ISSN: (3007-0384)
E-ISSN: (3007-0392)
Journal of Wahj Al-Ulom for Pure Sciences
available online at: https://uomosul.edu.iq/womeneducation/jwups/

Relationship of Angiotensin Converting Enzyme 2(ACE2) to Antioxidants and Lipid level in Patients with Heart Diseases

Lubna L.Ghazy [1]*, Nawal Th. Younis[2],

[a] **Bartella Intermediate School for Boys,Al-Hamdaniyah Education Directorate,Nineveh Governorate ,Iraq**
[b] **Department of chemistry, Collage of Education for Girls, University of Mosul, Mosul, Iraq**

***Corresponding author e-mail:**
dr.nawal.thanoon@uomosul.edu.iq

***Keywords*:**
ACE2, antioxidant, BMI, dyslipidemia, smoking.

ARTICLE INFO
Article history:
Received : 26/06/2023
Accepted : 03/07/2023
Available online : 01/08/2024

Email:
journal.purescience.ge @uomosul.edu.iq

ABSTRACT

The study included the relation between angiotensin-converting enzyme ACE2 and some of the biochemical parameters related to the heart diseases (reduced Glutathione (GSH), Arylesterase (ARE), ceruloplasmin, malondialdehyde (MAD), Cholesterol, Triglyceride (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), Atherogenic Index(AI), Antiatherogenic Index(AAI) in the serum of the patients compared with control group, the results demonstrated a significant increase in the activity of ACE2 in patients (0.69 ± 0.22 ng/ml) compared with the activity in control group (0.47 ± 0.17 ng/ml) , while the result showed a significant decrease in the concentration of GSH, HDL and ARE activity. A significant increase had been shown in the concentration of ceruloplasmin, TG, LDL, VLDL, and AI, AAI index. Non-significant difference in MAD and cholesterol in patients compared with the healthy group. The results also showed that there was an increase of ACE2 activity in smoking and non-smoking patients compared control group, also an increase of ACE2 activity with increasing body weight or BMI, the Correlation coefficient showed a non-significant correlation between ACE2 and the biochemical parameters measured in patients and control groups.

علاقة الانزيم المحول للانجيوتنسين 2(ACE2) بمضادات الاكسدة ومستوى الدهون لدى المصابين بالأمراض القلبية

لبنى لقمان غازي[1] أ.م.د. نوال ذنون يونس[2]

[1]متوسطة برطلة للبنين/مديرية تربية الحمدانية/ محافظة نينوى [2]قسم الكيمياء/كلية التربية للبنات/جامعة الموصل

الخلاصة:

تضمنت الدراسة العلاقة بين الانزيم المحول للانجيوتنسين 2(ACE2) وبعض المتغيرات الكيموحيوية المتعلقة بالأمراض القلبية (الكلوتاثايون المختزل (GSH), الاريل استريز(ARE) , السيريلوبلازمين, المالوندايالديهايد (MAD), الكوليسترول, الدهون الثلاثيةTG, البروتين الدهني الواطئ الكثافة LDL, البروتين الدهني الواطئ الكثافة جدا VLDL, البروتين الدهني العالي الكثافةHDL, معامل مسبب التعضدية (AI), معامل مضاد مسبب التعضدية (AAI), في مصل دم المرضى مقارنة مع مجموعة السيطرة, اظهرت النتائج زيادة معنوية في فعالية انزيم ACE2 في المرضى (0.69 ± 0.22 ng/ml) مقارنة مع فعاليته في مجموعة السيطرة (0.47 ± 0.17 ng/ml) بينما اظهرت النتائج انخفاض معنوي في تركيز انزيمات (GSH) و((ARE، HDL وظهرت زيادة معنوية في تركيز السيريلوبلازمين, A,AI , VLDL, LDL, TG واختلاف غير معنوي في تركيز (MAD) والكوليسترول في المرضى مقارنة مع مجموعة الاصحاء واظهرت النتائج ايضا زيادة في فعالية انزيم ACE2 لدى المدخنين وغير المدخنين في المرضى مقارنة مع مجموعة السيطرة وزيادة في فعالية انزيم ACE2 بزيادة مؤشر كتلة الجسم (BMI) واظهر معامل الارتباط الخطي علاقة غير معنوية بين ACE2 والمتغيرات الكيموحيوية المقاسة في المرضى مقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: ACE2, عسر شحميات الدم، مضادات الاكسدة، التدخين، BMI.

INTRODUCTION

Heart failure is inability of the heart to pump enough blood to meet the body's requirements of nutrients and oxygen, which causes irregular heartbeat, shortness of breath, chest pain, fatigue, body weakness, and a change in the pulse [1]. Oxidative stress is the state of imbalance in the system of oxidants and antioxidants towards the production of more oxidants reflecting an imbalance between the systemic manifestations of Radical Oxidative Stress ROS and the ability of the organism to easily detoxify the reaction intermediate or repair the resulting damage [2]oxidative stress is associated with ageing and with diseases such as cardiovascular disease, chronic kidney disease, neurodegenerative diseases and cancer[3]. ACE2 enzyme (Ec 3.4.17.23) was discovered in 2000 It is a monocarboxylate peptide and it is considered a metalloproteinase. It consists of (805) amino acids. It is a type-1 transmembrane protein, and molecular weight (110-120)KDa [4]. ACE2 is a major component of the renin-angiotensin-aldosterone system (RAAS) where it converts angII into ang(1-7) (RAAS)is regulates a range of physiological and pathological functions including cell proliferation, invasion and differentiation as well as inflammation and oxidative stress[5].ACE2 plays a protective role in organ damage by protecting pro-inflammatory actions of AngII, and ACE2 reduces oxidative stress caused by AngII through its production of Ang (1-7) when bound to the Mas receptor, reduces ROS, increases endothelial nitric oxide(NO) formation, improves the antioxidant capacity of tissues, and prevents endothelial dysfunction[6], Therefore, the RAAS system contains pro-inflammatory and anti-inflammatory actions. The pro-inflammatory actions are due to the effect of AngII, which acts on the AT1R receptor. It activates many pathways related to tissue injury, inflammation, fibrosis, inhibition of protein kinases and nuclear transcription factors, recruitment of inflammatory cells, adhesion of monocytes, and release of cytokines and chemokines, while the ACE2and Ang(7-1) axis suppresses inflammation by inhibiting leukocyte migration, cytokine expression and release, and fibrotic pathways[7]. Exposure to many environmental toxicants can affect ACE2 expression and this may increase the severity of infections [8]. Studies revealed an enhanced expression of ACE2 in polluted air that contains nanoparticles and exposure to NO2 and other substances. particulate matter enhances ACE2 expression after chronic exposure to these pollutants [9].ACE2 mRNA and protein levels increase in response to acute hypoxia. Increased cell migration and proliferation are associated with decreased ACE2 expression in hypoxia-exposed cells[10].

The Aim of Research:

The research Aims are to measure some of the biochemical parameters related to heart disease, such as lipid, some antioxidants and oxidants in the patient's blood serum, the ACE2 enzyme, and to study the effect of smoking and BMI on the incidence of heart disease, and then to find the linear correlation coefficient between the ACE2 enzyme and all the studied biochemical parameters to identify the effect of the enzyme and antioxidants, oxidants on disease, as well as a study of the mechanism of action of the ACE2 enzyme and its physiological effect on the body.

Materials and Methods

Forty blood samples were collected from heart patients in age (20-70) years. The samples should be divided into age groups in order to show differences in results and the effect of these factors and the results of the males should be isolated from females due to the different physiology of these two groups, males (22) and females (18), and (25) blood samples were collected from healthy people as a control group for both sexes, males (15) and females (10), the blood serum was separated from All blood samples were then estimated for some biochemical parameters

-Estimating of ACE2 enzyme: the enzyme was estimated using Fine Test kit (China)by enzyme-linked immunoassay ELISA technique [11].

-GSH: was estimated using Elman's reagent by modified method for researchers (Sedlak and Lindsy,1968) [12].

- ARE enzyme: was estimated by analyzing the substrate phenylacetate to phenol and acetic acid [13].

-Ceruloplasmin: it was estimated using (para-phenylenediamines) by a modified method for researchers (Menden et al,1977)[14].

-Malondialdehyde: it was estimated using Thiobarbituric acid (TBA) by modified method for researchers (Guidet and Shah;1989) [15].

-Cholesterol: it was estimated using BIOLABO Kit (France)[16].

-Triglyceride: it was estimated using BIOLABO Kit(France)[17].

-LDL: it was calculated according to the following equation:

LDL-C =TC-HDL-C-VLDL-C[18].

-VLDL: it was calculated according to the following equation: VLDL=TC/5 [19].

-HDL: it was estimated by using BIOLABO Kit (France) [20].

-Atherogenic Index (AI): It was calculated according to the following equation:

AI=Log (TG/HDL-C) [21].

-Antiatherogenic Index (AAI): it was calculated according to the following equation:

AAI=HDL-C×100/(TC-HDL-C) [22].

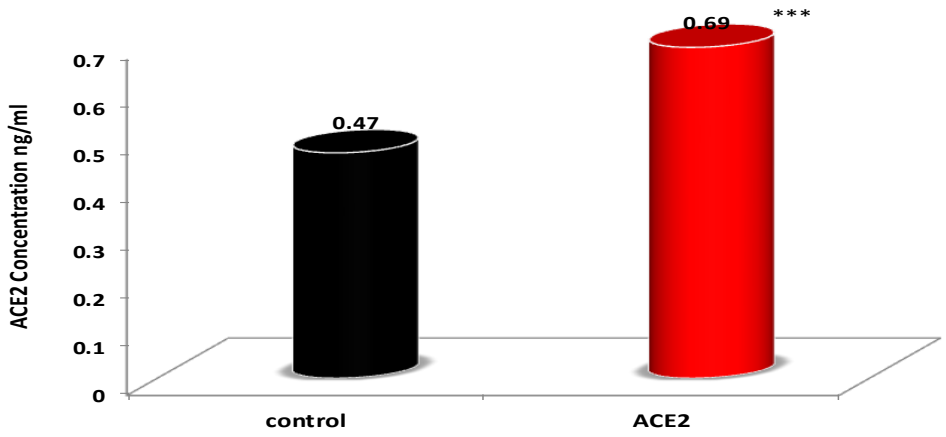
Data Analysis:

The data were analyzed statistically using the T-test method to find differences between two parameters, find the mean for the measured biochemical parameters and the standard deviation, the linear correlation coefficient(r)] was performed to identify the relation between different clinical parameters and ACE2 by using the statistical analysis program (SPSS 22) [23].

Results and Discussion

1- ACE2 concentration in heart patients compared with the control group:

Figure 1. shows that there is a significant increase in the activity of the ACE2 enzyme in heart patients, as it was (0.69 ± 0.22 ng/ml) compared with its activity in healthy group (0.47 ±0.17 ng/ml), where this result is consistent with [24], the activity of the enzyme is an indicator of the weakness of the lining of blood vessels, atherosclerosis, and that AngII binds to its receptors to constrict blood vessels and increase cardiac output, blood pressure [25].



***** Significant difference between heart patients and control at p<0.001**

Fig. 1. ACE2 activity ng/ml in heart patients and control group

2-Some Clinical parameter concentrations in heart patients compared with the control group:

The results in Table 1 show that there is a significant decrease in the concentration rate of GSH enzyme in heart patients compared with the control group, It is confirmed that both GSH and NO are regulators of the heart muscles and cardiac tissues, which are reduced in heart failure[26], the results also showed a significant decrease in ARE enzyme in the patients compared with healthy subjects, The reason may be due to the fact that the activity of the paraoxonase (PON1) and the ARE enzyme is less in coronary heart disease and that lipoprotein a (LPa) is oxidized in the wall of blood vessels and plays a role in complete occlusion coronary artery disease due to hyperlipidemia [27]. The same Table also noted a significant increase in the concentration of Ceruloplasmin in the patients. This is due to the action of ceruloplasmin as an oxidant, which is positively associated with coronary heart disease [28]. The results also showed in Table 1 a non-significant increase in the concentration of Malondialdehyde compared with healthy subjects, this is because MDA is considered a major factor in the molecular mechanisms of vascular wall damage in the development of heart disease, and it is a by-product of lipid peroxidation, free radicals, and increases in oxidized LDL [29].

while the same table showed a non-significant increase in the concentration of cholesterol, a significant increase in the LDL in patients compared to healthy subjects the reason is due to an increase in the ratio of LDL/HDL is associated with an increase in coronary artery stenosis, as LDL increases with an increase in body mass index (BMI) [30], also, the results showed a significant increase in the concentration of TG and VLDL. This result is consistent with what was stated by [31, 32] that the increase in triglycerides is an indicator associated with the risk of developing cardiovascular diseases and is a sign of insulin resistance in the elderly, VLDL also increases the risk of vascular disease, and the hydrolysis of triglycerides generates inflammation, causing the release of free fatty acids, which stimulate inflammation of the lining of blood vessels, while the results showed a significant decrease in HDL The reason may be that Cholesterol ester transfer protein (CETP) facilitates the exchange of triglycerides and cholesterol ester between HDL and apolipoproteins containing lipoproteins B100. Therefore, inhibition of CETP increases HDL and reduces LDL, A100, and non-HDL, resulting in a cardiovascular benefit [33].

Table 1: Some Clinical parameters concentration in heart disease patients and control group

Clinical Parameters	Patients	Control Group
GSH µmol/L	1.4 ± 0.44***	2.1 ± 0.59
ARE enzyme U/ml	97.3 ± 8.73***	116.6 ± 8.0
Ceruloplasmin µmol/L	240.6 ± 2.7***	228.1 ± 7.18
Malondialdehyde µmol/L	3.6 ± 0.5	3.4 ± 0.54
Cholesterol mg/dl	162.17 ± 18.49	149.33 ± 26.1
Triglyceride mg/dl	166.38 ± 35.69***	143.66 ± 26.11
LDL mg/dl	99.32 ± 19.79***	78.71 ± 25.99
VLDL mg/dl	33.27 ± 7.13***	28.73 ± 5.22
HDL mg/dl	29.58 ± 7.12***	40.82 ± 6.94
AI	0.75 ± 0.15***	0.54 ± 0.13
AAI	22 ± 6.3***	37.6 ± 9.47

***Significant difference at $p<0.001$

3-Correlation between ACE2 activity and measured Clinical parameters in heart patients comparing to the control group:

The results in Table 2 showed that the associations are not significant between ACE2 and the biochemical parameters measured, while other studies showed that AngII is associated with a significant positive correlation with cholesterol, TG, LDL, and VLDL, and a negative significant correlation with HDL, also smoking increases ACE synthesis and changes the inflammatory state [34]. ACE level rise due to lipid levels during oxLDL synthesis, smoking causes the release of reactive oxygen species, lipid oxidation, and reduces antioxidants [35, 36], also, the studies showed that genetic deletion of ACE2 causes a decrease in NO concentration, and that defect in ACE2 leads to a decrease in the activity of the superoxide Dismutase (SOD) enzyme and an increase in lipid peroxidation, which indicates a weak ability of antioxidants [37]. Also, the deficiency of the MAS receptor indicates a decrease in SOD and catalase activity, an increase in lipid peroxidation, and ROS levels, accompanied by impaired endothelial function and an increase in blood pressure [35]. Also, Ang (1-7) produced from ACE2 plays a role in inhibiting inflammatory signals and protein kinase C(PKC) and has an anti-inflammatory effect. Inhibiting neutrophil aggregation and leukocyte adhesion, inhibiting the inflammatory cytokines IL-1B, IL-6 and tumor necrosis factor (TNF) and enhancing the anti-inflammatory cytokine IL-10 [7].

Table 2: Correlation between ACE2 activity and Clinical parameters in heart patients comparing to the control group

Clinical Parameters	Patients r-value	Control Group r-value
GSH µmol/L	0.069	- 0.09
ARE enzyme U/ml	- 0.046	- 0.202
Ceruloplasmin µmol/L	- 0.019	0.12
Malondialdehyde µmol/L	- 0.016	0.345
Cholesterol mg/dl	0.032	0.215
Triglyceride mg/dl	0.059	- 0.105
LDL mg/dl	- 0.072	0.290
VLDL mg/dl	0.059	- 0.105
HDL mg/dl	0.223	- 0.152
AI	- 0.137	0.032
AAI	0.134	- 0.075

3-Effect of smoking on ACE2 activity in heart patients and control group

Table 3 shows that there is a significant increase in the activity of the ACE2 enzyme in smokers and non-smokers compared with healthy subjects. This may be due to the fact that smokers who suffer from coronary heart disease have a low level of antioxidants and a higher level of lipid peroxidation compared to non-smokers. Studies have found that the amounts of MDA emitted from the red blood cells of smoker heart patients are much higher than that of the healthy group and that oxLDL enhances platelet adhesion, destroys DNA strands and leads to cell death, and angiotensin-converting enzyme is also produced in lung endothelial cells resulting from smoking, the activity of angiotensin-converting enzyme It may increase the cardiovascular events associated with smoking [36].

Table 3: Effect of Smoking on ACE2 activity in heart patients compared with the control group

Smoking	ACE_2 Activity (ng/ml) (Mean $\pm$ S.D.)	
	Patients	Control
Smokers	0.76 $\pm$ 0.188***	0.34 $\pm$ 0.168**
Non-Smokers	0.68 $\pm$ 0.21***	0.4 $\pm$ 0.14

** Significant difference between smokers and non-smokers in the control group at $p \leq 0.01$

*** Significant difference between patients and control group at $p \leq 0.001$

4-Effect of Body Mass Index (BMI) on ACE2 activity in heart diseases and control group

The results show in Table 4 that there is a rise in the activity of the ACE2 enzyme with an increase in the body mass index (BMI), and this is consistent with [38] that the enzyme is positively associated with the body mass index and the percentage of fat, and that obesity caused by a high-fat diet (HFD) activates the renin-angiotensin system through the role of ACE2.

Table 4: Effect of BMI on ACE2 activity in patients compared to control.

BMI kg/m^2	ACE_2 Activity (ng/ml) (Mean ± S.D.)	
	Patients	Control
18.5-24.9	0.6 ± 0.1	0.5 ± 0.1
25 - 29.9	0.6 ± 0.13***	0.4 ± 0.1
30- 39.9	0.7 ± 0.28*	0.4 ± 0.2
≥ 40	0.9 ± 0.12**	0.7 ± 0.23

* Significant difference between patients and control group at p≤0.05

** Significant difference between the fourth category and the rest of the categories at p≤0.01

*** Significant difference between patients and control group at p≤0.001

Conclusion

It is concluded from the research that the ACE2 enzyme plays an important role as an antioxidant and reduces inflammation through its formation of (1-7) Ang and inhibition of the effect of AngII and that ACE2 is increased by increasing smoking, lipid parameters and body mass index (BMI), also ACE2 is increased by increasing antioxidants and decreases by increasing oxidants in cells.

Acknowledgement

The authors would like to express their appreciation to the presidency of the University of Mosul and the College of Education for Girls and the Department of Chemistry for the facilities and ethical approvals they provided to us for the completion of this research.

References

[1] M. K. Ames, C. E. Atkins, and B. Pitt, "The renin-angiotensin-aldosterone system and its suppression," *J. Vet. Intern. Med.*, vol. 33, no. 2, pp. 363–382, 2019, doi: 10.1111/jvim.15454.

[2] E. Wojtaszek, U. Oldakowska-Jedynak, M. Kwiatkows, T. Glogowski, and J. Malyszko, "Uremic toxins, oxidative stress, atherosclerosis in chronic kidney disease, and kidney transplantation," *Oxi. Med. Cell Longev.*, vol. 2021, 2021, doi: 10.1155/2021/6651367.

[3] L. Liguori *et al.*, "Oxidative stress, aging, and diseases," *Clin. Interv. Aging*, vol. 13, pp. 757–772, 2018, doi: 10.2147/CIA.S158513.

[4] J. Wysock, A. Schulze, and D. Batlle, "Novel variants of angiotensin converting enzyme-2 of shorter molecular size to target the kidney renin angiotensin system," *Biomolecules*, vol. 9, no. 12, p. 886, 2019, doi: 10.3390/biom9120886.

[5] S. Tamanna, E. R. S. Lumbers, K. S. Morosin, S. Delforce, and K. G. Pringle, "ACE2: A key modulator of the RAS and pregnancy," *Am. J. Physiol. Integr. Comp. Physiol.*, vol. 321, no. 6, pp. R833–R843, 2021, doi: 10.1152/ajpregu.00211.2021.

[6] L. A. Rabelo, N. Alenina, and M. Bader, "ACE2 – angiotensin-(1–7)–Mas axis and oxidative stress in cardiovascular disease," *Hypertens. Res.*, vol. 34, pp. 154–160, 2011, doi: 10.1038/hr.2010.235.

[7] A. C. Simoes e Silva, K. C. Silveira, A. J. Ferreira, and M. M. Teixeira, "ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis," *Br. J.*

Pharmacol., vol. 167, pp. 477–492, 2013, doi: 10.1111/bph.12159.

[8] S. X. Naughton, U. Raval, J. M. Harary, and G. M. Pasinetti, "The role of the exposome in promoting resilience or susceptibility after SARS-CoV-2 infection," *J. Expo. Sci. Environ. Epidemiol.*, vol. 30, pp. 776–777, 2020, doi: 10.1038/s41370-020-0232-4.

[9] B. Paital, "Nurture to nature via COVID-19, a self-regenerating environmental strategy of environment in global context," *Sci. Total Environ.*, vol. 729, p. 139088, 2020, doi: 10.1016/j.scitotenv.2020.139088.

[10] R. Zhang *et al.*, "Role of HIF-1α in the regulation ACE and ACE2 expression in hypoxic human pulmonary artery smooth muscle cells," *Am. J. Physiol. Lung Cell. Mol. Physiol.*, vol. 297, pp. 631–640, 2009, doi: 10.1152/ajplung.90415.2008.

[11] S. Li *et al.*, "ACE2 attenuates epithelial-mesenchymal transition in MLE-12 cells induced by silica," *Drug Des. Devel. Ther.*, vol. 14, pp. 1547–1559, 2022, doi: 10.2147/DDDT.S252351.

[12] G. Salbitani, C. Bottone, and S. Cafagna, "Determination of reduced and total glutathione content in extremophilic microalga Galdieria phlegrea," *Bio Protoc.*, vol. 7, no. 13, p. e2372, 2017, doi: 10.21769/BioProtoc.2372.

[13] R. T. Darwish, H. M. Megahed, M. H. Attia, and D. A. El-Neily, "Paraoxonase 1 gene polymorphism and enzymatic activity as a prognostic marker in cases of poisoning by cholinesterase inhibitor pesticides among Egyptians," *Ain Shams J. Forensic Med. Clin. Toxicol.*, vol. 28, pp. 88–98, 2017, doi: 10.21608/AJFM.2017.18282.

[14] K. N. Issa and F. M. Ahmed, "Oxidant-antioxidant status in postmenopausal osteoporotic women in Duhok city," *Duhok Med. J.*, vol. 12, no. 2, pp. 68–80, 2018, doi: 10.31386/dmj.uod.18.12.2.6.

[15] M. Senthilkumar, N. Amaresan, and A. Sankaranarayanan, "Estimation of malondialdehyde (MDA) by thiobarbituric acid (TBA) assay," in *Plant–Microbe Interactions, Springer Protocols Handbooks*, 2021, pp. 103–105, doi: 10.1007/978-1-0716-1080-0_25.

[16] C. A. Buritis, E. R. Ashwood, and D. E. Bruns, *Tiez Textbook of Clinical Chemistry and Molecular Diagnostics*. Saunders, an imprint of Elsevier Inc., USA, 2015, pp. 356–363.

[17] F. G. Menezes, A. C. O. Neves, F. D. Lima, S. D. Lourenco, L. C. Silva, and K. M. G. Lima, "Bioorganic concepts involved in the determination of glucose, cholesterol, and triglycerides in plasma using the enzymatic colorimetric method," *Quim. Nova*, vol. 38, no. 4, pp. 588–594, 2015. https://doi.org/10.5935/0100-4042.20150040

[18] A. Sajja, J. Park, and V. Sathiyakumar, "Comparison of methods to estimate low-density lipoprotein cholesterol in patients with high triglyceride levels," *JAMA Netw. Open*, vol. 4, no. 10, p. e2128817, 2021. https://doi.org/10.1001/jamanetworkopen.2021.28817

[19] S. Hwang, C. Gwon, M. D. Seo, J. Cho, J. Kim, and Y. Uh, "A deep neural network for estimating low-density lipoprotein cholesterol from electronic health records: real-time routine clinical application," *JMIR Med. Inform.*, vol. 9, no. 8, p. e29331, 2021. https://doi.org/10.2196/29331

[20] V. R. A. Silva, E. C. Albuquerque, A. P. C. dos Santos, J. C. A. Santos, L. S. S. Junior, W. A. Presada, C. G. C. Vinagre, and R. D. Couto, "HDL-particles separation employing different precipitating agents: functional properties of the impact of chemical

precipitation on lipoprotein particle-size and paraoxonase-1-activity," *Quim. Nova*, vol. 42, no. 8, pp. 859–865, 2019. https://doi.org/10.21577/0100-4042.20170410

[21] M. S. Bo, W. L. Cheah, S. L. Win, T. M. Nwe, T. T. Win, and M. Aung, "Understanding the relationship between atherogenic index of plasma and cardiovascular disease risk factors among staff of a university in Malaysia," *J. Nutr. Metab.*, vol. 3, pp. 1–6, 2018. https://doi.org/10.1155/2018/7027624

[22] O. Komolafe, A. Adeyemi, S. Adewole, and E. Obuotor, "Streptozotocin-induced diabetes alters the serum lipid profiles of adult Wistar rats," *Internet J. Cardiovasc. Res.*, vol. 7, no. 1, pp. 1–7, 2008. https://doi.org/10.5580/2251

[23] S. Patrick and V. Thomas, "Nonparametric statistical methods in medical research," *Anesth. Analg.*, vol. 131, no. 6, pp. 1862–1863, 2020. https://doi.org/10.1213/ANE.0000000000005101

[24] H. Y. Lim, S. K. Patel, P. Huang, M. Tacey, K. W. Choy, J. Wang, G. Donnan, H. H. Nandurkar, H. Prahlad, and L. M. Burrell, "Plasma angiotensin converting enzyme 2 (ACE2) activity in healthy controls and patients with cardiovascular risk factors and/or disease," *J. Pers. Med.*, vol. 12, p. 1495, 2022. https://doi.org/10.3390/jpm12091495

[25] E. Takimoto-Ohnishi and K. Murakami, "Renin-angiotensin system research: from molecules to the whole body," *J. Physiol. Sci.*, vol. 69, pp. 581–587, 2019. https://doi.org/10.1007/s12576-019-00679-4

[26] X. Chen, Y. Wu, X. Ge, L. Lei, L. Y. Nin, Q. Z. Yang, and L. Zheng, "In vivo imaging of heart failure with preserved ejection fraction by simultaneous monitoring of cardiac nitric oxide and glutathione using a three-channel fluorescent probe," *Biosens. Bioelectron.*, vol. 214, p. 114510, 2022. https://doi.org/10.1016/j.bios.2022.114510

[27] M. Ozdin and M. F. Gursu, "The relationship between paraoxonase, arylesterase, lipoprotein (a), and other lipid parameters in patients with coronary heart disease," *CMJ*, vol. 42, no. 3, pp. 271–276, 2020. https://doi.org/10.7197/cmj.vi.766384

[28] A. P. Arenas de Larriva, L. Limia-Perez, J. F. Alcala-Diaz, A. Alonso, J. Lopez-Miranda, and J. Delgado-Lista, "Ceruloplasmin and coronary heart disease—a systematic review," *Nutrients*, vol. 12, no. 10, p. 3219, 2020. https://doi.org/10.3390/nu12103219

[29] V. L. Lankin, A. K. Tikhaze, and A. M. Melkumyants, "Malondialdehyde as an important key factor of molecular mechanisms of vascular wall damage under heart diseases," *Int. J. Mol. Sci.*, vol. 24, no. 1, p. 128, 2023. https://doi.org/10.3390/ijms24010128

[30] T. Sun, M. Chen, H. Shen, P. Yin, L. Fan, X. Chen, J. Wu, Z. Xu, and J. Zhang, "Predictive value of LDL/HDL ratio in coronary atherosclerotic heart disease," *BMC Cardiovasc. Disord.*, vol. 22, p. 273, 2022. https://doi.org/10.1186/s12872-022-02706-6

[31] Q. Liu, H. Cui, Y. Ma, X. Han, Z. Cao, and Y. Wu, "Triglyceride-glucose index associated with the risk of cardiovascular disease: the Kailuan study," *Endocrine*, vol. 75, pp. 392–399, 2022. https://doi.org/10.1007/s12020-021-02862-3

[32] B. E. Heideman, C. Koopal, M. L. Bots, F. W. Asselbergs, J. Westerink, and F. L. J. Visseren, "The relation between VLDL-cholesterol and risk of cardiovascular events in patients with manifest cardiovascular disease," *Int. J. Cardiol.*, vol. 322, pp. 251–257, 2021. https://doi.org/10.1016/j.ijcard.2020.08.030

[33] J. Armitage, M. V. Holmes, and D. Preiss, "Cholesteryl ester transfer protein inhibition for preventing cardiovascular events," *J. Am. Coll. Cardiol.*, vol. 73, pp. 477–487, 2019. https://doi.org/10.1016/j.jacc.2018.10.072

[34] L. A. Rabelo, M. Todiras, V. Nunes-Souza, F. Qadri, I. A. Szijarto, I. Penninger, M. Bader, R. A. Santos, and N. Alenina, "Genetic deletion of ACE2 induces vascular dysfunction in C57BL/6 mice: Role of nitric oxide imbalance and oxidative stress," *PLOS ONE*, vol. 11, p. e0150255, 2016. https://doi.org/10.1371/journal.pone.0150255

[35] J. Ni, K. L. Ma, C. X. Wang, and J. Liu, "Activation of renin-angiotensin system is involved in dyslipidemia-mediated renal injuries in apolipoprotein E knockout mice and HK-2 cells," *Lipids Health Dis.*, vol. 12, no. 1, p. 49, 2013. https://doi.org/10.1186/1476-511X-12-49

[36] D. Nath and M. Shivasekar, "Role of cigarette smoking on serum angiotensin-converting enzyme and its association with inflammation and lipid peroxidation," *Cureus*, vol. 14, no. 8, p. e27857, 2022. https://doi.org/10.7759/cureus.27857

[37] L. A. Rabelo, P. Xu, M. Todiras, W. O. Sampaio, J. Buttgereit, M. Bader, R. A. Santos, and N. Alenina, "Ablation of angiotensin (1-7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction," *J. Am. Soc. Hypertens.*, vol. 2, pp. 418–424, 2008. https://doi.org/10.1016/j.jash.2008.05.003

[38] R. Ridwan, R. Natzir, H. Rasyid, I. Patellongi, M. Hatta, B. E. Linggi, A. Bukhori, and U. Bahrun, "Decreased renal function induced by high-fat diet in Wistar rat: The role of plasma angiotensin converting enzyme 2 (ACE2)," *Biomed. Pharmacol. J.*, vol. 12, no. 3, p. 702, 2019. https://doi.org/10.13005/bpj/1756