EFFECT OF CIGARETTE SMOKING ON SERUM AND SALIVALIVER ENZYMES FUNCTION.

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ABSTRACT

Background: Smoking may affect the liver through inflammatory pathways and may aggravate the pathogenic effects of smoking on the liver. The study has examined the relationship between cigarette smoking and liver enzymes function. Methods: The subjects consisted of 100 men aged 19-25 years with no history of any diseases. The saliva and blood was withdrawn from each subject. Biochemical assays for saliva and serum enzymes ALP, AST, ALT, LDH and GGT were performed in all subjects (control and smokers). All assays were performed at the college laboratory. Results: The results showed that serum and saliva enzymes ALP, ALT, AST, LDH, and GGT levels were significantly high (P value<0.05) in smoker group when compared with nonsmoker group. Cigarette smoking was significantly associated with increased levels of gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), (P < 0.05) and was inversely associated with increased aspartate aminotransferase (AST) after adjustment for smokers. Conclusions: Smoking affects the individual components of liver enzymes function in different ways on liver function tests. Therefore, their effects on enzymes liver function should be carefully interpreted, and further study on the mechanism of the effects is warranted.
KEYWORDS: Smoking, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) and Lactate dehydrogenase (LDH).

INTRODUCTIONS
Cigarette smoking is a major cause of preventable morbidity and mortality.\textsuperscript{[1]} Worldwide, more than 3 million people currently die each year from cigarette smoking.\textsuperscript{[2]} The risk of death in the smokers measured by the number of cigarettes smoked daily, the duration of smoking, the degree of inhalation and the age of initiation.\textsuperscript{[3, 4]} Cigarette smoke contains over 4000 different chemicals, 400 of which are proven to be carcinogenic; it also contains various oxidants such as oxygen free radicals and volatile aldehydes which are probably the major causes of damage to biomolecules.\textsuperscript{[5]} Cigarette smoking consists of many chemicals, including nicotine,\textsuperscript{[6]} tar with its many carcinogens, and gaseous compounds including carbon monoxide.\textsuperscript{[7]} Cigarette smoke also contains large numbers of free radicals that are capable of initiating or promoting oxidative injury.\textsuperscript{[8]} Cigarette smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancers, etc. Cigarette smoking causes a variety of adverse effects on organs especially liver organ.\textsuperscript{[9]} The liver is an important organ that has many tasks; such as responsibility for processing drugs, alcohol and other toxins to eliminate them from the body.\textsuperscript{[10, 11]}

Liver function enzymes are useful tools in clinical practice to assess potential liver diseases, to monitor treatment responses, and to predict prognosis of the patients with liver diseases. As a battery, Liver function tests consists most commonly of serum total cholesterol (TC), total protein, albumin, alkaline phosphatase (ALP), total bilirubin (TB), aspartate amino transferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT). However, the interpretation of Liver function tests should be comprehensive and careful because liver enzymes function can be influenced by many personal and environmental factors, including age, gender,\textsuperscript{[12]} body mass index (BMI),\textsuperscript{[13]} alcohol drinking,\textsuperscript{[14]} cigarette smoking, malnutrition, presence of extra hepatic diseases such as cardiac, musculoskeletal, or endocrine diseases, and status of liver health in itself.\textsuperscript{[15]}

Recently, it has been suggested that smoking increases GGT and boosts the alcohol-induced GGT elevations.\textsuperscript{[16]} Although smoking does not damage hepatocytes directly, it may change the effect of alcohol drinking on AST, ALT and GGT activities via the actions of numerous ingredients that alter the activities of enzymes found in the liver.\textsuperscript{[17]} Furthermore, smoking
increased serum ALP levels\textsuperscript{[18]} produced by the bone and the kidney, as well as by the liver. However, it is still not clear whether smoking is related to each component of liver function tests and whether the effects are independent of alcohol drinking, since the smoking habit itself is closely related to the alcohol drinking habit.\textsuperscript{[19]} Therefore, liver function tests changes in real clinical situations need to be interpreted carefully in the context of the interaction between various life style factors.

Most previous studies deal with the separate effects of alcohol, coffee, or smoking on one or a few components of liver function tests, and there has been a lack of data on the comprehensive effects of smoking on the commonly used all components of liver functions. This study was conducted in Jordanian students to evaluate the effects of smoking on the most commonly used sets of liver functions in order to consider the interactive association with major demographic factors.

**MATERIALS AND METHODS**

In the present study was carried out a survey on health-check examinee volunteers who were eager to be evaluated about their lifestyle and the effects on their health status, and would provide almost complete responses about smoking; this provided the opportunity to perform a comprehensive analysis of the relationship of behaviors with Liver function tests. The strengths of our study included an intensive data collection, and distinguished participants who had quitted smoking from those with no history and current users, which made it possible to gain information about lifetime consumption nicotine amounts. An additional strength was presented independent effects of smoking on the most commonly used comprehensive items of Liver function tests, adjusted by extensive confounding factors that included age, gender, and regular medications. Nevertheless, the lack of evidence of the causal-relationship between lifestyle and Liver function tests changes remained a limitation of our study due to its cross-sectional design. In addition, some smokers might be excluded from the present study since 50\% of eligible patients refused to respond to the questionnaire.

**Subjects**

Total of (100) healthy subjects were enrolled in the present study during their attendance to department at Zarqa University College. They were divided into two groups; 50 smokers and 50 non-smokers. Their ages were ranged from (19-24) years, their mean cigarette amounts 20 - 30 smoked per day over the previous 6 months and the number of years having smoked.
In this study, all health-check examinees were consecutively enrolled in the health promotion Center of Zarqa University College from October 2013 to December 2013. Of 100 examinees during this study period, all subjects agreed to participate to a comprehensive self-administered questionnaire survey about smoking. Moreover, they were requested to answer the questions about their current diseases undergoing medical care, their past histories of diabetes mellitus, of any other unspecified diseases and regular medications, in order to discern the effects of regular medication on serum liver functions. This study was approved that all participants provided written informed consent prior to this study.

**Samples Collection and biochemical measurements**

Five milliliter of saliva and blood was withdrawn from each subject. The blood was allowed to clot in a plain tube for 30 minute at room temperature. The serum was separated by centrifugation at 5000 rpm for 10 minutes, and then each subject serum was stored in plain tube, frozen at -20 °C until the analysis of assay. The pure saliva was separated from all impurities by centrifugation at 5000 rpm for 5 minutes and then each sample frozen at -20 °C until analysis of enzymes assay. Biochemical assays for saliva and serum ALP, AST, ALT, LDH and GGT were performed in all subjects (control and smokers). All assays were performed at the laboratory, which complied with the Clinical Chemistry and Laboratory Medicine in the College.

**Statistical analysis**

The liver function enzymes tests were recorded for enzymes ALP, AST, ALT, LDH and GGT. Student T tests and analysis of variance (ANOVA) for variables tests were analyzed.

**RESULTS**

Estimated means and standard deviation for each enzyme results were calculated. The enzyme results were measured in serum and saliva for both non smokers and smokers groups (Table 1, 2). To estimate independent effects of smoking on liver function enzymes, the same multivariable analyses were performed after adjustment for daily smoking amounts, separately. One hundred participants were involved in two group's smoker and non-smoke. The effects of cigarette smoking on saliva and serum activities of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) were investigated in 100 students attending at Zara University College, Jordan during the period of three months, after allowing same ages. As expected, all showed cigarette smoking produced a significant
increase AST, ALT, ALP, LDH and GGT activity for those smoking cigarettes in serum and saliva (Table 1 and 2). We postulate that the effects of smoking on enzymes activity are a result of induction of the enzyme by nicotine.

**Table 1 Serum enzymes (ALT, AST, ALP, LDH, GGT) in nonsmokers and smokers**

<table>
<thead>
<tr>
<th>Enzymes (IU/L)</th>
<th>Non-smokers Mean ±SD Serum No., = 50</th>
<th>Smokers Mean ±SD Serum No., = 50</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>20.21 ± 1.52</td>
<td>25.33± 5.76</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AST</td>
<td>23.66 ± 2.32</td>
<td>32.21± 4.46</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ALP</td>
<td>73.33 ± 3.17</td>
<td>77.34± 7.12</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>LDH</td>
<td>260.53±53.60</td>
<td>275.52± 57.67</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>GGT</td>
<td>28.19±11.23</td>
<td>41.44±10.75</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

**Table 2 Saliva enzymes (ALT, AST, ALP, LDH, GGT) in nonsmokers and smokers**

<table>
<thead>
<tr>
<th>Enzymes (IU/L)</th>
<th>Non-smokers Mean ±SD Saliva No., = 50</th>
<th>Smokers Mean ± SD Saliva No., = 50</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>15.40 ± 4.16</td>
<td>23.27 ± 4.92</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AST</td>
<td>20.11 ± 4.32</td>
<td>30.46 ± 5.37</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ALP</td>
<td>6.94 ± 2.11</td>
<td>31.48 ± 8.71</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>LDH</td>
<td>76.92 ± 6.98</td>
<td>132.58 ± 11.73</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>GGT</td>
<td>5.41 ± 1.89</td>
<td>11.22 ± 1.87</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The current study showed that smoking caused a significant increase in serum enzymes activity of AST, ALT, LDH and ALP has been observed in smoker volunteers. These results were in agreement with Dales et al. 1974[20], Robinson and Whitehead 1989[21] and Gordon 1993[22] and this may attributed to the fact that cigarette smoke contains a large variety of compounds including nicotine, benzopyrene, tar, carbon monoxide, many oxidants and free radicals such as polonium -210, potassium -40 and radium -226[23], that are capable of causing a pro-oxidant / antioxidant imbalance in the blood and tissues of smokers, so hepatocellular damage lead to release of enzymes into circulation.[24]

The liver is the organ responsible for removing toxins from our bodies and helping us digest our food. Through our day to day lives, we put our livers through a great deal of strain, so it takes a great deal of damage to the liver before symptoms begin to show. Elevated liver
enzymes are a symptom of too much strain being put on the liver, and they are a clear indication that changes need to be made. This is how to lower liver enzymes.

The results showed statically significant increase in serum and saliva enzymes (ALT, AST, LDH, ALP and GGT) activity in cigarette smokers when compared to control group (nonsmoker). This may occur due to nitrosative stress which is a condition that occurs when the production of highly reactive nitrogen-containing chemicals, such as nitrous oxide, exceed the ability of the human body to neutralize and eliminate them. Nitrosative stress can lead to reactions that alter protein structure thus interfering with normal body functions.\cite{25, 26} Cigarette smoke contains a large number of chemical substances with hepatotoxic potential including nicotine.\cite{27} The term extrahepatic cholestasis refers to bile duct blockage or injury outside of the liver and may occur in individuals with gallstones. GGT and ALP can seep out of the liver and into the bloodstream, but only with blockage or inflammation of the bile ducts.

As we know that the liver enzymes are found in normal plasma and serum and can be divided into different groups. Aspartate aminotransferase (AST or GOT) and alanine aminotransferase (ALT or GPT), together these enzymes are known as transaminases, Alkaline phosphatase (ALP) and gammaglutamyltransferase (GGT) are known as cholestatic liver enzymes. If these enzymes are elevated it can indicate the presence of liver disease, and secreted enzymes which are made in the liver and allocated to the blood plasma and saliva enzymes.\cite{28}

There are enzymes that enter into the blood from the tissues to perform intracellular functions. Some of the enzymes are in the cell cytosol, such as ALT, AST and LDH, and others are in the cell mitochondria, such as GGT and ALP. Any damage to the liver will cause the enzymes from the cells to enter the blood and their activity will increase. Amounts of ALT and AST are the greatest diagnostic value. In parenchymatous hepatitis serum transaminase ALT increases, sometimes 100 times or more and AST to a lesser extent. In addition to the liver, AST enzymes can be found in the heart, muscle, brain and kidney and is released into blood serum when these tissues become damaged. For example, a heart attack or muscle disorders will increase AST serum levels. Because of this AST isn’t necessarily an indicator of liver damage. ALT is almost specifically found in the liver. After liver injury it’s released into the bloodstream and therefore can be used as a fairly specific indicator of liver function. It’s common for high levels of AST and ALT in the liver to damage numerous liver
cells, called hepatic necrosis and can lead to death of the cells. The higher the ALT levels the greater the amount of cell death. Despite this ALT’s aren’t always a good indicator of how well the liver is functioning.

Diseases that can cause increased levels of liver enzymes AST and ALT are acute viral hepatitis A or B, as well as toxins caused by acetaminophen overdose, or a prolonged collapse of the circulatory system, which is called shock. It deprives the liver of fresh blood that brings oxygen and nutrients. Transaminase levels can be 10 times the upper limit. Sometimes elevated liver enzymes can be found in otherwise healthy individuals. In such cases they’re usually found to be twice the upper limit.

Liver cell damage is characterized by release of enzymes particularly (aspartate AST and alanine ALT transaminase) from damaged hepatocytes into the circulation.\[29,30\]

By comparing the mean of enzymes activities between male and female smokers, no significant difference in mean activities of serum AST, ALT and ALP was noted in (Tables 1 and 2). It concluded from the above finding that the cigarette smoking has a relative risk of liver diseases. Finally, the research recommended to quit smoking

The present study is the first to describe a detailed effect of smoking on the individual test of liver function tests. Previous studies showed that current smokers revealed high GGT compared to non-smokers\[31\] and people with high GGT levels smoked more\[32\], which was compatible with our results (Table 1 and 2). GGT is another enzyme that’s produced in the bile ducts and can become elevated if there is a problem with the bile ducts. High levels of GGT and ALP indicate a possible blockage of the bile ducts or a possible injury or inflammation of the bile ducts. This problem is characterized by an impairment or failure of bile flow and is known as cholestasis and the term refers to bile duct blockage or injury within the liver. As a rule, intrahepatic cholestasis will occur in individuals with primary biliary cirrhosis or liver cancer. It was controversial whether smoking could affect aminotransferase activities. Some investigators claimed ALT was increased by smoking\[33\], it was agreed with our results, while other studies argued that smoking did not influence AST or ALT, but GGT.\[34\] Although present results showed elevated enzymes levels in the current smoker compared to never having smoked, it was not confirmed in the daily or lifetime smoking amounts. Several studies concerning osteoporosis have documented increased serum ALP levels in current smokers, as a marker of bone turnover.\[35\] Therefore, the effects of smoking on ALP level
may be complicated with many extrahepatic mechanisms that showed a consistent association with smoking in our study after adjustment of many factors.

Alkaline phosphatase is an enzyme that’s produced in the bile ducts, kidney, intestines, placenta and the bone. If this enzyme is high and ALT and AST levels are pretty normal there could be a problem with the bile duct such as an obstruction. Some bone disorders may also cause alkaline phosphatase levels to increase. If there is an elevation of alkaline phosphatase, it could also indicate there is an injury to the biliary cells. This could be due to gallstones or certain medications. Under normal circumstances the enzyme is mainly allocated to the bile, but if pathology exists the norm is disturbed and the enzyme increases in blood and saliva.

It was interesting to observe the strong correlations between coffee consumption, alcohol drinking, and smoking habits in our study subjects. In addition, these correlations have been documented in most prior studies\[36\] suggesting that coffee drinkers are more likely to be alcohol drinkers or cigarette smokers. Therefore, the complete information about these habits is warranted to analyze or interpret reasonably the effect of each habit on liver function enzymes.

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Previous epidemiological studies suggested a hepato-protective effect of smoking\[37\] were well known factors to raise GGT levels. Although BMI is one of the most important factors resulting in GGT elevation\[38\], the present study is the first to describe a detailed effect of smoking on the individual test of liver enzymes. Previous studies showed that current smokers revealed high GGT compared to non-smokers\[39\] and people with high GGT levels smoked more \[40\], which was compatible with our results. It was controversial whether smoking could affect aminotransferase activities.

Previous studies demonstrated that total protein and albumin levels were decreased by smoking. The decrease of serum protein and albumin levels according to increase of daily smoking consumption was also confirmed in subjects who had never drunken alcohol nor
drinking coffee in the present study (data not shown). Although most previous studies had not mentioned total protein or albumin levels, a study documented that current or past smoking lower serum albumin, globulin, and all other protein fractions.\[^{41}\] Moreover, in the chronic hepatitis patients, current smokers were more likely to have lower albumin levels than nonsmokers.\[^{42}\] However, the biological mechanisms leading to decreased levels of serum protein and albumin by smoking have not been studied, yet.

The present study is the first to describe a detailed effect of smoking on the individual test of Liver function tests. Previous studies showed that current smokers revealed high GGT compared to non-smokers\[^{43}\] and people with high GGT levels smoked more \[^{44}\], which was compatible with our results. It was controversial whether smoking could affect aminotransferase activities. Some investigators claimed ALT was increased by smoking \[^{45}\], while recent studies argued that smoking did not influence AST or ALT, but GGT \[^{46}\] as our results support. Although our multivariable results showed elevated ALP levels in the current smoker compared to never having smoked or past-smokers, it was not confirmed in the daily or lifetime smoking amounts. Several studies concerning osteoporosis have documented increased serum ALP levels in current smokers, as a marker of bone turnover \[^{47}\] Therefore, the effects of smoking on ALP level may be complicated with many extrahepatic mechanisms that did not show a consistent association with smoking in our study after adjustment of many factors.

It was interesting to observe the strong correlations between smoking habits in our study subjects. In addition, this correlations have been documented in most prior studies \[^{48}\] suggesting that coffee drinkers are more likely to be alcohol drinkers or cigarette smokers. Therefore, the complete information about these habits is warranted to analyze or interpret reasonably the effect of each habit on Liver function tests.

In the present study, we carried out a survey on health-check examinee volunteers who were eager to be evaluated about their lifestyle and the effects on their health status, and would provide almost complete responses about smoking; this provided the opportunity to perform a comprehensive analysis of the relationship of behaviors with Liver function tests. The strengths of our study included an intensive data collection, and we distinguished participants who had quitted smoking from those with no history and current users, which made it possible to gain information about lifetime consumption nicotine amounts. An additional strength was that we presented independent effects of smoking on the most commonly used
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CONCLUSION
Cigarette smoking can affect liver functions, through its effect on serum and saliva ALP, ALT, GGT, LDH and AST enzymes. Smoking was associated with low enzyme levels, independently. Because smoking, showed strong interactions among each other, the association of those habits and liver function should be carefully analyzed and interpreted. Further studies on the mechanisms of these associations are warranted.

In the present study, strong correlations existed among smoking habits and level of enzymes. The associations between those lifestyle habits and liver function enzymes were investigated using statistical analyses: Smoking was significantly associated with high levels of AST, ALP, ALT, GGT and LDH in both serum and saliva. As our best knowledge, this is the first study that demonstrated independent effects of smoking on the comprehensive liver function enzymes commonly in humans. Several previous studies included only limited test items in liver function and their associations with smoking, but not with all of this common lifestyle habits were reported. Finally, the research recommended to quit smoking.

REFERENCES


