ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study of Biochemical Markers in Alcoholic Liver Disease: Hospital-Based Case Control Study

M Adak[1], AN Thakur[2], K Adhikari[3]

[1]Department of Biochemistry, National Medical College & Teaching Hospital, Birgunj, Nepal
[2]Department of Pathology, National Medical College & Teaching Hospital, Birgunj, Nepal
[3]Department of Community, National Medical College & Teaching Hospital, Birgunj, Nepal

ABSTRACT

Alcoholism is a chronic, progressive and potential cause of liver disease in the western world and it is most common in Nepal. Numerous data are available regarding alcoholic liver disease (ALD) with biochemical and hematologic indicators but very few works have been made in the Nepalese context. Hence, an effort has been made to evaluate the status of biochemical markers in ALD among the Nepalese subjects**.**166 ALD cases were enrolled in our OPD. Out of total ALD patients, 110 (66.27 %) patients were male and 56 (33.73%) patients were female. ALD patients had significantly low body weight ($p<0.05$) and low BMI ($p<0.05$) compared to control. Hyperbilirubinemia and hypoalbuminemia correlate with alcohol intake. Albumin / globulin ratio significantly decreased in ALD. The elevated levels of AST ($p<0.001$), ALT ($p<0.01$), ALP ($p<0.001$), GGT ($p<0.001$) and AST/ALT ratio > 1 were found with ALD patients respectively. The percentage of hemoglobin and total number of RBC were found to be significantly decreased, whereas mean corpuscular volume (MCV) significantly increased in ALD. The findings of the present study are consistent with previous studies, suggesting that hepatocytes damage causes leak of these enzymes into the circulation. This study concludes that biochemical and hematological parameters is dependable marker of ALD.

Key words: Alcohol liver disease, aminotransferase, γ-glutamyl transferase, alkaline phosphatase.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Alcohol use is rising rapidly in developing regions and is a major concern among indigenous people around the world, showing a higher prevalence of liver disease. However, levels and patterns of alcohol consumption do not fully explain the cause of alcoholic liver disease mortality [1]. The global burden of disease project estimated alcohol to be responsible for 1.5% of all deaths and 3.5% of those who live life with disability [2]. In the USA, 67.3% of the population over 18 years of age drinks alcohol each year [3]. Now a day, it is a common substance abused in Nepal [4, 5]. A group of researchers reported on 2000AD, about 60% of the Nepalese people had experienced alcohol and 41% had taken it during the last 12 months [6]. Alcoholism is a chronic, progressive and is one of the potential causes of liver disease [7]. Although alcoholism is more common in men, women are much more susceptible to the toxic effects of alcohol [8]. Recent evidence has shown that estrogen may increase the susceptibility of the liver to alcohol-related damage, rendering women more vulnerable to its toxic effects [9]. Cirrhosis mortality rates are very low in the younger population, but rise with increasing age. In fact, the rate of cirrhosis among people 75–84 years of age is as high as 31.1 per 100,000 individuals and contribution of cirrhosis to total deaths peaks between 45- 54 years of age, becoming the fourth leading cause of death in the US within this age group [9]. The prevalence of ALD, particularly cirrhosis, varies significantly with socioeconomic status and social class. Numerous studies have shown that individuals who are unemployed, have low income, or have low educational background exhibit higher rates of cirrhosis mortality [10]. Severity of liver damage is often associated with the amount of heavy alcohol consumption with a history of alcohol abuse [11]. However, the magnitude of ALD not only depends on the total amount of alcohol consumed; drinking patterns and type of alcoholic beverage intake [12].

Three major histological changes have been associated with chronic alcohol consumption: alcoholic fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Alcoholic fatty liver is a condition of accumulation of fat in the liver, reversible upon discontinuation of alcohol use. Alcoholic hepatitis is a second major histopathological lesion due to alcohol. Alcoholic cirrhosis is the third major histological pattern of liver injury due to alcohol. It occurs in about 15% of heavy drinkers. Acetaldehyde formed of ethanol oxidation stimulates collagen synthesis. It is an irreversible stage of alcoholic liver damage, and is of the micro nodular type [13]. ALD constitutes a significant number of patients in various countries around the world and presents serious health-related as well as economic problems [14]. The pattern of liver disease varies geographically, among various ethnic groups with different practices and time [15]. Alcoholic liver disease causes elevations of serum aspartate transaminase (AST) and alanine transaminase (ALT) [16]. More than 80% of patients with alcoholic liver disease have De Ritis Ratio (AST: ALT ratio) of 2 or more [17]. This ratio is a valuable diagnostic marker of ALD [18]. Hyperbilirubinemia is frequent in alcoholic liver disease. Tests for alkaline phosphatase, γ-glutamyl transpeptidase (GGT), serum albumin, and prothrombin time, are also indicator tests of altered hepatic activity [19]. Hematologic tests, namely, RBC counts, WBC counts, hemoglobin levels and mean corpuscle volumes are strong indicators of alcoholic liver disease as reported by several researchers [7].

ISSN: 0975-8585

However, numerous data are available regarding alcoholic liver disease with biochemical and hematologic indicators throughout globe but very few numbers of researches have been made about ALD in the Nepalese context. Hence, an effort has been made to evaluate the status of biochemical markers in alcoholic liver disease among the Nepalese subjects.

MATERIALS AND METHODS

This retrospective hospital-based case control study was conducted in the National Medical College and Teaching Hospital (NMCTH), Birgunj, Nepal, in collaboration with the department of central pathology laboratory. All clinically suspected patients attending at our outpatient department (OPD) with common symptomatic disease like fever, diarrhea, skin disease etc. from 16thSeptember 2009 to 15th August 2011 were selected for study. A total number of 166 cases of clinically diagnosed cases of ALD in the age group of 20-60 years with history of prolong alcohol intake. ALD was diagnosed with the help of clinical and biochemical findings [20]. 100 healthy patients and volunteers of medical college staffs who did not have any features suggesting abnormalities related to biochemical, liver or hematological parameters, were enrolled in the study as controls. Patients with diabetes mellitus, autoimmune disease, hemolytic anemia, or infections of the liver, renal problem were excluded from the study. Detailed present and past histories of the patients were collected from preset proforma. The proforma included name, age, sex, dietary habit, dirking habit, smoking habit, family history of disease ,socio-economic status , community and occupation.

Ethical approval was taken from Institutional Research Committee. 5ml of blood was drawn by venipuncture under aseptic precaution in the fasting condition in a plain tube. Serum was separated and biochemical parameters include total bilirubin, conjugated and unconjugated bilirubin, total protein, albumin, albumin: globulin ratio, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) were assayed by using Bio-Kit (Ranbaxy) by semi - automated clinical chemistry analyzer (Microlab 300 , Vital Scientific, USA) . The ratio of AST to ALT was calculated. The hematological measurements included total count of RBCs and WBCs, haemoglobin concentration , Packed Cell Volume (PCV) and mean corpuscular volume (MCV) were measured by an automated cell counter (MEK6410K,Nohon Kohdan ,China).

All results were expressed in Mean ± SEM. One-way analysis of variance (ANOVA) was used to test the significance of difference between all the parameters of controls and patients and p value < 0.05 was taken to indicate a statistically significant. The statistical evaluation was done using the Student's't' test. Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 166 adult subjects were enrolled in the outpatient department (OPD) in National Medical College and Teaching Hospital, Birgunj. Regarding sex out of total patients, 110 (66.27 %) patients were male and 56 (33.73%) patients were female. Age of the patients

ISSN: 0975-8585

was considered from 20-60 year and they were divided into four groups with age of 10 years interval. Maximum frequency of ALD has been found in 31-40 year age of both sex group patients. There were no statistically significant differences in age and sex between patients and controls (Table 1).

Table 1 Sex distribution of patients with ALD (n = 166) and non-ALD controls (n = 150).

Age Group Mean Age		Male			Female		
		Patients (n=110)	Control (n=100)	p-value	Patients (n=56)	Control (n=50)	p-value
21 - 30	24.91±2.72	32 (29.09%)	30 (30%)	> 0.05	19 (33.92%)	20 (40%)	> 0.05
31 – 40	33.55±2.71	44 (40%)	35 (35%)	> 0.05	21 (37.50%)	15 (30%)	> 0.05
41 - 50	44.33±2.51	21 (19.09%)	20 (20%)	> 0.05	11 (19.64%)	12 (24%)	> 0.05
51 - 60	55.00±3.03	13 (11.82%)	15 (15%)	> 0.05	5 (8.94%)	3 (6%)	> 0.05
N = 166		n = 110 (66.27%)			n = 56 (33.73%)		

p> 0.05 = statistically non-significant

Mean age, body weight, height and body mass index were determined of the total patients 166 (male =110 and female = 56) and healthy control (n=100) group (Table 2). Male as well as female ALD patients had significantly low body weight ($p<0.05$) and low BMI ($p<0.05$) compared to control.

Table 2. Demographic profile of patients with ALD and healthy control groups

Parameters	Male, Mean ± SEM		Female, Mean ± SEM	
	ALD (n=110)	Control (n=100)	ALD (n=56)	Control (n=50)
Age (yrs)	38.23 ± 1.23	40.01±0.89	35.23± 1.53	35.89±1.28
Bodyweight (kg)	43.08 ± 1.72*	41.28 ± 1.63	40.89 ± 1.65*	39.28±1.21
Height (cm)	162.23±0.27	162.11 ±0.28	160.22±0.51	160.01 ± 0.55
BMI (kg/m^2)	19.87±0.04*	22.62±0.63	19.03±0.34*	21.08±0.92

Table 3. Enzymatic parameters among ALD and control groups.

Parameters	Male, Mean ± SEM		Female, Mean ± SEM	
	ALD (n=110)	Control (n=100)	ALD (n=56)	Control (n=50)
GGT (IU/L)	327.57±13.27**	24.55±0.41	302.13±13.60**	25.02±0.62
ALP (IU/L)	197.78±8.25**	127.15±1.60	197.61±10.64**	125.11±2.11
AST (IU/L)	151.75±6.00**	32.38±0.68	147.46±3.29**	31.52±1.06
ALT (IU/L)	69.81±2.83	30.56±0.73	65.42±3.75	30.55±1.03
AST: ALT	2.64±1.54**	1.12 ± 0.31	2.46±1.97**	1.11±0.19

p value *<0.05, **<0.001 when compare Alcoholic liver disease (ALD) with healthy non-alcoholic liver disease as controls.

In the present study, the serum enzymatic parameters include AST, ALT, ALP, GGT and AST/ALT levels in the control group and alcoholic patients are given in Table 3. The elevated

ISSN: 0975-8585

levels of AST (p<0.001), ALT (p<0.01) and ALP (p<0.001) were found with ALD patients respectively. One point that is more interesting the increased ALP levels remain same in both male and female patients. The ratio of serum AST/ ALT in the male and female was 2.64±1.54 and 2.46±1.97 as compared to the controls (p<0.001) respectively. All patients with ALD had an AST/ALT ratio > 1, but no one in the group had an AST or ALT level >300 IU/L. GGT levels in the alcoholic patients of both gender was highly elevated as compared to healthy control (p<0.001). Patients with ALD were assessed by non-enzymatic biochemical parameters (Table 4). In the present study, there is significantly increase total bilirubin (<0.05) in male and female alcoholic patients respectively. They further observed that all patients showing elevated level of conjugated and unconjugated bilirubin. On closer look, it was found that conjugated bilirubin (p<0.05) elevated more than unconjugated bilirubin (<0.05) when compared with healthy control group. Total proteins including albumin and A: G ratio were significantly decreased in both gender. It was also found that prothrombin time of male alcoholic patients elevated more than female alcoholic patients and both values were statistically significant(<0.05).

Table 4. Non- enzymatic biochemical parameters among ALD and control groups.

Parameters	Male, Mean ± SEM		Female, Mean ± SEM	
	ALD (n=110)	Control (n=100)	ALD (n=56)	Control (n=50)
Total Bilirubin (mg/dl)	3.08 ± 0.5***	0.67 ± 0.02	2.21 ± 0. 06***	0.56 ± 0.19
Conjugated Bilirubin (mg/dl)	2.56 ± 1.15***	0.56 ± 0.05	1.98 ± 0.02***	0.50 ± 0.12
Unconjugated Bilirubin (mg/dl)	1.58 ± 0.87**	0.36 ± 0.02	1.01 ± 0.05**	0.07 ± 0.21
Total Protein (g/dl)	5.28 ± 1.18***	7.45 ± 0.89	4.59 ± 0.99***	6.87 ± 0.09
Albumin (g/dl)	2.22 ± 0.75**	3.12 ± 0.56	1.82 ± 0.71**	3.32 ± 0.88
Albumin: Globulin (A: G)	0.92 ± 0.26*	1.32 ± 0.14	0.82 ± 0.22*	1.09 ± 0.16
Prothrombin time (s)	25 .02 ± 0.04***	15.08 ± 0.18	24.07 ± 0.07***	14.13 ± 0.04

p value * < 0.05, ** < 0.01, *** < 0.001 when compare ALD with healthy non-alcoholic liver disease as controls.

Table 5 Hematological parameters among ALD and control groups

Parameters	Male, Mean ± SEM		Female, Mean ± SEM	
	ALD (n=110)	Control (n=100)	ALD (n=56)	Control (n=50)
Hb (g /dl)	12.43 ± 0.11*	13.69 ± 1.26	11.18 ± 0.08*	12.32 ± 0.87
RBC x 10^6 (cells/µl)	4477.23 ± 0.06*	5064.71 ± 0.08	4098.46 ± 0.21*	4638.66 ± 0.29
WBC x 10^3 (cells / µl)	4136.91 ± 0.08*	5124.57 ± 0.07	4119.68 ± 0.07*	5083.12 ± 0.01
PCV (%)	40.12 ± 0.03**	44.06 ± 0.21	35.09 ± 0.08**	38.19 ± 0.03
MCV (fl/l)	101.06 ± 1.01*	92.84 ±0.87	96.05 ±0.12*	93.09 ±0.09
ESR (mm/h)	2.75 ±0.01	2.55 ±0.08	2.69 ± 0.03	2.54 ± 0.06
Platelets x 10^3 (cells/ µl)	197099.46±0.09	206238.09±1.16	2078236.23±0.86	216348.39±1.04
Lymphocytes (%)	47.13 ±0.17	48.17 ±0.16	43.01 ±0.18	44.16 ±0.15
Neutrophils (%)	41.12 ±0.24	40.09 ±0.22	39.93 ± 0.18	41.01 ± 0.87
Eosinophil (%)	2.57 ± 0.06	2.59 ±0.10	2.55 ± 0.02	2.54 ±0.05

p value, * < 0.05, **<0.01 when compare ALD with healthy non-alcoholic liver disease as controls.

In the present study (Table 5), the percentage of hemoglobin, total number of RBC and WBC were found to be significantly decreased ($p<0.05$), whereas mean corpuscular volume (MCV, $p<0.05$)) significantly increased in alcoholic liver disease in comparison to control group. Pack cell volume (PCV) was found marginally decreased in both groups. Differential counts, platelet counts and ESR value remain almost same as healthy control.

DISCUSSION

Liver is a versatile organ of the body and it performs different kinds of biochemical function and host defense of the body. Therefore, liver disease is a collection of conditions, diseases, and infections that affect the cells, tissues, structures, or functions of the liver [16]. Chronic heavy alcohol drinking induces liver injury and results in ALD, even irreversible alcoholic liver cirrhosis [21]. ALD diagnosis is currently based on drinking history, related laboratory test [22]. We analyzed 166 ALD patients, of which maximum frequency of ALD has been found in 31-40 year age of both sex. Our study showed that male population (66.27%) is affected with ALD, which is corroborated with a previous study of western Nepal [23]. This may be associated with a variation in the drinking pattern or medical care seeking practice between sexes in these geographical regions. This could be due to the gender-dependent difference in the gastric and hepatic metabolism of alcohol added with hormonal factors along with delayed medical care among female patients.

In the present study showed that ALD patients had significantly low body weight and BMI. Reduced adipose tissue is one cause of lower body weights in such patients. Loss of adipose tissue in chronic alcoholics who continue to drink is probably due to simultaneous inadequate nutritional intake [24, 25]. In recent years, conventional biochemical markers and potential ones have evoked the interest of researchers to study the damages caused by ethanol in the liver [26].

An elevated serum AST in relation to serum ALT has been proposed as an indicator of alcohol induced organ damage [27]. The elevation in ALT was not as high as that of AST in ALD patients, thus reflecting the diminished hepatic activity of these enzymes, which made them to leak into the serum from damaged hepatocytes due to location of ALT in the cytosol [28]. The increase in AST may be due to increased cell membrane permeability, cell necrosis and mitochondrial leakage into the blood, caused by excessive alcohol consumption [29]. Since AST is located in both the cytosol and mitochondria, serum levels depend markedly on the degree of liver damage [30]. It was reported that most patients with high alcohol consumption but without severe liver disease do not have an AST/ALT ratio above one. A high AST/ALT ratio suggests advanced ALD [31]. Some interrelated reasons have been reported for the high AST/ALT ratio in ALD due to (i) decreased hepatic ALT activity [32], (ii) pyridoxal -5'- phosphate depletion in the liver of alcoholics [33] and (iii) mitochondrial damage leading to an increase in the serum activity of mitochondrial aspartate in patients with high alcohol consumption [31]. GGT located in several tissues of the kidney, pancreas and liver and plays a role in the metabolism of glutathione, facilitates amino acid transport. The activity of serum GGT is induced by ALD and cholestasis, not by renal disease. Several researchers have reported a

significant elevation in GGT in patients with ALD, and even in light and moderate drinkers [17]. Studies by Cushman et al.[34] and Poikolainen et al.[35] showed that elevated serum GGT levels in drinkers were related more closely to the biological effects of alcohol consumption rather than to the amount of alcohol consumed. Irie et al.[36] found that GGT synthesis and protein expression were increased in ALD, leading to elevated serum levels of GGT that was commonly noted in patients with the disease. We found in our study, level of GGT elevated significantly due to regular intake of alcohol. Many authors have shown that the determination of ALP is useful for the diagnosis and clinical evaluation of patients with different types of carcinomas [37]. Our results demonstrate that none of our patients with total ALP values lower than 100 IU/L presented ALD without any sign and symptom of cancer.

Patients with various forms of liver disorders showed hyperbilirubinemia. In the present study, there is an increase of total bilirubin, conjugated bilirubin and unconjugated bilirubin level of serum of alcoholic patients in male and female respectively. Uptake and excretory functions of liver is constrained depending upon the increment of bilirubin level of serum. Albumin level was found to be significantly decreased in all the tested groups when compared with normal group and is most common complication of ALD. Hypoalbuminemia was present about 86% patients of the hepatic encephalopathy in a study carried out in Pakistan [38] .Previous study carried out in central Nepal shows that reversal A/G ratio can help in the diagnosis of ALD [23].

Alcohol has a variety of pathologic effects to the bone marrow resulting in vacuolization of the bone marrow precursor cells, anemia, leukemia and thrombocytopenia. It also affects the function of the leukocytes and platelets [39]. It directly damages erythroid precursors, thereby contributing to macrocytosis and the anemic state of chronic alcoholics. Ethanol induces sideroblastic anemia, perhaps by direct interference with heme synthesis. Further, chronic ingestion of alcohol can lead to various types of hemolytic anemia caused by alterations in the erythrocyte membrane lipids that occur in association with alcoholic liver disease [40]. Because the red blood cell survive for 120 days after it has been released into the circulation, an MCV result may remain elevated for up to 3 months after a person has stopped drinking. However, increase in MCV has been reported in other conditions such as thyroid disease, folate deficiency, recent blood loss and a number of hematological conditions, and liver disease from other causes [41]. The values obtained for haematological parameters in alcohol drinkers irrespective of sex showed a significant difference ($p<0.05$) in the haemoglobin concentration , MCV and PCV when compared to those of the control this could be as a result of dehydration as reported by Kristensson et al.[42]. Prolonged PT is associated with increased mortality [43]. Our study showed that mean rise in PT was 6.94 s in both genders, which was supported with published work done by Pakistani researcher [38]. However, no significant variation in either of the groups tested was observed in case of polymorphonuclear cells, lymphocytes, eosinophil and ESR values.

ISSN: 0975-8585

CONCLUSION

The result of this study established that alcohol drinking is associated with a number of changes in cell functions, body weight, body mass index and hematological parameters. Hyperbilirubinemia, Hypoalbuminemia, high erythrocyte, mean corpuscular volume and pack cell volume are common features of alcoholics. Monitoring GGT, ALP, AST and ALT in combination is a sensitive means of detecting severity of alcohol induced liver damage. Internationally the AST/ALT ratio is accepted as dependable marker for diagnosis of ALD. Mostly affecting the productive age group of the male as well as female population. ALD has prime economic burden of the society as well. This provides reliable evidence for the liver injury caused by acute alcohol intoxication. We recommended screening for alcohol abuse in all adult patients presenting to the hospital as early detection of ALD could decrease morbidity and mortality due to ALD.

ACKNOWLEDGMENTS

Our sincere thanks go to Dr. JN Shivapuri, Professor and HOD, Dept. of Biochemistry, Principal, Vice-Principal and Board of Directors of National Medical College for their invaluable support and inspiration. The authors thank to all Central pathology laboratory technicians for their help without which this study could not have been possible.

REFERENCES

[1] Leon DA, McCambridge J. Lancet 2006b; 367: 1900.
[2] Mandayam S, Jamal MM, Morgan TR. Semin Liver Dis 2004; 24: 217-232.
[3] Sofair AN, Barry V, Manos MM, Thomas A, Zaman A, Terrault NA, et al. J Clin Gastroenterol 2010; 44(4): 301-307.
[4] Jhingan HP, Shyangwa P, Sharma A, Prasad KMR, Khandelwal SK. Addiction 2003; 98(3): 339 -343.
[5] Mishra AK, Shrestha P, Bista NR, Bhurtel P, Bhattarai S, Thakali K, et al. J Nepal Health Res Counc 2009; 7(14): 14-18.
[6] Shrestha SM. Kathmandu Univ Med J 2005; 3(2): 178-180.
[7] Ryback SR, Eckardt JM, Felsher B, Raw RR. J Am Med Assoc 1982; 248: 2261-2265.
[8] Sharma A, Khandelwal SK. Addiction 2000; 95(7): 1105-1108.
[9] Mann RE, Smart RG, Govoni R. Alcohol Res Health 2003; 27: 209-219.
[10] Singh GP, Hoyert DL. Hum Biol 2000; 72: 801-820.
[11] Nevins BS, Malaty CL, Velez H, Anand ME. Dig Dis Sci 1999; 44: 1236-1242.
[12] Bellentani S, Saccocio G, Masutti F, Gaicca M, Migilioli L, Monzoni A, Tiribelli C. Addict Biol 2000; 5: 261-268.
[13] Fickert P, Zatloukal K. Pathogenesis of alcoholic liver disease. In: Handbook of Alcoholism. CRC Press.2000, pp317-23.
[14] Mayers WC, Ricciard R. Liver function. Heart Court Publisher. A heart Court Asia PTE Ltd, 2001, pp1010.
[15] Pastor CM, Billiar TR, Losser MR, Payen DM. J Crit Care 1995; 10:183-197.

[16] Adak M, Shivapuri JN. Res J Pharma Biol Chem Sci 2010; 1(4):593-605.
[17] Daeppen JB, Schoenfeld-Smith K, Smith TL, Schuckit MT. J Stud Alcoh 1999; 60(5): 589-594.
[18] Majhi S, Baral N, Lamsal M, Mehta KD. Nepal Med Coll J 2006; 8(1): 40-42.
[19] Eckardt JM, Harford F, Kaelber TC, Parker ES, Rosenthal SE, Rybcak RS, Salmoiraghi GC, Vanderveen E, Warren KR . J Am Med Assoc 1981; 246: 648-666.
[20] You Ming LI, Jian GF, Bing YW, Lun GL, Jun PS, Jun QN, Wei S. J Dig Dis 2011; 12: 45-50.
[21] Reuben A. Curr Opin Gastroenterol 2007; 23: 283-291.
[22] Das SK, Vasudevan DM. Ind J Clin Bioch 2005; 20(1): 35-42.
[23] Pathak OK, Paudel R, Panta OB, Pant HP, Giri RB, Adhikari B. Saud J Gastro 2009; 15(3): 171-175.
[24] World MJ, Ryle PR, Jones D, Shaw GK, Thomson AD. Alcoh Alcoh 1984; 19(4): 281-290.
[25] Hart CL, Morrison DS, Batty GD, Mitchell RJ, Smith GD. Brit Med J 2010; 340: 1-7.
[26] Stickel F, Poeschl G, Schuppan D, Conradt C, Strenge-Hesse A, Fuchs FS, Hofmann WJ, Seitz HK. Eur J Gastroenterol Hepatol 2003; 15: 945-950.
[27] Sorbi D, Boynton J, Lindor KD. Am J Gastroenterol 1999; 94: 1018-1022.
[28] Sharpe PC, McBride R, Archbold GP. Quart J Med 1996; 89: 137-144.
[29] Cohen JA, Kaplan MM. Dig Dis Sci 1979: 24: 835-838.
[30] Assy N, Minuk GY. Am J Gastroenterol 2000; 95(6): 1545-1550.
[31] Nyblom H, Berggren U, Balldin J, Olsson R. Alcoh Alcoh 2004; 39: 336-339.
[32] Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF, Mezey E. Gastroenterol 1984; 86: 632-636.
[33] Nalpas B, VassaultA, LeGuillou A, Ferry N, Lacour B, Berthelot P. Hepatology 1984; 4: 893-896.
[34] Cushman P, Jacob G, Barboriak JJ, Anderson AJ. Alcoh Clin Exp Res 1984; 8: 253-257.
[35] Poikolainen K, Karkkainen P, Pikkarainen J. J Stud Alcoh 1985; 46: 383-387.
[36] Irie M, Suzuki N, Sohda T, Anan A, Iwata K, Takeyama Y, Watanabe H, Fischer P, Scherberich JE, Sakisaka S. Hepatol Res 2007; 37: 966-973.
[37] Moss DW. Prog Clin Biochem Med. 1989; 8: 47-62.
[38] Maqsood S, Saleem A, Iqbal A, Butt JA. J Ayub Med Coll Abbottabad, 2006; 18(4): 58-62.
[39] Chu YC. Alcoh Clin Exp Res 2000; 24: 117-122.
[40] Nordmann R, Rouach H. Gastroenterol Hepatol 1996; 32(3): 128-133.
[41] Whitfield JB, Hensley WJ, Bryden D, Gallagher H. Annals Clin Biochem 1978; 15: 297-303.
[42] Kristensson AAAS, Wallestedt S, Alling C, Cederblad G, Magnusson B. Eur J Clin Invest 2008; 16: 178-183.
[43] Maddrey WC, Boitnott JK, Bedine MS, Weber FL Jr, Mezey E, White RI Jr. Gastroenterol 1978; 75:193-199.