"A STUDY OF CHANGES IN HEMATOLOGICAL PARAMETERS IN ALCOHOLIC LIVER DISEASE"

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In partial fulfilment of the requirements for the award of the degree of

DOCTOR OF MEDICINE IN PATHOLOGY

Under the Guidance of

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I hereby declare that this dissertation entitled "**A STUDY OF CHANGES IN HEMATOLOGICAL PARAMETERS IN ALCOHOLIC LIVER DISEASE**" is a bonafide and genuine research work carried out by me under the guidance of DR. PRAKASH M. PATIL, Associate Professor, Department of Pathology, BLDE (Deemed to be University) Shri B. M. Patil Medical College, Hospital & RC, Vijayapura, Karnataka.

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…. To my parents

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LIST OF ABBREVATIONS USED

EDTA---------- Ethylene Diamine Tetra Acetate.

SD----------Standard Deviation

WHO----------World Health Organization

RBC----------Red Blood Cell

Hb----------Hemoglobin

HCT----------Hematocrit

PCV----------Packed Cell Volume

MCV----------Mean Corpuscular Volume

MCH----------Mean Corpuscular Hemoglobin

MCHC----------Mean Corpuscular Hemoglobin Concentration

WBC----------White Blood Cell

PT----------Prothrombin time

aPTT----------Activated partial Thromboplastin time

ABSTRACT

Background:

Alcohol is considered to be one the most commonly consumed drug substances which causes injury to the heath by altering the body metabolism. Alcohol consumption affects various systems which includes hepatobiliary system, gastrointestinal system, nervous system, cardiovascular system and genitourinary system.

Liver is one of the primarily affected organs due to alcohol consumption leading to alcoholic liver disease. Alcoholic liver disease may include alcoholic fatty liver, alcoholic hepatitis to the severe form of alcoholic liver cirrhosis. The changes in the hematological parameters is an important indicator of the severity of the disease process.

Aim:

To assess the changes in hematological parameters in patients with alcoholic liver disease.

Materials and Methods:

The study was done on a total of 70 patients clinically diagnosed as Alcoholic liver disease admitted in the Department of Medicine. The values of Hemoglobin (Hb) levels, Red blood cell (RBC) count, Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Total count, Differential count and

Platelet count were analysed using the fully automated hematology analyser (Sysmex XN1000- Sysmex Corporation, Kobe, Japan).

The values of coagulation profile- Prothrombin time (PT) and Activated partial thromboplastin time (aPTT) were also analysed using the coagulometer (ACL elite Pro).

Results:

Sample size of 70 patients clinically diagnosed as Alcoholic liver disease were evaluated for hematological changes and coagulation profile. The mean age was 44 years with maximum patients in the age group of 50-59 years which included 95.8% of males and 4.2% of females. The values of Hb, RBC count, PCV and Total count were found to be reduced in maximum patients. MCV values showed no significant change. Thrombocytopenia was a major significant finding in 68.57% of ALD patients with a mean value of 1.37 lakh/µl and median value of 1.02 lakh/µl. 31.43% patients showed neutrophilia, while 61.4% patients had reduced number of lymphocyte count. Significant derangement of coagulation profile was found with prolongation of PT in 71.43% patients and aPTT in 75.71% patients.

Conclusion:

Our study demonstrates that patients with Alcoholic liver disease due to alcohol abuse can manifest with hematological abnormalities due to direct or indirect toxic effects of alcohol on haematopoiesis. Therefore, laboratory investigations like complete blood count and coagulation profile are important in assessment of the severity of the disease, other complications and proper management of such cases.

Keywords: Alcoholic liver disease, Hematological changes, Chronic alcoholism, Alcohol abuse.

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INTRODUCTION

Alcoholism is one of the most commonly encountered problems in day to-day practice which not only affects the health but also hampers the socio-economic condition of the individual. Alcohol consumption affects multiple systems in the body which includes hepatobiliary system, gastrointestinal system, nervous system, cardiovascular system and genitourinary system.

"Chronic and excessive ingestion of alcohol is one of the major causes of liver disease in the industrialized nations. The spectrum of pathology of alcoholic liver injury ranges from fatty liver to alcoholic hepatitis and ultimately to cirrhosis. Fatty liver is present in over 90% of binge and chronic drinkers but 10 to 20% of alcoholics develop alcoholic hepatitis".[1] Various risk factors implicated in alcoholic liver disease include quantity and duration of alcohol intake, type of alcohol consumption and drinking pattern, gender (females susceptibility twice compared to males), co-existing HCV infection, gene polymorphism, nutritional factors such as malnutrition/obesity, smoking, iron over load and exposure to other hepatotoxins.[1]

Alcoholics may show variety of pathologic effects on haematopoiesis which can be due to direct or indirect toxic effects of alcohol. It directly damages erythroid precursors, thereby leading to alteration in the structure of RBCs leading to abnormaly enlarged erythrocytes contributing to macrocytosis and the anaemic state (moderate to severe anemia).[2] Other hematological changes can include mildly reduced numbers of leukocytes, neutrophils and moderately to severely reduced number of platelets. Although this generalized reduction in blood cell numbers (i.e., pancytopenia) is reversible with abstinence to alcohol and is usually neither

progressive nor fatal, complex aberrations of haematopoiesis can develop over time that may cause death. [7]

"Chronic alcoholism is associated with inflammatory changes and has toxic effects on the production of haematologic precursor cells and on red cell morphology, particularly in Alcoholic liver disease, unlike NAFLD which has limited effect on hematological parameters. Therefore, hematological examination might be useful for the identification of alcoholics or ALD patients". [2]

Alcohol consumption is known to cause liver injury in the form of alcoholic liver disease but it cannot be generalized for every alcohol consumer. It purely depends on the amount and duration of alcohol consumption. The deleterious effect on liver is actually unrelated to the type of beverage. Continuous or steady alcohol intake is more dangerous than intermittent consumption. Sometimes even after consumption of the "threshold dose", many do not develop the disease. Surprisingly, nearly 50% of individuals who ingest large amounts of ethanol (more than 80 grams per day) are spared of serious injury. It is seen that drinking of alcohol in a quantity more than 160 grams per day for a period of minimum 8 years leads to the development of alcoholic cirrhosis.

Diagnosis of ALD can be made based on history, clinical evaluation and laboratory findings. Also, distinguishing alcoholic from non-alcoholic liver disease is equally important as their treatment & management differ. Most of the times it is challenging as there is no single specific diagnostic test to detect ALD, since both the physical findings and laboratory evidence may be non-specific. Therefore, the clinician has to rely on other methods of indirect evidence of alcohol abuse, such as

questionnaires, information from family members and group of laboratory tests to strengthen or confirm a clinical suspicion of ALD. [1]

Fatty infiltrations of the liver can though be detected by certain other investigations like radiologic imaging with ultrasonography (USG), computed tomography (CT) or magnetic resonance imaging, used either singly or in combinations, however each of these modalities have an adequate threshold for the detection and has its own pitfalls. On the other hand, few readily available laboratory tests are extremely useful in acquiring a better understanding of the disease, and thereby allow thoughtful management decisions to be taken.[2]

Hence, this study is done to assess the various changes in hematological parameters associated with liver disease developed due to alcohol consumption which will not only help to identify the severity of the disease but also prevent progression of the disease process; considering alcoholism a serious health hazard worldwide.

AIM AND OBJECTIVE

To assess the changes in hematological parameters in patients with Alcoholic liver disease.

This in turn will identify the severity of the disease and prevent progression of the disease process reducing the morbidity and mortality.

REVIEW OF LITERATURE

Alcohol is amongst the commonest beverages consumed in the world. "Alcoholic beverages have been associated with human civilisation since time immemorial, and today, alcohol abuse is ubiquitous, with constantly changing patterns of alcohol intake around the world."[2] Alcoholism constitutes not only one of the major health issues but also a major socioeconomic issue worldwide.[1] The hazardous and harmful use of alcohol is a major global factor contributing to disease and injury and might lead to death: through health impacts, such as alcohol dependence, liver cirrhosis, cancers and other injuries.[3] Ingestion of alcohol in an excess amount for a longer duration is harmful and poses a great threat to human health.

"Alcohol consumption and the problems related to alcohol abuse vary widely around the world, but the burden of disease and death remains significant in most countries. Alcohol consumption is the third largest risk factor in the world for disease and disability; greatest risk in middle-income countries. Alcohol is a causal factor in 60 types of diseases and injuries and a component cause in 200 others. Yet, despite all these problems, the harmful use of alcohol remains a low priority in public policy, including health policy. Many other lesser health risks have higher priority".[3]

Chronic alcoholism is one of the major causes of liver injury, leading to a spectrum of liver disease known as Alcoholic liver disease (ALD). This entity comprises of three major lesions, with a progressive injury rarely existing in a pure form: (1) fatty liver (2) alcoholic hepatitis and (3) alcoholic cirrhosis. "Fatty liver is present in >90% of daily as well as binge drinkers. Only a smaller percentage of

heavy drinkers progress to alcoholic hepatitis, thought to be a precursor to cirrhosis. The prognosis of severe alcoholic liver disease is dismal; the mortality of patients with alcoholic hepatitis concurrent with cirrhosis is nearly 60% at around 4 years. Although alcohol is considered to be a direct hepatotoxin, only between 10 and 20% of alcoholics seem to develop alcoholic hepatitis. The explanation for this apparent paradox is unclear but involves the complex interaction of facilitating factors, such as drinking patterns, diet, obesity and gender. There is no specific diagnostic tool that can predict individual susceptibility to alcoholic liver disease".[4]

GLOBAL CONSIDERATION:

Alcohol is the third largest risk factor for disease burden in the world. The harmful use of alcohol results in 2.5 million deaths each year. Most of the mortality attributed to alcohol is secondary to alcoholic liver cirrhosis. Mortality from cirrhosis is declining in most Western countries, concurrent with a reduction in alcohol consumption, with exceptions in the United Kingdom, Russia, Romania and Hungary. This increase in alcoholic liver disease and its complications is closely correlated with increased volume of alcohol consumed per capita population and is regardless of gender.[4]

Worldwide recorded per capita consumption has remained stable at around 4.3–4.7 litres of pure alcohol since 1990, including relative stability in all WHO regions. Though, there was a slight decrease in the use of alcohol at the beginning of the 1990s in European Region, but again increased to around the same level of 9.5 litres. The initial decline in the 1990s in the region of the Americas stabilized in the new millennium at about 6.7 litres. An increase towards the end of the last century in the Western Pacific Region was seen, but then stabilized at around 4.7 litres.[3]

EPIDEMIOLOGY:

One of the major medical complications of alcohol abuse is Alcoholic liver disease (ALD). Alcohol is known to be a cause of its serious manifestation, liver cirrhosis. Alcohol accounts for 80% of all liver cirrhosis cases seen in district general hospitals in the UK. Alcoholic liver cirrhosis is increasingly seen in countries such as India and Japan which traditionally had a low prevalence of the disease.[8] The safe limits for alcohol intake are still controversial. Guidelines recommended by the Royal College of Physicians advise a weekly limit of 21 units (210 g) of alcohol in men and 14 units (140 g) in women. The 1994 'Office of Population Censuses and Surveys' General Household Survey found that 27% of men and 13% of women in the UK were exceeding these limits. A recent prospective Italian study in 6534 subjects showed the risk threshold for developing ALD is 30 g ethanol/day and this risk increases with increasing daily intake.[9] In men, 40–80 g/d of ethanol produces fatty liver; 160 g/d for 10–20 years causes hepatitis or cirrhosis. Alcoholic liver disease is found to develop in only 15% of alcoholics.[4]

Women exhibit increased susceptibility to alcoholic liver disease at amounts >20 g/day; in the range of 3–8 drinks daily assuming that 2 drinks per day is probably safe. A previous study in 13285 Danish subjects found that ALD was increased above a threshold of 7–13 drinks per week in females and 14–27 drinks in males.[10] There was a steep dose dependent increase in ALD risk above this threshold with increasing alcohol intake. Women are seen to have greater susceptibility to ALD at any given level of intake. The pattern of drinking was also important as ALD is increased in those who drink without accompanying food and also in those who drink multiple different alcoholic beverages.[8, 9] It is well known that food delays gastric emptying and intestinal absorption of alcohol and thus intake of food before drinking will

decrease the rise of blood alcohol concentrations. The absorption of alcohol is lower when consuming low concentration beverages such as beer compared with high concentration spirits.[8]

ETIOLOGY AND RISK FACTORS:

There are many risk factors involved in the development of alcoholic liver disease. The important ones include quantity and duration of alcohol intake. It is well known that the amount of alcohol consumption is correlated with evolution of liver cirrhosis. Although, the role of type of beverages i.e. wine, beer, or spirits and pattern of drinking (daily versus binge drinking) is less clearly understood, still these are thought to play a role in the disease development. The stage of the disease and progression beyond fatty liver seems to acquire additional risk to liver injury. Consumption of alcohol for a longer duration, 10–20 years causes hepatitis or cirrhosis. Hepatitis C (HCV) infection concurrent with alcoholic liver disease is associated with younger age for severity, more advanced histology, and decreased survival.[4]

Gender is a strong determinant for alcoholic liver disease. Women are at higher risk to develop similar degrees of liver disease than men and develop advanced liver disease with consuming significantly less alcohol. It is useful in estimating alcohol consumption to understand that one beer, four ounces of wine, or one ounce of 80% spirits all contain ~12 g of alcohol. Gender-dependent differences in susceptibility to liver injury may result from poorly understood effects of oestrogen, proportion of body fat and the gastric metabolism of alcohol. The pharmacokinetic mechanisms include differences in ethanol absorption or alternatively differences in the response

of the liver to alcohol induced injury such as that caused by oxidative by-products of ethanol metabolism in the liver.[8]

Chronic infection with hepatitis C virus (HCV) is an important comorbidity in the progression of alcoholic liver disease to cirrhosis in chronic and excessive drinkers. Even moderate alcohol intake of 20–50 g/d increases the risk of cirrhosis and hepatocellular cancer in HCV-infected individuals. Patients with both alcoholic liver injury and HCV infection develop decompensated liver disease at a younger age and have poorer overall survival.

Genetic factors in ALD is thought to play a role though incompletely defined. Studies of ethnic differences and twin studies also indicate that genetic factors affect the risk of ALD. The risk of ALD is 3-fold higher in monozygotic twins than control pairs of adults, independent of concordance for alcoholism. These findings indicate that there is a genetic predisposition to organ-specific complications of alcoholism. An analysis of a large cohort of drinkers with and without cirrhosis showed that per day and total lifetime alcohol consumption were significantly higher among drinkers without liver disease, suggesting that there is an existence of individual vulnerability factor. Mortality from ALD among men was highest in Hispanic whites (12.6 of 100,000) followed by non-Hispanic African Americans (7.4), non-Hispanic whites (5.2) and Hispanic African Americans (1.8). In the United Kingdom, mortality from ALD in black and Asian men is 3.8-fold greater than in whites.

The variations in the prevalence of genetic variants, such as the Patatin-like phospholipase domain-containing protein 3 (PNPLA3/Adiponutrin) on chromosome 22 is thought to contribute to this inter ethnic variation. Few gene studies have uniformly supported a role for PNPLA3 as a modifier of ALD.[11] Several other studies

have also confirmed that this variant predisposes towards all the stages of liver damage initiating from steatosis to steatohepatitis and progressive fibrosis and is also linked to increased risk of ALD.

Polymorphisms of genes involved in alcohol metabolism:

Alcohol is degraded into acetaldehyde in liver and other tissues which is further converted by aldehyde dehydrogenases (ALDH) to acetate which, after release from the liver, is metabolized by heart and skeletal muscle tissue. Different classes of ADH isoenzymes are known and all ADHs are dimeric zinc-containing enzymes classified according to their metabolic properties and sequence similarities. Class I ADH comprises isoenzymes with a, b and g subunits coded by corresponding gene loci termed ADH1, ADH2 and ADH3. Among the human ADH gene loci, two class I ADH genes are polymorphic with three alleles existing for either ADH2 or ADH3 which substantially reveal different enzymatic characteristics. ADH2 alleles are ADH2*1 and ADH2*2 detectable in Caucasians and Asians respectively and encodes for low activity b1 and high activity b2 subunits, respectively resulting in dimeric isoenzymes (b1b1 and b2b2). Similarly, ADH3 alleles- ADH3*1 and ADH3*2 produce g1 and g2 subunits, resulting in g1g1 and g2g2 isoenzymes. The g1g1 isoenzyme is twice as active as the g2g2 isoenzyme. A recently described ADH3*3 allele has not yet been enzymatically characterized. According to the differences in the capacity to metabolize alcohol to acetaldehyde, it has been speculated that individuals with the more active alleles, i.e, ADH2*2 and ADH3*1 alleles are at increased risk of developing alcohol-related organ damage because of higher acetaldehyde exposure.[13]

Fatty liver caused by alcohol injury does not necessarily require malnutrition, but obesity and non-alcoholic fatty liver are risk factors. The present conceptual understanding is that alcohol acts as a direct hepatotoxin and malnutrition does not play a major role.

Table 1: Risk factors for Alcoholic Liver Disease[4].

Risk Factor	Comment
Quantity	In men, 40–80 g/d of ethanol produces fatty liver; 160 g/d for 10–20 years causes hepatitis or cirrhosis. Only 15% of alcoholics develop alcoholic liver disease.
Gender	Women exhibit increased susceptibility to alcoholic liver disease at amounts >20 g/d; two drinks per day is probably safe.
Hepatitis C	HCV infection concurrent with alcoholic liver disease is associated with younger age for severity, more advanced histology, decreased survival.
Genetics	Gene polymorphisms may include alcohol dehydrogenase, cytochrome P4502E1, and those associated with alcoholism (twin studies).
Malnutrition	Alcohol injury does not require malnutrition, but obesity and fatty liver from the effect of carbohydrate on the transcriptional control of lipid synthesis and transport may be factors. Patients should receive vigorous attention to nutritional support.

SPECTRUM OF LIVER INJURY:

The three widely recognised forms of ALD are-

I) alcoholic fatty liver (steatosis),

II) acute alcoholic hepatitis and

III) alcoholic cirrhosis.

At least 80% of heavy drinkers develop steatosis, 10%–35% develop alcoholic hepatitis, and approximately 10% progress to cirrhosis.

PATHOGENESIS:

Not all individuals who abuse alcohol develop alcoholic hepatitis or cirrhosis. The pathogenesis of ethanol can differ in different individuals due to differences in human alcohol metabolism and differences in gender, genetic and environmental factors, diet and microbiome.[12]

Upon ingestion of ethanol, it comes in contact with the gastric mucosa. Here, it undergoes its first-pass metabolism and gets converted into acetaldehyde in the liver and other tissues by alcohol dehydrogenase (ADH) in the cytosol and cytochrome P4502E1 in microsomes. When ingested with a meal, ethanol's gastric absorption rates have been reported to fluctuate from 30% to 100% among healthy individuals, with a small amount transitioning to the distal small intestine. Metabolism of ethanol in the small and large intestine takes place by the microbiome. Along the way, the rapid transit of ethanol makes it bioavailable through the portal system, passing through the gut wall mucosa. The body's ability to metabolize it changes according to the duration and amount of ethanol ingestion, resulting in progressive hepatotoxicity through the production of acetaldehyde in hepatocytes, gastric mucosal cells and by intestinal bacteria.

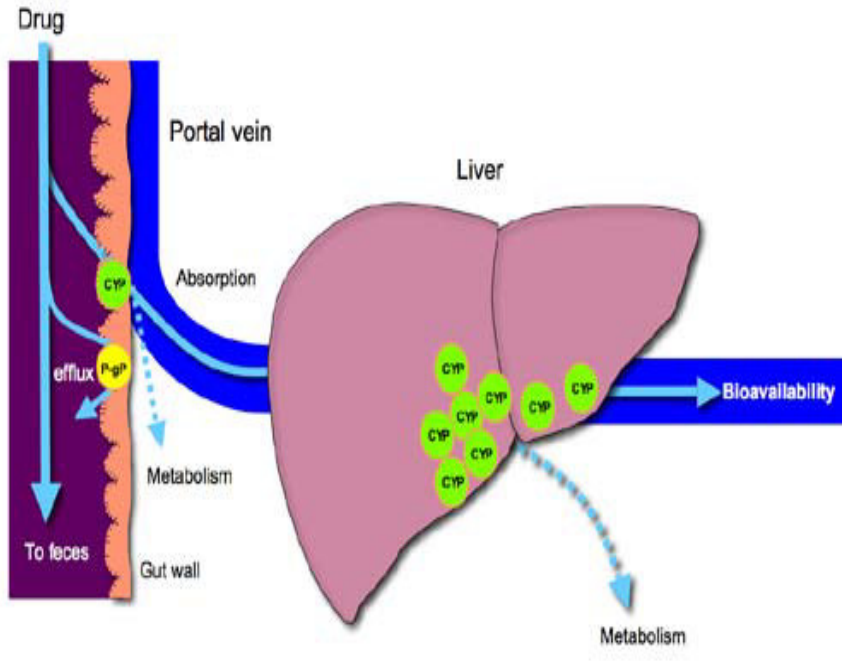

Figure 1: Bioavailability of ethanol.

The pathogenesis of ALD has some role in this interaction between the microbiome and the host's liver where alcohol has been shown to both change the composition of the microbiome and impair intestinal integrity and barrier function. The microbiome changes appear to cause leakage of lipopolysaccharide from gram-negative bacteria into the portal circulation, which further activates Kupffer cells that are responsible for initiating and sustaining subsequent inflammatory responses. The Kupffer cell activation is supposed to be the core of ALD. Intestinal derived bacterial endotoxin (lipopolysaccharide-LPS) activates the kupffer cells in the liver through CD14/Toll-like receptor 4 (TLR4) complex and tumor necrosis factor α (TNF-α), thereby increasing porto-systemic uptake of endotoxins by gut derived bacteria after excessive alcohol intake.[12] The cell injury and endotoxin release initiated by ethanol and its metabolites also activate innate and adaptive immunity pathways releasing proinflammatory cytokines (e.g., TNF-α), chemokines and proliferation of T and B

cells. The production of toxic protein-aldehyde adducts like the powerful mitogen platelet-derived growth factor (PDGF) and the most important profibrogenic cytokine transforming growth factor b1 (TGFb1), leads to transformation of quiescent hepatic stellate cells into an activated myofibroblast (MFB)-like phenotype leading to liver fibrosis. Hence, this battery of cytokine-chemokine release, generation of reducing equivalents and oxidative stress contribute to necro-inflammation of hepatocytes leading to hepatocyte injury and impaired regeneration.[13] Hence, chronic alcohol abuse determines the architectural derangement of the liver and associated pathophysiology.

CLINICAL FEATURES:

The clinical manifestations of alcoholic fatty liver are subtle and differentiating it from non-alcoholic fatty liver is difficult until and unless an accurate drinking history is ascertained. It is often an incidental finding. Previously unsuspected hepatomegaly is often the only clinical finding. Occasionally, patients might present with upper quadrant discomfort, nausea and rarely jaundice. An accurate and descriptive drinking history which includes a standard and validated questionnaire is very important in every patient suspected to have liver disease. Many patients are often asymptomatic while others may present with fever, spider nevi, jaundice and abdominal pain simulating an acute abdomen. Portal hypertension, ascites or variceal bleeding can also occur even in the absence of cirrhosis. Recognition of the disease in an early stage prompt towards initiation of an effective and appropriate diagnostic and therapeutic strategy.

Table 2: Clinical features of patients with Alcoholic Cirrhosis

SYSTEM OR ORGAN INVOLVED	CLINICAL MANIFESTATIONS
Hepatic	Jaundice, ascites, varices, fetor hepaticus
Digestive	Anorexia, vomiting, abdominal pain
Endocrine	Gynecomastia spider nevi, testicular atrophy
Hematologic	Coagulopathy, anemia, leukopenia, thrombocytopenia
Renal and electrolytes	Azotemia, alkalosis-acidosis, hypokalemia, hyponatremia
Circulatory	Cyanosis, clubbing, hyperkinetic circulation
Neurologic	Encephalopathy-coma (and various other neuropsychiatric sequelae of alcoholism)
Systemic	Wasting, fever

HISTOPATHOLOGY OF ALD:

ALD has three categories of histopathological changes: steatosis, steatohepatitis, and steatofibrosis or cirrhosis. These changes often invariably begin and are usually most prominent in the centrilobular region of the hepatic lobule. The centrilobular hepatocytes are enzymatically characterized to metabolize alcohol through cytosolic alcohol dehydrogenase and the induction of cytochrome p450 enzymes. Such

pathways are responsible for production of fatty acids which accumulate in the steatotic hepatocytes and create the toxic metabolites causing inflammation, hepatocyte injury and fibrosis.

ALCOHOLIC FATTY LIVER (STEATOSIS)-

Fatty liver is the initial and most common response to hepatotoxic stimuli, including excessive alcohol ingestion. It is common if consumption exceeds 80 g of alcohol per day. Liver function is often normal. A single large triglyceride occlusion in the affected hepatocytes is noted in a large proportion. The accumulation of fat within the perivenular hepatocytes coincides with the location of alcohol dehydrogenase, the major enzyme responsible for alcohol metabolism. Fat accumulation increases to throughout the entire hepatic lobule with continuation of alcohol consumption.[8] So, hepatocellular steatosis progresses from small-droplet fat (microvesicular fat) to large-droplet fat accumulation imparting almost a mixed pattern.[14] Most of the hepatocyte cytoplasm is occupied by lipid vacuoles, pushing the nucleus and other organelles to the periphery of the cell. These lipid vacuoles are composed of triglycerides, fatty acids, monoglycerides and diglycerides. The presence of intracellular fat acts as a trigger for lipid peroxidation and generation of its byproducts such as malondialdehyde. The importance of lipid peroxidation as one of several pathogenetic factors involved in the process of steatohepatitis has been recognized recently, particularly in studies of individuals with non-alcoholic fatty liver disease(NAFLD).[15]

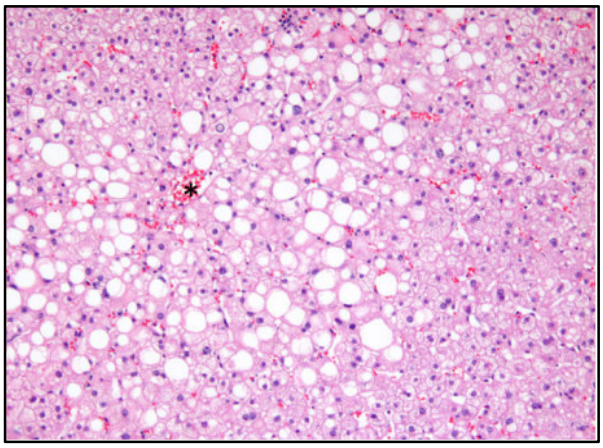

Figure 2: Alcohol-related steatosis- composed of fat accumalation, prominent near the central vein (asterisk) and extends towards the acinus zone 2 or the midzone of the hepatic lobule.

An uncommon form of ALD steatosis is alcoholic foamy degeneration. The pathological findings comprises of microvesicular steatosis in virtually all hepatocytes, similar to that seen in diseases with mitochondrial dysfunction, such as Reye syndrome and fatty liver of pregnancy. It usually occurs without any histological evidence of steatohepatitis or steatofibrosis despite chronic alcohol ingestion. Megamitochondria may be associated with steatosis, with or without other changes of ALD, further indicating the potential for alcohol to cause mitochondrial defects.[14, 15]

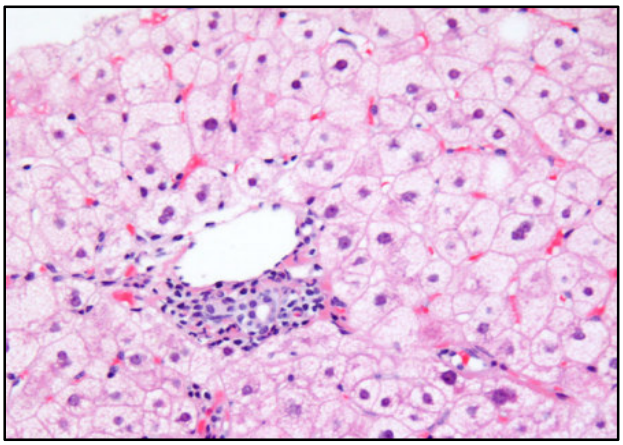

Figure 3: Acute alcoholic foamy degeneration- showing diffuse microvesicular steatosis.

Despite extensive fatty change and distortion of the hepatocytes with macrovesicular fat, the cessation of drinking results in normalization of hepatic architecture and fat content. Thus, it is reversible with abstinence but may progress to cirrhosis with persistence of excessive alcohol intake.[8]

ACUTE ALCOHOLIC HEPATITIS-

Alcoholic steatohepatitis (ASH) or alcoholic hepatitis includes all forms of hepatocyte injury and degeneration, which may or may not be associated with inflammation. It typically shows a picture of hepatocyte ballooning, cell swelling, rarefaction of the cytoplasm and disruption of the cytoskeleton, often with the formation of Mallory-Denk bodies.[14] This occurs due to increased intracellular water accumulation. The hallmark of alcoholic hepatitis is hepatocyte injury characterized by ballooning degeneration, spotty necrosis, polymorphonuclear infiltrate and fibrosis in the perivenular and perisinusoidal space of Disse. Mallory-Denk bodies or

Mallory's hyaline bodies are seen in the hepatocytes which are perinuclear eosinophilic inclusion bodies and are probably condensed and disorganised fragments of the cytoskeletal framework of the hepatocyte. They have been considered a ''sequestosome'', a product of cellular stress, whose constituents include not only polyphosphorylated intermediate filament-type cytokeratins, but also p62 (a phosphotyrosinein dependent ligand in the family of cytoplasmic kinases) and ubiquitin (cellular chaperone for proteins fated for degradation). Immunohistochemical staining for ubiquitin is thereby useful in corroborating the presence of Mallory bodies and differentiating it from other conditions like Wilson's disease and primary biliary cirrhosis, hence are not specific for alcoholic hepatitis.[8, 15] Inflammation in ASH is variable and ranges from scant/mild and is typically neutrophil-rich, predominantly infiltrating the portal tracts and hepatic parenchyma giving a ''satellitosis'' appearance in and around the hepatocytes containing Mallory bodies. They can either be focal around ballooned hepatocytes or courses diffusely through the lobule. Sometimes, lymphocytes may also be seen or can be the predominant inflammatory cells in few cases. Activated Kupffer cells, some of which contain lipid vacuoles, are also in the background inflammatory infiltrate of steatohepatitis, although they are relatively inapparent on routine microscopy.[14, 15]

It has been estimated that excessive drinking for a longer duration of about 15–20 years is necessary to develop alcoholic hepatitis. It is unrelated to pattern of drinking or type of beverage. It is more severe in females and also in Northern Europeans. It is potentially reversible with cessation of alcohol intake but in a few cases, patients can deteoriate after hospital admission despite abstinence and may even lead to death. The probable reason could be reperfusion liver injury.

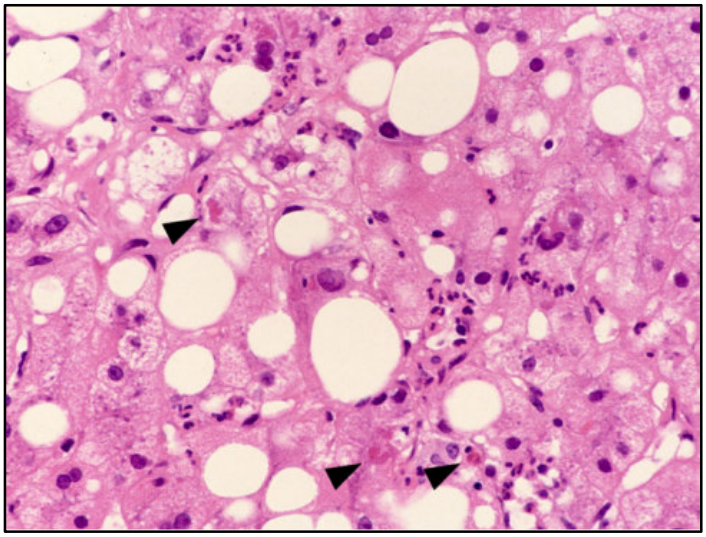

Figure 4: ALD with steatosis and steatohepatitis. The hallmark feature hepatocyte ballooning with Mallory-Denk bodies (arrows) and a diffuse inflammatory infiltrate rich in neutrophils can be seen.

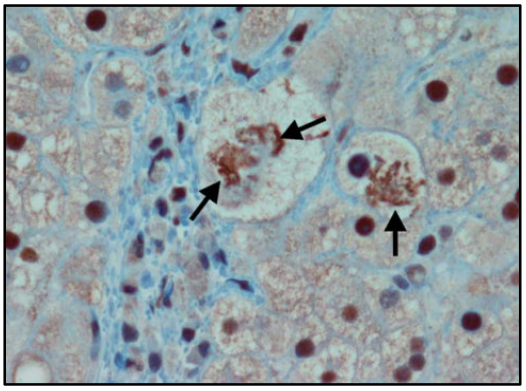

Figure 5: Ubiquitin seen as brown reaction product (arrows) within Mallory body material present in hepatocytes. Specific immunoperoxidase for ubiquitin.

ALCOHOLIC CIRRHOSIS-

The risk of cirrhosis is increased in continuous drinkers compared to binge drinkers.(8)It is the most severe form of alcoholic liver injury. The process of steatofibrosis begins with perivenular fibrosis of ASH which has been cited as an important predictor of progression to cirrhosis unless alcohol exposure is abated. Morphologic progression proceeds by two major routes: one characterized by fibrosis, which bridges between central veins and the second, by fibrosis linking central veins to portal tracts. The accumulation primarily takes place in the space of Disse followed by extension of this pericellular or perisinusoidal fibrosis outwards, giving a picture of classic "chicken-wire fence" pattern, sometimes all the way to the portal tract. Deposition of collagen around central veins can be termed as central hyaline sclerosis. Eventually, collagen bridges developing between the central veins and portal tracts tend to isolate groups of hepatocytes which form the regeneration nodules.[14, 15] The trichrome stain is useful in demonstrating the linkage of central veins to portal tracts. This progression of pericellular scarring to the end-stage liver disease of typical micronodular type Laennec's cirrhosis is complex. "Repeated cycles of hepatocellular regeneration within the fibrous webs lead to expansile hepatocyte nodules compressing adjacent hepatocytes and leading to their atrophy and the condensation of the fibrous scars into dense septa".[14] With subsequent scarring of these newer hepatocytic nodules, the net result is the subdivision of the liver parenchyma into units which are similar to the size of lobules or smaller by fibrosis, referred to as the future ''micronodular cirrhosis''. These cycles of injury and regeneration occur parallel to parenchymal extinction resulting from double-hit vascular injuries (to central veins and to one or both of the portal vessels.[16]

Cholestasis, not unusual but a major feature of simple perivenular steatohepatitis, often a prominent finding because of interrupted bile flow by fibrosis. “Broad areas of replacement fibrosis may obliterate contiguous lobules, and are frequently evident macroscopically in explanted livers or at postmortem examination. Evolution to macronodules or a macronodular cirrhosis may occur with prolonged abstinence”.[15]

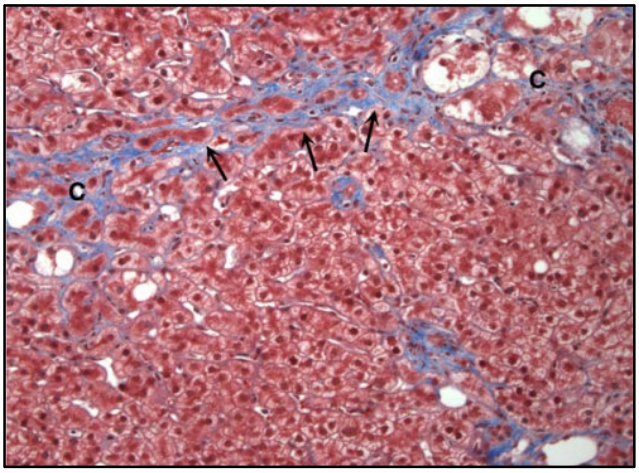

Figure 6: Central-to-central bridging fibrosis in progressive steatohepatitis. A bridge of fibrosis (arrows) links two centrilobular regions (C). [Masson trichrome stain]

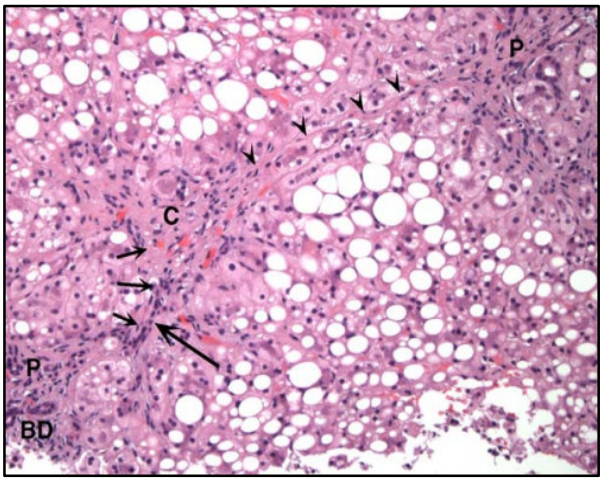

Figure 7: Central-to-portal bridging fibrosis. A centrilobular region is being linked to two portal tracts (P) by bridging fibrosis (arrows and arrowheads). In the portal tract at the lower left there is a native bile duct (BD) as well as a reactive structure (long arrow) migrating along the fibrous septum toward the centrilobular region (C). [Hematoxylin and eosin stain]

Cirrhosis is present in up to 50% of patients with biopsy-proven alcoholic hepatitis and its regression is quite uncertain, even with abstention. Survival for patients is 60%–70% at one year and 35%– 50% at five years.[8] Hepatocellular carcinoma (HCC) may also develop in some 5% to 15% of patients with ALD, often in the setting of macronodular cirrhosis.[17] In patients of ALD with cirrhosis, HCC is the most common fatal complication and has the second highest cancer incidence rate after kidney tumors.[18] Concomitant risk factors like chronic hepatitis C and iron overload may contribute to the risk of HCC.

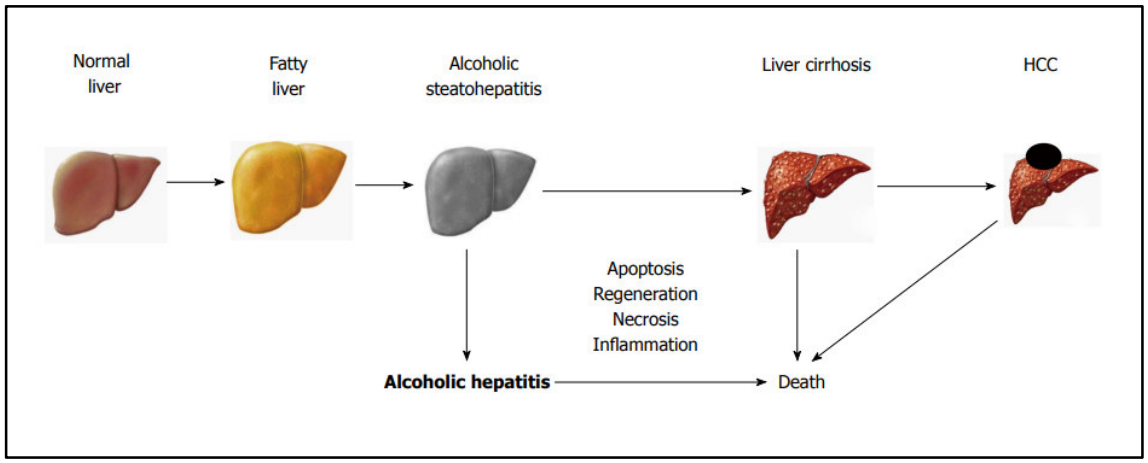

Figure 8: Course of Alcoholic liver disease with major end points.

OTHER CONDITIONS MIMICKING ALD:

Several conditions that may closely resemble ALD are listed below:[17]

1) Non alcoholic fatty liver disease (NAFLD)

2) Acute alcoholic cholestasis

3) Alcoholic chronic active hepatitis

4) Hepatocellular carcinoma (HCC)

5) Fetal alcohol syndrome

Distinguishing ALD from NAFLD:

The two major changes seen on biopsy which can be undoubtedly used to distinguish ALD from NAFLD could be:

i) Marked cholestasis- usually indicative of acute decompensation (hepatocyte keratin 7 immunostaining becomes particularly prominent) and

ii) Sclerosing hyaline necrosis- wherein the central vein is essentially obliterated.

Other changes may be present but differ only in degree. Though there is substantial overlapping in the findings between individual patients in both the disease categories, clinical history can be of utmost help in differentiating the two. Steatohepatitis may be far more prominent in ALD than NAFLD whereas, in populations of affected patients of NAFLD- neutrophilic infiltrates may be more widespread, Mallory-Denk bodies may be thicker and coarser and mega-mitochondria may be more plentiful. However, in the absence of a clinical history to indicate the nature of the patient's disease, the histology is not purely determinative. On the other hand, pathologists and clinicians should also keep in mind that few patients with NAFLD may also be drinking and should not be contented with a diagnosis of metabolic syndrome as the sole etiology. Also, labelling a patient as alcoholic in a written part of the medical record can serve to have a potential social, psychological and economic impact. Hence, it is probably wise for the diagnosing pathologist to personally contact the clinician so as to convey a heightened suspicion of alcohol use.[14]

Table 3: Morphologic differential diagnosis of Alcoholic liver injury.[17]

Serial no.	**Examples of diseases with similar Pattern of alcoholic injury**	**Morphology**
1.	Macrovesicular steatosis	Obesity, diabetes mellitus, corticosteroid therapy
2.	Microvesicular steatosis	Tetracycline or salicylate toxicity, acute fatty liver of pregnancy
3.	Acute intrahepatic cholestasis	Large duct obstruction (e.g., acute pancreatitis, choledocholithiasis), drug-induced cholestasis, Zieve's syndrome
4.	Alcoholic hepatitis	Pseudoalcoholic hepatitis (non-alcoholic steatohepatitis) associated with diabetes mellitus and/or obesity, jejuno-ileal bypass or drug injury (e.g., perhexilene maleate, amiodarone, nifedipine, diltiazem, 4,4-diethylaminoethoxyhexestrol, diethylstilbestrol)
5.	Alcoholic chronic active hepatitis (CAH)	Hypervitaminosis A, methotrexate therapy, diabetes mellitus
6.	Fibrosis (zone 3)	Non-A, non-B CAH, autoimmune CAH, drug induced CAH
7.	Fibrosis (zone 3) with venocclusive	Venocclusive disease secondary to pyrrolizidine alkaloids, therapeutic drugs (e.g., dacarbazine) or

		radiation injury
8.	Cirrhosis	Cirrhosis complicating conditions causing pseudoalcoholic hepatitis (non-alcoholic steatonecrosis)
9.	Cirrhosis with iron overload	Genetic hemochromatosis

Changes with abstinence of alcohol abuse:[14]

With cessation of drinking, steatosis tends to rapidly disappear, supposedly within weeks and is followed by subsidence of the inflammatory changes. However, Mallory-Denk bodies may persist for longer, say for upto 6 months. Eventually, some of the fibrotic changes can also regress. The early lesions of pericellular fibrosis may disappear altogether with the cessation of alcohol intake. Remodelling of the later stages of scarring and nodularity was also seen on comparison of end-stage cirrhotic livers from active drinkers and livers resected at transplant after more than 6 months. The nodules initially become densely compacted, then gets fragmented and coalesce with adjacent nodules, forming a macronodular pattern which keeps increasing as the fibrous scars regress. Whether alcoholic cirrhosis can regress completely still remains questionable.

LABORATORY FINDINGS:

Medically diagnosed alcoholic liver disease can be differentiated from non-alcoholic liver diseases using various laboratory tests which has important implications for treatment and management.[20] No single laboratory test definitely establishes alcohol consumption, therefore, a panel of routine tests are necessary to establish the diagnosis. The diagnosis of ALD is however complicated by a rather varied clinical presentation, underreporting by patients and the lack of good biomarkers for alcohol consumption. Thus, it is routinely underestimated by physicians.

The typical laboratory abnormalities seen in fatty liver are non-specific and include modest elevations of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGTP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) can indicate an early ALD and is often accompanied by hypertriglyceridemia and hyperbilirubinemia. Hyperbilirubinemia is accompanied by modest increase in the alkaline phosphatase level. In alcoholic hepatitis and in contrast to other causes of fatty liver, AST and ALT are usually elevated two- to-sevenfold. Advanced ALD is heralded by hypoalbuminemia, features suggestive of coagulopathy like prolonged prothombin time (>5 seconds) or thrombocytopenia and abnormal renal function tests.[19]

Heavy drinking induces changes in biological parameters such as γ- glutamyl transferase (GGT).[20] GGT is usually higher in ALD patients compared with those who have other liver diseases.[19] Though, GGT activity is not specific for alcohol intoxication and can also be seen in few other conditions like cholestastic liver

disease, cardiac insufficiency, drugs and many more. Furthermore, serum GGT loses its specificity for alcohol in more advanced stages because its activity is elevated in patients with extensive fibrosis regardless of the cause.[18, 19] Elevation of AST may be observed in all forms of ALD with a sensitivity of 50% and a specificity of around 80%. AST levels are rarely above 300 IU/ml, while serum alanine aminotransferase (ALT) levels are commonly lower.[19] An elevated serum AST in relation to serum ALT has been proposed to be an indicator of alcohol induced organ damage. The AST/ALT ratio is usually >1 in cases of alcoholic liver disease,[21, 22] although this finding is neither specific nor sensitive and it has also been shown to be an indirect marker of advanced fibrosis. AST/ALT ratio of >1.5 is considered as highly suggestive that alcohol is the cause of the patient's liver injury.[23]

Table 4: Laboratory diagnosis of Alcoholic fatty liver and Alcoholic hepatitis.[4]

Test	Comment
AST	Increased two- to sevenfold, <400 U/L, greater than ALT
ALT	Increased two- to sevenfold, <400 U/L
AST/ALT	Usually >1
GGTP	Not specific to alcohol, easily inducible, elevated in all forms of fatty liver
Bilirubin	May be markedly increased in alcoholic hepatitis despite modest elevation in alkaline phosphatase
PMN	If >5500/μL, predicts severe alcoholic hepatitis when discriminant function >32

Note: AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGTP, gamma-glutamyl transpeptidase; PMN, polymorphonuclear cells.

Apart from these hepatic enzymes, few cardiac markers like lactate dehydrogenase (LDH) and creatine kinase (CK) and serum cholesterol levels can also

sometimes show a significant change in alcoholics. The LDH and CK are slightly elevated in alcoholic patients compared to non-alcoholics which signifies destruction of the cardiac cells, equally pointing to the possibility of cardiac dysfunction. Increase in serum cholesterol levels is indicative of the possibility of atherosclerotic disorder due to alcohol induced lipolysis and liponeogenesis depending on the energy state of the body.[24]

HEMATOLOGICAL ABNORMALITIES IN ALD:

Alcoholism is associated with abnormalities of the red cells, white cells and platelets. Reduction in (white blood cell (WBC), red cell count (RBC), haemoglobin (Hb), haematocrit and platelet counts have been observed, while the mean corpuscular volume (MCV) appears to be increased compared to that normally seen in healthy individuals. The blood film report can also show increased macrocytic, microcytic, hypochromic and polychromic red blood cells among the alcoholics.[25] Red cells abnormalities can be due to defects in folate and iron metabolism, marrow erythroid disorders, including the ring sideroblasts seen in alcoholic patients or hemolytic anemias associated with stomatocytosis, acanthocytosis and Zieve's syndrome. [26]

- **Red cell changes:** "The evolution of anemia in the alcoholic patient proceeds through predictable stages:

1) Negative vitamin balance- a stage which begins when the dietary intake of the patient is reduced and significant amounts of alcohol is ingested. This period is characterized by a sharp fall in the serum folic acid level, with no abnormality in erythropoiesis;

2) Megaloblastic conversion- which may occur as early as one week after initiating alcohol intake and a poor diet;
3) Sideroblastic conversion- which may occur soon after the megaloblastic change and appears to be related either to pyridoxine deficiency or an intracellular enzyme defect;
4) Early resolution- a recovery stage in which rapid disappearance of megaloblastic change takes place with persistence of ring sideroblasts making marrow interpretation difficult;
5) Late resolution- in which marrow erythroid hyperplasia and reticulocytosis simulate a hemolytic state.

Differences in timing of the medical evaluation rather than differences in etiology may yield any one of the five red cell patterns in a given patient".[27]

- **White blood cell (WBC) changes:** The bone marrow depression with decrease in marrow granulocyte reserve (MGR) may be seen in chronic alcoholic patients even if cirrhosis is not a factor.[28] The reduction in total WBC count substantiates that alcohol abusers are at high risk to develop various diseases, including infections, or cancer, though the exact mechanism how alcohol is related to cancer is not yet well established.[29] So in the later stages where alcoholism is superimposed with infections, patients may even present with leucocytosis rather than leucopenia.
- **Platelet changes:** Moderate to severely reduced numbers of platelets leading to thrombocytopenia is a common finding in severe alcoholics. This can also be explained by the bone marrow suppression caused by alcohol abuse or cytotoxic effects of ethanol. Two factors thought to probably contribute to alcohol-induced thrombocytopenia are- first, reduction in platelet survival by more than 50% and

second, reduction in effective platelet production despite a normal megakaryocyte mass.

Thus, alcohol use especially in heavy drinkers can cause various metabolic derangements necessitating the importance of blood investigations like complete blood count (CBC). The main causes leading to changes of CBC are: myelosuppression that is accompanying with slight reduction in all blood cells, blood loss from gastrointestinal tract, malnutrition etc. Chronic excessive alcohol ingestion reduces the number of blood cell precursors in the bone marrow and causes characteristic structural abnormalities in these cells, resulting in fewer than-normal counts or alter their function resulting in non-functional mature blood cells.[29]

Folate or vitamin B12 deficiency in alcoholics:

Folate deficiency observed in severe alcoholics can be due to dietary deprivation or malabsorption of folate, liver dysfunction, inability to adequately store or utilize folate or other factors such as an increased folate requirement, perhaps related to alcohol.[31] Though, human folate storage lasts but little over a month, the "dedicated alcoholics," who deprive themselves of folate-containing foods by ingesting wine (which is low in folate)[32] or whiskey (which is devoid of folate)[32] to the exclusion of food for a period much in excess of a month, would seemingly develop folate deficiency. This may not be true for the beer-drinking alcoholics since beer is high in folate content.[32] Macro-ovalocytes and an increase in neutrophil segmentation were evident in the peripheral blood smears of all the patients with serum folate concentrations below 3ng/ml, and in majority of the patients with serum folate concentrations of 3 to 4.9 ng/ml. Bone marrow aspirates in such patients were either overtly megaloblastic or demonstrated large metamyelocytes and

"macronormoblasts," as further morphologic evidence for folate (or vitamin B12) deficiency. Finally, suppression of hematopoiesis by alcohol evidently suggests that it may occur via interference with folate metabolism, thus increasing folate requirement.[31]

USE OF IMAGING TECHNIQUES IN DIAGNOSIS OF ALD:

Ultrasonography is an useful non-invasive technique in detecting fatty infiltration of the liver and determining liver size. The advanced method- Colour Doppler US provides further information on hemodynamics of portal venous system, the hepatic artery and the hepatic veins by demonstration of portal vein flow reversal, ascites and intra-abdominal venous collaterals which points towards serious liver injury with less potential for complete reversal. Again, the reliability and reproducibility are the limitations for its daily usage as screening tool. In cases of ALD, early screening for steatosis can be carried out using hepatic imaging techniques such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). Among these methods, hepatic steatosis assessment via US, especially in patients with low fat deposition (below 30%) has poor analytical sensitivity and specificity. MRI and MR techniques are the imaging tools of choice for accurate steatosis assessment but limited by the lack of established standardization of sequence characteristics and their high cost. These methods are however helpful in excluding other causes of chronic liver disease and its complications independent of the etiology such as obstructive cholestasis, infiltrative or neoplastic liver diseases. With respect to fibrosis assessment, imaging techniques are not definitive and rely on so called sure morphological signs of cirrhosis.[18]

"Need for liver biopsy in histological diagnosis": [14, 19]

• Histological diagnosis of ALD may require a liver biopsy. It is an invasive procedure with potential mortality and morbidity, therefore it is not recommended for all patients with suspected ALD.

• Precise indications of liver biopsy are not well established in routine practice. However, it is indicated in "patients with aggressive forms of ALD such as severe steatohepatitis requiring specific therapies (e.g. corticosteroids and/or pentoxiphylline) and in patients with other cofactors suspected of contributing to liver disease".

• It can be done percutaneously in most of the patients and may require a transjugular approach in patients with a low platelet count and/or a prolonged prothrombin time.

• Assessment of liver histology by performing a liver biopsy is recommended for patients in the setting of clinical trials because liver biopsy ''allows the risk of long-term mortality prognosis of patients with ALD to be classified according to the severity of the histological lesions.'' Thus, allows a better prediction of the patient's outcome.

• Although the presence of acute steatohepatitis or acute-on-chronic hepatitis can be suspected on clinical and biochemical grounds, a definitive diagnosis of acute steatohepatitis requires liver biopsy. Thus, liver biopsy should be performed whenever possible to establish the correct diagnosis and to guide therapeutic decisions.

PROGNOSIS:

"In 2016, 2·8 million deaths (95% UI 2·4–3·3) were attributed to alcohol abuse. This corresponds to 2·2% (1·5-3·0) of total age-standardised deaths among females and 6·8% (5·8–8·0) among males". Among the population aged 15–49 years, alcohol use was the leading global risk factor for risk-attributable disease burden, accounting for 3.8% (3·2–4·3) of female deaths and 12·2% (10·8–13·6) of male deaths". [33]

"Given the high mortality rate for patients with severe disease, it is imperative to determine which patients require supportive treatment versus more aggressive care. Several scoring systems have been developed to determine prognosis in patients with ALD and aid in determining which patients will benefit from interventions beyond supportive care".[34] The common ones include:

1) Maddrey's discriminant function (DF): the DF has been the mainstay for determining the prognosis in which a score ≥32 is associated with significant short-term mortality and has been used as a cut-off for use of glucocorticoid treatment. A discriminant function is calculated as 4.6x (the prolongation of the prothrombin time above control [seconds]) + serum bilirubin (mg/dl).The main drawback of this scoring system is that it requires a prothrombin time (PT) for the calculation. Prothrombin time may vary in different laboratories and may lead to variations in the INR (international normalized ratio) between laboratories. This has led to the development of few other newer scoring systems.
2) Model for end-stage liver disease (MELD): this score was found to be an independent predictor of mortality in patients with Alcoholic hepatitis. A score of ≥21 is associated with significant mortality in alcoholic hepatitis. Cut-off

score of 21 has a sensitivity of 75% and specificity of 75% in predicting 90-day mortality. The MELD was also found to be comparable to the DF in predicting mortality within 30 days. The benefit of this score over the DF is INR, which is standardized across laboratories, whereas PT is not.

3) Glasgow alcoholic hepatitis score (GAHS): this score uses age, white blood cell count, urea level, PT ratio or INR and bilirubin level to calculate a score between 5 and 12, with scores of 9 and above indicating poor prognosis but showed reduction in mortality on treatment with corticosteroids.
4) The Lille model: this is used to predict the mortality in patients with severe alcoholic hepatitis who have received glucocorticoid treatment. It uses six variables- age, renal insufficiency INR >1.3 or creatinine clearance <40), albumin, PT, bilirubin level, and bilirubin level on day 7; used to determine mortality at 6 months. This score classified the patients into two categories- responders (Lille score 0.56) who had significant improvement in survival at 28 days when treated with corticosteroids compared with the other category of non-responders (Lille score > 0.56).
5) Age-bilirubin-INR-creatinine (ABIC) score: this score has similarities to the MELD score but also includes age as a variable. Additionally, it assigns different weighting for values of INR, bilirubin level and creatinine level.

Not much major differences were found between these scoring systems in predicting the mortality. Thus, none of these prognostic scores can be definitively recommended above another.[34]

The prognosis in ALD patients mostly relies on the degree and stage of pathological injury, the patient's nutritional status, the presence of complications of advanced disease and other comorbid conditions such as hepatitis C virus infection

along with patient's ability to eliminate destructive patterns of drinking.[35] " In studies that have examined the natural history of ALD on the basis of histological characteristics at diagnosis, patients with fatty liver have had the best outcome (70 −80% survival rate at 4−5 years); those with alcoholic hepatitis (AH) or cirrhosis, an intermediate outcome (50−75% survival rate at 4−5 years), and those with cirrhosis combined with AH, the worst outcome (30−50% survival rate at 4−5 years)". Considering all patients with ALD as a single group, the average 1- year and 5-year survival rates are approximately 80% and 50%, respectively. Alcoholic cirrhosis can also be considered as an independent risk factor for hepatocellular carcinoma, where men older than 50 years of age appear to be most vulnerable to development of hepatocellular carcinoma.[35]

General counselling needs to be provided to all ALD patients and they should be explained about the importance of the prognosis of the disease for particularly two outcomes:

(i) determining the need for specific therapy in patients with severe AH and

(ii) determining the need for liver transplantation in patients with alcoholic cirrhosis.[35]

TREATMENT:

- The cornerstone in the treatment of ALD is lifestyle modification, including abstinence from alcohol and treatment of decompensation, if appropriate. This has shown to improve survival and has the potential for reversal of histologic injury.[35]Abstinence is the most important aspect of treatment. Few newer drugs such as acamprosate and naltrexone are also used to reduce alcohol craving.[8]

- Nutrition intervention has been found to play a positive role on both in-patient and out-patient basis.
- Corticosteroids are effective in selected patients with alcoholic hepatitis. Data suggests that the pathogenic mechanisms in alcoholic hepatitis involve cytokine release and the perpetuation of injury by immunologic processes, therefore glucocorticoids have been extensively evaluated in the treatment of alcoholic hepatitis. Patients with severe alcoholic hepatitis, defined as a discriminant function >32 or MELD >20, should be given prednisone, 40 mg/d, or prednisolone, 32 mg/d, for 4 weeks, followed by a steroid taper. Exclusion criteria include active gastrointestinal bleeding, renal failure, or pancreatitis. Women with encephalopathy from severe alcoholic hepatitis may be particularly good candidates for glucocorticoids. A Lille score >0.45, uses pre treatment variables plus the change in total bilirubin at day 7 of glucocorticoids to identify patients unresponsive to therapy.
- Recent studies have indicated the role of anti-TNFα therapy, atleast for alcoholic hepatitis.[35] TNF-α expression and receptor activity in alcoholic liver injury has led to an examination of TNF inhibition as an alternative to glucocorticoids for severe alcoholic hepatitis. The nonspecific TNF inhibitor, pentoxifylline, demonstrated improved survival in the therapy of severe alcoholic hepatitis, primarily due to a decrease in hepatorenal syndrome and appears to be a promising anti-inflammatory therapy. Monoclonal antibodies that neutralize serum TNF-α should not be used in alcoholic hepatitis because of studies reporting increased deaths secondary to infection and renal failure.
- Some complementary and alternative medicinal agents, such as milk thistle and S-adenosylmethionine, may be effective in alcoholic cirrhosis.

- Other potential therapy which includes supplementation with either folic acid or betaine (thought to combat against the alcohol induced alteration in the methionine metabolism in the liver) may also be protective in ALD. Another potential dietary supplement is zinc because of the well known fact that patients with AH and cirrhosis may have zinc depletion in the circulating levels, mediated both at the level of absorption or excretion.[35]
- Also, treating the complications of ALD can lead to improvement in the quality of life and in some cases, decrease in the short-term mortality.[35]

Referral of patients to experienced counsellors and/or alcohol treatment programs should be routine in the management of patients with alcoholic liver disease. Special attention should be directed to the nutritional and psychosocial states during the evaluation and treatment periods.

ROLE OF LIVER TRANSPLANTATION:

Liver transplantation represents an accepted standard of care in selected and motivated patients with end-stage cirrhosis and shows excellent long term outcomes that are equal to or superior to other indications for transplantation. ALD is the second most common indication for liver transplantation, accounting for more than 20% of all cases.[37] In general, transplant candidacy should be re-evaluated after a defined period of sobriety. Patients presenting with alcoholic hepatitis have been largely excluded from transplant candidacy because of the perceived risk of increased surgical mortality and high rates of recidivism following transplantation. Recently, a European multidisciplinary group has reported excellent long-term transplant outcomes in highly selected patients with florid alcoholic hepatitis. General application of transplantation in such patients must await confirmatory outcomes by

others. "Thus, evaluation of alcoholic patients for liver transplantation should comprise a multidisciplinary program including medical, surgical and psychiatric assessment".[38]

Outcome of patients transplanted for alcoholic cirrhosis:

Based on data reported from different parts of the world, the patient survival rates after liver transplantation for alcoholic cirrhosis seem to be 81%-92%, 78%-86%, and 73%-86% at 1, 3, and 5 years respectively.[39] On assessment of the long–term outcome for patients receiving orthotopic liver transplantation (OLT) or any other kind of transplant, researchers and clinicians evaluate numerous other factors in addition to the survival rate. This includes how long the transplanted organ continues to function (i.e., graft survival) and the patient's quality of life. Out of concerns that ALD patients may resume drinking after OLT, thereby damaging the transplanted liver, investigators frequently keep assessing the graft survival in these patients. Studies have suggested that the ALD patients had similar patient and graft survival rates, if not better in some cases, compared to those of patients undergoing liver transplantation for other indications, though initially it was thought that ALD patients would have poorer graft survival.[40] Also, there are very few reliable predictors of relapse in ALD though these patients are not more likely to relapse (or that their alcohol consumption may not be likely to damage the transplanted liver). "Although not supported by all studies, abstinence of fewer than 6 months prior to transplantation may be a reasonable predictor of recidivism and is widely employed as a criterion for listing for liver transplantation. There are no good data to determine if some patients with sobriety fewer than 6 months might benefit from liver transplantation".[37] Patients with alcoholic cirrhosis showed post-transplant

improvement in the health related quality of life, mood status and cognitive functioning, with no difference compared to patients transplanted for non-alcoholic cirrhosis. Patients were able to lead active and prolific lives as well as return to society, irrespective of the indication for transplantation.[39]

MATERIALS AND METHODS:

Source of data:

The study was done in the hematology laboratory of Pathology department on patients clinically diagnosed as alcoholic liver disease in the Department of Medicine, Shri B. M. Patil Medical College, Hospital and Research Centre, B.L.D.E. (DEEMED TO BE UNIVERSITY), Vijayapura.

Study period: 1st November, 2018 to 30th May, 2020.

Methods of collection of data.

The study includes 70 patients clinically diagnosed with Alcoholic liver disease. The values of Haemoglobin (Hb) levels, Red blood cell count, Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Total count, Differential count and Platelet count will be analysed using the fully automated hematology analyser (Sysmex XN1000- Sysmex Corporation, Kobe, Japan). The coagulation profile which includes Prothrombin time (PT) and Activated partial thromboplastin time (aPTT) will also be analysed using the coagulometer (ACL elite Pro). It will be ensured that the quality control of the machines are well maintained under the standard protocols.

Sample collection and analysis:

• After taking informed consent, under aseptic precautions, venous blood sample was collected from all the cases.

• Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count parameters, that included Hb, PCV, MCV, MCH, MCHC, Total count, Differential count and Platelet count using an automated 5-part differential hematology analyzer (SYSMEX XN-1000). (Figure 9)

• Also, blood was collected in Citrate bulb for analysis of coagulation profile which included Prothrombin time (PT) and Activated partial thromboplastin time (aPTT) using the Coagulometer (ACL ELITE PRO). (Figure 10, 11)

Figure 9: Automated Hematology Analyzer (Sysmex XN-1000)

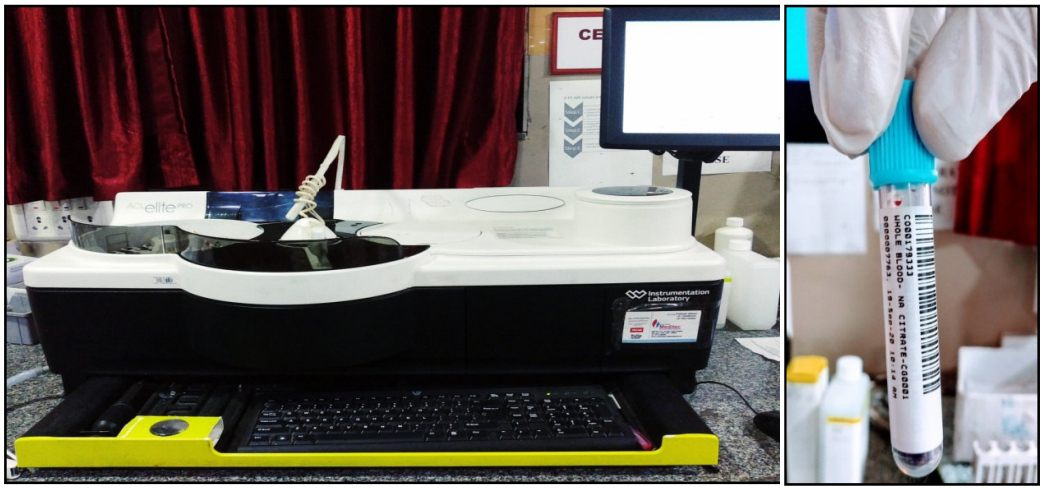

Figure 10 and 11: Coagulometer (ACL elite Pro) and Citrate bulb for coagulation profile analysis

Inclusion criteria:

- All clinically diagnosed cases of alcoholic liver disease admitted in Shri B. M. Patil Medical College, Hospital and Research Centre, B.L.D.E. (DEEMED TO BE UNIVERSITY), Vijayapura during the study period were included.
- All adult patients of alcoholic liver disease were evaluated.

Exclusion criteria:

- Patients receiving hepatotoxic drugs like Acetaminophen, Isoniazid, Rifampicin, Phenytoin, Allopurinol, Amiodarone, Ketoconazole, Methotrexate etc.
- Patients who have received blood transfusion in the last 3 months.
- Patients undergoing treatment for any other disease like Iron supplements.
- Patients suffering from any infectious diseases like Dengue etc.

The nature and purpose of the study were carefully explained to the patients and their attendants before obtaining their consent.

The parameters analysed were- Hb, RBC count, PCV, MCV, MCH, MCHC, Total count, Differential count, Platelet count, PT and aPTT.

STATISTICAL METHODS

Sample Size:

As in the study done by Deepak Jain *et al*[29], with anticipated mean ± SD of hemoglobin levels was 9.4 ± 2[29] (ref) at 99% level of significance and with precision 1.0, the calculated sample size was 56.

By the following formula,

$$n=\frac{z^2\,(SD)^2}{d^2}$$

where,

Z= Z statistic at α level of significance

d^2= Absolute error

SD= Standard deviation

Here 70 cases were included in the study.

Statistical analysis:

The following statistical analysis were done:

- The data obtained were entered in a Microsoft Excel sheet and statistical analysis was performed using statistical package for the social sciences (Version 17).
- Results were calculated by employing the independent t-test and Chi square test. A p-value was calculated by Mann Whitney test. For all tests, significance was achieved at $p<0.05$. The continuous data were given as mean value ± standard deviation.

Study design: PROSPECTIVE OBSERVATIONAL STUDY

RESULTS

This study was done at the Department of Pathology and Medicine, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka where ALD patients were being admitted and treated for clinical symptoms and manifestations of the disease. Hematological analysis was performed in the central laboratory of Department of Pathology.

A total of 70 patients were included in the present study. The blood sample was collected from all the patients and presented to be evaluated for hematological parameters. All these patients had a history of alcohol abuse and were found to be known cases of ALD. Here, in this study we present the results of the analysis of CBC parameters and Coagulation profile in all these patients.

1. AGE DISTRIBUTION IN CASES:

Majority of the patients diagnosed with Alcoholic liver disease were in the age group of 50-60 years with almost all being in the 30-60 years range. The mean age was observed to be around 44 years (44.62±10.79). (Table 5, Figure 12)

Table 5: Distribution of patients according to Age (in years)

Age (in years)	No. of patients	Percentage
< 30	4	5.8
30 – 39	18	26.1
40 – 49	20	29.0
50 – 59	25	36.2
60 – 69	1	1.4
>70	2	2.9
Total	70	100
Mean ± SD	44.62 ± 10.79	

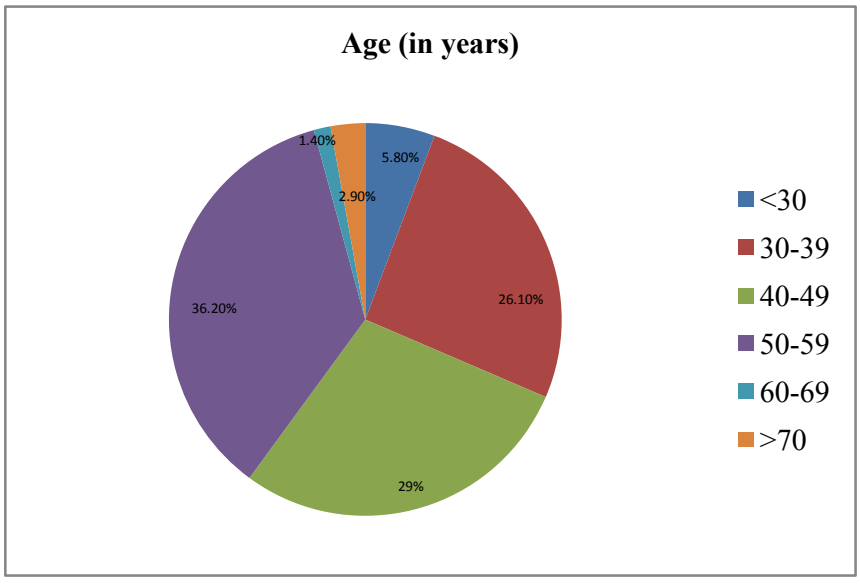

Figure 12: Pie chart showing ALD cases in different age groups

2. DISTRIBUTION OF CASES ACCORDING TO GENDER:

The total number of males diagnosed with Alcoholic liver disease among the total 70 cases were 67 (95.8%) and females were 3 (4.2%). (Table 6, Figure 13)

Table 6: Distribution of patients according to Gender

Gender	No. of patients	Percentage (%)
Males	67	95.8
Females	3	4.2
Total	70	100

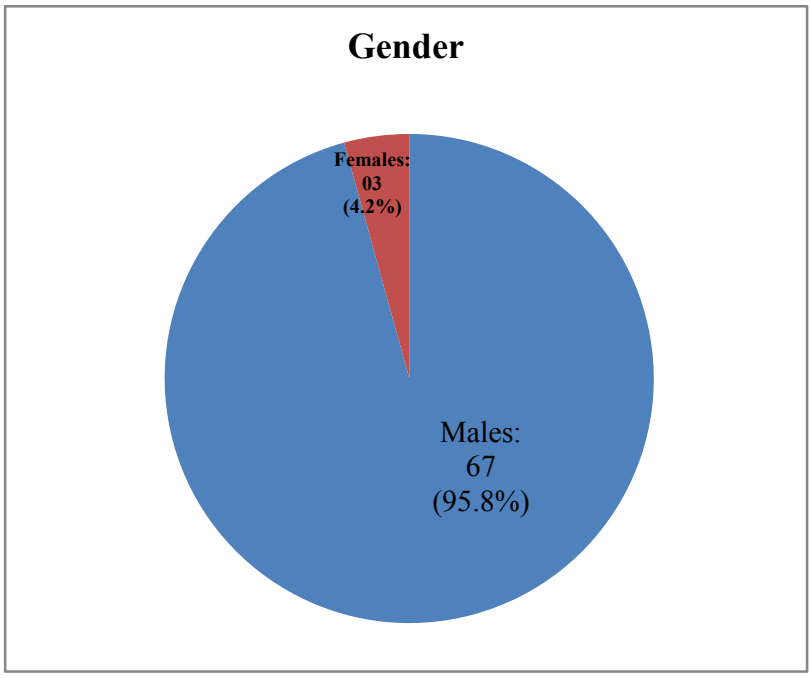

Figure 13: Pie chart showing ALD case distribution according to gender

3. DESCRIPTIVE ANALYSIS FOR THE CBC VARIABLES:

Table 7: Descriptive statistics for the CBC variables

Variables	Minimum	Maximum	Mean	Std. Deviation	Percentiles		
					25th	50th	75th
Hb (g/dl)	4.20	15.60	9.40	2.476	7.2	9.1	11.6
RBC count	1.70	6.13	3.06	0.908	2.3	2.9	3.7
PCV (%)	12.90	47.60	28.15	7.480	21.7	27.5	33.3
MCV (μm^3)	55.40	122.90	93.50	11.364	88.2	93.9	100.2
MCH (pg)	16.20	41.20	31.21	4.108	29.6	31.5	33.4
MCHC (g/dl)	25.50	37.60	32.87	4.207	32.1	33.3	34.9
Total WBC count	460.00	33720.00	10969.14	7286.761	5330	9020	15185
Platelet Count	0.32	9.68	1.37	1.307	0.60	1.02	1.7
Differential Count							
N	44.20	93.20	72.99	12.462	63.97	72.7	82.3
L	2.90	50.00	18.42	10.693	10.07	17.0	24.1
M	1.700	12.100	5.61	2.459	3.57	5.4	7.5
E	0.00	15.000	2.06	2.439	0.37	1.3	3.0
B	0.00	1.00	0.17	0.193	0	0.1	0.3

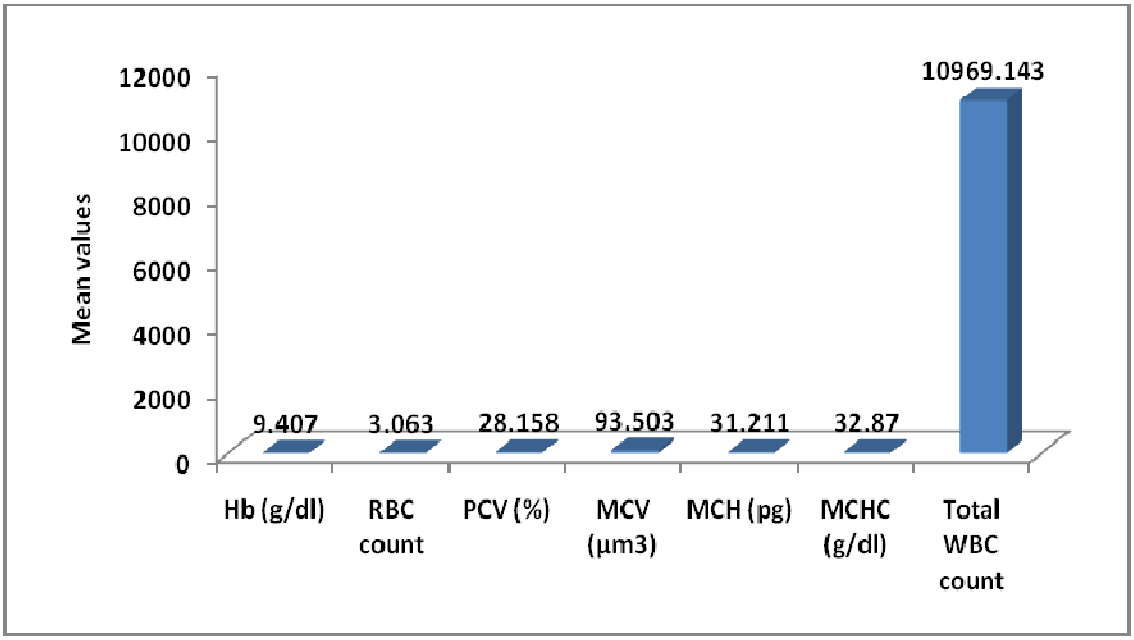

Figure 14: Mean values of different CBC parameters

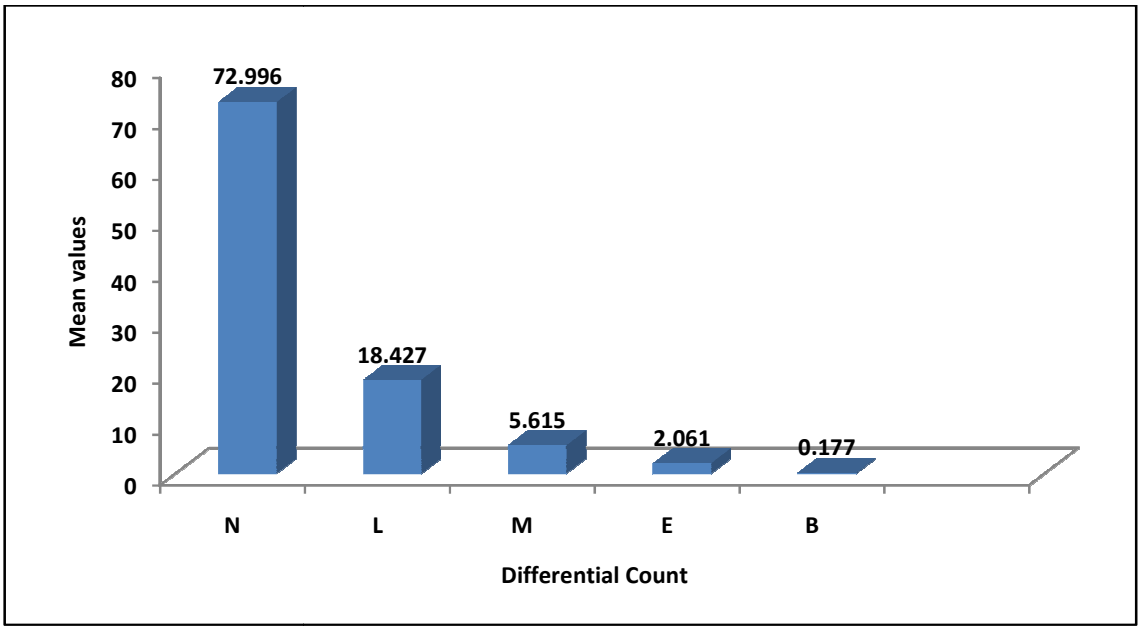

Figure 15: Mean values of type of leucocytes

On evaluation of the hematological parameters in all the cases, the following findings were noted: (Table 7; Figure 14, 15)

➢ Minimum Hb was 4.2 g/dl and maximum was 15.6 g/dl with an average of 9.40 ± 2.476 (Mean ± SD).

➢ Minimum RBC count was 1.70×10^{12}/l and maximum was 6.13×10^{12}/l with an average of 3.06 ± 0.908 (Mean ± SD).

➢ Minimum PCV was 12.90% and maximum was 47.60% with an average of 28.15 ± 7.480 (Mean ± SD).

➢ Minimum MCV was 55.40 μm^3 and maximum was 122.90 μm^3 with an average of 93.50 ± 11.364 (Mean ± SD).

➢ Minimum MCH was 16.20 pg and maximum was 41.20 pg with an average of 31.21 ± 4.108 (Mean ± SD).

➢ Minimum MCHC was 25.50 g/dl and maximum was 37.60 g/dl with an average of 32.87 ± 4.207 (Mean ± SD).

➢ Minimum Total WBC count was 460 and maximum was 33720 with an average of 10969.14 ± 7286.761 (Mean ± SD).

➢ Minimum platelet count was 0.32 lakh/µl and maximum was 9.68 lakh/µl with an average of 1.37 ± 1.307 (Mean ± SD)

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➢ Minimum relative neutrophil and lymphocyte count was 44.20%, 2.90% and maximum was 93.20%, 50% with an average of 72.99 ± 1.307 (Mean ± SD).

4. DESCRIPTIVE ANALYSIS FOR THE COAGULATION PROFILE:

Table 8: Descriptive statistics for the coagulation profile

Coagulation Profile	**Minimum**	**Maximum**	**Mean**	**SD**	Percentiles		
					25th	**50th**	**75th**
PT	11.20	59.60	22.191	8.445	14.67	21.6	27.65
aPTT	30.40	61.60	59.383	6.889	41.15	48.4	60.37

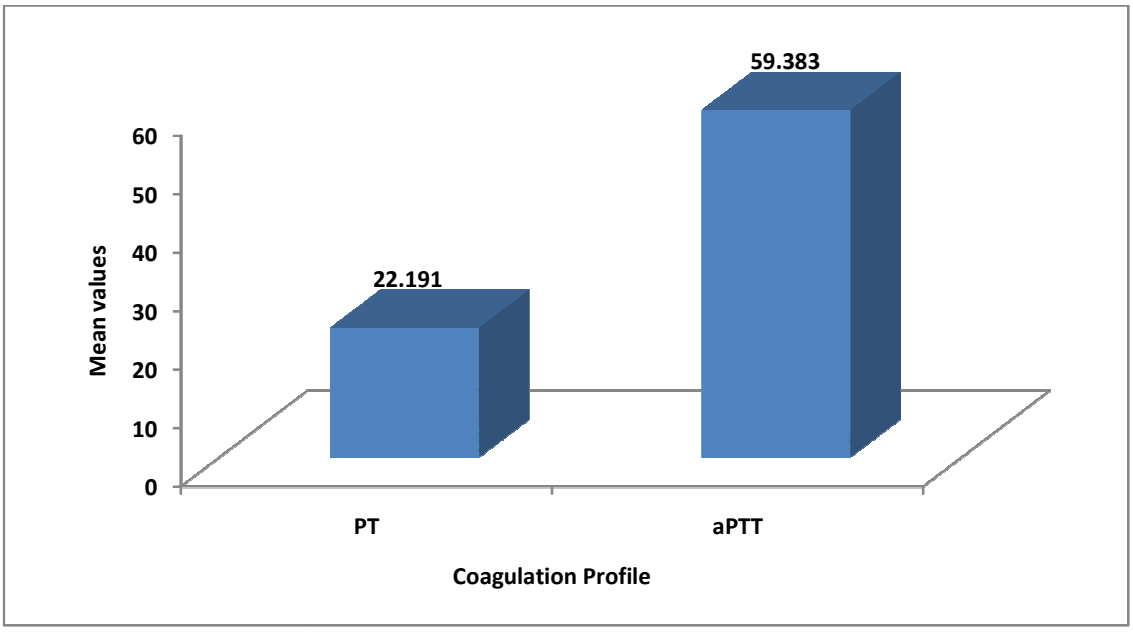

Figure 16: Mean values of PT and aPTT

On evaluation of the coagulation profile in all the cases, the values of PT and aPTT were noted as:

➢ Minimum PT was 11.20 sec and maximum was 59.60 sec with an average of 22.191 ± 8.445. (Mean ± SD).

➢ Minimum aPTT was 30.40 sec and maximum was 61.60 sec with an average of 59.383 ± 6.889

5. CORRELATION BETWEEN THE VARIOUS PARAMETERS:

Table 9: Correlation between the various parameters

Correlation between variables	Correlation coefficient	p values	Remark
Hb and Platelet count	r=0.309	p=0.009*	Mild positive correlation
Hb and Neutrophils	r=0.068	p=0.575*	No correlation
Hb and MCV	r=-0.086	p=0.481*	No correlation
Total WBC count and Neutrophils	r=0.588	p=0.001*	Moderate positive correlation
Platelet count and Neutrophils	r=0.588	p=0.077	Mild positive correlation
Platelet count and PT	r=-0.355	p=0.003*	Mild positive correlation

Platelet count and aPTT	r=-0.334	p=0.005*	Mild positive correlation
*:Correlation is significant			

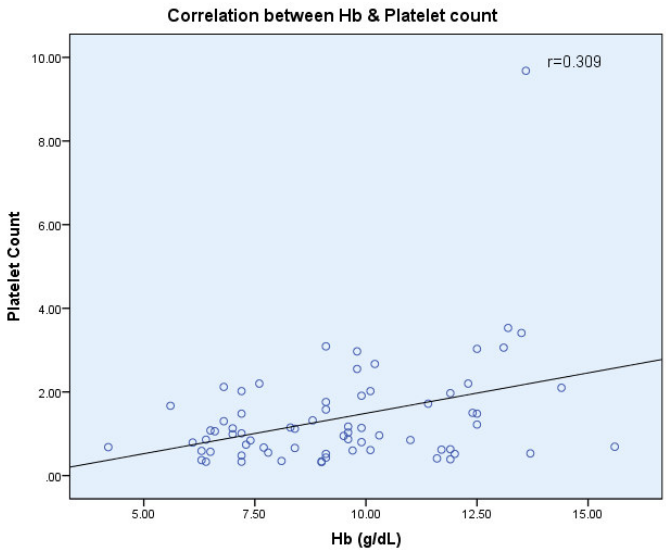

Figure 17 (Scatter plot-1): showing correlation between Hb and Platelet count

In the present study, statistically significant (p=0.009) mild positive correlation was observed between values of Hb and Platelet count. (Figure 17)

This implies the presence of anemia along with thrombocytopenia in the patients of ALD.

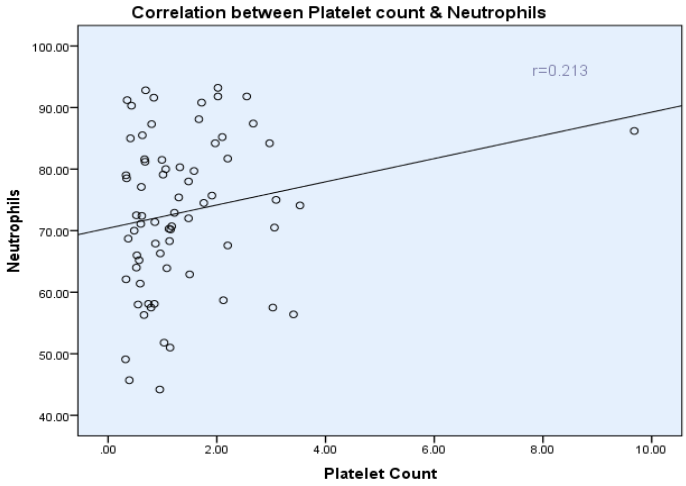

Figure 18 (Scatter plot-2): showing correlation between Platelet count and Neutrophils

Statistically significant (p=0.077) mild positive correlation was observed between Platelet count and Neutrophil count. (Figure 18)

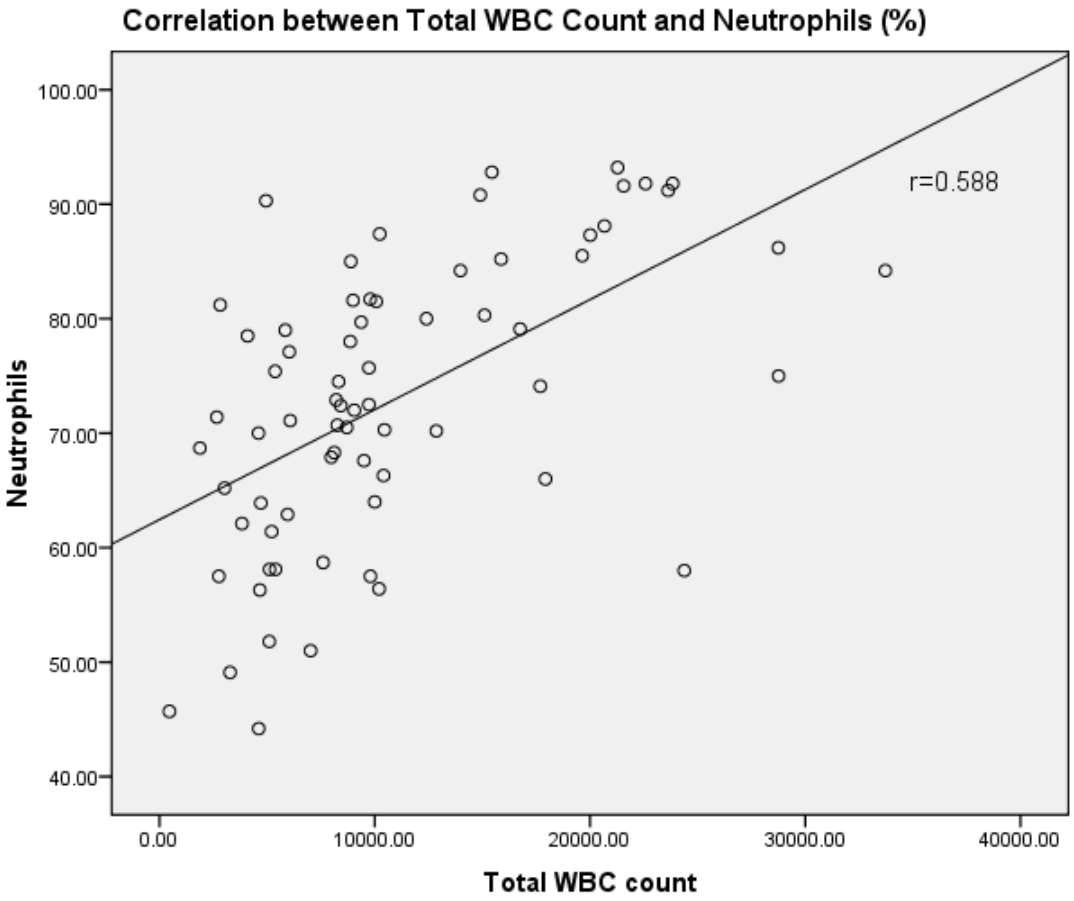

Figure 19 (Scatter plot-3): showing correlation between Total WBC count and Neutrophils

Statistically significant (p=0.001) moderate positive correlation was observed between Total WBC count and Neutrophil count. (Figure 19)

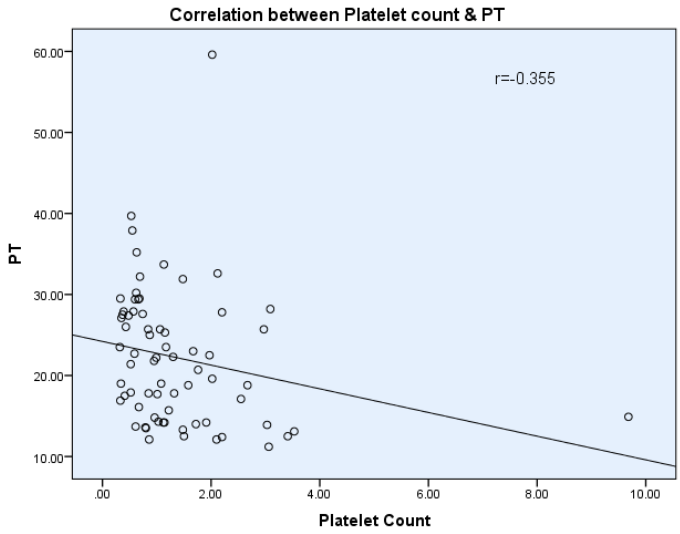

Figure 20 (Scatter plot-4): showing correlation between Platelet count and PT

Statistically significant (p=0.003) mild negative correlation (r=-0.355) was observed between Platelet count and Prothrombin time (PT). (Figure 20)

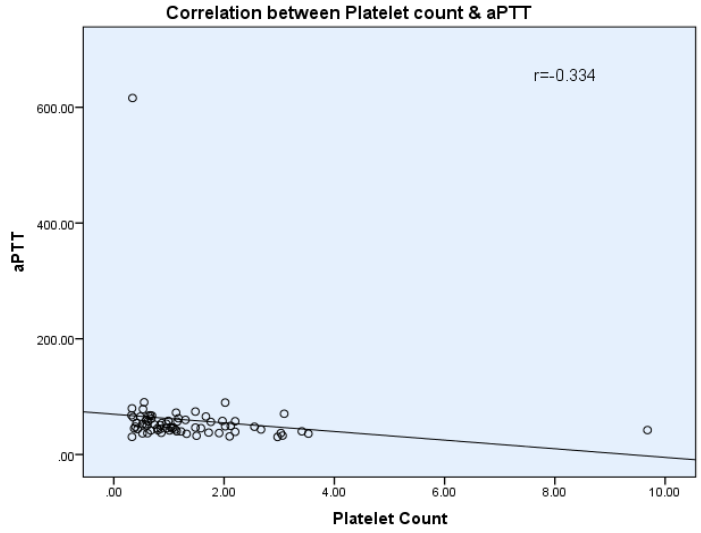

Figure 21 (Scatter plot-5): showing correlation between Platelet count and aPTT

Statistically significant (p=0.005) mild negative correlation (r=-0.334) was observed between Platelet count and activated partial thromboplastin time (aPTT). (Figure 21)

6. COMPARISON OF HEMATOLOGICAL VALUES IN MALE ALD PATIENTS WITH NORMAL VALUES:

Table 10: Comparison of Hematological values in Male ALD patients with Normal values

Hematological values	MEN					
	Normal		ALD patients		Unpaired t test	p values
	Mean	±SD	Mean	±SD		
Hemoglobin	15.0	2.0	9.35	2.49	t=16.333	p<0.001*
RBC count	5.0	0.5	3.04	0.91	t=18.769	p<0.001*
Packed cell volume (PCV)	0.45	0.05	27.90	7.47	t=36.665	p<0.001*
Mean cell volume (MCV)	92	9	93.40	11.52	t=0.8802	p=0.3800
Mean cell Hemoglobin (MCH)	29.5	2.5	29.96	2.98	t=0.3173	p=0.7517
Mean cell Hemoglobin Concentration(MCHC)	330	15	31.26	0.55	t=34.330	p<0.001*
Platelet count	280	130	1.38	1.33	t=3.694	p=0.004*
Total WBC Count			10932.68	7433.47		

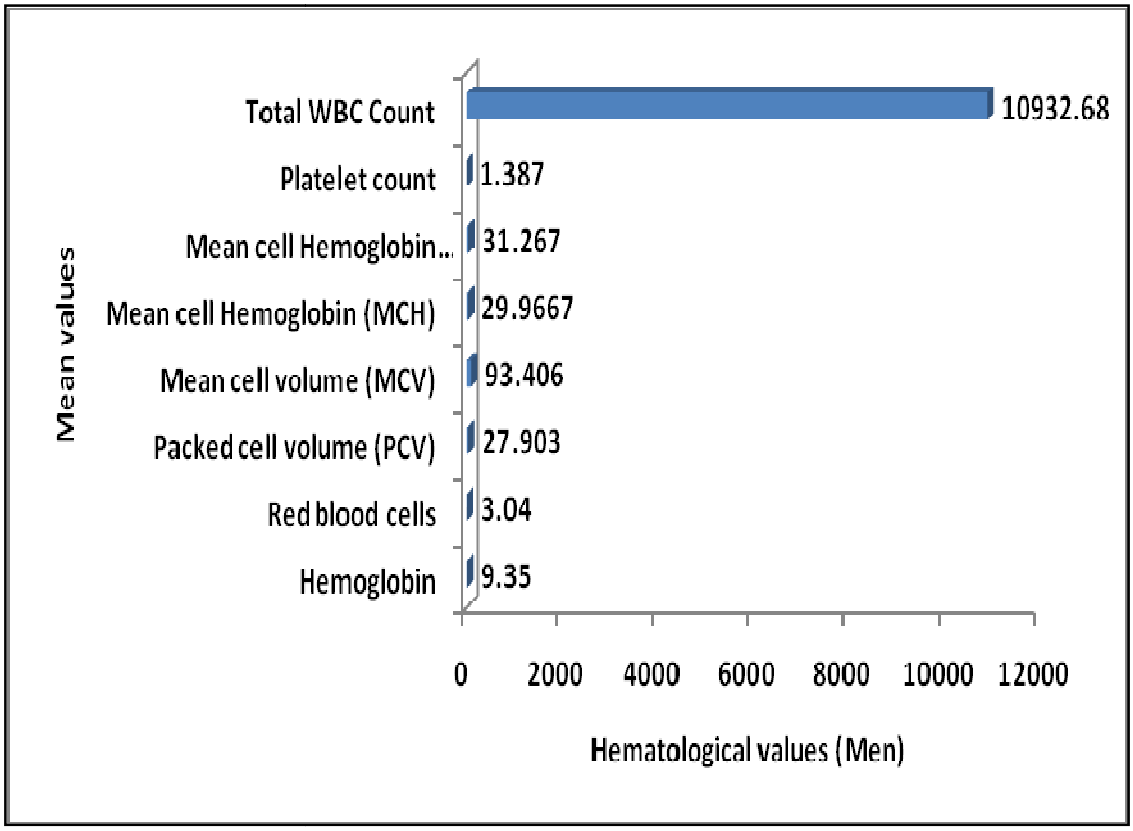

Figure 22: Mean values of different parameters in male ALD cases

Statistically significant decrease in the values of Hb, RBC count, PCV, MCHC and Platelet count were observed in the male patients of ALD. However, no significant changes were noted in the MCV and MCH values. Also, the mean Total WBC count was 10932 ± 7433.47 which reduced than the normal reference range. (Table 10, Figure 22)

7. COMPARISON OF HEMATOLOGICAL VALUES IN FEMALE ALD PATIENTS WITH NORMAL VALUES:

Table 11: Comparison of Hematological values of Female ALD patients with Normal values

Hematological values	WOMEN					
	Normal		ALD		Unpaired t test	p values
	Mean	±SD	Mean	±SD		
Hemoglobin	13.5	1.5	10.60	1.92	t=3.278	p=0.001
RBC count	4.3	0.5	3.57	0.79	t=2.454	p=0.015*
Packed cell volume (PCV)	0.41	0.05	33.87	6.12	t=66.123	p<0.001*
Mean cell volume (MCV)	92	9	95.66	8.05	t=0.696	p=0.487
Mean cell Hemoglobin (MCH)	29.5	2.5	31.26	4.15	t=3.429	p=0.008*
Mean cell Hemoglobin Concentration(MCHC)	330	15	32.94	4.28	t=157.71	p<0.001*
Platelet count	280	130	1.12	0.60	t=3.698	p=0.0004*
Total WBC Count			11783.33	2708.47		

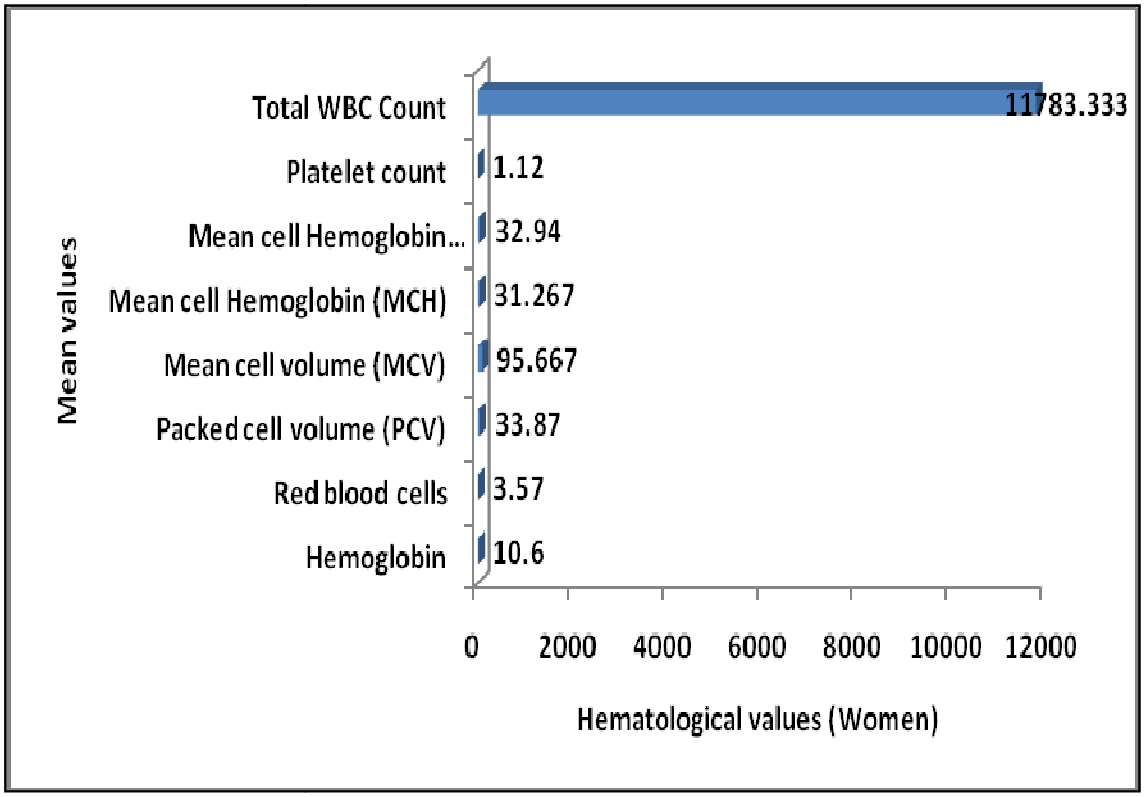

Figure 23: Mean values of different parameters in female ALD cases

Similar to male ALD patients, statistically significant decrease in the values of RBC count, PCV, MCHC and Platelet count were also observed in the female patients of ALD. But, unlike the male patients, females had a statistically significant mild increase in the MCH values. However, no significant changes were noted in the Hb and MCV values in the female patients of our study. Also, the mean Total WBC count was 11783.33 ± 2708.47 which was found to be normal. (Table 11, Figure 23)

8. DISTRIBUTION OF PATIENTS ACCORDING TO SEVERITY OF ANEMIA (WHO);

Table 12: Distribution of patients according to Severity of Anemia (WH0)

Severity of Anemia (in g/dl)	No. of patients	Percentage (%)
Non-anemic (>11)	20	28.57
Mild (9.5-10.9)	14	20
Moderate (8.0-9.4)	12	17.14
Severe (6.5-7.9)	17	24.28
Life-threatening (<6.5)	07	10
Total	70	100.0

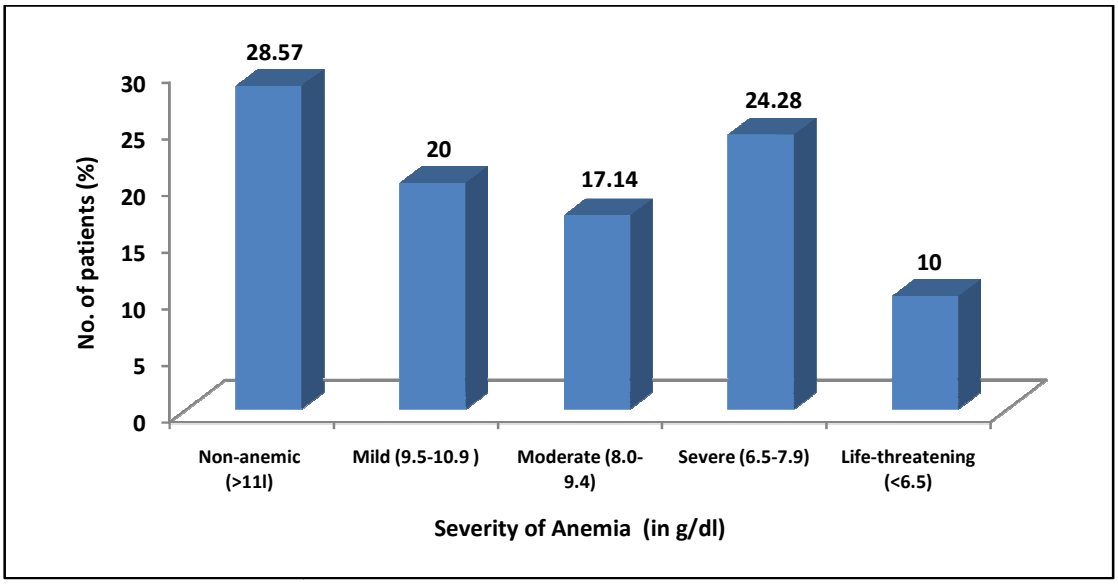

Figure 24: Distribution of patients according to severity of anemia

It was seen that maximum patients were non-anemic that is 28.57%. But, the anemic group of patients mostly had severe anemia (24.28%) or moderate anemia (17.14%).

7 patients (10%) had hemoglobin levels of <6.5 g/dl which contributed to life-threatening anemia indicating need for immediate intervention. (Table 12, Figure 24)

9. ALD PATIENTS IN DIFFERENT AGE GROUPS HAVING ANEMIA;

Table 13: ALD patients of different age groups with Anemia

Age (in years)	Severity					Total
	1.00	2.00	3.00	4.00	5.00	
< 30	1	0	1	0	2	4
	14.3%	0%	9.1%	0%	10.0%	5.8%
30 – 39	2	3	6	2	5	18
	28.6%	17.6%	54.5%	14.3%	25.0%	26.1%
40 – 49	0	7	1	6	6	20
	0%	41.2%	9.1%	42.9%	30.0%	29.0%
50 – 59	4	7	3	5	5	24
	57.1%	41.2%	27.3%	35.7%	25.0%	34.8%
60 – 69	0	0	0	1	0	1
	0%	0%	0%	7.1%	0%	1.4%
70+	0	0	0	0	2	2
	0%	0%	0%	0%	10.0%	2.9%
Total	7	17	11	14	20	69
%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

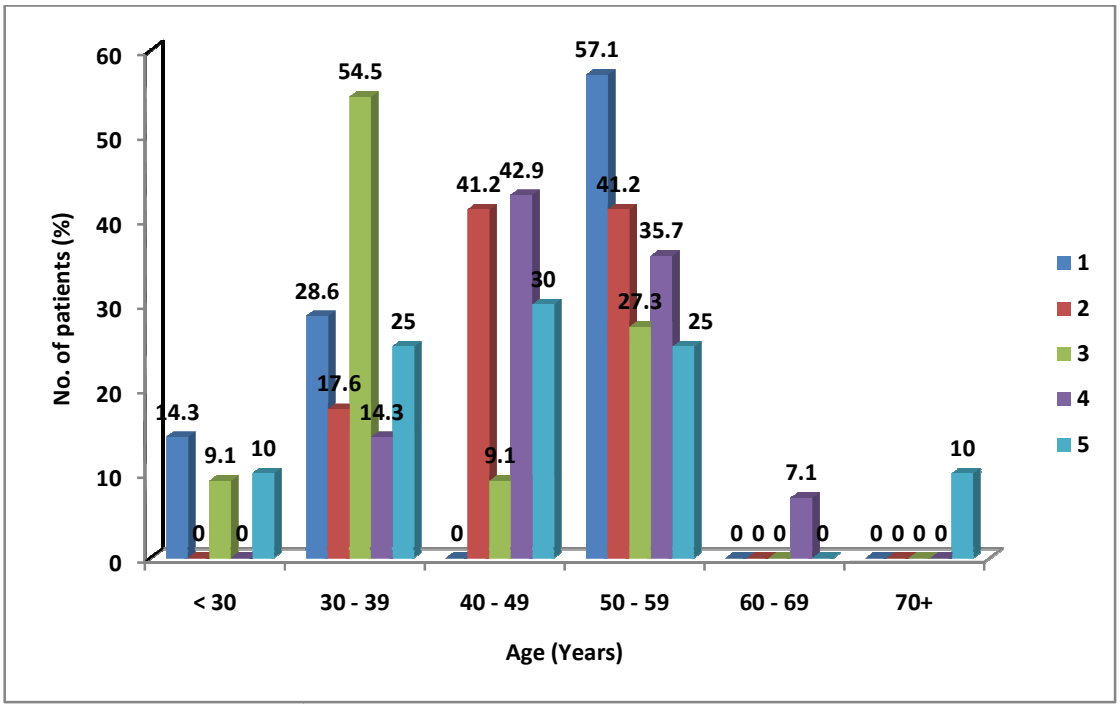

Figure 25: ALD patients of different age groups with Anemia

It was found that amongst all the ALD patients in the anemic group, maximum patients were elderly in the age group of 50-59 years followed by 40-49 years. In the age group of 30-39 years; 54.5%, 14.3%, 25% patients had moderate, severe and life-threatening anemia respectively. (Table 13, Figure 25)

10. PATIENTS OF ALD AND THROMBOCYTOPENIA;

Reduced platelet count was one of the major findings in ALD patients. Out of total 70 patients, 48 patients which is 68.57%, were found to have reduced number of platelets. The mean platelet count was 1.37 lakh/µl with an average of 1.37 ± 1.307 (Mean ± SD) as mentioned earlier and the median value was calculated to be 1.02 lakh/µl which indicates thrombocytopenia in majority of patients.

11. DIFFERENTIAL COUNT IN ALD PATIENTS;

Table 14: Distribution of Differential count in ALD patients

Hematological values	Number of patients	Percentage (%)
Differential Count		
Neutrophils		
<40	0	0
40-80 (normal)	48	68.57
>80	22	31.43
Lymphocytes		
< 20	43	61.4
20-40 (normal)	25	35.7
>40	2	2.9
Monocytes		
< 2	2	2.9
2-10 (normal)	66	94.3
>10	2	2.9
Eosinophils		
< 1	25	35.7
1-6 (normal)	42	60.0
>6	3	4.3

Basophils		
0-1 (normal)	69	98.6
1-2	1	1.4
Total	70	100

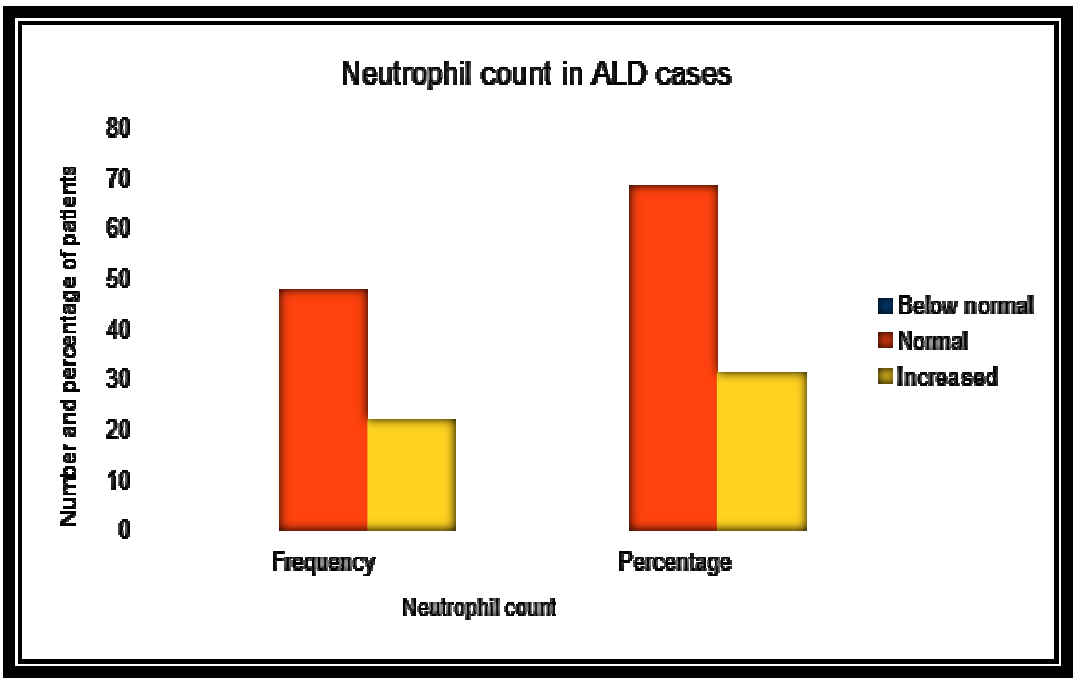

Figure 26: Distribution of neutrophils amongst ALD cases

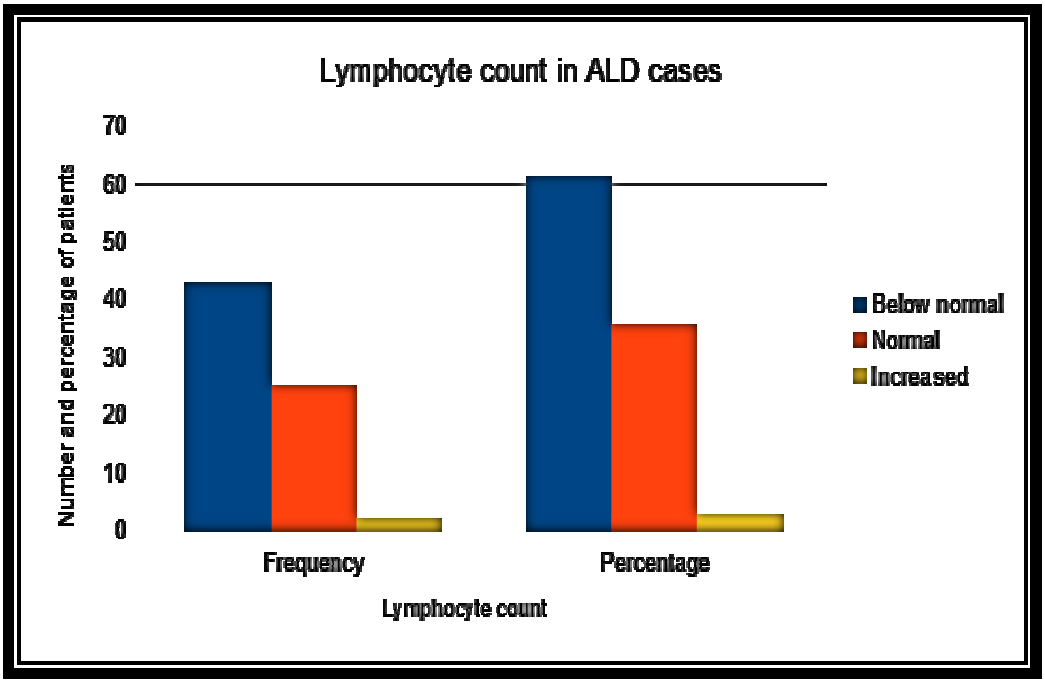

Figure 27: Distribution of lymphocytes amongst ALD cases

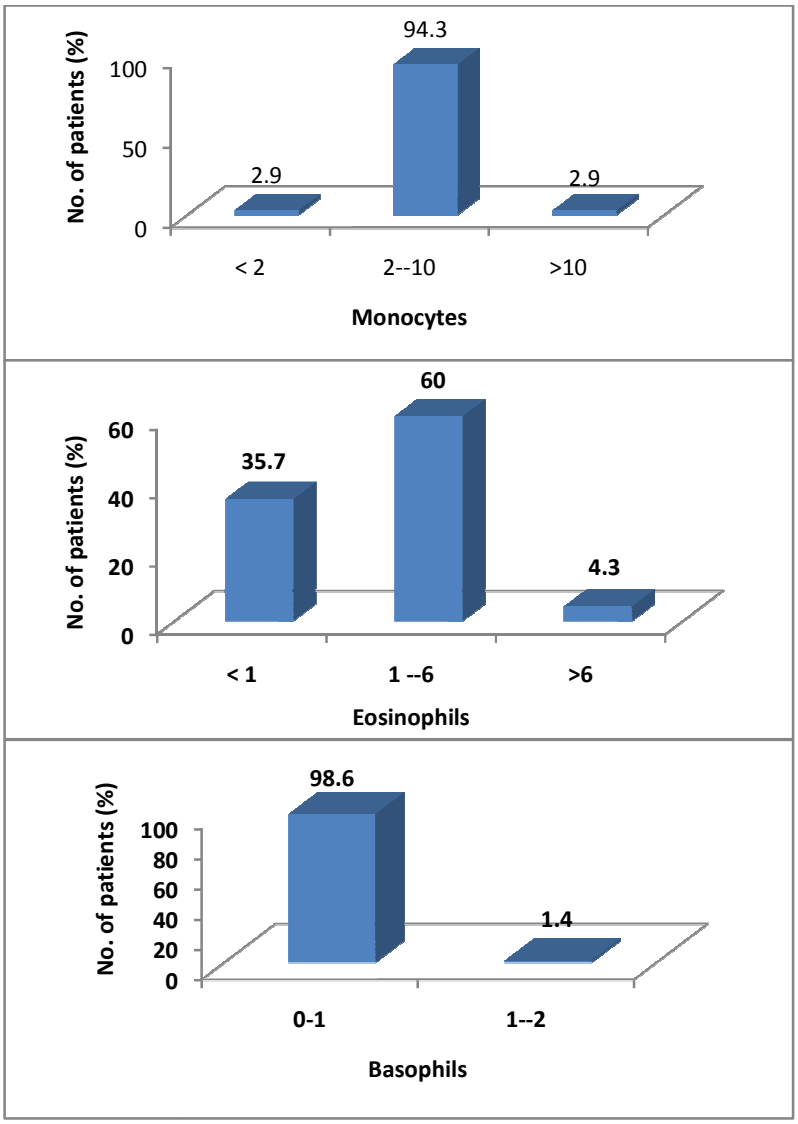

Figure 28: Distribution of monocytes, eosinophils and basophils amongst ALD cases

The major determinant type of leucocytes which play a role in ALD are neutrophils and lymphocytes. It was found that 31.43% of cases had neutrophilia and 61.4% cases had lymphopenia. Neutropenia is a significant finding in ALD, however our study showed no cases with neutropenia which may indicate towards presence of secondary infections. (Table 14, Figure 27, Figure 28)

12. COAGULATION PROFILE IN ALD PATIENTS;

Table 15: Coagulation profile in ALD cases

Coagulation profile		
	Number of patients	Percentage (%)
PT		
<11	0	0
11-16 (Normal)	20	28.57
>16	50	71.43
aPTT		
<26	0	0
26-40 (Normal)	17	24.29
>40	53	75.71
Total	70	100

Prolonged values of PT and aPTT were found in maximum cases, 50/70 patients which is 71.43% had prolonged PT and 53/70 patients which is 75.71% had prolonged aPTT. (Table 15, Figure 29, Figure 30). These changes clearly indicates the presence of hepatic dysfunction in ALD.

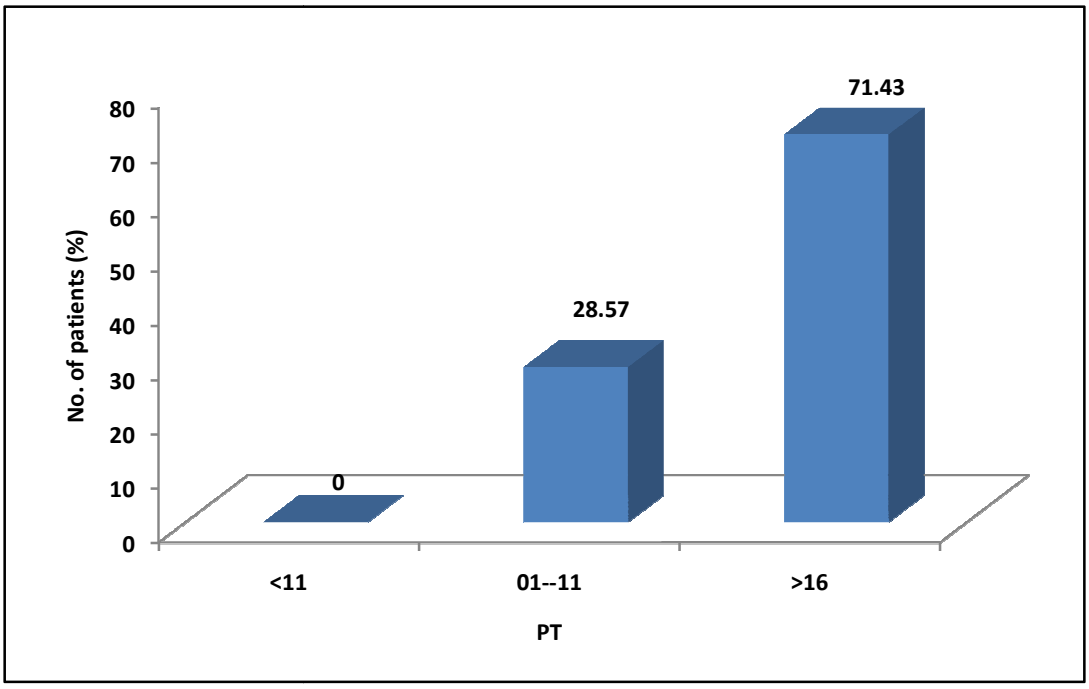

Figure 29: Distribution of PT

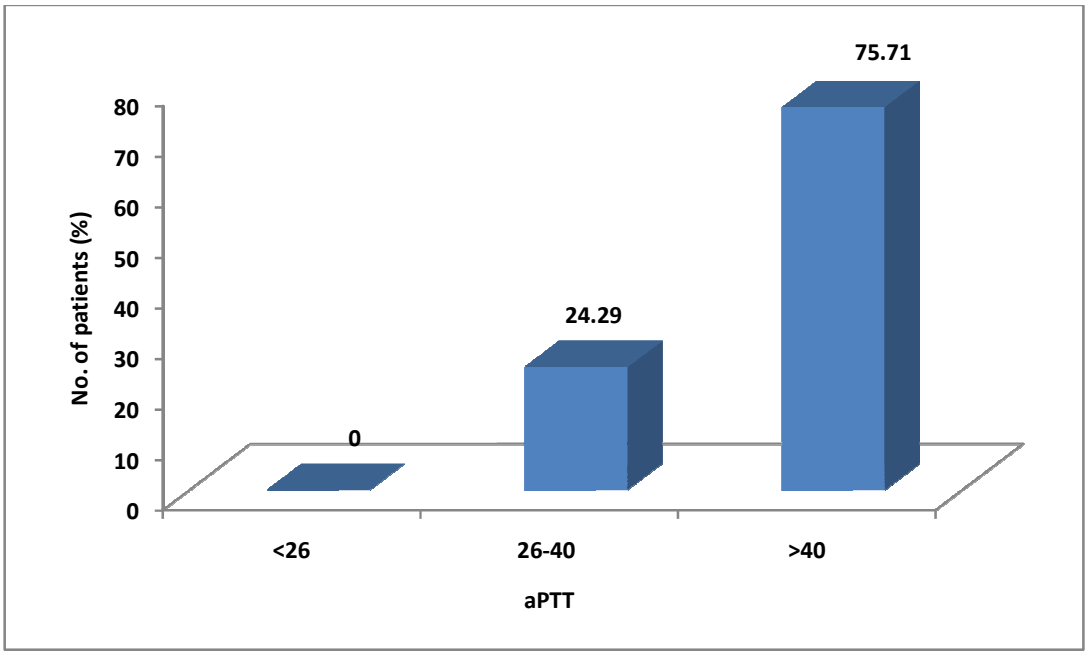

Figure 30: Distribution of aPTT

DISCUSSION

Abnormalities in hematological parameters are common in patients with alcoholic liver disease. The pathogenesis of abnormal hematological indices in ALD is multifactorial and includes alterations in bone marrow stimulating factors, viral and toxin-induced bone marrow suppression etc. Excessive alcohol intake itself directly causes bone marrow suppression leading to toxic effects on all the blood cell lines. Indirectly, it also affects the nutritional biology of the patient resulting in production of functionally immature cells. The derangement in hematological parameters, if not monitored regularly will lead to various complications like bleeding and infection, that may decrease the quality of life and the overall survival of these patients.[7] In recent years, several authors have reported a high incidence of mortality rates due to complications, predominantly in patients diagnosed with ALD. Therefore monitoring of hematological parameters is essential to assess the severity of the disease.

The present study was done on 70 patients who were diagnosed as Alcoholic liver disease admitted in the Department of Medicine, Shri B.M Patil Medical College and Research Centre. All the study subjects were diagnosed cases of Alcoholic liver disease on the basis of history, clinical evaluation and laboratory investigations.

In the present study, the mean age of the study population was 44.62 years similar to the studies done by Sarangi R. *et. al.*[1], Rao S. *et al.*[6], Deshpande N. *et al.*[41], Raviteja V. *et al.*[42] and Solomon R. *et al.*[44]. In the study done by Yoganandh T. *et al.*[29] and Sambyal V. *et al.*[43], maximum patients were found in the age group of 31-40 years and 41-50 years respectively, whereas in our present study most patients belonged to the age group of 50-59 years.

Table 16: Age incidence in study subjects compared to other studies.

Studies	**Mean age (in years)**
Present study	44.62
Sarangi R. *et al.*[1]	46.78
Rao S. *et al.*[6]	49.3
Deshpande N. *et al.*[41]	41
Raviteja V. *et al.*[42]	55.66
Solomon R. *et al.*[44]	48

Our study also showed that maximum ALD cases were males accounting for 95.8% and only remaining 4.2% were females. Similar findings were seen in the study done by Rao S. *et al.*[6], Lugos D. *et al.*[25] and Solomon R. *et al.*[44].

Table 17: Gender distribution in study subjects compared to other studies.

Studies	**Males (%)**	**Females (%)**
Present study	95.8%	4.2%
Rao S. *et al.*[6]	93%	7%
Lugos D. *et al.*[25]	55.9%	44.1%
Solomon R. *et al.*[44]	86%	14%

THE CHANGES IN THE CBC PARAMETERS

1) Hemoglobin:

In present study it was observed that mean hemoglobin was 9.40 ± 2.476 g/dl similar to Jain D. *et al.*[7] where the mean Hb was 9.4 ± 2.9 g/dl. Another study by Yoganandh T. *et al.*[29] showed mean Hb to be 9.33 ± 1.20 g/dl in moderate alcoholics and 9.37 ± 2.30 in severe alcoholics. In few other studies like that done by Shetty S. *et al.*[45] and Khare S. *et al.*[46], mean Hb was found to be 10.46 g/dl and 7.64 g/dl respectively suggesting the presence of anemia in ALD patients.

2) RBC count:

Our study population showed decrease in the mean RBC count with a value of 3.06 ± 0.908 similar to study done by Das S.K. *et al.*[2], where the mean RBC count was found to be 3.7 ± 1.00 in the ALD cases. Another study done by Seitz H.K. *et al.*[47] also showed reduced RBC count with the mean value of 3.2 ± 9.5.

3) PCV:

The mean value of haematocrit or PCV was found to be 28.15 ± 7.480% similar to Das S.K. *et al.*[2], with a mean value of 33.7 ± 8.40%. In the study of Khare S. *et al.*[46], it was found to be 26.67 ± 9.85%.

4) MCV:

Most of the studies show significantly high value of MCV suggesting macrocytosis induced due to alcohol abuse like in one of the studies done by Oduola T. *et al.*[20]. In a study done by Chauhan N. *et al.*[48], MCV level was increasing with increase in severity of the disease but in our study there was no significant change noted in the value of MCV.

5) MCH and MCHC:

MCH and MCHC values can also be deranged in ALD. In the present study, male patients showed no significant change in MCH values (p=0.751), but it was slightly increased in the female patients (p=0.008). This finding is correlating with the study done by Hanumanth R.G. *et al.*[49], where there was no significant change seen in the value of MCH. However, MCHC values showed mild significant decrease in both the sexes (p<0.001) with mean value of 32.87 ± 4.20 g/dl similar to the study done by Raviteja V. *et al.*[42] where it was 32.1 ± 2.68 g/dl.

6) Total count:

Leucopenia in alcoholic liver disease can be due to decrease in granulocyte and granulocyte monocyte colony stimulating factors.[42] In the present study, the total leucocyte count was reduced ranging from 460-33720 cells per mm^3 with a mean value of 10969.14 ± 7286.76 cells per cumm. Similar findings were found in the study done by Raviteja V. *et al.*[42], where the total count ranged from 900-24,900 cells per mm^3 with the mean of 7755 ± 4661 cells per mm^3 in the study population. In another study done by Thinnahanumaih M. *et al.*[50], the mean value was found to be 6500 ± 2000 cells per mm^3 amongst the short-term moderate alcohol drinkers.

7) Differential count:

In the present study, the major change in the differential count was seen as neutrophilia in 31.43% and lymphopenia in 61.4% of cases. This finding was similar to the study done by Raviteja V. *et al.*[42], where 29.2% cases had neutrophilia and 17.6% cases had lymphopenia.

8) Platelet count:

Reduced number of platelet count was a significant finding in our study with 68.57% of cases and the mean platelet value was found to be 1.37 ± 1.307 lakh/µl. Study done by Raviteja V. *et al.*[42] also demonstrated similar findings of 70% cases of thrombocytopenia and the mean value of 3.10 ± 7.422 lakh/µl.

Several causal factors of thrombocytopenia includes hypersplenism leading to splenic platelet sequestration, marrow suppression mediated by toxins like alcohol, hepatitis B, chronic hepatitis C infection or antiviral treatment with interferon-based therapy. Reductions in the level or activity of the hematopoietic growth factor-thrombopoietin (TPO) is also thought to play a role. TPO is produced by the liver, kidney, muscle and bone marrow but its synthesis is predominantly dependent on the hepatic function as it is constitutively expressed by hepatocytes.[51, 52] It stimulates the production and differentiation of megakaryocytes into mature platelets, hence defect in either of its synthesis or release can also lead to thrombocytopenia.[53] A raised level of platelet associated immunoglobulin is also found in patients with alcoholic cirrhosis. Overall, the mechanism of thrombocytopenia in liver disease could be due to:

i. Shortened mean platelet life span
ii. Platelet pooling due to hypersplenism
iii. Inability of the bone marrow to compensate
iv. Reduced production of TPO
v. Platelet associated immunoglobulin.

However, no clear relationship between the abnormalities of platelet kinetics and severity of liver disease could be established. Also, there is a growing evidence of

defective platelet function in ALD and possibility of impaired aggregation by intrinsic platelet defects and circulating inhibitors of aggregation.[54]

9) Severity of anemia:

In the present study, it was seen that 24.28% of patients had severe anemia while 17.14% of patients manifested with moderate anemia. This finding was similar to the study done by Patel N. *et al.*[55], where they found 18.2% and 56.1% patients with severe and moderate anemia respectively. Another study done *by* Khan F. *et al.*[56] showed similar findings with 26.1% cases each of moderate and severe anemia.

The presence of anemia in patients of ALD can be due to suppression of bone marrow by inflammatory cytokines, hemodilution, decrease in erythropoietin levels, folic acid and vitamin B12 deficiency or defective synthesis of coagulation factors by injured hepatocytes.[55] Occasionally, upper and lower GI bleeding due to portal hypertension resulting from alcoholic cirrhosis can also lead to anemia.[56]

Alcohol exerts a direct toxic effect on the bone marrow resulting in vacuolization of the bone marrow precursor cells leading to anemia. This finding is of utmost importance as it may mimic and obscure other disorders.[57]

THE CHANGES IN THE COAGULATION PROFILE

Defects in the coagulation system is not uncommon in Alcoholic liver disease. Alcohol contributes to derangement in the coagulation process by various factors that include thrombocytopenia, functional defects in platelets, decreased levels of circulating coagulation factors and endothelial dysfunction.[58] Reduced hepatic synthesis of all the coagulation factors (except factor VIII & von willibrand factor), deficiency of vitamin K, hyperfibrinolysis resulting from reduced hepatic synthesis of inhibitors and dysfibrinogenemia, all can contribute to increased bleeding tendency. Few studies have shown that in addition to the diminished synthesis of clotting factors, patients can also have deficiency of natural anticoagulants, like protein C (a protein synthesized by the liver) mainly, and also of anti-thrombin, which may counterbalance the bleeding tendency to lesser or greater extent caused by the deficiency in procoagulants. Therefore, the clinical status of the patient should be treated and not the laboratory parameters while correcting coagulopathy per se in a patient with liver disease. [44]

Alcohol abuse can directly induce anti-hemostatic changes in the body and indirectly worsen the coagulopathy of liver cirrhosis.[58] Thus, prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the most commonly used tests for identifying and monitoring coagulopathy. These values are of significance with respect to predicting risk of hemorrhagic event in chronic liver patients.[59]

1) Prothrombin time (PT):

PT is measured as the time needed for the platelet-poor plasma to clot after the addition of tissue extracts (thromboplastin) and calcium chloride. It is an indicator of the extrinsic pathway and determines the Vitamin K-dependent extrinsic factors

II,V,VII,X, and fibrinogen. The extent of derangement in PT correlates with the severity of liver injury.[60] In our present study the prolongation of PT value was seen in 71.43% of patients with a mean value of 22.191 ± 8.445 seconds, similar to study done by Raviteja V. *et al.*[42] where PT was prolonged in 71.5% cases with mean value of 18.7 ± 4 seconds.

2) Activated partial thromboplastin time (aPTT):

It is the time taken for the platelet-poor plasma to clot when mixed with an activator of the contact factors (factor XII, pre-kallikrein, and high-molecular-weight kininogen) and phospholipids. It is an indicator of intrinsic and common pathways of coagulation cascade, which depend on factor VIII, IX, XI and XII and those of the contact system.[60] Similar to PT, aPTT value was also prolonged in 75.71% of our study population with mean value of 59.383 ± 6.889 seconds. This correlated with the study of Raviteja V. *et al.*[42] where aPTT was prolonged in 46.2% cases with the mean value of 38.26 ± 7.51 seconds.

This study observed and highlighted the nature and diversity of changes and several correlations between few variables of complete blood count, which is one of the readily available laboratory tests helpful in determining and evaluating problems caused from alcohol abuse.[61] Liver being the site for extramedullary haematopoiesis, synthesis of coagulation proteins and the major storage site of iron, vitamin B12 and folic acid; liver disease is commonly associated with a wide range of haematological abnormalities. Pancytopenia, megaloblastic anemia, increased PT, aPTT are some characteristic hematological abnormalities in ALD.[62] These changes occur due to alteration in the normal bone marrow function. In a homeostatic condition, HSCs respond to infectious and inflammatory signals by delivering emergency immune

related cells through their proliferation and differentiation. In advanced cirrhosis, bone marrow HSCs are compromised and fail to react in their full capacity against persisting or acquired opportunistic infections, leading to complications of overwhelming sepsis in later stages of liver disease.[63]

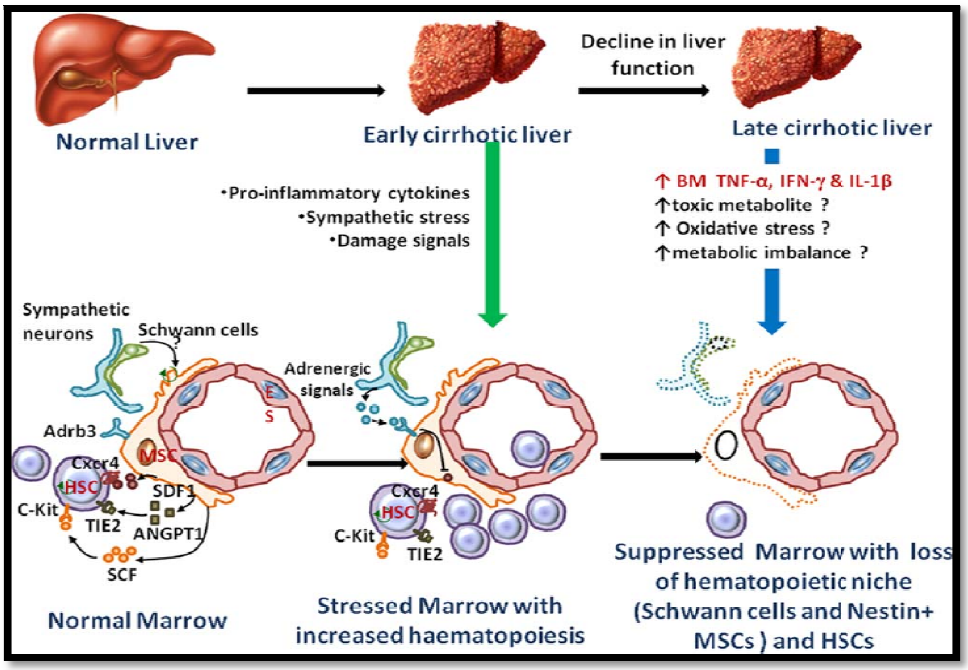

Figure 31: Schematic diagram of possible mechanism of cytokine stress-mediated suppression of marrow HSCs and niche cells in advanced cirrhosis.

Though in our present study, the type of anemia was not studied and the insignificant change in MCV values also did not confirm megaloblastic change.[64] Patients with ALD and cirrhosis can also suffer from both hemorrhagic and thrombotic complications. Few studies have described the changes in primary hemostasis in patients with ALD and cirrhosis but are still subject to ongoing debate.[65] However, the classical laboratory abnormalities may be minimal or even

absent in patients with established ALD. Liver biopsy is considered to be the gold standard in the diagnosis of ALD, but it is rarely performed in clinical practice.[64] Therefore, ALD should be accurately diagnosed and demands medical attention for improved survival which it can be best done by encouragement of alcohol cessation though brief interventions, providing specific nutrition and addition of medical therapy as required.[66]

SUMMARY

A prospective study was done to assess the changes in hematological parameters and coagulation profile in patients of Alcoholic liver disease that can help in providing timely correction of any deranged parameter, prevent progression of the disease, any severe complications and improve the quality of life in these patients

The study was undertaken during the period of 1st November, 2018 to 31st May, 2020 in the hematology laboratory of the Department of Pathology, B.L.D.E (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. All patients were admitted in the hospital and treated according to their manifestations.

Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, that included Hb, RBC count, PCV, MCV, MCH, MCHC, Total count, Differential count and Platelet count using an automated 5-part differential hematology analyzer (SYSMEX XN-1000). Two ml of blood sample was taken in citrate bulb and was analysed for both PT and aPTT using the coagulometer (ACL ELITE PRO).

The salient features observed in this study are:

- Patients diagnosed with Alcoholic liver disease were maximum in the age group of 50-60 years with almost all being in the 30-60 years range. The mean age was observed to be around 44 years.

- 67 patients amounting to 95.8% were males and only 3 patients (4.2%) were females.
- All the male patients had statistically significant decrease ($p<0.001$) in the values of Hb, RBC count, PCV, MCHC and statistically significant decrease ($p=0.004$) in the platelet count. No statistically significant difference were observed in the values of MCV and MCH.
- All the female patients had statistically significant decrease in the values of RBC count ($p=0.015$), PCV ($p<0.001$) and MCHC ($p<0.001$) with mild increase in the value of MCH ($p=0.008$). Also, statistically significant decrease ($p=0.0004$) in the platelet count was noted. The values of Hb and MCV showed no significant change.
- The mean Total WBC count was 10932.68 amongst the males which was lower than the normal range, while it was 11783.33 which was normal amongst the female patients.
- On correlation of Hb with platelet count, mild positive correlation was observed between both the variables. However, no correlation was seen with other parameters like neutrophil count and MCV.
- Neutrophil count also showed mild positive correlation with platelet count and moderate positive correlation with total WBC count.
- Negative correlation was observed between platelet count and the variables of coagulation profile (PT and aPTT).
- Anemia was one of the major findings in ALD patients. 10% cases had life threatening anemia, 24.28% had severe anemia and 17.14% had moderate anemia. Also, ALD patients who had anemia were maximum in the elderly age group of 50-59 years followed by 40-49 years.

- Reduced number of platelet count was noted in 48/70 patients which amounts to 68.57%, with mean and median value of 1.37 and 1.02 lakh/µl, respectively.
- The derangement in differential count also plays a significant role in ALD cases. In our study, 31.43% of cases had neutrophilia and 61.4% cases had lymphopenia,
- Coagulation profile analysis is a reliable clue of hepatic function. Prolonged values of PT and aPTT were seen in 71.43% and 75.71% patients, respectively indicating the degree of hepatic injury.

Hence, monitoring of hematological parameters in ALD patients not only helps to identify the disease but is also important in determining the other co-existing factors responsible for deteoriation of the patients. It also helps in differentiating alcoholic liver disease from other non-alcoholic causes of liver diseases. Hence, it is important to regularly assess these parameters for appropriate management of such cases in order to decrease complication associated mortality.

CONCLUSION

In a country like India, where alcoholism has become a lifestyle, development of Alcoholic liver disease is not uncommon. It is highly prevalent in the general population and the initial stages with less severity are mostly undetected and remains neglected unless complicated. So, it is mandatory to identify such cases as soon as possible and prevent them from advanced problems. Our focus lies on reducing the morbidity and mortality of the patients, hence improving the quality of life.

The increasing burden of alcohol abuse can deteoriate the heath of people in multiple ways. Thus, counselling of such patients in conjunction with medical treatment plays a very crucial role in reversal of disease process. Abstinence from alcohol and proper nutrition management is an unavoidable side-line therapy for these patients to prevent the disease progression.

However, in advanced cases like alcoholic cirrhosis with complications, proper treatment becomes the only saviour. Liver transplantation should be advocated, wherever possible as the last resort of survival in life-threatening cases.

The ALD patients show significant changes in their hematological parameters where the values of Hb, RBC count, PCV, MCV, MCH, MCHC, Total count, Differential count are usually deranged. Also, deranged values of prothrombin time and activated partial thromboplastin times indicates defect in hepatic function. Therefore, the above mentioned parameters should also be routinely evaluated in ALD patients to prevent associated complications.

It is very important to keep a check on the general health of patients with ALD apart from management in order to prevent the advancement of liver injury.

Furthermore, it necessitates the need to provide them with better quality and longer span of life that is free of treatment-related complications so that they can contribute to decrease the burden on society. Hence, we conclude that better management of ALD can be provided by early detection and continuous monitoring of the parameters evaluated in the present study so as to reduce severity of the disease and lead a normal life.

LIMITATIONS OF THE STUDY

1. The sample size was too small.

2. We could not categorize the study population into different stages of Alcoholic liver disease.

3. The peripheral smears were not evaluated to determine the type of anemia.

4. The INR (international normalized ratio) was not calculated, which could have provided a more clear picture of the coagulation system.

REFERENCE CHART OF NORMAL VALUES[67]

PARAMETERS (Unit)	NORMAL VALUES Mean ± SD
Hb (g/dl)	Men- 15 ± 2 Women- 13.5 ± 1.5
RBC count ($x10^6$ cells/mm^3)	Men- 5.0 ± 0.5 Women- 4.3 ± 0.5
PCV (%)	Men- 45 ± 5 Women- 41 ± 5
MCV (fl)	92 ± 9
MCH (pg)	29.5 ± 2.5
MCHC (g/dl)	33 ± 15
Total WBC count	4000-11000
Differential count Neutrophils Lymphocytes Monocytes Eosinophils Basophils	 40-80% 20-40% 2-10% 1-6% <1-2%
Platelet count (lakh/µl)	2.8 ± 1.3

PARAMETERS (in seconds)	NORMAL VALUES Mean ± SD
PT	11-16 seconds
aPTT	26-40 seconds

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ANNEXURES

ANNEXURE-I

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E (Deemed to be University)
SHRI.B.M.PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
VIJAYAPUR – 586103

Ec/No: 286/2018
17-11-2018

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2018 at 03-15 PM scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title : A study of changes in hematological parameters in alcoholic liver disease.

Name of P.G. Student : Dr Shilpy Bajaj.
Department of Pathology.

Name of Guide/Co-investigator: DrPrakash.M.Patil, Associate Professor of Pathology.

DR RAGHAVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
B.L.D.E(D) Shri B.M. Patil
Medical College, Bijapur-586103.

Following documents were placed before E.C. for Scrutinization:

1) Copy of Synopsis/Research Project
2) Copy of informed consent form.
3) Any other relevant documents.

ANNEXURE- II

B.L.D.E.U.'s SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: "A STUDY OF CHANGES IN HEMATOLOGICAL PARAMETERS IN ALCOHOLIC LIVER DISEASE"

PRINCIPAL INVESTIGATOR: Dr. Shilpy Bajaj
P.G.
Department of Pathology

P.G. GUIDE: Dr. Prakash M. Patil
M.D,
Associate Professsor
Department of Pathology

P.G. CO-GUIDE: Dr. Anand Ambali
M.D,
Professsor
Department of Medicine

RISK AND DISCOMFORTS:

I understand that, there are risks involved in the procedures performed like continued pain at the procedure site, infection.

BENEFITS:

I understand that my participation in the study will help in assessing the severity of Alcoholic liver disease and help prevent progression of the disease process.

CONSENT:

I, the undersigned,_____ , S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr _____ of _____ Hospital has examined me thoroughly on _____ at _____BLDE_____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following

diseases. Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to

treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE-111

PROFORMA FOR THE STUDY

NAME : OP/IP No. :

AGE :

SEX : D.O.A :

RELIGION : D.O.D :

OCCUPATION :

RESIDENCE :

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

General physical examination:

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphadenopathy present/absent

Edema present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

CBC

COAGULATION PROFILE

CBC:

PARAMETERS (Unit)	**NORMAL VALUES Mean ± SD**	**ALCOHOLIC LIVER DISEASE (n=70) Mean ± SD**	**p-value**
Hb (g/dl)			
RBC count ($x10^6$ cells/mm^3)			
PCV (%)			
MCV (µm3)			
MCH (pg)			
MCHC (g/dl)			
Total WBC count			
Differential count **Neutrophils** **Lymphocytes** **Monocytes** **Eosinophils** **Basophils**			
Platelet count			

(lakh/µl)			

COAGULATION PROFILE:

PARAMETERS (in seconds)	**NORMAL VALUES Mean ± SD**	**ALCOHOLIC LIVER DISEASE (n=70) Mean ± SD**	**p-value**
PT			
aPTT			

KEY TO MASTER CHART

M	Male
F	Female
Hb	Hemoglobin
RBC	Red blood cell
PCV	Packed Cell Volume
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
N	Neutrophils
L	Lymphocytes
M	Monocytes
E	Eosinophils
B	Basophils
PT	Prothrombin time
aPTT	Activated partial thromboplastin time

MASTER CHART

CASES

Sl. no.	IP no.	Name	Age (yrs)	Sex	Hb (g/dl)	RBC count (x10^6 cells/cu mm)	PCV (%)	MCV (µm^3)	MCH (pg)	MCHC (g/dl)	Total WBC count	Differential Count					Platelet Count	Coagulation Profile	
												N	L	M	E	B		PT	aPTT
1	1425/19	Tulajaramsingh Narasingh Rajput	45	M	11.9	3.91	36.3	92.8	30.4	32.8	19640	85.5	8.7	5.3	0.3	0.2	0.63	35.2	67.0

2	3021/19	Avvappa Madivalappa	38	M	7.0	1.70	20.9	122.9	41.2	33.5	10070	81.5	14.5	3.6	0.2	0.2	0.99	22.2	58.2
3	3204/19	Jagadesh Kaanchappa Konnur	40	M	7.2	2.58	22.4	86.8	27.9	32.1	16750	79.1	13.4	6.0	1.4	0.1	1.01	17.7	41.5
4	3821/19	Mallanna Basangouda Biradar	55	M	9.7	2.90	29.9	103.1	33.4	32.4	6070	71.1	19.3	6.9	2.5	0.2	0.6	29.4	50.0
5	5534/19	Ashfaq Sheikchand Tikoti	40	M	9.9	3.09	30.6	99.0	32.0	32.4	9730	75.7	15.8	6.9	1.4	0.2	1.91	14.2	37.1
6	5616/19	Laxman Chandru Pawar	32	M	10.1	3.06	31.9	104.2	33.0	31.7	21280	93.2	3.0	3.6	0.1	0.1	2.02	59.6	89.7
7	6005/19	Saleem Matabsab Kotnal	49	M	12.5	4.02	35.3	87.8	31.1	35.4	8860	78.0	13.3	7.7	0.7	0.3	1.48	13.3	46.5
8	6317/19	Shrikant Somanna Malmi	50	M	7.4	2.22	22.8	102.	33.3	32.5	21560	91.6	3.6	4.3	0.4	0.1	0.84	25.7	50.8
9	6320/19	Rajashekhar Shanmukhappa Shetagar	54	M	7.2	2.28	21.8	95.6	31.6	33.0	9050	72.0	17.5	9.9	0.3	0.3	1.48	31.9	74.2
10	7256/19	Goudappagouda Basavantray Potareddi	45	M	7.2	2.72	22.7	83.5	26.	31.7	4600	70	24	04	02	00	0.48	27.4	65.5
11	8984/19	Gurulingappa Malakajappa Rampur	38	M	7.0	2.20	21.4	97.3	31.8	32.7	8120	68.3	20.7	5.7	5.2	0.1	1.13	33.7	72.3
12	9408/19	Hanamanth Chandram Her	55	M	9.8	2.92	29.6	101.4	33.6	2.55	23840	91.8	4.9	2.9	0.2	0.2	2.55	17.1	47.9
13	10569/19	Irshad Bandagisab Bepari	35	M	14.4	6.13	47.6	77.7	23.5	30.3	15870	85.2	9.1	5.3	0.1	0.3	2.1	12.1	31.3

14	10670/19	Sabanna Fakirappa Mopagar	56	M	5.6	3.45	19.1	55.4	16.2	29.3	20670	88.1	6.4	4.9	0.5	0.1	1.67	23.0	65.6
15	10777/19	Somnath Revansiddappa Ijeri	24	M	13.1	4.2	39.3	89.6	28.2	32.5	8700	70.5	25.4	2.6	1.5	00	3.06	11.2	32.6
16	11147/19	Shivanand Revappa Malipatil	35	M	12.5	4.6	37.5	86.2	29.1	32.5	9800	57.5	36.8	3.5	2.2	00	3.03	13.9	37.1
17	11323/19	Prakash Guttappa Sajjan	38	M	13.5	4.1	40.5	91.7	28.3	32.5	10200	56.4	34.6	3.8	5.2	00	3.41	12.5	40.0
18	11690/19	Rajakumar Bhimaraya Biradar	40	M	13.2	5.3	42.2	79.6	24.9	31.3	17690	74.1	20.1	5.4	0.2	0.2	3.53	13.1	36.0
19	12024/19	Kallappa Basappa Sarwad	55	M	12.3	4.0	37.8	88.2	29.1	33.5	9500	67.6	28.9	1.8	1.7	00	2.20	12.4	39.2
20	12782/19	Rukamawwa Appanna Karalatti	70	F	12.0	3.71	37.7	101.6	32.3	31.8	10000	64.0	29.2	4.9	1.8	0.1	0.52	17.9	36.6
21	12811/19	Somaninga Gurusangappa Sakari	37	M	8.1	2.14	23.4	109.3	37.9	34.6	23630	91.2	2.9	4.6	1.2	0.1	0.35	27.1	64.4
22	12820/19	Umesh Siddappa Kolhar	40	M	6.8	4.1	20.4	71.8	22.6	24.8	7600	58.7	37.3	2.8	1.2	00	2.12	32.6	49.5
23	13397/19	Prakash Siddaraya Ainapur	27	M	15.6	5.23	46.7	89.3	29.8	33.4	15440	92.8	5.4	1.7	00	0.1	0.69	32.2	67.0
24	14894/19	Manjunath Appasaheb Nidoni	30	M	7.8	2.46	25.7	104.5	31.7	30.4	24380	58	10	05	00	00	0.55	37.9	90.2
25	14999/19	Basavaraj Gurulingappa Shetagar	46	M	7.7	2.16	21.8	100.9	35.6	35.3	8990	81.6	9.6	8.8	00	00	0.67	16.1	41.5

26	15561/19	Vittal Baburaya Biradar	54	M	7.2	2.35	21.4	91.1	30.6	33.6	5840	79	14	06	01	00	0.33	16.9	30.5
27	16430/19	Sharanamma Sidappa Harapad	55	M	6.3	2.14	19.3	90.2	29.4	32.6	1880	68.7	21.8	7.4	2.1	00	0.37	27.5	45.1
28	16692/19	Gururaj Somaling Teli	30	M	9.0	2.32	26.5	114.2	38.8	34.0	4090	78.5	16.1	5.4	00	00	0.34	19.0	61,6
29	17191/19	Mahantesh Basavaraj Tavase	40	M	11.0	2.84	33.0	116.2	38.7	33.3	5390	58.1	32.7	4.3	4.5	0.4	0.85	17.8	44.4
30	17579/19	Ravi Dasharath Kamble	35	M	10.2	3.23	31.0	96.0	31.6	32.9	10230	87.4	5.9	6.1	0.5	0.1	2.67	18.8	43.2
31	18146/19	Kallappa Bhimappa Uppar	55	M	9.9	3.24	31.4	96.9	30.6	31.5	7020	51.0	35.5	8.4	4.7	00	1.14	14.2	40.1
32	18238/19	Babugouda Shankargouda Patil	54	M	6.5	2.08	20.0	96.2	31.3	32.5	3020	65.2	23.2	6.3	5.3	00	0.57	27.9	52.2
33	18584/19	Prakash Mahadevappa Daded	36	M	6.4	1.89	18.9	100.0	33.9	33.9	3830	62.1	26.9	9.7	1.0	00	0.33	29.5	79.7
34	19403/19	Kumaresh Virupakashappa Kanur	40	M	10.3	3.45	29.7	86.1	29.9	34.7	10410	66.3	25.4	5.6	2.1	0.6	0.96	14.8	45.5
35	20534/19	Basavaraj Dundappa Bilagi	54	M	9.0	2.95	27.7	93.9	30.5	32.5	3280	49.1	38.4	5.8	6.7	00	0.32	23.5	67.8
36	20950/19	Anasuya Basappa Adahalli	30	F	11.4	4.29	37.1	86.5	26.6	30.7	14900	90.8	6.2	2.9	00	0.1	1.72	14.0	37.9
37	20919/19	Dattu Shivaji Pawar	49	M	6.5	2.16	21.3	98.6	30.1	30.5	4710	63.9	23.4	9.1	3.2	0.4	1.08	19.0	46.6
38	2096	Babugouda	54	M	6.1	1.87	19.1	102.1	32.6	31.9	2760	57.5	28.3	8.0	5.8	0.4	0.79	13.6	45.1

	3/19	Shankargouda Patil																	
39	21187/19	Iranna Hanamanth Hadapad	32	M	9.1	2.95	27.2	92.2	30.8	33.5	28760	75	11	04	01	00	3.09	28.2	70.4
40	21197/19	Chandarawwa Dannappa Gadanchi	50	F	8.4	2.71	26.8	98.9	31.0	31.3	10450	70.3	17.3	6.4	5.7	0.3	1.12	14.2	42.9
41	22360/19	Tikaram Sapshingh Rajaput	36	M	9.1	2.69	30.2	112.3	33.8	30.1	9730	72.5	17.1	8.4	1.6	0.4	0.52	21.4	52.21
42	23568/19	Banyappa Kalappa Talawar	28	M	8.8	2.61	25.1	96.2	33.7	35.1	15100	80.3	12.5	6.5	0.5	0.2	1.32	17.8	35.8
43	36851/19	Ravoosab Shrishail Biradar	39	M	8.3	2.72	24.0	88.2	30.5	34.6	12860	70.2	20.6	7.8	1.2	0.2	1.15	25.3	56.9
44	37649/19	Kaseem Chandasab Chandkavathe	58	M	9.1	2.72	26.0	95.6	33.5	35.0	4950	90.3	7.3	2.4	00	00	0.43	26.0	43.9
45	37830/19	Babu Sakru Rathod	46	M	9.8	4.12	28.0	68.0	23.8	35.0	33720	84.2	11.3	2.6	1.7	0.2	2.97	25.7	30.4
46	37923/19	Hanamanth Shivappa Shegunashi	78	M	11.7	3.51	34.1	97.2	33.3	34.3	8420	72.4	19.5	7.0	1.0	0.1	0.62	30.2	57.2
47	40890/19	Mallappa Baladandappa Sanapur	55	M	9.6	3.35	29.9	89.3	28.7	32.1	5100	51.8	31.6	8.4	7.6	0.6	1.03	14.3	47.3
48	41120/19	Jagadeesh Annappa Ballolli	40	M	9.5	2.33	27.2	116.7	40.8	34.9	4610	44.2	49.9	3.3	2.4	0.2	0.95	21.8	53.2
49	4237	Sureshshing	50	M	12.5	3.83	36.1	94.3	32.6	34.6	8210	72.9	15.0	10.	1.0	0.4	1.22	15.7	39.8

	2/19	Sujeetshing Shing												7					
50	4380 0/19	Basavaraj Shivanand Awaji	32	M	6.4	2.02	20.6	102.0	31.7	31.1	2660	71.4	17.7	7.5	3.4	00	0.86	12.1	37.6
51	4401 7/19	Ashok Revanasiddappa Malagar	45	M	11.9	3.21	34.1	106.2	37.1	34.9	460	45.7	50.0	4.3	00	00	0.39	27.9	48.1
52	341/2 0	Ashok Nagappa Agasar	45	M	10.1	3.21	29.6	92.2	31.5	34.1	6030	77.1	12.1	9.5	1.0	0.3	0.61	13.7	36.8
53	1143/ 20	Kashinath Gurupadappa Maddi	40	M	13.7	3.91	37.8	96.7	35.0	36.2	17930	66	16	02	02	0.1	0.53	39.7	78.5
54	1537/ 20	Maharaja Ramachandra Kalagi	42	M	6.6	1.96	18.6	94.9	33.7	35.5	12400	80	14	02	03	01	1.06	25.7	45.0
55	2176/ 20	Gurusiddayya Jambayya Hiremath	56	M	9.9	3.32	28.7	86.4	29.8	34.5	20010	87.3	10.1	2.5	00	0.1	0.8	13.5	42.1
56	3044/ 20	Subhash Kadappa Daduti	40	M	7.2	2.66	19.4	72.9	27.1	37.1	22580	91.8	5.1	3.0	00	0.1	2.02	19.6	48.7
57	3798/ 20	Raju Chandrakant Biradar	35	M	9.1	2.81	27.8	98.9	32.4	32.7	9370	79.7	17.0	2.9	0.2	0.2	1.58	18.8	45.5
58	4551/ 20	Vasant Shankareppa Biradar	50	M	12.4	4.02	35.9	89.3	30.8	34.5	5950	62.9	24.5	7.7	4.7	0.2	1.5	12.5	32.4
59	4616/ 20	Suresh Shivappa Oji	43	M	9.1	2.97	26.2	88.2	30.6	34.7	8320	74.5	17.4	6.6	0.8	0.7	1.76	20.7	56.3
60	5333/ 20	Keshu Hunnu Naik	54	M	8.4	2.41	24.5	101.7	34.9	34.3	4660	56.3	19.1	9.2	15.0	0.4	0.66	29.4	67.7

61	5354/20	Pradeep Shanamukappa Jogur	51	M	11.9	3.55	32.9	92.7	33.5	36.2	13980	84.2	8.9	4.6	1.9	0.4	1.97	22.5	58.0
62	5406/20	Laxman Mallangouda Hiredesai	54	M	7.6	2.87	23.1	80.5	26.5	32.9	9790	81.7	11.6	5.7	0.9	0.1	2.2	27.8	57.4
63	5644/20	Nagappa Shankreppa Yaranal	52	M	11.6	3.72	33.1	89.0	31.2	35.0	8890	85.0	9.1	2.6	3.1	0.2	0.41	17.5	54.6
64	5921/20	Arasagonda Parappa Yashavanta	57	M	6.3	1.99	17.9	89.9	31.7	35.2	5210	61.4	21.5	12.1	4.8	0.2	0.59	22.7	59.9
65	6342/20	Babagouda Shidagondappa Biradar	61	M	9.6	2.92	27.5	94.2	32.9	34.9	8260	70.7	23.8	3.4	2.1	00	1.17	23.5	62.6
66	9042/20	Siddaling Karalingappa Pujari	24	M	4.2	1.73	12.9	74.6	24.3	32.6	2820	81.2	10.6	7.1	1.1	00	0.68	29.5	61.8
67	9148/20	Kutbuddin Mohamadsab Yelapur	44	M	9.6	3.05	27.5	90.2	31.5	34.9	7980	67.9	21.9	8.3	1.6	0.3	0.87	25.0	54.4
68	10014/20	Shreeshail Yallappa Yaandigeri	56	M	6.8	1.97	18.1	91.9	34.5	37.6	5370	75.4	15.1	9.1	0.4	00	1.30	22.3	59.8
69	10159/20	Shivaji Gopal Kambale	30	M	13.6	4.15	39.0	94.0	32.8	34.9	28750	86.2	6.0	3.7	3.7	0.4	9.68	14.9	42.2
70	10775/20	Shrishail Ramappa Pated	50	M	7.3	2.46	19.6	79.7	29.7	37.2	5110	58.1	33.1	4.9	3.7	0.2	0.74	27.6	51.6