



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/6149
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/6149>



RESEARCH ARTICLE

BIOCHEMICAL AND HEMATOLOGICAL PROFILE OF FATTY LIVER PATIENTS FROM WESTERN NEPAL.

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Manuscript Info

Manuscript History

Received: 23 October 2017
 Final Accepted: 25 November 2017
 Published: December 2017

Key words:-

Fatty Liver Disease, Lipid profile, Liver function test, Ultrasonography, Hepatosteatosi.

Abstract

Background: Fatty liver disease may be the most common liver disease in the world. There are limited studies on biochemical and histological features of fatty liver disease. Different laboratory tests are extremely useful in achieving a better understanding of diseases, and thereby, allow making decision for better management. The examination of different biochemical parameters usually provides excellent clues to the cause of the disease. The rationale of the present study was to demonstrate the changes in biochemical and hematological changes in fatty liver patients compared with control groups.

Methods: About 49 patients with fatty liver and 27 healthy control groups from the outpatient clinics of the Internal Medicine Department, Fishtail Hospital and Research Centre Pokhara, Nepal during the period of April 2017 to September 2017 were enrolled in the study. An overnight fasting, blood samples were taken from the patients to analyze the lipid profile, liver function tests, renal function tests FBS, HbA1c and hematological tests Hemoglobin (Hb), WBC and Platelet counts. The anthropometric parameters: waist and hip circumferences were measured, and body mass index (BMI) and waist/hip ratio (WHR), hypertension were calculated and hepatosteatosi through abdominal ultrasonography.

Results: With the increased level of triglyceride, total cholesterol, LDL-C had a significantly increasing trend ($P < 0.05$); whereas HDL went down with significant differences between two groups. The levels of alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and GGT were noticed significantly difference in the cases as compared to those in the controls ($P < 0.05$). Other biochemical and hematological parameters also noticed significant difference.

Conclusions: Most of the patients of fatty liver disease were asymptomatic. The study revealed that fatty liver patients have dyslipidaemia, abnormal liver function tests, FBS, HbA1c and hematological parameters were significantly different than the control

group and required special attention. It's early detection will help in modifying the disease course and delaying further complications.

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..... **Introduction:-**

The liver plays a central role in lipid metabolism, which stores and exports lipids and lipoproteins [1]. Fat accumulation is one of the most common abnormalities of the liver. Fatty liver is a term applied to a wide spectrum of conditions characterized histologically by triglyceride accumulation within the cytoplasm of hepatocytes. The amount of fatty acid in the liver depends on the balance between the processes of delivery and removal. There are many causes of steatosis, like viral, toxic, genetic, and metabolic causes, or various combinations of these etiologies [2]. Fatty liver disease that develops in the absence of alcohol abuse is recognized increasingly as a major health burden. This condition is characterized by accumulation of fat in the liver exceeding 5-10% by weight, in the presence or absence of substantial alcohol consumption or other causes of liver disease such as viral liver disease [3, 4]. In addition, it is common in obese subjects and nonalcoholic fatty liver disease (NAFLD) has become widespread with the increasing prevalence of obesity [5]. In Nepal, the prevalence of NAFLD has been recently increasing [6], which may be attributable to the lifestyle changes including physical inactivity and an increase in daily fat consumption.

Potential pathophysiologic mechanisms of fatty liver include the following: Decreased mitochondrial fatty acid, beta-oxidation, Increased endogenous fatty acid synthesis or enhanced delivery of fatty acids to the liver and deficient incorporation or export of triglycerides as very low-density lipoprotein (VLDL). No single pathway of cause and effect has been found. Fatty liver disease comprises a wide spectrum of liver damage ranging from simple, uncomplicated steatosis to steatohepatitis to advanced fibrosis and cirrhosis. Liver fibrosis is the common endstage of most chronic liver diseases regardless of etiology and its progression leads to cirrhosis and liver cancer [7]. Estimates based on imaging and autopsy studies suggest that about 20% to 30% of adults in the United States and other Western countries have excess fat accumulation in the liver. This strongly supports the notion that fatty liver disease is the hepatic manifestation of the syndrome. Hence, liver steatosis is of major concern for worldwide health, especially since sedentary lifestyle, modern Western nutrition, genetic predispositions, a variety of pharmacological agents and hepatitis B and C infection have been identified as critical causes of NAFLD [8,9].

The etiology of fatty liver is multifactorial and has been suggested that fatty liver disease can be considered mainly due to two most common conditions are alcoholic liver disease and nonalcoholic fatty liver disease. Alcoholic liver disease is caused by excess alcohol consumption, whereas the nonalcoholic variant is related to be the hepatic consequence of metabolic syndrome or a cluster of metabolic disorders. The principal factor being obesity, in particular central obesity, but also Type 2 diabetes mellitus, dyslipidaemia and hypertension and excessive alcohol consumption. Each of these abnormalities carries a cardiovascular disease risk and together they are often categorized as the insulin resistance syndrome or the metabolic syndrome. [10-13]. The association of fatty liver with metabolic syndrome elements can be considered as secondary to central obesity and to the decrease in insulin sensitivity. Fatty metamorphosis in the liver is common in these obese subjects and nonalcoholic fatty liver disease (NAFLD) has become widespread with the increasing prevalence of obesity [5]. Diagnostic methods for fatty liver are various and include using laboratory tests with imaging methods or liver biopsy [13]. Some tests include: Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Triglyceride (TG), Hepatitis B or C serologies, autoimmune and Wilson's disease [14,15].

The goal of this study is to identify the patients with fatty liver before the stage of advanced fibrosis. The study of fatty liver disease prevalence was based on screening population studies using for diagnosis ultrasound and liver tests. The readily available laboratory tests are extremely useful for achieving a better understanding of the disease, and thereby allow thoughtful management decisions to be made. Examination of the biochemical parameters and hematological profile usually provides excellent clues to the cause of fatty liver even when there is more than one contributing factor.

Material and Methods:-

Patients group comprised of Fatty liver (n=49) were selected from the outpatient clinics of the Internal Medicine Department, Fishtail Hospital and Research Centre Pokhara, Nepal and consented by the Institutional Review Board

of the Hospital to participate in the study. Apparently healthy residents (n=27) of the region having either no health problem or not receiving any therapeutic treatment were used as the normal control group. At the time of sample collection information regarding demography, lifestyle and health/medical history was recorded in the predesigned questionnaire.

People who visited the department of Internal Medicine, Fishtail Hospital and Research Centre from 6th April 2017 to 23rd September 2017 for an abdominal ultrasound examination were initially selected for this study. All the Fatty liver cases were diagnosed by imaging tests, such as ultrasound, computerized tomography scan (CT), blood testing and clinical testing.

The following biochemical parameters were evaluated in the blood serum at the department of laboratory of Fishtail Hospital and Research Centre. Blood sample were collected aseptically. A total of 5.0 ml of venous blood was drawn from each subject. Blood samples were allocated in plane tube for biochemical tests and EDTA tube for hematological test and Glycosylated hemoglobin (HbA1c) test. Clotted bloods allocated in plane tube were then centrifuged at 2000 rpm for 10 minutes in a refrigerated centrifuge to separate serum samples from the cells. The following biochemical parameters were evaluated in the serum sample: Glucose, Urea, Creatinine, Total proteins, albumin, total cholesterol and its fractions, triacylglycerols, bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), C reactive protein (CRP) and aspartate transaminase (AST) to platelet ratio index (APRI) was calculated as $\{(AST\ (IU/l)/upper\ limit\ of\ normal)/platelet\ count\ (x10^9)\} \times 100$. EDTA blood was used to examine the complete blood cell count (CBC) and HbA1c test. CBC test were performed by fully automated five part hematological Beckman Coulter. White blood cells, hemoglobin and platelets counts were noted. HbA1c and CRP test were measured by Nephelometry method using Agappe, MISPA I2 Instruments. Glucose, Urea, Creatinine, Total proteins, albumin, total cholesterol and its fractions, triacylglycerols, bilirubin, AST, ALT, ALP, GGT were measured using standard protocol in a fully automated biochemistry analyzer.

Statistical analysis:-

The statistical analysis was under taken using SPSS version 20.0 software. All values are expressed as mean \pm SD. Student t- test was used to estimate the significant difference between the groups. The level of significance was considered when p value <0.05. was considered as cutoff value for significance.

Result:-

Total of 49 cases, ultrasonographically diagnosed as fatty liver and 27 healthy control groups was included in the study. Comparison of serum biochemical profile's means between fatty-liver and non-fatty liver groups of cases and control groups are shown in **table 3**. Most of the biochemical parameters were higher in fatty liver disease patients compared to healthy group and the variations between them were showing significant (P value <0.05) values. The comparison of anthropometric parameters age, SBP, DBP, BMI, Waist/Hip ratio, prevalence of hypertension, smoking habits are summarized in **table 1 and 2**. The mean age of the patient 47.04 ± 11.32 and control group 40.035 ± 10.44 showed a significant difference (P=0.000). Systolic blood pressure (SBP) was noted statistically significant between fatty liver and control group (133.27 ± 14.49 Vs 124.93 ± 15.43 , P=0.000). However, diastolic blood pressure (DBP) did not show any significant difference (P=0.800). BMI and waist/hip ratio were the risk factor of fatty liver, where mean BMI was noted 28.01 ± 3.82 in fatty liver group and 27.51 ± 4.42 in the control group (P=0.369). In addition, waist/hip ratio in test group 0.99 ± 0.10 as compared to control 0.96 ± 0.09 , P=0.027 showed a significant difference between the two groups. Out of 49 fatty liver patients 75.5% had hypertension and 9 subjects (24.5%) did not have hypertension, Odd ratio (95% CI) 1.40 (0.47-4.20) demonstrate that hypertension have positive correlation with the fatty liver disease. Similarly, we have noted 28.57% patients were smoker and 63.26 % fatty liver patients did not have alcohol drinking habit. In addition, 79.59% test groups were noticed overweight and 65.30% showed high waist/hip ratio (**Table 2**).

The biochemical and hematological parameters of all the cases and control groups are depicted in the **table 3**. Compared to normal subjects, those with fatty liver had significantly (p<0.05) higher levels of glucose (101.14 ± 17.70), HbA1c (6.09 ± 0.64), Urea (30.12 ± 4.07), Cholesterol (210.57 ± 38.45) triglyceride (207.51 ± 58.88) and LDL (122.06 ± 25.56). However, the value of HDL in subjects with fatty liver (47.80 ± 3.82) was significantly lower (p<0.05) than that of normal subjects (Table 3). In addition, liver enzymes were also found statistically significant when compared with control groups. Elevated level of AST, ALT and ALP levels were noticed in cases,

respectively. As it is shown, the mean \pm SD of serum AST, ALT, ALP, (IU/L) were 42.04 ± 10.72 , 45.12 ± 14.11 and 235.49 ± 48.28 were significantly higher in fatty liver patients as compared to control group AST (32.11 ± 10.38), ALT (31.22 ± 12.25) and ALP (220.81 ± 48.45). In addition, AST/ALT ratio 0.96 ± 0.15 and GGT 56.98 ± 37.48 , were also noticed highly significant in fatty liver group compared to control AST/ALT ratio 1.06 ± 0.15 and GGT 31.30 ± 9.16 AST/ALT ratio 0.96 ± 0.15 and GGT 56.98 ± 37.48 . Median APRI values in fatty liver patients was 0.543 ± 0.229 and 0.332 ± 0.154 in control group and was statistically significant ($P=0.00$).

Similarly, serum globulin 3.11 ± 0.26 , $P=0.012$ and direct bilirubin 0.49 ± 0.15 , $P=0.009$ in fatty liver group revealed significant difference with control groups globulin 3.21 ± 0.41 and direct bilirubin 0.44 ± 0.11 . However, the total protein, albumin, total bilirubin, indirect bilirubin were remain similar range between the group and did not show any significant difference.

Fasting blood sugar (FBS) was showing higher level in fatty liver group having 101.14 ± 17.70 and control with 95.30 ± 12.08 , which was noted statically significant ($P=0.025$). In addition, HbA1c was 6.09 ± 0.64 in fatty liver and 5.58 ± 0.58 in control groups and showed significant difference ($P=0.000$). Similarly, in our study there was significant relation noted in urea (36.12 ± 4.07 , Vs 29.83 ± 4.32 , $P=0.044$), uric acid 5.78 ± 1.28 , Vs 4.83 ± 1.12 , $P=0.000$ between fatty liver patients and control groups. On the other hand Creatinine value did not show any significant difference among the two groups $P=0.066$. The hemoglobin (Hb) concentration 13.35 ± 1.97 , $P=0.003$ and platelet count 209138.78 ± 71448.60 $P=0.000$ in fatty liver group were significantly lower than control group Hb 14.22 ± 1.74 and platelet count 264888.49 ± 78570.07 , while there was no significant change seen in the level of WBC count $P=0.082$. (Table 3)

Table 1:- Anthropometric parameters of fatty liver and healthy control persons

	Patient Mean \pm SD	Control Mean \pm SD	P-value
Age	47.04 ± 11.32	40.00 ± 10.44	0.000
Systolic	133.27 ± 14.49	124.93 ± 15.43	0.000
Diastolic	86.24 ± 7.50	86.52 ± 10.91	0.800
Height	161.53 ± 8.77	163.63 ± 7.70	0.100
Weight	73.06 ± 11.12	73.26 ± 9.76	0.901
BMI	28.01 ± 3.82	27.51 ± 4.42	0.369
Waist	43.67 ± 8.74	44.04 ± 7.47	0.772
Hip	44.33 ± 9.34	45.74 ± 5.29	0.295
Waist/Hip ratio	0.99 ± 0.10	0.96 ± 0.09	0.027

Table 2:- Risk analysis of general characteristics based on the presence or absence of fatty liver

Division		Presence of fatty liver		Total	Odd ratio (95% CI)
		Patient	Control		
Smoking	Non-smoking	35 (62.5%)	21 (37.5%)	56	1.40 (0.47-4.20)
	Smoking	14(70.0%)	6 (30.0%)	20	
BMI	Normal	10 (43.5%)	13 (56.5%)	23	0.276 (0.10 -0.77)
	Overweight	39 (73.6%)	14 (16.40%)	53	
Waist/Hip ratio	Normal	17 (63.0%)	10 (37.0%)	27	0.903 (0.34-2.40)
	High	32(65.3%)	17 (34.7%)	49	
Alcohol	Non-drinking	31 (72.1%)	12 (27.9%)	43	0.206 (0.07-0.61)
	Drinking	8 (34.8%)	15 (65.2%)	23	
Blood pressure	Normal	9 (39.1%)	14 (60.9%)	23	4.79 (1.68-13.61)
	High	40 (75.5%)	13 (24.5)	53	

Table 3:- Biochemical parameters of NAFLD and control healthy persons

Parameters	Patient Mean \pm SD	Control Mean \pm SD	P-value
Glucose	101.14 \pm 17.70	95.30 \pm 12.08	0.025
HbA1C	6.09 \pm 0.64	5.58 \pm 0.58	0.000
Urea	30.12 \pm 4.07	28.93 \pm 4.32	0.044
Creatinine	1.02 \pm 0.18	0.97 \pm 0.18	0.066
Cholesterol	210.57 \pm 38.45	195.81 \pm 28.19	0.010
Triglyceride	207.51 \pm 58.88	164.85 \pm 37.51	0.000
HDL	47.80 \pm 3.82	49.63 \pm 4.58	0.002
LDL	122.06 \pm 25.56	103.70 \pm 20.45	0.000
Total protein	7.01 \pm 0.29	7.03 \pm 0.39	0.518
Albumin	3.90 \pm 0.33	3.86 \pm 0.31	0.390
Globulin	3.11 \pm 0.26	3.21 \pm 0.41	0.012
Albumin/ Globulin ratio	1.26 \pm 0.18	1.23 \pm 0.22	0.158
Total bilirubin	0.93 \pm 0.27	0.87 \pm 0.18	0.096
Direct bilirubin	0.49 \pm 0.15	0.44 \pm 0.11	0.009
Indirect bilirubin	0.45 \pm 0.15	0.44 \pm 0.09	0.513
GOT	42.04 \pm 10.72	32.11 \pm 10.38	0.000
GPT	45.12 \pm 14.11	31.22 \pm 12.25	0.000
GOT/GPT ratio	0.96 \pm 0.15	1.06 \pm 0.15	0.000
ALKP	235.49 \pm 48.28	220.81 \pm 48.45	0.039
GGT	56.98 \pm 37.48	31.30 \pm 9.16	0.000
Uric acid	5.78 \pm 1.28	4.83 \pm 1.12	0.000
WBCs	7757.14 \pm 2022.58	7244.44 \pm 2169.43	0.082
Hemoglobin	13.35 \pm 1.97	14.22 \pm 1.74	0.003
Platelets	209138.78 \pm 71448.60	264888.89 \pm 78570.07	0.000
AST/Platelet ratio	0.543 \pm 0.229	0.332 \pm 0.154	0.000

Discussion:-

This is the first study that has been performed on biochemical and hematological changes on fatty liver disease and healthy subjects in the western part of Nepal. In the present study, we have evaluated lipid profile, liver function tests, renal function tests, FBS and HbA1c. In addition, we have measured systolic and diastolic blood pressure, BMI, waist/hip ratio in fatty liver and control groups. The incidence of fatty liver disease is increasing, and NAFLD has become a common chronic liver disease and a critical problem threatening the human health and is one of the global public health problems in the general population [16]. This disease is associated with various metabolic disturbances include type-2 diabetes mellitus, obesity, dyslipidaemia, hypertension, etc. Undiagnosed, this condition may progress silently and result, progressive liver disease, fibrosis and cirrhosis. [17] But there is scanty information about fatty liver and its impact on different biochemical and hematological parameters in the population of Nepal.

The mean age group of those having fatty liver was 47.04 \pm 11.32 and which is higher than the study done by Bajaj et al 40.11 \pm 1.1 [18]. However, our data agree with some previous studies that also reported a similar age range 49.67 \pm 9.30 [19] BMI measurement is helpful for evaluation of fatty liver disease. In our study, we have noticed that 73.6% of the patients were overweight, but BMI between fatty liver and control group did not show any significant difference. However, similar findings were not supported in previous study done by Rocha and Fassio [15,20]. In the present study, the waist/hip ratio 0.99 \pm 0.10 Vs 0.96 \pm 0.09, P=0.027 was significantly higher for fatty liver compared to control group (P<0.05). This result supports the ratio reflects the abdominal fat distribution. However, in accordance with our findings, it has been shown in a previous study that there is a significant correlation between waist/hip ratio and the degree of hepatic steatosis, even in patients with normal BMI [21]. In recent years, BMI, WHR, and abdominal circumference are changed significantly with the elevation of living standard and changes in the lifestyle, and the mean BMI in adults is increasing in both developed and developing countries. Central obesity may contribute to insulin resistance, and increased visceral adiposity might be relevant in the pathogenesis of

NAFLD [22]. The results of our study also agree with some previous studies that also reported deranged lipid profile and liver function tests. Our study revealed that fatty liver patients have a significant increase in total cholesterol, triglyceride and LDL cholesterol, whereas decreased HDL was noticed. An abnormal triglyceride level in fatty liver patients reflects insulin resistance in the liver and muscle.

Fatty liver disease is also associated with worsening lipid profile in individuals with metabolic syndrome, especially, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and high plasma triglyceride.[23]. Our data are also consistent with above mentioned reports. Higher levels of triglycerides, total cholesterol and LDL were observed in fatty liver patients, which may possibly reflect a greater accumulation of fatty acid in the liver, higher insulin resistance and a greater tendency to develop into fatty liver disease [24]. Increased lipid profile among NAFLD subjects had been reported in many studies. Clark et al [25] in the USA in a cross-sectional study found that NAFLD subjects were higher in high triglyceride levels. In another cross-sectional study in Brazil, subjects with NAFLD had a higher triglyceride [26]. However, Lizardi-Cervera et al [27] in Mexico found that the high level of cholesterol was found in 63 percent of the NAFLD subjects. Similar findings were also found by other researchers [28,29].

Similarly abnormal AST, ALT, ALKP and GGT were observed in greater percentages in patients with fatty liver than those without a fatty liver group. AST and ALT increases are closely related to the fatty liver [30]. On the other hand, some of the study reported that higher AST and ALT levels in the blood increases the risk of type-2 diabetes independent of BMI.[31] Higher level of alkaline phosphatase (ALKP) can also be considered as a marker relating to hepatic fibrosis in patients with steatohepatitis. In agreement with this, our study also demonstrates the significant difference in the level of ALKP between fatty liver and control group. In addition, GGT levels in fatty liver patients also noted significant difference then the healthy control. Patients with elevated GGT levels had the greatest likelihood of having fatty liver. We also evaluated the performance of the APRI score in the diagnosis of fibrosis and cirrhosis in fatty liver patients. Evaluation of liver fibrosis in fatty liver disease is of paramount importance. So, in fatty liver patients APRI values suggest the degree of fibrosis and could be useful. APRI values tend to increase with the degree of fibrosis. In our study APRI score of 0.543 which is close to the 0.5 value recommended by the other authors [32]. The lower the APRI score (less than 0.5), the greater the negative predictive value (and ability to rule out cirrhosis) and the higher the value (greater than 1.5) the greater the positive predictive value (and ability to rule in cirrhosis).

Similarly, the serum level of total protein and albumin did not show any significant difference in patients and control group. However, similar findings were not supported in previous studies by Suzuki et al [33] and Kaneda H, et al [34]. These studies reported that albumin and total protein were significantly lower in NAFLD patients. Hyperglycemia is also a significant risk factor for the development of NAFLD. In our study, we have noticed 27.65% people have impaired fasting blood glucose and 72.35% with normal fasting glucose. Fasting blood glucose (FBS) ($P=0.025$) and HbA1c ($P=0.000$) values in fatty liver disease were significantly elevated compared to normal participants.

The WBC count was higher in NAFLD than in control groups, which is a marker of inflammation, [35] and we observed higher levels of WBC count in the fatty liver group but did not reveal a significant difference. Similarly, significant difference was noticed in Hemoglobin (HGB) and platelet count among fatty liver and control group. In our study, hemoglobin level was significantly reduced than the control group, but various literature mentioned that high HGB significantly added to the risk of NAFLD[36]. Similarly, the platelet count is clinically important to predict the status of hepatic fibrosis in patients with chronic hepatic diseases [37]. We have reported significantly lower platelet count in patients, which is consistent with the study done by Suzuki et al [33].

Conclusion:-

Fatty liver disease is an appalling public health problem in the world. Early recognition would help not just by changing the disease course and postponing its complications, however, it will also play an important role to prevent complications as its relationship with metabolic disorder. It is now becoming clear that fatty liver disease is closely associated with marked metabolic derangements. Our study also revealed that there are deranged biochemical parameters in cases of fatty liver disease group. So, there is a need of conducting further studies in relation to this and awaring general population about the various clinical effects and fatality of fatty liver may cause in chronic conditions. An appropriate sample size is required to ensure the validity of the results.

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