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Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya

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ABSTRACT

Tobacco use is widely spread throughout the world. The effect of smoking on human health are serious and in many cases, deadly. The present study was done to investigate the effect of cigarette smoking on some liver functions in male population in El-beida city, Libya. The study was carried out on thirty Libyan male smokers, who smoked at least 10 cigarettes per day for at least 15 years. The group includes smokers with age range between 30-60 years. Non-smokers, (control, n= 30) group were collected with the same range of age for statistical comparison. The whole blood samples were drawn by venipuncture from each member and liver functions test were estimated by a kit method on automatic analyzer (Beckman BUN analyzer, USA). The results of the study revealed a significant increase of malondialdehyde (MDA), which is an indicator of lipid peroxidation and oxidative stress significantly increased in cigarette smokers in groups when compared with control group. While the plasma level of total protein, albumin and total bilirubin decreased during smoking. Cigarette smoking raised alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma total cholesterol and triglycerides in smoker group when compared with non-smoker group. To conclude, cigarette smoking leads to oxidative stress by free radical generation by the mechanism of lipid peroxidation. Smoking exerts negative influence on liver functions test should be carefully interpreted, and further study on the mechanism of the effects is warranted.

Key Words: Malondialdehyde, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, triglycerides.

INTRODUCTION

Although tobacco has dangerous effect on human health, it still highly consumed throughout the world (Benowitz *et al.*, 1988). Smoking is one of the most common addictions of modern times. It has been implicated as an etiological agent for various chronic diseases, including a variety of infection, cancers, heart diseases and respiratory illnesses (Mehta *et al.*, 2008; Zhong *et al.*, 2008). Cigarette smoke (CS) contains over 4000 compounds, including at least 200 toxicant, 80 known or suspected carcinogens, large quantities of oxidants and free radicals that induce oxidative stress (de Heens *et al.*, 2008; Abel *et al.*, 2005; Carel and Eviatar, 1985). Moreover, cigarette smoking generates many toxic and carcinogenic compounds harmful to the health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals (Hoffmann *et al.*, 2001).

Liver functions test 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. Liver functions test consists most commonly of serum total protein, albumin, alkaline phosphatase (ALP), total bilirubin (TB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Albumin is a well-known negative acute phase protein that acts as a marker of inflammation and has antioxidant properties (Pongpaew *et al.*, 2001), but the underlying biochemical mechanisms in many pathological conditions associated with smoking are not fully understood. Therefore, this study was conducted to investigate the effect of smoking

on the inflammatory markers and other biochemical parameters, specifically total protein. However, the interpretation of liver functions test should be comprehensive and careful because these parameters can be influenced by many personal and environmental factors including cigarette smoking (Nathwani *et al.*, 2005). Although in some earlier studies found relationship between smoking and decreased serum protein, albumin and bilirubin concentrations (Dass *et al.*, 2013; Jang *et al.*, 2012; Van Hoydonck *et al.*, 2001). Recently, it has been suggested that smoking does not damage hepatocytes directly, because it does not effect on ALT and AST activities (Dass *et al.*, 2013; Jang *et al.*, 2012). On other hand, Gordon, 1993 found the smoking increased serum ALP levels produced by the bone and the kidney as well as by the liver (Gordon, 1993). However, it still not clear whether smoking is related to each component of liver functions test. Therefore, liver functions test changes in real clinical situations need to be interpreted carefully in the context of the interaction between various life style factors. Moreover, those studies have been reported primarily from Western and Asian countries, while the study of the effects of the cigarette smoking on liver functions test for persons living in African countries have had limited study. Thus, this study was conducted in a Libyan population to evaluate the effects of smoking habits on the most commonly used sets of liver functions test on order to consider the interactive association with major demographic factors.

MATERIALS AND METHODS

Design of the study

This study was conducted among thirteen Libyan male voluntary cigarette smokers (test) and thirteen male non-smokers (control) aging between 30-60 years. The research project was approved by ethics review committee at Omar Al-Mukhtar University (ref. no. - HE26May2014-DO3343).

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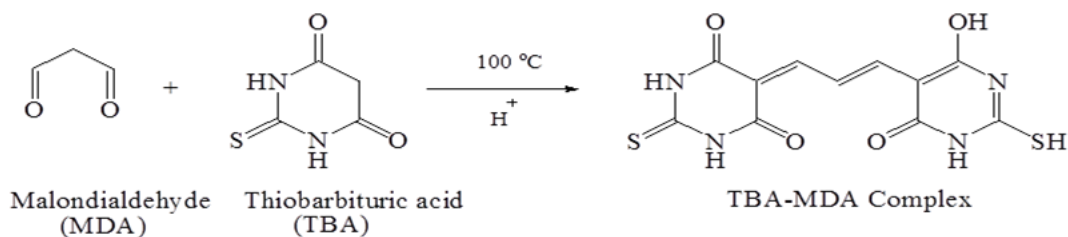


Figure 1: Thiobarbituric acid reaction (Gerard-Monnier *et al.*, 1997).

Two groups (smokers and non-smokers) were collected with the same range of age for statistical comparison. The smokers were regularly consuming minimum of 10 cigarettes per day for at least 15 years. The smokers were collected in El-beida city during the period (November 2013- February 2014). The enrolled subjects did not have any serious health problem; no history of drug usage and none had donated or received blood in last 6 months. The clinical data, medical history and other relevant information were collected from subjects by personal interview.

Evolution of Liver Function Tests

5 ml of whole blood samples were drawn by venipuncture from each member and placed in a heparinized tube and centrifuged at 3000 rpm for 15 minute. Plasma levels of total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma total cholesterol, triglycerides, total protein and albumin were estimated in all subjects by a kit method on automatic analyzer (Beckman BUN analyzer, USA). Commercially available test kits, products of Analyticon Biotechnologies, Germany were used and with the manufacturer's instructions strictly adhered to. Plasma globulin was determined as the difference between plasma total protein and albumin.

Determination of Plasma Malondialdehyde (MDA)

The assessment of the lipid peroxidation process is achieved via determining the end product MDA. The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to 150 μl serum sample the following were added: 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000rpm for 15 minutes, the supernatant was taken out for spectrophotometer assay at 532nm (figure 1) (Muslih *et al.*, 2002).

The concentration of MDA calculated as follow:

$$\text{MDA } (\mu\text{mol/l}) = \frac{\text{Absorbance at } 532 \text{ nm}}{L \times E_0} \times D \times 10^6$$

L: light path (1cm).

E_0 : Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor.

Statistical analysis

All data are expressed as mean \pm standard deviation ($M \pm SD$), and statistical analysis was carried out using statistically available software (SPSS) version 22.0. Unpaired t-test were applied to test the significance of variance ($p < 0.05$) of the parameters under study between control and smokers groups.

RESULTS AND DISCUSSION

Table 1 demonstrates general characteristics of Libyan male smokers and non-smokers group. The mean age of male smokers and non-smokers were 49.11 ± 10.53 and 47.97 ± 9.83 years, respectively. The non-smokers subjects were having weight of 73.13 ± 13.16 and smokers were having 71.82 ± 11.20 kg.

The level of MDA in smokers group significantly increased with mean value ($3.856 \mu\text{mol/l} \pm 0.213$) when compared with its control group ($2.637 \mu\text{mol/l} \pm 0.253$). This result is similar to Aula and Qadir, 2013 and Durak *et al.*, 2002 and Schmid *et al.*, 1996 published reports. CS is known to contain a large number of oxidants; it has been hypothesized that many of the adverse effects of smoking may result from oxidative damage to critical biologic substances (Skurnik and Shoenfeld, 1998). Two major phases were identified in CS: a tar phase and a gas phase; both phases are rich in oxygen-centered, carbon-centered and nitrogen-centered free radicals as well as non-radical oxidants. From the analysis of each phase, it was estimated that a single cigarette puff contains approximately 1014 free radicals in the tar phase and 1015 radicals in the gas phase. These include various compounds, which are capable of causing an increase in the generation of various ROS like superoxide ($\text{O}_2^{\cdot-}$) hydrogen peroxide (H_2O_2), hydroxyl (OH^{\cdot}) and peroxy (ROO^{\cdot}) radicals. These ROS, in turn, are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Pasupathi *et al.*, 2009). (Durak *et al.*, 2002) suggested that smoking creates a significant oxidant load in the erythrocytes. As a result, toxic free radicals and other oxidant substances in CS damages unsaturated fatty acids and some other oxidation-sensitive structures in the erythrocytes leading to MDA level increase.

In a recent study, increased MDA levels have been reported in blood samples from chronic smokers compared to non-smokers (Aula and Qadir, 2013). In some other studies it has been established that smokers need more antioxidant vitamins, suggesting that antioxidant supplementation is vital for smokers and scavenging potentially harmful free radicals produced by CS (Brown *et al.*, 1987).

Compared to never-smokers and current-smokers had higher levels of plasma total cholesterol, triglycerides, ALT, AST and ALP. However, plasma total protein, albumin and plasma bilirubin levels were lower in smokers than never-smokers (Table 2). All of these differences were statistically significant ($P < 0.05$).

Cigarette smoke contains high concentration of the gaseous compounds, like carbon monoxide (CO) Nitric oxide (NO) and Nitrogen dioxide (NO_2) and other substances in cigarettes such as aldehydes, hydrogen cyanide, lead, cadmium etc., In addition, it contains considerable number of free radicals that can be detected (Bridges, 1979). Free radicals are highly reactive atom, which damage the biological membrane through lipid

Table 1: General characteristic of smokers and non-smokers groups.

Characteristics	Control (Non-smoker; n=30)	Smokers (n=30)
Age (years)	47.97 ± 9.83	49.11 ± 10.53
Weight (kg)	73.13 ± 13.16	71.82 ± 11.20

Data are expressed as mean ± SD; (n) the number of subjects.

peroxidation. The heavy metal ions lead and cadmium present in smoke is also capable of inducing lipid peroxidation and hence provokes damage of organs propagating lipid peroxidation (Fraga, 1992). In the present study enhanced lipid peroxidation observed in smokers when compared with non-smokers.

Cigarette smoke propagates the lipid peroxidation, which damage the biological cell membrane of the liver and serum aminotransferases are enzymes that act as sensitive indicators of hepatocellular damage (Rochling, 2001). The enzymes are leaked out into blood and increased the level of AST and ALT in smokers when compared non-smokers (Table 2). In our study, we have observed the AST and ALT activities were significantly increased ($P < 0.05$) in smoker's plasma. The present study is the first to describe a detailed effect of smoking on the individual test of liver functions test. Previous studies showed controversial whether smoking could affect aminotransferase activities (AST and ALT). Some investigators claimed AST increased by cigarette smoking (Chan-Yeung *et al.*, 1981) as our results support, while other studies argued that smoking did not influence AST or ALT (Suriyaprom *et al.*, 2007; Whitehead *et al.*, 1996; Tajima *et al.*, 1988).

Although our results showed elevated ALP levels in the current smoker compared to never having smoked (Table 2). ALP was strongly influenced by smoking, consistent with other studies (Cheung *et al.*, 2009; Wannamethee *et al.*, 2005) concerning osteoporosis have documented increased serum ALP levels in current smokers, as a mainly marker of the liver and bones turnover, but is also present in the kidneys and leukocytes count (Friedman *et al.*, 1995).

The effect of cigarette smoking on biochemical parameters studied is shown in Table 2. The higher level of serum cholesterol, triglycerides, low plasma protein, albumin levels in smokers group. Various mechanism leading to lipid alteration by smoking are (a) Nicotine increased sympathetic adrenal system leading to increased secretion of catecholamines resulting in increased lipolysis and increased concentration of plasma free fatty acid which further result in increased secretion of hepatic free fatty acids and hepatic triglycerides along with VLDL-cholesterol in the blood stream (b) Presence of hyperinsulinaemia in smokers leads to increased activity of lipoprotein which leads to increased levels of lipid in smokers (Austin, 1991; Rustogi and Shrivastva, 1989). Frohlich *et al.*, 2003 and Effraim *et al.*, 2000 reported that usage of nicotine like products caused adverse effects like hypercholesterolemia in blood system. Plasma triglycerides determination was also one of the sensitive and detectable biomarkers to cigarette smoking (Kume *et al.*, 2009). Kume *et al.*, 2009 showed the triglyceride levels by cigarette smoking were increased for male smokers. On other hand, acute smoking was negatively associated with total cholesterol and triglycerides concentration (Suriyaprom *et al.*, 2007).

Our study demonstrated that cigarette smoking is associated with significantly lower ($P < 0.05$) concentrations

Table 2: Effects of cigarette smoking on liver functions of smokers and non-smokers group.

Parameters	Reference range	Control (n=30)	Smokers (n=30)
ALP (U/l)	80-306	139.0 ± 10.90	240.51* ± 63.84
ALT (U/l)	0-40	20.37 ± 4.33	36.24* ± 8.79
AST (U/l)	0-38	22.72 ± 3.26	38.06* ± 9.79
Total Bilirubin (mg/dl)	0.3-1.0	0.88 ± 0.10	0.30* ± 0.09
Total Protein (g/l)	4.6-8.0	6.91 ± 0.49	4.03* ± 1.10
Albumin (g/l)	3.8-4.7	4.29 ± 0.51	2.78* ± 0.97
Globulin (g/l)	2.3-3.5	2.51 ± 0.81	1.24* ± 0.71
Triglycerides (mg/dl)	40-160	97 ± 4.51	203* ± 12.65
Cholesterol (mg/dl)	0-240	218 ± 20.54	356* ± 58.12

Data are expressed as mean ± SD; (n) the number of subjects.

*Represents significant at $P < 0.05$. ALP: Alkaline phosphatase; AST:

Aspartate transaminase; ALT: Alanin transaminase.

of plasma total protein, albumin and bilirubin levels among healthy Libyan men (Table 2). The observation that the smokers in present study had lower plasma albumin concentrations in smokers than non-smokers also suggests the induction of an acute-phase response to smoking, since albumin is a negative acute-phase protein. The lower plasma albumin concentration in smokers agrees with certain (Dales *et al.*, 1974) but not all (Chao *et al.*, 1982). Moreover, there may be dietary effects on albumin synthesis, since smokers have been shown to consume less protein than non-smokers (Thompson *et al.*, 1992). The results of the present study in smoker's subjects confirm these finding and indicate that albumin concentrations were decreased. In addition, cigarette smoking is associated with increased oxidative stress. Albumin also has antioxidant properties by binding copper ions and scavenging hypochlorous acid (HOCl). The scavenging of HOCl by albumin may be due to the rapid reaction with SH-group rapidly from the circulation and degraded (Hu *et al.*, 1993; Halliwell, 1988). Cigarette smoking has a powerful influence on WBC count (Frohlich *et al.*, 2003; Hansen *et al.*, 1990), which may be a marker of exposure to oxidants, the inflammatory response to oxidants (Crowell and Sarnet, 1995). This study confirms higher WBC counts in smokers than never smokers.

Smokers groups resulted in a significant ($P > 0.05$) decrease in plasma total protein and globulin concentrations. We suggested that the decline in plasma protein and globulin was mainly due to the decrease in albumin level. Additionally, the protein depression in the blood may be due to loss of protein by reduce protein synthesis or increased proteolytic activity due to cigarette smoke exposure or via release of high levels of cellular oxidative free radicals could result increased proteolytic activity (Tetley, 2006). Also, the observed decrease in plasma protein of smokers could be attributed in part to the damaging effect of harmful compounds from cigarette smoking on liver cells as confirmed by the increase in the activities of plasma ALT, AST and ALP in this present study (Table 2).

Our results demonstrated that cigarette smoking is associated with lower concentrations of plasma bilirubin among healthy Libyan men (Table 2). In this study, these significant associations were independent of hemoglobin levels. The association between cigarette smoking and serum bilirubin concentration has been described previously (Schwertner, 1998; Hopkins *et al.*, 1996; Chan-Yeung *et al.*, 1981). However, cigarette smoking is also found to be positively associated with hemoglobin, which

regulates bilirubin concentrations (Milman *et al.*, 2001; Micozzi *et al.*, 1989). Therefore, differences in bilirubin levels may be due to hemoglobin concentrations. Still, this study showing the significant associations between total serum bilirubin and smoking were independent of hemoglobin. Regarding the relationship between smoking and bilirubin level have been consistently shown to be lower in current- smokers than never-smokers. Plasma bilirubin was significantly associated with initial stroke severity. It is still unclear why plasma bilirubin, especially direct bilirubin, was significantly associated with initial stroke (Pineda *et al.*, 2008). From a recent study, they found to be a more important determinant of serum bilirubin concentration which that was decline in smokers than never smokers groups (Jaeseong *et al.*, 2012). Bilirubin, which is a bile pigment, is generally regarded as a waste product of heme catabolism. However, it has been suggested that bilirubin might play an important role as an antioxidant, and its role as an antioxidant *in vitro* has been identified (Minetti *et al.*, 1998; Yamaguchi *et al.*, 1995; Hidalgo *et al.*, 1988). A number of recent *in vitro* studies have shown that bilirubin is more efficient than α -tocopherol at inhibiting low-density lipoprotein (LDL) oxidation and is a more efficient protector of human ventricular myocytes than either vitamin C or vitamin E (Wu *et al.*, 1996; Neuzil and Stocker, 1994; Wu *et al.*, 1994). In this study, low serum bilirubin levels could have been caused by increased level of free radicals produced mostly by cigarette smoking.

CONCLUSION

In conclusion, we show in the present study that smokers have higher plasma concentrations of MDA compared with non-smokers. Heavy smoking was associated with low total protein, globulin and albumin levels and raised AST, ALT and ALP levels. The levels of cholesterol and triglycerides were significantly increased in all smokers as compared to non- smokers. The association of smoking habits and liver functions test should be carefully analyzed and interpreted. Further studies on the mechanisms of these associations are warranted. Cigarette smoking decreases serum bilirubin in apparently smoking Libyan men. Bilirubin is inexpensive and easily measured in most clinical laboratories. Further studies are needed to determine if measuring bilirubin is effective in detecting free radicals produced by smoking, which contribute to serious diseases. Thus our study concludes that smokers have higher risk than that of non-smokers. Hence our primary aim is aware the smokers not to habitat or addict to smoking by explaining the harmful effects of smoking and thus protect them from various pathological disease.

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