



# EFFECT OF CIGARETTE SMOKING ON THE ACTIVITY OF LIVER ENZYME (AST,ALT,ALP)

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## Abstract

Smoking effect directly and indirectly on most organs of the body, this effect may occur in less than a second when inhaled and reach the alveoli and prevalence in the pulmonary veins to study the changes in the levels of liver enzymes (AST) Aspartate amino transferase, (ALT) alanine aminotransferase and (ALP) alkaline phosphatase in the blood serum of collection of (100) blood samples of smoker, which divided into three groups depending on age group.

1. First group of 10-20 years 2. Second group of 21-30 years 3. Third group of 31-40 years 4. Fourth group of 41 – 50 years 5. Fifth group of 51 and more

The above groups compared with 10 non smokers with age range 25-40 years old in Alrefaie General Hospital from period 1-2-2017 to 1-2-2018 in thiQar province. The results showed decreasing the activity of serum liver enzymes AST,ALT compared to control group while the of ALP increase significantly when compared with control. Also, there were decreasing in amount of (T.S.B. , blood urea , serum creatinin and serum albumin)

**Key words :** Liver enzyme, AST, alkaline phosphatase, alanine aminotransferase.

## Introduction

World health organization reports indicates that the annual number of dead's in the world resulted from smoking about (5) five millions persons and this number will be duplicated in the next twenty years (WHO, 2010), and the half of this number of deaths get before the end of seven decade of life as well as one – third of this number suffering from cardio – vascular problems which mostly lead permanent failure (Aurelio, 2005) and there is more than (1) one billion smoker in the world and this number will increase into ( 1.7) billion smokers in the 2015 (WHO, 2010). Liver is consider as one of most important body organ, which has many important functions as metabolism of drugs and alcohol, detoxification of toxins (Pessione *et al.*, 2001). Smoking has three effects: Toxic effects directly or indirectly on body organs, immunological effects and organic effects (EL-Zayadi, 2006). The toxic effects coming from the smoking contain toxic chemicals that cause oxidative stress through lipid peroxidation which lead to activation

of hepatic stellate cells, liver fibrosis and increase production of inflammatory cytokine (TNF, IL-6, IL1) (Moszczynski *et al.*, 2001). Also, smoking indirect causes increase carboxy haemoglobin which decrease oxygen loading capacity by R.B.C resulting in tissue hypoxia which lead to increase production of Erythropoietin leading to hyperplasia of bone marrow and this secondary polycythemia, increasing of R.B.C mass lead to increasing of (Fe) catabolism fromaged R.B.C and (Fe) resulting from polycythemia R.B.C catabolism added to that Erythropoietin stimulate (Fe) absorption from small intestine all these factors lead to accumulation of (Fe) macrophage and hepatocyte and with time lead to increasing of oxidative stress of hepatocyte (El- Zayadi, 2006) so that smoking lead to iron overload disease, thalassemia, porphyria curate trade and alcoholic cirrhosis (Nakanishis *et al.*, 2001) smokers are highly susceptible to cardiovascular system disease, respiratory system disease, tumor, gastric ulcer, gastro-esophageal disease, blindness, loss of bone matrix, liver toxicity and loss of hearing (Silvestri and Spiro, 2005).

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## Aim of study

Study the effects of smoking on the activity of liver enzyme (AST, ALT, ALP) in the serum.

## Materials and Methods

### 1. Place of sample collection

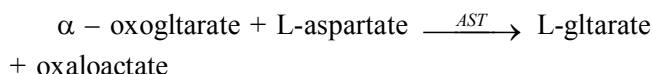
In case – control study, a total of (90) smoker were referred to general AL-Refaie Hospital at period extended from 2-January to 28-February / 2018

### 2. Blood sample collection

5 ml of venous blood were collected by using disposable syringe than put of this sample in non-heparinized tube for 30 min. at room temperature to get large quantity of serum when using os centrifuge (3000 / min.rpm) for 5 min, after that transport of serum to appendroff tubes and store at 20C until using measurement of AST enzyme by using diagnostic kit provided Randox company, United Kingdom.

### Principle

The activity of enzyme measured by oxaloacetate hydrazon which consist of 2-4 dinetrophenyl hydrazine which give purple colour can be measured by spectrophotometer at wave length (546) (Reitman and Frankel, 1957)



### (2 – 1) Reagent

R1	Buffers	
	Phosphate buffer	100 mmol/L, pH7.4
	L- aspartate	100 mmol/L
	$\alpha$ – oxaloacetate	2 mmol/L
R2	2-4 dinetrophenyl hydrazine	2.0 mmol/L
R3	Sodium hydroxide	4.0 mmol/L
	CAL. Pyruvate standerd	See lot specific insert

### (2 – 2) Procedure

	Reagent	Sample
Sample	————	0.1 ml
Buffer (R1 )	0.5 ml	0.5 ml
Distilled water	0.1 ml	————
Mix well and incubate at 37C/30 min.		
2-4 dinetrophenyl hydrazine (R2)	0.5 ml	0.5 ml
Mix well and allow to stand for 20 min. at 20-25C		
Sodium hydroxide (R3)	0.5 ml	0.5 ml

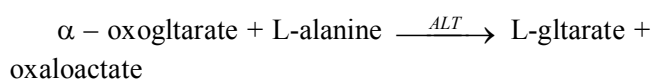
The reaction finished Sodium hydroxide and after 5 min. read by wave length 546 nm. And calculation according to company instructions ( normal value : up to 124 U/L )

### 1. ALT measurement

By using diagnostic kit provided Randox company, United Kingdom.

### Principle

The activity of enzyme measured by pyruvate hydrazon, which consist of 2-4 dinetrophenyl hydrazine which give purple colour can be measured by spectrophotometer at wave length (546) (Reitman and Frankel, 1957).



### (2 - 3) Reagent

R1	Buffers	
	Phosphate buffer	100 mmol/L, pH7.4
	L- alanine	100 mmol/L
	$\alpha$ – oxaloacetate	2 mmol/L
R2	2-4 dinetrophenyl hydrazine	2.0 mmol/L
R3	Sodium hydroxide	4.0 mmol/L
	CAL. Pyruvate standerd	See lot specific insert

### (2 – 4) Procedure

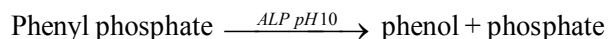
	Reagent	Sample
Sample	————	0.1 ml
Buffer (R1 )	0.5 ml	0.5 ml
Distilled water	0.1 ml	————
Mix well and incubate at 37C / 30 min.		
2-4dinetrophenyl hydrazine (R2)	0.5 ml	0.5 ml
Mix well and allow to stand for 20 min. at 20-25C		
Sodium hydroxide ( R3 )	0.5 ml	0.5 ml

The reaction finished Sodium hydroxide and after 5 min. read by wave length 546 nm. And calculation according to company instructions (normal value : up to 124 U/L)

### 2. ALP measurement

By using kit provided by Biomerieux company / France.

### Principle



Release phenol measured by presence of 4-antipyrine, potassium ferricyanide and sodium arsenate which found in the indicator which stop the reaction and measurement at 510nm (Kind and King, 1954).

### (2- 5) Reagent

R1	Substrate	Disodium phenyl phosphate Carbonate-bicarbonate buffer	5mmol/L
	Buffers	pH 10 Sodium merthiolate	50mmol/L 0.1g/L
R2	Standard	Phenol equal 20 king and king	U
R3	Reagent blocking	4-amino antipyrinesodium arsenate	60mmol/L 75g/L
R4	Color reagent	Potassium ferricyanide	150mmol/L

### (2-6) Procedure

	Serum sample	Serum	Standard Blank	Reagent Blank	
R1	2 ml	2 ml	2 ml	2 ml	
Incubate for 5min. at 37°C					
Serum	50µl	—	—	—	50µl
R2	—	—	—		
Incubate for 15min. at 37°C					
R3	0.5ml	0.5ml	0.5ml	0.5ml	
Mix well for exactly 15min. at 37°C					
R4	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Serum	—	—	—	—	—
D.W.	—	—	—	—	0.5ml

## Results and Discussion

### The effect of smoking on enzyme activity Aspartate, Alanine transaminase and alkaline phosphatase

In case of GPT (ALT) enzyme there was statistically significant differences in case of males (8.800±0.286) and in case of females (7.010±0.421) lower than control, in female with age group (10-20) years there was significant differences (7.210±0.312) and non-significant differences in case of males with same age group (11.220±0.256); in males and females with age group (21-30) years there was statistical differences (8900±0.546) and (9.600±0.452) respectively compared with control level; there was significant difference in case of age group (31-40) years in male and female which were (6.800±0.295) and (8.300±0.305) respectively ;statistical differences in case of age group (41-50) years in males and females, which were (8.700±0.404) and

(7.200±0.380) respectively. Also, significant level in case of age group (more than 51 years) to both males and females (23.700±0.521) and (8.600±0.186), respectively.

In case of GOT (AST) enzyme there was no statistically significant differences in case of males (11.900±0.264) and in case of females (10.800±0.687) lower than control; in female with age group (10-20) years there was no significant differences (10.800±0.673) and non-significant differences in case of males with same age group (12.100±0.094); in males and females with age group (21-30) years there was statistical differences (9.900±0.737) and (12.600±0.426) respectively compared with control level; there was no significant difference in case of age group (31-40) years in male and female which were (11.800±0.231) and (10.800±0.357), respectively. There was no significant difference in case of age group (41-50) years in males (11.100±0.253) but statistical differences in females (9.200±0.318) also, no statistical differences in case of age group (more than 51 years) of males and females (9.900±0.343) and (10.600±0.524), respectively. In case of ALP enzyme there was statistically significant differences in case of males (130.900±0.236) more than control and no significance difference in case of females (90.200±0.767); the remaining age groups to both sexes males and females there were no statistical differences in this enzyme compared with control except in females with age group (41-50) years was (53.900±0.857) and in males with age group more than 51 years was (129.100±0.102).

In case of T.S.B., there was statistically significant differences in case of males (0.760±0.058) and females (0.780±0.035) lower than control, in males with age group (21-30) years there was statistical differences (0.840±0.0305) compared with control level. There was no significant difference in case of age group (31-40) years in male (1.010±0.0.040) and statistical significance in case of female with same age group (0.950±0.073) ;statistical differences in case of age group (41-50) years in males was (0.560±0.040) and in case of age group (more than 51 years) in males was (1.940±0.057) compared with control level.

In case of *B. urea*, there was statistically significant differences in case of males (23.100±0.396) and females (21.100±0.458) lower than control.

In case of *S. albumin*, there was statistically significant differences in case of males with age groups (10-20), (21-30), (more than 51) were (3.980±0.183) , (18.470±0.614), (2.700±0.257) respectively more than control group but non-significant in case of males with age group (31-40) years which was (4.100±0.363).

In serum creatinin there was decreasing in its level compared with control group.

Cigarette smoking diminished serum total protein and albumin that current or past coffee consumption and smoking lower serum albumin, globulin, and all other protein fractions (Al-Khayat *et al.*, 2001). Moreover, in the chronic hepatitis patients, current smokers were more likely to have lower albumin levels than nonsmokers. However, the biological mechanisms leading to decreased levels of serum protein and albumin by coffee drinking and smoking have not been studied, yet.

Some investigators claimed ALT was increased by smoking (Astle, 2005), while recent studies argued that smoking did not influence AST or ALT, 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 (Awumey and Bukoski, 2006). Therefore, the effects of smoking on ALP level may be complicated with many extra hepatic mechanisms that did not show a consistent association with smoking in our study after adjustment of many factors. Many, but not all, epidemiological studies have suggested that high plasma uric acid is a risk factor for cardiovascular diseases, and they aimed at evaluating its prognostic implications and potential utility in the therapy monitoring (Ahmed and Weisberg, 2001; Al-Hamadani, 2010). This raised level of plasma uric acid, parallel to an increased risk of cardiovascular diseases, could be either primary or secondary to the underlying causes of the cardiovascular diseases (Al-Hamadani, 2007). However, the specific role of plasma uric acid in this constellation remains uncertain, although it may be involved in the platelet adhesiveness, aggregation, or inflammation, and it may be implicated in the genesis of hypertension (Al-Harbi, 2012). In contrast, there is some evidence that the increase of plasma uric acid is protective against the cardiovascular diseases, since uric acid acts as an endogenous antioxidant (Al-Harbi, 2012; Ali *et al.*, 2006) and the higher plasma uric acid levels found in cardiovascular diseases patients suggest that any protective antioxidant effect of uric acid is hidden by other negative effects in these pathogeneses. In this study, the plasma uric acid level in smokers was significantly lower than in nonsmokers both in men and in women. This could confirm the effect of cigarette smoking on uric acid levels independently of the gender. In addition, we noted a significant negative correlation with the smoking status, including the average number of

cigarettes smoked/day and the smoking duration. Moreover, we noted that the uric acid levels decrease when the smoking duration exceeds 5 years. This finding is in agreement with other studies showing a low plasma uric acid in regular smokers (Al-Khashab, 2004) and a reduction of antioxidants, including uric acid, in smokers, indicating that oxidative stress increases each time a cigarette is smoked (Al-Khayat *et al.*, 2001; Allen, 2002). Other studies proved that even nonsmokers exposed to cigarette smoke have a significantly lower plasma antioxidant status than unexposed nonsmokers, independently of the differences in the dietary antioxidant intake (Allen, 2002). Others studies proved that the administration of uric acid increases the circulating antioxidant defenses and allows the restoration of endothelium-dependent vasodilatation (Alsahen and Abdalsalam, 2014). A decrease of uric acid in smokers can be explained by the inactivation of xanthine oxidase by cyanide, which is eliminated as thiocyanate (Al-Shammaa *et al.*, 2011; Altinipik *et al.*, 2002). Therefore, high plasma uric acid concentrations might be protective in situations characterized by an increase of cardiovascular risk and oxidative stress, such as smoking (Al-Khashab, 2004) and a reduction of its level, which increases susceptibility to oxidative damage and accounts for the excessive free radical production (Allen, 2002). Therefore, the possibility that uric acid confers protection against the development of atherosclerosis, in view of its antioxidant properties, has been recognized (Allen, 2002). In this study, we found a significant decrease of plasma creatinine levels in smokers compared to nonsmokers, although these values are not pathological. This can confirm that all of the subjects studied are without any renal failure, since the determination of creatinine has been reported to be useful in evaluating the renal handling of uric acid and as concentrations of this parameter are highly dependent on endogenous production as well as on renal excretion (Altinipik *et al.*, 2002; Alwar *et al.*, 2013). Therefore, the low plasma uric acid level in smokers is attributed to a reduction of endogenous production. This finding is in agreement with other studies that proved that the reduction of antioxidants, including uric acid in smokers, is due to both the chronic exposure to cigarette smoke, which is a significant source of oxidative stress, and to the low intake of dietary antioxidants (Ambrose and Barua, 2004).

Some of the relationships between tobacco and urea or uric acid are very significant; however, they are all very weak. If these relationships have the same origin, a hypothetical renal mechanism must first be considered. In fact, the blood urea is a product of the catabolism of

proteins and their amino acids, whereas uric acid originates from the oxidation of purines. Moreover, the two molecules, while circulating in the blood, remain unlinked, either directly or by a common carrier. On the contrary, they are both excreted by the kidney and in the disease processes, they generally vary in the same way: a rise in blood uric acid is well known as an early sign of renal failure. An increase in the renal excretion of urea and uric acid under the influence of tobacco is, therefore, a reasonable hypothesis, and it is supported by the known action of nicotine on the metabolism of catecholamines and the effect of these substances on renal function (Anderson and Vulpe, 2009). Also many researches indicate the cigarettes vapor undergo metabolic activity by cytochrome enzymes p450 of reactive electrophiles which cause nitrosative stress which lead to cytotoxicity, mutation, cancer (Anderson and Vulpe, 2009). Also the cigarette vapor contain large number of toxic chemical materials which cause hepato cellular toxicity as nicotine (Bonkovsky *et al.*, 1997). Oxidative stress resulted from smoking lead to stimulation of NADPH oxidase which lead suppression of anti-oxidase and increase fat oxidation (Aurelio, 2005). These effects will lead increase damage of liver cells and activation of hepatic satellite cells and liver fibrocytes (Berg *et al.*, 2001). While other liver fibrosis cells as mesangial cells which stimulated by smoking vapour results as nicotine resulted in increase extracellular matrix proteins (Al-Khayat *et al.*, 2001). Other cause of liver fibrosis is iron deposition (Al-Khashab, 2004). All these results are corresponding with Ali *et al.* (2006), Al-Harbi (2012), Anderson and Vulpe (2009), who proved that the increased activity of liver enzymes resulted from hyper nitro sative stress.

AL-Hamdani (2007) indicate in his research which include (139) smokers to study the changes in the biochemical parameter of liver enzymes in smokers that the smoking lead to increasing in the activity of liver enzymes AST, ALT, ALP due to its content of nicotine tar, free radicals which lead to increase its concentration in blood. Abdrabo *et al.* (2013) in a study about the effect of smoking on the Biochemistry of serum between people of Sudan which include (105) smoker and (105) non-smoker at the ages rated 25-63 years old and approved that the smoking lead to increase the level of transported liver enzyme because of oxidative stress. Abdalsalam and Alsahen (2014) explained in their study about the effect of smoking on some of liver functions in smoker males that the smoking vapor has many effect on the liver functions because its contain free radicals, which lead to oxidative stress and increase fat oxidation and all studies explain that the increasing of ALP in the serum

of smoker as compared with non-smoker as in studies of Berg *et al.* (2001), Baccon and Britton (1990), Ali *et al.* (2006), Al-Khayat *et al.* (2001), Ahmed and Weisberg (2001).

Hassan *et al.* (2014) explain that the alkaline phosphatase has specific relationship with cigarette vapor also with smoking period.

Kahnameoi and Javid (2014) explained that the smoking lead to cardiovascular system diseases, Respiratory system disease, lung and mouth cancer, cancer don't related with nicotine, but related with carcinogenic agents in cigarettes vapor and other studies indicate that the smoking has greater effect on the serum ALP which consider as good marker to wounds or damage in bile ducts, added to that , the increasing the activity of AST and ALT in the serum indicate to the damage in hepatocytes (Bishop *et al.* (2005).

### Conclusion

An decreasing in activity of liver enzymes related with smoking.

1. There is significant relationship between ALP enzyme activity and smoking .
2. An decreasing in the amount of T.S.B , blood urea and serum creatinin related with smoking .
3. Cigarette vapor has dangerous effect on the liver and bile duct cells .

### Recommendation

1. Many researches should be made to explain the effect of smoking on serum electrolytes.
2. Study of pituitary and adrenal glands hormones and its relationship with liver enzymes .
3. Study of effect of oxidative stress in smokers at different smoking period .

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