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Original Research Article **Biochemistry**

A Study on Serum Hepatic Enzymes in Smokers and Nonsmoker's Adult Male

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Abstract

Background: Cigarette smoking is one of the greatest indirect causes of global death and disease. Cigarette smoke consists of many chemicals, including cytotoxic, carcinogenic and free radicals, therefore it affects many organs including liver either directly or indirectly. ***Objective:*** The aim of this study was to find out level of serum hepatic enzymes in smokers and non-smokers adult male. ***Method:*** This cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh, from January 2019 to December 2019. Total study subjects were one hundred and twenty with age ranging from 20 to 50 years of male gender, selected from attendance of admitted patients and third & forth class employees of Dhaka Medical College Hospital, Dhaka. Study subjects were grouped smokers as Group A and age and gender matched, nonsmokers as Group B. Group A smokers again divided into three groups. Group A_1- heavy smokers (who consume 20 cigarette sticks or more per day) consist of thirty study subjects, Group A_2- moderate smokers (who consume 11-19 cigarette sticks per day) consist of thirty study subjects and Group A_3- light smokers (who consume 1-10 cigarette sticks per day) consist of thirty study subjects. Serum Alanine Transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) level of all study subjects were estimated and recorded. ***Results:*** Mean age of the study subjects in group A_1, A_2, A_3 & B were 33.88 years, 34.60 years, 34.60 years and 29.80 years. There was a significant increase in serum ALT, AST and ALP level in heavy, moderate and light smokers when compared to nonsmokers, but the increase was more significant in heavy smokers when compared to moderate smokers, also moderate smokers when compared to light smokers. ***Conclusion:*** This study revealed that significantly higher level of serum hepatic enzymes with smokers when compared to that of non smokers. This significantly higher level was also seen in heavy smokers in comparison to moderate smokers, moderate smokers in comparison to light smokers.

INTRODUCTION

Cigarette smoke contains over 4000 different chemicals, 400 of which with bad effect on the Human body. It contains different compounds that work as an oxidant which including Oxygen free radicals and volatile aldehydes and which may be responsible of the bimolecular damage [1].

Besides, smoking induces three major adverse effects on the liver: direct or indirect toxic effects, immunological effects and oncogenic effects. Smoking yields chemical substances with cytotoxic potentials which increase necro-inflammation and fibrosis. In addition, smoking increases the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) that would be involved in liver cell injury [2].

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 is 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 enzymes [3].

Another study found that liver functions test changes in real clinical situations need to be interpreted carefully in the context of the interaction between various life style factors including cigarette smoking [4]. In present study they measure the important enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in adult cigarette smoking male. The results show a significant increase in levels of activity of the liver enzymes ALT, AST and ALP in smokers compare to non-smokers and increase in proportion with duration of smoking.

Thus, several reports show interest in the liver function and especially the enzymes Alkaline Phosphates (ALP), Aspartate and Alanine Transferase (AST and ALT) in cigarette smoking cases. All these studies recommend that these enzymes can be used as indicators for predicting different kind of clinical results for the patients and healthy groups, and also to indicate if there is any damage or disorder in the function of the liver by smoking [5].

Objective

The aim of this study was to find out level of serum hepatic enzymes in smokers and nonsmokers adult male

METHODOLOGY

Study Design: Cross sectional study (Analytical).

Time of Study: January 2019 to December 2019.

Place of Study: Department of Biochemistry, Dhaka Medical College, Dhaka.

Study Population: Adult healthy male smokers with the age ranging from 20-50 years from attendance of admitted patient and third- & fourth-class employees of Dhaka medical college hospital, Dhaka and age and gender matched non smoker subjects.

Sample Size: 120 (One hundred and twenty). Sample size should be at least 30 (Appendix- V). For better assessment total one hundred and twenty samples were taken (30 in each group).

Sampling Technique: Purposive sampling. It was followed as per inclusion and exclusion criteria.

Grouping of Samples: All the study subjects were grouped into group A (smoker) and comparison group B (non smoker). Smoker group was again subdivided into heavy smokers (Group A_1), moderate smokers (Group A_2) and light smokers (Group A_3) on the basis of number of consumed cigarette sticks per day.

Group A: Ninety smokers from attendance of admitted patient and third & fourth class employees of Dhaka medical college hospital, Dhaka with
Thirty heavy smokers as Group A_1
Thirty moderate smokers as Group A_2
Thirty light smokers as Group A_3

Group B: Thirty age & gender matched apparently non smoker subjects from attendance of admitted patient and third- & fourth-class employees of Dhaka medical college hospital, Dhaka.

Selection criteria:
Inclusion criteria:
For all groups:

1. Age: between 20 to 50 years and apparently healthy persons
2. Gender: male.

For Group A

1. Smoker
2. Smoking duration: at least last 1 year and still continuing

For Group (A_1) (Heavy smoker)

1. A smoker who reports consuming 20 cigarettes or more per day.

For Group (A_2) (Moderate smoker)

1. A smoker who reports consuming between 11-19 cigarettes per day

For Group (A_3) (Light smoker)

1. Smoker who reports consuming between 1-10 cigarettes per day.

For Group B (Comparison group)

1. Age and gender matched non smoker subjects.

Exclusion criteria:

1. Age: < 20 years and > 50 years.
2. Patients with acute and chronic liver disease.
3. Other co morbidities
4. Patients taking any drug that impair liver function.

Biochemical variables:
Serum alanine transaminase (ALT) level
Serum aspartate transaminase (AST) level
Serum alkaline phosphatase (ALP) level

Hepatic enzymes with reference value:
Alanine amino transferase (ALT, SGPT): <45U/ L
Aspartate amino transferase (AST, SGOT): <35U/ L
Alkaline phosphatase (ALP): 53-128U/L

Data collection methods:

This cross sectional study was conducted from January 2019 to December 2019. Total 120 (one hundred and twenty) individuals were enrolled. Among them 90 smokers (group A) which divided into three groups in 30 heavy smokers (group A_1), 30 moderate smokers (group A_2) and 30 light smokers (group A_3) were selected from attendance of admitted patient and third- & fourth-class employees of Dhaka medical college hospital, Dhaka. Another 30 age and gender matched non smoker subjects (group B) from same place were selected. Initial evaluation of the subjects by history were performed and recorded in the preformed data collection sheet. The objectives, nature, purposes and potential risks of all procedures used for the study were explained in details to each subjects and informed written consent was taken from both smokers and non smokers. Blood pressure was measured.

Collection and preservation of blood samples:

After all aseptic precaution 5 ml of venous random blood sample was collected from each study subject in a disposable plastic syringe and immediately transferred to a fresh red capped clean test tube (Guangzhou ASPO Medical Eqipment co., Ltd), which was allowed to clot at room temperature and clear serum was separated into a sterile eppendorf tube. The sample was separated after centrifuging at 3000 rpm for 10 minutes and the separated serum was used for biochemical assay or was stored at -20ºC if the analysis was delayed. All the biochemical tests were performed in the department of Pathology, Dhaka Medical College, Dhaka. After collection of all samples, serum was used for the measurement of serum ALT, AST & ALP

Laboratory procedure:

All the tests were performed in the laboratory of pathology department of Dhaka Medical College, Dhaka. Liver enzymes were measured by a semi auto analyzer instrument (Avolation 3000 made in USA) for all smokers and nonsmokers.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel methods (Appendix V and VI). Alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method (Appendix VII). Primary IFCC reference procedures were available for the measurement of catalytic activity concentrations of AST, ALT and ALP at 37 degree Celsius.

Data Analysis:

After meticulous checking and rechecking all the data was recorded in a predesigned data collection sheet. Continuous variables were expressed as mean ± SD and compared between two groups of study subjects by unpaired student's t-test and among four groups by analysis of variance (ANOVA). Bonferroni test was done between the groups. Correlation was done by Pearson's correlation coefficient (r) test. All p values were two-tailed with significance defined as $p < 0.05$ at the level of 95% confidence interval (CI). All analysis was done using the SPSS 20.0 (Statistical Package for Social Science) package for windows.

RESULTS

Table shows no significant difference of mean age of the study subjects among the groups.

Table I: Age (years) distribution of the study subjects (N=120)

Age (years)	Group A (n=90)	Group B (n=30)	p-value
Mean ± SD (years)	34.22±8.50	29.80±5.21	0.008
Range (years)	22-50	20-38	

Table II shows significant difference of ALT, AST and ALP of the study subjects between group A and group B that is significantly increased in group A than group B.

Table II: Serum hepatic enzymes (U/L) level in smokers and non-smokers (N=120).

Serum hepatic enzymes	Group A (n=90) Mean±SD (Range)	Group B (n=30) Mean±SD (Range)	p-value
ALT (U/L)	55.5 ± 10.0 (40.0-80.0)	28.8 ± 6.2 15.0-38.0)	<0.001
AST (U/L)	45.5 ± 10.0 (30.0-66.0)	26.9 ± 4.3 (20.0 -36.0)	<0.001
ALP (U/L)	118.4 ± 11.3 (101.0-140.0)	94.6 ± 5.6 (72.0-100.0)	<0.001

Table III shows there was significant difference of serum ALT level among the groups.

Table III: Serum ALT (U/L) level of the study subjects in different groups (N=120)

Group	Mean ± SD (U/L)	Range (U/L)	p-value
Group A_1 (n=30)	67.13 ±5.16	60-80	<0.001
Group A_2 (n=30)	54.10 ±4.17	47-60	
Group A_3 (n=30)	45.23 ±3.35	40-50	
Group B (n=30)	28.80 ±6.24	15-38	

One way ANOVA test was done among the groups.

Serum ALT level between the groups.

Group	ALT
A_1 vs A_2	<0.001
A_1 vs A_3	<0.001
A_1 vs B	<0.001
A_2 vs A_3	<0.001
A_2 vs B	<0.001
A_3 vs B	<0.001

Bonferroni test was done between the groups.

There was significant difference in serum ALT level between the groups.

Table IV shows there was significant difference in serum AST level among the groups.

Table IV: Serum AST (U/L) level of the study subjects in different groups (N=120).

Group	Mean ± SD (U/L)	Range (U/L)	p-value
Group A_1 (n-30)	57.30 ±3.91	52-66	<0.001
Group A_2 (n-30)	44.70 ±3.34	39-50	
Group A_3(n-30)	34.43 ±3.10	30-40	
Group B(n-30)	26.93 ±4.27	20-36	

One way ANOVA test was done among the groups

Serum AST level between the groups.

Group	AST
A_1 vs A_2	<0.001
A_1 vs A_3	<0.001
A_1 vs B	<0.001
A_2 vs A_3	<0.001
A_2 vs B	<0.001
A_3 vs B	<0.001

Bonferroni test was done between the groups

There was significant difference in serum AST level between the groups.

Table V shows there was significant difference of serum ALP level among the groups.

Table V: Serum ALP (U/L) level of the study subjects in different groups (N=120)

Group	Mean ± SD (U/L)	Range (U/L)	p-value
Group A_1 (n-30)	131.93 ±4.76	122-140	<0.001
Group A_2 (n-30)	115.80 ±3.69	110-122	
Group A_3(n-30)	107.50 ±6.19	101-120	
Group B(n-30)	94.57 ±5.58	72-100	

One way ANOVA test was done among the groups

Serum ALP level between the groups.

Group	ALP
A_1 vs A_2	<0.001
A_1 vs A_3	<0.001
A_1 vs B	<0.001
A_2 vs A_3	<0.001
A_2 vs B	<0.001
A_3 vs B	<0.001

Bonferroni test was done between the groups
There was significant difference in serum ALP level between the groups.

DISCUSSION

Serum hepatic enzymes (ALT, AST & ALP) levels were done to observe the harmful effect of smoking on the liver of the study subjects. Mean serum ALT, AST & ALP in group A were 55.5 U/L, 45.5 U/L & 118.4 U/L respectively, these were significantly higher than group B (P <.001). These results were consistent with other study [6].

Mean serum ALT level of group B was compared with group A1, A2 & A3 separately. Mean serum ALT among group A1, A2 & A3 were 67.13 U/L, 54.10 U/L and 45.23 U/L respectively that was significantly higher than group B (P <.001). The study subjects in group A1 had poor hepatic function as reflected by their increased serum ALT level. Another study found mean serum ALT is significantly higher in heavy smoker, moderate smoker and light smoker compared to non smoker [7].

Mean serum AST level of the study subjects in group A was 45.5 U/L that was significantly higher than group B (P <.001). These results were close to the findings of other study. 6 Mean serum AST level of group B was compared with group A_1, group A_2 and group A_3 respectively. Mean serum AST among group A_1, A_2 & A_3 were 57.30 U/L, 44.70 U/L and 34.43 U/L respectively that was significantly higher than group B (P <.001). Another study found mean serum AST is significantly higher in heavy smoker, moderate smoker and light smoker compared to non smoker [7].

According to this study mean serum ALP level of the study subjects in group A was 118.4 U/L and 94.6 U/L in group B. Mean serum ALP level that was significantly higher in group A than group B (P <.001). Mean serum ALP among 131.93 U/L in group A_1, 115.80 U/L in group A_2 & 107.50 in group A_3. There was significant increase of serum ALP in group A_1, A_2 & A_3 when compared to group B. But the increase was more significant in group A_1 when compared to group A_2 and group A_2 when compared to group A_3. Other study evaluated serum ALP is higher in heavy smoker, moderate smoker and light smoker compared to non smoker [7].

Small increase in AST & ALT may be as a result of wide range of liver disease. It has been suggesting that ALP can seep out of the liver and into the blood stream, but only with blockage or inflammation of the bile ducts [3].

Other study found serum hepatic enzymes of smokers were higher with longer duration of smoking compared to shorter duration of smoking. They conclude that significant increase in levels of activity of the liver enzymes ALT, AST & ALP in smokers compare to non smokers and increase in proportion with duration of smoking [8].

Another report found mean±SD of AST, ALT & ALP of smoker were (14±59 U/L), (27±13 U/L) and (105±18.9 U/L) respectively and of diabetic (25±7.8 U/L), (28±5.8 U/L) and (130±20 U/L) respectively, which were higher compared to control groups (15.5±4.6 U/L), (23±5.1 U/L) and (88.3±16.7 U/L) respectively, p value=0.000. They observed that both cigarette smoking and diabetes have effects in liver function leading to variable alteration of liver enzymes activity [9]. Cigarette smoke propagates the lipid peroxidation, which damage the biological cell membrane of liver and serum aminotransferase are enzymes that act as sensitive indicators of hepatocellular damage [10].

Another study investigated the effect of cigarette smoking on some liver function in libyan male population. They evaluated that the enzymes are leaked out from damaged hepatocytes into the blood and increase the AST & ALT in smokers when compared to non smokers. In that study they observed the AST & ALT activities were significantly increased (P <0.005) in smokers plasma. 11 Although elevated ALP levels in the current smoker compared to never having smoked. ALP was strongly influenced by smoking. Smoking increases the production of proinflammatory cytokines (IL-1, IL-6 & TNF) that would be involved in liver cell injury. It causes the development of secondary polycythemia and in turns to increased red cell mass and turnover which might be a contributing factor to secondary iron overload disease promoting oxidative stress of hepatocytes. Smoking also increases serum and hepatic iron which induce oxidative stress and lipid peroxidation that leads to activation of stellate cells and development of fibrosis. Smoking yields chemicals with oncogenic potential that increase the rise of hepatocellular carcinoma (HCC) in patients with viral hepatitis and are independent of viral infection as well.

Other study also found that enhanced activities of the serum enzymes viz, AST, ALT, ALP in smokers were compared to non smokers [12]. Other studies also observed that significant higher level of liver enzymes such as Aspartate transaminase (AST), Alanine tranaminase (ALT) and Alkaline phosphatase (ALP) in all smokers compared to non smokers [6-9]. All these findings were similar to present study indicating serum hepatic enzymes increased in smokers.

Several studies have observed significantly higher levels of liver enzymes such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP) in smokers compared to non-smokers. For example, research by [13] demonstrated enhanced activities of AST, ALT, and ALP in smokers, indicating a possible association between smoking and liver enzyme elevation. Similarly, studies by [14-17], corroborate these findings, revealing significantly higher serum enzyme levels in smokers, which suggests that smoking may exacerbate liver dysfunction and potentially influence overall metabolic health.

The elevated triglycerides and low HDL cholesterol levels observed in the current study among non-diabetic obese individuals echo the findings of [18], which reported high triglyceride levels and low HDL cholesterol as prevalent among obese populations. This dyslipidemic profile is consistent with other studies, such as [4], which found similar lipid abnormalities in populations at risk of metabolic syndrome. Additionally, [19, 20] found that elevated LDL cholesterol and reduced HDL cholesterol are common in individuals with metabolic syndrome, underscoring the importance of addressing these lipid disturbances.

CONCLUSION

This study revealed that significantly higher level of serum hepatic enzymes with smokers when compared to that of non smokers. This significantly higher level was also seen in heavy smokers in comparison to moderate smokers, moderate smokers in comparison to light smokers.

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