A CLINICAL STUDY TO INVESTIGATE LIVER ENZYMES AND MINERALS IN ALCOHOLIC AND CIGARETTE SMOKER AMONG IRAQI MALES

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ABSTRACT

Minerals participate in the most cellular efficacy and showed a key role in metabolism, also they work as a cofactor in a large number of important cellular enzymatic reactions and they have many functions such as blood clotting and muscle contractions. The aim of our work was to explain the effect of minerals modifications on serum enzymes in alcohol consumption and chronic cigarette smoking. Five groups of male patients were selected as experimental subjects aged between 20-50 years, each group consists of 20 patients compared with a control group (20 male who didn’t smoke or drink alcohol). Venipuncture blood samples were drawn from patients and were used immediately for analysis. People drinking alcohol and not smoker showed marked increased in GOT and GPT level only, Mg was reduced and Vit-D3 also and no effect on Iron level. While in all groups using alcohol with few smokers showed a significant increased in all enzymes and reduced Mg only. Iron and Vit-D were significantly different. Heavy and moderate smokers showed again increased on all enzymes. Iron was high while no significant effect on Vit-D3. Study outcomes demonstrated an elevation of all enzymes, Iron, in contrast to Mg and Vit-D3. To conclude our study, alcohol, and smoker have a potential role in effecting these indicated biomarkers. Abuse alcohol and smoking lead to the liver enzyme and protein damage with a significant effect and these effects are increased with increasing amounts and periods of abuse alcohol and smoking.

Keywords: Smoking, Minerals, Alcohol, Liver function test.

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INTRODUCTION
The dangerous of chronic cigarette smoking are well-known worldwide\textsuperscript{1}; however, huge numbers of persons continue smoking till in progressing countries\textsuperscript{2}. Smoking is a risk factor for a lot of diseases that attacked to different body parts such as relating to the organs of respiration\textsuperscript{3}, or relating to the heart and blood vessel diseases\textsuperscript{4}, of or relating to neoplasia\textsuperscript{5}, and other diseases\textsuperscript{6}. Alcohol consumption is also dangerous as smoking and has various effects on all body parts like secondary osteoporosis\textsuperscript{7}, mineral density\textsuperscript{8}, bone density\textsuperscript{9}, liver cirrhosis\textsuperscript{10}, and other diseases. There have been opposite articles mention that chronic and too much alcohol consumption might minimize the density of the bone\textsuperscript{8,11}, and the other denies that\textsuperscript{12,9}, while other articles mention that moderate alcohol consumption might elevate the density of the bone\textsuperscript{7,13}.

Liver is a very important member of the human body, which performs many important functions for human health, including the detoxification of harmful substances such as alcohol, nitrogen in the form of urea or ammonia, tobacco, free radicals and other harmful substances to the body, so liver function tests are an important stuff in clinical exercise to estimate possibility liver diseases, to keep an eye on treatment response and to foretell assessment of the patients with liver diseases\textsuperscript{14}. Therefore, it is necessary to maintain its health through liver function tests, which are considered a good observer of the liver, especially in chronic smokers and drink alcohol where LFTs may be effective by many factors such as age, sex\textsuperscript{15}, weight\textsuperscript{16}, lifestyle (smoking and alcohol consumption), malnutrition, existence of many diseases and healthy state to the liver itself\textsuperscript{17,18}.

Minerals participate in most cellular efficacy and showed a key role in metabolism, also they work as a cofactor in a large number of important cellular enzymatic reactions and they have many functions such as blood clotting, blood pressure, nerve function and muscle contractions\textsuperscript{19,20}. However, there is no enough job has been done to explain the effect of lifestyle (alcohol consumption and chronic smoking) on minerals, Vit.D3 and liver enzymes. Minerals disordered may affect the metabolism of several others and could lead to many acute and even life-menacing metabolic abnormalities like liver disease, lung infection and coronary heart disease\textsuperscript{21,22}. Also, alcohol consumption and chronic smoking lead to a decrease in Vit.D3 level has been correlated to an increase in fracture of the bone-like spine, etc\textsuperscript{23,24}.

Newly, the previous study demonstrated that smoking increases gamma-glutamyltransferase ($\gamma$-GT) and supports the alcohol-induced $\gamma$-GT elevation\textsuperscript{25}. Nevertheless, hepatocyte damage does not affect directly smoking, might alter the effect of alcohol consumption on GPT, GOT and $\gamma$-GT activities through the actions of a lot of components that alter the activities of liver enzymes\textsuperscript{26}. On the other hand, smoking raised levels of ALP in serum produced by the kidney, bone, and liver\textsuperscript{27}. Moreover, still not obvious if smoking has linked to each component of liver function tests and if the effects are separate from alcohol consumption because smoking behavior is specifically associated with alcohol user behavior\textsuperscript{28}. Consequently, liver function test changes in actual clinical conditions need to explain more in the context of the interaction between different lifestyle factors. The aim of our work was to explain the effect of minerals modifications on serum enzymes in alcohol consumption and chronic cigarette smoking.
MATERIALS AND METHODS

One hundred human blood male samples have been involved in this study, samples designed into five groups according to the specific conditions. Again, one hundred human male samples aged between 20-50 years, were classified into five groups (20 little alcohol drink and not a smoker, 20 low alcoholic smokers, 20 males who moderate smoker with high abuse alcohol (daily alcohol intake), 20 high alcoholics with a heavy smoker and 20 control groups, didn’t smoke or drink alcohol).

Blood samples were collected from five groups of people. The people's sample was taken from the males attended for treatment at Al-Ramadi Teaching Hospital/ Al-Anbar / Ramadi between the periods of May 2017 to December 2018. The written informed aspect was gained from every patient giving blood samples for the study. IEC approval number 47/13-03-2019

Venipuncture blood samples were drawn from patients and put them in suitable containers for analysis at the same time. Serum protein, albumin, concentrations of liver enzymes viz (GOT, GPT, ALK, TSB, Tp, Alb. and γ-GT) and minerals concentration in serum include (Mg, Iron, Ca, Vit.D3 and PO4) were measured using Linear reagents (Spain).

Subjects

Experimental subjects aged between 20-50 years smoked for 10 years at least, divided into five groups, each group consists of 20 patients compared with a control group.

**Group1**: control group (males who didn’t smoke or drink alcohol).

**Group2**: males who little alcohol drinks and not a smoker.

**Group3**: males who low alcoholic smokers.

**Group4**: males who moderate smokers with high abuse alcohol (daily alcohol intake).

**Group5**: males whose high alcohols with heavy smokers.

Biochemical Profile:

Biochemical, Enzymatic parameters and Vit.D3 are analyzed using standard procedures.

Statistical Analysis:

Statistical meaning of variances among means values from control and treated groups applied by specific (ANOVA) test via Graph Pad Prism® Version 5.0 software or Bonferroni Multiple Comparison test P<0.05established as significant.

RESULTS

The Effect of Liver Enzyme, Minerals, and Vitamin D3 Among Little Alcohol Drink and Not Smoker People
To assess the potential effect of alcohol and cigarette on males, a little alcohol drink and not smoker male group were utilized. In Fig. 1 was initially tested for effect upon the liver enzymes, minerals and vitamin D3 among the mentioned above group. Results demonstrated a significant effect upon GOT and GPT respectively with no effect upon the others panel (A) (mean ± SEM 12.54 ± 0.46 versus 40.81 ± 0.20 and 11.86 ± 0.40 versus 37.00 ± 0.23 control; low alcohol respectively, \( p = < 0.001 \)). The results in panel (B) showed a significant effect upon the magnesium only with no changes on the other parameters (mean ± SEM 2.55 ± 0.04 versus 1.44 ± 0.05, control; low alcohol respectively, \( p = < 0.001 \)). Fig. 1 panel (D) also demonstrated that little alcohol users decrease vitamin D3 levels more than the control group while no effect upon an iron level (mean ± SEM 52.6 ± 2.04 versus 32.28 ± 2.14, control; low alcohol respectively, \( p = < 0.001 \)).
Characterization of Liver Enzyme, Minerals, and Vitamin D3 Among Males Who Low Alcoholic With A Smoker

The serum of indicated parameter concentrations has been considered in response to males who low alcoholics with a smoker as shown in (Fig. 2) a strong increased in GOT, GPT, ALK. Phosphatase and γ-GT level in male on low alcoholic with a smoker (mean ± SEM 43.81 ± 2.11; 40.55 ± 3.10; 86.52 ± 3.07; 55.84 ± 3.07) respectively. In contrast, healthy control group (C) represents a substantial normal value of above mentioned parameters as expected (mean ± SEM 12.54 ± 1.46; 11.86 ± 3.40; 66.38 ± 3.03; 19.36 ± 3.32). This panel was indifferent in Fig. 1, which was different and restricted to GOT and GPT only. Further significant reduction in magnesium levels was observed in panel (B) (magnesium = mean ± SEM 2.55 ± 0.04 versus 1.06 ± 0.07). Panel (C) shows amazing outcomes in iron concentration. Iron levels were extremely increased. While significant reduction showed in Vit.D3, (iron= mean ± SEM 88.56 ± 3.04 versus 110.33 ± 4.21, Vit.D3= mean ± SEM 52.6 ± 2.04 versus 28.50 ± 2.45. **p < 0.01.
FIG. 2: Males who low alcoholic with a smoker group 2: (a) liver enzymes, (b) liver proteins and (c) minerals iron and VIT-D3. The value represents the mean ± S.E.M. The results are representative of at least three independent experiments with similar findings. ** and *** indicates p <0.01 compared to control group.
Characterization of Liver Enzyme, Minerals, and Vitamin D3 Among Males Who Moderate Smoker With High Abuse Alcohol (Daily Alcohol Intake)

Having established that from the previous figure the low alcoholic users increased the liver enzymes level, the effect of high abuse alcohol on the liver enzymes and minerals level was examined in the same manner. In Fig. 3 shows the increasing effect of high abuse alcohol upon liver enzymes panel (A), and there was a little but not significant reduction in magnesium level (magnesium = mean ± SEM 2.55 ± 0.04 versus 0.82 ± 0.17) as shown in panel (B). Surprisingly, the same group showed a marked effect of decreasing in vitamin D3 which was approximately 50 % panel (C) (Vit.D3= mean ± SEM 26.48 ± 2.08). While the marked effect of increasing iron level which was also approximately 50 % (Iron= mean ± SEM 134.43 ± 6.26). Under these conditions, high abuse alcohol with moderate smoker caused a significant increased in all liver enzymes and iron in contrast decrease in magnesium and vitamin D3 levels at all established samples. Which was not notable decreased by group one.
The Effect of Liver Enzyme, Minerals, and Vitamin D3 Among Males Who High Alcoholic With A Heavy Smoker

Having established that alcohol and smoking could increase the activity of liver enzymes and minerals biomarkers, the effects of high alcoholic and heavy smoker people on liver enzymes and minerals biomarkers were evaluated. In Fig. 4 shows the effect of high alcoholic and heavy smoker upon all mentioned markers above in response to alcohol users. Liver enzyme levels increased by the same group were much more and highly significant compared to the control group panel (A). Again, high alcoholic and heavy smoker reduced the magnesium and phosphorus a little but not significant than the other estimated minerals as shown in panel (B). Panel (C) showed an increased iron level compared to the control group. While the same reduced in vitamin D3 with no significant value iron= mean ± SEM 18.83 ± 11.58; Vit.D3= mean ± SEM 20.49 ± 5.43).
FIG. 4: Males who high alcoholics with heavy smoker group 4: (a) liver enzymes, (b) liver proteins and (c) minerals iron and VIT-D3. The value represents the mean ± S.E.M. The results are representative of at least three independent experiments with similar findings. ** and *** indicates p <0.01 compared to control group.

DISCUSSION

Alcohol consumption is major common health trouble worldwide. Alcohol consumption causes a lot of hurtful effects on the important internal organs, especially with chronic alcohol abuse and smoking.

The results of this study explained that subjects who are a little alcoholic without smoking have a significant effect on main liver enzymes especially in GPT and GOT. However, the results of groups 3 and 4 explained that liver enzymes remainder (ALK and γ-GT) also have a significant increase which rising with increasing amounts and periods of abuse alcohol and smoking. Also, this study showed that little alcohol drinking affect the bone contents through the strongly reduced magnesium level in serum of patients with a significant effect because of less appetite and decreased absorption due to disturbances of digestive system, lower magnesium leads to hypertension, and cardiovascular diseases, while the other minerals (PO4 and Ca) have very little fall (none appear) change, also liver protein which they are albumin, total...
protein, and iron while vitamin D3 has a significant decrease in its level as compared with a healthy group which has been correlated to an increase in fracture of bone-like spine, etc.\textsuperscript{24,33}.

Moreover, the results of this study also showed that males who are low alcoholic smoker have a significant effect on all main liver enzymes GPT, GOT, ALK and γ-GT as compared to healthy controls and the same results were obtained in heavy drinker moderate smoker in comparison to control group (Fig. 2 and 3 respectively). Again our results explained that low alcoholic smoker has a significant effect on minerals (Mg, Vitamin D3 and Fe) while liver proteins (Alb., TP, PO4 and Ca) have a small fall in their values as compared to control group (non-significant effect). Also, Fig. 3 shows the increasing effect of high abuse alcohol upon liver enzymes panel (A) and there was a little but not significant reduction in magnesium level as shown in panel (B).

Surprisingly, the same group showed the marked effect of decreasing in vitamin D3 which was approximately 50\% panel (C) which means alcohol drinking reduces bone mineral density due to the inhibition of bone formation\textsuperscript{13,21,34}. While marked effect of increasing iron level which was also approximately 50\% due to alcohol abuse with smoking enhance hypoxia which stimulates production of erythropoietin which develop hyperplasia of the bone marrow, the development of secondary polycythemia and increased red cell mass this will lead to increased erythropoiesis and inducing increased number of red cells destruction in normal turnover process leading to increased iron overload this finally causes deposition of excessive iron in parenchymal cells of the liver which causes hepatocellular damage\textsuperscript{35}. Under these conditions, high abuse alcohol with moderate smoker caused a significant increased in all liver enzymes and iron in contrast decrease in magnesium and vitamin D3 levels at all established samples.

This study as well shows the effect of high alcoholic and heavy smoker on all liver enzymes levels which too much increased by the same group was much more and highly significant as compared to control group panel (A) in response to alcohol drinking. Again, high alcoholic and heavy smoker reduced the magnesium and phosphorus a little but not significant than the other estimated minerals as shown in panel (B). Panel (C) showed an increased iron level compared to the control group. While the same reduced in vitamin D3 with no significant value iron showed an increased in iron level compared to the control group. Furthermore, the overload of iron may lead to clinical problems such as damage to myocardial, problems of the joints and diabetes\textsuperscript{36-38}.

\textbf{CONCLUSION}

Abuse alcohol and smoking lead to the liver enzyme and protein damage with a significant effect and these effects are increased with increasing amounts and periods of abuse alcohol and smoking. Also, our results showed that subjects whose alcohol consumption has a significant reduction in their magnesium levels regardless of the amount of alcohol drinker Vit.D3 deficiency mean they may have brought down in their bone density and decrease bone density loss which may lead to easily bone fractures.
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ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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