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Gyawali, Prajwal; Oguoma, Victor Maduabuchi

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Review Article

Current research on cigarette toxicity: critical appraisal in view of clinical laboratory

Prajwal Gyawali[1]*, Victor Maduabuchi Oguoma[2]

[1]School of Biomedical Sciences, School of Community Health, Charles Sturt University, New South Wales, Australia
[2]School of Psychological and Clinical Sciences, Charles Darwin University, Northern Territory, Australia

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***Correspondence:**
Dr. Prajwal Gyawali,
E-mail: clbioprajwal@gmail.com

ABSTRACT

Cigarette smoking has been implicated as a potential risk factor for development and progression of chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD), including ischemic heart disease. Although, several methods are in existence to measuring cigarette toxicity, evidence regarding adoption of a gold standard technique is still imprecise. In this study, we reviewed articles describing methods of measuring cigarette toxicity in relation to clinical laboratory practice. A critical analysis of the benefits and limitations of each method in relation to low-middle income countries is discussed.

Key wards: Biomarkers, Cigarette, Toxicity testing

INTRODUCTION

Cigarette smoking has been implicated as a potential risk factor for development and progression of chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD), including ischemic heart disease.[1,2] It has been reported that up to 3 million people die each year from smoking in economically developed countries, 50% of them die before attaining the age of 70 years.[3] However, a substantial majority of the 1.1 billion smokers in the world in the year 1995 lived in low-middle income countries.[4] Over the last two decades the rate of cigarette smoking has increased significantly in these countries, in contrast to the decline in overall consumption in high income countries. The resulting higher rates of tobacco use in girls aged 13-15 years in these low-middle income countries is a reflection of aggressive tobacco industry marketing to girls, particularly in these part of the world.[5]

Cigarette smoking in humans is known to enhance fatty acid turnover, which is expected to increase synthesis of very low density lipoprotein, an important cardiovascular risk factor.[6] More so, chronic cigarette smoking has also been implicated to be strongly linked to insulin resistant and hyperinsulinaemia, with associated increases in plasma triglycerides and decreases in plasma high density-lipoprotein-cholesterol concentration.[7] Nicotine and carbon monoxide are the main toxic products of cigarette. They circulate in the bloodstream, interfering with the activities of the endothelium, which in turn elicit blood lipid abnormalities and impairing glucose regulation.[8]

Increased oxidative stress and apoptosis due to cigarette smoke has been demonstrated in human fibroblast.[9] Cigarette smoking produces remarkably high concentration of free radicals such as nitric oxide, hydrogen peroxide, peroxynitrate, etc.[10] These free radicals are known to elicit the prooxidant-anti-oxidant

imbalance, which plays a critical role in damaging enzymes, nucleic acids and cell membranes. Several studies have associated smoking with oxidative stress.[11-14] Oxidative stress has been considered to be one of the important factors for the development of metabolic syndrome, CVD, chronic obstructive pulmonary diseases (COPD) and cancer. Hence, generation of oxidative stress due to excessive free radicals present in the cigarette smoke could be one of a possible link between smoking and its systemic effects.

Cigarette smoking initiates systemic inflammatory response, via the release and inhibition of pro-inflammatory and anti-inflammatory mediators.[8,15] This increases the production of endotoxin, one of the most potent inflammatory agents which contributes to an elevated IgE and the subsequent development of atopic diseases and asthma.[15]

It is noted that its influence on the immune system is orchestrated by the increased inflammatory allergic and immune responses, and decreased systemic activity against infections.[15,16] The dangers associated with cigarette smoking is widely known and debated upon. However, the measure of cigarette toxicity is not well known or documented in routine clinical laboratory practice.

In the wake of strong campaign to ban cigarette smoking and the emergence of e-cigarettes, a (routine) measure of level of cigarette toxicity in smokers can be a sentinel for prevention/intervention on the corresponding risk factors or a useful strategy to advocate and advise smokers to quit smoking, especially when the threshold indicating high toxicity has been reached.

This critical appraisal will mainly focus on the methodological approach to cigarette toxicity in the clinical laboratory practice and its significance to low-middle income countries where higher cigarette smoking is on the increase.

What is known?

Methods of measuring cigarette toxicity (smoking status)

Smoking status of the research participants are generally assessed through a questionnaire on smoking behavior. This method relies upon self-reported cigarette consumption level which is not always scientifically valid, and often underestimates the true prevalence.[17,18] This point to the need for proper laboratory methods for accurate validating of self-reports that is of value to low-mid-income populations, in order to establish the precise association of smoking and its health effects.

Measurement of the level of exposure to cigarette smoking and toxicity has evolved from the use of self-reports to human matrices. Major technological advances have been developed over the last decade for analysis of cigarette toxicity in human matrices such as serum, plasma, urine, saliva, toenail, breath, breast milk and other body fluids. These methods are largely based on detection of nicotine and other metabolites such as cotinine, norcotinine, or trans-3'-hydroxycotinine to name a few.[19]

The aim of this work was to review available methods for measuring cigarette toxicity and discuss their availability for clinical laboratory adoption especially as it relates to low-mid-income populations. We discretionally searched on PubMed using keywords 'cigarette toxicity' OR 'smoking toxicity' and 'methods' for articles published over the last five years. Search was started in late March 2015 and ended on 10th September 2015. The search yielded a total of six hundred and fifty one articles. All articles were reviewed and those reporting work on smoking/cigarette toxicity, and methods of assessing smoking status/exposure were selected. References of selected articles were also assessed for other potential articles on the subject irrespective of the year of study. Discretional selection of an article on comparative measurement of tobacco smoke exposure was used for the critical appraisal.

Quantitative techniques used for measuring cigarette toxicity

Several techniques have been developed to measure metabolites related to cigarette toxicity in body fluids. Mass spectrophotometry coupled with gas or liquid chromatography has been "considered" to be standard reference method for measuring nicotine and its metabolite in the body fluids.[19,20] Both sensitivity and specificity of the mass spectrophotometry technique is high.[21] Researchers have also used high performance liquid chromatography, radio immunoassay and enzyme immunoassay for measuring markers of cigarette toxicity in body fluids.[22-25] Non-invasive techniques (such as Pulse Oximetry based on absorbance at particular wavelengths) are also commonly used for measuring carboxyhaemoglobin and carbon monoxide concentration apart from classical measurement of these analytes in blood by spectrophotometric techniques.[26,27]

Metabolites use in detecting smoking status

Expired Carbon monoxide in breathe

carbon monoxide level measurement in the breathe sample fairly indicates the smoking habit.[28] It was one of the earliest biochemical method used in determining smoking status of the individuals.[29] Carbon monoxide levels, measured by breathe analyser, in expired air, showed 96% sensitivity and 100% specificity in determining the smoking status among smokers and non-smokers.[30] It confirmed smoking cessation (quitters) in 74% of the past smokers.[30] However, half-life of carbon monoxide is very short (only 4-6 hours) and could not be

used in confirming smoking abstinence over more than a few hours.[31]

Carboxyhaemoglobin and Methaemoglobin

Carboxyhaemoglobin is formed when carbon monoxide combines with haemoglobin. Aniline, one of the major toxic agents in cigarette smoke, forms methaemoglobin in the blood.[32] Carbon monoxide level and carboxyhaemoglobin and methaemoglobin levels were found to be higher in the blood of smokers when compared to non-smokers.[33-35] Moreover, the level of carbon monoxide in blood was increased with the number of cigarettes smoked per day.[33] Mean percentage of carboxyhaemoglobin level was found to be significantly reduced after controlled reduction in per-day cigarette smoking.[36] However, a carboxyhaemoglobin level is the marker if elevated carbon monoxide is in the blood, which in turn also could be due to several environmental reasons apart from smoking.[34]

Oxidative stress and inflammatory markers

Increased oxidative stress and apoptosis due to cigarette smoke extract was demonstrated in human lung fibroblast.[9] Erythrocyte activity of the anti-oxidant enzyme catalase, superoxide dismutase and glutathione peroxidase were found to be decreased in smokers when compared to non-smokers. Hydrogen peroxide and superoxide dismutase were shown to be increased whereas reduced glutathione was shown to be decreased in smokers compared to non-smokers.[37] In contrast, the concentration of lipoperoxide was increased in smokers when compared to non-smokers.[37] Similarly, increased concentration of lipid peroxidation marker, f2-isoprostanes, has been shown among smokers when compared to age and sex matched healthy non-smokers.[38]

Cigarette smoking has been constantly associated with the systemic inflammation.[14,39,40] C-reactive protein (CRP) concentration has been associated with life time exposure to smoking among elderly subjects without any cardiovascular diseases.[41] Increased plasma concentration of inflammatory markers such as hsCRP, interleukin-6, E-selectin, P-selectin and soluble intercellular adhesion molecule type 1 were reported to be higher among apparently healthy women smokers in comparison to women non-smokers after adjusting for the effect of age, sex, body mass index, alcohol use, dyslipidaemia and history of diabetes and hypertension.[42] Similarly, inflammatory markers were shown to be further higher in current smokers than the former smokers and also inflammatory markers showed significant increase in their concentration parallel to the level of cigarette smoke exposure in the same study.[42]

Plasma level of adhesion molecules that initiate atherosclerosis are increased due to smoking.[42,43] Reduction of inflammatory markers in blood after cessation of smoking for a period of one month shown by a recent study also suggest the role of smoking on systemic inflammation.[44]

Increased Nitric oxide bioavailability is reduced and endothelium dependent vasodilation is decreased as a results of cigarette smoking.[45,46] Activity and expression of the enzyme endothelial nitric oxide synthase is altered by cigarette smoke.[47] The availability of nitric oxide is also reduced by the reaction between superoxide and nitric oxide that results in the generation of peroxynitrite, further aggravating oxidative stress.[48] Reduced bioavailability of nitric oxide could possibly leads to thrombotic events and cardiovascular diseases.[49]

Whole blood viscosity (WBV)

Increased WBV has been reported among smokers when compared to non-smokers.[50,51] Moreover, an increased in WBV in metabolic syndrome was shown to be independently predicted by smoking habit.[52] The increase in WBV depends on the numbers of cigarettes smoked per day and WBV has been reported to be normal after cigarette abstinence for certain period of time.[53-55] The direct mechanisms altering WBV due to cigarette toxicity is not very clear but cigarette smoking has been hypothesized to be one of the link between elevated WBV and metabolic diseases.[56] The increased level of fibrinogen among smokers may account to increase in WBV to a certain extent.[57] Also, the exact effects of nicotine on red blood cells are unknown but the increase aggregation of white blood cells in smokers has been attributed to the effect of nicotine.[58]

Salivary thiocyanate

Cigarette smoke contains hydrogen cyanide which is metabolised by the liver to thiocyanate. Salivary thiocyanate measurement in saliva was developed as an alternative to expired carbon monoxide measurement.[29] However, several limitations were found in measuring this analyte in biological fluids. The main limitation was the sensitivity and specificity of the assay, which was found to be lowered compared to expired carbon monoxide and other new methods.[30] Salivary thiocyanate, measured by colorimetric method, showed only 67% sensitivity and 95% specificity in determining the smoking status among smokers and non-smokers.[30] The other important limitation of the measurement is that the thiocyanate is present in several human diets and, therefore, there is always a chance of introducing false positive error in its measurement.[59]

Qualitative detection in urine

Detection of nicotine metabolite (diethylthiobarbituric acid) in urine by a change in colour after mixing urine with certain chemicals forms the basis of this test.[60] This is a non-specific method and is subjected to lot of errors.[61]

Cotinine level

Towards the end of 1980's, measurement of cotinine level became the method of choice for assessing smoking status.[31] Tobacco contains nicotine and it is the primary source of nicotine in human body. Though there are dietary sources of nicotine, they are insignificant compared to tobacco use.[62] Nicotine has a half-life of about two hours and is metabolized rapidly into different metabolites.[63] Nicotine could be measured in a biological fluid by chromatographic and immunoassay but due to its short half-life, its measurement is not useful in determining smoking status prior to 8 to 12 hours.[64,65]

Table 1: Response to critical appraisal checklist.

Thematic questions	Specific appraisal checklist	Answer	Comment
Is the study method valid?	Clarity of question for the study	Yes	Compared toenail nicotine biomarkers and self reports
	Comparison with an appropriate reference standard	Unknown	There is still debate regarding what the gold standard is across populations, ethnicity and socio-economic background especially in relation to cost, validity, reliability and level of misclassification
	Inclusion of samples with all the common presentations	Yes	Patients with self-reports were spread across never smokers, past smokers and current smokers, whose sum corresponds to the total studied toenail patients
	Whether assessors of the index diagnostic test were blinded to results of reference standard	Unknown	The two index methods were compared in order to arrive at an agreement.
	Whether the reference standard applied regardless of the index test result	Yes	The toenail nicotine levels were compared with self-reports of tobacco exposure
	Rationale for the reference standard	Yes	Detection of nicotine in body matrices is widely reported as more efficient than self-reports, although its utility is limited owing to its own flaws. The validation of a novel detection of nicotine in toenails forms the rationale of the study
Is the study result valid?	Whether cases without results for the index or reference test were explained	Not applicable	All 2,485 cases were accounted for. Choice of inclusion and exclusion of other confounders were explained.
	How equivocal results, and discrepancies between index and reference test were handled	Not applicable	The results from both methods were clearly presented. However, authors emphasized the superiority of the toenail nicotine detection over self reports
	Whether there are clear criteria for defining the severity of positivity	No	This was not established
	Tabulation of index test results based on the reference standard results	Yes	The associations and or relationships between the two methods (where applicable) were clearly outlined
	Whether the results include estimates of diagnostic test accuracy	No	The results were mainly presenting associations and prediction potential of each method
Is the study significant to low-mid income countries?	Whether clientele/samples from low-mid income countries are similar to those in the study	Yes	Nicotine exposure is same at all levels of economic status
	Whether the index test is affordable and available, as well as reflects current practice	inconclusive	Use of self-reports is largely common in LMICs as compared to quantitative detection of nicotine using high throughput techniques
	Whether the test result will change the way a patient is managed	inconclusive	As there is yet no gold standard for assessing smoking exposure, validation and adoption of other cost effective techniques that would benefit the LMICs is imperative

Cotinine is the major metabolite of nicotine with half-life much longer than that of nicotine (around 11 to 37 hours).[66] It can be measured in biological specimens.[64] However, several studies have recommended the measurement of cotinine instead, in biological fluid (urine, plasma or saliva) in assessing smoking status.[17,65,67] Cotinine level in biological fluid is measured by advanced chromatographic and immunoassay techniques.[68,69] A cut off of 14 ng/mL or 15 ng/mL in plasma and 50 ng/mL in saliva have been used in differentiating smokers from non-smokers in general population.[65,70]

Salivary cotinine, measured by gas chromatography method, showed 99% sensitivity and 100% specificity in determining the smoking status among smokers and non-smokers. The salivary cotinine confirmed smoking cessation (quitters) in 55% of the past smokers. Measurement of serum cotinine level by radioimmunoassay showed that 32.2% of the cohorts were current smokers in contrast to 30.9 % self-reported smokers in the same cohort.[71] Cotinine concentration has been shown to be associated with the number of cigarettes smoked per day.[72]

There are, however, some limitations associated with cotinine measurement in determining smoking status. The test is not valid if the individual is in nicotine-replacement therapy and the test also can not verify the long term smoking cessation.[31]

Human matrices for measuring smoking exposure

Decreased in-vivo synthesis of collagen and destruction of matrix collagen has been reported among smokers when compared to non-smokers.[73,74] Markers of collagen metabolism plasma pro-collagen 1-N propeptide and degradative enzymes matrix metalloproteinase 9 has been reported to be higher in blood among smokers. The abnormal production of matrix metalloproteinase due to smoke toxin causes connective tissue damage among smokers.[75,76] Also, nicotine has been shown to impair the extracellular matrix metabolism and growth factor signalling system on human osteoblasts affecting bone differentiation.[77] Effect of cigarette toxin is also seen on extracelluar matrix related gene of rat cerebral arteries.[78] Thus, abnormal extracellular matrix gene signalling and metabolism due to cigarette toxin has a potential to be a modern quantitative marker of cigarette toxicity.

However, in both clinical and research studies, other human matrices have been demonstrated as potential carriers of nicotine and its metabolites. Quantitative analysis of nicotine in blood has been performed using both paper spray and liquid chromatography MS.[79] A review of other human matrices other than blood, urine and saliva; such as dried blood spots, hair, toenail, breast milk placenta, sweat, and breath use in analysis nicotine and its metabolites is presented by El-Khoury JM et al.[19]

DISCUSSION

Several studies have arrayed the benefits of using biomarkers via high throughput techniques in detecting smoking status and toxicity.[80-82] The distinctions of these techniques over self-reports are centred on issues with validity, reliability and misclassifications.[80] In this appraisal, we would analyse the study of Al-Delaimy WK et al, which compared toenail nicotine biomarkers and self-reports in view of clinical laboratory practice. Responses to the critical appraisal questions are succinctly presented in Table 1. The overreaching finding of the study shows that toenail nicotine levels picked up overall burden of tobacco smoke and provided further information on exposure not picked by self-reported history.[83]

High performance liquid chromatography (HPLC) was the method employed for detection of nicotine in the nail samples. This technique has limitation of less separation efficiency, albeit its precise and highly reproducible potential.[84] However, its objective measure of exposure to cigarette smoking highlights its precision as compared to self-reports.[83] The innovative advancement of HPLC to incorporate mass spectrometry (MS) is shown to be a powerful qualitative and quantitative analytical technique. Its strengths as being highly sensitive and specific, but marred with high instrument cost has been outlined.[19] This is one of the limitations restraining its adoption in clinical laboratories in low-middle income countries. Other limitations such as low throughput, inadequate service support from manufacturers and the need for extensive training and highly skilled personnel are some of the reasons restricting its usage in clinical laboratories.[19]

The fact that there is no ‘gold standard’ technique for measuring the level of cigarette toxicity, choice of method has largely rested upon the clinician, technician and or researcher. Evidence has suggested imprecise detection of cotinine in human matrices like urine, saliva and blood. This has led to novel development and validation of alternatives such as toenails, hairs, and detection of multiple metabolites of nicotine exposure.[19,83,85,86] However, what has not been largely discussed and adopted for the benefit of LMICs is the potential of haematological and haemorheological parameters as possible clinical diagnostic markers for cigarette toxicity.

Haustein KO et al argued that plasma fibrinogen, reactive capillary flow and transcutaneous partial oxygen tension were improved in smokers who abstained for about 26 weeks.[87] Again haematocrit and white blood cell count decreased extensively in abstainers.[87] On a different account, Galea G et al observed highly significant differences in whole blood viscosity, plasma viscosity, plasma fibrinogen concentrations, packed cell volume and caboxyhaemoglobin concentrations between smokers and non-smokers.[88] Smoking cessation leads quickly to

improvement of clinical, functional and laboratory parameters such as reduced carboxyhaemoglobin, increased oxyhemoglobin and increased average expiratory flow between 25 – 75% of vital capacity.[89] These suggest that adoption and validation of common routine haematological and haemorrheological parameters would be of value in LMICs for assessment of cigarette toxicity.

CONCLUSION

The results from this review show that there is no established gold standard method for clinical laboratory assessment of cigarette toxicity. The available hi-tech techniques largely used in research laboratories are not readily available in many clinical laboratories of the developing countries that are currently experiencing significant increase in cigarette smoking; and consequential COPD and CVD morbidity and mortality. As the world is transiting to the use of e-cigarettes amid the surge in non e-cigarette usage in developing countries; it's imperative that affordable, reliable and readily accessible clinical laboratory methods for assessing cigarette status/toxicity should be validated for the benefit of the LMICs.

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