OXIDATIVE STRESS INDUCED BY ELECTRONIC CIGARETTE IN THE MALE SMOKERS

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ABSTRACT:

Recently emerged, a new form of smoking known as electronic cigarettes. It is being promoted as a healthy alternative to quitting tobacco smoking, is gradually increasing in popularity among teenagers and young adults. The study examines the relationship of electronic smoking to the occurrence of oxidative stress in chronic male smokers by measuring a number of blood parameters and indicators of oxidative stress. The study was conducted on (100) person of males (in good health), ages ranged between (15-44 years), divided into two groups, the first group included (75) ECsmoker. the second group included (25) nonsmoker. The results showed that there was a significant increase (P < 0.05) in (RBC), (WBC) and (PLT) in ECsmokers, while there was no significant difference (P > 0.05) with respect to (HBG-HTC) and showed a significant (P < 0.05) decrease in levels of (TAC), (GSH) and (SOD) while indicated a significant (P < 0.05) increase in levels of (TPP),(MDA) and (OSI) in EC smokers compared to the control group. It also showed the extent to which the effect of electronic cigarettes is related for the age of the user, the period of smoking and the percentage of nicotine used. We conclude that e-cigarettes increase the production of oxidative stress, slightly affect a number of blood parameters and These signs may be a good indicator to impact on health of user.

I. INTRODUCTION:

E-cigarettes, has emerged which are modern electrochemical devices that deliver nicotine to the brain in a different way to compensate for tobacco combustion(1), which are powered by rechargeable batteries(2), atomizer, heating coil, cotton wick(3);(4). and refillable cartridges or containers, containing a liquid mixture consisting primarily of propylene, ethylene glycol, vegetable glycerin, nicotine, and common flavor additives (5);(6);(7). The e-liquid mixture is heated through heating coils to produce vapor that simulates the action of smoking(8);(9). E-liquid can contain potentially harmful chemicals, including nicotine, heavy metals, VOCs, other chemicals, added flavorings (Centers for Disease Control and Prevention(10)), and many other chemicals. One of the components of the liquid mixture has the ability to induce the generation of reactive oxygen species (ROS). There are increasing concerns about emissions from e-cigarettes and the health effects of these devices and they are hotly debated among studies, as a result of their widespread use among adolescents and young adults and being promoted as a healthy alternative to smoking cessation (American Thoracic Society);(11). It has become necessary to know the effect of these devices on the physiological processes inside the body by knowing the levels of oxidative stress, which is the real and clear indicator of what the body is exposed to from external oxidants that upset the balance and increase the production of free radicals, which cause irreversible damage to cells that can cause molecular targets carcinogenic (eg, DNA, proteins and lipids)(12);(13)(14). Therefore, knowledge of antioxidant levels within the body is important in the ability to predict, quantify the level of exposure, the body’s ability to resist stress, and the extent to which oxidative stress-induced damage from e-cigarette smoking is repaired(15);(16).

II. METHOD:

The current study was conducted on 100 healthy males (whose health record is free from any chronic diseases or other diseases) Volunteers are rated to two groups, the first group included 75% of those who used electronic cigarettes only, as the period of their use of electronic cigarettes ranged between (1-4) years and included different ages ranged between (15-44 years), using different percentages of nicotine that ranged between (0.3%)- (50%), regardless of the type of device used, and the second group included 25% of non-smokers (the control group), all volunteers were healthy. The method of collecting samples was by taking a quantity (5 ml) of blood for all members of the study sample, using special tubes for each purpose by standard laboratory conditions. These tests included
blood count (CBC) including (RBC, WBC, HGB, HCT, PLT), and measuring indicators of Oxidative stress which included (SOD, TAC, TPP, GSH, MDA).

**Laboratory tests:**

1. **Hematological analysis:**

The hematological parameters were performed on EDTA blood use of an automated auto-analyzer xp-300 (symex), measurement of the blood parameters.

2. **Antioxidants and oxidative stress analysis:**

Estimation of Serum Super oxide dismutase (SOD): measured by using a spectrophotometer method(17), Serum Reduced glutathione (GSH): measured by using a spectrophotometer method(18), Serum Malondialdehyde (MDA): by using a spectrophotometer method(19), Serum total plasma peroxide (TPP): measured by using a spectrophotometer method(20), Serum Total antioxidant capacity(TAC): by using a spectrophotometer method(21), oxidative stress index (OSI): measured was the oxidative stress index through the ratio of TPP to TAC an indicator of the degree of oxidative stress: OSI (%) = ((TPPμM × 100) ÷ TAC μM) (22).

**Statistical analysis**

The data was performed using the statistical software package SPSS (Statistical Package for the Social Sciences). This study used (T-test) for an independent sample between two groups for quantitative variables, and (F-test) (ANOVA) (Analysis of Variance).Pearson’s correlation was used to determine associations between variables all values are expressed as ± the mean value of the standard deviation, P- value of less than 0.05 and 0.01 was considered statistically significant.

**Results:**

Table (1): Differences in hematological markers between smokers and control groups.

<table>
<thead>
<tr>
<th>Indicators</th>
<th><code>Smokers (No. = 75)</code></th>
<th>Control (No. = 25)</th>
<th>T test</th>
<th>P value (Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (Cell*10^6/mm³)</td>
<td>5.44 ± 0.44</td>
<td>5.25 ± 0.30</td>
<td>2.01</td>
<td>0.04 (S)</td>
</tr>
<tr>
<td>WBC (Cell*10^9/mm³)</td>
<td>8.2 ± 1.92</td>
<td>7.32 ± 1.50</td>
<td>2.09</td>
<td>0.03 (S)</td>
</tr>
<tr>
<td>HBG (g/dl)</td>
<td>14.5 ± 1.09</td>
<td>14.31 ± 0.92</td>
<td>0.12</td>
<td>0.98 (NS)</td>
</tr>
<tr>
<td>HTC (%)</td>
<td>43.6 ± 2.87</td>
<td>43.41 ± 2.72</td>
<td>0.32</td>
<td>0.74 (NS)</td>
</tr>
<tr>
<td>PLT (Cell*10^3/mm³)</td>
<td>270.12 ± 56.11</td>
<td>239.53 ± 64.42</td>
<td>2.27</td>
<td>0.02 (S)</td>
</tr>
</tbody>
</table>

SD : Standard Deviation ; NS : Non-significant at P value >0.05 ; S : Significant at P value <0.05 ; RBC : Red Blood Corpuscles ; WBC : White Blood Cells ; HBG : Hemoglobin ; HTC : Hematocrit ; PLT : Platelets.

Table (2): Differences in oxidative stress markers between smokers and control groups.

<table>
<thead>
<tr>
<th>Indicators</th>
<th><code>Smokers (No. = 75)</code></th>
<th>Control (No. = 25)</th>
<th>T test</th>
<th>P value (Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (micromollar/l)</td>
<td>1574.37 ± 498.33</td>
<td>1819.51 ± 407.14</td>
<td>2.22</td>
<td>0.02 (S)</td>
</tr>
<tr>
<td>TPP (µmol H2O2/L)</td>
<td>27.16 ± 11.09</td>
<td>13.06 ± 4.88</td>
<td>6.15</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>MDA (µmol/ml)</td>
<td>25.74 ± 10.64</td>
<td>11.17 ± 3.74</td>
<td>6.69</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>GSH</td>
<td>63.36 ± 24.69</td>
<td>148.25 ± 20.94</td>
<td>15.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Markers</td>
<td>Age</td>
<td>Smoking Duration</td>
<td>Nicotine Ratio</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>-0.30 *</td>
<td>0.15</td>
<td>0.23 *</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>-0.12</td>
<td>- 0.27 *</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>HBG</td>
<td>-0.20</td>
<td>0.00</td>
<td>-0.05</td>
<td></td>
</tr>
<tr>
<td>HTC</td>
<td>-0.20</td>
<td>0.14</td>
<td>-0.09</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>0.23 *</td>
<td>0.07</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>0.02</td>
<td>0.15</td>
<td>-0.12</td>
<td></td>
</tr>
<tr>
<td>TPP</td>
<td>-0.14</td>
<td>0.245 *</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.01</td>
<td>- 0.043</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>0.18</td>
<td>0.04</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>-0.05</td>
<td>- 0.234 *</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>OSI</td>
<td>0.18</td>
<td>0.16</td>
<td>-0.06</td>
<td></td>
</tr>
</tbody>
</table>

* Significant correlation at p value <0.05

Table (1) is about the Differences in hematological markers between ECsmokers and control groups. According to this table, there is significant increase (P <0.05) in RBC count. The same table reveals that there is significant increase (P <0.05) in WBC count. According to platelets count, there is significant increase (P <0.05) in platelets count in ECsmokers compared to control group, while no significant difference (P >0.05) was seen regarding HBG (g/dl) HTC (%). Table (2) show the differences in oxidative stress markers between ECsmokers and control groups. According to this table, there is a significant decrease (P <0.05) in serum TAC compared to control group. The same table reveals that there is a significant increase (P <0.05) in TPP and there is a significant increase (P <0.05) in MDA and OSI in smokers compared to control group. While there is a significant decrease (P <0.05) in GHS and SOD compared to control group. Table (3) is about the Pearson Correlation Coefficient (r) between studied markers in smokers and their age, smoking duration and nicotine ratio. According to this table, there is significant negative correlation (P <0.05) between smokers age and RBC count (r= - 0.30) as in figure (1); while there is a significant positive correlation (P <0.05) between smokers age and platelets count (r= 0.23) as in figure (2). The same table reveals that there is a significant negative correlation (P <0.05) between smoking duration and WBC count (r= -0.27) as in figure (3), and SOD (r= - 0.234) as in figure (4); while positive correlation was seen with TPP (r= 0.245) as in figure (5).

![Figure (1) Scatter plot and regression equation for the correlation between RBC count and age of ECsmokers.](image-url)
Figure (2) Scatter plot and regression equation for the correlation between platelets count and age of ECsmokers.

\[ y = -0.1731x + 8.0487 \]

\[ R^2 = 0.048 \]

Figure (3) Scatter plot and regression equation for the correlation between WBC count and duration of smoking in ECsmokers.

\[ y = -0.22x + 19.111 \]

\[ R^2 = 0.045 \]

Figure (4) Scatter plot and regression equation for the correlation between Super oxide dismutase (SOD) and duration of smoking in ECSmokers.

\[ y = 0.0341x + 19.108 \]

\[ R^2 = 0.0695 \]
III. DISCUSSION:

The study dealt with measuring blood parameters and showed that there was a significant increase in the total red blood cells in ECsmoker as shown table (1). This increase can be attributed to hypoxia, depending on the frequency of use, duration of inhalation, depth and temperature used (23). We suggest that polycythemia may be caused by the fluids used in vaping that contains different textures of different chemical ingredients, flavors and metals, so (24), extrapolate this increase to the presence of metals in e-liquids such as nickel and others that affect the oxygen sensing mechanism by affecting on the proteins that bind oxygen and this leads to an increase Erythrocytes (25); (26).

The study also showed a significant increase in the number of platelets, and the reasons for this slight and significant increase can be attributed to the vascular effect through the consumed amount of nicotine or chemicals vaporized into the bloodstream. It has been proven inhaled nicotine resulting in a decrease in the level of EV mobilization in platelets will activate platelets, (27), high amounts of e-cigarette consumption may increase platelet activation (28) or this effect is due to nanoparticles in liquid (29). the presence of nicotine in the extract can cause persistent changes in platelet function. In addition, a significant increase in the total number of white blood cells was recorded as we attribute this increase to the stimulation of the hematopoietic component to release lymphocytes into the bloodstream, and the reason for the stimulation of these cells may be under the influence of infections, increased airway resistance and increased bacterial colonization (30) as a result of inhaling saturated aldehydes resulting from heating chemicals, flavors and metals in e-liquid, which leads to a decrease in the number of neutrophils and a decrease in immune function (31) regardless of the presence or absence of nicotine (32), and cause dose-dependent impairment and loss of endothelial barrier function in the respiratory tract due to oxidative stress and inflammation. The value of hemoglobin and hematocrit was not affected in this study, this could be due to the difference in the behavior of volunteers who use these devices in terms of the number of puffs, depth of inhalation, temperature, percentage of nicotine and flavors, the amount of e-liquid consumption per day and the type of device used. As well as the same reasons that lead to different results from one study to another, in addition
to the difference in studies conducted in vivo and in vitro, it can be noted that the lack of influence of these values is an indication that the changes that occur in blood parameter have little effect and can fade. The results of showed in the Table (2), indicated a decrease in the total antioxidants capacity (P < 0.05) in the users of electronic cigarettes compared to the control group. It is known that (TAC) is a reliable biomarker for diagnoses and predictions for many diseases and various pathophysiological conditions(33), the decrease in the capacity of TAC leads to the weakening of the body’s resistance in defense against foreign bodies and DNA damage in cells and decreased ability of antioxidants which indicates oxidative injury(34), and there was a significant increase (P < 0.05) in the value of the total plasma peroxide index (TPP), and this increase in the value TPP index is a clear evidence of an increase in oxidative stress through the increase Excessive free radicals, which lead to cells oxidation and damage because exposure of these cells to e-liquid vapor. The Malondialdehyde index showed high significant (P < 0.05) in level (MDA) of electronic cigarette users when compared to the control group, and this increase in the Malondialdehyde index is a vital sign in assessing the level of oxidative stress in addition to the increase in lipid peroxidation in the blood caused by the increase in free radicals that Leading to an imbalance between oxidants and antioxidants, which leads to cell membrane harm and damage(35) Several studies in vitro have noted that exposure to e-cigarette vapor has resulted in led to an increase in the level of MDA in the blood(36), it has been shown that e-cigarettes (with nicotine) can cause increased levels of (MDA) and inflammations(37). Furthermore there was a significant increase (P <0.05) in (OSI) index, where OSI is a comprehensive measure of the relationship between oxidants and antioxidants in the human body and a marker of the occurrence, development and prediction of many diseases. and indicating the status and level of exposure of free radicals and oxidants on body cells, an increase in the OSI indicates an increase in the state of oxidative stress as a general, and showed a significant (P < 0.05) decrease in the levels of decreased glutathione index among e-cigarette users, and GSH is one of the body's defense mechanisms or endogenous antioxidants that remove free radicals, and a reliable indicator of cellular health, about 98% of glutathione is found inside healthy cells in its reduced form, and upon exposure to an oxidizing source a reduction occurs and a rapid decrease in the GSH index, this decrease in GSH levels indicates an increase in oxidative stress through an increase in free radicals due to exposure to e-liquid vapor) 38) this suggests that PG/VG + nicotine derived from exposure to ROS can overwhelm the antioxidant system, leading to oxidative stress with increased protein oxidation, and increases pro-inflammatory cytokines and reduces glutathione (GSH) levels, which are essential for maintaining cellular redox balance (39);(40), manner Dose dependent leading to cytotoxicity. Due to oxidative stress caused by toxic substances (nanoparticles and chemicals) present in EC aerosols, that nicotine has a role in inhibiting glutathione levels in the body, which indicates that e-liquids containing nicotine contribute significantly to increasing the production of oxidative stress and thus reducing the effectiveness of antioxidants (41), The results also indicated a significant (P < 0.05) decrease in superoxide dismutase values, SOD are the major antioxidant defense systems where first line defense enzymes play an important role in Inhibition of oxidative stress in the extracellular and intracellular compartments. It is a true indicator and important biomarker of oxidative stress production. Several studies have concluded that exposure to e-cigarettes can reduce SOD activity(35). The results of our current study indicated the knowledge and understanding of the effect of electronic cigarettes on the user by knowing the effect of the percentage of nicotine, the smoking period used and through the known statistical correlations with Pearson's correlation coefficient as shown in Table (3). Indicating that the age of the user had a clear effect as for the red blood cells and the number of platelets in the body, there was a significant negative correlation between the age of smokers and the number of red blood cells meaning that the older the age leads to a decrease in the number of red blood cells in the body. It showed that there was a significant positive correlation between the age of smokers and the number of platelets. That is, the higher the age, the higher the number of platelets in smokers, noting that there was no effect or correlation on the rest of the physiological parameters used in this study. In addition, the period of cigarette use affected the white blood cell count (WBC) in an inverse relationship, as well as the antioxidant indices (SOD) in an inverse relationship and (TPP) in a positive relationship, we conclude that an increase in the period of use leads to a decrease in the number of White blood cells and a decrease in inflammatory immunity. With a decreased level of the enzyme (SOD) first line of defense against free radicals, and an increase in the level of plasma oxidation in cells, all these changes indicate an increase in the production of oxidative stress and a deterioration in the physiological state of cells within the body.
Conclusion:
We conclude that e-cigarettes have the potential to influence the physiological parameters of the blood and on oxidative indices but this the effect can be fading faster than other forms of smoking, depending on the use behavior in terms of the number of puffs, quantity and quality of consumption (Frequency of use, depth and duration of inhalation per day and temperature used). but it must be taken into account that electronic cigarettes are a less harmful form it is another form of smoking and it can affect the health status and physiological processes inside the body, as the results of the study showed.

REFERENCES

www.turkjphysiotherrehabil.org


