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REVIEW ARTICLE

SALIVA – A BIOMARKER FOR ORAL CANCER

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ABSTRACT

Oral cancer has emerged as an alarming public health problem with increasing incidence and mortality rates all over the world. The implementation of newer screening and early detection approaches are of utmost importance which could reduce the morbidity and mortality associated with this disease. Sensitive and specific biomarkers for oral cancer are likely to be most effective for screening, diagnosis, and staging and follow-up for this dreaded and lethal malignancy. Direct contact between saliva and the oral cancer lesion makes measurement of tumour markers in saliva a viable alternative to serum testing. Recent research has revealed that a large panel of human RNA, DNA and protein molecules derived from the malignant cells can be obtained from saliva. Thus, saliva being non invasive, accessible, and protective can be used as an efficient diagnostic medium.

INTRODUCTION

Saliva is a complex fluid rich in organic and inorganic constituents and plays a vital role in many necessary physiological functions in the oral cavity. A healthy adult individual produces about 500-1500ml of saliva per day with an average rate of about 0.5ml/min (Chicharro *et al.,* 1990). The main inorganic constituent of saliva which helps in salivary pH homeostasis are sodium, potassium, calcium and bicarbonates (Edgar *et al.,* 1996). The salivary pH could be defined as the negative logarithm of the hydrogen ions concentration. It plays a significant role in most chemical reactions occurring in the oral cavity, especially the equilibrium between the calcium phosphate of the tooth and the surrounding liquid phase. Tobaccos are used by millions of people worldwide. Apart from cigars and cigarettes, tobacco is also available in smokeless forms which are either chewed or placed in the vestibule. Arecanut is one such element of smokeless tobacco (Trivedy *et al.,* 2002). The use of tobacco products elicits various oral manifestations starting from simple attrition of teeth to malignant oral lesions and carcinomas. These pre-malignant lesions include leukoplakia, erythroplakia, lichen planus, oral submucous fibrosis etc. Arecanut contains four major alkaloids: arecoline, arecaidine, guvacoline and guvacine. A mixture of lime, tobacco and arecanut are commonly used. Arecanut can be chewed separately or placed in a betel leaf and chewed (Chu *et al.,* 2001). Nowadays, artificial flavour, saffron etc are added to the mixture of arecanut. In India mawa, kahaini and zardha are the other lesser products being used which are highly capable of initiating malignant lesions and hence early screening and diagnosis of the high risk group is of paramount importance in the management of oral cancer.

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Saliva can be a very potential biomarker that can be used in the diagnosis of oral cancer in vulnerable individuals (Chadda *et al.,* 2002).

SALIVARY BIOMARKERS

Saliva is not a passive "ultrafiltrate" of serum (Rehak, *et al.,* 2000), but contains a distinctive composition of enzymes, hormones, antibodies, and other molecules. In the past 10 years, the use of saliva as a diagnostic fluid has been successfully applied in diagnosis and for predicting populations at risk for a variety of conditions (Streckfus *et al.,* 2002). Diagnostic biomarkers in saliva have been identified for monitoring caries, periodontitis, oral cancer, salivary gland diseases, and systemic disorders, e.g., hepatitis and HIV (Lawrence, *et al.,* 2002). Human genetic alterations are detectable both intracellularly and extracellularly (Sidransky *et al.,* 1997). Nucleic acids have been identified in most bodily fluids, including blood, urine, and cerebrospinal fluid, and have been successfully adopted for use as diagnostic biomarkers for diseases (Anker *et al.,* 1999). Recent, investigators have become interested in detecting nucleic acid markers in saliva. To date, most of the DNA or RNA in saliva was found to be of viral or bacterial origin (Rieger-Christ, *et al.,* 2003). There are limited reports demonstrating tumour cell DNA heterogeneity in the saliva of oral cancer patients. The potential presence of mRNA in saliva may expand the domain of diagnostic assays for translational and clinical applications (Mercer *et al.,* 2001).

SALIVARY RNA

Although the presence of RNA in saliva was discovered a long time ago, researchers have not paid much attention to salivary RNA as a diagnostic tool until recently due to the absence of methods sensitive enough to work with saliva (Stramey *et al.,*

2003). Salivary RNA provides information about conditions in the oral cavity and expression of the analysed genes. Sampling is easy and cheap and is particularly useful for population screening. Diseases such as Sjögren′s syndrome or oral carcinoma are hard to detect at early stages. Therefore, it would be interesting to develop cheap and easy methods for large numbers of individuals (Wei *et al.,* 208). Salivary RNA is a promising tool due to the fact that it can provide information not only on the presence of genes, but also on their expression. The fact that saliva is a plasma ultrafiltrate indicates that it might also be used for diagnosing diseases not linked to the oral cavity.

Different people have varying concentrations and types of long RNA in their saliva. This is most likely due to the diversity of the microflora in saliva, and the fact that micro-organisms have diverse amounts and types of exonucleases and endonucleases. Therefore, the length of salivary RNA must be considered whenever working with it. The presence of partially degraded RNA suggests that endonucleases activity is more significant (Park *et al.,* 2007). Salivary RNA is a free nucleic acid and its stability is therefore quite surprising. Stabilising mechanisms have been studied and are summarised later in this review. It is supposed that the majority of salivary RNA is from damaged or dead cells. Nevertheless, studying RNA in cell-free saliva can provide valuable information about the studied individual and their health status (Wong *et al.,* 2008). cDNA libraries of salivary RNA from 10 people were created and compared with sequences in the human genome. Each healthy person was found to have a set of salivary RNA labelled the Normal salivary core transcriptome. Identification of such a set eased further work, analyses and utilisation of salivary RNA in diagnosis (Lia *et al.,* 2000). As other projects, like sequencing of the human microbiome, emerged, knowledge about salivary RNA was indirectly increased.

SOURCES OF SALIVARY RNA

Construction of cDNA libraries and their comparison with human genome sequences suggested the following. Saliva is excreted from three major glands and a number of minor ones. Acinary cells are one of the sources of salivary RNA. One of the sources of salivary RNA is gingival crevicular fluid (GCF), through which blood cells reach the oral cavity. Other sources are cellular shedding from the oral epithelium. RNA can also reach the saliva with blood from wounds in the oral cavity (Park *et al.,* 2006). Saliva is a plasma ultrafiltrate, creating a unique environment in the oral cavity. The fact that one of the sources of saliva salivary RNA is the oral epithelial cells might be used when studying oral carcinomas. Overall, saliva contains trace amount of RNA from the blood, RNA from the micro-organisms creating a biofilm in the oral cavity, RNA from epithelial cell and miRNA enclosed in vesicles (El nagger, *et al.,* 2001).

ISOLATION OF SALIVARY RNA

Salivary RNA is mainly isolated from supernatant due to the fact that cell-free samples are demanded for most of the analyses. The amount of salivary supernatant used for isolation of salivary RNA varies from 200 to 560 µl in the reviewed studies. Salivary RNA can be isolated using the RNeasy® Mini Kit (Kumar *et al.,* 2006).

RNA coexists with ribonucleases in the saliva. Despite this, salivary RNA is relatively stable. Salivary ribonucleases are still active after sampling and therefore need to be inhibited before processing the samples. This can be achieved by adding RNAase or commercially available stabilisation solutions. The best stabilisation reagent is RNAprotect® Saliva Reagent included in RNeasy® Protect Saliva Mini Kit (Qiagen). This was determined by comparing the Ct values of RNA samples treated with stabilisation solutions and stored for 10 weeks at room temperature (Hu *et al.,* 2008). The isolate of RNA needs to be treated with DNAases and RNAases, and properly stored before amplifying the RNA.

STABILITY OF SALIVARY RNA

The major problem with the isolation and working with RNA is its instability. The samples need to be kept at a specific temperature, and the characteristics of the salivary RNA have to be taken into account when evaluating the results obtained. Human RNA is not the only RNA occurring in saliva as microbial RNA is also present. Endonucleases and exonucleases of micro-organisms in the oral cavity affect the length and stability of salivary RNA. Saliva contains RNA from viruses, but its stability has not been well examined (Park *et al.,* 2006). On the other hand, many proteins in eukaryotes bind to RNA, directly or indirectly stabilising it. More than 30% of the mRNA found in human saliva contain areas rich in adenine and uridine (increasing the stability of RNA), which is five times more than that found in other mRNAs in the human organism (Palaniswamy *et al.,* 2008). Areas rich in A and U at the 3′ end of mRNA stabilise the mRNA before translation. Due to all of the listed mechanisms, the average salivary mRNA retains 42% of its original length (Khabar *et al.,* 2005). The methods used for characterising salivary RNA are mainly RT-PCR and microarrays.

PROFILING OF mRNAs IN SALIVA

It is necessary to inhibit RNAases for the treatment of saliva in vitro (Fabain *et al.,* 2008). Park *et al* performed RT-qPCR after various time periods of saliva storage to find an optimal room temperature stabilising reagent for the salivary transcriptome. Saliva stored at 4°C increased the stability of salivary RNA. After testing three stabilising reagents, RNAprotect® Saliva Reagent (RPS) proved to be the most appropriate after the incubation and comparison of Ct values obtained from the RT-PCR reactions. This reagent stabilised the sample at room temperature and enabled its transport under non-specific conditions. This finding led to the use of this kit in an extended screening of a population (Park *et al.,* 2006).

MOLECULAR COMPONENTS OF SALIVA

Some of the molecular components of saliva are used for diagnosis and screening, and the procedures are already available in kits or diagnostic reagents .The molecular composition of saliva reflects the levels of therapeutically, hormonally, immunologically or toxicologically interesting molecules. While proteins are the logical choice for salivary diagnostics, nucleic acids have emerged as highly discriminatory markers as well. The trend in studying molecular components of saliva was offset by a development of methods such as whole genome microarrays (Zimmermann *et*

al., 2007). Saliva contains DNA, RNA and proteins of human, bacterial and viral origin. Obtaining information about these components and their comparison and systematization could be used in future diagnoses of some diseases. Salivary transcriptome and proteome could be a source of relevant information about genes and their activity and the presence of micro-organisms in the oral cavity (Wong *et al.,* 2008).

TRANSCRIPTOME

Salivary supernatants from healthy people were seen to contain 185 types of transcripts, which were found in each individual. Microarray analysis showed that the transcripts of β-actin, RPS9, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), IL8 and spermidine/spermine N1-acetyltransferase were present at high concentrations in saliva (Li *et al.,* 2004). Data obtained from the transcriptome analysis using the SOLiD™ system and compared with the Human Oral Microbiome database revealed that more than 400 different microbial species occurred within a single sample of sputum (Spielmann *et al.,* 2012). These experiments are important for the examination of the oral transcriptome because the knowledge resulting from them will be used to overcome the problems involved with working with salivary RNA. They also proved that the salivary transcriptome was a complex network of all of these transcripts and could provide information about either the oral microflora (important in diseases such as caries or periodontitis) or the health status of the individual. This brings us closer to the utilisation of salivary RNA markers in the diagnosis of diseases occurring in the oral cavity or elsewhere.

It is supposed that exogenous miRNA degrades faster than endogenous one (Park N.J, *et al.,* 2009). It was shown that miRNA in saliva do not circulate freely, but are enclosed in vesicles (exosomes). In addition to the methods mentioned above, several experiments were conducted to characterise salivary RNA. The first work employed a microarray to identify salivary transcripts (Gallo A, *et al.,* 2012). The next investigation characterised salivary RNA using microarrays to study the relationship between RNA in saliva and gene expression. Conducting experiments using microarrays and salivary RNA is important in exploring the NSCT and obtaining an overall hint about the transcripts occurring in saliva in physiological conditions. Microarrays are an accurate method as they provide information about many transcripts in one performed working cycle.

ADVANTAGES AND DISADVANTAGES OF WORKING WITH SALIVARY RNA

The collection of saliva is easier than that of blood and does not require trained personnel. On the other hand, the contents of saliva may not be stable, which may limit the usefulness. As well as with the other compounds in saliva, one of the major issues with using salivary RNA for diagnostics is the stability (Park *et al.,* 2006). Even though saliva contains compounds present in blood, these compounds are not present abundantly enough in saliva. Therefore, the method commonly used with blood cannot be applied without optimising it for saliva. Methods with higher detection sensitivity need to be developed for accurate results when working with salivary compound, such as RNA (Spielmann *et al.,* 2012).

It is crucial that the human transcriptome found in saliva is described and examined thoroughly. Saliva contains microbial RNA that needs to be distinguished from that of human origin. It may also contain traces of food, some of which might interfere with the result from sample analysis. It is important to consider other facts that could affect the transcripts present in saliva. One of them might be the transcripts originating from other individuals.

DIAGNOSTIC USES OF SALIVARY RNA

Most compounds occurring in blood are present in the saliva as well. Saliva is a part of the environment in the oral cavity; therefore, it is accurate for oral carcinoma detection. It is possible to detect oral squamous cell carcinoma using salivary transcripts. Oral squamous cell carcinoma is the most common carcinoma occurring in the oral cavity (Brinkman *et al.,* 2006). The RNA biomarkers of this disease are those of IL8, IL1B, DUSP1, HA3, OAZ1, S100P and SAT. Tests combining all of these markers proved to have 91% specificity and 91% sensitivity. Salivary RNA is stable and complex enough to be used as a biomarker of oral carcinoma and other diseases (Park *et al.,* 2006). It is essential to use more than one marker in screening or diagnosis to select false positive and false negative results. The different levels of the salivary microRNAs miR-200a and miR-125a in healthy and squamous cell carcinoma patients proved that miRNA from saliva can be used as a marker in oncology (Park *et al.,* 2009).

CONCLUSION

The ability to control oral and oro-pharyngeal cancer will depend on two cornerstones: prevention and early diagnosis. Saliva meets the demand for non-invasive, accessible, and highly efficient diagnostic medium. Hence can be used as a biomarker in the early detection of oral cancer.

REFERENCES

Anker, P., Mulcahy, H., Chen, X.Q. and Stroun, M. 1999. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. *Cancer and Metastasis Reviews*, *18*(1), pp.65-73.

Brinkman, B.M. and Wong, D.T. 2006. Disease mechanism and biomarkers of oral squamous cell carcinoma. *Current opinion in oncology*, *18*(3), pp.228-233

Chadda, R.K. and Sengupta, S.N. 2002. Tobacco use by Indian adolescents. *Tob Induc Dis*, *1*(2), pp.111-119.

Chicharro, J.L., Lucía, A., Pérez, M., Vaquero, A.F. and Ureña, R. 1998. Saliva composition and exercise. *Sports medicine*, *26*(1), pp.17-27.

Chu, N.S. 2001. Effects of betel chewing on the central and autonomic nervous systems. *Journal of biomedical science*, *8*(3), pp.229-236.

Edgar, W.M., O'Mullane, D.M. and Dawes, C. eds. 1996. *Saliva and oral health* (pp. 81-94). London: British Dental Association.

El-Naggar, A.K., Mao, L., Staerkel, G., Coombes, M.M., Tucker, S.L., Luna, M.A., Clayman, G.L., Lippman, S. and Goepfert, H. 2001. Genetic heterogeneity in saliva from patients with oral squamous carcinomas: implications in molecular diagnosis and screening. *The Journal of Molecular Diagnostics*, *3*(4), pp.164-170

Fabian, T.K., Fejerdy, P. and Csermely, P. 2008. Salivary genomics, transcriptomics and proteomics: the emerging concept of the oral ecosystem and their use in the early diagnosis of cancer and other diseases. *Current genomics*, *9*(1), pp.11-21.

Gallo, A., Tandon, M., Alevizos, I. and Illei, G.G. 2012. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PloS one*, *7*(3), p.e30679.

Hu, Z., Zimmermann, B.G., Zhou, H., Wang, J., Henson, B.S., Yu, W., Elashoff, D., Krupp, G. and Wong, D.T. 2008. Exon-level expression profiling: a comprehensive transcriptome analysis of oral fluids. *Clinical chemistry*, *54*(5), pp.824-832.

Khabar, K.S. 2005. The AU-rich transcriptome: more than interferons and cytokines, and its role in disease. *Journal of interferon & cytokine research*, *25*(1), pp.1-10

Kumar, S.V., Hurteau, G.J. and Spivack, S.D. 2006. Validity of messenger RNA expression analyses of human saliva. *Clinical Cancer Research*, *12*(17), pp.5033-5039.

Lawrence, H.P. 2002. Salivary markers of systemic disease: non-invasive diagnosis of disease and monitoring of general health. *Journal-Canadian Dental Association*, *68*(3), pp.170-175.

Li, Y., John, M.A.S., Zhou, X., Kim, Y., Sinha, U., Jordan, R.C., Eisele, D., Abemayor, E., Elashoff, D., Park, N.H. and Wong, D.T., 2004. Salivary transcriptome diagnostics for oral cancer detection. *Clinical Cancer Research*, *10*(24), pp.8442-8450.

Li, Y., Zhou, X., John, M.S. and Wong, D.T.W. 2004. RNA profiling of cell-free saliva using microarray technology. *Journal of dental research*, *83*(3), pp.199-203.

Liao, P.H., Chang, Y.C., Huang, M.F., Tai, K.W. and Chou, M.Y. 2000. Mutation of p53 gene codon 63 in saliva as a molecular marker for oral squamous cell carcinomas. *Oral oncology*, *36*(3), pp.272-276.

McMaster-Kaye, R. and Taylor, J.H. 1958. Evidence for two metabolically distinct types of ribonucleic acid in chromatin and nucleoli. *The Journal of biophysical and biochemical cytology*, *4*(1), pp.5-11.

Mercer, D.K., Scott, K.P., Melville, C.M., Glover, L.A. and Flint, H.J. 2001. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. *FEMS Microbiology Letters*, *200*(2), pp.163-167.

Palanisamy, V., Park, N.J., Wang, J. and Wong, D.T. 2008. AUF1 and HuR proteins stabilize interleukin-8 mRNA in human saliva. *Journal of dental research*, *87*(8), pp.772-776.

Park, N.J., Li, Y., Yu, T., Brinkman, B.M. and Wong, D.T. 2006. Characterization of RNA in saliva. *Clinical chemistry*, *52*(6), pp.988-994.

Park, N.J., Yu, T., Nabili, V., Brinkman, B.M., Henry, S., Wang, J. and Wong, D.T. 2006. RNAprotect saliva: An optimal room-temperature stabilization reagent for the salivary transcriptome. *Clinical chemistry*, *52*(12), pp.2303-2304.

Park, N.J., Yu, T., Nabili, V., Brinkman, B.M., Henry, S., Wang, J. and Wong, D.T. 2006. RNAprotect saliva: An optimal room-temperature stabilization reagent for the salivary transcriptome. *Clinical chemistry*, *52*(12), pp.2303-2304.

Park, N.J., Zhou, H., Elashoff, D., Henson, B.S., Kastratovic, D.A., Abemayor, E. and Wong, D.T. 2009. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. *Clinical Cancer Research*, *15*(17), pp.5473-5477.

Park, N.J., Zhou, X., Yu, T., Brinkman, B.M., Zimmermann, B.G., Palanisamy, V. and Wong, D.T. 2007. Characterization of salivary RNA by cDNA library analysis. *Archives of oral biology*, *52*(1), pp.30-35.

Rehak, N.N., Cecco, S.A. and Csako, G. 2000. Biochemical composition and electrolyte balance of" unstimulated" whole human saliva. *Clinical chemistry and laboratory medicine*, *38*(4), pp.335-343.

Rieger-Christ, K.M., Medina, A. and Lee, P.J. 2003. Rieger-Christ KM, Mourtzinos A, Lee PJ, *et al*. Identification of fibroblast growth factor receptor 3 mutations in urine sediment DNA samples complements cytology in bladder tumor detection. Cancer.(2003) 98 (4): 737–44. *Cancer*, *98*(9), pp.2000-2000

Sidransky, D. 1997. Nucleic acid-based methods for the detection of cancer. *Science*, *278*(5340), pp.1054-1058.

Spielmann, N., Ilsley, D., Gu, J., Lea, K., Brockman, J., Heater, S., Setterquist, R. and Wong, D.T. 2012. The human salivary RNA transcriptome revealed by massively parallel sequencing. *Clinical chemistry*, *58*(9), pp.1314-1321.

Stamey, F.R., DeLeon-Carnes, M., Patel, M.M., Pellett, P.E. and Dollard, S.C. 2003. Comparison of a microtiter plate system to Southern blot for detection of human herpesvirus 8 DNA amplified from blood and saliva. *Journal of virological methods*, *108*(2), pp.189-193.

Streckfus, C.F. and Bigler, L.R. 2002. Saliva as a diagnostic fluid. *Oral diseases*, *8*(2), pp.69-76.

Trivedy, C.R., Craig, G. and Warnakulasuriya, S., 2002. The oral health consequences of chewing areca nut. *Addiction biology*, *7*(1), pp.115-125.

Wei, F., Wang, J., Liao, W., Zimmermann, B.G., Wong, D.T. and Ho, C.M. 2008. Electrochemical detection of low-copy number salivary RNA based on specific signal amplification with a hairpin probe. *Nucleic acids research*, *36*(11), pp.e65-e65.

Wong, D.T. 2008. Salivary Diagnostics Amazing as it might seem, doctors can detect and monitor diseases using molecules found in a sample of spit. *Am Sci*, *96*(1), pp.37-43.

Zimmermann, B.G., Park, N.J. and Wong, D.T. 2007. Genomic targets in saliva. *Annals of the New York Academy of Sciences*, *1098*(1), pp.184-191.

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