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EVALUATION OF THE LEVEL OF SIRTUIN7 AND SOME BIOCHEMICAL PARAMETERS IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Abstract:

The regulatory protein SIR7 Homolog 7, also known as Sirtuin7, is one of the seven important types of sirtuins found in mammals. It is primarily located in the nucleus and is released from the nucleus into the nucleoplasm in response to stress. The aim of this study is to study the relationship between the level of sirtuin7 and some biochemical variables in patients with cardiovascular disease, in addition to studying the effect of smoking on all these variables in both groups of patients and healthy smokers. Blood samples were collected from 45 patients suffering from cardiovascular diseases and 45 blood samples from healthy individuals as a control group. The following clinical tests were conducted for both the cardiovascular patients and the control group: Sirtuin7 enzyme (SIRT7), tumour necrosis factor alpha (TNF-α), lipoxygenase enzyme (LOX) , superoxide dismutase (SOD), albumin, glutathione peroxidase (GPx) , ceruloplasmin (Cp) , glutathione (GSH), malondialdehyde (MDA), vitamin C (VIT.C), vitamin D3 (VIT.D3), zinc (Zn), and copper (Cu). . Recorded results that low levels of the enzyme sirtuin7 in blood serum (2.6885 ± .88859) occurred in patients with cardiovascular disease compared to control group (13.5435 ± 2.19009) . A significant difference was recorded at the probability level ($p \leq 0.01$) in the levels of Sittuin7, tumour necrosis factor-α, lipoxygenase enzyme, lipoxygenase enzyme, superoxide dismutase, albumin, glutathione peroxidase, ceruloplasmin, glutathione, malondialdehyde, vitamin C, vitamin D3 , zinc ,and copper , in the blood . The results showed that the level of serum Sirtuin 7 in the blood of smokers with cardiovascular disease was lower (2.7944 ± .84794 ng / ml) compared to its level in the blood of healthy smoker (13.8697 ± 2.10722 ng / ml). Additionally, the results indicated that the levels of both TNF-α and LOX in the serum of smokers with cardiovascular disease were higher (320.08 ± 46.15937 pg/ ml, 120.55 ± 69.22078 U / L) respectively, compared to healthy smokers (7.4403 ± 1.45344 pg / ml , 51.6600 ± 5.05426 U / L) respectively . Furthermore, the albumin level in the blood serum of smokers with cardiovascular disease was lower (31.0288 ± 6.69500 g / dl) compared to its level in the blood of healthy smokers (54.9569 ± 3.17341 g / dl). The levels of SOD and GPx were also lower in the group of smokers with cardiovascular disease (2.4072 ± .57731 U / L , 75.2484 ± 13.30765 U / L) respectively , compared to healthy smokers(4.1730 .99011 U / L , 125.80 ± 18.41078 U / L) respectively . The results also revealed that the GSH hormone levels were lower in smokers with cardiovascular disease (9.8628 ± 1.95769 µmol / L) compared to levels in healthy smokers (39.5963 ± 6.40043 µmol / L). Moreover, the results indicated no significant difference in the levels of ceruloplasmin between smokers with cardiovascular disease (.8704 ± .39122 g / L) and healthy smokers (.9776 ± .51751 g / L) , while there was an indication of a decrease in the level of malondialdehyde in smokers with cardiovascular disease(2.6698 ± .62370 µmol / L) compared to healthy smokers(.1914 ± .17540 µmol /

L). Finally, the results showed that the levels of vitamin C, vitamin D, zinc, and copper were lower in smokers with cardiovascular disease (.0679 ± .02536 mg / dl , 14.9664 ± 1.74178 nmol / L , 89.7560 ± 7.62834 µg / dl and 158.62 ± 17.73731 µg / dl) respectively , compared to their levels in healthy smokers (.2573 ± .15179 mg / dl , 22.7314 ± 1.29645 nmol / L , 99.0095 ± 5.06978 µg / dl , 211.37 ± 23.11915 µg / dl) respectively .

Keywords: sirtuin7 enzyme, cardiovascular patients, effect of smoking.

INTRODUCTION:

The enzyme sirtuin7 is the least studied and well-known of the seven sirtuins found in mammals, but some studies have shown that it participates in many cellular processes and that its biological function has gradually become clear in health and disease. Sirtuin 7 consists of 400 amino acids that act as a class III histone deacetylase in humans. It is expressed in various organs and tissues of the body. Its highest expression is found in hyperplastic tissues such as the spleen and liver, and its lowest expression is found in skeletal muscles, the brain, and the heart [1]. The sirtuin7 enzyme is located primarily in the nucleus [2] and is released from the nucleus to Nucleoplasm in response to stress [3]. It acts as a dynamic nuclear master regulator of mitochondrial function through its ability to deacetylate GABPB1 and stimulate the formation of the GABP complex [4]. The GABP complex is a master regulator of mitochondrial biogenesis, which is the process of forming new mitochondria inside cells, as this complex activates nuclear-encoded genes involved in biogenesis. For mitochondria [5]. Cardiovascular disease (CVD) is a group of disorders and problems that affect the cardiovascular system and is considered the first and main cause of death around the world [6]. Mitochondrial dysfunction contributes to cardiovascular diseases [7], as mitochondria are the main sources for the production of reactive oxygen species (ROS) in cells [8] Excessive ROS promotes increased oxidative stress [9] Oxidative stress occurs when the rate of ROS production is higher than the capabilities of antioxidants and is an important risk factor for cardiovascular disease [10]

AIM OF THE STUDY: This research aims to conduct a biochemical study of cardiovascular patients and study the effect of gender on all measured variables.

MATERIALS AND WORKING METHODS:

The current study was conducted by taking 45 blood samples from cardiovascular patients who were diagnosed by specialized doctors and 45 blood samples from healthy people as a control group. Both groups were male and female.

PREPARATION OF BLOOD SERUM:

Blood samples were collected from patients with cardiovascular diseases (10ml), then the serum samples were separated and divided into four parts in small dry plastic tubes and kept in covered tubes at a temperature of (-20°C) until it is used in measuring the specified variables in the research .

Clinical variables

The levels of Sirtuin7 and TNF-α were assessed using ELISA. The ELISA kit employed the Sandwich-ELISA method [11] and [12] respectively. The activity of serum lipoxygenase was determined based on the method followed by the researchers in the study [13]. The level of albumin in serum was measured using the bromocresol green method, with standard analysis tools from the French company BIOLABO [14]. The activity of superoxide dismutase enzyme was estimated using the modified method (modified chemiluminescent nitro blue tetrazolium (NBT) method) [15]. Glutathione peroxidase (GPx) activity was estimated according to the method used in the research [16]. The concentration of glutathione in serum was estimated based on a modified method using an Ellman's reagent [17]. The level of ceruloplasmin was estimated according to the method adopted by the researchers in the study [18]. The concentration of malondialdehyde in serum was estimated according to the method used by the researchers in the study [19]. Vitamin C was estimated by oxidizing ascorbic acid using copper, following the method approved in the research [20]. Vitamin D was estimated using the method followed in the study [21]. The levels of zinc and copper were estimated according to the method followed in the research [22], [23] respectively.

STATISTICAL ANALYSIS :

The data collected from the study was analysed using a t-test, which is a type of statistics used to determine whether there is a significant difference between the averages of the two groups .

RESULTS AND DISCUSSION :

The study included, as shown in Table (1), the measurement of 13 variables in patients suffering from cardiovascular diseases and their comparison with healthy individuals. The results showed a significant decrease in the concentration level of Sirtuin7 at a probability level ($P \leq 0.01$) in the serum of patients with cardiovascular diseases compared to its concentration level in healthy individuals. Additionally, the results demonstrated a significant increase in the concentration level of Tumour Necrosis Factor Alpha (TNF-α) and a significant increase in the activity of Lipoxygenase enzyme in the serum of patients with cardiovascular diseases compared to healthy individuals at a probability level (P

≤ 0.01). Furthermore, the results indicated a significant decrease in the levels of antioxidants (catalase, SOD, GPx, GSH) in the serum of patients with cardiovascular diseases compared to healthy individuals at a probability level (p≤0.01). As for the level of serum and plasmin, the results showed no significant difference in their concentration levels between patients and healthy individuals at a probability level (p≤0.01). The results also pointed to a significant increase in the level of Malondialdehyde at a probability level (p ≤ 0.01) in the serum of patients with cardiovascular diseases compared to its concentration in healthy individuals. Regarding the levels of Vitamin C, Vitamin D, Zinc, and Copper, the results showed a significant decrease at a probability level (p≤0.01) in their concentration levels in the blood serum of patients with cardiovascular diseases compared to healthy individuals.

Table (1): Levels of biochemical variables measured in the blood serum of people with cardiovascular disease compared to the control group.

Biochemical variable	Patient group (No.45)	Control group (No.45)
	Mean ± Std .Deviation	Mean ± Std .Deviation
Sirtuin7 (ng / ml)	2.6885 ± .88859 **	13.5435 ± 2.19009
Tumor necrosis factor-α(pg / ml)	325.57 ± 46.88302 **	7.6453 ± 1.43800
Lipoxygenase (U / L)	129.39 ± 69.87575 **	52.5016 ± 5.19309
Albumin (g / dl)	31.2545 ± 6.94866 **	54.3705 ± 4.53965
Superoxide dismutase (U / L)	2.3567 ± .64348 **	4.0283 ± 1.00445
Glutathione peroxidase (U / L)	73.1519 ± 12.89980 **	122.15 ± 21.27557
Ceruloplasmin (g / L)	.9309 ± .44416 **	.9198 ± .43345
Glutathione (µmol / L)	10.1639 ± 1.93200 **	0.5360 ± 7.37368
Malondialdehyde (µmol / L)	2.5445 ± .63196 **	.1739 ± .13924
Vitamin C (mg / dl)	.0680 ± .02536 **	.2522 ± .13544
Vitamin D3 (nmol / L)	14.8693 ± 1.78565 **	22.5244 ± 1.46072
Zinc (µg / dl)	88.6965 ± 7.67925 **	98.1926 ± 5.14482
Copper (µg / dl)	153.28 ± 28.31724 **	207.84 ± 23.21803

**It means that there is a significant difference at the probability level (p ≤ 0.01) .

Effect of smoking:

The impact of smoking on all the biochemical variables measured was studied in both the patient group and the control group. The results shown in tables (2) indicated that there was a significant decrease in the level of sirtuin7 concentration at the probability level (p<0.01) in the group of smokers with cardiovascular diseases (2.7944 ± .84794 ng / ml) compared to the control group of smokers(13.8697 ± 2.10722 ng / ml). This decrease is attributed to cell senescence, as Sirtuin7 plays a role in regulating cell senescence, a process of cell cycle arrest associated with aging and age-related diseases. According to a previous study, smoking increases and accelerates cell aging. Therefore, the

decrease in sirtuin7 levels in patients with cardiovascular disease who smoke compared to a control group of smokers may be related to cellular senescence characterized by high levels of oxidative stress and inflammation [24] .

The results indicated a significant elevation in the level of tumour necrosis factor-alpha at a probability level ($p \leq 0.01$) among smokers with cardiovascular disease (320.08 ± 46.15937 pg / ml) compared to the control group of smoker(7.4403 ± 1.45344(pg / ml). This increase is attributed to inflammation, as a previous study has shown that smoking leads to inflammation, activating inflammatory genes, including those that cause elevated levels of inflammatory cytokines such as TNF-alpha. Considering that the inflammatory response in cardiovascular diseases is irregular, and smoking exacerbates inflammation, the combination of smoking and cardiovascular diseases may lead to synergistic effects, resulting in elevated levels among smokers with the disease [25] .

The results also indicated a significant elevation in the concentration level of the enzyme lipoxygenase at the probability level ($p \leq 0.01$) in smokers with cardiovascular diseases(120.55 ± 69.22078) compared to the control group of smoker (51.6600 ± 5.05426 U / L). This elevation is attributed to endothelial dysfunction, as it is associated with increased expression of lipoxygenase enzymes in the vascular endothelium. According to a previous study, smoking contributes to endothelial dysfunction. Thus, the results demonstrate that smokers with cardiovascular diseases exhibit more pronounced and severe endothelial dysfunction compared to healthy smokers, leading to higher levels of lipoxygenase in their bodies than in healthy individuals [26]

It has also been shown that there is a significant decrease in the levels of antioxidants (Albumin , SOD , GPx ,GSH and Vit.C) at the probability level ($p \leq 0.01$) in smokers with cardiovascular diseases (31.0288 ± 6.69500 g / dl , 2.4072 ± .57731 U / L , 75.2484 ± 13.30765 U / L , 9.8628 ± 1.95769 µmol / L and .0679 ± .02536 mg / dl) respectively , compared to the control group of smoker(54.9569 ± 3.17341 , 4.1730 ±.99011 U / L, 125.80 ± 18.41078 U / L , 39.5963 ± 6.40043 µmol / L and.2573 ± .15179 mg / dl) respectively. This decrease is attributed to oxidative stress, as cardiovascular diseases are associated with oxidative stress and inflammation. According to a previous study, smoking is a major cause of oxidative stress and inflammation. These smoking patients may experience exacerbation of oxidative stress and inflammation due to the synergistic effects of smoking and cardiovascular diseases. Consequently, the increased burden of oxidative stress in patients leads to a faster and greater depletion of antioxidant reserves, resulting in lower levels compared to healthy smokers [27].

The results indicated a significant elevation in the level of malondialdehyde at the probability level ($p \leq 0.01$) in smokers with cardiovascular diseases compared (2.6698 ± .62370 µmol / L) to the control group of smoker (.1914 ± .17540 µmol / L). This elevation is attributed to metabolic disturbances contributing to lipid peroxidation and malondialdehyde formation. Previous studies have shown that smoking induces dyslipidaemias, which is a characteristic feature of cardiovascular diseases and exacerbates with smoking. Therefore, smokers with cardiovascular diseases experience greater dysregulation of lipid metabolism and have higher levels of malondialdehyde compared to healthy smokers [28].

Table (2): Levels of biochemical variables measured in the blood serum of smokers of cardiovascular patients compared with smokers of a control group.

Biochemical variable	Patient group (No:28) (Smoker)	Control group (No:22) (Smoker)
	Mean ± Std. Deviation	Mean ± Std. Deviation
Sirtuin7 (ng / ml)	2.7944 ± .84794 **	13.8697 ± 2.10722
Tumor necrosis factor-α(pg / ml)	320.08 ± 46.15937 **	7.4403 ± 1.45344
Lipoxygenase (U / L)	120.55 ± 69.22078 **	51.6600 ± 5.05426
Albumin (g / dl)	31.0288 ± 6.69500 **	54.9569 ± 3.17341
Superoxide dismutase (U / L)	2.4072 ± .57731 **	4.1730 .99011
Glutathione peroxidase (U / L)	75.2484 ± 13.30765 **	125.80 ± 18.41078
Ceruloplasmin (g / L)	.8704 ± .39122 N.S	.9776 ± .51751
Glutathione (µmol / L)	9.8628 ± 1.95769 **	39.5963 ± 6.40043

Malondialdehyde (µmol / L)	**2.6698 ± .62370 ****	**.1914 ± .17540**
Vitamin C (mg / dl)	**.0679 ± .02536 ****	**.2573 ± .15179**
Vitamin D3 (nmol / L)	**14.9664 ± 1.74178 ****	**22.7314 ± 1.29645**
Zinc (µg / dl)	**89.7560 ± 7.62834 ****	**99.0095 ± 5.06978**
Copper (µg / dl)	**158.62 ± 17.73731 ****	**211.37 ± 23.11915**

**It means that there is a significant difference at the probability level ($p \leq 0.01$) .
N.S It means there is no significant difference.

The results also indicated a slight but statistically significant decrease in the levels of serum ceruloplasmin, zinc, and copper at the probability level ($p \leq 0.01$) among smokers with cardiovascular disease (.8704 ± .39122 g / L , 89.7560 ± 7.62834 µg / dl and 158.62 ± 17.73731 µg / dl) respectively , compared to the control group of smoker (.9776 ± .51751 g / L , 99.0095 ± 5.06978 µg / dl and 211.37 ± 23.11915 µg / dl) respectively . This decrease may be attributed to the depletion of antioxidants, including ceruloplasmin. Zinc and copper are essential factors that assist antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and ceruloplasmin. According to a previous study, smoking can increase the depletion of these antioxidant reserves due to elevated oxidative stress, leading to increased utilization of both copper and zinc in antioxidant defense mechanisms. Since oxidative stress is a hallmark of cardiovascular diseases, smokers with cardiovascular disease are likely to experience greater oxidative stress, resulting in a larger depletion of antioxidants such as ceruloplasmin. Consequently, they exhibit lower levels of ceruloplasmin as well as decreased levels of zinc and copper compared to healthy smokers [29, 30] respectively.

CONCLUSION:

There was a significant decrease in the level of the concentration of the enzyme sirtuin7 in patients with cardiovascular disease compared to control, while there was a significant increase in the level of tumour necrosis factor-alpha, the enzyme lipoxygenase, and malondialdehyde in patients with cardiovascular disease compared to control, while there was a significant decrease in the level of concentrations of Antioxidants (SOD, GPx, GSH) in cardiovascular patients compared to control group , but there was no significant difference in albumin and ceruloplasmin levels , also was a significant decrease in the level of the concentration of the vitamin C , vitamin D3 , zinc ,and copper, in the blood serum of cardiovascular patients . However, regarding the impact of smoking on both cardiovascular patients and the control group, there were clear results that showed a significant effect on the levels of the clinical tests conducted. A notable decrease in serotonin levels was recorded in smoking cardiovascular patients compared to healthy smokers, while the concentrations of both alpha-naphthylamine and lipoxygenase enzyme were found to be higher in smoking cardiovascular patients compared to healthy smokers. As for the antioxidant enzymes (SOD, GPx), a significant decrease in their levels was recorded, along with a decrease in the concentrations of antioxidants (albumin and glutathione) in smoking cardiovascular patients compared to healthy smokers. Regarding the levels of serotonin and plasmin, the results did not show any difference in their levels between patients and healthy individuals, while there was a significant increase in malondialdehyde in smoking cardiovascular patients compared to healthy smokers. Finally, the results indicated a significant decrease in the levels of vitamin C, vitamin D3, zinc, and copper in the serum of smoking cardiovascular patients compared to healthy smokers.

REFERENCES:

1. Wu, D., Li, Y., Zhu, K. S., Wang, H., & Zhu, W. G. (2018). Advances in cellular characterization of the sirtuin isoform, SIRT7. Frontiers in endocrinology, 9, 420642.
2. Michishita, E., Park, J. Y., Burneskis, J. M., Barrett, J. C., & Horikawa, I. (2005). Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Molecular biology of the cell, 16(10), 4623-4635.
3. Chen, S., Seiler, J., Santiago-Reichelt, M., Felbel, K., Grummt, I., & Voit, R. (2013). Repression of RNA polymerase I upon stress is caused by inhibition of RNA-dependent deacetylation of PAF53 by SIRT7. Molecular cell, 52(3), 303-313.
4. Ryu, D., Jo, Y. S., Sasso, G. L., Stein, S., Zhang, H., Perino, A., ... & Auwerx, J. (2014). A SIRT7-dependent acetylation switch of GABPβ1 controls mitochondrial function. Cell metabolism, 20(5), 856-869.
5. Yang, Z. F., Drumea, K., Mott, S., Wang, J., & Rosmarin, A. G. (2014). GABP transcription factor (nuclear respiratory factor 2) is required for mitochondrial biogenesis. Molecular and cellular biology, 34(17), 3194-3201.
6. Gaidai, O., Cao, Y., & Loginov, S. (2023). Global cardiovascular diseases death rate prediction. Current Problems in Cardiology, 48(5), 101622.
7. Stamerra, C. A., Di Giosia, P., Giorgini, P., Ferri, C., Sukhorukov, V. N., & Sahebkar, A. (2022). Mitochondrial dysfunction and cardiovascular disease: pathophysiology and emerging therapies. Oxidative Medicine and Cellular Longevity, 2022.

8. Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. Biochemical journal, 417(1), 1-13.
9. Nita, M., & Grzybowski, A. (2016). The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative medicine and cellular longevity, 2016.
10. Dubois-Deruy, E., Peugnet, V., Turkieh, A., & Pinet, F. (2020). Oxidative stress in cardiovascular diseases. Antioxidants, 9(9), 864.
11. Kohl, T. O., & Ascoli, C. A. (2017). Immunometric double-antibody sandwich enzyme-linked immunosorbent assay. Cold Spring Harbor Protocols, 2017(6), pdb-prot093724.
12. ELISA, A. H. T. A. Human TNF-α ELISA Kit.
13. Shastry, B.S.; Rao, M.A.R. (1975). Studies on lipoxygenase from rice bran. Cereal Chemistry. 52(5):597-603.
14. Hill, P. G. (1985). The measurement of albumin in serum and plasma. Annals of clinical biochemistry, 22(6), 565-578.
15. Gordon, A. M., Rowan, R. M., Brown, T., & Carson, H. G. (1973). Routine application of the nitroblue tetrazolium test in the clinical laboratory. Journal of Clinical Pathology, 26(1), 52-56.
16. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science, 179(4073), 588- 590.
17. Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry, 25, 192-205.
18. Sunderman Jr, F. W., & Nomoto, S. (1970). Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clinical chemistry, 16(11), 903-910.
19. Muslih, R., Al-Nimer, O., & Al-Zamely, M. (2002). The level of Malondialdehyde after activation with H2O2 and CuSO4) and inhibited by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infection. J. chem, 5, 148.
20. Omaye, S. T., Turnbull, J. D., & Sauberlich, H. E. (1979). [1] Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. In Methods in enzymology (Vol. 62, pp. 3-11). Academic press.
21. Heijboer, A. C., Blankenstein, M. A., Kema, I. P., & Buijs, M. M. (2012). Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clinical chemistry, 58(3), 543-548.
22. Eliasson, R. (1987). Evaluation of a commercially available kit for the colorimetric determination of zinc in human seminal plasma. In International Journal of Andrology (Vol. 10).
23. Noma, A. (1989). Abs. 0.2 0.1 Ascorbic acid SOS 0 Sensitive, Direct Colonmetric Assay for Copper in Serum. In CLINICAL CHEMISTRY (Vol. 35, Issue 4).
24. Nyunoya, T., Monick, M. M., Klingelhutz, A., Yarovinsky, T. O., Cagley, J. R., & Hunninghake, G. W. (2006). Cigarette smoke induces cellular senescence. American journal of respiratory cell and molecular biology, 35(6), 681-688.
25. Lee, J., Taneja, V., & Vassallo, R. (2012). Cigarette smoking and inflammation: cellular and molecular mechanisms. Journal of dental research, 91(2), 142-149.
26. Hahad, O., Arnold, N., Prochaska, J. H., Panova-Noeva, M., Schulz, A., Lackner, K. J., ... & Muenzel, T. (2021). Cigarette smoking is related to endothelial dysfunction of resistance, but not conduit arteries in the general population—results from the gutenberg health study. Frontiers in Cardiovascular Medicine, 8, 674622.
27. Addissouky, T. A., El Sayed, I. E. T., Ali, M. M., Wang, Y., El Baz, A., Elarabany, N., & Khalil, A. A. (2024). Oxidative stress and inflammation: elucidating mechanisms of smoking-attributable pathology for therapeutic targeting. Bulletin of the National Research Centre, 48(1), 16.
28. Behl, T. A., Stamford, B. A., & Moffatt, R. J. (2023). The effects of smoking on the diagnostic characteristics of metabolic syndrome: a review. American Journal of Lifestyle Medicine, 17(3), 397-412.
29. Astori, E., Garavaglia, M. L., Colombo, G., Landoni, L., Portinaro, N. M., Milzani, A., & Dalle-Donne, I. (2022). Antioxidants in smokers. Nutrition Research Reviews, 35(1), 70-97.
30. Seo, Y. S., Park, J. M., Kim, J. H., & Lee, M. Y. (2023). Cigarette smoke-induced reactive oxygen species formation: a concise review. Antioxidants, 12(9), 1732.