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Salivary Inflammatory Mediators in Cancer Diagnosis

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Abstract

Cancer is the second leading cause of death worldwide; it can be successfully treated in the early stages. Screening for cancer should be performed in safe, accurate, cost-effective, and non-invasive techniques and therefore achieved the patient convenience. The salivary diagnosis could be a promising era in oncological fields, which have some correlations with serum biomarkers in certain cancers. In this paper, we reviewed some of the salivary biomarkers in detecting different cancers and their origins [genomics, epigenomics, transcriptomics, metabolomics, proteomics, and microbiota].
Keywords: Cancer screening; Salivary diagnosis; Salivaomics; Inflammatory biomarker.

1. Introduction

According to the world health organization (WHO), the second cause of death in the world is cancer which was in charge of 9.6 million deaths in 2018 [1]. The success in cancer cure is linked with the detection of the premature stages, the earlier discovered tumors have enormous opportunities to be eliminated, especially with the highly sensitive diagnostic technologies such as magnetic resonance imaging (MRI) and computed tomography scan (CT scan), but they have some limitations of use because of the high cost, large exposure to the radiation and not-portable [2], this creates a clinical demand for looking at a new effective painless and non-invasive approach for prognosis and screening cancers. Saliva is an extracellular biological fluid that is secreted from major salivary glands which are the parotid, submandibular, and sublingual glands. The importance of saliva comes from its ability to supply lubrication, support digestion, and mastication, as well as prevent dental caries via preventing teeth demineralization [3]. Gingival crevicular fluid (GCF) is a fluid produced from the periodontal tissues in the case of inflammation, which contains enzymes, organic ions, epithelial cells that resulted from the desquamation of tissues and bacterial products. Previously, GCF was proven to be a diagnostic test for periodontal diseases [4]. Salivary diagnostics are considered as non-invasive, and convenient in sampling collection thus dramatically limiting stress associated with needle phobia and pain in blood sampling and therefore facilitate multiple sampling [5]. Saliva biological mediators are being used to diagnose dental problems and recently they have been tested for their ability to assess kidney and liver function and achieved promising results [6]. This paper aims to discuss the possibility of applications salivary inflammatory biomarkers in the local and systemic cancer diagnosis.

1.1. Salivaomics

It is a term used to describe a group of technologies that investigates several components in saliva, such as genomics, epigenomics, transcriptomics, metabolomics, proteomics, and microbiota. Salivary **Genomics** is suitable to be developed as a biomarker, due to its high quality and stability, it includes both host and microbial DNA. Sampling from saliva can provide adequate DNA to be sequenced in the polymerase chain reaction (PCR) or the quantitative PCR (qPCR) assays [7]. Tumorigenesis is the process of aggregation of genetic mutations and therefore loss of cells functions and subjected to malignant characteristics [8]. Salivary **Transcriptome** includes the whole RNA components such as mRNA, microRNA (miRNA), tRNA, rRNA, and piwi-interacting RNA (piRNAs), thus salivary transcriptome analysis can predict the disease condition [9]. Salivary piRNAs are emerging probable mediators for cancers screening because their sizes are small enough to be avoided from degradation via ribonucleases (RNases) [10]. Based on the previous studies which were performed on cancer patients by employing a quantitative real-time PCR (qRT-PCR), and gene microarrays with sufficient specificity and sensitivity, the miRNA and mRNA were detected in three cancer types: breast [11], lung [12], and pancreatic [13] cancers, some of them are illustrated in table 1.

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Table-1. Some of salivary mRNAs biomarkers and their role in OSCC [14]

Salivary mRMA biomarkers	Role in cancer cell
H3 Histone Family 3A [H3F3A]	Activate Cell Proliferation.
Interleukin-8 [IL-8]	Promote cell adhesion and arresting of the cell cycle.
Ornithine decarboxylase antizyme 1 [OAZ1]	Cell proliferation via commanding intracellular polyamine.
Spermidine/Spermine N1-Acetyltransferase 1 [SAT1]	Angiogenesis.
Dual specificity protein phosphatase 1 [DUSP1]	Radioresistance and chemoresistance in solid tumors.
Matrix Metallopeptidase (1 and 9) [MMP-1 and MMP-9]	Develop the dysplasia into cancer.

Salivary **Proteome** has confirmed its ability in detection protein biomarkers which are related to different systemic and local disorders as in the recent clinical research which was published in 2020 to identify a potential inflammatory biomarker for late-stage (III or IV) laryngeal cancer in African American and white American males by the employment of salivary proteome analysis by WebGestalt [15]. Mass spectrometry (MS) is performed in salivary protein detection, and can be combined with two-dimensional gel electrophoresis (2DE) in lung cancer monitoring [16]. The salivary proteins biomarkers in an oral squamous cell carcinoma (OSCC) patients are elucidated in Table 2.

Table-2. Some of salivary protein biomarkers and their role in OSCC [14]

Salivary protein biomarker	Role in cancer cell
Endothelin-1 [EDN1]	Activate tumorigenesis process.
Interleukin-8 [IL-8]	Promote inflammatory mediator (T-cell, neutrophils, basophils).
CD59 Molecule	Promote of T-cell production.
Cyclin D1	Metastasis process and cell proliferation.
Lactate Dehydrogenase	Use pyruvate to form lactate in anaerobic conditions.
Tumor Necrosis Factor-Alpha [TNF-α]	Prevention of apoptosis and activation cell proliferation.

The tumor metabolism alteration is based mainly on endogenous glucose, purine, pyrimidine, glutamine, and lipids [17]. The glucose utilization in cancer tissues is recognized as Warburg effect which would tend to the glycolysis even with a sufficient level of oxygen [18]. Salivary **metabolites** can be employed as a feasible diagnostic tool for cancer monitoring [14]. A clinical study was carried out by Sugimoto et al. to compare the salivary metabolites in cancer patients and healthy standards [19]. Some of the salivary metabolites can be a distinguishing tool among cancer and healthy tissues, for example, valine, pyrroline, cadaverine, taurine, tryptophan and leucine, Table 3 [20].

Table-3. Some of salivary metabolite biomarkers and their role in OSCC

Salivary metabolite biomarker	Role in cancer cell
Glutamic acid	Activate cell proliferation.
Ornithine	Promote the differentiation of tumor cells .
γ-aminobutyric acid (GABA)	Promote of production the p38 and therefore block apoptosis.
Carnitine	Cell differentiation in early stages.

The salivary **microbiota** has been studied in patients with OSCC who have plenty of periodontitis that correlated to *Prevotella tannerae*, and *P. intermedia* [21]. The impaction of radiotherapy on salivary microbiota in OSCC patients has been studied and longitudinally estimated, it has been shown an elevation in salivary *Lactobacillus* species that have a strong effect on salivary pH levels during the radiotherapy sessions [22]. Another study has proven that mucositis -which is a side effect of chemotherapy- can diminish salivary bacterial levels in health-correlated genera such as *Streptococcus*, and *Veillonella*, as well as an elevation of periodontitis-correlated genera such as *Prevotella*, and *Fusobacterium* [23].

2. Salivary Inflammatory Biomarkers in the Cancer Screening

2.1. Lung Cancer

Based on the WHO, the mortality from lung cancer in 2018 was 1.76 million deaths in the world [1]. Lung cancer can be screened by low-dose computed tomography (LDCT), which has some risks should take into considerations, for examples: it can give the false-positive result and therefore more follow-up checking and surgeries that are gratuitous, it can create *overdiagnosis* condition thus LDCT can detect problems that may not cause health issues and therefore have unnecessary therapy, as well as the continuous exposure to radiation [24]. These limitations create a demand for looking for some diagnostic non-invasive biomarkers to identify cancer. Electric field-induced release and measurement (EFIRM) are able to find out the mutations in epidermal growth factor receptor (EGFR) in saliva, this method is depending on immobilized DNA probes in order to catch the mutated sequences and therefore force an electrical field to expedite the hybridization. This technique is widely used in oncogenic clinics [25]. Salivary biomarkers could be an alternative option to detect the malignant tissues, there are

sixteen salivary diagnostic biomarkers while three proteins (glycoprotein, haptoglobin, calprotectin) achieved sufficient specificity and sensitivity, and there are five of salivary mRNA biomarkers (cyclin I [CCNI], EGF receptor [EGFR], fibroblast growth factor 19 [FGF-19], growth regulation by estrogen in breast cancer 1 [GREB1], and fibroblast growth factor receptor substrate 2 [FRS2]) that achieved 82.81% specificity and 93.75% sensitivity in lung cancer detection [26].

2.2. Pancreatic Cancer

Pancreatic cancer (PC) cases in 2018 were 458,918 in the world. The majority of pancreatic cancer patients will progress to the metastases, due to the deficiency of the early detecting techniques and therefore diminish the potency of treatment protocols [27]. Eighteen salivary transcriptomic biomarkers were being studied to detect pancreatic cancer, miR-21 is recognized as the most accurate miRNAs in an oncological screening, it is working by diminishing phosphatases production and thus restricting the protein kinase B (PKB) and MAP kinase (MAPK) pathways, the upregulated miR-21 in pancreatic cancer is indicated for metastatic disease and unresponsive therapy [28]. In addition, the salivary miR-3679-5p and miR-940 are working as a distinguishing diagnostic tool in pancreatic cancer with 72% sensitivity and 70% specificity [29]. Also, the elevation in urinary and salivary MIR1246 was confirmed in the diagnosis of pancreatic cancer [30]. The salivary microbiota can be performed in the screening of PC by the presence of two species of bacteria in the saliva (*Streptococcus mitis*, and *Neisseria elongate)* with 96.4% sensitivity and 82.1% specificity. Furthermore, the salivary ratio of *Leptotrichia* / *Porphyromonas* was higher in a patient with PC compared with healthy subjects. PC can be differentiated from another chronic pancreatitis by the salivary levels of N1- acetylspermidine, and N1-acetylspermine metabolites that create sufficient accuracy [31]. Non-coding RNAs play an important role in distinguishing PC, thus increment in the salivary HOTAIR and PVT1 is correlating with Pancreatic Ductal Adenocarcinoma [32].

2.3. Breast Cancer

WHO reported 2.09 million cases of breast cancer and 627 000 deaths in 2018 [1]. Breast cancer is currently diagnosed by a mammogram that has inadequate sensitivity to be considered as a golden diagnostic technique [33]. So it is important to search for non-invasive, accurate, and safe screening tests in body fluids like saliva. Cancer antigen 15-3 (CA 15-3) is a glycoprotein which is upregulated in cancer cell surface and help in cell adhesion, the high concentration of serum and salivary CA 15-3 is diagnosed as breast cancer [34]. It is applicable to identify the salivary autoantibodies (IgM and IgG) which are against of HER2 (human epidermal growth factor receptor 2) which is responsible for progressing a breast cancer, and creates a resistance regarding chemotherapy agents, but MUC1 (Mucin 1, Cell Surface Associated) is responsive for immunotherapy [35]. Also some of salivary transcriptional biomarkers play significant role in screening the breast cancer, such as: hexose-6-phosphatehexose-6 phosphate dehydrogenase (H6PD), ionotropic, kainate 1 (GRIK1), tumor protein translationally- controlled (TPT1), glutamate release (GRM1), and IGF2BP1 (Insulin Like Growth Factor 2 MRNA Binding Protein 1) [36]. Recently in 2020, a study provided the possibility of applying the Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR) spectroscopy to the first time to analyze saliva samples and find out the breast cancer samples which create high absorbance at wavenumber 1041 cm−1 in a comparison with normal samples, also the receiver operating characteristic curve (ROC curve) showed a sufficient precision to screen the breast cancer in salivary samples [37].

2.4. Gastric Cancer

In 2018, about 1.03 million cases were reported worldwide, and 783,000 deaths from gastric cancer [1]. The current use of diagnostic techniques is invasive like the upper digestive endoscopy, and therefore the diagnostic research tendency is going to explore a non-invasive, and accurate biomarker to detecting gastric cancer in the early stages [38]. Gastric cancer can be screened by salivary glycoproteins biomarkers through observing the variations of salivary N- and O-linked glycan, this can be carried out by incorporation of MALDI-TOF MS (matrix-assisted laser desorption/ionization-time of flight mass spectrometry) and isolation of lectin [39]. The salivary miRNA biomarkers are MIR301a, and MIR140-5p, and the salivary mRNA are SEMA4B, and SPINK7 which used in gastric cancer screening [40]. Salivary amino acids also had proven in the diagnosis of gastric cancer, Surface-enhanced Raman scattering (SERS) can perform to differentiate gastric cancer from healthy control with more than 80% sensitivity, and more than 87.7% specificity [41]. Lectin microarrays achieved 96%sensitivity, and 80% specificity in probing salivary glycopatterns to detect gastric cancers [42]. Helicobacter pylori (H.pylori) which causes a stomach inflammation can be used in detecting gastric cancer by cross-reactive sensitivity of its salivary metabolites (CO2, and NH3) in part per million (ppm) levels. [43]. Three of the salivary proteins create 93% accuracy in the diagnosis of gastric cancer, which are Malignant Brain Tumors-1 protein (DMBT1), cystatin B, and triose-phosphate isomerase (TPI) with 80% specificity, and 80% specificity [44].

3. Conclusion

Screening for cancer aimed to detect tumors in early stages to achieve successful complete therapy. The screening and monitoring techniques should be accurate, safe, and non-invasive. The salivary diagnostic could be potentially promising tools in cancer screening, that are away from oral cavities, such as lung cancer, breast cancer, pancreatic cancer, and gastric cancer. This elucidates that salivary biomarkers can be an auspicious diagnostic approach in oncological clinics. A deep understanding of the correlation among salivary and serum inflammatory

biomarkers and develop sufficient clinical studies in all cancer types will allow widespread applicability of salivary diagnosis.

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