Influence of the type of smoked cigarettes on indicators of oxidative stress in surgical and non-surgical specialists

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Introduction

Oxidative stress is defined as an imbalance between the activity of reactive oxygen species (ROS) and the activity of antioxidants. The disturbances can occur both at the cellular and the whole body levels. Long-term oxidative stress is one of the key risk factors for the development of diseases, including atherosclerosis, Alzheimer's disease, Parkinson's disease and heart diseases. Factors that may increase the risk of long-term oxidative stress include smoking, alcohol, obesity, and a diet high in sugar and fat.

- Tobacco kills up to half of its users.
- Tobacco kills more than 8 million people each year. More than 7 million of those deaths are the result of direct tobacco use while around 1.2 million are the result of non-smokers being exposed to second-hand smoke.
- Over 80% of the world's 1.3 billion tobacco users live in low- and middle-income countries.
In 2019, more than one fifth of Poles - 21%, admit to heavy (everyday) smoking.

HNBs are tobacco products that produce aerosols containing nicotine and other chemicals. HNB tobacco products heat tobacco to 350 degrees Celsius using battery-powered heating systems.
HNB tobacco products are not e-cigarettes. HNBs heat tobacco to produce nicotine, while e-cigarettes heat an e-liquid, which may or may not contain nicotine and, in most cases, is tobacco-free.
Like the electronic cigarette, HNB cigarettes are promoted by their manufacturers as a much safer alternative for smokers.
Smoking is a risk factor for many diseases. Tobacco smoke is highly carcinogenic and contains nearly 4,000,000 chemicals, many of which are carcinogenic.

Aim

The aim of my project is to prove that the use of stimulants such as tobacco and the method of their consumption have an impact on the human body. One of the tasks of the project is to demonstrate the relationship between smoking and an increase in oxidative stress. The aim is to test the levels of reactive oxygen species in smoking and non-smoking physicians taking into account surgical and non-surgical specialties, including resident physicians

Methodology

The study is conducted prospectively in the Łódź agglomeration. A control group consisting of non-smokers was created.
Each of them was asked to complete a questionnaire and was subjected to a blood test, which was taken from the basilic vein.
10 ml of whole blood from the basilic vein will be used to assess the level of fluorescence coefficient of free radicals: hydrogen peroxide which is a reactive form of oxygen (ROS).
Tested material:
- peripheral blood collected into a tube with heparin anticoagulant
Method Principle: Peripheral blood diluted with PBS is layered over a defined volume of the density gradient. After centrifugation, interphase rich in lymphoid cells is collected.
At the bottom of the tube, the erythrocytes and granulocytes that have passed through the Gradisol layer are collected.
 Execution:
- Dilute the blood collected for heparin in a 50 ml tube with PBS solution in a ratio of 1:3.
- Introduce Gradisol L and blood suspended in PBS in a 1:1 (3:1) ratio into a 15 ml tube so as to maintain the phase boundary.
- Centrifuge the tubes for 20 min at 20°C at 2000 rpm. - Collect a ring of mononuclear cells from the interphase.
- Rinse twice in PBS. Centrifuge at 4°C at 200 g.
- collect the cell pellet supernatant and resuspend in 0.5 ml PBS.
-Stain cells in 1X probe buffer (H2DCFDA working solution) for final effect
concentration of ~1-50 µM dye (100 µl per well for 96-well format). The optimal working concentration for a given application must be determined empirically.
Incubate at 37°C for 30-60 minutes. Wash cells once in 1X assay buffer. Treat test cells with ROS or H2O2 inducing agents.
Incubate for the desired time. Proper incubation time may require experimental determination. Signal intensity was measured with a fluorescent microplate reader at Ex/Em = 485/530 nm.
The questionnaire included questions on: sex, age, type of specialization performed, stage of specialization training, chronic diseases and smoking time, possible chronic and allergic diseases.
 In addition, they were asked to solve the Fagerström Test.

Work progress and preliminary results

The average age of the doctors in the study is 41 years. 52.46% are doctors of non-surgical specialties. Most of the participants - 77% smoked classic cigarettes. The average Fagerstrom test score is 5.34482759. The highest average test scores were reported by e-cigarette and IQOS smokers. (Fig.1)
The average ROS fluorescence score is 17255.2167. The results indicate that ROS levels are higher in smokers than in non-smokers. The highest fluorescence coefficient was shown by e-cigarette and IQOS smokers. (Fig.2)
The collected data will then be analyzed using STATISTICA 13.1 statistical software (StatSoft Inc., USA). Detailed results and conclusions will be developed.
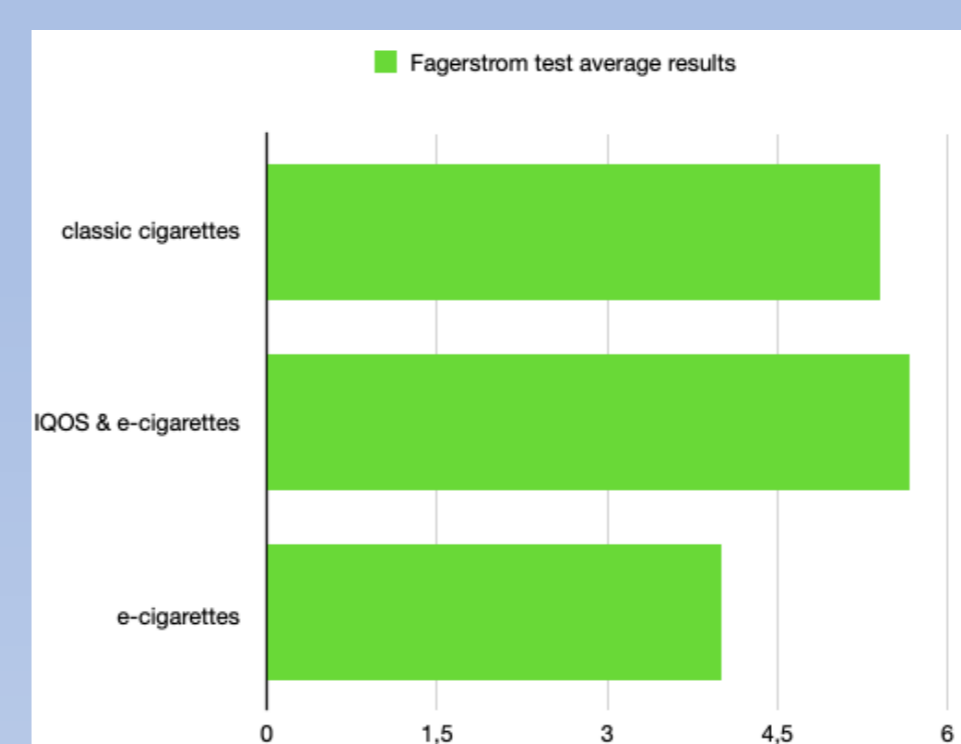There are plans to prepare a publication based on the results.
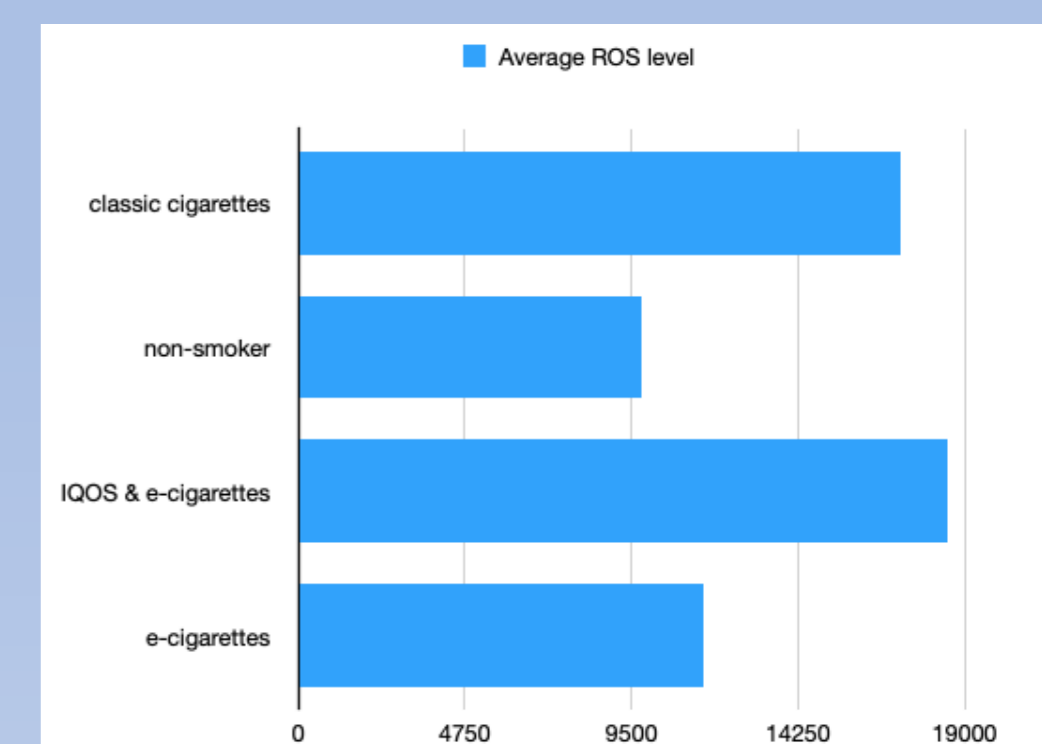

Figure 1

Figure 2

Bibliography
1. Chaumont.M De Becker.B, Zaher.W, Culié.A, Deprez.G, Christian Mélot.C, Reyé.F, Van Antwerpen.P, Delporte.C, Debbas.N, Boudjeltia.KZ, Van de Borne.P. Differential Effects of E-Cigarette on Microvascular Endothelial Function, Arterial Stiffness and Oxidative Stress: A Randomized Crossover Trial. Sci Rep. 2018 Jul 10;8(1):10378.
2. Guanghe.W, Wenjing.L, Weimin.S. Toxicity Assessment of Electronic Cigarettes . Inhal Toxicol. 2019 Jun;31(7):259-273.
3. Kopa PN, Pawliczak R. IQOS - a heat-not-burn (HnB) tobacco product - chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. Toxicol Mech Methods. 2020 Feb;30(2):81-87
4. Lorgis.L, Zeller.M, Dentan.G, Sicard.P, Richard.C, Buffet.P, Huillier.I.L, Beer.J.C, Cottin.Y, Rochette.L, Vergely.C. The free oxygen radicals test (FORT) to assess circulating oxidative stress in patients with acute myocardial infarction. Atherosclerosis.2010 Dec;213(2):616-21.
5. Garelnabi.M.O, Brown.W.V, Le.N-A . Evaluation of a novel colorimetric assay for free oxygen radicals as marker of oxidative stress. Clin Biochem. 2008 Oct;41(14-15):1250-4.