Review Article

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Dentistry Section

Diagnostic Methods for Detection of Cotinine Level in Tobacco Users: A Review

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ABSTRACT

The greatest disease-producing product known to man is tobacco. It is a cause of many oral diseases and adverse oral conditions. In India, tobacco is available in smokeless and smoking form. Tobacco contains nicotine which metabolises to form a toxic alkaloid i.e. cotinine. It stimulates autonomic ganglia and central nervous system. Cotinine is the best indicator of tobacco smoke exposure. Various methods are used to measure cotinine level in blood, saliva and urine such as high performance liquid chromatography, colorimetric assay, gas chromatography, NicAlert saliva test, etc.

Thus such wide range of methods for cotinine detection in tobacco users requires a detailed discussion regarding their utility. This review will help readers to compare various methods for cotinine detection and enable them to make scientifically informative decision.

Keywords: Nicotine, Tobacco products, Toxic alkaloid

INTRODUCTION

Tobacco is a harmful, addictive chemical responsible for many oral diseases and adverse oral conditions [1]. It can be consumed in two forms, smoking and non smoking [2]. Tobacco consumption in any form is responsible for diseases like oral cancer, adult periodontal diseases, and congenital defects such as cleft lip and palate in children whose mother consumed tobacco during pregnancy [1].

Virtually all tobacco products contain nicotine in substantial concentration [3]. Cotinine, a major metabolite of nicotine, can be easily detected in various body fluids like blood, urine and saliva [4]. It is most commonly used marker to distinguish between tobacco users and non users because of its greater sensitivity and specificity than other biochemical tests [5,6].

There is a high correlation between blood and saliva cotinine concentrations. A widely used biomarker is urine cotinine level since cotinine concentrations are four to six times higher in urine than that in blood or saliva [3,6]. This makes quantitative methods (like gas chromatography/mass spectrometry or high performance liquid chromatography, colorimetric assays and immunoassays) which measure urine cotinine more valid and reliable. The disadvantages of higher cost and time consumption have been addressed by recently developed semi quantitative methods [5,7].

COTININE: Every individual is exposed to nicotine either directly or indirectly [8].

Nicotine's major metabolite is cotinine which is oxidized in the liver by CYP2A6 (Cytochrome P450, Family 2, Subfamily A, Polypeptide 6) and is distributed in various body fluids including the blood, saliva and urine [9]. Cotinine is the main biomarker which is used to distinguish tobacco users from non users and reflects the extent of exposure [10]. Longer half-life of cotinine makes it a useful short-term marker of nicotine exposure. During tobacco smoking its level fluctuates relatively to lesser extent than other tobacco products [11].

Correlation exists between urinary cotinine and daily tobacco use. A value of urine cotinine ranges from 20-550 ng/ml. A change in the cut-off value is directly proportional to sensitivity of detection [6].

Pharmacokinetics and Pharmacodynamics Properties: In 24 hour urine sample analysis it is found that 5% of nicotine dose is excreted as it is while 10% in the form of cotinine and 35% as hydroxy cotinine. It is stable in body fluids, low plasma protein binding, has a long half-life 15-40 hour, it is directly proportional to the quantity of nicotine absorbed and dose independent disposition kinetics [8]. Thus cotinine is useful marker as it helps in estimation of exposure to active as well as passive smoke [8].

There are certain limitations in measuring cotinine level in tobacco users: it requires expensive laboratory instrumentation requires relatively large sample volume; time to store, ship and analyse; and well-trained personnel, the high cost of analysing the samples, the cost of collecting, handling and arranging for shipment of specimens [5].

Assessment of Cotinine Level in Saliva, Blood and Urine

Cotinine in Saliva: The small molecules, minimal protein binding in blood and water solubility increases concentration of cotinine in saliva by 15% to 40%. Thus cotinine measurement in saliva becomes a non invasive, easy and well tolerated collection procedure when multiple samples are required over a limited period [3].

Various studies were conducted by authors to detect cotinine in saliva to distinguish tobacco users from non users. [Table/Fig-1] shows studies conducted on salivary cotinine.

Cotinine in blood: Cotinine and 3-HC (3- hydroxycotine), nicotine can all be measured in blood but cotinine has longer half-life compared to others therefore it is considered as a preferred [3]. [Table/Fig-2] shows studies conducted on blood cotinine.

Advantages: Long half life, no requirement for adjusting hydration difference among individuals, uniform matrix measurement though it has a lower sensitivity compared to urine cotinine [3].

Cotinine in urine: The most widely used biomarker in tobacco users is urine cotinine.

Advantages: High sensitivity compared to blood cotinine, collecton is non- invasive, relatively acute exposure [3]. [Table/Fig-3] shows studies conducted on urine cotinine.

Very few studies have been conducted on COT one step cotinine test device a lateral flow chromatographic immunoassay which is used to detect human urine cotinine at a cut off value of 200ng/ml. There are two lines in the device one is test line and other is control line. Test line consist of mouse monoclonal anti cotinine

Authors	Year	Findings and conclusion
Montalto NJ, Wells WO	2007	Saliva NicAlertR assay found to be highly specific, sensitive and valid method for verifying smoking status [12].
Nuca C, Amariei C, Badea V, Zaharia A, Bucur L, Arendt C	2012	By using NicAlert™ Saliva tests it was found that 44.06% were active smokers, 16.43% were non-smokers and 39.50% were passive smokers [11].
Kulza M, Wozniak A, Przybylowska SM, Czarnywojtek A, Flisykowska KA, Florek E	2012	The concentration of salivary cotinine was detected using high performance liquid chromatography with diode array detection. Mean concentrations of cotinine was found to be 240.9 ng/ml of saliva determination of saliva cotinine is useful in the assessment of tobacco [4].
Asha V, Dhanya M	2015	Immunochromatographic assay using NicAlert saliva test was used to detect salivary cotinine in tobacco chewer. Levels found were as follows: 0 for 2 subjects (2.67%), 1 for 16 subjects (21.33%), 2 for 16 subjects (21.33%), 3 for 18 subjects (24.00%), 4 for 21 subjects (28.00%) and 5 for 2 subjects (2.67%). For tobacco chewers this method is convenient and useful for studying the nicotine dependence [13].

[Table/Fig-1]: Studies conducted by authors on salivary cotinine.

Hsieh SJ, Ware LB, Eisner MD, Lisa Yu, Jacob P, Havel C, Goniewicz ML, Matthay MA, Benowitz NL, Calfee CS	2011	More active smokers were identified by using the combination of serum cotinine and urine 4-(methylnitrosamino)-1-(3pyridyl)-1-butanol than did smoking history, secondhand smoke exposure prevalence was high in a critically ill population [14].
Xu X, Su Y, Fan ZH	2014.	Micellar electro kinetic chromatography with enrichment techniques was used to measure cotinine in serum samples of mice. It was found that there was an association between the concentration in serum cotinine and tobacco smoke-induced emphysema in mice and in future this method can be used to detect cotinine [15].

[Table/Fig-2]: Studies conducted by authors on blood cotinine.

Balhara YPS, Jain R	2013	Value of urinary cut off was kept greater than or equal to 2.47 ng/ml to detect the highest sensitivity and specificity of 100% for smoking using ELISA kits of Calbiotech. Inc., USA. Receiver operating characteristic (ROC) curve. Authors concluded that urinary cotinine cut off value was used to distinguish tobacco users and nonusers [6].
Kotandeniya D, Carmella SG, Ming X, Murphy SE, Hecht SS	2015	Liquid chromatography-electrospray ionization-tandem mass spectrometry method was used for detection of total cotinine (cotinine + glucuronide) and total NNAL (NNAL + glucuronide). Thus authors concluded that this method quantifies naturally occurring (13C) cotinine [16].

[Table/Fig-3]: Studies conducted on urinary cotinine.

antibody coupled particles and cotinine protein conjugates while goat antibody is present on control line. Urine sample must be stored at least 2-8 degree Celsius for 48 hours before assay [17], If two lines appear in control and test region the result is said to be negative which means that patient's urine cotinine concentration is below cut- off value of 200ng/ml, if only one coloured line appears in control and no line in test line region the result is said to be positive and if no line appears means the result is invalid that may be due to incorrect procedure [17]. [Table/Fig-4] shows different diagnostic method for assessing tobacco/nicotine.

CONCLUSION

A variety of techniques has been used to measure cotinine including gas chromatography, high performance liquid chromatography and colorimetric assays Immunoassays, NicAlert™ Saliva tests etc. Cotinine can be widely used in future compared to other diagnostic tools because of its higher sensitivity, specificity, long half life as well as it is the best indicator for distinguishing the tobacco users from non users.

Cotinine	Fagestrom Test for Nicotine Dependence (FTND)	Tobacco Dependence Screener (TDS)	Cigarette Dependence Scale. (CDS)	Nicotine Dependence Syndrome Scale (NDSS)
The major metabolite of nicotine is cotinine, has low plasma protein binding, long half-life and is a good marker for assessment of nicotine dependence in saliva, blood, urine in tobacco users [10,11].	Fagerström Test for Nicotine Dependence (FTND) is a six-item questionnaire that has scoring from 0-10 which is classified according to patient nicotine addiction as very low (0–2), low (3–4), medium (5), high (6–7), or very high (8–10) [18,19].	Tobacco dependence screener was developed on 10 symptoms its score was correlated with the years of smoking, the number of cigarettes smoked per day, breath carbon monoxide levels [20].	CDS is a valid and reliable method of cigarette. Dependence criteria are: withdrawal symptom, compulsion to smoke, loss of control, allocation of time to smoking, neglect of other activities to smoke, and persistence of use despite harm, except tolerance [21].	NDSS is 19 item questionnaire which includes five factors drive (craving and withdrawal to smoke), priority, tolerance continuity and stereotypy [22].

[Table/Fig-4]: Different diagnostic method for assessing tobacco/nicotine dependence.

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