

Comparison of Total Chromium (Cr) Levels in Blood Samples of Conventional Cigarettes and Electric Cigarettes Users

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Abstract: The extensive levels of heavy metals in the environment constitute a significant toxicity hazard to humans. Cigarettes contain heavy metal ions. Cigarettes that are generally consumed by the public are conventional cigarettes and e-cigarettes. In conventional cigarettes, heavy metals come from tobacco, while in e-cigarettes they come from the constituent components and liquids. One of the metal ions found in the two cigarettes is Chromium (Cr). Normal blood Cr levels are below 1.4 $\mu\text{g/L}$. The purpose of this study is to determine the difference Cr levels in the blood of conventional cigarette users and e-cigarettes. This study used a cross-sectional approach on 11 blood samples of conventional cigarette users and 11 blood samples of e-cigarette users. The level Cr was tested using an Atomic Absorption Spectroscopy (AAS) method. The results of the Cr level obtained by the Mann Whitney non-parametric test obtained a significant value of 0.005 ($p < 0.05$) which shows that there is a significant difference in Cr levels between the blood of conventional cigarette users and e-cigarettes.

Keywords: Blood, chromium, conventional cigarettes, e-cigarettes.

Introduction

The extensive levels of heavy metals in the environment constitute a significant toxicity hazard to humans (Felix & Ntarisa, 2024). Toxic heavy metals do not serve any biological role in humans, plants, or other living organisms. Even at low concentrations, these metals pose significant health risks. The most hazardous heavy metals include lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), and chromium (Cr) (Genchi *et al.*, 2020; Ilusanya *et al.*, 2024). Human exposure to metals occurs through various pathways, including ingestion or inhalation of contaminated soil and dust, and bioaccumulation via food, water, pesticides, and other sources (Rastian *et al.*, 2022).

Certain plants, including tobacco, are capable of accumulating heavy metals by absorbing metal ions and compounds from the soil through their roots and transporting them to the leaves (Ozcan *et al.*, 2019; Felix & Ntarisa, 2024). Several studies analyzing heavy metal content in conventional tobacco cigarettes, have

reported that Cr is among the heavy metals detected at significant levels, alongside other metals such as Pb, Cd, and Ni (Hossain *et al.*, 2018; Sing & Yadav, 2023; Sandal *et al.*, 2025). During cigarette combustion, accumulated metals are partitioned into mainstream smoke inhaled by smokers, sidestream smoke released from the burning tip, and residual ash and cigarette filters (Papas *et al.*, 2006; Lisboa *et al.*, 2020).

Pb and Cd levels are significantly elevated in four of the five lung lobes of smokers compared to nonsmokers, whereas Cr concentrations are significantly higher across all five lobes in smokers's lungs (Hossain *et al.*, 2018). Combustion temperatures can reach approximately 800°C between puffs and up to 950°C during puffing, providing sufficient energy for metal volatilization and transformation into more toxic forms, such as the oxidation of Cr compound to Cr hexavalent (Cr(VI)) (Sogor *et al.*, 1998; Lisboa *et al.*, 2020). Cr occurs naturally in trivalent (Cr(III)) and hexavalent (Cr(VI)) forms. While Cr(III) is

essential for certain metabolic processes, Cr(VI) is highly toxic and potentially carcinogenic, with its adverse effects primarily mediated through oxidative stress, free radical generation, and disruption of cellular antioxidant defenses (Milnerowicz et al., 2015).

The shift in smoking trends in society and the development of cigarette types have begun to enter Indonesia. In today's era, a new trend has emerged in cigarette consumption, namely electronic cigarettes. Electronic cigarettes or e-cigarettes are a type of cigarette that uses batteries as an energy source (Widyantari, 2023). E-cigarette generated aerosol through resistive heating of a metal coil that contacts a viscous e-liquid stored in the device reservoir. The e-liquid typically consists of organic solvents, such as propylene glycol and glycerol, along with varying amounts of nicotine and flavoring agents. When the coil is activated, it is heated to several hundred degrees celcius, vaporizing the e-liquid during inhalation (Fuoco et al., 2014; Rastian et al., 2022). As air is drawn through the device, the vapor cools and condenses into an aerosol that is subsequently inhaled, delivering nicotine as well as other potentially harmful constituents. Common coil materials include Nichrome, a nickel-chromium alloy, and Kanthal, composed of iron, chromium, and aluminum (Farsalinos et al., 2015; Son et al., 2019).

Research finding by Aherrera et al. (2017) demonstrated that Ni and Cr, key components of e-cigarette heating coils, as evidenced by positive associations with Ni and Cr levels in urine, saliva, and exhaled breath condensate Prokopowicz et al., 2020. Another study reported significantly lower blood Cd levels in e-cigarette user compared with conventional cigarette smokers (Prokopowicz et al., 2019; Prokopowicz et al., 2020). Another research found that elevated serum concentrations of selenium, silver, and vanadium in e-cigarette users, along with more frequent detection of beryllium, europium, and lanthanides compared with cigarette smokers (Badea et al., 2018; Prokopowicz et al., 2020). Serum Cr level normally less than or equal to 1.4 $\mu\text{g/L}$ or 26.92 nmol/L according UCSF (2023). Increased Cr level may result if humans are overexposed to the substance.

Although electronic cigarettes are widely perceived as a safer alternative to conventional

cigarettes due to the lack of combustion product such as tar and carbon monoxide (Gong et al., 2023), existing evidence on internal heavy metal exposure among e-cigarette users remains limited. Many existing studies primarily focus on the concentrations of metals in e-liquids or aerosol. Assessing data of heavy metal levels in biological samples, particularly blood, are still scarce. Moreover, direct comparisons between conventional cigarette smokers and electronic cigarette users are limited. The objective of this study is to determine the differences in blood Cr concentrations between conventional cigarette smokers and electronic cigarette users. The urgency of this research lies in its potential to provide a strong scientific basis for assessing the health risks associated with electronic cigarette use and increasing awareness of the potential heavy metal exposure arising from different smoking behaviors.

Materials and Methods

Ethical Clearance

This research has received approval from Ethics Committee of Universitas Muhammadiyah Purwokerto (KEPK-UMP) with registration number KEPK/UMP/120/VIII/2024.

Study Area and Period

This study was conducted in Surakarta and Sukoharjo, Indonesia. Blood samples were collected from residents of Ngruki Village and members of the vape-user community in Surakarta. Analysis Cr levels in blood samples were performed at the Health and Calibration Laboratory Center (Balai Laboratorium Kesehatan dan Kalibrasi, BLKK) Yogyakarta. This study was carried out from August 2024 to May 2025.

Study Design

This study used a cross-sectional approach to compare the levels of the heavy metal Cr in the blood between conventional cigarette and e-cigarette users. The study sample consisted of male respondents who were active users of conventional cigarettes and members of the e-cigarette community. The respondents must not be a dual user cigarette. Sampling was carried out using purposive sampling by applying predetermined inclusion and exclusion criteria.

According to Nursalam (2016), purposive sampling is widely applied in cross-sectional health research to select participants who meet predefined inclusion criteria. Venous blood samples were collected from the antecubital vein into EDTA tubes to prevent coagulation and preserve integrity for Cr analysis. Sample were processed and stored according to standard protocols for trace metal analysis (Ahmed et al., 2015).

Instruments and Material

The instruments used included a Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) system, an electric heating plate, measuring pipettes (10 mL and 1 mL), volumetric flasks (500 mL, 100 mL, 50 mL, and 10 mL), a glass stirring rod, a funnel, and forceps. The material used consisted of venous blood samples, a Cr stock solution (1000 ppm), concentrated nitric acid (HNO_3), and distilled water (aquabidest).

Measurements Cr Levels

The Cr levels were analyzed using a GFAAS. Sample preparation involved acid digestion, in which 2 mL of venous blood was digested with 10 mL of concentrated HNO_3 under heating until a clear solution was obtained. The digested sample was then diluted to a final volume of 10 mL with distilled water.

A Cr standard solution was prepared from a 1000 ppm stock solution, followed by the preparation of calibration standards at concentrations of 10, 20, 30, 50, and 100 ppb. Cr measurements were performed at the appropriate wavelength for Cr using GFAAS. Calibration curves generated from standard solutions, and Cr concentrations were calculated based on the linear regression equation obtained from the calibration curve. Results were expressed in $\mu\text{g/L}$.

Statistical analysis

In this study, quantitative data consisted of total Cr concentrations obtained from blood samples analyzed using GFAAS. All numerical data were categorized based on the type of cigarette use (conventional cigarettes and e-cigarettes). Normality testing, Shapiro-Wilk was performed for each group prior to statistical analysis. An independent T-test, Mann-Whitney

U test, was subsequently applied based on the results of the normality test that assumed non-normally distributed.

Result And Discussion

Result

Based on Table 1 below, the average Cr content of electric cigarette users is 3.657 $\mu\text{g/L}$ and the average Cr content of conventional cigarette users is 1.644 $\mu\text{g/L}$. The difference between the two levels is 2.013 $\mu\text{g/L}$.

Table 1. Average Cr content

Type of cigarette	Average Cr ($\mu\text{g/L}$)
Conventional Cigarettes	1,644
E-cigarettes	3,657
Average difference	2,013

In order to assess whether a significant difference exists Cr levels between users of conventional and electronic cigarettes, statistical analysis was conducted using IBM SPSS version 25. Prior to performing the T-test, a normality assessment was carried out to determine if the data followed a normal distribution. The outcomes of the Shapiro-Wilk normality test are summarized in Table 2.

Table 2. Normality Test of Cr Levels in Conventional dan E-cigarettes

Cr levels	Type of cigarette	Shapiro-Wilk		
		Statistic	df	Sig.
Conventional Cigarettes	Conventional	0,814	11	0,014
	E-cigarettes	0,913	11	0,263

Data analysis continued with the Non-Parametric Mann-Whitney test.

Table 3. Mann Whitney Test

Chromium levels
Asymp. Sig. (2 tailed)
Exact Sig. [2*(1-tailed Sig.)]
a. Grouping variable : Type of cigarette
b. Not corrected for ties

Discussion

Shapiro-Wilk test was used since the number of samples was less than 50. This test obtained sig. 0.263 for the type of e-cigarette and 0.014 for the type of conventional cigarette. Since the significance value obtained for e-cigarettes was more than 0.05, it can be concluded that the Cr levels of e-cigarettes are normally distributed. Conversely, since the significance value of conventional cigarettes is less than 0.05, it can be concluded that the Cr levels of conventional cigarettes are not normally distributed. Since one of the variables is not normally distributed, it is assumed that the research data is not normally distributed. Therefore, data analysis was continued with the Mann Whitney non-parametric test.

Based on the results of the Mann-Whitney test, the asymptotic significance value (Asymp. Sig. 2-tailed) was 0.006, and the exact significance value (Exact Sig.) was 0.005. Since both of these significance values are smaller than the significance limit of 0.05, it can be concluded that there is a significant difference in Cr levels between the groups of e-cigarette smokers and conventional smokers. The significant difference in Cr levels between the groups of conventional cigarette users ($1.644 \mu\text{g/L}$) and e-cigarette users ($3.657 \mu\text{g/L}$), with an average difference of $2.013 \mu\text{g/L}$, can be explained by various factors, including differences in Cr sources, release mechanisms, and chemical forms of Cr produced by each type of cigarette.

In e-cigarettes, Cr can come from a combination of e-liquid and a metal coil that functions as a heating element. The coil heating process takes place at a temperature of around $200\text{--}300^\circ\text{C}$ and has the potential to cause degradation of metal materials which then release Cr ions into the aerosol (Omaiye et al., 2021). The size of aerosol particles produced by e-cigarettes tends to be smaller than conventional cigarette smoke, so it has a deeper penetration ability into the respiratory tract and is easier to diffuse into the blood circulation (Mulder et al., 2020). In addition, Cr^{3+} released from the coil can be oxidized to Cr^{6+} under imperfect heating conditions (Sawicka, et al., 2021). Cr^{6+} has higher solubility, so it is absorbed into the blood more quickly and has a stronger toxic effect than Cr^{3+} . This heating process has the potential to produce Cr in hexavalent form (Cr^{6+}) which is more toxic

and difficult to excrete by the body (Reif & Murray, 2024)

In conventional cigarettes, Cr primarily originates from tobacco raw materials, which naturally contain heavy metals, including Cr at concentrations of approximately $2.35 \mu\text{g/L}$ (Caruso et al., 2014). During tobacco combustion at temperatures ranging from 600 to 900°C , pyrolysis occurs and generates various chemical compound, including Cr species (Muzyka et al., 2022). This process creates a highly reactive environment characterized by both reductive and oxidative zones. In oxygen-deficient reductive zones, trivalent chromium (Cr^{3+}) predominates due to its greater stability, whereas in oxidative zones, a portion of Cr^{3+} may be oxidized to hexavalent chromium (Cr^{6+}) (Tripathi & Chaurasia, 2020). Although Cr^{3+} is more commonly detected in mainstream cigarette smoke, studies have demonstrated that Cr^{6+} can still be formed and distributed within both the gas phase and fine particulate fractions of cigarette smoke (IARC, 2009).

However, blood Cr levels in conventional smokers tend to be lower than in e-cigarette users. This can be explained by the body's ability to excrete Cr^{3+} more efficiently. Cr^{3+} has low solubility in water, so most of the Cr particles in cigarette smoke are retained in the filter or remain in the ash, and only a small portion enters the body. The Cr^{3+} that does enter is also more easily eliminated through urine (Tripathi & Chaurasia, 2020). Previous studies have shown that Cr levels in the urine of smokers are higher than those of non-smokers ($33.41 \pm 14.99 \mu\text{g/L}$ vs. $27.45 \pm 10.49 \mu\text{g/L}$), which indicates the body's efficiency in eliminating Cr through the kidneys (Tian et al., 2014).

In addition to the source and chemical form of chromium (Cr), smoking behaviors such as smoking frequency and the amount of daily consumption also influence blood Cr levels. Individuals who smoke more than 10 cigarettes per day or consume more than 60 mL of e-liquid per week tend to exhibit higher Cr concentrations in blood compared to those with lower consumption levels. This finding indicates that consumption intensity has a direct relationship with the degree of exposure to heavy metals, including chromium, as higher smoking frequency and volume result in increased inhalation and systemic absorption of metal-

containing particles and aerosols (Bernhard et al., 2005; Aherrera et al., 2017). Similar associations between smoking intensity and elevated blood or biomarker metal concentrations have been reported in population-based studies, supporting a dose-response relationship between tobacco product use and metal exposure (CDC, 2020).

Conclusion

Based on the results of the study, a significance value of 0.005 ($p < 0.05$) was obtained, indicating that there was a significant difference in chromium (Cr) levels between the blood of conventional cigarette and e-cigarette users. The average Cr level in e-cigarette users (3.657 $\mu\text{g/L}$) was higher than in conventional cigarette users (1.644 $\mu\text{g/L}$), indicating that exposure to heavy metals from e-cigarettes is potentially higher. Therefore, it is recommended that further researchers use a larger sample size and conduct in-depth analysis of specific forms of chromium such as Cr^{3+} and Cr^{6+} , so that the research results are more representative and able to provide a more comprehensive understanding of the toxicological risks associated with chromium exposure from e-cigarette use.

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