IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 1 Ser.3 (January. 2024), PP 01-05
www.iosrjournals.org

Salivomics And Pilot Study On Salivary Thiocyanate

Dr. Moharil Rohit
Assist Prof., Dept.Of Oral & Max. Pathology RDDCRC, (VSPM)Nagpur

Dr. Dive Alka
Professor & Head, Dept.Of Oral & Max. Pathology RDDC RC(VSPM) Nagpur

Dr Shubhangi Khandekar
Professor, Dept.Of Oral & Max. Pathology RDDC RC(VSPM), Nagpur

Abstract
Saliva plays a vital role in the integrity of oral health. Saliva has great potential as a diagnostic fluid and offers advantage over serum and other biological fluids by an economic and noninvasive collection method for monitoring of systemic health and disease progression.
The term "Salivaomics" reflects the rapid development ofknowledge about saliva's various constituentslike Proteome, transcriptome, miRNA,metabolome and microbiome etc.These components in saliva can act as biomarkers for diagnosis of various systemic and local diseases by the use of emerging biotechnologies. This article discusses on importance of saliva as a useful biomarker and also the result of pilot study of salivary Thiocyanate in tobacco chewers and smokers. We found increase in salivary thiocyanate levels almost in all other groups (8/23 Cases) as compare to non smokers and non chewers group (3/15).This concludes that salivary components can act as a early markers in diagnosis of oral lesions.
Key Words:*Saliva, Biomarker, Salivary Thiocynate, Oral diseases*

Date of Submission: 01-01-2024Date of Acceptance: 09-01-2024

I. Introduction

Saliva is a mixtureof the secretions of the major and minor salivary glands.Saliva is approximately 99% water and 1% protein andsalts. It is a complex fluid containinghormones, proteins, enzymes, antibodies,antimicrobial constituents, exfoliated cells, inflammatory markers, microorganism and cytokines as part of normal flora**.**(1, 2)

Saliva can be easily collected and stored by noninvasive method, greater sensitivity, as compare to serum and ideal for early detection of disease as it contains specific soluble biological markers.(1, 2, 3)

Saliva has been considered as a diagnostic tool for enormous conditions. As Saliva remains directly in contact with the premalignant lesions and oral carcinomas, which makes easy detection of DNA, RNA and proteins released by these lesions. It is also useful in the early diagnosis, prognostics, and post-treatment monitoring of various diseases. (3,4)

Advantages of saliva as a diagnostic tool (5)
-Non-invasive
-Easy to store & transport
-Cost-effective
-Samples of saliva can be collected multiple times
-Useful in challenging situations where blood sampling could be difficult to perform.

Saliva collection methods

There are various methods to collect the whole saliva such as draining, spitting, and absorbent methods. Shannon (2008) concluded that it is ideal to collect saliva, while the subject is sitting upright with the head slightly tilted forward and the eyes open.

Draining method /spitting method: The patient is asked to accumulate saliva in the mouth and then expectorate into a pre-weighed test tube.

Absorbent method: Gauze is placed in the patient mouth for a predetermined amount of time and this gauze is preweighed, following which the gauze is weighed again, and the volume of saliva is determined. (4,5)

Characteristics of an ideal biomarker (4,5)
-Should be Easy and inexpensive to measure in body fluids.
- Should predict the recurrence of the disease, before they are clinically detection.

Normal Composition and properties of Human Saliva (6,7)
(Fig: 1)

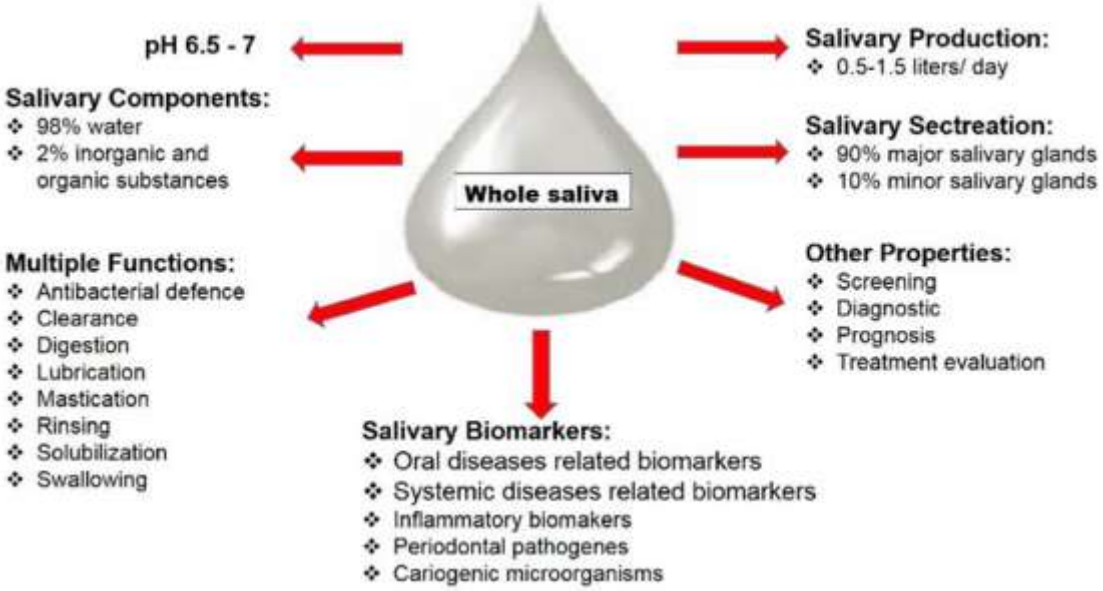

Fig :1- Courtesy: Metabolites 2022, 12, 638. (https://doi.org/10.3390/metabo12070638)

SALIVA AS A BIOMARKER

1. Salivary diagnostics in oral diseases (2,7,8,9)

Saliva is a watery product of salivary glands that maintains healthy oral mucosa by lubricating, protecting, moistening, ease of swallowing and also limiting the growth of pathogenic bacteria. Saliva also provides a number of enzymes that initiate food digestion, there by starting carbohydrate and fat metabolism

Many of the salivary or Gingival cervicular fluid (GCF) derived molecules are used as diagnostic biomarkers for oral diseases including oral cancer, and conditions caused by fungi ,viruses such as HPV, EBV, Cytomegalovirus [CMV]) andbacteria.

Table 1 – Salivary Biomarkers (1,2)

Sr.No	Biomarker	Clinical application
1	Streptococcus mutans and lactobacilli count, aspartate aminotransferase, alkaline phosphatase, uric acid, albumin, , CA VI, IL-1Ra, PLS-2,	Dental caries and periodontal diseases
2	Lactoferrin, beta-2 microglobulin, lysozyme C, cystatin C, Albumin serum, actin, alpha-actin-1, Ig gamma-1 chain C region, B2-microglobulin, Ig receptor polymeric salivary amylase, carbonic anhydrase, polymeric Ig receptor, prolactin-inducible protein, cystatin SN	Autoimmune diseases • Sjogren's syndrome

2 .Salivary proteases as biomarkers for premalignant and malignant oral lesions.

Oral squamous cell carcinoma (OSCC) accounts for more than 90 percent of oral cancers worldwide. Its five-year survival rate is below 50 percent .Its survival rates increase significantly when it is detected and treated early. Unfortunately, clinicians lack tests which easily and reliably distinguish pre-malignant oral lesions from those already transitioned to malignancy.

Salivary biomarkers can be useful for the diagnosis, monitoring, and prognosis of various oral diseases such as oral leukoplakia, Oral Submucous Fibrosis, oral lichen planus, Sjögren's syndrome, Pemphigus, and Pemphigoid also to assess their malignant transformation potential.(1,2,5)

Table 2-Biomarker of Oral Leukoplakia (1.2,5)

Sr.No	Biomarker	Clinical application
1	TNF- α TGFβ	To detect malignant transformation of leukoplakia into OSCC
2	IL6 IL8	**Prognosis**

Table 3-Biomarkers for Oral Submucous Fibrosis (1.2,5)

Sr.No	Biomarker	Clinical application
1	IgA	To detect OSMF transforming to oral carcinoma.
2	IgG	Diagnosis

Table 4-Biomarkers for Oral lichen planus (1.,5)

Sr.No	Biomarker	Clinical application
1	**Cortisol**	**Diagnosis**
2	**Nitric Oxide, IL6**	**Prognosis**
3	**IL1α** **IL1β** **IL8**	**Immune modulators**

3. Salivary biomarkers for oral cancer detection

Early detection is the key to good prognosis in almost all types of cancer. Saliva has been used as a diagnostic medium for oral squamous cell carcinoma (OSCC),and salivary analytes such as proteins,mRNA, andDNA havebeen used in their diagnosis. (1.2,5)

Table 5-Potential salivary biomarkers for oral cancer detection (1,2,5,10)

Sr.No	Biomarker	Molecules affected
1	P53 gene codon 63	DNA
2	IL8	mRNA
3	IL-1 IL-6 IL-1β TNF-α Thiocyanate ,amylase Carcinoembryonic antigen (CEA) cortisol, lactate dehydrogenase, , and salivary adenosine deaminase, glycoprotein etc	Proteins
4	Reactive nitrogen species – nitric oxide (NO), nitrites (NO2) Glutathione S-transferase 8-hydroxy-2-deoxyguanosine	Oxidative stress-related molecules

Table 6-Salivary biomarkers in systemic conditions (5,11,12)

Sr.No	Biomarker	Clinical application
1	C-reactive protein Myeloperoxidase Tumor necrosis factor α Matrix metalloproteinase–9 Lysozyme	Cardiovascular diseases
2	Cortisol Nitrite Alpha-amylase Lactoferrin	Renal condition
3	Glucose	Diabetes mellitus
4	Cortisol Proinflammatory cytokines (IL-6, IL-1b, TNF- α) Catecholamines (dopamine, norepinephrine, and epinephrine)	Psychological Stress
5	Measles virus-specific IgM HIV – HIV-1, HIV-2 – antibodies, salivary	Infections

	proteins, MUC 5B, and MUC 7 *Candidiasis* immunoglobulins, , histatins, mucins	• Viral infections • Bacterial infections • Fungal infections

Advantages of Saliva As A Diagnostic Fluid (4,5,7)

1. Noninvasive diagnostic tool.
2. Painless, less discomfort to patient in the collection process.
4. Relatively cheap method as compared to other tests.
5. Easy for screening of large population.
6. Where blood sampling is a problem e.g. children, anxious, handicap,elderly patients, salivary diagnostic methods can be used to study this special population
7. Convenient for multisampling.
8. Compared to blood and urine, saliva is also cheaper to store and transport.

Limitations (7)

1. Levels of certain markers in saliva are not always a same as levels of these markers in serum. Hence reliability is not predicted accurately.
2. Method of collection and degree of stimulation of salivary flow may influenceSalivary composition.
3. Changes in salivary flow rate may affect the concentration of salivary markers.
4. Variability in salivary flow rate is expected between individuals and in the same individual under different conditions.
5. Some systemic disorders, medications and radiation may affect salivary gland function, quantity and composition of saliva.

Short report of Pilot Study:

One of the biomarkers is salivary thiocyanate which is found in the saliva.

Thiocyanate (SCN) is a normal constituent of body fluids such as serum, saliva, urine, and tears. Diet and tobacco are its main sources in the human body. It has a long half life of 10-14 days in normal adults and is in continuous contact with oral epithelium through saliva. The long biologic half-life of thiocyanate makes it an ideal substrate to determine habitual smoking behaviour (13,14)

Salivary SCN concentration in normal nonsmokers ranges from 0.5 to 2 mM with an average of 1 mM. However, heavy smokers may have a salivary concentration range as high as 6 mM.

Hence, Thiocyanate (SCN) is an important biomarker in the saliva to know about the SCN being liberated in different forms of tobacco consumed by humans. (14,15,16)

Hence pilot study was carried out to Evaluate and Correlate Salivary Thiocyanate Levels in Tobacco Smokers and Tobacco Chewers.

II. Methodology:

Participants were divided in to different groups as follows. (17,18)

- **Group I - Individuals habituated with Tobacco smoking**
- **Group II - Individuals habituated with tobacco chewing**
- **Group III - Individuals habituated with both Tobacco smoking and Tobacco chewing**
- **Group IV - Individuals without any habits**

Participants were asked to rinse their mouth gently with water, and 2 ml of unstimulated saliva was collected by spitting method in clean sterile container. Saliva was then transferred into test tubes and used for thiocyanate analysis.Each sample was analyzed on the same day of collection by spectrophotometric method. Saliva samples were centrifuged at 3000 rpm for 10 min and clear supernatant solution was collected. Then, the solutions were measured at 447 nm wavelength using ultraviolet spectrophotometer for estimation of salivary thiocyanate. (6,7)

Result of Pilot Study:

We found increase in salivary thiocyanate levels almost in all other groups (8/23 Cases) as compare to non smokers and non chewers group (3/15).

Table: 7- Result of pilot study on salivary Thiocyanate biomarker.

Study Parameters	Smokers Group I cases 8	Tobacco chewers Group II Cases- 10	Smokers & Tobacco chewers Group III Cases- 5	Control (Non smokers & Non Chewers) Group IV Cases- 15
Salivary Thiocyanate	3 	2 	3 	3 

III. Conclusion

The components of saliva act as amirror of the body's health, and its widespread use and acceptability as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public.We also got positive correlation of salivary thiocyanate marker in tobacco chewers and smokers as compared to non smokers and non chewers in our pilot study to test the salivary markers efficacy

With the development of novel, sensitive detection technology platforms, and the innovation, will make saliva diagnostic a reality will be one of the important componenent of diagnosis and treatment plan.

References:

[1]. Malathi N,Mythili S, And Vasanthi Hr. Salivary Diagnostics: A Brief Review Hindawi Publishing Corporation Isrn Dentistry Volume 2014, , 1-8 Pages.

[2]. Narang D, Kaur M, Shishodiya S, Khan F. Saliva As A Medium: A New Tool In Diagnosis. Int J Res Health Allied Sci 2018; 4(3):56-58.

[3]. Ranganath Lm, Shet Rgk, Rajesh Ag. Saliva: A Powerful Diagnostic Tool For Minimal Intervention Dentistry. J Contemp Dent Pract 2012;13(2):240-245.

[4]. Mittal S, Bansal V, Garg S, Atreja G, Bansal S. The Diagnostic Role Of Saliva — A Review. J Clin Exp Dent. 2011;3(4):E314-20.

[5]. Saraswathi K. Gopal Et Al. Salivary Biomarkers – A Modern Approach To Diagnosis. J. Pharm. Sci. & Res. Vol. 13(11), 2021, 677-680

[6]. Papale, F.; Santonocito, S.; Polizzi, A.; Giudice, A.L.; Capodiferro, S.; Favia, G.; Isola, G. The New Era Of Salivaomics In Dentistry: Frontiers And Facts In The Early Diagnosis And Prevention Of Oral Diseases And Cancer. Metabolites 2022, 12, 638.

[7]. Madalli V, Shrinivas M Basavaraddi,Burde K, Horatti P. Saliva- A Diagnostic Tool Iosr Journal Of Dental And Medical Sciences (Iosr-Jdms) E-Issn: 2279-0853, P-Issn: 2279-0861. Volume 11, Issue 6 Nov.- Dec. 2013

[8]. Shantala Arunkumar S, Arunkumar. J S, Krishna N Burd K, Shakunthala G K. Developments In Diagnostic Applications Of Saliva In Oral And Systemic Diseases- A Comprehensive Review. Journal Of Scientific And Innovative Research 2014; 3(3): 372-387

[9]. Farnaud Sj, Kosti O, Getting Sj, Et Al. Saliva: Physiology And Diagnostic Potential In Health And Disease. Scientificworldjournal 2010;10:434–456.

[10]. T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner, And C. Punyadeera, "Diagnostic Potential Of Saliva: Current State And Future Applications," Clinical Chemistry, Vol. 57,No. 5, Pp.675–687, 2011.

[11]. Y.-H. Lee Andd. T.Wong, "Saliva: Anemergingbiofluidfor Early Detection Of Diseases," American Journal Ofdentistry, Vol. 22,No. 4, Pp. 241–248, 2009

[12]. Herenia P, Lawrence Dm. Salivary Markers Of Systemic Disease: Noninvasive Diagnosis Of Disease And Monitoring Of General Health, March 2002;68(3). Retrieved, Feb 29, 2008, From Journal De L" Association Dentaire Canadienne: Http://Www.Cda-Adc.Ca/ Jadc/Vol-68/Issue-3/170.Pdf

[13]. Baral R, Ojha B, Bajracharya D, Silwal G. Correlation Of Salivary Thiocyanate Levels And Oral Cytological Changes In Smokers. Jcms Nepal. 2020; 16(4):259-266

[14]. Prakruthi Bv, Nandini Db, Donoghue M, Praveen Sb, Kumar Kp, Ashwini R. Effects Of Salivary Thiocyanate Levels On Oral Mucosa In Young Adult Smokers: A Biochemical And Cytological Study. J Oral Maxillofac Pathol 2018; 22:204-9

[15]. Baldawa Ps, Kulkarni Vv, Koshy Av, Shaikh Ss, Varu R, Srivastava G. Levels Of Salivary Thiocyanate And Its Relation With Occurrence Of Micronuclei Using Exfoliative Cytology In Smokers And Nonsmokers. Indian J Dent Res 2016;27:568-73

[16]. Jurel Sk, Gupta Ds, Singh Rd, Singh M, Srivastava S. Genes And Oral Cancer. Indian J Hum Genet 2014;20:4‑9.

[17]. Sivakumar Kokila Et.Al Evaluation Of Micronuclei And Cytomorphometric Changes In Patients With Different Tobacco Related Habits Using Exfoliated Buccal Cells Asian Pac J Cancer Prev,2021 : 22 (6), 1851-1855.

[18]. Upadhyay M, Verma P, Sabharwal R, Subudhi Sk, Jatol-Tekade S, Naphade V, Et Al. Micronuclei In Exfoliatedcells: A Biomarker Of Genotoxicity In Tobacco Users. Niger J Surg 2019; 25:52-59.