ORIGINAL ARTICLE

Understanding the Correlation of Cotinine on Early Vascular Function Decline via Monocyte Chemotactic Protein-1 Activation in Smokers

Comprendre la corrélation entre la cotinine et le déclin précoce de la fonction vasculaire via l'activation de la protéine chimiotactique monocytaire-1 chez les fumeurs

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Abstract

Introduction: Oxidative stress describes an imbalance of pro-oxidant and antioxidant levels in cells. This study aimed to investigate the significant correlation of Cotinine on Monocyte Chemotactic Protein-1 (MCP-1) and Nitric Oxide (NO) as an indicator of vascular function.
Methods: The research, designed as a comprehensive cross-sectional study, included a sample of non-smokers (n=100) and smokers (n=100). The study utilised Cotinine, MCP-1 Human MCP-1 ELISA kit, and Nitric Oxide Colorimetric Assay kit, with data analysis conducted using Wrap Partial Least Square.
Results: This study showed a significant effect between Cotinine with MCP-1 (p-value <0.001) and Cotinine with NO (p-value <0.029). The impact of MCP-1 on NO resulted in a p-value of <0.001. Indirectly, it can be known that the effect of Cotinine on NO through MCP-1 produces a p-value of <0.001. The study concluded a significant direct association between Cotinine and MCP-1, Cotinine and NO, and MCP-1 and NO. Meanwhile, the indirect relationship of Cotinine on NO through MCP-1 is paramount.
Conclusion: These findings underscore Cotinine's direct and indirect results on vascular function decline, contributing significantly to our understanding of vascular inflammation and providing a new perspective on the impact of smoking on cardiovascular health.

Keywords: Cardiovascular risk; MCP-1; nitric oxide; cotinine; smokers

Résumé

Introduction : Le stress oxydatif décrit un déséquilibre des niveaux de pro-oxydants et d'antioxydants dans les cellules. Cette étude visait à examiner la corrélation significative de la cotinine sur la protéine chimiotactique des monocytes-1 (MCP-1) et l'oxyde nitrique (NO) comme indicateur de la fonction vasculaire.
Méthods: La recherche, conçue comme une étude transversale complète, comprenait un échantillon de non-fumeurs (n = 100) et de fumeurs (n = 100). L'étude a utilisé la cotinine, le kit ELISA MCP-1 humain MCP-1 et le kit d'analyse colorimétrique de l'oxyde nitrique, avec une analyse des données réalisée à l'aide de la méthode des moindres carrés partiels Wrap.
Résultats : Cette étude a montré un effet significatif entre la cotinine avec MCP-1 (valeur p < 0,001) et la cotinine avec NO (valeur p < 0,029). L'impact de MCP-1 sur NO a donné lieu à une valeur p de < 0,001. Indirectement, on peut savoir que l'effet de la cotinine sur le NO via MCP-1 produit une valeur p de < 0,001. L'étude a conclu à une association directe significative entre la cotinine et le MCP-1, la cotinine et le NO, et le MCP-1 et le NO. Parallèlement, la relation indirecte de la cotinine sur le NO via MCP-1 est primordiale.
Conclusion : Ces résultats soulignent les résultats directs et indirects de la cotinine sur le déclin de la fonction vasculaire, contribuant de manière significative à notre compréhension de l'inflammation vasculaire et offrant une nouvelle perspective sur l'impact du tabagisme sur la santé cardiovasculaire.

Mots clés : risque cardiovasculaire ; MCP-1 ; oxyde nitrique ; cotinine ; fumeurs

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Introduction

Cardiovascular Disease (CVD) is a group of blood vessel and heart disorders, such as rheumatic heart disease, coronary heart disease, cerebrovascular disease, and other conditions. Heart attacks and strokes cause one-third of the deaths to occur in people under 70 years (1). Cardiovascular disease consists of coronary artery disease (CAD) and acute coronary syndrome (ACS) (2). According to WHO (2021), heart disease is the leading cause of death globally. It is estimated that as many as 17.9 million people die each year (1). According to primary health research data in 2018 in Indonesia, the prevalence of CVD in Indonesia is 15 out of 1000 people, or currently, 4.2 million people have CVD.

Smoking is one of the significant risks for CVD. The pathological of CVD is related to oxidative stress that causes mitochondrial changes (3). Oxidative stress describes an imbalance of pro-oxidant and antioxidant levels in cells. Oxidative stress can cause cell damage and disrupt the state of proteins, lipids, and DNA (4). The nicotine metabolite is Cotinine, the primary degradation product of nicotine metabolism. Cotinine has a serum half-life of about 17 hours, so researchers use it to estimate exposure to tobacco smoke in blood serum in passive and active smokers (5). About 70 to 80% of nicotine is rapidly metabolised to Cotinine in the liver. Therefore, blood cotinine is considered a sensitive, direct, and specific marker of tobacco exposure (6). Tobacco smoke contains >200 toxins, including nicotine, carbon monoxide (CO), and oxygen-free radicals that can induce oxidative stress on vascular walls, which in turn will cause vascular inflammation and arteriosclerosis (7). Inflammation in response to oxidative stress will result in the synthesis of chemokines, including Monocyte Chemoattractant Protein-1 (MCP-1), whose primary source is endothelial cells, smooth muscle cells, and macrophages (8,9). MCP-1 is essential in vascular inflammation and endothelial dysfunction, attracting or increasing the expression of other inflammatory factors/cells (10).

Long-term smoking has increased MCP-1 and pro-inflammatory cytokines (11). MCP-1-deficient mice have been shown to produce less ROS (12). ROS plays an essential role in the occurrence of vascular inflammation. Increased ROS levels can reduce the bioavailability of Nitric Oxide (NO) and cause endothelial dysfunction (13). NO is a signaling molecule that modulates many cardiomyocyte functions, from ATP formation to sarcomere contraction. NO is synthesised in the blood vessels by nitric oxide synthases (NOS). NOS is a family of enzymes that includes endothelial NOS, neuronal NOS, and inducible NOS (14). The eNOS isoform is constitutively expressed in the vascular endothelium. It maintains vascular tone through intrinsic NO synthesis, blocking the adhesion of platelets and leukocytes to the endothelium, thereby inhibiting the pro-inflammatory state. One study reported that NO regulates MCP-1 gene expression, whereas NO suppresses MCP-1 expression by blunting redox changes associated with cytokine-induced endothelial cell activation (15). Cotinine as a marker of exposure to cigarette smoke is not yet known—the role of its metabolites on vascular inflammatory conditions is assessed from MCP-1 and NO status.

Endothelial dysfunction contributes to atherosclerotic plaques and subsequently causes plaque rupture, reducing blood flow due to thrombosis and vasospasm, leading to heart disease (6). As a result, NO production is reduced, and ROS increases (16). Exposure to cigarette smoke can also cause inflammation of the walls of blood vessels in response to oxidative stress, resulting in the synthesis of chemokines, including MCP-1, by endothelial cells. In addition, MCP-1 induces monocyte migration to the vessel wall and activates monocytes during the development of atherosclerosis. MCP-1 has a crucial role in the pathogenesis of metabolic syndrome, a collection of conditions that elevate the likelihood of developing heart disease, stroke, and diabetes. It is associated with various metabolic parameters, such as obesity and diabetes (17,18). This study aims to explain the mechanism of Cotinine's direct and indirect relationship to MCP-1 and NO in smokers.

Methods

Study design and patient selection

The design of this study was cross-sectional, a meticulous approach that allowed for a comprehensive understanding of the correlation between Cotinine and vascular function decline. Blood serum samples were collected from non-smokers (n=100) and smokers (n=100) at the Central Laboratory of Saiful Anwar General Hospital. The participants were men who met the inclusion criteria: 20-40 years old and did not have dyslipidemia, diabetes mellitus, or hypertension.

The absence of diabetes and dyslipidemia was confirmed through a meticulous process, ensuring the reliability and validity of our findings. The respondent's medical history and venous blood tests at the Central Laboratory-Saiful Anwar Hospital were thoroughly reviewed. The respondent's blood sugar level was confirmed by testing the fasting and the haemoglobin A1c (HbA1c) levels using the high-performance liquid chromatography method. The average value of HbA1c is 4%-5.6%. Fasting blood glucose was counted using the hexokinase enzyme reference method. The standard fasting blood glucose level is below 100 mg/dL. The analytical method was used to calculate cholesterol using an enzyme assay with a standard value of less than 200 mg/dL in adults. Researchers confirmed those without a history of hypertension by taking a medical history and measuring blood pressure with a digital manometer. Respondents were asked to lie down and measure their blood pressure. Standard blood pressure criteria for systolic in this study ranged from 110-120 mmHg, and for diastolic, 70-80 mmHg.

The research variables measured were carefully chosen to understand the subject comprehensively. To ensure the accuracy and reliability of the biochemical

measurements, stringent quality control (QC) procedures were implemented throughout the analysis. All assays were performed in duplicate, and mean values were used for statistical analysis to minimize intra-assay variability. These included participant traits (age, smoking status and duration, type of cigarette, level of addiction), Cotinine serum, MCP-1, and NO. Each variable is crucial to the investigation and contributes to the study's findings.

The researcher confirmed the respondents' age by checking each subject's identity cards. Smoking status was established based on urine cotinine measurements using the Human Cotinine Enzyme-Linked Immunosorbent Assay (ELISA) kit with Cat. No. E2043Hu. Respondents were identified as non-smokers if the cotinine concentration was 0-10ng/mL. The duration of smoking was measured using a questionnaire, starting from the beginning of smoking until the study was conducted. Types of cigarettes included mild (0.8-1.1 mg nicotine and 10-18 mg TAR/cigarette), kretek (60 mg nicotine and 40 mg TAR/cigarette), and Electric (16 mg nicotine/mL). The level of addiction in smokers was measured by The Fagerström Test for Nicotine Dependence (FTND). It consists of six questions that are designed to determine the level of nicotine addiction, with a score of 4-6 indicating mean nicotine addiction.

Detection of Cotinine

Human Cotinine Enzyme-Linked Immunosorbent Assay (ELISA) kit with Cat No E2043Hu was used to analyse Cotinine levels in blood serum samples. Plates have been coated with Human Cotinine antibodies. The Cotinine sample is added to the coated plate and binds to the antibody on the well plate. The plate was washed five times, and then substrate solutions A and B were incubated for 10 minutes at 37°C. A stop solution was added, and the optical density value was read for 10 minutes. The quality control procedures were done in Cotinine level measurement by calibration curves which were generated using manufacturer-provided standards, and samples with coefficients of variation (CV) >10% were reanalyzed. The ELISA plates included internal controls (low, medium, and high Cotinine concentrations) to verify assay precision, with inter-assay CV maintained below 8%.

Detection MCP-1 Levels

Human MCP-1 ELISA Kit from Elabscience with Catalogue No. E-EL-H6005 was used to detect MCP-1 levels in the respondent's blood serum. The principle of this ELISA kit uses Sandwich-ELISA, which has been provided with a specific antibody on Human MCP-1. First, the sample was placed in the micro-ELISA plate wells and merged with the specific antibody. Next, 100 µL of the sample was added to the healthy plate and incubated for 90 minutes at 37°C. After that, the liquid was removed, and 100 µL of Biotinylated Detection Ab was added and incubated for 1 hour at 37°C. Next, samples were aspirated and washed thrice, 100 L of HRP conjugate was added, and 30 minutes were incubated at 37°C. Next, the sample was aspirated and soaked for 5 minutes, and then 90 µL of substrate reagent was incubated for 15 minutes at 37°C. Finally, the stop solution was added to 50 µL. To maintain the quality of the data in MCP-1 detection, pre-coated plates were validated using spike-and-recovery experiments, demonstrating recoveries of 90–110% in pooled human serum. Absorbance readings were normalized to blank wells, and outliers beyond ±2 standard deviations from the mean were excluded. The assay's sensitivity (limit of detection: 4.69 pg/mL) and specificity (no cross-reactivity with related cytokines) were confirmed per the manufacturer's protocol.

Detection NO Levels

Detection of NO levels in the respondent's blood serum using a Nitric Oxide Colorimetric Assay kit from Elabscience with Catalogue No. E-BC-K035-S. The instruments used are a Spectrophotometer (550 nm), vortex mixer, Centrifuge, Analytical Balance, and Micro-pipettor. Fresh blood samples were collected at 25°C for 30 minutes into blood clots—centrifuge at 2000g for 15 min at 4°C. NO measurement followed a standardized colorimetric protocol, with fresh Griess reagent prepared daily to avoid degradation. A nitrate/nitrite standard curve (0–100 µM) was included in each run, and samples were assayed in batches to reduce inter-day variability. Hemolyzed or lipemic samples were excluded to prevent interference. Instrument calibration (spectrophotometer and centrifuge) was performed before each use, and QC samples (10% of total) were interspersed to monitor precision (CV <5%).

Data Analysis

In the analysis, we have presented the characteristics of the respondents descriptively, using proportions for the categorical measurement scale and the mean ± standard deviation for numerical data. Differences in Cotinine, MCP-1 and NO levels in the participant group were analysed using an independent t-test. Meanwhile, Wrap Partial Least Square tested the relationship between Cotinine, MCP-1, and NO levels. The analysis was conducted with a p-value ≤ 0.05 (95%).

Ethical Clearance

The research outlined above has received permission from the Health Research Ethics Committee-Saiful Anwar Hospital under Ref. No. 400/097/K.3/302/2021. Before participation, all individuals involved were requested to provide their signature on an Informed Consent form. Furthermore, according to the Declaration of Helsinki, all patients who participated in this study provided informed consent.

Results

Characteristics of non-smokers and smokers: age, cigarette classification, smoking period, level of nicotine dependency, and age can be seen in Table 1. The table shows that the most significant percentage appears in the productive age with an age range of 35-40 years (28%) of smoker respondents, then the age of 31 -35 years (27%), 25-30 years (23%) and the lowest percentage is 20-25 years (22%). Meanwhile, the highest rate of non-smoking respondents was in the 20-25 years (34%). Judging from the type of cigarettes, most smokers (50%) use mild kinds of cigarettes, then kretek (30%), and a small portion (20%) use e-cigarettes. According to the highest level of addiction among respondents, there was mild addiction (40%) in 40 respondents, then severe addiction (37%) in 37 respondents, and moderate addiction (23%) in 23 respondents. According to the length of smoking, most (36%) of respondents with a period of 6-10 years, then (28%) within a range of < 5 years, then at least (1%) more than 20 years.

Table 1. Participant Characteristics Among Non-Smoker and Smoker Group

Participant Characteristics	Non-Smoker (n=100)	Smoker (n=100)
Age (years)		
20 - 25	22 (22.0%)	34 (34.0%)
25 - 30	23 (23.0%)	25 (25.0%)
31 - 35	27 (27.0%)	19 (19.0%)
35 - 40	28 (28.0%)	12 (12.0%)
Cigarette Classification (n, f%)		
Mild cigarette		50 (50%)
Kretek (Clove) Cigarette		30 (30%)
E-cigarette		20 (20%)
Duration of Smoking (years)		
≤ 5		28 (28%)
6-10		36 (36%)
11-15		20 (20%)
16-20		15 (15%)
>20		1 (1%)
Nicotine Dependency Level (n, f%)		
Low		20 (20%)
Low-Moderate		30 (15%)
Moderate		19 (19%)
High		31 (31%)

Table 2 showed that the Cotinine and MCP-1 levels in non-smoker participants were lower than in the smoker group. On the other hand, the non-smoker group had higher NO levels than the smoker group.

The effect of Cotinine on MCP-1 activity and vascular function as measured by NO was tested using PLS Wrap. The Goodness of fit Model results in the PLS analysis used the coefficient of determination (R-squared) and Q-squared predictive relevance (Q2). For example, in Figure 1, The coefficient of determination (R-square) of the MCP-1 variable is 0.32 or 32%. Therefore, the value can indicate that Cotinine can explain the diversity of the MCP-1 variable by 32%. On the other hand, the NO variable's coefficient of determination (R-square) is 0.13 or 13%. Therefore, the Cotinine variable can explain the variability of the NO variable by 13%. Then, the Q-square of the NO variable is 0.12.

Table 2. Differences in Cotinine, MCP-1, and NO levels among participant groups

Variables	Non-Smoker (n=100)	Smoker (n=100)	p-V
Cotinine (ng/mL)	9.25 ± 3.25	32.84 ± 24.97	<0.001
MCP-1 (pg/mL)	5.70 ± 2.64	89.87 ± 51.87	<0.001
NO (µmol/L)	73.96 ± 14.52	59.22 ± 20.72	0.042

* Tested using t-independent at a significance level of 0.05

The results of hypothesis testing directly in Table 3 show a significant effect between Cotinine with MCP-1 p-value <0.001 and Cotinine with NO p-value <0.029. The impact of MCP-1 on NO resulted in a p-value of <0.001. Indirectly, in Table 4, it can be seen that the effect of Cotinine on NO through MCP-1 produces a p-value of <0.001. In this study, the characteristics of the respondents included age, length of smoking, level of addiction, and type of cigarette. All respondents are male, ranging from 20 to 40 years. Older age may affect the high levels of Cotinine because of the length of exposure to second-hand smoke. Increasing the number of cigarettes smoked each day causes increased Cotinine levels, which has been widely reported in previous studies (19). Increased Cotinine levels are also influenced by decreased Cotinine metabolic function in the liver with increasing age (20).

Table 3 quantifies the annual cigarette consumption stratified by nicotine dependency level, reporting both daily averages and cumulative yearly figures in two standardized units: individual sticks and 20-stick packs. The data indicate a strong positive relationship between nicotine dependency severity and consumption volume.

Table 3. Annual Cigarette Consumption Among Smoker Participants

Nicotine Dependency Level	Daily Average	Annual Consumption	Annual Consumption (1 pack = 20 sticks)
Low	8 sticks	2,920 sticks	146 packs
Low-Moderate	13 sticks	4,745 sticks	237 packs
Moderate	18 sticks	6,570 sticks	329 packs
High	22.5 sticks	8,212.5 sticks	411 packs

Based on the data presented in Table 3, participants with high nicotine dependency exhibited significantly greater cigarette consumption (averaging 22.5 sticks/day or 8,212.5 sticks/year) compared to the low-dependency group (8 sticks/day). This elevated tobacco intake directly increases systemic cotinine levels (a primary nicotine metabolite), as stated in Table 4. Table 4 demonstrates that cotinine exerts a direct positive effect on MCP1 elevation (path coefficient = 0.567; p < 0.001) and a direct negative effect on nitric oxide (NO) (path coefficient = −0.132; p = 0.029). Additionally, Table 5 demonstrates a statistically significant indirect effect of cotinine on NO mediated through MCP1 (β = −0.15; p <

0.001). Consequently, the heavy cigarette consumption observed in high-dependency smokers is likely to induce pro-inflammatory responses (via MCP1 upregulation) and impair vasodilatory function (via NO reduction), representing critical pathophysiological mechanisms in cardiovascular dysregulation as modeled in Figure 1.

Table 4. Hypothesis Testing the Direct Effect of Cotinine on MCP-1 and NO

Exogen	Endogen	Path Coefficient	SE	p-Value
Cotinine	MCP-1	0.567	0.063	<0.001
Cotinine	NO	-0.132	0.069	0.029
MCP-1	NO	-0.265	0.067	<0.001

Table 5. Hypothesis Testing the Indirect Effect of Cotinine on NO through MCP-1

Exogen	Intervening	Endogen	Indirect Coefficient	SE	p-Value
Cotinine	MCP-1	NO	-0.15	0.049	<0.001

Abbreviations: ACS, acute coronary syndrome; CAD; coronary artery disease; CVD, Cardiovascular Disease; ELISA; Enzyme-Linked Immunosorbent Assay; MCP-1, Monocyte Chemotactic Protein-1; NO, Nitric Oxide; PKG, Protein Kinase G; PLS, Partial Least Square; PMPs, positive platelet-derived microparticles; ROS, Reactive Oxygen Species; SGC, Soluble Guanylate Cyclase; WHO, World Health Organization;

The regression analyses revealed distinct explanatory power among the studied biomarkers. For MCP-1, the model explained 32% of the variance (R^2 = 0.32), indicating a moderate effect size and suggesting that the included predictors meaningfully influence MCP-1 levels. In contrast, both Cotinine and NO exhibited lower explained variances (R^2 = 0.13 for each), reflecting small effect sizes. Despite the statistical significance of their predictors, as evidenced by β-values and p-values, the modest R^2 values imply that unaccounted factors contribute substantially to the variability in Cotinine and NO levels. These findings underscore the comparatively stronger predictive capacity of the model for MCP-1, while highlighting the need for further investigation into additional determinants of Cotinine and NO variability (Figure 1).

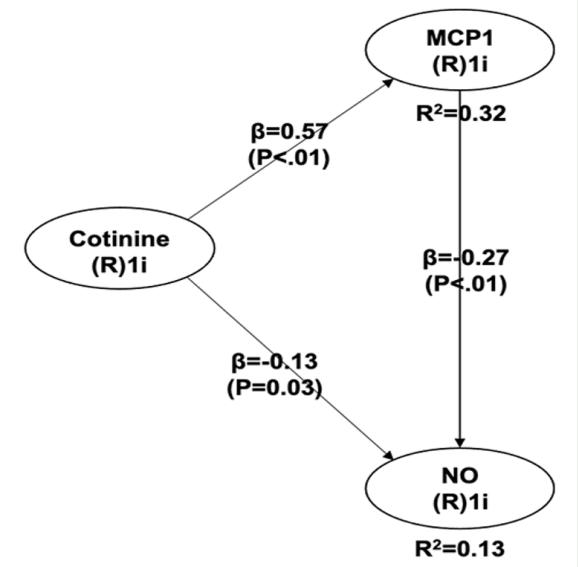

Figure 1. Path diagram of the relationship between Cotinine, MCP-1, and Nitric Oxide

Discussion

This study showed that Cotinine in smokers was very high compared to respondents who did not smoke. In addition, the study found that Cotinine can be detected in the serum of smokers and non-smokers, and it was found that serum cotinine levels in smokers significantly increased. However, the Cotinine level test has limitations in determining the length of cigarette smoke exposure and the number of cigarettes consumed per day (21). Still, the Cotinine test is a person's smoking habit.

Exposure to cigarette smoke triggers inflammation of the blood vessels in response to oxidative stress and synthesises chemokines, including MCP-1. In a previous study, smokers' MCP-1 concentrations were associated with smoking duration and high blood pressure. In addition, MCP-1 contributes to decreased vascular elasticity function and increased vascular resistance induced by long-term smoking (17). This study found a direct correlation between Cotinine and NO. NO molecule plays a vital role in every cell and organ function (22). NO also regulates the cardiovascular through 2 different pathways: Soluble Guanylate Cyclase (SGC) and Protein Kinase G (PKG) (23). Cigarette smoke extract alters the endothelial-mediated NO function under culture conditions, increasing the production of superoxide anion (O2-) through nicotinamide adenine dinucleotide phosphate (NADPH) stimulation, thereby reducing NO bioactivity that triggers endothelial dysfunction (16).

In addition, this study also found a significant effect of MCP-1 on NO. MCP-1 is essential in monocyte-induced inflammation in cardiac and vascular tissue (24). Monocyte recruitment is tightly regulated by the interaction between MCP-1 and the MCP-1 receptor gene polymorphism in myocardial damage (25). However, no research explains the effect of MCP-1 on NO. Previous studies have demonstrated that oxidative stress is essential in promoting the activation of several kinases in inducing inflammatory states (26). The potential implications of these findings for cardiovascular health are promising, offering hope for improved prevention and treatment strategies. Clinically, these findings reinforce the need for early vascular monitoring in young smokers, as even modest MCP-1 elevation predicts future cardiovascular risk in longitudinal cohorts. For research, three key implications emerge. First, the partial variance explanation warrants investigation of additional pathways (e.g., oxidative stress, epigenetic changes). Second, the β coefficients suggest cotinine may serve as a quantitative exposure marker for intervention studies. Third, the demographic limitations highlight the urgency of replicating these assays in women and older smokers.

While our findings demonstrate significant associations between cotinine and vascular biomarkers, the modest explanatory power (R^2=0.32 for MCP-1 and R^2=0.13 for NO) suggests these relationships are mediated by additional biological pathways not captured in our current model. The incomplete variance explanation particularly for NO implies that other oxidative stress mechanisms beyond cotinine's direct effects may contribute to vascular dysfunction. To advance mechanistic understanding,

future studies should incorporate a broader panel of inflammatory mediators (e.g., IL-6, TNF-α, CRP) and oxidative stress markers (e.g., superoxide dismutase, myeloperoxidase, F2-isoprostanes). Such comprehensive profiling could help a mechanistic understanding by identify key secondary pathways amplifying cotinine's vascular effects, clarify potential interactions between inflammatory and oxidative processes, and reveal biomarkers with greater predictive value for smoking-related vascular damage.

While this study provides valuable insights into the relationship between cotinine and vascular biomarkers, several limitations should be acknowledged. First, the participant cohort consisted exclusively of men aged 20–40 years from a single center, which may limit the generalizability of our findings to women, older populations, or individuals with comorbid conditions such as diabetes or hypertension. Additionally, the cross-sectional design precludes causal inferences. Future research should address these limitations by employing longitudinal designs to establish temporal relationships, incorporating multi-center cohorts with broader demographic representation to enhance external validity, and utilizing comprehensive assessments of potential confounders (dietary patterns, physical activity, and environmental exposures) through validated questionnaires or standardized instruments. Furthermore, stratified analyses by smoking intensity, duration, and cigarette type could help identify critical effect modifiers and vulnerable subpopulations.

Conclusion

In conclusion, Cotinine is associated with decreased vascular function directly and indirectly by activating vascular inflammation. Therefore, circulating Cotinine levels can be used to mark smoking-induced vascular inflammation.

Author contribution

Conceptualisation: KK, TAW; Formal analysis: HS; Funding acquisition: KK, TAW; Investigation: HS; Resources: KK, TAW; Validation: INC, WSH, AKH; Interpretation of data: INC, WSH, AKH; Writing– original draft: KK, INC, WSH, AKH; Writing – review &editing: KK, INC, WSH, AKH. All authors agree to be accountable for aspects of the work.

Disclosure of interest

No potential conflict of interest was reported by the author(s).

Data Availability

The data supporting this study's findings are available from the corresponding author, KK, upon reasonable request.

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References

1. Cardiovascular diseases. Available at: https://www.who.int/health-topics/cardiovascular-diseases. Accessed February 17, 2023.
2. Sanchis-Gomar F, Perez-Quilis C, Leischik R, et al. Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med. 2016;4(13):256. doi:10.21037/atm.2016.06.33.
3. Dikalov S, Itani H, Richmond B, et al. Tobacco smoking induces cardiovascular mitochondrial oxidative stress, promotes endothelial dysfunction, and enhances hypertension. Am J Physiol Heart Circ Physiol. 2019;316(3):H639-H646 . doi:10.1152/ajpheart.00595.2018.
4. Tang MS, Wu XR, Lee HW, et al. DNA damage, DNA repair and carcinogenicity: Tobacco smoke versus electronic cigarette aerosol. Mutat Res Rev Mutat Res. 2022;789:108409. doi:10.1016/j.mrrev.2022.108409.
5. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev. 1996;18(2):188-204. doi:10.1093/oxfordjournals.epirev.a017925.
6. Kumboyono K, Widyawati MN, Kusumawardani LH, et al. Factors affecting the serum cotinine level of male smokers in Malang. Int J Med Toxicol Forensic Med. 2023;13(3):E40130 . doi:10.32598/ijmtfm.v13i3.40130.
7. Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. Nat Rev Cardiol. 2013;10(4):219-230. doi:10.1038/nrcardio.2013.13.
8. Yu R, Li Y, Zhang Y, et al. Correlation between monocyte to high-density lipoprotein ratio and major adverse cardiovascular events in patients with acute coronary syndrome after percutaneous coronary intervention. Pak J Med Sci. 2021;37(3):1-6. doi:10.12669/pjms.37.3.3914.
9. Lin J, Kakkar V, Lu X. Impact of MCP-1 in atherosclerosis. Curr Pharm Des. 2014;20(28):4580-4588. doi:10.2174/1381612820666140522115801.
10. Ohman MK, Eitzman DT. Targeting MCP-1 to reduce vascular complications of obesity. Recent Pat Cardiovasc Drug Discov. 2009;4(3):164-176. doi:10.2174/157489009789152237.
11. Kastelein TE, Duffield R, Marino FE. Acute immune-inflammatory responses to a single bout of aerobic exercise in smokers: The effect of smoking history and status. Front Immunol. 2015;6:634. doi:10.3389/fimmu.2015.00634.
12. Zamara E, Galastri S, Aleffi S, et al. Prevention of severe toxic liver injury and oxidative stress in MCP-1-deficient mice. J Hepatol. 2007;46(2):230-238. doi:10.1016/j.jhep.2006.08.019.
13. Matsubara K, Matsubara Y, Hyodo S, et al. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. Int J Mol Sci. 2015;16(3):4600-4614. doi:10.3390/ijms16034600.
14. Walford G, Loscalzo J. Nitric oxide in vascular biology. J Thromb Haemost. 2003;1(10):2112-2118. doi:10.1046/j.1538-7836.2003.00345.x.
15. Desai A, Zhao Y, Lankford HA, et al. Nitric oxide modulates MCP-1 expression in endothelial cells: implications for the pathogenesis of pulmonary granulomatous vasculitis. Inflammation. 2003;27(4):213-223. doi:10.1023/a:1025093615755.
16. Kaplan HM. Molecular basis of vascular damage caused by cigarette smoke exposure and a new approach to the treatment: Alpha-linolenic acid. Biomed Pharmacother. 2018;102:458-463. doi:10.1016/j.biopha.2018.03.096.
17. Komiyama M, Shimada S, Hori S, et al. Association between monocyte chemoattractant protein-1 and blood pressure in smokers. J Int Med Res. 2018;46(3):965-974. doi:10.1177/0300060517749131.
18. Bai XY, Liu J, Zhang Q, et al. Association of monocyte chemoattractant protein-1 (MCP-1)-2518A>G polymorphism with susceptibility to coronary artery disease: a meta-analysis. Ann Hum Genet. 2015;79(3):173-187. doi:10.1111/ahg.12100.
19. Rapp JL, Alcala K, Brody JG, et al. Serum cotinine levels and nicotine addiction potential of e-cigarettes: an NHANES analysis. Carcinogenesis. 2020;41(10):1454-1459. doi:10.1093/carcin/bgaa060.
20. Molander L, Lunell E, Fagerström KO. Pharmacokinetics of nicotine in healthy elderly people. Clin Pharmacol Ther. 2001;69(1):57-65. doi:10.1067/mcp.2001.113181.
21. Duque A, Martínez-Pabón MC, Isaza-Guzmán DM, et al. Accuracy of cotinine serum test to detect the smoking habit and its association with periodontal disease in a multicenter study. Med Oral Patol Oral Cir Bucal. 2017;22(4):e425-e431 . doi:10.4317/medoral.21682.

22. Thomas DD, Ridnour LA, Isenberg JS, et al. The chemical biology of nitric oxide: implications in cellular signaling. Free Radic Biol Med. 2008;45(1):18-31. doi:10.1016/j.freeradbiomed.2008.03.020.
23. Maron BA, Tang SS, Loscalzo J. S-Nitrosothiols and the S-Nitrosoproteome of the cardiovascular system. Antioxid Redox Signal. 2013;18(3):270-287. doi:10.1089/ars.2012.4744.
24. Kumboyono K, Widyawati MN, Kusumawardani LH, et al. Analysis of monocyte chemoattractant protein-1 and interleukin-1β levels with adrenocorticotropic hormone levels in farmers: early detection of vascular inflammation due to pesticide exposure. Environ Pollut Bioavailab. 2024;36(1):2391352. doi:10.1080/26395940.2024.2391352.
25. Majmudar MD, Keliher EJ, Heidt T, et al. Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. Circulation. 2013;127(20):2038-2046. doi:10.1161/CIRCULATIONAHA.112.000116.
26. Peña E, Brito J, El Alam S, et al. Oxidative stress, kinase activity and inflammatory implications in right ventricular hypertrophy and heart failure under hypobaric hypoxia. Int J Mol Sci. 2020;21(17):6421. doi:10.3390/ijms21176421.