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REVIEW ARTICLE

SALIVARY BIOMARKERS FOR DRUG AND TOXIN DETECTION

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Abstract

Saliva is an easily accessible and non-invasive diagnostic fluid that gains significant attention in the biomedical field, especially for drug and toxin detection. This review explores the potential of salivary biomarkers, detailing the types of biomarkers, mechanisms of drug deposition, and their diagnostic applications [1]. The role of salivary biomarkers for discovery and improvement has gained precedence over the years. So biomarkers become integrated into drug development and clinical trials, and quality assurance and assay validation become essential with the need to establish standardized guidelines for analytic methods used in biomarker measurements [2]. The Nobel protein method helps in stabilization and proteomic analysis of the extracted sample. The article also discusses recent advancements [3], limitations, and future perspectives of salivary diagnostics in clinical and forensic sciences [4]. DNA libraries with BAB modifications can achieve more diverse conformations for various targets compared with natural DNA libraries, which is an important advantage for aptamer development. Similarly, chronic kidney disease has been identified by studying the correlation between creatinine and uremic toxin (UT) [6], which impaired kidneys cannot filtrate and By replacing the traditional biomarkers of druginduced toxicity, they have improved the value of the potential for use of circulating miRs in the field of drug-safety assessment [7].

Introduction:-

Saliva is basically known as a diagnostic fluid which plays a crucial role in the emerging field of drug and toxin detection as a biomarker due to its minimal risk of infecting agents, real time reflection of individual physiological state[3]. saliva collection can be an easy-handled sample as rapid screening under the filed of forensic toxicology and drug testing with detection[6]. Saliva offers a non-invasive alternative for drug testing compared to blood or urine[8]. A Saliva sample is basically composed of components like enzymes, antibodies, hormones and slight range of toxins and drug materials. Saliva doesn't require any kind of medically trained personnel for undergoing any sort of collections and by this criterion the chances of contaminations are expected to be low, So the benefits in the forensic Investigations while testing drugs, therapeutic drug managements and others are quite higher[9]. Few techniques like biosensors saliva based, chromatographic techniques and immunoassays along with specified sensitivity of wide spectrum of substances from narcotics, alcohols and prospection on drugs[10].Through the

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passive diffusion method, correlation with the pharmacological active free fraction present in the blood plasma[5]. Drugs are highly lipid soluble and low at protein binding which is helpful in dynamic view of Drug Analysis[6]. This section explains its composition, role of drug and toxin detection, focusing on its advantages, Drug incorporation, analytical methods with current challenges and why it's emerging in diagnostics.

Anatomy And Physiology Of Salivary Glands:-

Describes the structure of major and minor salivary glands, the process of saliva production, and its relevance to biomarker studies[11]. Describes the salivary gland composed of Major and Minor Glands which are further classed into sub-components like Parotid Glands, Submandibular Glands, Sublingual Glands and the Minor Glands with stimulation on nervous systems of Parasympathetic and Sympathetic[12]. This Glands are functionally collective to secrete saliva and the oral health biomarkers can be used in the analysis of clinical and other forensic related studies, the process of saliva production, and its relevance to biomarker studies[13].

Composition of Saliva:-

Saliva is composed of 99% water with 1% inorganic and organic substances, electrolytes like Sodium, Potassium, Chlorine, Mucins- Immunoglobulins and Anti-microbial peptides, Protein binding with ionization, Enzymes like Amylase and Lipase[14]. Hormones which are Estrogens, Progestin,
Testosterone, Cortisol, Corticosteroids, Thyroid and Growth Hormones,
DNA, RNA, Drugs and Toxins[15]. There secretions can also depend on the Acinar secretion and Ductal modifications which results in the reabsorption of Hypotonic final saliva[16]. These components make it suitable for biomarker detection.

Types of Salivary Biomarkers:-

Covers proteins, Enzymes, Cytokines, Nucleic acids (miRNA, mRNA),
Microbial, Hormonal, Immunological, Xenobiotic, Electrolyte and Mineral Biomarkers, Metabolomic, Proteomic and Genomic Biomarkers, and Exogenous compounds such as drugs and toxins[17].

Mechanisms of Drug Entry into Saliva:-

Drugs were entered saliva through passive diffusion by the factors of Lipid solubility, Molecular weight, Ionization, Plasma protein by concentration gradient among the plasma and interstitial fluid, were drugs move across lipid bilayer membranes[15]. Active transport induces the drugs to transport by membrane transporters and occurs when drug structure enacts the physiological substrates, Saliva to Plasma pH Gradient were in theweak bases become ionized in acidic saliva to trap and accumulate know to be “Ionic Trapping” or by other method of Ultrafiltration in which the paracellular movement are accomplished through small pores between acinar of cells[13].

Sampling and Storage of Saliva:-

Unstimulated saliva- the sample been collected by stimulation used for physiological state, Stimulated Saliva- Taken after chewing or sensual stimulation which has the capacity to increase the volume, Gland-specific Saliva- Collected from submandibular glands mainly used in the field of specific study of saliva[15]. Collection methods are of major Absorbent devices, spitting method, Parotid cup which is completely dependent on the Time of collection, Recent drug taken, Hydration level, Circadian rhythm which are the factors that can affect the saliva composition[18]. Saliva samples can be stored under 3 temperature conditions like 4℃ (<24 hours) ,-20℃(Days to Weeks),-80℃ (Weeks to Months), Lyophilization (Freeze- drying saliva)[18]. moreover, when kept under repeated freezing the components like proteins RNA and others with lesser quantity of molecules can be Degraded.

Analytical Techniques for Detection:-

Includes ELISA that can detect specific antigens and antibodies in saliva which can label detection in enzymes and majorly used for Cocaine, THC, Amphetamines since they are highly rapid and good screening with low-cost efficiency but, sometimes they can give a false positive result[19]. GCMS(Gas Chromatography-Mass Spectrometry) used for confirmatory test for drugs in the saliva sample due to there separation of volatile compounds by the identification of Mass, some of the drug materials detected in GC-MS are Opioids, Cocaine, Methamphetamine[9]. LC-MS(Liquid Chromatography-Mass Spectrometry helps to detect non-volatile and thermolabile drugs that can in turn detect wide range of substances under availability of lower sampling due to their high sensitivity and Multi Drug Analysis[14]. Some others techniques like biosensors, andmicrofluidic labon-chip devices can also be used for analyzing drugs and toxins in saliva[2].

Diagnostic and Forensic Applications:-
Saliva is used in drug screening (roadside, workplace), Clinical toxicology(toxin exposure to occupational and environmental health), Doping control, Therapeutic drug monitoring, medical diagnosis, Substance abuse screening in healthcare forensic investigations and other drug-related disorders[20]. In the field of Forensic Applications, they play a key role in Drug test kits, Crime scene and assault, Postmortem Toxicology, Forensic Drug and DNA Traces[12].

Recent Advances in Salivary Diagnostics:
Explores nano-sensors, AI-driven detection, portable saliva analyzers, and multi-omicsapproaches like saliva omics[21].recent trends in the field of Lab-on-a-chip devices, Aptamer Biosensors, Smartphones-Integrated Tools, SERS Technology.

Advantages Over Blood and Urine Testing:
Saliva is non-invasive, easy to collect, has minimal infection risk, less expectations of contaminations and provides real-time drug monitoring by which they are mainly focused in the field of clinical and forensics because of their high reliability, broader detection and greater analytical concentration level[22]. They are also detected better in Pharmacokinetics for the study of absorption, distribution and metabolic activity of clinical studies[23]. Limitations include low concentration and variability.

Limitations and Challenges:
Highlights variability in flow rate, Contamination risk, Difficulty in detecting low concentration drugs, Influencing Physiological Factors, Lack of Standardization which are collected and handled[24].Legal and Forensic acceptance, Limited Detection of Metabolites, Technical and Cross Barriers[25]. Several biological techniques and analytical limitations are universal for the toxin detection which can lead to clinical contexts[26].

Conclusion:-

Summarizes the potential of saliva as a diagnostic tool for drug and toxin detection, it has served as a non-invasive biological matrix under the detection of drugs and toxins by offering various advantages like ease to collection of samples, minimal infectious risk, sustainability to field applications, growth in the integration of advanced analytical techniques which are LC-MS/MS, Saliva omics with improved sensitivity and specificity of diagnostics under salivary analysis[9]. These also include the limitations like low analytics concentrations, short detections windows, biological variability along with legal acceptance, in few cases were the lack of standardization in the obtained protocol, processes to store continues challenge reproducibility and comparability[10].

By comparing the saliva samples with urine and blood it is still limited by its ability to detect metabolites to provide an quantitative levels for drugs[27]. Moreover, the researchers continue to refine the sensitivity of detection and technologies for the development of AI- integrated portable systems[28]. The diagnostics under the filed of forensic potentially focus on the validation of Legal framework, Clinical standardization of the evidence, Biomarkers to serve alternative methods to traditional matrices apart from other biological samples like Blood and Urine[29].The oral fluid plays an alternative role in enhancing the drug analysis apart from urine and plasma components, Most commonly the cigarette smoke consist of saliva traces that can be reliable, They can also rely on the carcinogenic components by tobacco smoke[30]. These criteria are important for emphasizing the need for further research and standardization.

Conflict of Interest:
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