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THE COMPARISON OF OXIDATIVE STRESS LEVELS BETWEEN E-CIGARETTE SMOKERS AND CONVENTIONAL SMOKERS IN YOUNG ADULT IN SURABAYA

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ABSTRACT

Background: *Smoking is one of the leading causes of morbidity and mortality worldwide. Previously, e-cigarettes were considered safe, but later some studies have reported that e-cigarette can increase oxidative stress and induce an inflammatory response. Isoprostane is a biomarker of oxidative stress, which is thought to play a role in the pathogenesis of COPD. The purpose of this study was to determine the level of oxidative stress between e-cigarette and conventional smokers by measuring the isoprostan level in the two group and comparing them.* ***Method:*** *This research is an observational analytic study conducted in Surabaya. Anamnesis, physical examination, chest X-ray, and complete blood were carried out, followed by urine isoprostane. The research subjects were 28 e-cigarrete smokers, 27 conventional smokers, and 14 controls who never smoked. Urine isoprostane levels were measured by the ELISA method using a random urine sample and then corrected with urine creatinine from the same sample. Differences in urine isoprostane levels in the three groups used the Mann-Whitney test.* ***Result:*** *The statistical analysis results showed no significant difference in urine isoprostane levels between e-cigarette and conventional smokers (p = 0.054). The mean and median of urine isoprostane in e-cigarette and conventional smokers tended to be higher than controls.* ***Conclusion:*** *The levels of urine isoprostane for e-cigarette and conventional smokers tended to be higher than controls, but there was no statistically significant difference. It can be concluded that the level of oxidative stress in the two groups did not have significant difference but tended to be higher than controls.*

Keywords: *Oxidative stress, E-cigarette, urine isoprostane, ROS*

ABSTRAK

Latar Belakang: Merokok merupakan salah satu penyebab utama morbiditas dan mortalitas di seluruh dunia. Sebelumnya, rokok elektrik dianggap aman, namun belakangan beberapa penelitian melaporkan bahwa rokok elektrik dapat meningkatkan stres oksidatif dan memicu respons peradangan. Isoprostane adalah biomarker stres oksidatif, yang diduga berperan dalam patogenesis PPOK. Tujuan dari penelitian ini adalah untuk mengetahui tingkat stres oksidatif antara rokok elektrik dan perokok konvensional dengan mengukur kadar isoprostan pada kedua kelompok dan membandingkannya. **Metode:** Penelitian ini merupakan penelitian observasional analitik yang dilakukan di Surabaya. Dilakukan anamnesis, pemeriksaan fisik, rontgen dada, dan pemeriksaan darah lengkap, diikuti isoprostan urin. Subjek penelitian adalah 28 perokok elektrik, 27 perokok konvensional, dan 14 kontrol yang tidak pernah merokok. Kadar isoprostan urin diukur dengan metode ELISA menggunakan sampel urin acak dan kemudian dikoreksi dengan kreatinin urin dari sampel yang sama. Perbedaan kadar isoprostan urin pada ketiga kelompok menggunakan uji Mann-Whitney. **Hasil:** Hasil analisis statistik menunjukkan tidak ada perbedaan kadar isoprostan urin yang signifikan antara rokok elektrik dengan perokok konvensional (p = 0,054). Rerata dan median isoprostan urin pada rokok elektrik dan perokok konvensional cenderung lebih tinggi daripada kontrol. **Kesimpulan:** Kadar isoprostan urin pada rokok elektrik dan perokok konvensional cenderung lebih tinggi dibandingkan kontrol, namun tidak terdapat perbedaan yang bermakna secara statistik. Dapat disimpulkan bahwa tingkat stres oksidatif pada kedua kelompok tidak memiliki perbedaan yang bermakna tetapi cenderung lebih tinggi dibandingkan dengan kontrol**.**

Kata kunci: *Oxidative stress, E-cigarette, urine isoprostane, ROS*

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BACKGROUND

Smoking is one of the leading causes of morbidity and mortality globally. Indonesia is the third country with the largest cigarette consumption in the world.[1,2] E-cigarettes have been discovered since 2003 and growing rapidly as they are considered a safer alternative to conventional tobacco cigarettes. The 2018 Indonesian Tobacco Atlas states that e-cigarette users over ten years of age in Indonesia reach 2.8%, and 13 provinces have a prevalence above the national prevalence average.[3] In 2009 FDA detected the presence of tobacco-specific nitrosamines at low levels in e-cigarettes.[4] The impact of e-cigarette use on human health is not known with certainty due to a lack of data.

Liquids used in e-cigarettes, or called e-liquids, contain nicotine and propylene glycol, vegetable glycerol, and artificial flavorings. After going through the heating process, propylene glycol and vegetable glycerol will be converted into strong irritants and can cause inflammation of the lung tissue.[4,5] Previous studies have shown that cells exposed to e-cigarette vapor develop inflammation. E-cigarettes induce pro-inflammatory cytokines and mediators and also cause ROS and oxidant reactivity.[5,6] There are very few studies measuring oxidative stress in e-cigarettes. Roberts and Morrow in 1990 found that F2-isoprostane can be relied upon as a biomarker of oxidative stress because it is a stable compound that is formed in large numbers in vivo after oxidative damage and can be detected even though the tissue is not injured. Morrow et al. demonstrated increased levels of F2-isoprostane in the urine of smokers compared to non-smokers.[7,8]

In this study, we want to find out that e-cigarettes can cause tissue damage due to oxidative stress similar to conventional cigarettes by measuring urine isoprostane levels in the two groups and comparing them.

METHOD

This research is an observational analytic study with a cross-sectional design conducted in Surabaya. The research sample was e-cigarette smokers and conventional smokers in Surabaya who met the inclusion and exclusion criteria and were willing to participate in the study. The inclusion criteria for this study were e-cigarette smokers or conventional smokers who smoked for more than one year, 20 to 40 years old, normal complete blood count, and normal chest radiographs read by the same radiologist. The exclusion criteria of this study were a history of asthma, a history of allergies, a history of diabetes

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mellitus and heart disease, have an illness that has respiratory symptoms (autoimmune disease, malignancy), currently have respiratory symptoms caused by acute infection, active e-cigarette and conventional smoking simultaneously, pregnancy and breastfeeding.

RESULT

This research was conducted from July to September 2020 in Surabaya. The initial subjects of the study were 80 people, while subjects who appropriated inclusion and exclusion criteria were 69 people. The research subjects consisted of 28 e-cigarette smokers, 27 conventional smokers, and 14 controls. The characteristics of subjects are shown in table 1.

Table 1. Characteristics of subjects based on gender, age, education, occupation, and nutritional status.

Characteristics	**E-cigarette smokers** (n=28)	**Conventional smokers** (n=27)	**Controls** (n=14)	**p**
Gender				
Male	27 (96,4%)	27 (100%)	12 (85,7%)	0,102
Female	1 (3,6%)	-	2 (14,3%)	
Age (years)				
20 – 29	15 (53,6%)	9 (33,3%)	5 (35,7%)	0,273
30 – 40	13 (46,4%)	18 (66,7%)	9 (64,3%)	
Mean ± SD	29,64 ± 6,19	31,85 ± 4,05	31,71± 4,34	
Range	20 s/d 40	24 s/d 40	20 s/d 40	
Nutritional status based on BMI (WHO)				
Underweight - normal (< 25)	10 (35,7%)	19 (70,4%)	7 (50%)	0,036
Overweight - obesity I (≥ 25)	18 (64,3%)	8 (29,6%)	7 (50%)	
Mean ± SD	26,52 ± 3,68	23,53 ± 3,50	24 ± 3,38	
Range	16,5 s/d 31,6	18,7 s/d 30,7	17,8 s/d 29,7	
Last education				
SD	-	1 (3,7%)	-	
SMP	1 (3,6%)	-	-	
SMA	12 (42,9%)	8 (29,6%)	-	
D3/S1	14 (50%)	17 (63%)	14 (100%)	
S2	1 (3,6%)	1 (3,7%)	-	
Occupation				
College student	2 (7,1%)	-	-	
Administrative officer	2 (7,1%)	1 (3,7%)	1 (7,1%)	
E-cigarette store	3 (10,7%)	-	-	
Other indoor work	11 (39,3%)	5 (18,5%)	-	
Health workers	2 (7,1%)	11 (40,7%)	12 (85,7%)	
Couriers and expeditions	2 (7,1%)	2 (7,4%)	1 (7,1%)	
Technician	2 (7,1%)	-	-	
Other outdoor work	3 (10,7%)	6 (22,2%)	-	

The majority of research subjects were male and consisted of 66 men and 3 women. The mean age of the research subjects was 30,93 ± 5,12 with the lowest age being 20 years, and the highest age being 40 years. Characteristics of leukocytes, eosinophils, and ESR results of subjects can be seen in table 2.

Table 2. Characteristics of subjects based on the value of leukocytes, absolute eosinophils, and ESR

Group	Leukocytes	Absolute eosinophils	ESR
E-cigarette smokers	7303,57 ± 1370,18	172,14 ± 69,73	8,39 ± 6,43
Conventional smokers	7470,37 ± 1594,42	218,89 ± 126,32	7,37 ± 5,08
Controls	6478,57 ± 983,85	217,86 ± 148,28	7,57 ± 3,48

The characteristics of the e-cigarette smokers group are shown in table 3 and conventional smokers group in table 4.

Table 3. Characteristics of the e-cigarette smokers group

Characteristics	Total (n)
Duration of smoking	
1 - 3 years	12 (42,9%)
4 - 6 years	14 (50%)
7 - 9 years	2 (7,1%)
Current smoking degree	
Mild	14 (50%)
Moderate – severe	14 (50%)
Previous smoking history	
Yes	19 (67,9%)
No	9 (32,1%)
Previous smoking degrees (Brinkman)	
Mild	17 (89,5%)
Moderate	2 (10,5%)
Types of e-cigarette cigarettes	
Mods	13 (46,4%)
Pods	8 (28,6%)
Mixed	7 (25%)
Type of liquid flavor	
Creamy	16 (57,1%)
Fruity	6 (21,4%)
Mixed	6 (21,4%)
Liquid packaging used	
Liquid 100 ml	8 (28,6%)
Liquid 60 ml	17 (60,7%)
Liquid 30 ml	3 (10,7%)
Amount of nicotine per day	
Less than 0,5 mg	16 (57,1%)
0,5 to 1 mg	10 (35,7%)
More than 1 mg	2 (7,1%)

Table 4. Characteristics of the conventional smokers group

Characteristics	Total (n)
Current conventional smoking degrees (Brinkman)	
Mild	18 (66,7%)
Moderate	9 (33,3%)
Kind of cigarettes	
Cigarette	21 (77,8%)
White cigarette	6 (22,2%)
Filter	
Filter	27 (100%)
Duration of smoking time	
1 - 10 years	9 (33,3%)
11- 20 years	16 (59,3%)
More than 20 years	2 (7,4%)

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Corrected urine isoprostane levels in the three groups can be seen in table 5. There was no significant difference in corrected urine isoprostane levels in the e-cigarette smoker, conventional smoker, and control groups (p> 0.05).

Table 5. Differences in corrected urine isoprostane in the e-cigarette, conventional smokers, and control groups

	Group	N	Median	p value
Corrected urine isoprostane (pg/mg creatinine)	E-cigarette smokers	28	75,39 (28,47-236,83)	0,054
	Conventional smokers	27	93,49 (37,09-322,08)	
	Controls	14	72,02 (32,79-120,92)	

Based on BMI, corrected urine isoprostane levels in the three groups were not significantly different (p> 0.05). It can be seen in table 6.

Table 6. Corrected urine isoprostane in the group of e-cigarette smokers, conventional smokers, and controls based on BMI

BMI	N	Median corrected urine isoprostane (pg/mg creatinine)
Underweight – normal		
E-cigarette smokers	10	54,93 (28,47-155,33)
Conventional smokers	19	89,19 (37,09-322,08)
Controls	7	82,99 (47,04 – 120,92)
p-value		0,622
Overweight – obesity I		
E-cigarette smokers	18	76,32 (29,5-236,83)
Conventional smokers	8	98,87 (43,47 – 171,1)
Controls	7	76,19 (29,5 – 236,83)
p-value		0,145

There was no significant difference in corrected urine isoprostane levels based on current smoking degrees in the e-cigarette smokers and conventional smokers groups (p> 0.05), shown in table 7.

Table 7. Corrected urine isoprostane levels in the e-cigarette smokers and conventional cigarette smokers groups based on current smoking degrees

Current smoking degree	N	Median corrected urine isoprostane (pg/mg creatinine)
E-cigarette smoker		
Mild	14	76,31 (28,47-189,81)
Moderate – severe	14	74,05 (31,15-236,83)
p-value		0,713
Conventional smoker		
Mild	18	84,9 (37,09-322,08)
Moderate	9	103,43 (43,47-171,1)
p-value		0,719

There was no significant difference (p> 0.05) in corrected urine isoprostane levels based on previous smoking history in the e-cigarette group, and it can be seen in table 8.

Table 8. Corrected urine isoprostane levels in e-cigarette smokers based on previous smoking history

Previous smoking history	N	Median corrected urine isoprostane (pg/mg creatinine)
No	9	76,44 (40,01-189,81)
Yes	19	75,39 (28,47-236,83)
p-value		0,446

There was no significant difference (p> 0.05) in corrected urine isoprostane levels based on the duration of smoking time in the e-cigarette smoker group, shown in table 9.

Table 9. Corrected urine isoprostane for the current smoking duration in the e-cigarette group

Duration of smoking time	N	Median corrected urine isoprostane (pg/mg creatinine)
1 - 3 years	12	68,18 (29,13-200,48)
4 - 6 years	14	81,22 (28,47-236,83)
7 - 9 years	2	52,28 (34,12-76,44)
p-value		0,816

DISCUSSION

Characteristics of the study subjects

The study subject is according to conditions in Indonesia. According to BPS data in 2018, the prevalence of conventional cigarette users is mainly in the 30 to 34 year age group as much as 36.66% and followed by the 35 to 39 year age group as much as 36.23%.[9] Elsa and Nadjib's study reported that e-cigarette users in 2017 based on SUSENAS (National Socio-Economic Survey) data in 2017 were dominated by men with the age group of 25 to 45 years.[10]

Corrected urine isoprostane levels in the study subjects

In this study, the sample taken was random urine so that the urine isoprostane levels obtained were then corrected with the urine creatinine value of the subject. This is in accordance with several previous studies. Correction is done by dividing the urine isoprostane by the urine creatinine level in the same sample. This correction is done because the random urine sample can be affected by variations in the diuresis and hydration level of the subject. Five of twenty studies reported by the meta-analysis of Van der Plas et al used 24-hours urine samples, and it was stated that 24-hour storage of urine was not affected by variations in diuresis, so there was no need to correct it with urine creatinine.[11–14]

The highest median of corrected urine isoprostane was found in the conventional cigarette group, while the lowest was in the control group. The median of corrected urine isoprostane in the e-cigarette smokers and conventional smokers tended to be higher than the median of the controls. These conditions are in accordance with the report of Obata et al. which stated that urine isoprostane levels in male smoker or with history of smoking were higher than non-smokers.[15] Research in Japan by Sakano et al. stated that urine isoprostane levels in healthy non-smokers were 680 ± 30 pg/mg creatinine; the reagent used was the enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI). This study's median values were below previous studies due to differences in reagent brands.[16]

Conventional cigarettes and e-cigarettes can produce ROS, which contributes to oxidative stress. Conventional cigarettes make $5x10^{14}$ ROS in each smoke blast.[17] ROS in the tar phase resulting from burning tobacco in conventional cigarettes contains semiquinone radicals. The semiquinone radicals present in the tar phase are not volatile and cause prolonged ROS production.[18] The levels of ROS detected in aerosols produced by the mixture of propylene glycol and VG are higher than in pure air.[5] Several studies have stated that e-cigarette cigarettes contain more ROS per exhale slightly compared to conventional cigarettes.[19]

The difference in corrected urine isoprostane

This study found that corrected urine isoprostane levels in the three groups were not significantly different ($p > 0.05$). The obtained p-value = 0.054, which is almost significant. When viewed from median values between the three groups, urine isoprostane levels in the e-cigarettes and conventional smokers groups were higher than the controls.

Singh et al. reported that e-cigarette users had higher urine isoprostane levels than non-smokers.[6] The same was written by Morrow et al. and Obata et al. that the urine isoprostane levels of active smokers were more elevated than non-smokers.[7,15] Morrow et al. state that oxidants found in cigarette smoke can increase isoprostane levels. The levels of free and esterified F2-isoprostane/F2-IsoPs in the plasma of

active smokers were significantly increased compared to non-smokers.[7,8] Two meta-analyzes by Van der Plas et al. reported that conventional smokers had higher urine isoprostane levels than non-smokers.[13] Czekala et al. study on in-vitro tissue reported tissue isoprostane levels after exposure to 27 conventional cigarette puffs resembling 80 e-cigarette puffs with or without flavor. Previous studies that directly compared corrected urine isoprostane levels between e-cigarette and conventional smokers have not been found.[20]

Several previous study reported that obesity can affect isoprostane levels.[21,22] Bougoulia et al. in 2006 reported that isoprostane levels were influenced by obesity levels. Bougoulia et al. study measured levels of isoprostane in obese women and compared with women who had a normal BMI. The isoprostane levels in obese women were significantly higher. After doing regular exercise to lose weight, it was found that isoprostane levels decreased significantly in women who were previously obese.[23] Sakano et al reported that urinary isoprostane levels had a positive correlation with BMI in 320 healthy Japanese people.[16] In our study there was no significant difference in corrected urine isoprostane levels based on BMI in the electronic cigarette, conventional cigarette and control groups. This study has a small number of samples for each group and the BMI range is not too large. Researchers limit the subject's maximum BMI is 31. Most of the study subjects fit into the normal criteria so it is difficult to assess the relationship between urine isoprostane levels and BMI.

The corrected isoprostane levels in the e-cigarette smoker group who had a history of previous conventional smoking and those who did not have a previous history of conventional smoking in this study did not have a statistically significant difference. Researchers have difficulty finding the same research so it is difficult to find comparisons. In this study, the number of samples of electronic smokers was very small. After being categorized based on previous smoking history, the number of samples in each category was smaller and the statistical distribution is not normal so that it could affect data analysis.

There was no significant difference in corrected urine isoprostane levels based on current smoking duration in the electronic cigarette group. According to theoretical basis, chronic exposure to irritants can increase ROS levels and increase oxidative stress so as to increase isoprostane levels.[5,6] Researchers find it difficult to find previous comparative studies, because of the small number of studies on e-cigarettes.

CONCLUSION

Urine isoprostane levels in e-cigarettes and conventional smokers based on median tend to be higher than controls. There was no significant difference in corrected urine isoprostane levels between the e-cigarette and conventional smokers. It can be concluded that the level of oxidative stress in the two groups did not have a significant difference but tended to be higher than the non-smoker group.

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