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Gender and Alcohol Consumption Affect Human Serum Enzymes, Protein and Bilirubin

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Abstract: The effects of gender and alcohol consumption on serum enzymes, protein and bilirubin in heavy, moderate and non-drinkers were investigated. Seven-two healthy human subjects were divided equally into males and females. They were categorized as heavy, moderate and non-drinkers, using carefully structured questionnaires. Whole blood was taken via puncture of the cubical vein and serum protein, albumin, total and direct bilirubin concentrations were determined using Synchron CX 5 autoanalyzer. The activities of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase were assayed. The age and body mass index of the males and females were 45.60±1.80 years, 22.16±0.83 kg m^{-2} and 43.87±2.46 years, 20.78±1.03 kg m^{-2}, respectively. Serum protein, albumin and bilirubin levels were significantly different in both male and female heavy and moderate drinkers. The activities of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase in the non-drinkers were significantly lower than in moderate or heavy drinkers of alcohol in both males and females. These findings indicate that alcohol consumption either as chronic or moderate, elevated the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase in both males and females, but more pronounced in the females. Serum protein, albumin and bilirubin levels were impaired by alcohol consumption in both males and females and may provide additional information in the diagnosis and management of alcoholism.

Key words: Alcohol consumption, gender, serum enzymes, protein, albumin, bilirubin

INTRODUCTION

Worldwide adults consume on average 5 L of pure alcohol from beer, wine and spirits per year. The average alcohol consumption is highest in Europe, followed by the America and by Africa. It tends to increase with economic development. However consumption remains low in some regions where the majority of the population is muslim (WHO, 2002, 2004).

Alcohol consumption has been reported to be associated with cardiovascular morbidity and mortality in a dose-dependent manner (Kalousova *et al*., 2004). Alcohol appears capable of inhibiting platelets function, enhancing fibrinolytic activity altering vascular tone and hindering proliferation and migration of arterial smooth muscle cells (Belleville, 2002; Redmond *et al*., 2000). Intake of moderate alcohol has protective effects by decreasing coronary heart disease mortality, while excessive alcohol misuse has detrimental effects on the cardiovascular system, which can result in cardiomyopathy, coronary heart disease or hypertension and haemorrhagic stroke (Sierksma *et al*., 2002; Baer *et al*.,

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2002; Burger *et al*., 2004; Klatsky *et al*., 1990). Alcohol may accelerate oxidative stress directly or indirectly, which may increase cell death modification of biological structures and tissue damage. Free radicals are formed in chain reactions with the contribution of cytochromes, as a result of mitochondrial damage and due to decreased antioxidant defence mechanisms (Sun *et al*., 2001).

There is an increasing interest in the potential effects of alcohol consumption on human hearts worldwide (Oyama *et al*., 2000). For instance, acute or chronic alcohol consumption causes degeneration in different internal organs and systems of adults (Brailowsky and Garcia, 1999). Maternal alcohol consumption affects different organs and systems of the developing fetus (Dencker and Eriksson, 1998). Due to these adverse effects, some functional disorders of these organs occur frequently. For example, alcohol consumption is known to cause diarrhea, other gastro-intestinal symptoms and in advanced states, decreased body weight (Zakhari, 1997; Redmond *et al*., 2000; Belleville, 2002).

It has been reported that high consumption of alcohol results in malnutrition, depending on the possible changes in intestinal absorption mechanisms and dysfunction of some organs, such as, the liver and pancreas (Carey, 2003; Marway *et al*., 1993). In addition, maternal alcohol consumption during gestation is known to cause fetal growth retardation in humans and laboratory animals (Lin, 1991), an effect persisting for a long period affect parturition (Oyama *et al*., 2000). The exact mechanism by which alcohol causes growth retardation is not known, evidences indicate that ethanol interacts with nutrients (Lin, 1991).

Despite the extensive literature on the hepatic effects of ethanol (Dixon *et al*., 2002; Burger *et al*., 2004; Heathcote, 2000), its influence on gender or sex and serum enzymes, bilirubin and proteins in human is not clearly understood. There has been an increase in alcohol consumption in recent years apparently among women, men and adolescents of both sexes and has posed a major public health concern. Alcohol consumption is the leading risk factor for disease burden in low mortality developed countries (Rehm and Eschmann, 2002). Alcohol causes 1.8 million deaths (3.2% of total) and a loss of 58.3 million (4% of total of disability-adjusted life years (WHO, 2002; WHO, 2004). The aim of our study was to determine whether gender and alcohol consumption affect serum enzymes, bilirubin and proteins in humans.

MATERIALS AND METHODS

Study Design

The subjects used in the study were males and females, who were heavy drinkers, moderate drinkers and non-drinkers of alcohol (Table 1). They were categorized as heavy, moderate and non-drinkers after interview and administration of carefully structured questionnaires, containing general questions on age, sex, height, weight, type, quantity, duration of drinking alcohol, attitudes to health issues and alcohol habits. The Body Mass Index (BMI) was estimated as body weight (in kilograms) divided by height squared (in meters). The amount of self-reported alcohol intake per week was estimated from the answers to the following questions in the questionnaires, such as how much beer, wine or spirits do you drink on average during an ordinary week.

The heavy drinkers goes beyond what is considered moderate or socially acceptable. They admitted to a daily intake which ranged from 4-6 pints of beer to 3-5 bottles of whisky or other spirits, for over 20 years. Heavy drinking was defined in terms of exceeding a certain daily volume (e.g., three drinks a day) or quantity per occasion (e.g., five drinks or an occasion at least once a week) or daily drinking. The moderate drinkers were those who drinks alcohol in very small quantities and irregularly, such as once or twice weekly, for over 20 years. The non-drinkers served as controls and have not drank any alcoholic beverages, such as beer, wine, distilled spirit and liquors, containing ethyl alcohol.

Table 1: Characteristics of heavy, moderate and non-drinkers of alcohol

Characteristic	Male	Female
No. of participants	36	36
Heavy drinker	12	12
Moderate drinker	12	12
Non-drinker	12	12
Age of participants (years)	45.10±1.84	43.87±2.46
Body Mass Index (kg m^{-2})	22.75±1.38	20.47±1.69

The subjects consisted of 24 heavy drinkers with history of continuous or periodic heavy alcohol abuse, 24 moderate drinkers and 24 non-drinkers. Each category comprises 12 males and females and the age brackets of the males and females were 45.60±1.84 and 43.87±2.46 years, respectively (Table 1). The nutritional status of the subjects and controls were assessed based on the dietary history, body weight, BMI, serum protein and albumin levels (Wardlaw and Kessel, 2002). None of the subjects suffered from obesity, liver cirrhosis or alcohol hepatitis in the history. All patients were otherwise healthy (no diabetes mellitus, nor alteration of liver and renal function) and had no signs of acute infection. All the subjects and controls were drug free prior to a minimum of two weeks before the study commenced. The study was approved by the Local Institutional Ethical Committee and all subjects gave their informed consents prior to entering this study.

Blood Chemistry and Enzyme Assay

Blood was collected via puncture of the cubital vein between 0830 and 0930 h after an overnight fast and centrifuged at 3000 g, 4°C for 10 min. Serum was extracted and the concentrations of protein, albumin, total and direct bilirubin of the heavy drinkers, moderate drinkers and non-drinkers were determined, using Synchron CX5 autoanalyzer. The activities of serum aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), alkaline phosphatase (EC 3.1.3.1) and gamma-glutamyl transferarse (GGT, EC 2.3.2.2.), were analyzed at 37°C according to the recommended principles (Steffensen *et al*., 1977) and using commercial kits manufactured by Boehringer, Mannheim, Germany and Roche, Switzerland.

Statistical Analysis

The Student's t-test and analysis of variance were used to analyze the data for significant differences (Snedecor and Cochran, 1969). Microsoft Excel XP 2002 software was utilized.

RESULTS

The characteristics of heavy drinkers, moderate drinkers and non-drinkers of alcohol, used for the study are presented in Table 1. The age brackets of the males and females were 45.60±1.84 and 43.87±2.46 years. The body mass index of the males and females were 22.16±0.83 and 20.78±1.03 kg m^{-2}. The BMI of the males was higher than in females.

The concentrations of serum protein, albumin, total and direct bilirubin of heavy drinkers, moderate drinkers and non-drinkers of alcohol are shown in Table 2. There were significant ($p<0.01$) differences in the levels of serum protein, albumin, total bilirubin and direct bilirubin in the male and female heavy, moderate and non-drinkers. The serum protein and albumin levels of the males and females, were significantly higher in the non-drinkers as compared to the moderate or heavy drinkers. The serum protein and albumin levels of the heavy drinkers were significantly lower than in the moderate drinkers. Total and direct bilirubin levels significantly higher in the heavy and moderate drinkers than in non-drinkers, in both males and females (Table 2).

The activities of serum enzymes of heavy, moderate and non-drinkers of alcohol are presented in Table 3. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT),

Table 2: Serum protein, albumin and bilirubin concentrations of heavy drinkers, moderate drinkers and non-drinkers of alcohol*

Alcohol bilirubin status	Protein (g dL^{-1})	Albumin (g dL^{-1})	Total bilirubin (mg dL^{-1})	Direct (mg dL^{-1})
Heavy drinker				
Male	6.34±0.09	3.31±0.02	0.91±0.04	0.33±0.03
Female	5.71±0.04	2.62±0.06	1.03±0.02	0.41±0.05
Moderate drinker				
Male	7.01±0.02	3.92±0.05	0.63±0.08	0.21±0.07
Female	6.62±0.01	3.57±0.01	0.52±0.03	0.15±0.01
Non-drinker				
Male	7.44±0.04	4.52±0.01	0.42±0.01	0.12±0.03
Female	7.25±0.05	4.36±0.08	0.25±0.02	0.10±0.01

*Values are means±SE of three determinations

Table 3: Serum enzymes activities of heavy drinkers, moderate drinkers and non-drinkers of alcohol*

Alcohol status	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	GGT (U L^{-1})
Heavy drinker				
Male	19.4±0.72	17.6±2.08	41.4±5.62	69.1±4.08
Female	33.6±1.18	25.7±1.62	49.7±6.03	84.5±5.12
Moderate drinker				
Male	13.2±0.41	10.50±0.56	36.4±3.07	52.6±5.83
Female	12.1±0.61	8.40±0.72	32.3±4.21	50.4±4.05
Non-drinker				
Male	6.90±0.06	4.60±0.08	5.23±0.06	38.2±1.40
Female	8.10±0.05	5.31±0.02	6.10±0.09	24.1±0.85

*Values are means±S.E. of three determinations (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT)

alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in the non-drinkers were significantly lower than in moderate or heavy drinkers of alcohol, in both males and females.The activities of AST, ALT, ALP and GGT of the heavy drinkers were significantly higher than in moderate drinkers of both males and females. However the variation in the activities of these enzymes were more pronounced in the female heavy or moderate drinkers as compared to the male counterparts (Table 3).

DISCUSSION

The Body Mass Index (BMI) of the heavy, moderate and non-drinkers of alcohol were less than 25, which indicates that they were in no risk for body weight-related health disorders, such as obesity. The BMI corresponded within acceptable range of 18.50-24.90 (Wardlaw and Kessel, 2002). Serum albumin levels of the heavy, moderate and non-drinkers of alcohol were within the recommended limits indicating that they were not malnourished. However, alcohol consumption impaired the levels of serum protein, albumin, total and direct bilirubin in both males and females. The adverse effects of alcohol intake were more pronounced in both total and direct bilirubin levels in both males and females. Our results are in agreement with previous reports, which indicated that acute alcohol caused a significant reduction in the concentrations of plasma total protein and albumin in humans (Marway *et al*., 1993; Whitehead *et al*., 1978; Zakhari, 1997; Sun *et al*., 2001).

Data suggest that alcohol consumption may inhibit protein synthesis, especially in the heavy drinkers. The binding and transport of substances by albumin, such as bilirubin could be affected by heavy or moderate alcohol consumption, especially in females. Bilirubin is transported to the liver by binding non-covalently to albumin (Wardlaw and Kessel, 2002). From the present study, both conjugated (direct) and total bilirubin levels were more reduced than albumin or protein levels in both male and female heavy or moderate drinkers than in non-drinkers.

The activities of AST, ALT, ALP and GGT, which are serum liver derived enzymes in the heavy and moderate drinkers in both males and females, were elevated as compared to the non-drinkers. This finding suggests the possibility of alcohol abuse, which might pose a threat to liver damage and disease. The results concur with previous reports (Patel and O'Gorman, 1975; Steffenson *et al*., 1997; Nishimura *et al*., 1980; Nemesanezky *et al*., 1988; Sun *et al*., 2001). The activities of these serum liver enzymes were more raised in females than in males. This may be due to certain differences in the physiological and endocrine systems in both males and females.

Biochemical diagnostic tests of liver function are numerous, but the activities of AST, ALT and GGT are frequently used in general practice for assessing liver function in health screening and in patients with non-specific symptoms (Nishimura *et al*., 1980; Steffensen *et al*., 1997). Since usage of questionnaires and self-reporting are commonly unreliable laboratory tests of the activities of AST, ALT and GGT, have been proposed as indicators of alcohol abuse. For instance, GGT catalyzes the transfer of gamma glutamyl group from peptides containing it to other peptides and to L-amino acids. It has been reported to be sensitive and fairly specific indicator of liver disease (Patel and O'Gorman, 1975; Nishimura *et al*., 1980).

Excessive alcohol consumption is widely associated with liver damage (Heathcole, 2000; AGA, 2002). Alcohol can cause physical, mental and social effects which are determined by both quantity consumed and pattern of drinking. Alcohol affects practically every organ in the body and its consumption has been linked to more than 60 disease conditions (Ridolfo and Stevenson, 2001; Zakhari, 1997; Ebuehi *et al*., 1997).

Too little is known about the influence of ethanol, whether heavy or moderate, on the synthesis and release of liver enzymes at the cellular level. The toxic effects of ethanol on hepatocytes, pancreatic and aciner cells and so on, are well documented (Nemesanesky *et al*., 1988; Whitehead *et al*., 1978). Some of the present findings in this study are in harmony with previous workers (Patel and O'Gorman, 1975; Steffenson *et al*., 1997; Nishimura *et al*., 1980; Nemesanezky *et al*., 1988; Sun *et al*., 2001), but data on the effects of heavy or moderate alcohol consumption in the females, are relatively new contributions to the existing body of knowledge.

CONCLUSIONS

Data of the present study indicate that serum protein, albumin, total and direct bilirubin levels are impaired by heavy or moderate alcohol consumption. The activities of serum AST, ALT, GGT, ALP are good indicators of alcohol abuse. These effects are gender-dependent. However, the adverse effect of alcohol consumption is more detrimental to the females than in males, therefore, gender or sex factor may be relevant in the diagnosis and management of alcoholism.

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