

Influence of age, body fat distribution, alcohol consumption and smoking on liver enzymes activity in apparently healthy western Indian males

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Abstract

This prospective study was conducted to assess the effect of demographic and clinical parameters on liver enzymes in apparently healthy western Indian male population. The study population consisted of 669 males attending blood donation camps during 2011 to 2013. Anthropometric measurement was taken and history for alcohol consumption and smoking was noted. Biochemical parameters including AST, ALT, and GGT activities were determined using auto analyzer (AU-400 by Olympus). Mann-Whitney Test and Kruskal-Wallis One Way ANOVA on Ranks Test applied. P value of < 0.05 was considered to be statistically significant. All analyses were conducted using the SPSS-13.0. According to alcohol habit, 97 (14.5%) were alcoholic and 572 (85.5%) were non-alcoholic. According to smoking habits, 171 (25.6 %) were smokers and 498 (74.4 %) were non-smokers. Median activity of serum AST, ALT and GGT were 27.00 IU/L, 17.00 IU/L and 18.80 IU/L respectively. By univariate analyses, there were significant associations between increasing AST, ALT, or GGT tertiles and age, body mass index, and waist and hip circumferences, Waist-Hip ratio ($p < 0.05$). AST and GGT activity were significantly higher in alcoholic men compared to non alcoholic ($p < 0.05$). BMI, AST, ALT and GGT were significantly higher in smokers compared to non-smokers ($p < 0.05$). These data suggest that slight to moderate increase in BMI and light alcohol intake and smoking affected liver enzymes levels in apparently healthy western Indian males. These factors should be considered in the definition of normal limits for liver enzymes.

Keywords: γ -glutamyltransferase; Alanine aminotransferase; Aspartate aminotransferase; alcohol; smoking, Western Indian Male.

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INTRODUCTION

Liver enzyme tests 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. Aspartate amino transferase (AST), alanine aminotransferase (ALT), and γ -glutamyl transferase (GGT) are tested routinely in current clinical settings¹. These enzymes are commonly elevated in patients with liver diseases and thus may reflect the status of liver injury². Physicians generally use significant elevations of liver enzyme levels as complementary markers to aid the

diagnosis of various diseases. For example, elevations of ALT and AST may indicate the presence of hepatocellular predominant disorders while elevations of ALP and GGT may implicate cholestatic predominant diseases³. However, the interpretation of these tests should be comprehensive and careful because these enzyme levels can be influenced by many personal and environmental factors, including age, gender, body mass index (BMI), alcohol drinking, cigarette smoking, and malnutrition¹. Liver enzymes levels can be modulated by the pattern of body fat distribution. Presently, body mass index (measure of total body fat), waist circumference and waist-to-hip ratio (the measures of regional body fat) are employed for classifying the pattern of abdominal fat accumulation. In the absence of other causes, excess body weight increases the risk of liver disease^{4,5}. Health risks associated with body weight are of greater concern depending on the pattern of fat distribution in the body⁶. Alcohol is another factor associated with increased liver enzymes activity. Studies have indicated a gradual effect of alcohol on GGT enzyme induction, which may be initiated at rather low levels of ethanol intake in a gender-

dependent manner^{7,8}. It has also been suggested that smoking increases GGT levels and can boost the alcohol-induced GGT elevations⁹. Therefore, liver enzymes alterations in real clinical situations need to be interpreted carefully in the context of the interaction with various clinical, demographic and environmental factors. This is one of the reasons that the healthy ranges of liver enzymes has recently been brought into question¹⁰. The interaction of liver enzymes with clinical, demographic and environmental factors should be implicated in the definition of normal ranges for such an important biomarkers. There is paucity of data regarding evaluation of the effects of body fat distribution, alcohol consumption and smoking on the level of liver enzymes in a prospectively systematic way among apparently healthy West Indian male people. So, the aim of this study was to determine serum aminotransterases and γ -glutamyltransferase, activity and to investigate their relationship with body fat distribution, alcohol and smoking in apparently healthy Western Indian male population.

MATERIAL AND METHODS

Study population

This cross sectional study was conducted by the Department of Biochemistry, Seth G S Medical College, Mumbai. The population chosen for this study comprised of individuals who attended blood donation camps held by the institute during July 2011- March 2013. This study was approved by the Institutional Ethics Committee. Total of 750 apparently healthy men were interviewed and finally 669 were enrolled after applying exclusion criteria. Written informed consent was obtained from all enrolled men. Information regarding age, sex and anthropometric parameters (Height, Weight, Waist circumference and Hip circumference) smoking and alcohol were noted. Exclusion criteria used were- pathophysiological states (Any concurrent acute or chronic illness including acute febrile illness, thyroid disease, hypertension, diabetes, renal failure, congestive heart disease, chronic respiratory diseases, liver diseases, malabsorption syndromes, and nutritional anemia), acute viral hepatitis in last six months, intake of pharmacologically active agents (tobacco, oral contraceptives, replacement or supplementation therapy such as insulin intake), family history of jaundice, h/o hepatotoxic drugs like aspirin, acetaminophen, ibuprofen etc, any other concurrent alternative medications (Ayurvedic, Homeopathic anticoagulant drugs etc.), blood transfusion in recent past (6 months), alcohol consumption (> 1.2 drinks per day) obese (BMI > 30), modified physiological states (psychological and mental disorders such as severe stress and depression, exercise or

physical training in previous days). Alcohol consumption was assessed as the type of beverage, frequency of consumption and the amount of drinks consumed. A dose of 10 g of pure ethanol was considered as one standard drink. Alcohol drinking status was classified as non drinkers and light drinker (<1.2 drinks per day)¹¹. Past drinkers were excluded. Smoking status was classified as smokers and non-smokers. For smokers, the amount of smoking was not taken into account and past smokers were not included in the study.

Physical and Biochemical Measurements

Physical measurements included waist circumference, hip circumferences, height and weight. Standing waist circumference was measured at the high point of the iliac crest. For hip circumference, the tape horizontally placed around the hips at the biggest circumference point (maximum protrusion) between the iliac crest and the head of the femur. Heel to head-crown length was measured as height. Weight was measured using a self-zeroing weight scale with the participant wearing no shoes. The waist-to-hip ratio was calculated as the waist measurement divided by the hip measurement. Venous blood samples were collected in appropriate evacuated tubes in aseptic condition. Serum AST, ALT, GGT, ALP, total protein, albumin, TB and DB were carried out using standard clinical methods. The analysis was performed on fully automated analyzer (AU-400 by Olympus).

STATISTICAL ANALYSIS

All the biochemical parameters revealed non-Gaussian distribution (as tested by D'Agostino-Pearson test). Data was represented as the mean \pm SD and Median and IQR (Interquartile range). For categorical variables, number and percent were used. Analysis of Quantitative data between two subgroups was done using Mann-Whitney Test. Study population was subdivided into AST, ALT, GGT tertiles and data were compared. For associations of them with numerical variables, Kruskal-Wallis One Way ANOVA on Ranks Test applied, and for analyzing statistical difference among categorical variables in relation to AST, ALT, and GGT levels, chi-square tests were performed. P value of < 0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for Social Sciences (SPSS-13.0, Chicago, IL).

RESULTS

Baseline characteristics of study population

The median age of all participants (n=669) was 25 years. Median BMI was 22.60 Kg/m² and median waist-hip ratio was 0.89. Median serum AST, ALT, GGT levels were 27.00, 17.00 and 18.80 IU/L respectively. (**Table-1**) A total of 97 (14.5%) were alcoholic and 572 (85.5%) were

non-alcoholic at the time of study. According to smoking habits, 171 (25.6 %) were smokers and 498 (74.4 %) were non-smokers.

Comparative demographic and clinical characteristics in AST, ALT and GGT tertile groups

Table-2 reports median value of clinico-demographic factors according various AST subgroups. Univariate analysis showed significant associations between increasing AST tertile and age, BMI, WHR and smoking habits ($p < 0.0001$). On pair wise group comparison, median age, BMI, WC and WHR were significantly higher ($p \leq 0.05$) in group-3 (AST > 25 IU/L) compared to group-1 and 2 (AST < 21 IU/L, AST 21-25 IU/L respectively). **Table-3** reports median value of clinico-demographic factors according various ALT subgroups. Univariate analysis showed significant associations between increasing ALT tertile and age, BMI, WC, HC, WHR and smoking habits ($p < 0.0001$). On pair wise group comparison, median BMI, WC, HC and WHR were significantly lower ($p \leq 0.05$) in group-1 (ALT > 22 IU/L) compared to group-2 and 3 (ALT < 22-34 IU/L, ALT > 34 IU/L respectively) while median age of group 1 and 2 were significantly lower compared to group-3 ($p \leq 0.05$). **Table-4** reports median value of clinico-demographic factors according various GGT subgroups. Univariate

analysis showed significant associations between increasing ALT tertile and age, BMI, WC, HC, WHR, alcohol and smoking habits ($p < 0.0001$). On pair wise group comparison, median BMI, WC and WHR were significantly lower ($p \leq 0.05$) in group-1 and 2 (GGT < 15 IU/L, GGT 15-25 IU/L respectively) compared to group-3 (GGT > 25 IU/L respectively) while median age and HC of group 1 were significantly lower compared to group-3 ($p \leq 0.05$).

Effect of alcohol consumption on liver enzymes and demographic variables

In univariate analysis, median AST and GGT levels were significantly higher in alcoholic group, compared to non-alcoholic group while no significant difference could be observed in age, BMI, WC, HC and WHR between both groups. (**Table-5**)

Effect of smoking on liver enzymes and demographic variables

In univariate analysis, median AST, ALT and GGT levels were significantly higher in smoker group, compared to non smoker group. For demographic variables median BMI was significantly higher in smoker compared to non-smoker group while no significant difference could be observed in age, WC, HC and WHR between both groups. (**Table-6**)

Table 1: Baseline characterizes of study population

Variables	Mean	SD	Median	IQR	Minimum	Maximum
Age (years)	27.67	6.92	25.00	11.00	21.00	48.00
BMI (Kg/m ²)	22.94	2.92	22.60	4.15	16.40	29.90
Waist Circumference (cm.)	84.43	8.98	85.00	14.00	62.00	105.00
Hip Circumference (cm)	95.59	7.10	96.00	10.00	76.00	115.00
Waist/Hip ratio	0.88	0.05	0.89	0.07	0.69	0.99
AST (IU/L)	27.55	7.79	27.00	10.00	13.00	81.00
ALT (IU/L)	19.65	10.63	17.00	13.00	5.00	64.00
GGT (IU/L)	21.19	9.27	18.80	11.05	6.60	70.00
ALP (IU/L)	99.48	39.81	93.50	41.00	8.00	412.00
TP mg %	7.27	0.66	7.30	0.80	5.40	9.90
Sr. Albumin mg %	4.23	0.33	4.20	0.40	2.80	5.40
Sr. Bilirubin (Total) mg/dl	0.71	0.27	0.72	0.39	0.10	1.30
Sr. Bilirubin (Direct) mg/dl	0.25	0.11	0.24	0.16	0.10	0.50

Table 2: Comparison of clinico-demographic variables between different AST sub-groups

Variable	AST {Median (IQR)}			Chi-square (p)	p value for pair wise group comparison (Dunn's method)
	< 21 (Group 1) n=125	21-25 (Group 2) n=168	> 25 (Group 3) n=376		
Age (years)	25.00 (8.50)	23.00 (10.00)	27.00 (10.00)	11.87***)	≤ 0.05(Group 1,2 vs 3)
BMI (kg/m ²)	22.14 (3.94)	22.17 (3.54)	23.30 (4.16)	18.97***)	≤ 0.05(Group 1,2 vs 3)
Waist Circumference (cm)	84.00 (14.00)	84.00 (14.00)	85.00 (14.00)	8.56***)	≤ 0.05(Group 1,2 vs 3)
Hip Circumference (cm)	96.00 (10.50)	95.00 (9.00)	96.00 (10.00)	3.44 NS	NS
Waist/Hip ratio	0.87 (0.07)	0.87 (0.09)	0.90 (0.07)	18.43***)	≤ 0.05(Group 1,2 vs 3)
Alcohol habit (% positive)	12.40	23.70	63.90	3.71 NS	-----
Smoking habit (% positive)	4.20	15.20	80.60	56.30***)	-----

Table 3: Comparison of clinic-demographic variables between different ALT sub-groups

Variables	ALT {Median (IQR)}			Chi-square (p)	p value for pair wise group comparison (Dunn's method)
	<22 (Group 1) n= 429	22-34 (Group 2) n=173	>34 (Group 3) n=67		
Age (years)	25.00 (9.00)	27.00 (12.00)	30.00 (12.00)	31.03***	≤ 0.05(Group 1,2 vs 3)
BMI (kg/m ²)	22.10 (3.50)	23.66 (4.52)	25.20 (4.97)	73.09***	≤ 0.05(Group 2,3 vs 1)
Waist Circumference (cm)	83.00 (14.00)	87.00 (12.00)	89.00 (11.00)	48.99***	≤ 0.05(Group 2,3 vs 1)
Hip Circumference (cm)	95.00 (10.00)	98.00 (11.00)	98.00 (9.00)	40.87***	≤ 0.05(Group 2,3 vs 1)
Waist/Hip ratio	0.88 (0.08)	0.90 (0.06)	0.91 (0.06)	25.61***	≤ 0.05(Group 2,3 vs 1)
Alcohol habit (% positive)	55.70	27.80	16.50	6.19*	-----
Smoking habit (% positive)	49.10	32.70	18.20	26.30***	-----

Table 4: Comparison of clinico-demographic variables between different GGT sub-groups

Variables	GGT {Median (IQR)}			Chi-square (p)	p value for pair wise group comparison (Dunn's method)
	< 15 (Group 1) n= 183	15-25 (Group 2) n= 372	> 25 (Group 3) n= 174		
Age (years)	24.00 (10.00)	25.00 (10.00)	28.00 (10.25)	9.25***	≤ 0.05 (Group 1 vs 3)
BMI (kg/m ²)	22.41 (3.38)	22.50 (4.46)	23.30 (4.13)	13.23***	≤ 0.05 (Group 1,2 vs 3)
Waist Circumference (cm)	84.00 (12.00)	84.00 (14.00)	89.00 (12.00)	23.57***	≤ 0.05 (Group 1,2 vs 3)
Hip Circumference (cm)	95.00 (9.00)	95.50 (11.00)	97.00 (10.00)	6.75***	≤ 0.05 (Group 1 vs 3)
Waist/Hip ratio	0.88 (0.09)	0.88 (0.06)	0.91 (0.06)	30.35***	≤ 0.05 (Group 1,2 vs 3)
Alcohol habit (% positive)	11.30	28.90	59.80	68.10***	-----
Smoking habit (% positive)	9.10	38.80	52.1	87.01***	-----

Table 5: Comparison of clinico-demographic variables between alcoholic and non-alcoholic

Variables	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Z-value
	Alcoholic n=97		Non-alcoholic n=572		
Age (years)	27.68 ± 6.84	26.00(10.00)	27.66 ± 6.94	25.00(11.00)	-0.074NS
BMI (Kg/m ²)	23.49 ± 2.94	23.50(4.55)	22.84 ± 2.91	22.59(3.99)	-1.904 NS
Waist Circumference (cm.)	86.01 ± 9.35	87.00(16.50)	84.16 ± 8.90	85.00(16.50)	-1.781 NS
Hip Circumference (cm)	96.54 ± 7.35	98.00(11.00)	95.43 ± 7.05	96.00(10.00)	-1.571 NS
Waist/Hip ratio	0.89 ± 0.05	0.90(0.07)	0.88 ± 0.05	0.89(0.07)	-1.547 NS
AST (SGOT) (IU/L)	29.66 ± 8.71	29.00(10.00)	27.19 ± 7.58	26.00(11.00)	-2.556*
ALT (SGPT) (IU/L)	22.78 ± 13.85	19.00(18.00)	19.12 ± 9.90	17.00(12.00)	-1.936 NS
GGT (IU/L)	28.80 ± 11.41	29.40(17.05)	19.91 ± 8.19	17.90(9.48)	-7.590***

Table 6: Comparison of clinico-demographic variables between smokers and nonsmokers

Variables	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Z-value
	Smoker n=165		Non-smoker n=504		
Age (years)	27.72 ± 6.64	27.00 (10)	27.64 ± 6.98	25.00 (11.00)	-0.419NS
BMI (Kg/m ²)	23.51 ± 3.10	23.30 (4.57)	22.74 ± 2.83	22.50 (3.96)	-2.588*
Waist Circumference (cm.)	84.96 ± 9.07	84.00 (14)	84.25 ± 8.95	85.00 (14.00)	-0.673NS
Hip Circumference (cm)	96.43 ± 7.39	94.00 (11)	95.30 ± 6.98	96.00 (10.00)	-1.77 NS
Waist/Hip ratio	0.87 ± 0.04	0.88 (0.07)	0.88 ± 0.05	0.88 (0.07)	-0.867 NS
AST (SGOT) (IU/L)	31.70 ± 7.43	31.00 (8.5)	26.18 ± 7.42	25.00 (10.00)	-8.67***
ALT (SGPT) (IU/L)	23.29 ± 12.61	22.00 (17.5)	18.45 ± 9.61	16.00 (11.00)	-4.416***
GGT (IU/L)	28.35 ± 11.41	27.60 (17.1)	18.84 ± 7.01	17.30 (8.78)	-10.016***

DISCUSSION

The liver associated enzymes, (ALT), (AST), and (GGT) are measures of liver homeostasis¹². The aminotransferases are sensitive indicators of liver cell

injury and GGT as a clinical marker of alcohol abuse are most helpful in recognizing hepatocellular diseases. How the healthy ranges of these liver enzymes are defined is a critically important decision making tool. Elevations in

ALT activity usually reflect the presence of non alcoholic fatty liver disease (NAFLD), if other causes have been excluded¹³. Further variation within the normal ranges of AST, ALT, and GGT in healthy subjects can be associated with clinical and demographic factors. This study was conducted to evaluate the association of liver enzymes with clinical and demographic factors in the western Indian male population. In this study, significant associations between increasing AST, ALT, or GGT tertiles and age were observed. We found that increased AST, ALT and GGT activity were positively related to age for the age range of 21-48 years. (**Table-2, 3, 4**) In similar studies by Piton A *et al*, (1998) it was observed that Serum ALT levels in men steadily rise up to the fifth decade and then decline¹⁴. Lee J K *et al*, 2010 studied the healthy population comprising of 643 men of Korea with biopsy proven histological normal livers. They concluded that age was independently correlated with ALT levels in male¹⁵. While study has also reported increased GGT activities with increasing age¹⁶. Liver enzymes levels can also be modulated by the body weight. In our study, with increasing AST, ALT and GGT enzymes values, the BMI (measure of total body fat) showed significant increase indicate important association of body fat with liver enzymes levels. (**Table-2,3,4**) Our findings are in general agreement with a previous study¹⁰. where univariate analyses showed significant associations between increasing AST, ALT, or GGT tertiles and body mass index for healthy Iranian men population. Other studies also demonstrated that BMI is strongly associated with increased serum activities of liver enzymes, such as ALT, AST, and GGT^{17,18}. In contrast, Mala H *et al* 2000 reported that there was no effect of decrease of BMI on serum ALT and AST activities. These inconsistencies may reflect the differences of subject populations among the studies¹⁹. In addition to BMI, liver enzymes levels also modulated by the pattern of regional body fat distribution⁶. Waist circumference and waist-to-hip ratio can be employed for classifying the pattern of regional fat accumulation¹⁴. The waist circumstance (WC) and WHR are reported to be closely linked to the liver enzymes elevation in general population^{4,5}. In our study significant positive association of AST, ALT and GGT tertiles with WC and WHR was observed while HC showed positive association with ALT and GGT tertiles only. (**Table-2, 3, 4**) Our results correspond to the data of Khedmat H *et al*, 2007 with respect to the association of liver enzymes with WC and HC but we also noted significant increase in WHR with increase in AST, ALT and GGT tertile levels¹⁰. Satish K *et al*, 2013 also observed the positive correlation of ALT with WHR in male²⁰. Liver enzymes activity in male is found to be associated with factors such as alcohol consumption, and smoking. Studies have

indicated a gradual effect of alcohol on GGT enzyme induction, which may be initiated at rather low levels of ethanol intake⁷. Same was observed in our study where serum GGT levels were significantly higher in moderate drinkers compared to non-drinkers. Other than GGT, serum AST showed weak association and ALT had no significant association with alcohol consumption in our study. (**Table-4**) Same was observed for studies from Japan and Korean population^{21,22}. In our study no significant alteration could be observed in BMI, WC, HC and WHR in alcoholic and non-alcoholic groups showing no association of anthropometric parameters with alcohol intake. (**Table-4**) Confirming our observation, in study by Alatalo P *et al*, 2009 the groups of abstainers and moderate drinkers showed essentially similar BMIs (24.3 ± 3.5 and 24.0 ± 2.9 , respectively)⁸. In spite of non-significant relation of body fat distribution with alcohol, overweight when occurring together with alcohol drinking could, however, aggravate the metabolic burden and hepatic enzyme responses^{8,23}. In our study we attempted to measure the influence of moderate smoking on liver enzymes and observed that serum AST, ALT and GGT levels were significantly higher in moderate smokers compared to non-smoker men. (**Table-6**) Some studies showed that current smokers have high serum GGT levels compared to non-smokers²⁴ and people with high GGT levels smoked more²⁵, which was compatible with our results. The effect of smoking on aminotransferase activities was observed controversial. Some investigators claimed ALT was increased by smoking²⁶, while recent studies argued that smoking did not influence AST or ALT, but GGT²⁷.

CONCLUSION

From the present study it can be concluded that body fat distribution pattern, smoking and smoking may be the important variables affecting liver enzymes levels in healthy western Indian male population. Due to the possible effects of these clinic-demographic factors on hepatic the clinical value of liver enzymes measurements in the assessment of NAFLD, excessive ethanol intake and other liver related diseases can be further improved if factors (clinical and demographic factors) affecting these liver enzymes levels in healthy population are efficiently controlled when defining liver enzymes normal ranges.

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