Initial Association Study of Exon 4 of P-53 Gene Polymorphisms with the use of Open System Device Electronic-Cigarette/Mods

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Abstract

Recently the use of e-cigarette is increasing very rapidly, but studies on the impact of the e-cigarette use behavior on gene mutations are still very limited. This research described the profile of the e-cigarette use behavior by users and evaluate the association with the incidence of exon 4 P53 gene polymorphisms.

The study with a cross-sectional design was conducted on 87 research subjects that met the inclusion criteria and were taken from the population using the purposive/judgmental sampling technique consisting of 27 e-cigarette users, 29 smokers as positive controls, and 31 non-both (non-smokers and non-e-cigarette users) as negative controls. The study was conducted by DNA sequencing analysis of all blood samples for analysis of the P-53 gene polymorphism mutation, and also the socio-behavioral factor assessment instrument was employed.

The behavior profile showed that 92.6% of the e-cigarette group stated using it every day, 44.4% of the e-cigarette group stated that the nicotine concentration of e-cigarettes used was 3 mg/bottle; 55.6% of the e-cigarette group used a closed system and 44.4% used an open system, and 59.3% of the e-cigarette group spent 1-time refill over 5 hours, with a maximum period of using the coil between 2 days - 1 week (48, 1%). The results of the P53 gene sequencing showed polymorphism mutation in exon 4 comprising C124R TGC>CGC and rs1042522 CGC>CCC in most of the smoking group (positive control), in some groups of e-cigarette users, and in most of the non-both groups (negative control).

Behaviors using open-system e-cigarette associated with polymorphism rs1042522 CGC>CCC was fourteen times greater than behaviors using closed system e-cigarette with 74.1% of contribution. This research requires further research and study.

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Introduction

The electronic cigarette (e-cigarette) is a new class of electronic nicotine delivery system, introduced in 2004[1]. E-cigarettes are products that typically vaporize liquid substance containing nicotine, flavoring agents, propylene glycol, and/or glycerol.[2] Electronic cigarettes (e-cigarettes) have grown in popularity in recent years, however there is some public health controversy surrounding their use.[3] The e-cigarette is highly controversial from scientific, political, financial, psychological, and sociological ideologies.[4] Despite their widespread usage, little is known about their health impacts, and opinions on the hazards and benefits of e-cigarette usage vary greatly among the general public, e-cigarette users, health-care providers, and the public health community.[5]

The scientific information on the impact of e-cigarettes on human health is minimal.[6] Most discussion of the health effects of e-cigarettes has focused on cancer.[7] Future studies will provide more information about the relationship between short-term and long-term consequences

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of e-cigarettes.[8] The long-term effect of e-cigarettes are currently unknown. It's known that electronic cigarettes generate aerosol containing metal contaminants that may leach from several parts of an e-cigarette, including the heating coil, joints, and wires.[9] Chromium and nickel are the leading contributors to these risks, with cadmium, lead, manganese, and arsenic as minor contributors. The device design and heating elements appear to be the main source of metals in e-aerosols.[10] Levels of harmful metals in aerosols were found to be largely dependent on device power and type. Most notably, higher device power was associated with higher aerosol metal levels. Open-system device/mods may place users at increased health risks due to exposure to high levels of toxic metals as compared to closed system devices (ciga-likes and pods).[9] Electronic cigarettes are powered by a battery or accumulator device with a heating element that heats the fluid (the e-liquid) to a temperature of about 200–300°C to form an aerosol (vapor) which is inhaled into the lung.[8] How individuals use these products has strong implications for nicotine intake and exposure to other potential toxicants.[11] Information on e-cigarettes use behaviors can provide understanding based on sociodemography characteristics and the patterns of usage related to e-cigarette use.

Besides to the metal content, there is substantial evidence that some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis.[5] The p53 gene is a well-studied tumor suppressor gene, and are commonly accompanied by wild-type TP53 inactivation.[12] In several types of human carcinomas, the p53 tumor suppressor gene is often altered. Exon 4 codon 72 is a frequent polymorphism, with two alleles encoding either arginine (CGC) or proline (PCG) (CCC). According to reports, this p53 polymorphism is linked to lung cancer susceptibility. Previous studies have indicated that the *TP53* gene Arg72Pro (rs1042522 C>G) polymorphism is associated with susceptibility to various types of cancer.[13]

To gain a better understanding of the e-cigarette use behavior profile and its impact on polymorphism on exon 4 of p53 gene mutation that has not been widely studied as a cancer predictive factor preliminary, we conducted a study which has been carried out on e-cigarettes user, smoker, and non-smoker/e-cigarettes user. The study objectives were to describe the profile of the e-cigarettes use based on socio-behavioral factors and to investigate the association between the e-cigarette use behavior and p53 polymorphism gene mutation, especially the association of the e-cigarette use behavior (either using open system/mods or using closed system/pods) on exon 4 of *TP53* gene rs1042522 C>G and C124R TGC>CGC polymorphism.

Materials and methods

A preliminary study with a cross-sectional design was carried out on e-cigarette users, smokers, and non-both. The research variable was the behavior factor using an e-cigarette as the independent variable, and the dependent variable was the p53 gene. The recruited population for the group of e-cigarettes users was the community located in Jakarta and Bandung regions and meets the inclusion and exclusion criteria. The group of e-cigarettes users in this study was the intervention group, while the smoking group was the positive control group, and the non-both of them was the negative control group. Affordable population groups of smoker and non-smoker/non-user of e-cigarette are local smoking communities.

The study inclusion criteria for the entire group were over 25 years of age, the East Asian ancestry group with Deuteromalay sub-race with good health. The inclusion criteria for the group of e-cigarettes users are users of e-cigarettes at least the last 3 years, with a minimum daily rotation of 25 days in the last 30 days. The inclusion criteria for the smoker group were cigarette users for at least the last 5 years, at least smoking cigarettes for 25 days in the last 30 days, smoking more than 10 cigarettes per day. The inclusion criteria for the group of non-smokers/e-cigarette users were never smoking/using e-cigarettes. Samples that met the inclusion criteria and were willing to participate in the entire study series were taken from the population using the purposive/ judgmental sampling technique. The total number of samples as the research object was 87 people, comprising 27 e-cigarettes users, 29 smokers as a positive control group, and 31 non-smokers/e-cigarettes users as a negative control.

This study has obtained ethical approval from the Research Ethics Committee of Universitas Padjadjaran, Bandung No.1504/UN6.KEP/EC/2019. All respondents have received a complete explanation of the research procedures and have expressed their consent to be involved in the study.

Collection of socio-behavioral factor data

The data collection process was carried out in stages. The first stage is the collection of socio-behavioral factor data through questionnaires and integrated interviews (*guided interviews*) to all respondents. The questionnaire as a measuring tool was created and developed by a researcher through review and discussion with several experts through *Focus Group Discussions* (FGD). Experts involved in preparing the dimensions of the questionnaire are experts in molecular biology, public health, molecular epidemiology, and representatives of community members who are concerned with public policies/regulations on smoking. The FGD resulted in 4 dimensions include socio-demographic characteristics, knowledge, attitudes, and actions of respondents related to the use of e-cigarettes.

DNA Extraction

Genomic DNA was extracted from the peripheral blood lymphocytes using Wizard Genomic DNA Purification Kit (Promega). Extracted DNA quality and quantity were determined by agarose gel electrophoresis and NanoDrop 1000 (Thermo Fisher Scientific; USA). Blood samples were collected by competent health personnel. A written informed consent was signed by all participants and then 3cc peripheral venous blood sample was obtained.

P53 sequencing analysis

Initially, SNP-containing fragment was amplified by using site-specific primers as follows: forward, 5'- GGT CCA GAT GAA GTC CCA GAA -3' and reverse, 5'- CGT GCA AGT CAC AGA CTT GGC-3'. As much as 200 ng of DNA template was taken for the Polymerase Chain Reaction (PCR) step. PCR reaction system: 2 mmol dNTP 2 μL, 5 × buffer 4 μL, 25 mmol MgCl2 2 μL, Taq enzyme 1 U, 10 μmol primers 0.5 μL each, template 2 μL, and plus ultra-pure water to 20 μL. Amplification conditions: 95 °C for 5 min; 95 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s , 35 cycles; and 72 °C for 5 min. Electrophoresis analysis was performed and the purified electrophoresis products then sent for Sanger sequencing. DNA sequencing was performed by using dideoxy Sanger sequencing method in the First Base Laboratory, Malaysia. The sequencing result was aligned afterwards with the reference sequence from the gene bank used the Bioedit® software.

All statistical analyses were performed with SPSS software (version 22; SPSS Inc., Chicago, IL). The data obtained from the results of data collection are then processed, analyzed, and presented in the form of a frequency distribution table. Univariate analysis in the form of frequency distribution and bivariate (chi-square test, Spearman correlation, logistic regression) is used in this study.

Results

Socio-behavioral factor analysis

Results of the analysis of socio-demographic characteristics and the overall knowledge of the research group can be seen in table 1. Based on table 1, it can be seen that the last education level majority of non-smoking respondents was masters/undergraduates by 87.1%, while the majority of respondents using e-cigarettes and conventional cigarettes was high-school leavers by 55.6% and 82.8% with a value of $p = 0.000$ ($p < 0.001$). This shows that there was a very significant relationship between education level and non-smoking behavior, the higher the level of education, the higher the non-smoking behavior.

Based on table 1, it can be seen that 51.6% of the non-smoking/e-cigarette group had good knowledge about the impact of nicotine on health, while 33.3% of the e-cigarette and smokers group (31.0%) were still a lot who did not know that nicotine affected health with a value of $p = 0.004$ ($p < 0.05$). This shows that there was a relationship between non-smoking/e-cigarette behavior and knowledge, the better knowledge about the impact of nicotine on health, the higher the non-smoking behavior. A total of 88.9% of e-cigarette users and 29.9% of the non-smoker/e-cigarette group stated that cigarettes were more harmful than e-cigarettes while the smokers (65.5%) and non-smokers/e-cigarette groups (58.1%) stated that e-cigarettes are more harmful or just as harmful than cigarettes with a value of $p = 0.000$ ($p < 0.001$). This shows that there was a relationship between non-smoking/e-cigarette behavior and knowledge, the better knowledge about the impact of nicotine on health, the higher the non-smoking behavior.

Variables	Groups			P-value
	Non-Smoking	E-cigarette	Smoker	
	n (%)	n (%)	n = (%)	
Last Education				
• Master/Bachelor Degree	27 (87.1)	12 (44.4)	5 (17.2)	0.000*
• SLTA (Senior High School)	3 (9.7)	15 (55.6)	24 (82.8	
• SLTP (Junior High School)	1 (3.2)	0 (0)	0 (0)	
Knowledge of the Health Risks of Nicotine due to Smoking				
• More than half risk	16 (51.6)	2 (7.4)	8 (27.6)	0.013**
• Less than half or nearly all at risk	12 (38.7)	16 (59.3)	12 (41.4)	
Not know	3 (9.7)	9 (33.3)	9 (31.0))	
Knowledge about Comparison between E-cigarette and cigarette				
• Cigarettes are more harmful than e-cigarettes	9 (29.0)	24 (88.9)	0 (0)	0.012**
• E-cigarettes are more harmful or just as harmful than cigarettes	18 (58.1)	0 (0)	19 (65.5)	
• Not know	31 (12.9)	27 (11.1)	29 (34.5)	
Total n=87 (100%)	31 (100)	27(100)	29 (100)	

Table 1. Characteristics of Socio-behavioral factor.
Spearman test: *significant p < 0.001, **significant p < 0.05

p53 gene polymorphism analysis

The results of the mutation analysis of the p53 gene comprising polymorphisms C124R TGC>CGC and rs1042522 C> G can be seen in table 2, and the result of agarose gel stained presented in figures 1 and the sequence of exon 4 p-53 gene in figures 2. Based on table 2, results showed that 12 rs1042522 G>C polymorphism (38.7%) which changed the amino acid from arginine (CGC) to proline (CCC), 7 rs1042522 G>C polymorphisms from 27 e-cigarette group (25.9%) and 12 rs1042522 G>C polymorphisms from 29 tobacco smoker group (41.4%). Whereas the results for C124R TGC>CGC polymorphism, 31 participants of the non-smoker/e-cigarettes group showed 3 polymorphism TGC>CGC (9.7%).

There were 2 polymorphisms TGC>CGC from the 27 e-cigarettes group (7.4%). Tobacco smoker group consisting of 6 polymorphisms TGC>CGC from 29 participants (20.7%) (Table 2). Based on the results of the Kruskal-Wallis test, there was no difference in p53 gene polymorphism in the three treatment groups for both C124R TGC>CGC (p = 0.274) and rs1042522 G>C (p = 0.441). This means that the p53 genotype and alleles polymorphism can occur in all study groups.

Variables	Groups			P-value
	Non-Smoking	E-cigarette	Smoker	
	n (%)	n (%)	n = (%)	
C124R TGC>CGC				
• TGC (wild type)	28 (90.3)	25 (92.6)	23 (79.3)	0.274
• CGC	3 (9.7)	2 (7.4)	6 (20.7)	
rs1042522 C>G				
• CGC (wild type)	19 (61.3)	20 (74.1)	17 (58.6)	0.441
• CCC	12 (38.7)	7 (25.9)	12 (41.4)	
n = 87				

Table 2. Results of the p53 gene polymorphism analysis.
*abnormal distribution, **Kruskal-Wallis test.

To determine the differences in the occurrence of p53 gene mutations in the e-cigarette and cigarette groups, a chi-square test was conducted with the results as shown in table 3 and seen that there was a p53 gene mutation comprising a C124R TGC>CGC polymorphism (p = 0.000) and a polymorphism rs1042522 C>G (p = 0.016) at codon 4, both in the e-cigarette user group and smoker. The risk factors for polymorphism in the e-cigarette group can be seen in table 4., and be seen that 81.5% of the e-cigarettes group were cigarette smokers before using e-cigarettes, with the time to cease smoking cigarettes over 36 months (40.7%). Some 92.6% of the e-cigarettes group stated that they use e-cigarettes every day. The highest concentration of nicotine in e-cigarettes was 3 mg/bottle (44.4%). Some 55.6% used a closed system so the volume of cigarette cartridges was known, and the remaining 44.4% used an open system. A total of 59.3% spent 1-time refill over 5 hours, with a maximum period of using the coil between 2 days - 1 week (48.1%).

Variables	Observed	Expected	Residual	P-value
C124R TGC>CGC				
e-cigarette	48	28.0	20.0	0.000*
Cigarette	8	28.0	-20.0	
N 56				
rs1042522 G>C				
e-cigarette	37	28.0	9.0	0.016**
Cigarette	19	28.0	-9.0	
n = 56				

Table 3. The p53 gene polymorphisms in the e-cigarette and cigarette groups.
Chi-square test, *p<0.001, **p < 0.05.

Based on table 4, it can be seen that there was a significant relationship between the use of open system and the polymorphism rs1042522 C>G (p = 0.009). To find out more about the strength of the relationship between the use of the open system e-cigarette and the polymorphism rs1042522, a Spearman correlation test was conducted and the r-value was 0.5 with p = 0.009. The effect of the open system e-cigarette on polymorphism rs1042522 was tested using logistic regression with test results can be seen in table 5.

		P53 gene polymorphisms	
Risk-behavior factor related to e-cigarettes use	n (%)	C124R	rs1042522
Using conventional cigarettes before using rotric			
□ Yes	22 (81.5)	0.6920	0.439
□ No	5 (18.5)		
Frequency of using e-cigarettes			
□ Not every day	2 (7.4)	0.883	0.977
□ Every day	25(92.6)		
Time to cease conventional cigarettes			
□ above 36 months	11 (40.7)	0.059	0.509
□ 24-36 Months	8 (29.6)		
□ 12-24 Months	8 (29.6)		
The concentration of nicotine in the e-cigarette liquid used			
□ 3 mg/bottle	12 (44.4)		
□ 6 mg/bottle	11 (40.7)	0.439	0.883
□ 9 mg/bottle	2 (7.4)		
□ 16 mg/bottle	1 (3.7)		
□ Not know	1 (3.7)		
The volume of the rotric cartridge used			
□ closed system	15 (55.6)	0.108	0.009*
□ open system	12 (44.4)		
Time to spend 1-time refilling cartridge (hour)			
□ 0 - 1 hour	3 (11.1)		
□ 1 - 5 hours	8 (29.6)	0.502	0.088
□ above 5 hours	16 (59.3)		
Duration of using coils			
□ under 2 days	4 (14.8)		
□ 2 days - 1 week	13 (48.1)	0.768	0.110
□ above 1 week	10 (37.0)		

Table 4. Risk-behavior factor related to e-cigarettes use.
*Spearman test (p< 0.01), r=0.5.

Based on the logistic regression results as shown in table 5, it can be seen that there was a significant influence between behaviors using an open system in the e-cigarette group with the incidence of polymorphism rs1042522 C>G (p = 0.026), OR = 14.0 with 95% CI (1.372-142,888,) and a contributing factor was 74.1%.

Open system	polymorphism rs1042522		P	Exp (B)	Confidence Interval (CI)	
	No	Yes			Lower	Upper
No	14	6	0.026	14.00	1.372	142.888
Yes	1	6				

Table 5. The effect of open system on polymorphism rs1042522.
Overall percentage: 74.1%.

The results showed a mutation in the p53 gene. The smokers cigarette group is the largest group that have mutation of p53 gene. In table 6 we can see that TGC changes to CGC.

Sample code	F113 del CTT	C124 R TGC> CGC
P1	normal	normal
P2	normal	normal
P3	normal	normal
P4	normal	normal
P5	normal	normal
P6	normal	normal
P7	normal	cgc
P8	normal	normal
P9	normal	cgc
P10	normal	normal
P11	normal	normal
P12	normal	normal
P13	normal	normal
P14	normal	normal
P15	normal	normal
P16	normal	cgc
P17	normal	normal
P18	del	normal
P19	normal	normal
P20	normal	normal
P21	normal	cgc
P22	normal	normal
P23	normal	normal
P24	del	normal
P25	normal	normal
P26	normal	cgc
P27	normal	cgc
P29	normal	normal
P30	normal	normal
V01	normal	normal
V02	normal	normal
V04	normal	normal
V05	normal	normal
V06	normal	cgc
V07	normal	normal
V08	normal	normal
V09	normal	normal
V10	normal	normal
V11	normal	normal
V12	normal	normal
V13	normal	cgc
V14	normal	normal
V15	normal	normal
V16	normal	normal
V17	normal	normal
V18	normal	normal
V19	normal	normal

V20	normal	normal
V21	normal	normal
V23	normal	normal
V24	normal	normal
V25	normal	normal
V26	del	normal
V27	normal	normal
V28	normal	cgc
V29	normal	normal
V30	normal	normal
V31	normal	normal
V32	normal	normal
V33	normal	normal
N1	normal	normal
N2	normal	normal
N3	normal	normal
N4	normal	normal
N5	normal	normal
N6	normal	cgc
N7	normal	normal
N8	normal	normal
N9	normal	normal
N10	normal	normal
N11	normal	normal
N12	normal	normal
N13	normal	normal
N14	normal	normal
N15	normal	normal
N16	normal	normal
N17	normal	normal
N18	normal	normal
N19	normal	cgc
N20	normal	normal
N21	normal	normal
N22	normal	normal
N23	del	normal
N24	normal	normal
N25	normal	cgc
N26	normal	normal
N27	normal	normal
N28	normal	normal
N29	normal	normal
N30	normal	normal
N31	normal	normal

Table 6. Polymorphism Result p53 exon 4.

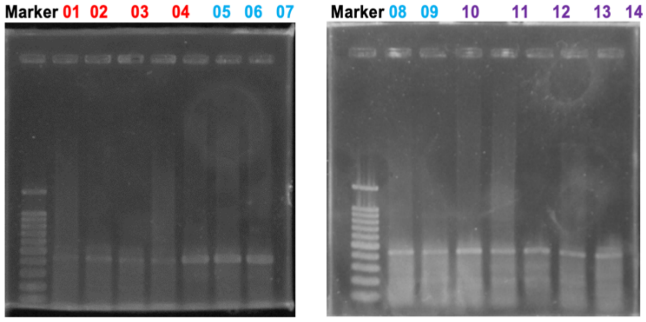

Figure1. Agarose Gel Stained with GelRed Showing p53 200bp bands of 4 sample non-smoker, 5 sample tobacco smoker and 5 sample e-smoker.

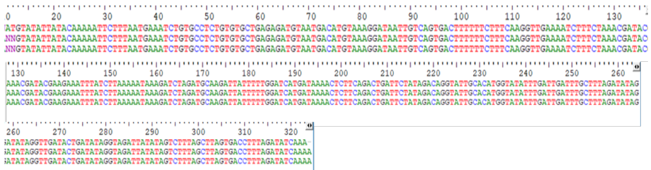

Figure 2. Sequence of exon 4 p53 gene.

Discussion

Research on the relationship between smoking behaviors and gene mutations has been widely published, but research on the relationship between e-cigarette user behaviors in association with molecular aspects is still very limited. Accumulated evidence regarding the impact of e-cigarettes on human health shows several gaps and this is understandable, given a short history of the habit. This research that have been done on e-cigarettes user behaviors and its relationship with exon 4 p53 gene polymorphism is the initial stage of studying the impact of the e-cigarette use behavior on molecular aspects.

In this study, the e-cigarette use behavior was described through the socio-demographic characteristics and risk-behavior factor related to the e-cigarettes use. Results of this study illustrated that most of the respondents especially the non-smoker and the e-cigarette user groups had a high level of education as a bachelor/master, while the education level in the group of most smokers were senior high school. This is in line with research by Paavola et al.[14] stating that the socioeconomic status consisting of levels of employment, education, and income had a significant relationship with smoking behavior. The level of education possessed by respondents in this study led to a better knowledge of respondents about the hazards of nicotine as a health risk factor. This is in line with research[15] stating that high educational attainment lowered the prevalence, severity, duration, and consequences of conventional smoking and e-cigarette.

The research results showed the smoker group stated that e-cigarettes were the same or even more harmful than cigarettes, while the e-cigarette user group stated that the hazards of smoking were greater when compared to the hazards of e-cigarettes. This is in line with the study[16] researching the perceived risk of electronic cigarettes compared with combustible cigarettes based on the Tobacco Products and Risk Perceptions survey data that was asked through indirect questions to e-cigarettes users. The research results[16] stated that 42.1% of e-cigarette participants were identified as less harmful than cigarettes, 23.8% said they were of equal harm, 7.1% perceived e-cigarettes to be more harmful and 27.1% did not know. The findings of this study were comparable to those of a prior study that underlined the advantages of e-cigarette use, such as ease of quitting smoking, less addictiveness, and less harm.[17]

The research results showed that most (81.5%) adult respondent groups were smokers before starting to use e-cigarettes, this is in line with research[18] stating that use of e-cigarettes without a prior history of smoking is currently a rare phenomenon in adults, but is increasingly common among youth. The percentage of respondents using e-cigarettes every day was 92.6%. This rate was higher than reported by Brown et al 2014 that 45.9% of ex-smokers in Great Britain use e-cigarette daily.[19]

The research results showed that 40.7% of respondents using e-cigarettes had ceased smoking over 36 months, and 29.6% had ceased since 24-36 months ago. A total of 29.6% of e-cigarette user respondents had also ceased smoking 12-24 months ago. The research results of the study[20] found that 31.0% of respondents did not smoke in the past 6 months, and of those who did not smoke, 56.7% used e-cigarettes, 9.0% used tobacco-free nicotine products, and 34.3% were completely nicotine free.

The research results showed that 44.4% of respondents used a liquid containing 3 mg of nicotine/mL, this result is in line with research[21] stating that most electronic (83.33%) cigarette user participants smoked electronic cigarette liquid containing 3 mg/mL of nicotine. Some 55.6% of respondents used e-cigarettes with a closed system and 44.4% used e-cigarettes with an open system. E-cigarettes can be broadly divided into two distinctive categories: closed system and open system devices. Closed system devices are commonly disposable or can be reloaded with a prefilled cartridge or tank of their own brand with limited choices of flavors and nicotine concentrations, and limited ability to change power. Open-system devices resemble a pen or tank, which allows users to refill an “atomizer” with an increasingly wide variety of e-liquids differing in flavor, nicotine content, and manufacturer.[22] Based on research, [9] open-system devices generate aerosol with higher metal concentrations than closed system devices. These findings inform tobacco regulatory science, policymakers and health professionals on potential mental health risks associated with e-cigarette use, design, and manufacturing.

In our study, smokers and e-cigarette users had an impact on changing the gene profile comprising C124R TGC>CGC and rs1042522 C>G polymorphisms, as well as a positive correlation of using the open system e-cigarette with polymorphism rs1042522 C>G. The finding of a positive correlation from the use of an open system e-cigarette with this rs1042522 C>G polymorphism seems to be related to open system devices generating aerosols with higher concentrations of metal,[9] and probably also with high concentrations of aldehydes or acrolein that will trigger mutations. This study has a tendency towards e-cigarette users. Open system users tend to have lower polymorphism values than other cigarette user groups.

Many studies have shown mutations in the P53 gene to the incidence of lung tumors in smokers along with an increase in tobacco consumption. The common polymorphism in tobacco smokers is Arg72Pro (rs1042522 C>G), which is the CGC codon changes to CCC codon (missense mutation) that converts the amino acid arginine into proline and results in changes to the final structure of the resulting protein. Changes in protein structure lead to changes or loss of function of these proteins. The loss of function of the P53 gene contributes to genetic instability and also the potential for metastasis in various types of cancer. Our findings in the initial study indicated an association between the polymorphism of rs1042522 CGC>CCC and the behavior of using the open system e-cigarette. Larasputri et al (2018) reported that many factors can contribute to mutation, and Chromium metals plays a role increasing the level of TP53 mutant.[23] The polymorphism was fourteen times greater in the behavior of using open system compared to closed system e-cigarettes, with 74.1% of contribution. However, due to the effect of exon 4 variants on the function of p53 protein, they may also influence tumor behavior in response to environmental carcinogens. In addition to mutations, some polymorphisms may also affect p53 function and increase the risk of cancer.

Conclusion

Overall, observation results of the mutation analysis on the p53 gene comprising C124R TGC>CGC and rs1042522 C>G polymorphisms at codon 4 were observed in the three research groups (smokers, e-cigarette users, and non-users of both) with a special note that the percentage of polymorphisms in the e-cigarette user group is far below that of smokers and below that of the non-smoking and e-cigarette groups. This interesting finding hints at the need for further and in-depth research on e-cigarette especially about the possible mechanism of e-cigarette in inhibiting the occurrence of mutations. However, given this study is preliminary study and has many limitations such as insufficient sample size, the research design is not case-control so that further research and study are required.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Drummond MB, Upson D. Electronic cigarettes: Potential harms and benefits. Ann Am Thorac Soc. 2014;11:236-242.
2. Nayir E, Karacabey B, Kirca O, Ozdogan M. Electronic cigarette (e-cigarette). J Oncol Sci. 2016;2:16-20.
3. McKeganey N, Barnard M, Russell C. Vapers and vaping: E-cigarettes users views of vaping and smoking. Drugs Educ Prev Policy. 2018;25:13-20.
4. Palazzolo DL. Electronic cigarettes and vaping: A new challenge in clinical medicine and public health. A literature review. Front Public Heal. 2013;1:56.
5. Academies of Sciences, Engineering, and Medicine, et al. Public Health Consequences of E-Cigarettes. Edited by David L.Eaton et. al. National Academies Press (US). 2018; doi:10.17226/24952.
6. Callahan-Lyon P. Electronic cigarettes: Human health effects. Tob Control. 2014;23:ii36-ii40.
7. Glantz SA, Bareham DW. E-Cigarettes: Use, Effects on Smoking, Risks, and Policy Implications. Annu Rev Public Health. 2018;39:215-235.
8. Jankowski M, Brozek G, Lawson J, Skoczyński S, Zejda JE. E-smoking: Emerging public health problem?. Int J Occup Med Environ Health. 2017;30:329–344.
9. Zhao D, Navas-Acien A, Ilievski V, Slavkovich V, Olmedo P, Adria-Mora B, et al . Metal concentrations in electronic cigarette aerosol: Effect of open-system and closed-system devices and power settings. Environ Res. 2019;174:125-134.
10. Fowles J, Barreau T, Wu N. Cancer and non-cancer risk concerns from metals in electronic cigarette liquids and aerosols. Int J Environ Res Public Health. 2020;17: 2146.
11. Cahours X, Prasad K. A review of electronic cigarette use behavior studies. Beitr Tab Int. 2018;28:81-92.
12. Pillai RM, Nair SA. Polymorphism of p53 in cancer prognosis. Indian J Med Res 2016;144:314–6.
13. Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, Wiencke JK, Christiani DC. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2020;9:1037-42.
14. Paavola M, Vartiainen E, Haukkala A. Smoking from adolescence to adulthood: the effects of parental and own socioeconomic status. Eur J Public Health. 2004;14:417-21.
15. Assari S, Mistry R, Bazargan M. Race, Educational Attainment, and E-Cigarette Use. J Med Res Innov. 2020;4:e000185.
16. Churchill V, Nyman AL, Weaver SR, Yang B, Huang J, Popova L. Perceived risk of electronic cigarettes compared with combustible cigarettes: direct versus indirect questioning. Tob Control. 2020;0:1-3.
17. Alzalabani AA, Eltaher SM. Perceptions and reasons of E-cigarette use among medical students: an internet-based survey. J Egypt Public Health Assoc. 2020;95:1-6
18. Besaratinia A, Tommasi S. An opportune and unique research to evaluate the public health impact of electronic cigarettes. Cancer Causes Control. 2017;18:1167-71.
19. Brown J, West R, Beard E, Michie S, Shahab L, McNeill A. Prevalence and characteristics of e-cigarette users in Great Britain: findings from a general population survey of smokers. Addict Behav. 2014;39:1120-5.
20. Siegel MB, Tanwar KL, Wood KS. Electronic cigarettes as a smoking-cessation tool: Results from an online survey. Am J Prev Med. 2011;40:472-5.
21. Lestari DA, Tandelilin RT, Rahman FA. Degree of Acidity, Salivary Flow Rate, and Caries Index in Electronic Cigarette Users in Sleman Regency, Indonesia. J Indones Dent Assoc. 2020;3:37-41.
22. Chen C, Zhuang YL, Zhu SH. E-Cigarette Design Preference and Smoking Cessation: A U.S. Population Study. Am J Prev Med. 2016; 51:356-63.
23. Larasputri I, Berniyanti T, Diyatri I. Analysis of TP53 Mutants Due to Chromium Metal Exposure on Dental Technicians at Surabaya Laboratory. Journal of International Dental and Medical Research 2018; 3(11):950-954.