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Research Article

Evaluation of oxidative stress and whole blood viscosity for clinical laboratory testing of smoking toxicity

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ABSTRACT

Background: There are no clearly established clinical biochemical markers for cigarette smoking despite the knowledge that cigarette smoking is a risk factor for various diseases, especially cardiovascular complications of respiratory pathologies. However, there are reports of significant increases in blood viscosity and oxidative stress among smokers. The main objective of the study was to ascertain the association of toxicity from cigarette smoking on whole blood viscosity in our data.
Methods: This study analysed the archived clinical data of 20 cigarette smokers and 20 apparently healthy individuals.
Results: The data show that on average, oxidative stress levels are relatively the same between groups, while whole blood viscosity is statistically significantly lower in non-smokers compared to smokers.
Conclusions: This report suggests that oxidative stress induced whole blood hyper-viscosity could be a valid biomarker for laboratory testing of smoking toxicity among cigarette smokers.

Keywords: Clinical biomarkers, Smoking toxicity, Oxidative stress, Whole blood viscosity

INTRODUCTION

There are no clearly established clinical biochemical markers for cigarette smoking despite knowledge of smoking-induced stress, yet it is conventionally accepted that cigarette smoking is a risk factor for various diseases including cancer, cardiovascular disease (CVD) and type II diabetes. For instance, smoking is one of the modifiable factors in risk assessment of heart disease, as well as death and diabetes progression.[1-3]

In vitro methods are useful for screening toxicity from tobacco smoked, but better methods are needed for today's context of regulation and evaluation of health claims as well as to evaluate claims by manufacturers on modified tobacco products.[4,5] This need is superimposed on the use of therapeutic smoking that is now increasing. That is, the smoker's paradox acknowledges the substantial benefits of therapeutic smoking, which has contributed to adoption of therapeutic smoking and development of new drugs; but the concept of evaluation of efficacy and toxicity has yet to be seriously discussed.[6]

Also, industrial and household smoke could cause oxidative stress, which would go on to induce hyperviscosity.[7-9]

Therefore, there is an obvious need to develop a unified approach for assessment of cigarette toxicity, especially for non-smokers, because this group is exposed to second-hand smoke and also third-hand smoke that is the accumulation of first-hand smoke over a period becoming more toxic in the process.[5,10] Also, since tobacco causes a duration-dependent increase in oxidative stress, this

impacts on toxicity.[11] Studies demonstrate that quitting smoking is associated with improvement in measurable biomarkers that pre-dispose to diseases such as CVD.[12]

For instance, it has been shown that compared with subjects who continued to smoke, quitters had higher plasma HDL cholesterol concentrations.[13] Other studies also report of improved plasma total homocysteine concentrations in quitters.[12,13]

It has been reported that age and smoking are two independent factors that affect the deformability of human RBCs, and nicotine has been shown to inhibit the efficacy of antihypertensive treatment on RBC deformability.[14,15]

It is therefore important to understand the association of nicotine vis-à-vis toxicity from tobacco ingested and/or smoked (recreational, industrial or domestic) on haemorheology including whole blood viscosity.

The clinical symptoms of tobacco toxicity include dizziness, malaise, nausea and vomiting, bradycardia and dilated pupils as well as organ damage.[16] It is therefore important that in addition to assessment of these clinical features there are pathology biomarkers for smoking and tobacco toxicity.

Assessment of tobacco toxicity by mere count of number of cigarettes is inaccurate because there are differences in metabolism due to ethnicity and method of smoking and the sizes and types of the cigarettes also differ as well as the volumes of inhaled smoke.[17]

Hence, assessment of nicotine metabolism has become an integral part of some nicotine dependency treatment programs, whereby reduced and zero level of nicotine metabolites and anabasine are used to validate reduction or abstinence from tobacco and its products.[18] The metabolites; nicotine, cotinine, trans-3′-hydroxy cotinine, nornicotine, and anabasine in various biological fluid such as urine, serum, and plasma can be tested using liquid chromatography-tandem mass spectrometry.[19]

The hypothesis

We reported that WBV varies between individuals with different stages of macrovascular pathogenesis and recommended that the criteria for WBV could be redefined on the basis of underlying oxidative stress and/or consequential stasis.[20-22] Smoking is a risk factor in assessment of heart disease.[3]

The associated factors which constitute a vasculopathy triad (homocysteinaemia, hypercoagulability and hyperviscosity) are some of the emerging laboratory markers for assessment of CVD.[23,24]

The hypothesis in this study is that the levels of oxidative stress biomarkers and WBV are higher in cigarette smokers relative to a non-smokers' group. A positive finding will be valuable to further the discussion on early identification of subclinical macrovascular complications of respiratory diseases, especially in smokers.

METHODS

This work analysed archived clinical data (N=20) of cigarette smokers, which constituted the main 'smokers' group. Another 20 apparently healthy individuals who did not smoke were then selected to constitute the control 'non-smokers' group. The archived data were collected as part of doctoral research, but now being analysed in a different context.[20,25]

All participants had results for malondialdehyde (MDA), methaemoglobin (metHb), reduced glutathione (GSH) and WBV.

Some participants in the smokers group were individuals without any obvious health conditions, while some were taking medications for various reasons.

All participants in the non-smokers group were without any known health condition nor were they taking any medication and had normal blood glucose, blood pressure and cholesterol profile levels.

RESULTS

A review of statistics appears to indicate some discrepancies, especially in relation to our hypothesis.

The data show that on average, oxidative stress level are higher (lower erythrocyte GSH in conjunction with higher erythrocyte MDA and metHb) while whole blood viscosity was lower in non-smokers compared to smokers (Figure 1).

A critical review of the results also indicates no statistically significant differences between the groups in the level of oxidative stress biomarkers, excepting WBV level which is significantly different (Table 1).

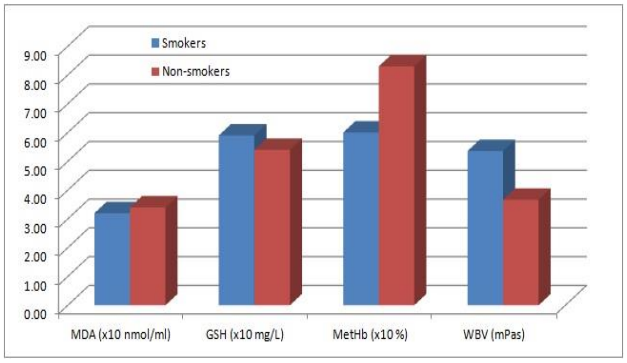

Figure 1: Comparative levels of oxidative stress and WBV levels between groups.

Further review of the smokers group indicate 25% (4/20) have pre-existing health conditions and are taking medications; another 10% (2/20) were be probably prediabetes from our laboratory screening (Table 2).

Table 1: Descriptive statistics.

	Age (Years)		MDA (nmol/ml)		GSH (mg/L)		MetHB (%)		Viscosity (mPas)	
	Smoker	Non-smoker	Smoker	Non-smoker	Smoker	Non-smoker	Smoker	Non-smoker	Smoker	Non-smoker
Mean	56.80	55.55	0.32	0.34	0.59	0.54	0.60	0.83	5.36	3.67
Median	60.00	54.00	0.31	0.23	0.56	0.58	0.53	0.59	4.25	3.89
SD	12.02	9.78	0.18	0.32	0.23	0.18	0.36	0.70	3.37	1.22
Min	37.00	38.00	0.05	0.17	0.10	0.21	0.16	0.17	1.11	0.63
Max	74.00	74.00	0.84	1.58	1.27	0.81	1.36	2.69	12.42	6.32
Kurt	-1.02	-0.78	3.66	12.71	3.78	-1.12	-0.61	2.77	0.56	1.74
P- value	0.72		0.81		0.50		0.21		0.05	

Table 2: State of health among the smokers' group.

State of health	Percentage of cohort
Apparently healthy	65
Cardiovascular disease (CVD)	5
Diabetes & CVD co-morbidity	20
Probable prediabetes	10

DISCUSSION

There is no dispute that cigarette smoking is a major cause of modifiable morbidity and premature mortality.[1,2] According to data from the American Lung Association in a 2011 report, cigarette smoking was responsible for approximately one in five (20%) deaths in the United States.[26]

In particular, respiratory diseases rank third on the list of health complications attributable to deaths due to cigarette smoke and the report estimated that reduction of smoking rate to 15% by 2023 could save about USD 31.4 billion on pulmonary conditions and raise productivity by about USD 79 billion.[26] What is yet to be seriously discussed is how to access subclinical pulmonary conditions, in order to improve early identification, before obvert clinical symptoms and complications occur.

In this albeit small retrospective study, the hypothesis 'that the levels of oxidative stress biomarkers and WBV are higher in cigarette smokers relative to non-smokers' group' has been investigated.

The result shows that WBV is statistically significantly higher in smokers compared to the non-smokers group. This observation is in agreement with the report of a prospective study that had sample size similar to our (n=20), which showed high significant difference in WBV of smokers.[27]

Although the observation from this study does not indicate statistical significant differences between smokers and non-smokers on oxidative stress biomarkers, this cannot be translated to be lack of smoking effect on clinical biochemistry parameters. Rather, it may be that smoking-induced oxidative stress is inseparable from the stress associated with the clinical conditions of non-smokers including but not limited to a complex of secondary smoking, environmental smoke pollution and/or chronic diseases.

For instance, there have been mixed reports some indicating that methaemoglobin level being higher smokers, and other reports indicating greater levels in in non-smokers.[28,29] Further, oxidative damage has been implicated in various diseases and it seems that the mechanism of air pollution-induced health effects involves oxidative stress.[30] Increased malondialdehyde levels in breath after experimentally exposing adult human subjects to wood smoke in a controlled environment is an example of this effect.[31]

Further studies are needed to discretely compare apparently healthy populations of non-smokers versus smokers. In making this recommendation, it must be pointed out that there is difficulty in recruiting apparently healthy smokers. Perhaps, it may be necessary to consider using teenagers and young adults who are newly initiated into smoking. In the cohort of smokers in this study, 25% reported having pre-existing health conditions and were taking medications but another 10% were identified as probable prediabetes from our laboratory screening (Table 2).

A confounding effect of this is that the ongoing medical treatments may be ameliorating oxidative stress among those who have pre-existing health conditions. This effect may be insignificant to WBV, which is quite specific for blood stasis.[22,32] Further, the sample size was small in this

preliminary study and this was a limiting factor. A larger population may give us some more significant stats.

CONCLUSION

This report provides additional evidence that WBV is significantly increased in smokers compared to non-smokers. It also provides indication that oxidative stress indices may be varied among cigarette smokers relative to non-smokers.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Angrave D, Charlwood A, Wooden M. Working time and cigarette smoking: evidence from Australia and the United Kingdom. Soc Sci Med. 2014;112:72-9.
2. Cena H, Tesone A, Niniano R, Cerveri I, Roggi C, Turconi G. Prevalence rate of Metabolic Syndrome in a group of light and heavy smokers. Diabetol Metab Syndr. 2013;5(1):28.
3. D'Agostino RB, Vasan RS, Pencina MJ, et al. General Cardiovascular Risk Profile for Use in Primary Care: The Framingham Heart Study. Circulation. 2008;117(6):743-53.
4. Johnson MD, Schilz J, Djordjevic MV, Rice JR, Shields PG. Evaluation of in vitro assays for assessing the toxicity of cigarette smoke and smokeless tobacco. Cancer Epidemiol Biomarkers Prev. 2009;18(12):3263-304.
5. Thielen A, Klus H, Muller L. Tobacco smoke: unraveling a controversial subject. Exp Toxicol Pathol. 2008;60(2-3):141-56.
6. Aune E, Roislien J, Mathisen M, Thelle DS, Otterstad JE. The "smoker's paradox" in patients with acute coronary syndrome: a systematic review. BMC Med. 2011;9:97.
7. Granados-Castro LF, Rodriguez-Rangel DS, Montano M, Ramos C, Pedraza-Chaverri J: Wood smoke exposure induces a decrease in respiration parameters and in the activity of respiratory complexes I and IV in lung mitochondria from guinea pigs. Environ Toxicol. 2015;30(4):461-71.
8. Kurmi OP, Dunster C, Ayres JG, Kelly FJ. Oxidative potential of smoke from burning wood and mixed biomass fuels. Free Radic Res. 2013;47(10):829-35.
9. Rosa MJ, Yan B, Chillrud SN, Acosta LM, Divjan A, Jacobson JS, et al. Domestic airborne black carbon levels and 8-isoprostane in exhaled breath condensate among children in New York City. Environ Res. 2014;135:105-10.
10. Martins-Green M, Adhami N, Frankos M, Valdez M, Goodwin B, Lyubovitsky J, et al. Cigarette smoke toxins deposited on surfaces: implications for human health. PLoS One. 2014;9(1):e86391.
11. Samal IR, Maneesh M, Chakrabarti A. Evidence for systemic oxidative stress in tobacco chewers. Scand J Clin Lab Invest. 2006;66(6):517-22.
12. Stein JH, Bushara M, Bushara K, McBride PE, Jorenby DE, Fiore MC. Smoking cessation, but not smoking reduction, reduces plasma homocysteine levels. Clin Cardiol. 2002;25(1):23-6.
13. Tonstad S, Urdal P. Does short-term smoking cessation reduce plasma total homocysteine concentrations? Scand J Clin Lab Invest. 2002;62(4):279-84.
14. Salbas K. Effect of acute smoking on red blood cell deformability in healthy young and elderly non-smokers, and effect of verapamil on age- and acute smoking-induced change in red blood cell deformability. Scand J Clin Lab Invest. 1994;54(6):411-6.
15. Salbas K, Gurlek A, Akyol T. In vitro effect of nicotine on red blood cell deformability in untreated and treated essential hypertension. Scand J Clin Lab Invest. 1994;54(8):659-63.
16. Furer V, Hersch M, Silvetzki N, Breuer GS, Zevin S. Nicotiana glauca (tree tobacco) intoxication-two cases in one family. J Med Toxicol. 2011;7(1):47-51.
17. Benowitz NL, Hukkanen J, Jacob PI. Nicotine Chemistry, Metabolism, Kinetics and Biomarkers. In: Nicotine Psychopharmacology. Edited by Henningfield J, London E, Pogun S, vol. 192: Springer Berlin Heidelberg; 2009: 29-60.
18. Moyer TP, Charlson JR, Enger RJ, Dale LC, Ebbert JO, Schroeder DR, et al. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolic profiles. Clin Chem. 2002;48(9):1460-71.
19. Yue B, Kushnir MM, Urry FM, Rockwood AL. Quantitation of nicotine, its metabolites, and other related alkaloids in urine, serum, and plasma using LC-MS-MS. Methods Mol Biol. 2010;603:389-98.
20. Richards RS, Nwose EU. Blood viscosity at different stages of diabetes pathogenesis. Br J Biomed Sci. 2010;67(2):67-70.
21. Nwose EU, Cann NG. Whole blood viscosity issues VI: Association with blood salicylate level and gastrointestinal bleeding. North Am J Med Sci. 2010;2(10):457-60.
22. Nwose EU, Butkowski EG. Algorithm for whole blood viscosity: Implication for antiplatelet bleeding risk assessment. Austr J Med Sc. 2013;34(2):50-5.
23. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation. 2004;109(25 Suppl 1):IV6-19.
24. Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. Br J Haematol. 1997;96(1):168-73.
25. Nwose E, Richards R, Kerr P, Tinley R, Jelinek H. Oxidative damage indices for the assessment of

subclinical diabetic macrovascular complications. Br J Biomed Sc. 2008;65(3):136-41.
26. American Lung Association. Trends in tobacco use [http://www.lung.org/ finding-cures/our-research/ trend-reports/ Tobacco-Trend-Report.pdf]
27. Galea G, Davidson RJ. Haematological and haemorheological changes associated with cigarette smoking. J Clin Pathol. 1985;38(9):978-84.
28. Imbriani M, Melotti A, Ghittori S. Methemoglobin and carboxyhemoglobin levels in smokers and non-smokers. G Ital Med Lav. 1987;9(1):11-4.
29. Borland C, Harmes K, Cracknell N, Mack D, Higenbottam T. Methemoglobin levels in smokers and non-smokers. Arch Environ Health. 1985;40(6):330-3.
30. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. Journal of toxicology. 2011; 2011:487074.
31. Barregard L, Sallsten G, Andersson L, et al. Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. Occup Environ Med. 2008;65(5):319-24.
32. Nwose EU, Cann NG, Butkowski E. Whole blood viscosity assessment issues III: Association with international normalized ratio and thrombocytopenia. North Am J Med Sci. 2010;2(7):301-5.

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