Human Urinary Metabolomics as Biomarkers in Tobacco Users: A Systematic Review

Abstract

Aim: Urine as a biofluid has been rarely used as a diagnostic fluid in oral diseases. The article aims to systematically review the utility of human urinary carcinogen metabolites as an approach for obtaining important information about tobacco and cancer. **Materials and Methods:** The following article reviews the use of urine and its metabolites as biomarkers in various lesions of the oral cavity including oral squamous cell carcinoma and as a screening method in evaluating tobacco and its components. A bibliographic comprehensive search was carried out in the main databases: PUBMED, SciELO, Google Scholar, VHL, and LILACS for articles that were published from 1985 to 2020. The inclusion criteria were "urinary metabolites," "oral cancer/HNSCC," "body fluids," "tobacco," and "metabolomics." A total of 55 articles were collected which included laboratory studies, systematic reviews, and literature of urinary metabolites in tobacco users. **Results:** Most of the studies carried out show accurate results with high sensitivity of urinary metabolite biomarkers in individuals with tobacco-based habits and lesions caused by them. **Conclusion:** The review indicates that urinary metabolite analysis demonstrates its applicability for the diagnosis and prognosis of disease. Urine is a remarkable and useful biofluid for routine testing and provides an excellent resource for the discovery of novel biomarkers, with an advantage over tissue biopsy samples due to the ease and less invasive nature of collection.

Keywords: *Biomarkers, body fluids, carcinogenesis, metabolites, urine*

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Introduction

The use of body fluids can be an interpretative tool in the diagnosis of various diseases.[1] Body fluids such as blood and urine have been used in pathology for quite some time.[2] Laboratory tests carried out in these body fluids can help know the presence or absence of disease to its severity and prognosis in a patient.[1] Recent advances in the field of biologic science have sparked new interest in the area of identifying biomarkers in body fluids. It has been shown that mutations present in the primary tumors or disease can also be identified in the body fluids of the affected patients.[2,3] Cancer-related analysis in blood, urine, and cerebrospinal fluid has been used successfully as cancer biomarkers.[4]

The oldest known test on body fluids was done on urine in ancient times (before 400 BC).[5] Urine is a very useful biofluid for routine testing and a wonderful means for discovering novel biomarkers. It has an advantage over tissue biopsies due to the ease and noninvasive nature of collection.[6] The current article is an attempt to review such urinary biomarkers that can be studied in tobacco users and oral squamous cell carcinoma (OSCC) patients.

Tobacco kills half of its users. More than 8 million tobacco users are killed annually. Eighty percent of the world's tobacco users are from developing nations. Smoked and smokeless tobacco forms are the two main ways to consume tobacco. Both are equally damaging to health and addictive.[7] These forms of tobacco contain toxic chemicals and constituents that are carcinogenic having cancer-promoting substances. Vast epidemiological studies conclude the risk of oral cancers and premalignant conditions attributed to tobacco use and dependence.[8] Other possible causative risk factors such as age, gender, alcohol, diet, and human papillomavirus may be associated, but tobacco is the most common.[9,10]

The incidence of head-and-neck (H and N) cancer exceeds half a million cases annually worldwide.[11] Oral cancer is the sixth most

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common human cancer, with a high morbidity rate and an overall 5-year survival rate of <50%.[12] About 75% of H and N cancers are oral cancers, and 90% of oral cancers are diagnosed as OSCCs.[13] It has been proven that the development of OSCC is associated with tobacco use. Various studies show how tobacco can cause epigenetic alteration of oral epithelial cells through its toxic metabolites and induce OSCC.[8] H and N cancers tend to be malignant in nature with chances of recurrence. Their prognosis has not improved despite technological and therapeutic advances.[14,15] There is an urgent need to discover more biomarkers for diagnosis, prognosis, therapeutic response prediction, and population screening of human cancers, which can hopefully improve treatment and reduce cancer mortality. Pathophysiological stimuli by such risk factors such as tobacco can alter the metabolic profile of biofluids such as urine, serum, saliva, and blood which can be used as an advanced and precise screening modality in disease state.[16] Urinary metabonomic analysis demonstrates its applicability for the diagnosis and prognosis of disease[17-19] and can be used as a complementary approach for early detection of oral cancer (OSCC)[20,21] [Figure 1].

Materials and Methods

The present systematic review was conducted according to the guidelines provided by the PRISMA statement. Published literature was searched to discuss the use of urinary metabolites as a biomarker for oral lesions as a screening tool in tobacco users. A comprehensive bibliographic search in the main electronic databases: PubMed (www.pubmed.gov), SciELO (www.scielo.org), Google Scholar (www.scholar.google.com.br), BVS (http//bvsalud.org/), and LILACS (http://lilacs.bvsalud.org) was performed using the inclusion criteria "urinary metabolites," "tobacco," "biomarkers," "body fluids," and "metabolomics." We collected papers with cross-references that were published from 1985 to 2020. The search included original laboratory studies, systematic reviews, and literature that were developed on human species. The search included a total of 55 articles from wherein we reviewed the utility of urinary metabolites as biomarkers in tobacco users.

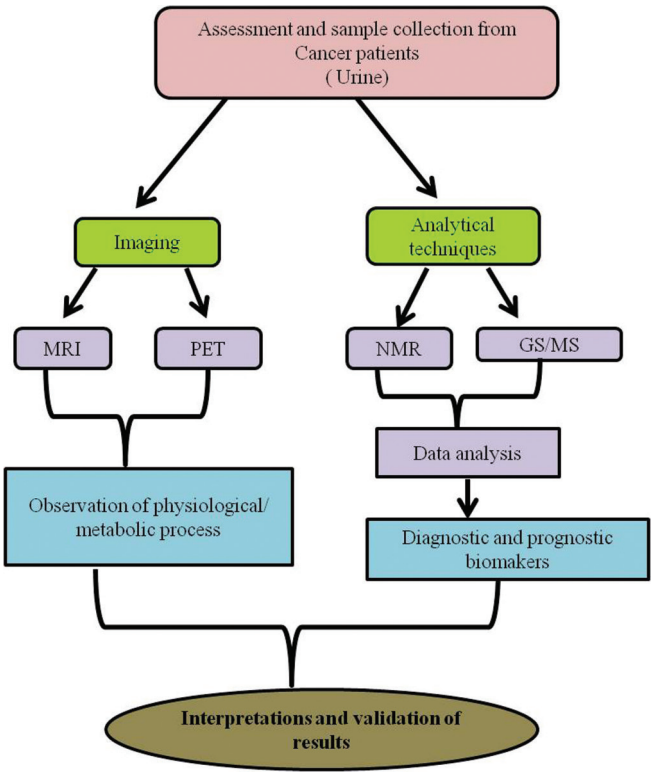


Figure 1: Step-wise method to use urinary metabolites as biomarkers in cancer screening

Urinary carcinogenic metabolites

Measurement of human urinary carcinogen metabolites is a practical approach for obtaining important information about tobacco and cancer.[22] Studies and research material show that OSCC and precancerous lesions are not only because of aberrant expression of genes and proteins but also abnormal concentrations of endogenous metabolites. Urine samples are not commonly used in H and N cancer metabolomic studies as compared to other body fluids, whereas it is widely used by metabolomic researchers for other conditions or diseases as it is easy to obtain and has a wide metabolic cover.[23]

Several types of carcinogenic biomarkers have been used till date. Human urinary carcinogenic metabolites can be an alternative means of detecting toxicity and carcinogenesis in subjects with tobacco habits [Figure 2]. Biomarkers from urine can help us understand tobacco-related cancer mechanisms. This will also help us develop preventive strategies which may decrease the toll of cancers[24] [Figure 3].

Urinary metabolites in tobacco smokers and nonsmokers

Carcinogenesis links nicotine addiction and cancers. Tobacco is consumed in various forms by millions of people in India. Individuals who do not smoke are exposed to passive smoking. These tobacco products contain thousands of chemical constituents including major alkaloids (nicotine) and minor alkaloids (noricotine, anabasine, anatabine, etc.). These alkaloids can react with nitrite to form nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which are called tobacco-specific nitrosamines. These tobacco constituents are important progenitors in the formation of tobacco-specific nitrosamines.[25] The major constituents of tobacco such as nicotine, cotinine, and nitrites + nitrates are excreted in the urine of tobacco-exposed individuals and used as the markers of tobacco exposure. Methods such as thin-layer chromatography, high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrophotometry (MS) can be used to estimate urine cotinine and nicotine levels in urine. Tobacco exposure to electrophilic moieties increases

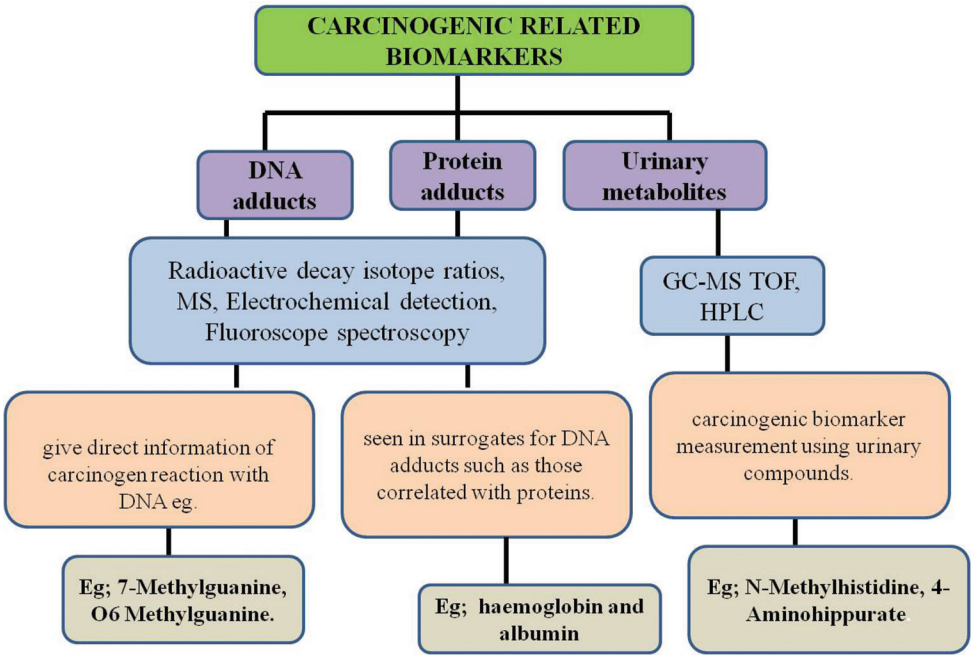


Figure 2: Types of carcinogenic biomarkers

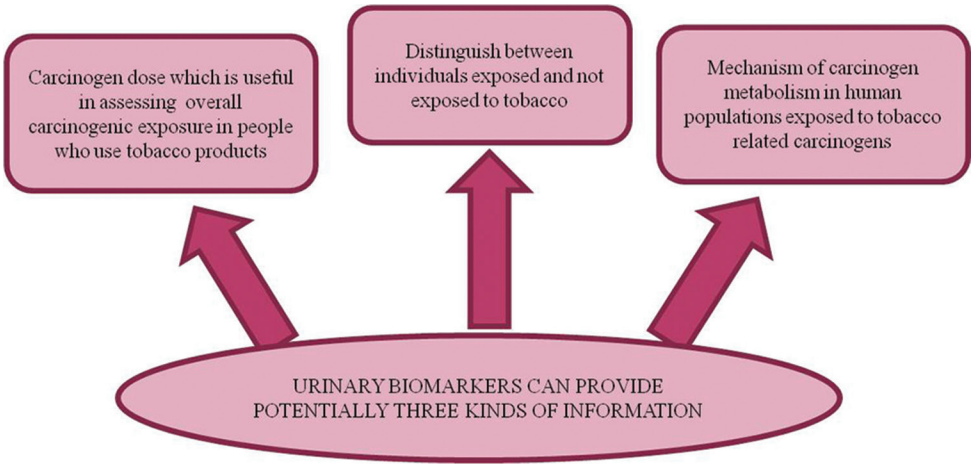


Figure 3: Uses of urinary biomarkers

urinary thioether levels which is another useful biomarker of tobacco exposure.[16,24-26]

Measurements of urinary compounds have many advantages. Important among these is their quantity which is sufficient enough with the use of modern analytical methods giving reliable data. Urine is simple to obtain in large quantity, and compliance is not a problem.[5,22,27]

Results

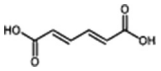
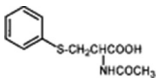
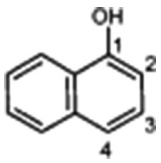
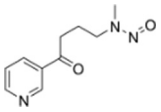
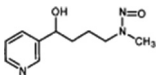
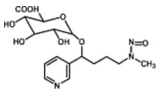
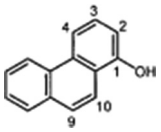
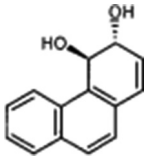
While reviewing the articles and literature, we came across various studies where urinary metabolites were assessed in smokers and nonsmokers. The studies showed results which supported and favored the presence of biomarkers in the urine of individuals with varying tobacco habits. The results of all those studies are summarized in Table 1 and further talked over in the discussion.

Discussion

Nuclear magnetic resonance (NMR) spectroscopy is a commonly used analytical method to analyze the small molecule composition that is the metabolome of body fluids such as urine and blood serum. Metabolite concentration is associated with the biochemical state of the organism. Conditions such as disease state and response to chemical treatment can change these metabolic concentrations. Recent studies demonstrate the applicability of NMR-based metabolomics using serum and urine samples for the diagnosis and prognosis of disease.[21]

Multiple researches on tobacco demonstrate that nicotine-derived nitrosamines such as NNK and NNN as well as nornicotine-, anabasine-, and anatabine-derived nitrosamines significantly contribute to tobacco carcinogenesis.[41] Wei

Table 1: Common Urinary Biomarkers

Chemical compound	Chemical structure of the urinary biomarker studies	Results in various studies and type of tobacco habit	
		Smokers	**Nonsmokers**
Benzene metabolite	 tt-MA	Significantly elevated levels in the urine of smokers. 1.4–4.8 times greater than nonsmokers[28] Boogaard *et al*.; no significant difference between tobacco habits[29]	No significant levels detected Mixed results obtained by environmental tobacco smoke-exposed subjects[22]
	 S-PMA	Significantly higher in smokers[30]	No significant levels detected
	 1-and 2-naphthol	Various studies suggest elevated levels in smokers[31,32] Nan *et al*. reported almost twice the levels in smokers and significant results (P<0.01)[33]	No significant levels were detected in studies
N-nitrosamine	 NNK	Strongest detected carcinogen that plays an important role in cancer induction (oral cancer, leukoplakia, and lung cancer)[34] Ratio of the following two metabolites of NNK have been intensively studied and detected in the urine of tobacco habit users in assessing exposure and screening oral cancers	
	 NNAL	Identified in urine along with NNAL-O-Gluc as NNAL is not present in cigarette smoke[35]	Not excreted in urine[35] Exceptionally high levels of both NNAL and NNAL-O-Gluc excreted[36]
	 NNALgluc (conjugated glucuronide form of NNAL-O-Gluc)	Present in urine, comprise 50±25% of total NNAL-0-Gluc[37] Both NNAL and NNAL-O-Gluc found[34]	Present in urine, comprise 24±12% of total NNAL-0-Gluc[37] Both metabolites of NNK are readily determined in tobacco chewers and snuff dippers. Same levels as smokers[34]
Polyaromatic hydrocarbon	 1-HOP	Significantly higher levels in smokers in most studies. Many studies show twice and even more levels of 1-HOP in smokers than nonsmokers[22,38]	Nonsignificant levels detected
	 Phenenthrene-1,2-dihydrodiol and phenanthrene-3,4-dihydrodiol	The ratio between these two metabolites decreases further with an increase in smoking	Studies done by Jacob *et al*.[39] and Heudorf and Angerer[40] show similar results of lower ratio of the two metabolites in both habit groups

tt-MA: Trans,trans-muconic acid; S-PMA: S-phenylmercapturic acid; 1-HOP: 1-hydroxypyrene; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL: 4-(methylnitrosamino)-1-(-3pyridyl)-1-butanol

et al. in their study established that NNK levels in the urine of tobacco-exposed individuals can help investigators ascertain the best means to protect people's health and help prevent cancer.[42] As mentioned in Table 1, Murphy *et al*. in their study using urine samples analyzed NNAL, and its glucuronide NNAL-O-Gluc along with 1-hydroxypyrene and anatabine found a positive correlation between anatabine and urinary NNAL and NNAL-O-Gluc (both metabolites of the tobacco-specific carcinogen NNK) showing their usefulness as a biomarker of tobacco exposure.[36,43] Kresty *et al*. concluded

a potentially important finding and relationship between the levels of NNAL, NNAL-Gluc along with cotinine in urine, and the presence of oral leukoplakia in their study.[34] Experiments have shown the induction of oral tumors in rat mucosa on applying a mixture of NNK metabolites validating the role of these products in inducing precancerous lesions of the oral cavity.[44]

One of the first attempts to study urine metabolites in screening oral lesions was done by Xie *et al*. using GC-MS in a combination of three differential metabolites. They used 6-hydroxynicotic acid, cysteine, and tyrosine to segregate between OSCC and OLK. Their study had a sensitivity and specificity of 85% and 89.7%, respectively, with an accuracy of 92.7%. Their study clearly indicated that urinary metabolite profiling can be a promising diagnostic tool for the early stages of OSCC and for differentiating between other oral lesions and conditions.[16]

As discussed in Table 1, benzene metabolites such as trans, trans-muconic acid (tt-MA) and S-phenylmercapturic acid (S-PMA) have been studied extensively to assess tobacco exposure and significantly high levels of these metabolites have been found in the urine of smokers.[28,29] Both tt-MA and S-PMA are the most sensitive biomarkers, the latter being more according to Boogaard.[29] Similarly, Nan *et al*. using benzene metabolites 1- and 2-naphthols came to the conclusion of the presence of high levels of these metabolites in smokers although no significant levels were noted in nonsmokers.[33]

The presence of polycyclic aromatic hydrocarbon metabolites in urine was first demonstrated by Jongeneelen[38] using HPLC, and since then, many variations to this method have been used to study the presence of these metabolites in the urine of tobacco-exposed individuals.

Besides the metabolites discussed above, studies to assess the role of tobacco habits as a risk factor for the development of oral cancer have been done by evaluating other urinary metabolites such as nicotine, cotinine, thioether, nitrite, and nitrate levels that have been compared in subjects with and without tobacco habit as discussed in the following section. Modified HPLC using a UV detector is used to analyze urinary nicotine and cotinine levels. Levels of nitrites + nitrates in tobacco and urine and urinary thioether levels are estimated by spectrophotometry. It has shown that tobacco chewing and smoking habits are prominent risk factors for the development of oral cancer. Urinary nicotine, cotinine, nitrite + nitrate, and thioether levels can be helpful for screening programs for oral cancer.[24,26]

Patel *et al*. in a study done in Gujarat, India, evaluated urinary nicotine, cotinine, thioether, and NO2 + NO3 levels in healthy individuals without habits of tobacco, healthy individuals with habits of tobacco, patients with oral precancers, and oral cancer patients; their results confirmed that tobacco chewing and smoking habits are prominent risk factors for the development of oral cancer. Urinary nicotine, cotinine, NO2 + NO3, and thioether levels can be helpful for screening programs for oral cancer.[24] In another study, Behera *et al*. using HPLC assay analyzed the urine of smokers and chewers for nicotine and cotinine levels and found these components as useful markers to assess the effects of different tobacco types.[45] Similarly, a study by Oberoi and Oberoi concluded a significant increase in urinary cotinine levels among smokers and smokeless tobacco individuals compared to nonsmokers.[46] Urinary cotinine is a widely used biomarker as it has the advantage of being 4–6 times more than blood or salivary cotinine and is highly sensitive when assessed.[47,48] Both smoked and chewed forms of tobacco are highly linked to the induction of OSCC. Higher risk is associated with greater amounts and longer duration of tobacco use.[24,41]

Urine metabolomics has emerged as an outstanding noninvasive realm in discovering biomarkers that can detect the slightest of metabolic discrepancies in response to a specific disease or therapeutic interpretation.[49] The development and advances in LC-MS/MS have revolutionized analytical studies of biomolecules including urine metabolome by enabling their accurate identification and in an unprecedented manner.[50] A study done by the Internal Radiation and Clinical Oncology Department, Maria Sklodowska-Curie Institute-Oncology Center, Poland, showed that metabolic alterations using NMR can be detected already at the beginning of the treatment, making it possible to monitor the patients with a higher risk.[51] MS-based metabolomic technology has provided exciting opportunities in the field of health and medical science. It is believed that, with the continuous progress in technologies, there will be more and more effective biomarkers for the diagnosis of clinical diseases and treatment.[52]

Conclusion

Urinary metabolomics is an efficient and accurate means to retrieve data about tobacco exposure and oral cancer. The biomarkers discovered and obtained from urine can enhance our knowledge and understanding of tobacco-related cancer mechanisms, which can help us evolve new strategies and action plans to help combat the loss of life to cancers. Various studies performed demonstrate that this robust and noninvasive profiling approach can be a promising screening tool for the early diagnosis of oral cancer. Furthermore, studies can highlight the applicability of urinary metabolite markers that can be used as a stratification tool in the diagnosis of different oral conditions, complementary to the existing clinical procedures.

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Conflicts of interest

There are no conflicts of interest.

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