Comparison of haematological parameters in patients with non-alcoholic fatty liver disease and alcoholic liver disease

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ABSTRACT

Introduction: Readily available laboratory tests are extremely useful in achieving a better understanding of diseases, and thereby, allow thoughtful management decisions to be made. The examination of peripheral blood smears usually provides excellent clues to the cause of the disease. The main objective of this study was to compare the haematological parameters of patients with non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD), and assess whether these tests have any discriminant value between the two conditions.

Methods: The haematological parameters were investigated in 105 NAFLD patients, 40 ALD patients, 32 alcoholics and 77 normal participants.

Results: The haemoglobin, red blood cell, haematocrit, lymphocyte count and platelet count were significantly reduced, while the mean corpuscular volume, mean corpuscular haemoglobin and prothrombin time expressed as an international normalised ratio (PT/INR) were significantly elevated in ALD patients compared to the other groups. The platelet count was significantly reduced, while the PT/INR and erythrocyte sedimentation rate (ESR) were significantly elevated in alcoholics compared to normal participants. ESR was also significantly elevated in ALD patients compared to normal participants and NAFLD patients. Compared to the control group, the NAFLD patients have significantly higher haematocrit and significantly lower platelet count .

Conclusion: Chronic alcoholism is associated with inflammation and haematotoxic effects, while NAFLD has limited effect on haematological parameters.

Keywords: alcoholic liver disease, fatty liver, haemoglobin, mean corpuscular volume, platelet count

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INTRODUCTION

Alcoholic beverages have been associated with human civilisation since time immemorial, and today, alcohol is ubiquitous, with constantly changing patterns of alcohol intake around the world.[1] Although research has contributed substantially to our understanding of the relation of drinking to specific disorders, the effect of alcohol consumption on health outcome is complex and multidimensional.[1] It is estimated that 3.5% of the global burden of disease is attributable to alcohol, which accounts for as much death and disability as tobacco and hypertension.[2,3] Alcohol is not only causally related to more than 60 medical conditions,[1] but is also linked to categories of disease whose relative impact on the global burden is predicted to increase.[4]

It has also been observed that with modernisation, a sedentary lifestyle and a lack of exercise are associated with an increased prevalence of diabetes mellitus (DM), obesity, hypertension and hypertriglyceridaemia. These are considered to be important causes of non-alcoholic fatty liver disease (NAFLD) and its more aggressive, non-alcoholic steatohepatitis (NASH) form,[4] which histologically resembles alcohol-induced liver damage without a history of significant alcohol consumption.[5] Although liver biopsy is the gold standard for the diagnosis of NAFLD and NASH, ethical considerations as well as the inherent risks associated with this procedure limit its widespread applicability.[4] In contrast, radiologic imaging with ultrasonography (US), computed tomography (CT) or magnetic resonance imaging, used either singly or in combination, have an adequate threshold for the detection of fatty infiltrations of the liver. However, each of these modalities has its own pitfalls.[6] On the other hand,

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 peripheral blood smear usually provides excellent clues to the cause of NAFLD/NASH even when there is more than one contributing factor. However, it is not clear if chronic liver disease affects haematological parameters. Therefore, the present study was undertaken to find out whether haematological parameters can differentiate between alcoholic liver disease (ALD) and NAFLD.

METHODS

Several questionnaires have been developed for the diagnosis of alcohol misuse.[7] The Michigan Alcoholism Screening Test and the CAGE questionnaire have withstood the test of time. In this study, alcoholics, and patients with ALD and NAFLD were selected from among those who had visited the Gastroenterology Department of the Amrita Institute of Medical Sciences, Cochin, India, on the basis of an oral questionnaire (Appendix), laboratory investigations, clinical findings and US/CT imaging or biopsy, where applicable. Patients with elevated liver-specific aminotransferases in serum but no findings on investigation that were suggestive of viral, metabolic or other specific aetiologies of liver diseases were included in this study.[8] However, patients who had undergone gastrointestinal surgery recently, who were consuming drugs known to result in steatosis, such as glucocorticoids, synthetic oestrogens, aspirin, tamoxifen, amiodarone, calcium channel blockers and methotrexate, as well as those who were suffering from malignancy or were pregnant were excluded from the study.[8] Older age is described as an important risk factor for fatty liver disease;[9,10] ageing affects immune function and increases susceptibility to infection in older individuals. Moreover, alcohol metabolism changes with age, and the elderly are thus more sensitive to its toxic effects.[11] Therefore, participants older than 65 years of age were also excluded from this study.

In our study, 32 alcoholic participants (all male) were identified during the course of various health check-up programmes. They were regular consumers of alcohol and all were actively drinking until shortly before presentation. None had any abnormal liver function test (LFT). A total of 40 ALD patients (all male) were identified. These patients had an average daily alcohol consumption of ≥ 80 g for at least five years; at presentation, they had abnormal LFTs, typical imaging (US or CT) and endoscopic findings. The NAFLD group consisted of 105 patients (89 male and 16 female), including eight biopsy-proven NASH patients; all alcohol consumers were exempted from this group. All the NAFLD patients had moderately severe to severe fatty liver on imaging/biopsy. The NAFLD fibrosis score was determined based on six variables, as previously described: $-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ body mass index (kg/m^2) $+ 1.13 \times$ impaired fasting glucose/DM (yes = 1, no = 0) $+ 0.99 \times$ aspartate aminotransferase/alanine aminotransferase ratio $- 0.013 \times$ platelet ($\times 10^9$/l) $- 0.66 \times$ albumin (g/dl).[12] The normal group consisted of 77 participants (45 male and 32 female) who had attended routine health check-up programmes at our institution; no specific problem or abnormality had been detected in their clinical and laboratory findings.

The institutional ethics committee approved the procedures according to the ethics guidelines of the 1975 Declaration of Helsinki, and all the patients provided their written informed consent for the study. Fine chemicals were purchased from Sisco Research Laboratory (Mumbai, India), Sigma Chemical Co. (St Louis, MO, USA) and E Merck (Mumbai, India).

Venous blood was used for the haematological examinations. Red blood cells (RBC) and white blood cells (WBC) were counted using a haemocytometer or Neubauer's counting chamber. Platelets and eosinophils were counted with an RBC pipette and a WBC pipette, respectively. The erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) were both determined by Wintrobe's method. The RBC count, haemoglobin content and PCV were used to obtain certain RBC indices or absolute values of blood index. These indices indicated the size of the RBC and the haemoglobin concentration within the RBCs. The mean corpuscular volume (MCV), i.e. the volume of a single RBC in cubic microns (μm^3), was computed using the following formula: PCV per 100 ml blood/RBC count in million per cumm × 10. The mean corpuscular haemoglobin (MCH), i.e. the average amount of haemoglobin in a single RBC in picogram (10^{-12} g), was computed using the following formula: haemoglobin in g%/RBC count in million per cumm × 10. The mean corpuscular haemoglobin concentration (MCHC), i.e. the amount of haemoglobin expressed as a percentage of the volume of a RBC or the haemoglobin concentration in a single RBC, was calculated using the following formula: haemoglobin in g%/PCV per 100 ml blood × 100.

Different types of leucocytes (granulocytes such as neutrophil, eosinophil and basophil and non-granulocytes such as lymphocytes and monocytes) were determined by staining a blood smear with Leishman's stain. For haemoglobin estimation, 5 ml cyanmeth

Table I. Haematological profile of the study groups.

Parameter	Mean ± SD				F value	p-value
	NAFLD (n = 105)	ALD (n = 40)	Alcoholic (n = 32)	Normal (n = 77)		
WBC	8.1 ± 2.00 (+9.45)	9.1 ± 4.80[c] (+22.7)	7.8 ± 4.00 (+5.4)	7.4 ± 1.70	3.103	< 0.001
RBC counts × 10^6 (cells/cu mm)	5.03 ± 0.57[eg] (+5.89)	3.7 ± 1.00[ad] (−21)	4.6 ± 0.99 (−4)	4.7 ± 0.49	33.114	< 0.001
Hb (g%)	15.0 ± 1.45[beg] (+8)	12.0 ± 2.87[ae] (−13.6)	13.6 ± 3.19 (−2.1)	13.9 ± 1.53	22.356	< 0.001
HCT (%)	43.2 ± 4.49[bdg] (+8)	33.7 ± 8.40[af] (−15.72)	37.5 ± 8.33 (−6.1)	39.9 ± 4.41	27.771	< 0.001
MCV (fl)	85.9 ± 4.83[eg] (+2.15)	91.6 ± 4.60[ad] (+8.84)	82.2 ± 5.30 (−2.28)	84.1 ± 5.23	26.219	< 0.001
MCH (pg)	29.9 ± 2.08[g] (+1.9)	32.3 ± 2.48[ad] (+10)	29.8 ± 2.69 (+1.46)	29.4 ± 2.13	16.316	< 0.001
MCHC (g%)	34.8 ± 1.57[di] (−0.2)	35.6 ± 1.47 (+2.11)	36.3 ± 2.15[a] (+3.89)	34.9 ± 1.27	8.993	< 0.001
RDW (%)	14.6 ± 1.40[fg] (-3.63)	16.3 ± 2.49[c] (+7.66)	15.8 ± 3.01 (+4.56)	15.1 ± 1.77	8.652	< 0.001
MPV	8.6 ± 1.14 (+1.65)	8.9 ± 1.46 (+6.02)	8.4 ± 1.25 (−0.2)	8.5 ± 0.97	2.192	0.109
ESR	16.4 ± 11.60[fg] (+10.8)	25.9 ± 12.20[a] (+75)	23.0 ± 19.90[b] (+55.4)	14.8 ± 6.10	10.233	< 0.001
Neutrophil	54.5 ± 9.60[i] (+0.6)	60.8 ± 15.40[c] (+12.3)	55.6 ± 11.98 (+2.8)	54.1 ± 9.00	3.879	0.004
Eosinophil	3.7 ± 3.28 (−16.6)	4.7 ± 5.23 (+5.33)	3.9 ± 2.33 (−12)	4.5 ± 3.00	1.135	0.336
Basophil	0.7 ± 0.34 (+5.6)	0.9 ± 0.72[c] (+35.2)	0.8 ± 0.48 (+12.6)	0.7 ± 0.38	3.091	0.028
Lymphocyte	34.7 ± 9.25[g] (+0.05)	24.8 ± 11.10[af] (−28.3)	31.3 ± 11.50 (−9.6)	34.7 ± 8.20	11.789	< 0.001
Monocyte	6.8 ± 1.94[g] (+3.3)	9.6 ± 4.15[a] (+44.56)	8.2 ± 3.96[c] (+23.4)	6.6 ± 1.85	13.212	< 0.001
Platelet count	237.9 ± 51.30[bfg] (−10.8)	142.2 ± 73.80[ad] (−46.7)	205.6 ± 66.10[a] (−22.9)	266.8 ± 59.40	40.924	< 0.001
Prothrombin time index ratio	1.0 ± 0.14[eg] (+0.0)	1.6 ± 0.59[ad] (+56.2)	1.2 ± 0.17[b] (+18.1)	1.0 ± 0.10	56.016	< 0.001

Level of significance: [a] < 0.001, [b] < 0.01, [c] < 0.05 compared to normal group; [d] < 0.001, [e] < 0.01, [f] < 0.05 compared to alcoholic group; [g] < 0.001, [h] < 0.01, [i] < 0.05 compared to ALD group.
The figures in parentheses are percent increase (+) or decrease (−) compared to the normal participants.
SD: standard deviation; NAFLD: non-alcoholic fatty liver disease; ALD: alcoholic liver disease; WBC: white blood cells; RBC: red blood cells; Hb: haemoglobin; HCT: haemocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; ESR: erythrocyte sedimentation rate

reagent (0.0016 *M* KCN, 0.0012 *M* $K_3Fe[CN]_6$, 0.0238 *M* $NaHCO_3$) was mixed thoroughly with 20 µl whole blood, and absorbance was read at 540 nm after three minutes. A blank was prepared using only cyanmeth reagent. The haemoglobin concentration was determined using a standard (0, 5, 10, 15 g%) calibration curve.[13]

All the data was analysed using the Statistical Package for the Social Sciences version 11.0 (SPSS Inc, Chicago, IL, USA). The results were expressed as mean ± standard deviation. The sources of variation for multiple comparisons were assessed by the analysis of variance (ANOVA), followed by the post hoc test with Bonferroni's multiple comparisons test. The differences were considered to be significant at $p < 0.05$.

RESULTS

A total of 254 participants were included in this study. The patients were further classified into three subgroups according to the degree of disease severity, i.e. mild (I), moderate (II) and severe (III). The disease severity of the ALD patients was based on the modified Child-Pugh classification method.[14,15] Among the ALD patients, 17 were characterised as mild (I or Grade A), 11 were moderate (II or Grade B) and 12 were severe (III or Grade C) cases. Except for eight biopsy-proven NASH patients (who were considered to be severe cases), the remaining NAFLD patients were classified into two subgroups according to the degree of severity of fibrosis. A fibrosis score of –1.455 to 0.676 was considered mild

Table II. Analysis of the haematological parameters according to the degree of severity of the liver disease.

Parameter	Mean ± SD					
	NAFLD			ALD		
	I (n = 62)	II (n = 35)	III (n = 8)	I (n = 17)	II (n = 11)	III (n = 12)
WBC	8.4 ± 2.2	7.5 ± 1.5	8.1 ± 1.6	9.4 ± 4.4	9.5 ± 6.9	8.2 ± 3.2
RBC counts × 10^6 (cells/cu mm)	5.0 ± 0.6	5.1 ± 0.5	5.1 ± 0.4	4.0 ± 1.0	3.9 ± 0.9	3.2 ± 0.9
Hb (g%)	14.8 ± 1.5	15.3 ± 1.4	15.2 ± 0.8	12.7 ± 2.9	12.8 ± 2.4	10.1 ± 2.4[b]
HCT (%)	42.8 ± 4.9	43.5 ± 4.1	43.9 ± 2.7	36.0 ± 8.8	35.9 ± 7.3	28.3 ± 6.9[b]
MCV (fl)	86.1 ± 5.3	85.3 ± 4.1	86.9 ± 3.8	89.0 ± 3.4	92.8 ± 6.1	94.0 ± 2.8
MCH (pg)	29.9 ± 2.1	30.0 ± 2.1	30.1 ± 1.6	31.6 ± 2.0	33.3 ± 2.6	32.5 ± 2.4
MCHC (g%)	34.6 ± 1.2	35.1 ± 2.0	34.6 ± 1.0	35.5 ± 1.6	35.9 ± 1.4	35.5 ± 1.3
RDW (%)	14.5 ± 1.5	14.8 ± 1.0	13.9 ± 1.3	15.7 ± 1.9	15.9 ± 1.8	17.3 ± 3.4
MPV	8.5 ± 1.1	8.6 ± 1.2	8.6 ± 0.8	8.7 ± 1.6	9.3 ± 1.4	9.1 ± 1.2
ESR	17.9 ± 12.1	13.7 ± 10.9	16.2 ± 9.1	22.4 ± 14.0	27.9 ± 10.3	28.2 ± 10.6
Neutrophil	56.2 ± 9.7	51.0 ± 8.9	55.9 ± 8.0	57.3 ± 14.3	65.7 ± 15.3	61.3 ± 16.7
Eosinophil	3.9 ± 3.9	3.3 ± 1.8	3.9 ± 2.2	6.0 ± 5.4	2.7 ± 3.6	7.0 ± 6.0
Basophil	0.7 ± 0.3	0.8 ± 0.3	0.9 ± 0.5	0.7 ± 0.4	1.3 ± 0.9[a]	1.0 ± 0.7
Lymphocytes	33.2 ± 9.6	37.6 ± 8.6	33.2 ± 6.3	28.1 ± 10.4	23.2 ± 13.1	21.8 ± 9.6
Monocytes	6.8 ± 1.9	7.0 ± 2.0	6.3 ± 1.5	9.9 ± 3.3	7.0 ± 3.1	11.4 ± 5.0[c]
Platelet count	243.7 ± 52.5	226.4 ± 51.3	242.7 ± 34.9	161.7 ± 88.8	137.3 ± 61.5	118.9 ± 56.5
Prothrombin Time Index ratio	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.5 ± 0.6	1.8 ± 0.8	1.7 ± 0.4

Stages of liver disease are based on clinical findings; Stage I: mild; II: moderate; III: severe. Patients on Stage III of NAFLD are also NASH patients.
Level of significance: [a] < 0.01, [b] < 0.05, compared to stage I patients; [c] < 0.01, compared to stage II patients
SD: standard deviation; NAFLD: non-alcoholic fatty liver disease; ALD: alcoholic liver disease; WBC: white blood cells; RBC: red blood cells; Hb: haemoglobin; HCT: haemocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; ESR: erythrocyte sedimentation rate

(I, n = 62) and a score > 0.676 was considered moderate or significant (II, n = 35) NAFLD.[12]

The haemoglobin (Hb) concentration, RBC count, haematocrit (HCT), lymphocyte count and platelet count were significantly lower, while MCV, MCH and prothrombin time expressed as international normalised ratio (PT/INR) were significantly higher in ALD patients compared to the three other groups (Table I). The platelet count was reduced (22.9%) significantly ($p < 0.001$), while PT/INR (18.1%) and ESR (55.4%) were significantly elevated in alcoholics compared to normal participants (Table I). ESR was significantly elevated ($p < 0.001$) in ALD patients compared to normal participants and NAFLD patients (Table I). The HCT level was significantly ($p < 0.01$) higher (8%) and the platelet count was significantly ($p < 0.01$) lower (10.8%) in NAFLD patients compared to the control group (Table I). The Hb concentration and HCT levels showed a significant reduction in the severe stages compared to the milder form of the liver disease (Table II).

DISCUSSION

It has been well established that many haematological and biochemical abnormalities occur in chronic liver diseases.[16] A number of studies have shown that chronic ALD is associated with significant decreases in RBC count,[17] haemoglobin concentration,[17,18] HCT,[17] lymphocytes[17,19] and platelets,[17] while significant increases are seen in MCV,[18,20-22] MCH[20] and red cell distribution width.[17,22] The results of our study also support these observations. The elevated MCHC and reduced platelet counts in our alcoholic participants also concur with an earlier report.[23] Interestingly, although haemoglobin and HCT levels were elevated (8% in each case) significantly ($p < 0.01$) in the NAFLD participants compared to the normal participants, both remained within the normal limits. Some studies have reported that most patients with NAFLD are asymptomatic,[24-28] and are only diagnosed incidentally during the course of assessment of unrelated symptoms or the associated metabolic syndrome.[4]

In the current study, four out of 32 (12.5%) alcoholic participants and 18 out of 40 (45%) ALD patients had anaemia. Alcohol has a variety of pathologic effects on haematopoiesis. It directly damages erythroid precursors, thereby contributing to macrocytosis (enlarged erythrocytes) and the anaemic state. It also interferes with haeme synthesis and induces sideroblastic anaemia.

Furthermore, chronic alcohol ingestion can lead to various types of haemolytic anaemia due to alterations in erythrocyte membrane lipids.[29,30]

The MCV estimates the average erythrocyte volume and serves as an indicator of macrocytosis.[31] The significant elevation ($p < 0.001$) of MCV in ALD patients compared to NAFLD patients seen in this study is in agreement with an earlier study that showed significantly higher MCV levels in alcoholic hepatitis patients compared to NASH patients.[32] Increased MCV has long been used as part of the screening procedure for detecting alcohol abuse.[33-35] Higher MCVs reflect the severity of underlying liver disease.[36] Another study has suggested the strongest correlations between MCV and the amount of recent alcohol intake.[37] MCV responds slowly to abstinence;[38] as an RBC survives for 120 days after it has been released into circulation,[39,40] its normalisation may require 2–4 months.[38] An elevated MCV correlates closely with the duration and extent of drinking episodes; however, it is a relatively insensitive indicator of alcoholism.[41] An increase in MCV has also been reported in thyroid disease, folate deficiency, recent blood loss and a number of other conditions.[39,40] Anti-epileptics and non-alcoholic liver disease may also elevate MCV levels.[19,42]

A prolonged prothrombin time is usually indicative of severely impaired hepatic function in alcoholic patients,[43] as observed in the PT/INR in the present study (Table I), and it usually remains normal in NAFLD.[4] A significant increase in ESR, a marker of inflammation, in our alcoholic participants and ALD patients indicates that chronic alcohol consumption is associated with inflammation.

Under normal conditions, circulating platelets, whose lifespan is about ten days, exist in the resting form with a stable surface membrane structure.[16] During tissue damage or inflammation, the platelets stick to the lesions of the blood vessels and adhere to the exposed endothelial tissues.[16] The HCT is one of the main factors influencing platelet adherence to the vessel wall, and the elevation of the HCT causes an increase in platelet accumulation.[44] Although low platelet count is another common abnormality that may accompany heavy ethanol intake,[31,38,45,46] the platelet count was reduced significantly in all three tested groups compared to the normal group in this study (Table I).

An appropriate sample size is required to ensure the validity of the results. A lower margin of error and higher confidence level requires a larger sample size. As the sample size increases, the power of the study increases. However, populations with small tails or little skewing do not require too large a sample, while populations with very wide tails or those that are very skewed require a much larger sample size. If the sample size is too small, it will not yield valid results. Time and cost are also influencing factors. It is generally believed that a minimum sample size of 30 takes the sample closer to the normal distribution. A sample size of 30 is the threshold between using the Student's *t*-test statistics and the normal distribution.

A major limitation of the study was the small number of patients. The lack of a validated questionnaire was another limitation. Moreover, different procedures were adopted to further classify NAFLD and ALD patients according to the degree of severity. In conclusion, our data primarily suggests that chronic alcoholism is associated with inflammation and has toxic effects on the production of haematologic precursor cells and on red cell morphology, particularly in ALD, while NAFLD has limited effect on haematological parameters. Therefore, haematological examination might be useful for the identification of alcoholics or ALD patients, but not for NAFLD patients. However, repeated studies with larger sample sizes are required in order to come to a final conclusion.

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Appendix

Questionnaire for patients attending hospital

Do you drink alcohol?	☐ Yes	☐ No
If NO, did you ever drink?	☐ Yes	☐ No
How old were you when you started? (Age in yrs)	_____	
How often do/did you drink? (Circle)	Daily/ 2–3 times a week/ Once a week/ Weekend only/ Once a month/ Less	
What do you drink?	Beer/ Spirit/ Sherry/ Wine/ Cider	
How much do you drink?	_____	
Were you used to drinking more than this regularly?	☐ Yes	☐ No
Have you ever deliberately cut down on your drinking?	☐ Yes	☐ No