administration as samples obtained just before the BCG instillation were always

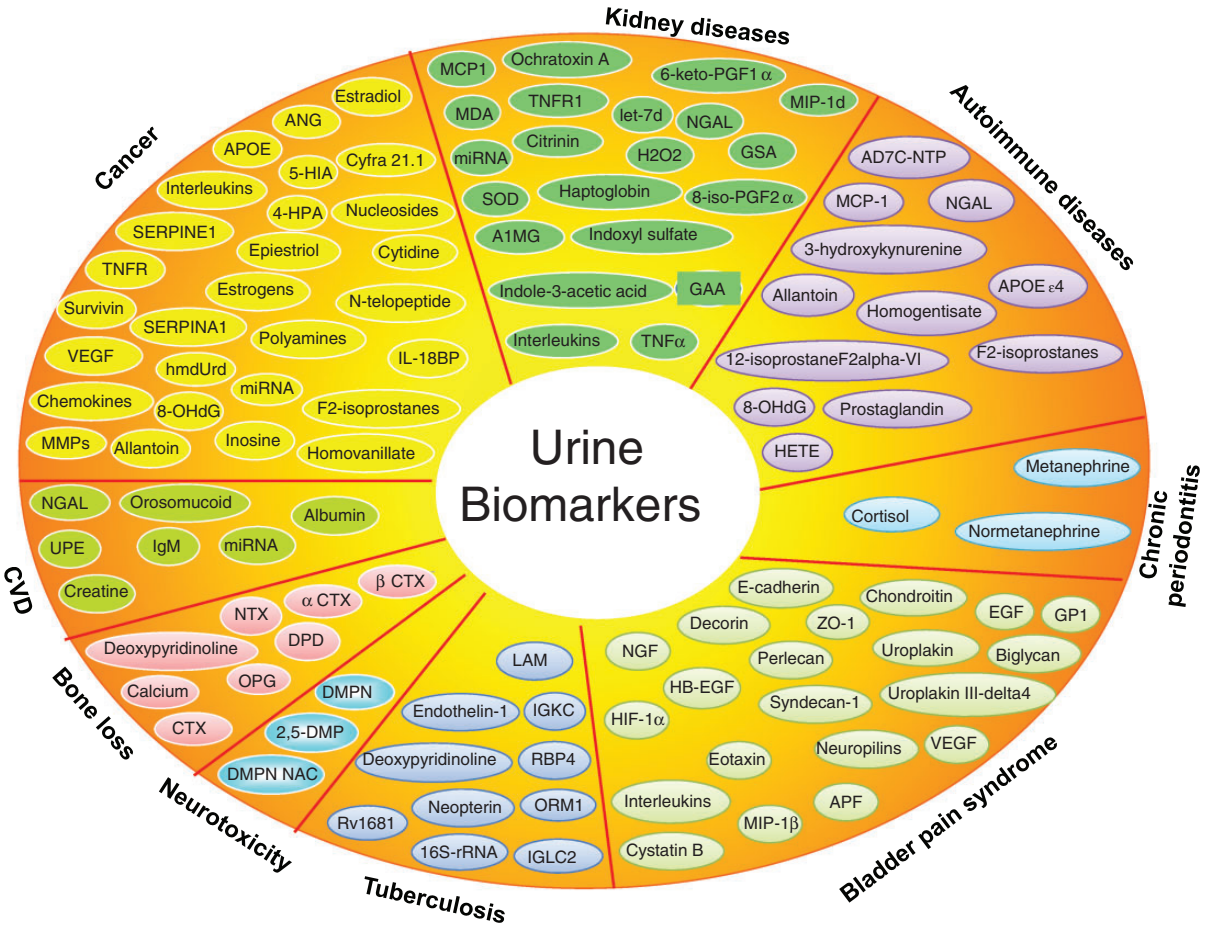

Figure 2 Urinary biomarkers linked with diagnosis, prevention, and treatment of various diseases. (A color version of this figure is available in the online journal.)

negative and urine became positive after the third or fourth BCG instillation.[215] An increase in the number of granulocytes, monocytes/macrophages, and T-lymphocytes in urine after intravesical BCG administration was also demonstrated in bladder cancer patients, indicating a role for urine biomarkers in the determination of local activation of the immune system.[216] Recently, the biomarkers cytokeratin 18, M30, and M65 have been investigated in urine to determine the response to BCG therapy of patients with bladder cancer. High levels of both M30 and M65 were found in all patients within 24 h after treatment; however, the return of both M30 and M65 levels in the urine to normal levels within five days or more was also observed as associated with complete remission.[217]

Neurological disorders

Increased levels of Alzheimer-associated neuronal thread protein (AD7c-NTP) are often detected in urine in the early in the course of Alzheimer's disease. The level of urinary AD7c-NTP and apolipoprotein E ϵ4 in patients with mild cognitive impairment was also found higher than in healthy people, which suggests that the level of urinary AD7c-NTP may be an important biomarker for the early diagnosis of mild cognitive impairment and Alzheimer's disease.[167,218] The detection of drugs in urine samples from patients of Parkinson's disease undergoing therapy with pramipexole, R(−)-apomorphine, (R−)-apocodeine, and R(−)-norapomorphine also indicates the association of urine in neurological diseases.[167,219] Another study showed that a higher concentration of 5- and 6-hydroxydopamine in the urine of dopamine treated Parkinson's patients than the normal controls.[220] This further associates urine and disease treatment. Chronic exposure of manganese has also shown to contribute to the development of neurological syndrome. The detection of elevated levels of manganese in urine can be used as a primary marker, which can be helpful in the prevention of neurological diseases.[221]

Diabetes

It has been found that the amounts of urea and creatinine decreased from normal to low risk and high risk of diabetes, and the amount of glucose increased in the urine of diabetic patients compared to healthy individuals. Thus, it can be concluded that urine provides diagnostic information of possible complications and a better disease prognosis.[222] Also, the presence of urine miRNAs,[223] haptoglobin

Table 2 Detection of inflammatory biomarkers in urine of patients with various chronic conditions

Biomarker	Reference
Cancer	
Bladder Cancer	
IL-6, IL-8	(131)
Chemokine 18	(132)
VEGF, IL-8, MMP-9, MMP-7, Survivin, Cyfra 21.1	(133)
MMP-9, MMP-10, SERPINE1,	(134)
IL8, APOE, SERPINA1, ANG	(134)
Breast Cancer	
IL-8, TNF receptor p55	(135)
Allantoin, F2-isoprostanes	(136)
Estrogens, metabolites	(137)
Estradiol, 16-epiestriol,	(138)
17-epiestriol, 16-ketoestradiol	(138)
5-hydroxymethyl-2-deoxyuridine,	(139)
8-hydroxy-2-deoxyguanosine,	(139)
Homovanillate 4-hydroxyphenylacetate	(140)
5-hydroxyindoleacetate, Urea	(140)
Nucleosides	(141)
Cytidine, methylcytidine, Inosine	(142)
Polyamines	(143)
IL-1α, -8, VEGF, MMP-9	(144)
Non-Hodgkin's Lymphoma	
IL-8	(145)
Prostate Cancer	
IL-18BP	(146)
miRNA-483-5p, miRNA-1275, miRNA-1290	(147)
N-telopeptide	(148)
miR-16, miR-21, miR-222	(149)
Lung Carcinoma	
NNAL	(150)
Kidney Disease	
MCP-1, TNF-α	(151)
MIP-1d, IL-1β	(152)
TNFR1	(153)
IL-8	(154)
let-7d, miR-203, miR-320	(155)
Ochratoxin A, Citrinin	(156)
NGAL	(157)
TNF-α, IL-8, IL-10, SOD, MDA,	(158)
6-keto-PGF1α, 8-iso-PGF2α, H2O2	(158)
Guanidinosuccinic acid	(159)
Guanidinoacetic acid, Indoxyl sulfate,	(159)
Indole-3-acetic acid	(159)
A1MG, Haptoglobin	(160)
Autosomal Dominant Polycystic kidney	
IL-18	(161)
NGAL	(162)
Focal Segmental Glomerulosclerosis	
MCP-1	(163)
HIV-associated Nephropathy	
NGAL	(164)
Neurotoxicity	
2,5-DMP, DMPN, DMPN NAC,	(165)
Autoimmune Diseases	
Alzheimer's Disease	
AD7C-NTP	(166)
APOE ϵ4	(167)
3-hydroxykynurenine, Homogentisate, Allantoin	(168)
8-Hydroxy-2'-deoxyguanosine	(169)
Amyloid beta protein	(170)
F2-isoprostanes, Prostaglandin	(171)
Parkinson Disease	
12-isoprostaneF2alpha-VI	(172)
Dopamine	(173)
8-hydroxy-2'deoxyguanosine	(174)
Hydroxyeicosatetraenoic acid	(175)
Systemic Lupus Erythematosus	
MCP-1	(176)
NGAL	(177)
Osteoclastogenesis	
DPD, CTX, NTX	(178)
αCTX, βCTX	(179)
OPG	(180)
Calcium	(181)
Deoxypyridinoline	(182)
Oral Diseases	
Chronic Periodontitis	
Creatinine-adjusted cortisol,	(183)
Metanephrine, Normetanephrine,	(183)
Bladder Pain Syndrome	
E-cadherin, ZO-1, uroplakin Chondroitin	(184)
Epithelial cell growth factors	(185)
GP51	(186)
Uroplakin III-delta4	(187)
biglycan, decorin, perlecan, Syndecan-1	(188)
Neuropilins, VEGF	(189)
IL-6, IL-8	(190)
NGF	(191)
HB-EGF, APF	(192)
Tuberculosis	
IGKC, RBP4, ORM1, IGLC2,	(193)
Lipoarabinomannan	(194)
16S-rRNA	(195)
Rv1681	(196)
Deoxypyridinoline	(197)
IP-10	(198)
Neopterin	(199)
Endothelin-1	(200)
Cardiovascular Disease	
NGAL	(201)

(continued)

Table 2 Continued

(continued)

Table 2 Detection of inflammatory biomarkers in urine of patients with various chronic conditions

Biomarker	Reference
Urine phosphorus	(202)
Creatinine, Albumin	(203)
IgM	(204)
Orosomucoid	(205)
miRNAs	(206)

IL, Interleukin; TNF, Tumor Necrosis Factor; IL-18BP, Interleukin-18 Binding Protein; α: Alpha VGEF, Vascular Endothelial Growth Factor; MMP, Matrix Metalloproteinases; MCP, Monocyte Chemoattractant Protein; MIP, Macrophage Inflammatory Protein; NGAL: Neutrophil Gelatinase-Associated Lipocalin; TNFR1, Tumor Necrosis Factor Receptor-1; PET PIB, positron emission tomography, radiotracer Pittsburgh Compound-B; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; DHBA, 3, 5-dihydroxy-benzoic acid (DHBA); DHPPA, 3-(3, 5-dihydroxyphenyl)-1-propanoic acid; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; DPD, deoxypyridinoline (lysylpyridinoline); NTX, N-terminal cross-linking telopeptide of type I collagen; IGKC, immunoglobulin kappa chain C; RBP4, retinol binding protein 4; ORM1, α-1-acid glycoprotein 1; IGLC2, immunoglobulin lambda-2 chain C; A1MG, Alpha-1-microglobulin; DMPN, dimethylpyrrole norleucine; DMPN NAC, cysteine-pyrrole conjugate; DMPN GSH, glutathione-pyrrole conjugate; 2,5-DMP, 2,5-dimethylpyrrole; AD7c-NTP, Alzheimer-associated neuronal thread protein.

levels,[224] and several other proteins can be used as diagnostic tools for diabetes.

The American Diabetic Association recommended aspirin therapy as a primary prevention of the disease mellitus. However, it has several limitations. The presence of microalbumin and macroalbumin in urine has reported in the patients of type 2 diabetics treated with aspirin as preventive measure,[225] indicating its role in prevention of diseases. Albuminuria in the urine of diabetic patients has also determined for prevention and control of diabetic nephropathy.[226] Urine biomarkers are also linked to the treatment of numerous other diseases. In case of diabetes treatment by amiloride, urinary plasminogen and albumin excretions were found to be significantly reduced. Urokinase activity was detectable in macroalbuminuric urine, with a tendency toward reduction in activity after amiloride treatment.[227]

Renal diseases

The urine marker neutrophil gelatinase-associated lipocalin has been demonstrated to be useful in the early diagnosis of acute kidney injury.[228] High urine levels of annexin A1 (ANXA1) protein have been demonstrated as an index for glomerular injury. Thus, ANXA1 could be universal biomarker for the early diagnosis, prediction, and outcome monitoring of glomerular injury.[229] Chronic exposure to cadmium causes renal proximal tubular dysfunction, and the level of cadmium in urine is generally regarded as an indicator of cadmium body burden. Therefore, timely determination of urinary cadmium levels and proteinuria can prevent the incidence of diseases caused by cadmium exposure.[230]

Other

Tight junction breakdown, with loss of the important sealing protein claudin-3, is an early event in the development of intestinal damage. Both experimental and clinical studies showed an increased urine claudin-3 protein, indicating a strong relation between intestinal tight junction loss and urinary claudin-3 levels that is useful in diagnosis.[231] These findings suggest that measurement of urinary claudin-3 can be used as non-invasive marker for intestinal tight junction loss. It has been also observed that patients with endometriosis have a unique cluster of peptides in urine, and peptide proteomic profiling provides a novel method for non-invasive diagnosis of endometriosis.[232] Sylvester et al.[233] identified seven urine proteins capable of providing highly accurate diagnostic and prognostic information for infants with suspected necrotizing enterocolitis, which could be helpful in improving the efficiency and early diagnosis of this disease.

Saliva- and urine-based biosensors for diagnosis

Over the last 20 years, the development of rapid, selective, sensitive, and cost effective sensors have been a major interest in the bioanalytical sciences. Currently, much effort is being invested in the research of new materials toward the development of electrochemical biosensors with low detection limits.[234] Because of their unique specificity and detection of low concentrations of specific biomarkers, electrochemical biosensors are attractive for analytical applications.[235] Saliva and urine have been used as diagnostic media in recent years. The biomarkers contained in saliva and urine can be detected by biosensors and used for the determination of various specific biochemical parameters to diagnose correlated diseases.

Basically, urine is considered an ideal biofluid for clinical investigation without any harm to patients. The determination of high concentration of the protein cathepsin D in urine can be used as a tumor marker in the diagnosis of bladder cancer. Recently, Gorodkiewicz et al.[236] reported that cathepsin D concentration in the urine of bladder cancer patients was very high (1.35–7.14 ng/mL) compare to healthy donors ($N = 54$) (0.32–0.68 ng/mL). A gas chromatography-sensor device was used for the diagnosis of bladder cancer based on volatile metabolites in urine. Urine samples were taken from 24 patients with urothelial cell carcinoma and 74 controls presenting with urological symptoms, but without a urological malignancy. By using the leave-one-out cross-validation method, 95.8% of cancer cases were correctly predicted with 93.2% of controls.[237] Analysis of the volatile metabolites in urine headspace by selected ion flow tube mass spectrometry was used for the profiling of gastro-esophageal cancer. Samples were retrieved from patients with gastro-esophageal cancer, non-cancer diseases of the upper gastro-intestinal tract, and a healthy cohort. Seven volatile organic compounds (acetaldehyde, acetone, acetic acid, hexanoic acid, hydrogen sulfide, methanol and phenol) were found to be significantly increased in the cancer cohort compared to positive control

and healthy groups.[238] High urinary MMP-7 levels revealed the presence of lymph node metastases, and an antibody-based electrical biochip technology was also developed for the quantitative detection of urinary MMP-7.[239] In this study, urine samples were acquired from 30 bladder cancer patients and 15 healthy individuals of similar age. The concentration of MMP-7 was significantly higher in patients with metastatic bladder cancer compared to others. Electrochemical sensing of prostate-specific membrane antigens, which is well known as a prostate cancer biomarker in urine, could revolutionize cancer diagnosis and treatment.[240]

Successful measurement of salivary markers reveals that saliva samples can play diagnostic roles in various diseases. As cancer is caused by the accumulation of mutations in various genes that inactivate tumor suppressor genes and activate proto-oncogenes, tumor-specific genetic markers for cancer cells were studied in patients' saliva. The p53 gene mutation in HNSCCs was sequenced and used retrospectively as tumor-specific genetic markers in saliva, and determined that tumor-specific mutations in preoperative saliva samples were 71% (5 out of 7 patients).[241] Because of constitutive activation and high mutations rate in EGFR, it is very common abnormal expression in non-small cell lung carcinoma (NSCLC), therefore noninvasive, accurate, rapid, user-friendly, inexpensive detection and monitoring of EGFR mutations in NSCLC patients is highly desired. Electric field-induced release and measurement was approved as a saliva-based EGFR mutation detection technique in saliva samples from 40 patients with NSCLC.[242] Saliva is used for the detection and quantification of cytokine level to treat various diseases such as OSCC. In a study, the quantification of picomolar IL-8 concentrations in human saliva was determined by using Biacore surface plasmon resonance in a microfluidic channel. IL-8 protein in the saliva of healthy individuals, and patients with OSCC were 30 pmol/L and 86 pmol/L, respectively.[243]

Generally, a surface-immobilized optical protein sensor is applied to detect oral cancer marker IL-8 with a limit of detection (LOD) at 1 pmol/L in buffer. Confocal optics-based sensor for further reducing the optical noise and extend the LOD of the surface immobilized optical protein sensor has been developed to detect IL-8 protein at 4.0 fmol/L in buffer.[244] In this report, saliva samples of 20 oral cancer patients and 20 controls were determined to compare the sensitivity and accuracy of optical protein sensor with traditional enzyme-linked immunosorbent assay values. Nano-biochips (the integration of semiconductor nanoparticle quantum dots into a modular and microfluidic biosensor) were also used for the determinations of cancer biomarkers, carcino-embryonic antigen, cancer antigen 125, and Her-2/Neu in saliva.[245]

Author contributions: SP and AKT participated in collecting data and reviewing all articles cited in the paper. SP wrote the manuscript. BBA developed concept and supervised in structuring the manuscript. All authors read and approved the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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