

## SALIVA DIAGNOSTIC AND CANCER MONITORING: OVERVIEW

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### Abstract

Saliva is a biological fluid consisting of a large number of biomarkers and secretions which can help in the diagnosis of many diseases. Indeed, saliva has known a particular popularity in biomedical field since the nineties.

Its wide popularity is mainly due to the ease of collection. This could fit the screening of large population. In cancers monitoring, saliva has also shown a potential in the detection of tumor markers which are detectable in cancer patients blood and could be easily quantified by immunoassays. This could be a proof of possible use of salivary markers in prevention, diagnosis and monitoring in many cancers without necessarily using high invasive methods used in practice.

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### Introduction

Saliva is a complex biological fluid consisting of a multiple secretions and useful biomarkers in the diagnosis of many diseases.<sup>1,2</sup> It has known a particular interest in biomedical field since the nineties.<sup>2</sup> Indeed, it could be a convenient tool of prevention, diagnosis and treatment assessment of systemic diseases.<sup>1,2</sup> In practice, saliva has already found a place in evaluation of some illnesses such as dental caries, markers of infection by mushrooms as candida or salivary gland diseases and hormone dosage. Also, several studies have suggested the diagnostic potential of saliva in viral, bacterial infections, and several cancers.<sup>3,4,5,6</sup>

Saliva was widely popular because of its ease of collection with non-invasive methods. It can be taken many times without patient discomfort and can even be collected by people not necessarily professional and stored at home.<sup>3</sup>

In addition, saliva is also a cost-effective approach suitable for screening in large populations. Moreover, obtaining saliva samples from children or anxious patients is much easier than obtaining other samples (blood ...).<sup>7</sup>

The whole saliva includes a complex mixture of secretions from salivary glands, gingival fluids and other secretions.<sup>8</sup> In fact, the gingival fluids are plasma exudations.<sup>9</sup> This could explain why most of biomarkers found in serum could possibly be quantified in saliva.<sup>9</sup>

In cancer diseases context, saliva also has shown an interest in the detection of tumor markers, detectable in the circulation of patients and easily quantified by immunological methods such as ELISA.<sup>10,11</sup>

The aim of this review is to provide an overview of the possible use of saliva as a non-invasive method to monitor cancers.

### Origin and Saliva Composition

Whole saliva in oral cavity includes a complex mixture of fluids from the salivary secretions of 3 major glands (parotid, submandibular and sublingual gland). Besides, other minor salivary glands, gingival fluids, excretions of oral mucosa, nasal and pharyngeal

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secretions, non-adherent bacteria, food and dead epithelial cells debris and possibly traces of drugs and chemicals.<sup>9</sup>

The amount of saliva produced daily by a healthy adult is approximately 0.5-1.5L with a flow rate of 0.5ml/min. Salivation is strongly controlled by the sympathetic and parasympathetic nervous system.<sup>12</sup> Furthermore, saliva flow rate increased under the action of several factors such as circadian rhythm, sex, age, nutritional and emotional state and the category of stimulus (smell, taste, chewing, hormonal disturbances related to pregnancy). Similarly, there are factors act by decreasing the flow rate of salivation as hormonal changes associated to menopause or stress.<sup>13</sup>

Saliva is composed 99% of water and 1% includes molecules such as mineral salts, proteins.<sup>12, 14, 15</sup> Moreover, almost all the organic substances and electrolytes (sodium, potassium, calcium, magnesium, bicarbonate, phosphate), immunoglobulin, proteins, enzymes, mucins, found in plasma could be detected in saliva at low concentrations.<sup>2, 13, 9</sup>

### Detection of Tumor Biomarkers in Saliva

Tumor biomarkers are substances that are detected in blood of patients with malignant tumors. In fact, the detection of those biomarkers are useful in all stages of cancer care, including early detection, diagnostic, evaluation of chemotherapy efficiency and the prediction of metastasis dissemination.<sup>10</sup> Besides, an increased number of researchers are interested in tumors detection using saliva medium. The common point in all those studies is the possible use of saliva in cancers monitoring Table 1. Actually, biomarkers could be oncoproteins, proteins encoded by tumor suppressor genes or simply other molecules indicators of oxidative stress.<sup>16</sup>

**Breast Cancer:** In breast cancer, many biomarkers such as VEGF, EGF and CEA were significantly elevated in saliva of patients with breast cancer compared to healthy women.<sup>17, 18</sup> Same as HER2 and CA15-3, which are the most important biomarkers in breast cancer diagnosis and monitoring.<sup>19, 20</sup> Case-control studies have shown that the salivary concentration of these markers was elevated in patients with breast

cancer compared to patients with benign tumors in the breast and healthy women.<sup>21, 22, 23</sup>

Authors	Cancer type	compared groups			biomarkers	Analysis method	P Value	Correlation
Balan et al. <sup>20</sup>	OCC	60 healthy volunteers	60 OCC patients	-	CA 125	ELISA	< .001	-
BROOKS et al. <sup>17</sup>	BC	49 healthy volunteers	49 BC patients	-	VEGF EGF CEA	ELISA	< .0001 < .0001 .0106	-
Streckfus et al. <sup>21</sup>	BC	57 healthy volunteers	41 women with BT	30 BC patients	HER2 CA 15-3	ELISA	< .001	r= .51
Navarro et al. <sup>18</sup>	BC	52 cases with active BC	22 BC in follow-up	33 healthy volunteers	EGF	RIA	-	-
Chen et al. <sup>21</sup>	OC	55 healthy volunteers	92 avec PBT	41 MPT	CA 12-5	RIA	< .005	r= .5
Streckfus et al. <sup>21</sup>	BC	15 healthy volunteers	12 BC patients	-	CA15-3 HER2 EGFR Cathepsin-D p53	ELISA	< .05 < .001 - -	-
Shpitzer et al. <sup>22</sup>	OCC	19 CT	19 healthy volunteers	-	MMP-9 Carbonyles OGG1 Src Ki67 Maspin LDH CycD1	ELISA	.014 .012 .007 .010 .015 .001 .002 < .00001	-
Agha-Hosseini et al. <sup>24</sup>	BC	25 healthy volunteers	24 untreated BC	23 treated BC	CA125	EIA	.01	r= .38
Laidi et al. <sup>23</sup>	BC	10 BC patients (HER2+)	17 BC patients (HER2-)	-	HER2	ELISA	> .05	-
Bernardes et al. <sup>27</sup>	OCC	46 patients OCC	46 healthy volunteers	-	HER2 EGFR EGF	ELISA	> .05 < .05 > .05	-
Laidi et al. <sup>23</sup>	BC	29 BC cases	31 healthy volunteers	-	CA 15-3	EIA	.03	r= .27
Pardis et al. <sup>23</sup>	HNC	28 HNC	25 healthy volunteers	-	HER2	ELISA	-	-
Agha-Hosseini et al. <sup>22</sup>	BC	35 healthy volunteers	26 untreated BC	-	CA 15-3	EIA	.01	r= .614

#### List of abbreviations :

**OCC:** oral cavity cancer, **BC:** Breast cancer, **OC:** ovarian Cancer, **BT:** benign tumors, **HNC:** head and neck cancer, **PBT:** pelvic benign tumors, **MPT:** malignant pelvic tumors, **CT:** cancer of the tongue.

**Biomarkers:** **VEGF:** vascular endothelial growth factor, **EGF:** epidermal growth factor, **CEA:** carcinoembryonic antigen, **MMP-9:** Matrix metalloproteinase 9, **OGG1:** 8-Oxoguanine glycosylase, **Src:** Proto-oncogene tyrosine-protein kinase, **Maspin:** mammary serine protease inhibitor, **LDH:** lactate dehydrogenase, **CycD1:** Cyclin D1, **CA 15-3:** cancer antigen 15-3, **HER2:** human epidermal receptor 2, **EGFR:** epidermal growth factor receptor.

**Analysis methods:** **ELISA:** enzyme-linked immunosorbent assay, **RIA:** radio immunoassays.

**Table 1.** Salivary biomarkers in cancers Detection

Also, the correlation found between serum and saliva was positive and varies from low to moderate correlations.<sup>21, 22, 23</sup> The breast cancer detection by the salivary marker CA 125 is also possible. Indeed, quantification of this marker in saliva in patients with untreated breast cancer was significantly higher comparing to patients treated and healthy women. Moreover, the correlation between this marker in saliva and serum was low but positive.<sup>24</sup>

Diagnosis and therapeutic evaluation of breast cancer biomarkers involves in practice mainly 2 biomarkers, HER-2 and CA 15-3.<sup>25</sup> The receptor HER-2, CA15-3 additionally to the protein p53 were detected in saliva of patients with benign tumors, malignant tumors and healthy women.<sup>25</sup> In fact, salivary levels of HER2 and CA 15-3 were elevated in cancer patients. However, the high levels of the protein p53 were observed in healthy women comparing to women with malignant or benign tumors.<sup>25</sup> Furthermore, saliva may also be useful tool in evaluation of therapeutic efficacy of breast cancer.<sup>26</sup> Indeed, quantification of tumor markers CA 15-3 and HER2 in the saliva of patients with breast cancer at different stages (before treatment and during treatment), has shown the potential of salivary HER-2 in the evaluation of the therapeutic efficacy. However CA 15-3 showed no interest at this level.<sup>26</sup>

Besides if all markers already mentioned revealed an interest, in breast cancer detection, other data did not show any usefulness such as cathepsin-D and EGFR.<sup>25</sup> Other data has shown no interest of saliva in detection of HER-2 marker in breast cancer, oral cavity cancer or head and neck cancer. Indeed, some authors did not find any association between overexpression of the HER-2 receptor in mammary tissues and its concentration in saliva.<sup>27, 28, 29</sup>

**Ovarian Cancer:** CA 125 protein is known for its role in some cancers such as ovarian, breast cancer and oral cavity cancer.<sup>30</sup> This marker was significantly elevated in patients with oral cavity cancer.<sup>30</sup> In ovarian cancer, CA 125 was also investigated. In fact, according to a comparative study of saliva in patients with benign and malignant tumors and healthy women, this marker could be useful in the detection of ovarian cancer. Salivary level of CA125 in patients was significantly higher compared to healthy women. Also, the authors found a low rate of false positives (13%) and the false negatives were totally absent.<sup>31</sup>

**Oral Cavity Cancer:** In oral cavity cancer, 8 salivary markers linked to oxidative stress, DNA repair, the process of carcinogenesis and cell death were analyzed in patients with cancer of the tongue.<sup>32</sup> 5 markers were elevated in patients: carbonyls, lactate dehydrogenase, the MMP9 (MMP-9), the Ki67

and Cyclin D1 (CycD1). Whereas the 3 markers: 8-oxoguanine (OGG1), phosphorylated protein Src and serine protease inhibitor (Maspin) were elevated in healthy volunteers.<sup>32</sup>

## Conclusion

The use of saliva as a diagnostic tool could be very advantageous in detection and monitoring of many cancers. The number of studies showing a potential, in all stages of cancer care, is superior to the studies that have not demonstrated any interest. However, the analysis of salivary cancer biomarkers is influenced by several factors such as saliva collection protocol and the degree of stimulation of the salivary flow rate. Consequently interpretation of results is sometimes difficult. Moreover, there is a need to study collection methods of saliva and analysis method as well as encouraging more studies including more biomarkers could be very useful in screening and diagnosis of many cancer illnesses.

## Declaration of Interest

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