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Effect of Different Types of Smoking (Cegarette, hookah and vape) on Neuregulin-1 and Other Biochemical Parameters of Cardiac Health for Al-Mustansiriyah University Students

Zaman Ahmed Hussain[1*a] and Mustafa Taha Mohammed[1]

[1]*Department of chemistry, College of science, Al-Mustansiriyah University, Baghdad, Iraq*

[*a] Corresponding authors e-mail: zamenalshmry@uoAl-Mustansiriyah.edu.iq

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***Abstract*—Tobacco smoking remains a significant contributor to preventable illness and premature mortality worldwide, posing a substantial risk factor for various cardiovascular diseases (CVDs). This study aimed to determine the impact of different smoking methods on cardiac function and oxidative stress parameters, focusing on cigarette smoking, hookah, and vape. A total of 120 male participants were splitted into four groups: healthy controls, cigarette smokers, hookah users, and vape users. Blood samples were collected and analyzed for oxidative stress markers, cardiac function indicators, and neuregulin-1 (NRG-1) levels, a peptide with cardioprotective potential. When compared to controls, the results showed that smokers had significantly higher concentrations of total oxidative stress (TOS) and lower concentrations of total antioxidant capacity (TAC), with hookah having the most noticeable impact. Both the hookah and cigarette groups had noticeably higher levels of neuregulin-1, suggesting possible heart stress. Moreover, smokers showed increased Creatine kinase-MB (CK-MB) levels and lactate dehydrogenase (LDH), which may indicate cardiac injury. The results of correlation analysis showed a strong relationship between LDH and NRG-1, indicating the possibility of using both of them together as markers of smoking-related cardiac risk. The diagnostic usefulness of TOS, TAC, Neuregulin-1, LDH, and CK-MB in differentiating smokers from non-smokers was shown by receiver operating characteristic (ROC) analysis; CK-MB showed the highest sensitivity and specificity. These results highlight how smoking hurts heart health, emphasizing the importance of comprehensive preventive strategies and smoking cessation interventions to mitigate the burden of CVDs associated with tobacco use.**

***Keywords*— Hookah, E-cigarette, Vape, Cardiovascular disease, Oxidative stress.**

I. INTRODUCTION

Despite mitigation in prevalence in recent years, smoking still one of the major preventable reasons for ill-health and precocious death around the world[1]. Smoking is one of the factors of lifestyle which ordeal the health of people and have been killing millions of people in most of countries. It is one of the main risk factors of the expansion of atherosclerosis, coronary heart disease, acute myocardial infarction and sudden cardiac death [2]. The major components of most kinds of smoking tools and methods is tobacco, it is a kind of drugs that is legal in use that when taken as prescribed by the manufacturer, kills a large number of consumers [3]. The World Health Organization's (WHO) 2015 global report on tobacco smoking trends starts with that. Despite being much less common, tobacco products like cigars and tobacco pipes show a similar pattern. In Middle Eastern countries, water pipes (hookah, shisha) are still a common way to inhale tobacco smoke [4]. There is a different way to smoke, the most known methods are (cigarettes, hookah, and vapor vape). Smoking can cause a variety of heart diseases and conditions. These include: Atherosclerosis, which increases the risk of heart disease, heart failure, or a heart attack, raise blood pressure, which raise the risk of stroke, Irregular heart rhythm, Coronary heart disease and Stroke [5].

Cigarettes are the most commonly utilized tobacco products, and despite falling use, the cigarette industry is intrinsic (6.35 trillion cigarettes smoked around world in 2012). Tobacco smoke involve a mixture of gases, aerosolized liquids, and tiny particles, the composition is quite complex, and contains more than 4,000 chemicals, of which 250 are famous to be harmful and 50 more are famous to cause cancer [6].

Hookah even known as waterpipe, narghile, argileh, shisha, and Hookah smoking is connected to various hurtful health impacts including complications of lung function, like chronic obstructive pulmonary disease (COPD) and bronchitis. raised risk of heart factors, like heart disease and heart attack. raised risk of cancer, particularly throat, lung, and mouth cancer. Bacteria or against present in the mouths of fellow hookah smokers could be "shared," containing microbes such as the oral herpes virus. Other serious conditions, such as lung diseases and carbon monoxide poisoning [7].

vapor vape is the newest type of smoking that have been start to use 10 years ago, It involves inhaling vapor over the mouth from a commonly battery-operated electronic

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apparatus that heats up and vaporizes a liquid or solid, vapes can cause respiratory issues, such as coughing, wheezing, and shortness of breath. This is because the smoke from hookah vapes contains harmful chemicals and toxins that can irritate the lungs and airways[8]. Although the vape used for a lot of years, there are not enough researches talking about the effect of vape on it is users. Figure 1 shows the different types of smoking tools.

Fig. 1: Shape of hookah, vape, and Cigarettes

Oxidative stress (OS) is defined as an imbalance between the production of free radicals and their metabolites with the elimination of them by antioxidant defense systems, which favor the oxidants. This discrepancy causes injury to key molecules and cells, potentially impacting the whole body[9]. The oxidative mechanism that happens on an orderly basis in cells is crucial for cell life and death. Cigarette smoke contains several organic components like hydrocarbons, nitric oxide, aldehydes, phenols and quinone radicals. These components linearly or indirectly drive to the formation of free radicals caused by oxygen[10]. the body can normally administer free radicals, but if anti-oxidants are unavailable or if the free-radical formation becomes more than normal, tissue harm can happen[11]. Free radicals react with biological components, like lipids, DNA, carbohydrates, and proteins, that cause metabolic and structural differences in cells. This results in tissue damage in vital organs, such as the heart, kidneys, lungs, stomach, liver, and brain [12].

Neuregulins (NRGs) are multipotent polypeptide growth factors and organs of the epidermal growth factor family, that can activate members of the Receptor tyrosine-protein kinase erbB-3 (ErbB) family of receptor tyrosine kinases, NRG-1has the capability to dampen cell death by improving mitochondrial membrane potential[13], suppressing calcium overload, suppressing endoplasmic reticulum stress, alleviating the inflammatory response and ultimately maintaining cellular viability throughout myocardial IR injury. Although NRG-1 has a substantial turn in cardiac development and in tissue healing following heart ischemia, potentially containing it in adjunctive therapy in the treatment of cardiac diseases is still a challenge [14].

The current study aims to find the effect and the connection between smoking tools and cardiac diseases through the study of the activities of cardiac enzymes and the level of new peptide (neuregulin-1) in serum.

II. MATERIALS & METHODS

a) The Study Population

One hundred and twenty participants were split into four groups for the study: group A consisted of thirty healthy individuals, group B of thirty cigarette smokers, group C of thirty traditional hookah smokers, and group D of thirty vape smokers. Between October and December 2023, the majority of the samples were taken from Al-Mustansiriyah University, although others were taken from other locations in Baghdad. The study's laboratory component was conducted at Al-Mustansiriyah University, Department of Chemistry Science, Biochemistry Research Laboratory.

b) Exclusion Criteria

In this study, the participant are men,their age are under 50 years old and most of the them are young and healthy.. People that consume more than wan type of smoking were excluded, people with chronic diseases like CVD, DM or other diseases were also excluded .

c) Sample Collection

Five ml of venous blood was collected from all participants then put into gel tubes and left for 15 minute minutes at room temperature to form a clot, the blood were centrifuged for 8 minutes at 3500 rpm, the serum then divided into four eppendorf tubes containing nearly 1.25 mL of serum and stored at -20 ºC until further biochemical analyses.

d) Biochemical parameters

1) Determination of Total Oxidant Status (TOS)

The samples' total oxidant status value was determined using the Erel technique [15]. The ferrous ion–o-dianisidine complex is oxidized to ferric ion by oxidants found in the sample. Glycerol molecules, which are prevalent in the reaction media, promote the oxidation reaction. In an acidic media, the ferric ion forms a colored complex with xylenol orange. The total number of oxidant molecules in the sample is correlated with the color intensity, which is quantifiable using spectrophotometry. The assay is calibrated using hydrogen peroxide, and the micro molar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L) is the unit of expression for the data.

2) Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was calculated using Erel's methodology[16]. Through a Fenton-type reaction, a standardized solution of Fe2+–o-dianisidine complex and a standardized solution of hydrogen peroxide combine to form $OH^{\cdot}$. Under low pH, these strong ROS oxidized the reduced, colorless o-dianisidine molecules to produce yellow-brown dianisidyl radicals. Additional oxidation processes arise as the dianisidyl radical oxidation reactions advance. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. This reaction can be monitored by spectrophotometry.

3) ELISA and colorimetric analysis

The level of LDH and CK-MB were assessed by the colorimetric method utilizing the company kits by (Biolabo, France) while Neuregulin-1 was assessed by (ELISA) method, the ELISA kit supplied by(Sun Long Biotech, China).

e) Statistical Analysis

The data has been subjected to statistical analysis using SPSS statistics. The descriptive statistics for each parameter included the calculation of the mean and the standard deviation (SD). The one way ANOVA was employed to compare the variables between smokers and control groups at a significance level of probability ($P < 0.05$)[17].

f) Study design

The study model has been designed to show the study map that used in this study

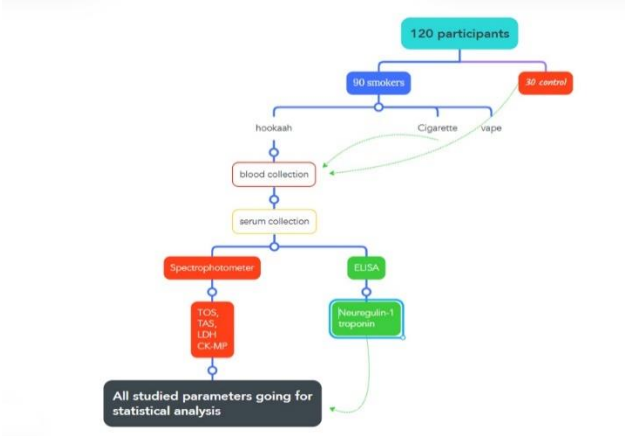

Fig. 2: Study design .

III. Results

a) ANOVA test for the studied parameters

The results of oxidative stress and antioxidant parameters for controls, cigarettes, hookah, and vape are listed in Table 1, by using the mean ± standard deviation (SD).

Table 1: Mean ± standard deviation results of oxidative stress and antioxidant parameters

Parameters	Controls, N=30 (mean ± SD)	cigarettes N=30 (mean ± SD)	hookah N=30 (mean ± SD)	vape N=30 (mean ± SD)
TOS (µmole/L)	81.88 ± 5.74	92.37 ± 10.66	94.37 ± 13.04	90.10 ± 5.54
TAC (µmole/L)	1.75 ± 0.133	1.41 ± 0.150	1.30 ± 0.194	1.38 ± 0.138
P-value	< 0.001*			

There is a highly significant increase ($p < 0.001$) between cigarettes, hookah and vape as compared with control in TOS among the four groups controls (81.88 ± 5.74), cigarettes (92.37 ± 10.66), hookah (94.37 ± 13.04), and vape(90.10 ± 5.54).

For TAC, there is a highly significant decrease ($p < 0.01$) between cigarettes, hookah and vape as compared with control among the four groups controls (1.75 ± 0.133), cigarettes (1.41 ± 0.150), hookah (1.30 ± 0.194), and vape (1.38 ± 0.138).

The results of Neuregulin-1 and cardiac function test for controls, cigarettes, hookah, and vape are listed in Table 2, by using the mean ± standard deviation (SD).

Table 2: Mean ± standard deviation results for Neurgulin-1 and cardiac function test.

Parameters	Controls, N=30 (mean ± SD)	cigarettes N=30 (mean ± SD)	hookah N=30 (mean ± SD)	vape N=30 (mean ± SD)
Neuregulin-1 (ng/mL)	2.97 ± 1.77	7.58 ± 1.06	6.07 ± 0.78	2.589 ± 1.05
LDH (IU/L)	196.7 ± 17.85	218.73 ± 17.02	198.2 ± 24.2	192.5 ± 25.7
CK-MB (IU/L)	119.62 ± 8.22	149.79 ± 1.287	150.0 ± 1.76	149.52 ± 4.15
P-value	< 0.001*			

For Neuregulin-1, there is a high significant increase ($p < 0.01$) between cigarettes, hookah and vape as compared with control among the four groups controls (2.97 ± 1.77), cigarettes (7.58 ± 1.06), hookah (6.07 ± 0.78), and vape(2.589 ± 1.05).

There is a high significant increase ($p < 0.01$) between cigarettes, hookah and vape as compared with control in LDH activity among the four groups controls (196.7 ± 17.85), cigarettes (218.73 ± 17.02), hookah (198.2 ± 24.2), and vape(192.5 ± 25.7).

For CK-MB, there is a high significant increase ($p < 0.01$) between cigarettes, hookah and vape as compared with control among the four groups controls (119.62 ± 8.22), cigarettes (149.79 ± 1.287), hookah (150.0 ± 1.76), and vape (149.52 ± 4.15).

b) Correlation between parameters

Correlation study for all parameters has been done to identify the correlation between parameters with each other to monitor the connection between smoking and cardiac diseases, the results are shown in Table 3.

Table 3: The correlation for the studied parameters.

Variables		TOS	TAC	Neuregulin-1
TOS	r	-	-0.12	0.151
	p	-	0.06	0.131
TAC	r	0.069	-	0.180
	p	0.672	-	0.267
Neuregulin-1	r	0.151	0.180	-
	p	0.131	0.267	-
LDH	r	.137	-.118	**.424****
	p	.171	.241	.000
CK-MB	r	-.001	.032	.053
	p	.992	.748	.598

The result in Table3 showed that there is a significant correlation between Neurgulin-1 and LDH, for the other parameters, there are no correlation between each other

c) ROC analysis for the studied parameters

ROC analysis study for all parameters represented in this study has been done to identify the ability of these

parameters to monitor the effect of smoking on heart, the results are shown in Table 4.

Table 4: Roc analysis for the studied parameters.

Parameter	AUC	SE	*p*-value	Cut-off value	Sensitivity	Specificity
TOS	0.824	0.038	<0.001	85.9	70%	70%
TAC	0.971	0.012	<0.001	1.54	90%	88%
Neuregulin-1	0.795	0.044	<.0.001	3.3	75%	72.5%
LDH	0.565	0.052	0.260	194	60%	62.5%
CK-MB	0.971	0.014	< 0.001	135	90%	90%

The result represented in Table 4 showed that most of the studied parameters (TOS, TAC, Neuregulin-1, LDH and CK-MB) work as a parameter to show the different between smokers and control people.

IV. Discussion

The significance of smoking behavior was uncovered in the risk of coronary heart disease in the youth population that current smoking was discovered to be the most important factor in differentiating between heart conditions among the traditional cardiovascular risk factors.

From the results obtained for TOS and TAC study, it is clear that the smoking with different tools causes an elevation in the level of total oxidative stress in the body as well as decreasing in the level of total antioxidants, the effect for increasing TOS is nearly the same between all smoking tools with a little more effect for hookah than the other types. While, the level of antioxidants decreased in hookah more than cigarette and vape. The vape has the minimal effect as it is compared with hookah and cigarette.

As an effect of smoking, long-time smoking could drive significant changes in the enzymatic antioxidant defense systems of smokers, and that will cause a rise in oxidative stress condition, This results are in agreement with Joshi *et al* [18] who said that Smokers' blood and tissues' antioxidant defense systems are disrupted and subjected to oxidative stress as a result of the prolonged inhalation of reactive free radicals found in tobacco's gas and tar phases. The study shows a quite decrease in Glutathione peroxidase-1(Gpx) and Superoxide dismutases (SOD) enzymes among tobacco users.

The study of Ahmed *et al* [19] showed that the antioxidant capability in all smokers (active and inactive) was lower than the group of control (non-smokers). The results of this study clarify that smoking decrease the activity of the antioxidant defense system and activates the oxidative stress system in the body because of the harmful components (carbon monoxide) which increase free radicals and need more anti-oxidant to neutralize.

Badran and his collagenous [20] showed the effect of Hookah on antioxidant system and said that the lifestyle and environmental risk factors that cause in oxidative stress can cause additional hurts in smokers of hookah.

For vape effect, Kuntic *et al* [21] agreed with the result in this study regarding the elevation of oxidative stress, kuntic said that Vape smoke exposure raises vascular, cerebral, and pulmonary oxidative stress by NOX-2-dependent mechanism, One important mediator of the documented detrimental vascular effects is hazardous aldehyde acrolein. Thus, there is a chance that using e-cigarettes could have serious negative effects on the heart, lungs, and brain [22].

As a net of the set of results obtained from the study, it is clear that the use of smoking tools leads to an increase in the percentage of oxidative stress in the body's cells due to the harmful components like (hydrogen cyanide, carbon monoxide, and ammonia) found in smoking tools. Therefore, the high percentage of oxidative stress leads to a lowering the percentage of anti-oxidants in the body and leads to a group of serious diseases of the lungs and heart. The mean and SD for TOS and TAC for cigarette hookah and vape shown in Figure 3.

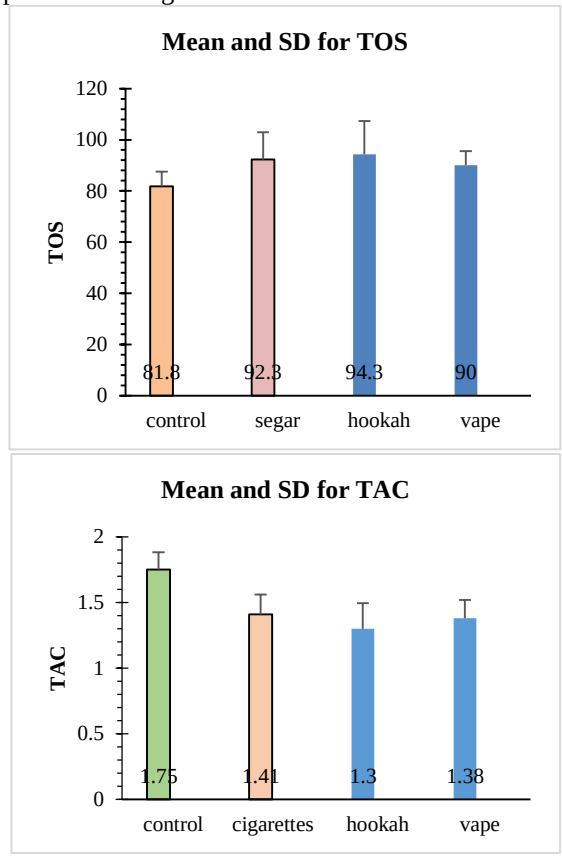

Fig. 3: Mean and SD for TOS and TAC for cigarette hookah and vape

For Neuregulin-1 the results showed that there is a hyper expression for the Neuregulin protein in smoking people as compared as nonsmoker . Neuregulin-1 was elevated in both cigarette and hookah groups, but it was non-significantly increased in vape group.

These results agreed with the study of Aliska *et al* [23] who said that NRG-1 can increase the myocardial angiogenesis, probably through the direct effects of NRG-1 and through the increased expression of Vascular endothelial growth factor (VEGF). Smoke from cigarettes causes the ligand of (ErbB3), Neuregulin (NRG) 1β, to activate, causing the airway epithelia to secrete mucus. This also causes the expression of Mucin 5AC (MU5AC) in human bronchial epithelial cells [24]. Smoking cigarettes activates (NRG1β/ErbB3) signaling, which in turn activates many signal cascade pathways and causes human

bronchial epithelial (16HBE) cells to produce mucin[25]. Smoking cigarettes enhances the synthesis of (MUC5AC), (ErbB3) phosphorylation, and NRG1β release. ErbB3-neutralizing antibodies and NRG1β-specific siRNA knockdown prevent these effects[24], NRG1β-dependent (ErbB3) activation is responsible for the reactions. NRG1β/ErbB3 signaling regulates the pathways activated by cigarette smoke, including extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinases (MAPKs), and phosphatidylinositol 3-kinase (PI3-K). These pathways are also inhibited by an ErbB3-neutralizing antibody and NRG1β siRNA [25].

Since Neuregulin-1 is elevated in smokers, and the previous papers show that there are no effect for age and gender on the level of Neuregulin-1.. This study ensures the effect of smoking on the heart and shows that elevating levels of Neuregulin-1 because of smoking may be considered as an avoidance and risk factor of smoking on cardiac health, and an important reason of CVD. Figure 4 shows the mean and SD for Neuregulin-1 for cigarette, hookah and vape.

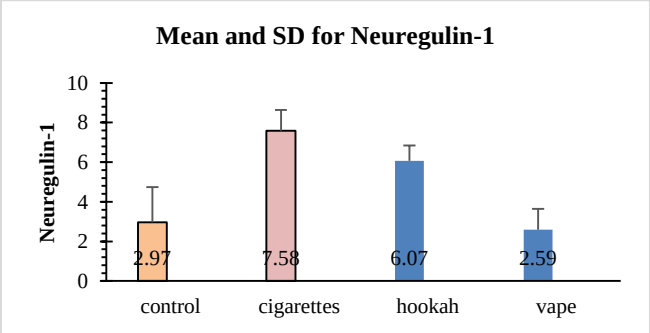

Fig. 4: Mean and SD for Neuregulin-1 for cigarette hookah and vape

For the study of cardiac function test, the level of LDH was showed to be elevated in the groups of cigarette and hookah but there are non-significant differences between vape group as compared with the control.

For cigarettes, the results are in agreement with Rao *et al* [26] who showed that smoking cigarettes raises salivary and blood LDH levels, which are markers of oral cavity tissue injury.

Iqbal and his colleagues [27] said that both cigarette and shisha smokers have abnormal lipid profiles suggesting dyslipidemia. Prediction of early atherosclerosis through the atherogenic indexes was observed to be significantly higher in shisha smokers than in cigarette smokers. Therefore, shisha smoking has more adverse effects on the health status of adult smokers [28].

About the vape, there is disagreement with the results of Pandarathodiyil *et al* [29] who showed that level of LDH is also elevated in vape group as compared with no smokers. This difference may be back to the time of use and difference of tobacco used among the participant in the two studies.

The level of CK-MB was shown to be elevated in all groups of smoking (cigarette, hookah and vape) as compared with non-smoker control group. Creatine kinase (CK) is an enzyme that occurs naturally inside muscle cells throughout the body especially the heart [30] . this result is in agreement with Zuurbier *et al* [31] who showed that CK-MB level increased as a response of smoking, smoke pump a lot of toxic and harmful components to the blood stream and it is shown to elevate the blood pressure, an elevation of BP causes more load on heart muscle so as a response of this load the level of CK-MB raised, mean and SD for LDH and CK-MB for all groups are shown in figure 5.

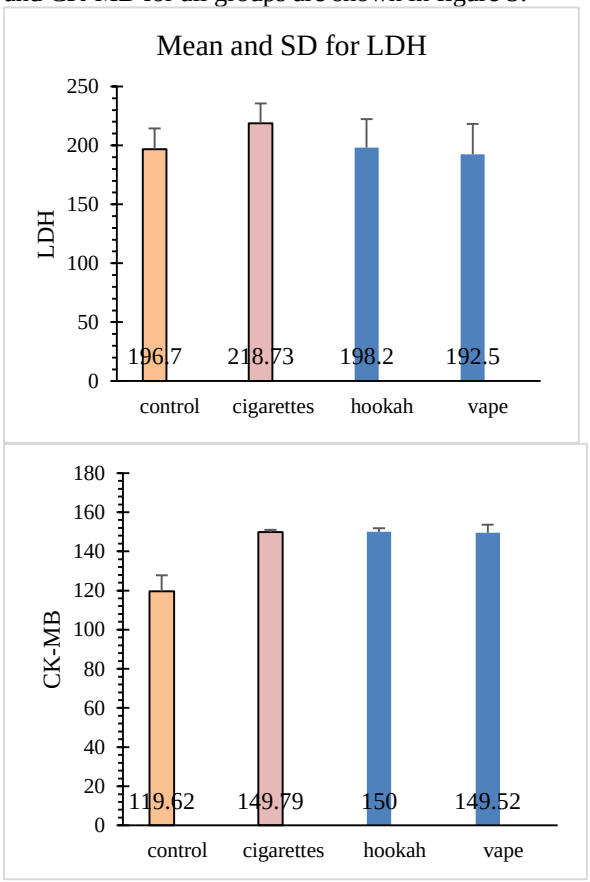

Fig. 5: mean and SD for LDH and CK-MB for cigarette hookah and vape.

The correlation study of the studied parameters shows a significant correlation between LDH and Neuregulin-1. Because of the therapeutic effect of Neuregulin-1 on the heart to prepare the damage. and the raise in the LDH connected with heart infections, this study revealed that there is a strong correlation between these two parameters and they could be used together to show the risk factor of smoking on heart, this is the first study which shows this kind of correlation between LDH and Neuregulin-1. The correlation between LDH and Neuregulin-1 is shown in Figure 6.

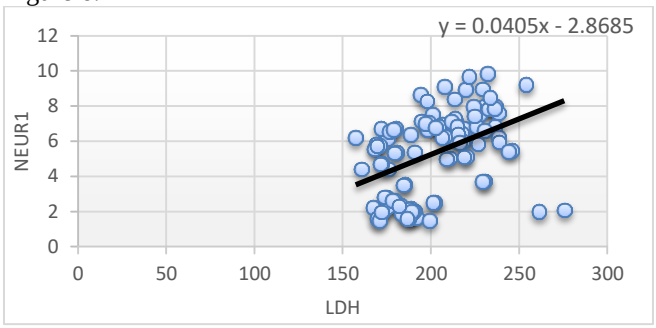

Figure 6: Correlation between LDH and Neuregulin-1.

For the result obtained from the ROC analysis study, it shows that most of parameters used in this study could work as a biochemical factor to diagnose and monitor the effect of smoke and it is risk factor on heart and CVD. CK-MB

shows the best monitoring capacities with 100% specificity and sensitivity for smoking people, TAC is the second one with 88% specificity and 90% sensitivity. The ROC analysis for parameters is shown in Figure 7.

The result of ROC for TOS and TAC is in agreement with other studies like Hocanli *et al* [32], who found that TOS increasing and TAC decreasing could be distinguishable for smokers than nonsmokers. Also, Ciftci *et al* [33] who found that TOS and TAC could be used in the diagnose of acute myocardial infarction.

For Neuregulin-1, the ROC analysis shows that at 3.3 ng/ml and it has 75% Sensitivity and 72.5% specificity for the detection of smokers. This is given an evidence that smoking is a real risk factor of CVD because in the study of Huang *et al* [34] who found that at 7.75 ng/ml, Neuregulin-1 generated 80.95% sensitivity and 73.85% specificity for the prediction of coronary collateral formation, that is a good clue to show the role of smoking in CVD.

For LDH, most of studies show that smokers have higher level of LDH than nonsmokers, and in ROC studies, results show that LDH consider as a good parameter to monitor for smoking people [35], [29].

For CK-MB, the result obtained is in agreement with Ahmed *et al* [36] who found that CK-MB is a good predictor for heart diseases and CVD.

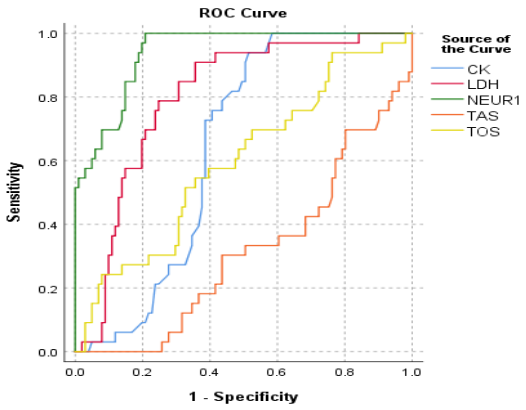

Fig. 7: ROC analysis of the studied parameters.

V. Conclusion

In conclusion, this study clarified how smoking different types of tobacco with different hazardous chemical components affect heart health. Smoking cigarettes, using a traditional hookah, and using an vape all increased oxidative stress and lowered antioxidant capacity, with hookah having the most significant effect. Increased levels of neuregulin-1 (NRG-1) in smokers suggested a connection between smoking and myocardial injury and highlighted possible cardiac stress. Moreover, smokers showed increased LDH and CK-MB levels, which are suggestive of myocardial damage. A substantial correlation between LDH and NRG-1 was found using correlation analysis, indicating the potential usefulness of both variables alone as indicators of smoking-related cardiac risk. The diagnostic efficacy of oxidative stress markers and cardiac function indicators in differentiating smokers from non-smokers was validated by ROC analysis, wherein CK-MB demonstrated the best level of specificity and sensitivity. These results highlight the critical need for comprehensive programs to help people quit smoking and public health campaigns to reduce the burden of tobacco-related CVDs and enhance cardiovascular outcomes for at-risk groups. Reducing the incidence of smoking-related cardiac problems and improving global cardiovascular health are possible outcomes of effective smoking cessation therapies.

Acknowledgment

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Availability of Data

None.

Competing Interests

The authors declare that they have no competing interests.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All procedures performed in studies involving human participants followed the ethical standards of the research committee of “Al-Mustansiriyah University” and with the 1964 “Helsinki Declaration” and its later amendments or comparable ethical standards.

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