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Original Article

Flow-Mediated Arterial Dilatation, Hypertension, and ACE2 in Adolescent Obesity: A Cross-Sectional Study

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ABSTRACT

Background: Endothelial dysfunction is a significant mechanism underlying various cardiovascular diseases. In the context of COVID-19, soluble angiotensin-converting enzyme 2 (sACE2) and flow-mediated dilation (FMD) are linked to endothelial dysfunction. This study aimed to evaluate sACE2 and FMD levels among obese and non-obese Indonesian adolescents.

Methods: This study involved 72 Indonesian adolescents, with a mean age of 195 months (range = 178–217). The majority of participants were male (61%). They were divided into 3 groups: those with obesity and hypertension (n=21), those with obesity without hypertension (n=19), and non-obese individuals (n=32). FMD assessment involved measuring the brachial artery using supra-systolic pressure applied to the forearm for 5 minutes. Further, sACE2 levels were determined using the Sandwich ELISA method.

Results: The median level of sACE2 was 25.4 pg/mL, with a range of 0.25 to 181.8 pg/mL. Nonetheless, no statistically significant differences in sACE2 levels were observed between the three groups (P = 0.464). Although descriptive statistics indicated that FMD values were higher in normal adolescents, no significant differences were found in FMD values between the groups: those with obesity and hypertension (9.1 ± 5%), those with obesity without hypertension (8.3 ± 3.1%), and normal adolescents (10.9 ± 6.2%) (P = 0.159). Furthermore, the comparison of sACE2 levels between the groups with and without endothelial dysfunction revealed no differences (P = 0.95). There was also no statistically significant correlation between sACE2 levels and FMD (P = 0.59, r = 0.064).

Conclusions: Our results showed no differences in sACE2 and FMD values between obese and non-obese Indonesian adolescents, regardless of hypertension status. Additionally, the correlation between endothelin-1 and FMD is not significant in this population of Indonesian adolescents. ***(Iranian Heart Journal 2025; 26(3): 51-58)***

KEYWORDS: Endothelial dysfunction, Flow-mediated dilatation, Obese

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Angiotensin-converting enzyme 2 (ACE2) is a key enzyme in the renin-angiotensin-aldosterone system (RAAS). [1] Beyond its role in RAAS activation, ACE2 also helps maintain endothelial integrity. It is predominantly expressed as a membrane protein in the endothelium, with the highest concentrations found in pulmonary capillaries. [1]

During the COVID-19 pandemic, research highlighted ACE2's involvement in SARS-CoV-2 infection. [2, 3] Studies linked ACE2 expression to COVID-19 severity, [3, 4] with elevated soluble ACE2 (sACE2) levels correlating with endothelial dysfunction in infected patients. [2] Comorbid conditions like obesity and hypertension may further exacerbate endothelial impairment in COVID-19 cases. [4-6]

Flow-mediated dilatation (FMD) has emerged as a cost-effective, noninvasive marker for endothelial dysfunction. [7] In patients with COVID-19, FMD correlates with disease severity, [8] but its relationship with sACE2 in obese adolescents without COVID-19 remains unexplored.

This study primarily aimed to evaluate sACE2 levels and FMD values between obese and non-obese adolescents. In addition, we classified the obese group into those with or without hypertension.

METHODS

Subjects

This study employed an analytical observational design with a cross-sectional approach. We categorized participants into 3 groups: those with obesity and hypertension, those with obesity but without hypertension, and a normal adolescent group. The normal group, defined as adolescents who did not have obesity or hypertension, served as the comparator in this research. Subjects were recruited from senior high school students in the Surabaya and Sidoarjo districts, based on their voluntary participation. The research was conducted from June through November 2023. For participants under 18 years old, parents or guardians provided representation. Adolescents with a history of hormonal therapy, smoking, alcohol consumption, endocrine disorders, dyslipidemia, liver disease, or renal disease were excluded from the study.

Anthropometric Measurements

Weight (kg) was measured using a digital electronic scale with an accuracy of 0.1 kg. Height was assessed with a wall-mounted Harpenden stadiometer. Body mass index (BMI) was calculated based on the recorded weight and height, and the resulting value was plotted according to the pediatric BMI centile chart. A BMI value exceeding the 95th percentile was classified as obesity. [9] Blood pressure (BP) was measured under relaxed conditions using a standard sphygmomanometer fitted with a cuff on the subject's arm. [10] To minimize measurement bias, we took an average of at least 2 BP readings. [10]

ACE2 and Metabolic Parameters

An automated analyzer (Auto Chemistry Analyzer TMS 24i Premium) was utilized to assess the metabolic parameters of the subjects, including triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, and random plasma glucose. We also calculated the total cholesterol/HDL ratio. Plasma ACE2 levels were measured using a sandwich ELISA (Bio Rad iMark Microplate Reader, USA). Blood analyses were conducted on the same day as the blood sampling.

FMD Measurement

FMD was defined as the percentage change in the brachial artery diameter from baseline in response to increased flow and was calculated accordingly. [7] The measurement

of the brachial artery diameter (baseline measurement) was initially performed at the one-third lower humerus while the arm was in a relaxed position. The pneumatic cuff was then inflated until supra-systolic pressure was achieved and maintained for 5 minutes. The cuff was positioned on the forearm, distal to the ultrasound probe. The brachial artery was measured after the deflation of the pneumatic cuff. FMD measurements were conducted by an experienced cardiologist using echocardiography (E Healthcare-Ultrasound Vivid IQ V204).

Statistical Analysis

All statistical analyses were conducted using SPSS, version 23. The Kolmogorov-Smirnov test was employed to determine whether the data distribution was normal or non-normal. The mean ± standard deviation (SD) was used to describe the distribution of continuous data with a normal distribution. For data with a non-normal distribution, the median (quartile 1–quartile 3) was employed to represent the data distribution. Chi-square analysis was utilized to evaluate differences between categorical data, presenting the results as proportions.

For data with a normal distribution, the ANOVA test was conducted to investigate differences between groups, followed by the post-hoc least significant difference (LSD) test to compare differences between pairs of groups for variables that showed significant differences in the ANOVA test. The Kruskal-Wallis test was used instead of the ANOVA test when the data distribution was non-normal. In addition, a comparison of the groups was performed based on the FMD cutoff (7.1%) to differentiate between those with endothelial dysfunction and those without. A cutoff of 7.1% was established based on a previous study conducted by Maruhashi et al. [11] A Spearman test was performed for variables with a non-normal data distribution to evaluate the correlation between FMD and ACE2. Results were considered significant when the *P*-value was less than 0.05.

RESULTS

This study involved 72 adolescents, stratified into 3 groups: obese with hypertension (n = 21), obese without hypertension (n = 19), and normal-weight controls (n = 32). Males comprised the majority (61%), and the median age across all participants was 195 months (IQR, 178 to 217). Age and sex distributions did not differ significantly between the groups.

As expected, systolic and diastolic BP values were significantly elevated in the obese hypertensive group compared to both the obese non-hypertensive and normal-weight groups ($P < 0.05$). In contrast, no significant differences in BP were observed between the obese non-hypertensive and normal-weight groups (systolic BP: 125 ± 11 vs. 119 ± 14 mm Hg; $P = 0.11$; diastolic BP: 79 ± 7.3 vs. 74 ± 11 mm Hg; $P = 0.69$).

Metabolic parameters

Plasma glucose levels were significantly elevated in the hypertensive obese group compared to the normal-weight group ($P < 0.001$), but showed no significant difference when compared to the non-hypertensive obese group ($P = 0.81$). While total cholesterol levels did not differ significantly between the three groups (ANOVA $P = 0.286$), HDL concentrations were markedly lower in both hypertensive ($P < 0.001$) and non-hypertensive obese groups ($P < 0.001$) relative to controls.

Notably, HDL levels did not differ significantly between the 2 obese groups (42.8 ± 6.9 mg/dL vs. 41 ± 7.6 mg/dL; $P = 0.04$). Similar patterns were observed for the total cholesterol-to-HDL ratio. No significant intergroup differences were

found in either triglyceride or LDL concentrations.

Endothelial dysfunction parameters

No significant differences in FMD were observed between the groups (P = 0.159), with values of 9.1 ± 5% in the hypertensive obese group, 8.3 ± 3.1% in the non-hypertensive obese group, and 10.9 ± 6.2% in the normal-weight group. Notably, while not statistically significant, FMD values were numerically higher in normal-weight participants than in both obese groups, regardless of hypertension status.

The median ACE2 level across all Indonesian adolescents in this study was 25.4 ng/mL (range = 0.25–181.8). Group-specific analyses revealed comparable ACE2 concentrations: 24.4 pg/mL (13.3–55.6) in the hypertensive obese group, 26.3 ng/mL (0.25–181) in the non-hypertensive obese group, and 44.5 pg/mL (26–62) in controls (P = 0.464). Secondary analysis using a 7.1% FMD cutoff to classify endothelial dysfunction showed no significant differences in ACE2 levels (P = 0.175). We also found no correlation between endothelin-1 concentration and FMD values (r = −0.064; P = 0.59).

Table 1. The baseline characteristics of the study population

Variables	Total (n= 72)	Obesity + HT (n= 21)	Obesity + Non-HT (n= 19)	Normal (n= 32)
Age, months *	195 (178-217)	195 (178-217)	196 (186-217)	194 (179-217)
Male, %	44 (61%)	17 (53%)	14 (74%)	13 (62%)
Systolic blood pressure, mm Hg	131 ± 16	142 ± 12.)	125 ± 11.2	119 ± 14
Diastolic blood pressure, mm Hg	82 ± 11.5	89 ± 9.7	79 ± 7.3	74 ± 11
Random blood glucose, mg/dL	85 ± 7.1	87 ± 5.8	86 ± 8.4	82 ± 7
Total cholesterol, mg/dL	175 ± 38	183 ± 36.4	169 ± 47	168 ± 41
LDL, mg/dL*	112 (54-201)	123 (66-188)	106 (83-201)	100 (52-160)
HDL, mg/dL	45.5 ± 10	42.8 ± 6.9	41 ± 7.6	54 ± 11
Total cholesterol- HDL ratio	4 ± 1.1	4.3 ± 0.92	4.3 ± 1	3.2 ± 0.9
Triglycerides, mg/dL*	96 (38-247)	119 (38-247)	95 (45-178)	71 (39-134)
FMD, %	9.1 ± 5	8.3 ± 3.1	8.5 ± 5.8	10.9 ± 6.2
sACE2, ng/mL*	25.4(0.25-181.8)	24.4 (13.4-114.4)	26.3 (0.25-181)	24.7 (13.3-55.6)

HT: hypertension, HDL: high-density lipoprotein, LDL: low-density lipoprotein, FMD: flow-mediated dilatation, sACE2: soluble angiotensin-converting enzyme 2

Table 2. ANOVA and post-hoc LSD test

Variables	Uji-ANOVA	Obesity + HT	Obesity + Non-HT
Age, months *	0.854*	N/S	N/S
Systolic blood pressure, mm Hg	<0.001	Normal= <0.001 Obesity + non-HT= <0.001	Normal= 0.114
Diastolic blood pressure, mm Hg	<0.001	Normal= <0.001 Obesity + non-HT= 0.002	Normal= 0.69
Random blood glucose, mg/dL	<0.048	Normal= 0.019 Obesity + non-HT= 0.81	Normal= 0.06
Total cholesterol, mg/dL	0.286	N/S	N/S
LDL, mg/dL	0.02*	Normal= 0.16 Obesity + non-HT= 0.59	Normal= 0.57
HDL, mg/dL	<0.001	Normal= <0.001 Obesity + non-HT= 0.4	Normal= <0.001
Total cholesterol- HDL ratio	<0.001	Normal= <0.001 Obesity + non-HT= 0.794	Normal= 0.001

Triglycerides, mg/dL	0.016*	Normal= 0.13 Obesity + non-HT= 0.435	Normal= 0.75
FMD, %	0.149	N/S	N/S
ACE-2, ng/mL	0.464*	N/S	N/S

HT: hypertension, HDL: high-density lipoprotein, LDL: low-density lipoprotein, FMD: flow-mediated dilatation, sACE2: soluble angiotensin-converting enzyme 2, N/S: not significant for variables with ANOVA or Kruskal-Wallis *P*-value >0.05
* The Kruskal-Wallis test was used for data with a non-normal distribution.

Table 3. The Spearman analysis

Variable	Correlation Coefficient (*r*)	*P*-value
FMD – ACE-2	0.064	0.59

FMD: flow-mediated dilatation, sACE2: soluble angiotensin-converting enzyme 2

Table 4. Comparison between the 2 study groups based on endothelial dysfunction status

Variables	FMD <7.1%	FMD ≥7.1%	*P*-value
Age, months *	195 (184-217)	195 (178-217)	0.792*
Male, %	18 (69%)	26 (67%)	0.62
Systolic blood pressure, mm Hg	132 ± 17.7	123 ± 15.3	0.662
Diastolic blood pressure, mm Hg	83 ± 11.8	81 ± 11.6	0.529
Random blood glucose, mg/dL	85 ± 7.3	85 ± 7.1	0.799
Total cholesterol, mg/dL	177 ± 46.6	174 ± 33.4	0.746
LDL, mg/dL*	116.5 (54-147)	110 (54-188)	0.729*
HDL, mg/dL	42.2 ± 7.8	47.1 ± 10.8	0.037
Total cholesterol- HDL ratio	4.3 ± 1.1	3.9 ± 1.1	0.107
Triglycerides, mg/dL*	108 (38-247)	87 (38-247)	0.3*
ACE-2, ng/mL	24.8 (13.1-181.8)	26 (0.25-114)	0.95*

HDL: high-density lipoprotein, LDL: low-density lipoprotein, sACE2: sACE2: soluble angiotensin-converting enzyme 2

* The Mann-Whitney test was used for data with a non-normal distribution.

DISCUSSION

Cardiovascular disease is currently the primary cause of morbidity and mortality worldwide. [12] Endothelial dysfunction is one of the primary underlying processes in various cardiovascular diseases. [7,13] Numerous pro-inflammatory, pro-oxidizing, and vasoconstrictive molecules are linked to the degree of endothelial dysfunction. [7] However, the role of sACE2 in endothelial dysfunction remains unclear. Recent reports have indicated that sACE2 is associated with increased mortality in COVID-19 patients and reflects the extent of endothelial dysfunction. [3]

Obesity and hypertension are recognized as cardiovascular risk factors, [10, 14] and their presence is linked to endothelial dysfunction when compared to individuals without these conditions. In this study, we found that sACE2 concentrations did not differ between obese and normal-weight adolescents, regardless of hypertension status.

Our findings contrast with those of previous studies, which indicate that sACE2 is closely related to endothelial dysfunction in COVID-19 patients. [2] sACE2 functions as a receptor or co-receptor that enables viral infection into cells through endocytosis, resulting in a higher viral load. [2] A high viral load during the initial days of infection is associated with a more severe hypersensitivity response and disease severity, ultimately leading to severe

endothelial dysfunction. [15] Endothelial dysfunction disrupts endothelial integrity and causes the release of ACE2 into circulation, [2] highlighting sACE2's role as a biomarker of endothelial dysfunction rather than a causative factor. Nevertheless, the smaller degree of endothelial dysfunction in adolescents may explain the similar sACE2 levels between obese and normal subjects in this study.

In addition, a previous study by Nagatomo et al. [5] demonstrated higher sACE2 levels in the obese group than in the non-obese group. Similarly, a study conducted by Emilsson et al. [16] also found elevated sACE2 levels in the obese group compared to the non-obese group.

Endothelial dysfunction disrupts endothelial integrity, leading to increased sACE2 levels in plasma. Be that as it may, it is important to note that the participants in these previous studies were considerably older than those in our study, with an average age of 75 ± 6 years. Therefore, it appears that sACE2 levels may be elevated in older individuals with obesity but not necessarily in adolescents.

Interestingly, when comparing sACE2 levels between our study and that of Nagatomo et al., [5] we found that their reported levels were approximately 3 times higher (68.7 pg/mL5) than those observed in our study (25.4 [0.25–181.8]). The difference in sACE2 levels may be attributed to peak steroid hormone levels, such as testosterone and estrogen, during adolescence, [17, 18] known to have protective effects on the cardiovascular system, including the reduction of endothelial dysfunction. [18–20] Furthermore, the increased sensitivity of steroid hormone receptors during early puberty could potentially contribute to a protective effect against endothelial dysfunction in obese adolescents. [11] Racial factors may also play a significant role, as the study by Emilsson et al. 1[6] exclusively included Caucasian subjects and the Nagatomo et al. [5] study focused solely on the Japanese population.

Notably, this is the first publication that compares sACE2 levels between obese and non-obese individuals. Our study also revealed no significant difference in sACE-2 levels between individuals with and without endothelial dysfunction based on FMD examinations, regardless of their obesity and hypertension status. These findings suggest that sACE2 concentrations may not be elevated in adolescents with endothelial dysfunction. As previously mentioned, this could be attributed to the potential protective effects of high steroid hormone concentrations and other factors in adolescents. Notably, FMD, a clinical parameter of endothelial dysfunction, also showed no significant correlation with sACE2 levels in adolescents, indicating that FMD results may not accurately reflect sACE2 levels in this population.

CONCLUSIONS

We observed no significant differences in sACE2 levels or FMD values between obese and normal-weight adolescents, regardless of their hypertension status. Furthermore, sACE2 levels showed no significant correlation with FMD in Indonesian adolescents.

Declarations

Ethical Approval and Consent to Participate

This research was approved by the Ethics Committee of Dr. Soetomo General Hospital (No. 271/EC/KEPK/FKUA/2023).

Consent for Publication

Not applicable.

Availability of Data and Materials

All data used in the manuscript are available upon reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

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Authors' Contributions

MA: conceptualization, methodology, investigation, writing—original draft, resources, review and editing, supervision (writing and final approval of manuscript)
NAW: conceptualization, methodology, formal analysis, writing–original draft, data curation, review and editing, supervision (writing and final approval of manuscript)
AF: data acquisition, data analysis, writing–original draft, project administration, review and editing (writing and final approval of manuscript)
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