Bob Barrett: This is the podcast from *'Clinical Chemistry'*. I am Bob Barrett. Saliva is a unique fluid that contains a spectrum of proteins and peptides, nucleic acids, electrolytes, and hormones. As a result, interest in saliva as a diagnostic medium has increased over the past decade.

However, the diagnostic potential has been hindered by our lack of understanding of the mechanisms by which biomolecules are released into oral fluid and poorly characterized reference values.

In addition, the diurnal and circadian variations in saliva do not always reliably match those in serum. Salivary composition can also be influenced by the method of collection and the degree of stimulation of salivary flow. Finally, there are analytical challenges as analytes found in saliva may appear at concentrations a thousand fold less than those in blood.

In a review published in the May issue of *'Clinical Chemistry'*, Dr. Chamindie Punyadeera, a Fellow at the Tissue Engineering and Microfluidics Laboratory at the Australian Institute for Bioengineering and Nanotechnology at the University of Queensland, explored the diagnostic potential of saliva with regard to diseases such as cardiovascular disease and oral cancer. Dr. Punyadeera is our guest in this podcast.

Dr., why is it important to explore human saliva as a possible diagnostic biological fluid?

Dr. Chamindie Punyadeera: Human saliva is just not the water in our mouth, but human saliva mirrors our body's health and wellbeing. Biomolecules that are circulating in blood are also found in human saliva.

Human saliva protium consists of approximately about 2,000 proteins, and most importantly, 26% of these proteins are also found in blood. This therefore emphasizes saliva's importance as another biological source for disease diagnosis and monitoring, as well as an ideal diagnostic medium to determine a person's response to treatment.

In comparison to blood, saliva has the following advantages. Saliva collection is easy and cost-effective, and even a layperson with minimal training can perform this task, which means that

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you and I can collect and process saliva for disease detection.

Unlike blood, where either centrifugation or filtrations are applied to remove blood cells to obtain either serum or plasma, saliva does not require preprocessing, which means that saliva is an ideal diagnostic fluid for point-of-care test devices.

In addition, saliva sample is noninvasive, and people who have a phobia for needles, like myself, can easily utilize saliva to obtain necessary tests.

As an example, if one wishes to determine adrenaline and know adrenaline levels in people who have a phobia for needles, and these hormones are elevated during fear and anxiety, a simple saliva test would yield true hormone levels as opposed to elevated blood levels that are measured due to anxiety for needles.

In addition, unlike blood test, there is a minimal and no risk of contracting infection agents such as HIV, Hepatitis B and C, and ideal for third world countries and for remote site testing. These key advantages make saliva as a good diagnostic medium for disease detection and monitoring.

Bob Barrett: So what are the mechanisms by which biomolecules are transported from blood into saliva?

Dr. Chamindie Punyadeera: There are three types of major salivary glands, namely parotid gland, submandibular glands, and sublingual glands. In addition, minor salivary glands are located throughout the oral cavity.

Most of the organic compounds in saliva are produced locally in the salivary glands, and these include mucins, which are high molecular weight proteins and high abundant proteins in saliva. And in addition, proline-rich peptides, histatins, cystatins are also produced in the salivary glands. Most importantly, an enzyme, alpha amylase, that aids in carbohydrate digestion, is also secreted from salivary glands.

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Several pathways, both intra and extracellular, enable molecules to be transported from blood

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capillaries into saliva. Molecules that are hydrophilic and small, such as steroid hormones, enter saliva from the blood capillaries through passive diffusion.

Molecules such as soluble immunoglobulin E enter into saliva as ligand receptor binding and the process is known as active transport.

Molecules such as sulfated steroids that are hydrophobic enter into saliva through a process known as ultrafiltration, which means these molecules get into saliva through the gap junctions on the plasma membrane.

Bob Barrett: What other types of molecules or diagnostics could be performed using human saliva?

Dr. Chamindie Punyadeera: If you look at the saliva diagnostic about ten years ago, saliva was mainly utilized by researches in the dental field to diagnose periodontal disease as well as to determine the severity of dental carriers. But now research has expanded and grown into other clinical applications such as detection of breast cancer and prostate cancer, as well as detection of heart diseases.

In particular, for early detection of oral cancellations, researchers are using both protein marker panels as well as gene expression profiles.

Gene expression profiles especially can be used to predict the development of cancer in people who are at the risk of developing cancer.

Research in my group focused on identifying and characterizing gene protein targets for early detection of oral and head and neck cancers with the utilization of saliva.

In addition, we are also investigating and validating biomarkers for early detection of heart diseases, such as heart attack or acute myocardial infarction or heart failure.

Human saliva can be used and applied to many diseases that are common to mankind and I believe that saliva diagnostics will one day revolutionize the current clinical practice.

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Bob Barrett: Well, this all sounds very positive, but is there a downside to using such specimens? What are the limitations of saliva diagnostics?

Dr. Chamindie Punyadeera: Like any other laboratory test, saliva has its own limitations.

Firstly, one of the main roadblocks hindering the progress in this filed lies on the limitation within current detection technologies.

To elaborate upon this point further, biomolecules are present in saliva at 100-1,000 fold lower as compared to blood. With the advent of improved detection technologies, we will be able to overcome this problem.

Secondly, biomolecules present in saliva display circadian rhythms and this may have an impact on kinetics and dynamics of these molecules in saliva.

As an example, for diagnosing Cushing's syndrome, doctors use salivary cortisol levels, but the salivary cortisol levels are low in the morning, and if one is to use a live saliva diagnostic for this purpose, a morning saliva sample would not be ideal.

Thirdly, before saliva diagnostic become a reality within the current clinical workflow, especially for cardiovascular disease detection and cancer screening, salivary biomarker discovery needs a greater attention with regard to development, validation, especially in relation to which biomarker or biomarker panels correlate with disease onset and progression.

Bob Barrett: Are there commercial sources for oral fluid diagnostics and who are the major players in the field?

Dr. Chamindie Punyadeera: Human saliva is currently being utilized to detect drugs-of-abuse and the players are Oasis Diagnostic, with their Sali•Chek device, Concateno-Philips hand-held Drugs-of-Abuse Test, which can detect five types of drugs-of-abuse within 90 seconds in saliva. So literally, it's a roadside testing, which I had the privilege of working on the bioassay part during my employment at Royal Philips Electronics in the Netherlands.

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So if you look at for infectious disease detections such as HIV detection, Orasure platform is the only FDA approved platform for detecting HIV virus in suspected AIDS patients.

There are also test for hormone tests as well as cancer screening tests.

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Bob Barrett: Well, Dr., what do you think the future will hold for oral fluid diagnostics?

Dr. Chamindie Punyadeera: The field of saliva diagnostics began in the early 60s when salivary calcium levels were found to be elevated in cystic fibrosis patients, and 50 years on now how the field has expanded. It has expanded into cancer detection, heart disease detection, as well as infectious disease detection.

Today we are using human saliva to detect illicit drugs, alcohol, to measure hormone levels, especially estrogen levels in women suffering from hormone misbalance, endometriosis, and to diagnose HIV virus in patient suspected of having AIDS.

In addition, there are home-based saliva tests that one can order over the Internet to determine one's cholesterol levels and also to determine the risk of developing prostate cancer whereas screening for prostate cancer antigens present in saliva.

With the development of novel, more sensitive detection technology platforms, and the advent of standardized analytical tools, establishment of reference intervals will make saliva diagnostic a reality in the near future in the areas of population-based screening programs, confirmatory diagnostics, risk stratification, and therapy response monitoring.

Bob Barrett: Dr. Chamindie Punyadeera is a Fellow at the Tissue Engineering and Microfluidics Laboratory at the Australian Institute for Bioengineering and Nanotechnology at the University of Queensland, and she has been our guest in this podcast from *'Clinical Chemistry'*.

I am Bob Barrett. Thanks for listening.

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