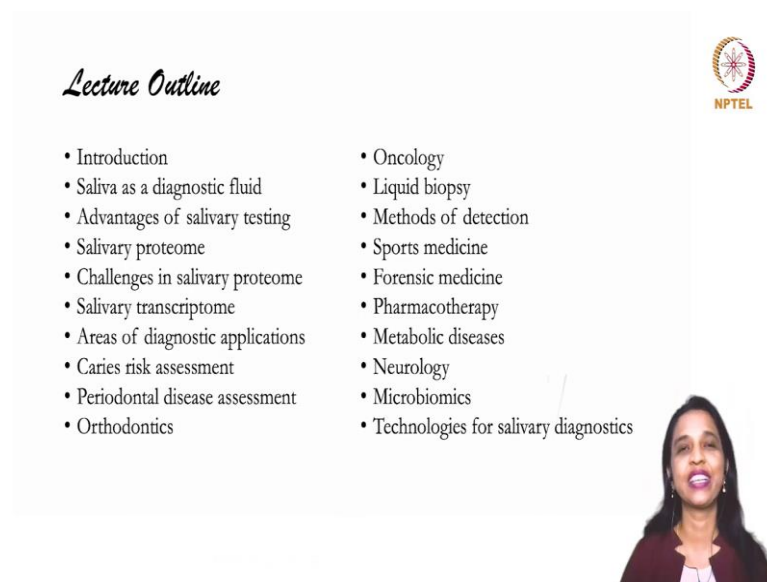


Oral Biology
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Lecture - 07
Saliva Diagnostics

Salivary diagnostics is a very interesting topic. Having read about the basics of saliva we would now look upon the most interesting applications of saliva in the salivary diagnostics.

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The slide displays the lecture outline for 'Saliva Diagnostics'. It features a title 'Lecture Outline' in a cursive font, an NPTEL logo in the top right corner, and two columns of bullet points. A small video inset of Dr. Ramya is visible in the bottom right corner of the slide.

Lecture Outline

- Introduction
- Saliva as a diagnostic fluid
- Advantages of salivary testing
- Salivary proteome
- Challenges in salivary proteome
- Salivary transcriptome
- Areas of diagnostic applications
- Caries risk assessment
- Periodontal disease assessment
- Orthodontics

- Oncology
- Liquid biopsy
- Methods of detection
- Sports medicine
- Forensic medicine
- Pharmacotherapy
- Metabolic diseases
- Neurology
- Microbiomics
- Technologies for salivary diagnostics

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Here I am again Dr. Ramya with you to talk about salivary diagnostics. So, this is the lecture outline about the salivary diagnostic topic.

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Saliva



- Saliva is an acidic (pH = 6–7) biological fluid composed of secretions from the three major salivary glands (parotid, submandibular, sublingual) and from minor glands (i.e. labial, buccal, lingual, and palatal tissues)
- Saliva plays an important role in many biological functions such as perception of oral sensations (i.e. taste, temperature and touch), lubrication, chewing, swallowing, and digestion. In addition, it enhances remineralization of tooth enamel and prevents demineralization due to its buffering capacity.
- Saliva also protects oral mucosa against biological, mechanical, and chemical factors, as well as against bacterial, viral, and fungal infections, thus maintaining the oral cavity ecosystem remain in balance

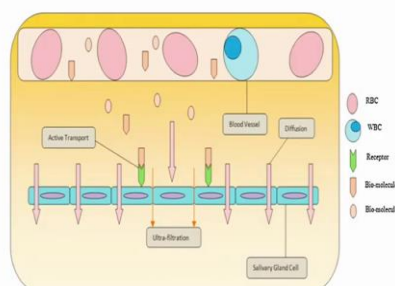


We have already read about saliva and we know that saliva is a very interesting biological fluid which is secreted from the major salivary glands and the minor salivary glands as well. And saliva has a lot of interesting roles to play which includes mastication, chewing, digestion, remineralization activity and buffering activity.

In addition, it also provides a protective role against all microorganisms like fungi, bacteria and viruses. As we say that it offers a lot of protective activity then why do we actually get into having lot of diseases? Saliva does protect only when the balance is lost. Otherwise, nature has bestowed upon us a very perfectly kept balance which if maintained would really keep you in perfect health and wellness.

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Saliva as a diagnostic fluid



Key routes through which serum molecules enter saliva. This movement of constituents makes saliva functionally equal to serum for potential diagnosis of various diseases.

Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. *J Oral Bio Craniofac Res.* 2016;6(1):66-75. doi:10.1016/j.ioocr.2015.08.006



And how is that saliva can be used as a diagnostic fluid? Because most of the biomolecules from the serum actually come into the saliva through many types of methods like ultra diffusion and active transport diffusion. And we also have the biomolecules of serum in saliva also. Because of that availability of biomolecules, saliva can be used as a diagnostic fluid.

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Advantages of salivary testing for diagnosis

- Non-invasive, easy to use, inexpensive
- Safer to administer than serum sampling (no needles)
- Real-time diagnostic values
- No need for trained medical staff
- Multiple samples can be obtained easily
- Collection and screening can be done at home
- Minimal risks of cross-contamination
- More economical sampling, shipping and storage compared to serum
- Requires less manipulation during diagnostic procedures compared to serum
- Commercial availability of screening assays

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The next interesting thing about saliva which we have to be aware of is that the advantages of salivary testing is that it is non-invasive. This particular point is the most important deterministic factor about salivary diagnostic because the moment we say non-invasive it means that there is no drama involved in collection, there is no prick or no pain to the patient.

So, in that way this can be tested even in a newborn child without causing any harm. So, it is non-invasive and it is very easy to use and inexpensive as well. Additionally the other factors which add on to the advantages is that it is more safer when compared to serum. Because where serum is collected there are lot of pinpricks involved to the person who is collecting and saliva can be collected with a person with lesser expertise than what the serum collection is requires.

So, that is a very important factor. And other important things are it is not as sensitive in storage requirements that it makes less expensive. So, it is non-invasive, more safer and allows multiple testing and it also is non-expensive.

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The slide features a central diagram titled "Salivary Proteome" with a water droplet icon in the center. Surrounding the center are eight colored segments, each representing a functional category of salivary proteins. To the left of the diagram is a list of three bullet points. In the top right corner is the NPTEL logo. In the bottom right corner, a woman is visible, likely the presenter.

Salivary Proteome

- Anti-bacterial**
Amylases, cystatins, histatins, mucins, peroxidases, lactoferrin, lysozyme, agglutinin
- Anti-viral**
Mucins, lactoferrin, cystatins, immunoglobulins, peroxidases
- Anti-fungal**
Lactoferrin, peroxidases, immunoglobulins, mucins, histatins
- Tissue coating**
Amylases, cystatins, mucins, proline-rich proteins, statherins
- Buffering**
Carbonic anhydrases, histatins
- Digestion**
Amylases, mucins, lipases
- Mineralization**
Cystatins, histatins, proline-rich proteins, statherins
- Viscoelasticity and lubrication**
Mucins, statherins

- Studying the proteome, the protein complement of the genome, in bodily fluids is valuable due to its clinical significance as source of disease markers
- Salivary proteins not only play a role in maintaining oral and general health but may also serve as biological markers to survey normal health and disease status.
- As a consequence, analysis and cataloguing of the human salivary proteome will be of great interest to researchers within the fields saliva-based diagnostics and oral biology

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And coming to the importance of what is present in saliva, the most common thing which is actually validated or the most common thing which is tested is the salivary proteome. So, in the salivary proteome we have multiple options which the saliva offers. So, as the word proteome is is nothing but a what genomic expression of what is there in a particular cell. So, as the cellular components of the body puts out its proteomic profile all that is manifested in saliva as well.

in the saliva we have elements which can confer antibacterial properties, elements those which can confer buffering properties, those which have antiviral properties, those which have digestive properties, those which confer antifungal, anti mineralization properties and those which have tissue coating properties. So, all this put together actually contributes to the proteome and definitely that sounds really offering a multitude of.

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Challenges in Salivary Proteome


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- Protein kinetics and its concentrations in saliva are influenced by several factors.
- Quantity and composition of extracted saliva are affected by the time of day, degree of hydration, body position, psychological stimuli, drug intake, health-related behaviours, systemic/oral health, and other factors.
- Deficits in sample collection, sample handling, and sample transport to the laboratory can trigger pre-processing problems




Challenges in salivary proteome; the main challenge is always that the quantity of the biomolecules present in saliva is lesser when compared to serum that is the very important challenge. So, we saw that most of the biomolecules are present in saliva as well in compared to serum, but the amount which is present is lesser. As the amount is present in is lesser what happens is you need very high sensitive technique to detect them.

And in addition there are other factors which contribute to difficulties in obtaining or validating the salivary proteome that is it can be affected by the time of collection and the degree of the hydrated status. For example how much of water do you take at that particular day of testing, the position of the body, psychological stimuli, drug intake, health related behavior and the basic systemic and oral health of the individual all have a definitive effect on the salivary proteome.


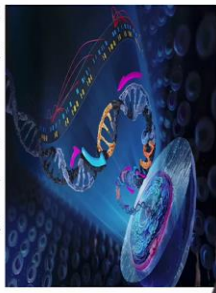
So, all these have to be kept in mind when you are deciding on the inclusion and diagnostic testing. And another important thing is the entire laboratory setup should be well equipped with whatever is required for salivary collection handling and processing.

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Salivary Transcriptome


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- Messenger (m) RNA is the direct precursor of proteins and in general the corresponding levels are correlated in cells and tissue samples
- Nucleic acids such as DNA and RNA are now much easier to screen and candidate disease markers can be verified by sensitive and specific PCR based methodologies allowing a relatively efficient level of throughput.
- Saliva has 3000 species of mRNA opening the avenue of salivary transcriptome diagnostics.



And next we move on to salivary transcriptome. So, transcriptome is where the nucleic acids that is your DNA transcribes into an RNA and then RNA becomes protein, but because salivary proteome is the one which is frequently done, and is being discussed now.

we have around 3000 micro RNAs which are there waiting to be measured and validated in the saliva and that is definitely an interesting number. So, in addition to the mRNAs we also have DNAs which can be isolated which can be very suggestive of specific disease conditions.

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Areas of diagnostic application of saliva



- Saliva reflects both local and general health of the human body, and thus it has the potential to be used for the detection of essential biomarkers for both oral and systemic diseases.
- There are many major areas where salivary diagnostics can be applied, including the fields of medicine, dentistry, pharmacotherapy & epidemiology




Now, we move on to what are the areas which diagnostic applications of saliva can give us our saliva. Very broad areas which saliva can offer, the first one is medicine dentistry and pharmacotherapy, additionally we also have epidemiology where the fields which saliva can be used to a greater deal. If you see the field of medicine you can see that the entire course of sub-specialties of medicine can utilize saliva as a diagnostic tool, dentistry as well.


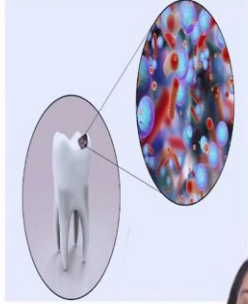
Dentistry is the one which actually brought out salivary diagnostics into the world and then pharmacotherapy. pharmacotherapy is again a very interesting area where we can use saliva for testing the pharmacovigilance or the pharmacokinetics and the dynamics. What the drug is doing to the body and what the body is doing to the drug both the ways it can be tested and in addition drug abuse can be tested by in pharmacotherapy aspects. Epidemiology again has a very huge scope in providing with the help of saliva.

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Caries risk assessment



- The use of salivary diagnostics for caries risk assessment includes microbiome, proteomic, genomic, and transcriptomic approaches.
- The most common human dental caries-associated pathogens are *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), and *Lactobacilli*.
- Caries risk assessment can be also managed by means of analyzing host-related factors in saliva including salivary flow rate, salivary pH, and buffer capacity.
- Diagnostic tools include culture-based methods such as mitis salivarius bacitracin broth (MSBB), dip-slide methods as well as newly emerging molecular technologies such as checkerboard DNA–DNA hybridization, genomic fingerprinting, 16S rRNA gene cloning and sequencing, T-RFLP and DNA sequencing including analysis of bacterial genome data.



Next is caries risk assessment these are specific topics related to dentistry. Caries is actually the most common disease in the human beings. It affects 70 to 80 percent of the whole world population.

So, when we are deriving or thinking or planning of a preventive regimen we need to know the process of caries because that again is multifactorial. So, we have to decipher it in varied components like assess the etiological factors. The microbiome can be assessed the microbial factors or the proteomic signatures the genome part of it and the transcriptome part of it.

any of these can be used to assess the caries risk so, that we can actually derive very effective preventive programs. When I say microbiome approach in caries risk we have the microbes associated like the streptococcus mutants, streptococcus sobrinus and lactobacillus which are very important caries risk microorganisms which can be easily tested.



And then we have the advanced testing using the proteome signatures and the genomic signatures. So, with all these we have very advanced testing methods like testing kits which are available as dip slide methods, broth method and the DNA hybridization method genomic fingerprinting using DNA and RNA and you also have a RFLP and DNA sequencing methods.

this can be done in very young children. So, that the parents can be advised on how to manage with the caries or how to avoid or how to prevent caries advanced techniques. There are very simple things which saliva can offer as far as caries risk assessment is concerned that is to monitor salivary flow rate to monitor the salivary pH and to monitor the buffer capacity.

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Periodontal disease assessment

- Analysis of saliva aids in assessment of current periodontal status, monitoring response to treatment and prediction of disease progression.
- Salivary biomarkers for periodontal diseases include proteins of host origin (i.e. enzymes and immunoglobulins), phenotypic markers, host cells, hormones, bacteria and bacterial products, ions, and volatile compounds.
- The most common periodontal pathogens implicated in periodontal diseases include *Tanarella forsythensis*, *Porphyromonas gingivalis* and *Treponema denticola*, so called "red complex" of bacteria.
- Host response and inflammatory mediators in saliva include: IL- β , IL-6, IL-8, TNF- α , elastase, aspartate, and aminotransferase, bone-specific markers of tissue destruction and connective tissue breakdown comprise: collagen telopeptides, MMP-9, osteocalcin, proteoglycans, or fibronectin.
- Metabolic profiling of saliva can provide a global outlook of the changes associated with periodontal diseases, particularly host enzymes (alkaline phosphatase, esterase, glucuronidase and aminopeptidase), prostaglandin E₂, matrix metalloproteinase-8, 8-hydroxy-deoxyguanosine, dipeptides (leucylisoleucine, phenylphenol and serylisoleucine), as well as the fatty acids (arachidonate, arachidate and dihomolinolate).



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Again I told you that caries is the most commonest or highly prevalent disease in human beings, next common human disease is periodontitis. So, periodontitis again is very common disease and having said that it is common we need to derive every possible step to prevent and give effective treatment regimens. This particular disease as you can see the picture as the tartar or as the calculus keeps depositing on the tooth surface there are millions of bacterial accumulation in this particular area and that causes inflammatory episodes.



The inflammatory episodes can cause connective tissue or tissue destruction and it can cause destruction of the hard tissues as well or bone that is bone destruction. So, that is what you can see over here there is redness of the gum region or the gingival region that is because of tissue breakdown. In case if we take a X-ray for this particular patient we can very well understand that there will be bone loss. So, there is tissue destruction and there would be bone loss as well.

So, this process is very nicely monitored by either assessing the microbial population or assessing the inflammatory mediators which are very inflammatory mediators. Inflammatory mediators can be monitored. So, with all this in mind periodontal disease can be very easily monitored using saliva.

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Orthodontics

- Saliva can be used to monitor the risk and the development of root resorption during orthodontic treatment.
- Changes in saliva, include: interleukin-1 β and interleukin-1 β receptor antagonist, proteoglycans, regulatory subunit of type II (RII) of cyclic AMP-dependent protein kinase (PKA) or anti-HDE sIgA antibodies



And, next we move on to another interesting area of dentistry all of us know and all of us have seen people wearing braces and these braces are used for alignment of teeth. When the teeth are crooked to align it in proper place, so that you have a very beautiful smile that is what orthodontics is the main part of orthodontics is all about there are other multi very deeper areas, but.

So, as the teeth are realigned using fixed orthodontic treatment what happens is there is a sequence of events which happen behind the alignment. When the force is applied there is either resorption or deposition. So, to monitor how much of resorptive activity is happening how much of deposition of activity is happening can be more in depth observed or can be more effectively monitored.

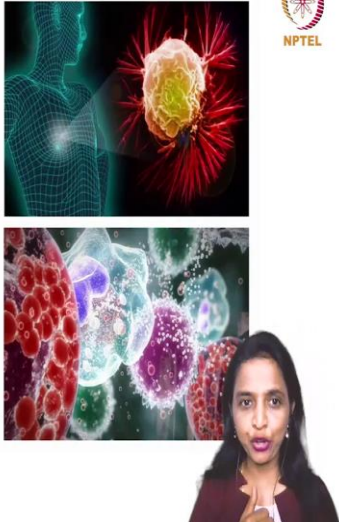
So, monitoring the move tooth movement with inflammatory mediators makes it a more sensitive method than monitoring it through clinical or radiographical methods. Because by the time you can see a clinical manifestation or by the time you see a radiographic manifestation, the changes are there witnessed in the inflammatory mediators. So, if you can observe the inflammatory mediators in orthodontics during inflammatory mediators

during orthodontic treatment if it can be observed. Then it would be a great help to the patient and that in saliva again reduces a lot of burden in orthodontic management.

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Salivary biomarkers - Oncology

- Saliva serves as a useful diagnostic mean in the early detection of various cancers such as oral cancer, pancreatic cancer, breast cancer, lung cancer, or gastric cancer.
- Multiple molecular alterations occur during cancer development. Deciphering the molecular networks distinguish progressive disease from non-progressive.
- Binding of transcription factors, chromatin structure modification, post-transcription -e.g., nucleocytoplasmic export or splicing of messenger RNA, differential ribosomal loading, and post-translation -protein degradation or export can be monitored
- Anti-p53 -esophageal cancer, stomach cancer, and cancer of the large intestine ,oral squamous cell carcinoma (OSCC).
- CA-125 - ovarian, endometrial, lung, breast, and gastrointestinal cancers.
- C-erbB-2 - breast cancer, while overexpression of EGFR receptor is observed in pancreatic cancer.



Next we move on to a more crucial area in diagnosis and that is oncology, also well established in monitoring pancreatic cancer, breast cancer lung cancer and gastric cancer. Most of the etiopathological mechanisms or the pathogenetic mechanisms to be more specific the molecular basis of cancer are all reflected in saliva.

So, that having that promising statement in mind, so, this salivary biomarkers there are a very big list which are specific for oncology related monitoring can be used for monitoring in cancer patients. As I say monitoring, so, at which stages is it helpful? It can be used as a preventive detection.

for those patients whom we think are more at risk can be you they can check their salivary status and see if there is a cancer risk and for those patients who are actually just recently detected it can be used for diagnosis that is detection at that particular time. And then it can also be used for a prognostic follow-up or a progressive follow-up which is very important in cancer patient management.

Because as the patient is subjected to therapeutic stages we have to see whether the cancer is coming down. So, there has to be effective monitoring tool at various stages. So, at that particular stage the patient in spite of just giving multiple pricks and then



multiple samples if they can just give saliva it would be a great help for them. So, that because we are offering a non-invasive tool.

And the most common markers which are used for oncological monitoring of salivary biomarkers are anti-p53, CA-125, C-erbB-2. So, these are the common markers.

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Methods of detection of Salivary Biomarkers

Type of biomarkers	Technology behind
Non-organic compound biomarkers	Identified by flame photometry, atomic absorption, and spectrophotometry
Peptide or protein biomarkers	High-performance liquid chromatography (HPLC) Enzyme-linked immunosorbent assay (ELISA) Radio-immunoassay Two-dimensional gel electrophoresis (2DE) followed by mass spectrometry (MS) 2DE and reverse-phase liquid chromatography (LC) followed by LC-tandem MS Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) DE followed by MALDI-TOF MS
DNA, mRNA or microRNA biomarkers	Polymerase chain reaction (PCR) Quantitative PCR (qPCR) Microarrays followed by qPCR
Metabolomic biomarkers	Capillary electrophoresis TOF MS HPLC with quadrupole/TOF MS Hydrophilic interaction chromatography-ultraperformance LC-MS analysis Reversed phase liquid and hydrophilic interaction chromatography/TOF-MS
Miscellaneous biomarkers (chemical and enzyme activity)	HPLC Colorimetric (mostly commercially available) assays

And the methods of salivary detection of salivary biomarkers methods of detection of salivary biomarkers listed here all sound very big. They are not very simple because of the main reason that the biomolecules present in saliva are very less than number. So, as the amounts amount is less again to reinforce we must remember the fact that most of the biomolecules present in serum are present in saliva that is the important statement.

Having said that, but the challenge lies in the amount of biomolecules present in the saliva, so, that is lesser when compared to serum. But all of them are present. Considering the advantages which saliva can offer, so, what should we do? We have to definitely go in for very highly sensitive very advanced diagnostic methods.




So, that is why you have a range of very high-end detection methods, but I am sure if the government machineries are identifying the importance of this very interesting field and if they can fund in enough sources then salivary diagnostics will really make a very big mark.

So, how do we detect these salivary biomarkers? So, what are all are detected? So, we can see that there are organic non-organic markers there are peptide and protein biomarkers there are DNA there are metabolic biomarkers and there are other miscellaneous markers. So, one thing we have to remember here again is that there are very advanced techniques which are required.

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Sports medicine

- Saliva analysis enables to monitor the metabolic response of sportsmen during physical training.
- This monitoring can avoid overtraining and lessen the risk of injuries.
- It helps to modify their plan of training, in terms of duration, frequency, and intensity of exercises.



And, now we move on to a very interesting area of salivary diagnostic sports medicine. So, this sounds really very this really arouses lot of curiosity. So, how do how does saliva help in person is involved in lot of physical activity, a very active physical active lifestyle? What happens is the person is subjecting the musculoskeletal system into a lot of action.


So, as the muscle musculoskeletal system is put into lot of action there are some enzymes which are released into the systemic circulation and they can be monitored very effectively by serum if its serum it is going to be saliva also. So, with that what can we do to the sportsmen?

So, those who are having a very rigorous practice they can be told that you can lift so much of weights and this is going to be your markers of what you are doing to the muscle and do not go into a weight lifting more than this particular level because that is not doing good to your body. Something like that can be very easily derived by using saliva.



And all this can play a very important role in fitness testing and in training young children and young adults who will be representing the country at national and international levels.

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Forensic medicine


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- Saliva is a useful diagnostic tool in forensic sciences, where there is a possibility to differentiate individuals, who are still alive, from dead bodies.
- Interestingly, due to the fast oral tissue turnover, DNA extracted from the OFs is much more valid compared to other possible DNA sources.



And then we have forensic medicine. Forensic medicine as the name suggests is again a very vital field where there is lot of investigations which are happening and it also identifies the victims as well. So, saliva offers multitude of options in forensic medicine also.




So, it helps to differentiate individuals who are still alive and those who are dead as well interestingly there are other areas which it can offer. DNA can be extracted from saliva and that can be used in individual identification. So, that makes a very big impact especially in mass disaster victims and in many other situations where the victim or the criminal leaves a trace of salivary sample in the area of the crime.

So, if the particular forensic team can identify that trace of saliva it can actually make a very big difference.

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Pharmacotherapy

- Monitoring the therapeutic drug level and poisoning as well as to study the biochemical and physiological effects of drugs such as carbamazepine, cisplatin, diazepam, digoxin, ethosuximide, irinotecan, lithium, metoprolol, paracetamol, phenytoin, primidone, procainamide, quinine, theophylline, or valproic acid. Also, cotinine can be monitored in saliva of smoking subjects.
- Drug abuse - Saliva plays an important role in detection of various drugs in the blood such as amphetamine, cocaine, methadone, phencyclidine, marijuana, or opiates.
- Alcohol consumption - Salivary diagnostics serves as a diagnostic tool in alcohol consumption



Pharmacotherapy has already mentioned what the drug is doing to the body and what the body is doing to the drug everything can be monitored with the help of saliva. And these are the drug list of drugs which actually have been proven to be present in the saliva and which can be efficiently monitored.

Very simple example is that if you keep smoking cotinine levels even how much of cotinine is still present in your saliva after a single cigarette smoke, so that all that can be monitored. And that can be very effectively used for advocating tobacco cessation programs. And in addition drug abuse is one very drug abuse is one very serious area drug abuse is most of the opioid addictives addictive agents. Most of the opioid addictive agents can be easily monitored by using saliva.

We know that drunken driving is a crime. So, drunken driving in can be very easily monitored by breath test additionally saliva also can offer as a supporting test.

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Metabolomics

- Metabolome is the complete set of small molecular metabolites of living tissues including metabolic intermediates such as carbohydrates, lipids, amino acids, nucleic acids, hormones, and other signaling molecules.
- Salivary metabolites are important in elucidating the pathways underlying different diseases, thus making it ideal for the early detection of a wide range of diseases, including oral cancer and periodontal diseases.
- The major role of metabolomics is to identify novel metabolic biomarkers from cells, tissues, or body fluids by means of high-performance liquid chromatography-mass spectrometry (HPLC-MS) or two-dimensional gas chromatography MS and nuclear magnetic resonance spectroscopy in conjunction with pattern recognition methods.

WU HQ, J Dent Res 2011

Then we move on to how much of metabolic activity can be monitored in saliva and as we say metabolome or metabolic activity we know that the most key areas of the salivary metabolome or the metabolic activity in the body revolves around the carbohydrates, the lipids and the proteins. If all of them can be evaluated or can be assessed in saliva then it is offering a huge scope which actually can be monitored.

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Metabolic diseases

- Salivary biomarkers have been recently explored as a useful screening tool in patients diagnosed with metabolic disorders such as obesity or diabetes mellitus.
- Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels and it is one of the most important health problems faced by the humankind today. It leads to morphological changes in the salivary glands and in the composition of saliva.
- Salivary glucose levels have been evidenced to serve as a reliable diagnostic tool similar to serum.
- Myeloperoxidase and IgA were correlated with a poor periodontal status in diabetic patients.
- In addition, in diabetes mellitus type 2, positive correlation between HbA_{1c} and total amount of proteins, and an indirect correlation between HbA_{1c} and pH in saliva.
- Since diabetes is a common chronic disease with many associated comorbidities, measuring the qualitative and quantitative salivary alterations could be a promising and cheaper way of monitoring affected patients.

III

As we say metabolic activity we know that if this metabolic programming or this metabolic activity in the body is not balanced it is going to lead to a disease state. The

most commonest metabolic disease in the body is diabetes mellitus and in India we know that we are the diabetic capital of the world and we have the most highest number of diabetes patients and that is a best example of a metabolic disease. And saliva can be used as a diagnostic tool in measuring and monitoring glucose levels.




So, this glucose levels we know that there are many patients who have to keep checking glucose levels by pricking and we know that there are kits which are available. There have been a multiple number of studies in literature which suggests that salivary glucose levels can be used as a reliable measuring device to monitor diabetes mellitus.

In addition, we also have other suggestive markers which are indicative of diabetes mellitus. So, the other things which can actually help in monitoring the disease are your myeloperoxidase IgA and it also helps to detect HbA 1 which is a more reliable testing than the salivary glucose. And also, it also helps in monitoring the pH as how diabetes is altering the pH.

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Neurology

- Salivary diagnostics is used in neurodegenerative diseases like Alzheimer's disease, with elevated levels of phosphorylated and total TAU proteins (Microtubule associated protein - neuron)
- Increased levels of nerve growth factor (NGF) and sensory neuropeptides (including substance P and calcitonin gene-related peptide [CGRP]) strongly correlated with the severity of pain in patients diagnosed with chronic migraine.



So, and now we move on to neurology. So, as we see this particular area and everything revolves around the brain. So, as we say such a statement, so, what happens? There are some diseases related to the IQ levels of the patient. We have done in our own lab about the patients who are having attention deficit syndrome, we have measured biomarkers in kidney urine pathway and serotonin pathway and we have found that every these pathways which are functioning in the brain can be effectively monitored in saliva.

So, as we say attention deficit syndrome can be monitored. You know that ADHD is very common in very young children who are having learning difficulties. So, if they can actually be detected at the early stage and inform the parents and informed the educators the lesson plan for them would really make a very big difference in that child's life.


And we move on to other serious problems related to the CNS. We know that the brain is subjected to neurodegenerative mechanisms as we keep ageing. The most common disease here is Alzheimer's disease which is a more common neurodegenerative disease and that involves elevated levels of phosphorylated and total microtubule associated proteins related to a mature neuron. So, all these can be effectively monitored.

So, in case if a patient is feeling that he is having memory loss and so on or if the parent is suffering from Alzheimer's disease and if that the son or the daughter wants to know if he or she will be vulnerable to Alzheimer, we can definitely check with the help of saliva.



And we also have other interesting suggestive markers like nerve growth factor, sensory neuropeptides which strongly correlates with severity of pain in patients diagnosed with chronic migraine. So, we know that mind is a very important that is brain is an important.

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Microbiomics



- New technologies have allowed the scientists to start to unravel the complex interactions between the microorganisms and the human body.
- It was reported that salivary microbiome could be used in the detection of early resectable pancreatic cancer by means of microbial profiling (the Human Oral Microbe Identification Microarray), where two microbial markers (*Neisseria elongata* and *Streptococcus mitis*) were successfully developed
- Currently, newer microbiome-based technologies have also become available, such as study of microbial sequences by means of RNA or DNA sequencing.






And as already mentioned in caries risk assessment and periodontal risk assessment, microbiome we know that interestingly here we have microbial population which can be

detected in early stage of pancreatic disease. Bacteria such as nisseria and streptococcus mites play a very important role during early stage of pancreatic cancers. So, such important statements make a very big important role in prevention as well as in deriving therapeutic strategies.

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Technologies for salivary diagnostics

- Currently, a great need exists for convenient and accurate point-of-care diagnostics that can serve as a non-invasive diagnostic tool.
- Novel point-of-care salivary technologies are being developed, which can facilitate biomarker identification without any pre-processing, screening, and non-invasive diagnostic testing
- Oral Fluid NanoSensor Test (OFNASET) for oral cancer detection, my PerioPath (OralDNA Labs) for diagnosing of periodontal disease, or the OraRisk HPV test (OralDNA Labs)



And, now we move on to; what are the technologies available for salivary diagnostics? We already discussed about the methods of salivary diagnostics. So, those are the methods which are involved what if we can have them as point of care testing devices. Point of care testing devices are very vital for disease management because all that we have to do is it should be a self assessment kit. Is that possible for saliva? Yes, there are many many kits which are available and many kits are in the making.



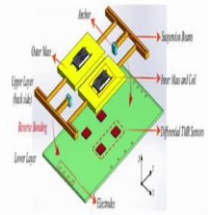
So, the ones which are right now available are your salivary fluid nanosensor test for cancer detection and perio path for OralDNA assessment we have the oral risk oral cancer risk HPV testing. So, we have multiple options of point of care devices available that is OFNASET for oral cancer, PerioPath for periodontal disease and oral risk for oral.

We have multiple point of care devices which are already available that is used for cancer detection, perio risk detection and an oral cancer HPV detection.

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Current Technologies

- There are several currently available or newly emerging technologies based on salivary diagnostics and development of microfluidics or micro/nanoelectromechanical systems (MEMS/NEMS).
- They are composed of mechanical, electrical, and functional elements such as sensors, actuators, and microelectronics that are made using the techniques of microfabrication.
- Currently developed tools include electrochemical sensing, on-chip qRT-PCR, fiber optic microsphere-based arrays, high-throughput DNA microarrays, surface plasmon resonance-based fiber optic sensors, and microchip-based electrophoretic immunoassay.



So, how does this point of care devices work? So, they are based on micro fluidics or they are based on micro and nanoelectro mechanical system. So, this is a very simple schematic diagram which actually tells about the interface which is involved. In addition, there are other interfaces which are commonly used for salivary diagnosis that is your RT-PCR, other fibro optic microsphere based arrays, DNA microarray, surface plasmon resonance based fiber optic sensors and microchip based electrophoretic assays.

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So, with this we end the salivary diagnostic lecture. So, what I would like to you to know is young minds to bring in more advanced interfaces to detect that interesting biological fluid and to make the world a better place.

Thank you.