"ASSOCIATION BETWEEN ALDEHYDE DEHYDROGENASE 2 (G1U504LYS) POLYMORPHISM AND ALCOHOLIC LIVER DISEASE"

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LIST OF ABBREVIATIONS

ALD	:	Alcoholic liver disease
ALDH	:	Alcohol dehydrogenase
CVD	:	Cardiovascular disease
CNNAC	:	Culture negative non-neutrocytic bacterascites
FAEE	:	Fatty acid ethyl esters
GLU	:	Glutamic acid
HRS	:	Hepato renal syndrome
LYS	:	lysine
MNB	:	Monobacterial non neutrocytic ascites
PCR	:	Polymerase chain reaction
SAAG	:	Serum ascitic albumin gradient
SBP	:	Spontaneous bacterial peritonitis
SNP	:	Single nucleotide polymorphism
TNF	:	Tumor necrosis factor
UTR	:	Untranslated region
WHO	:	World Health Organization

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ABSTRACT

TITLE: ASSOCIATION BETWEEN ALDEHYDE DEHYDROGENASE 2 (GLU504LYS) POLYMORPHISM AND ALCOHOLIC LIVER DISEASE.

NEED FOR THE STUDY:

Based on data from the Globe Health Organization report on alcohol, alcoholic liver disease (also known as ALD) is one of the most significant issues affecting the world today and is the principal cause of at least sixty of the most significant forms of systemic disorders. Consuming between 60 and 80 grammes of alcohol on a daily basis for males and 20 grammes of alcohol for women over a period of ten years or longer is associated with an advanced type of liver disease developing in approximately forty percent of individuals who develop liver illnesses (Alba et al. 2014).

The metabolic breakdown of alcohol into acetaldehyde, which is mostly catalysed by alcohol dehydrogenase, is the means by which alcohol is removed from the body (ADH). A following reaction involving aldehyde dehydrogenase (ALDH) results in the formation of acetate from acetaldehyde.

A SNP in the ALDH2 exon 12 gene predicts LYSINE rather than GLUTAMIC ACID at position 504.The acetaldehyde metabolism of an isoenzyme produced by the 504LYS allele is constrained by its catalytic inactivity. [(4)] .

Due to decreased acetaldehyde clearance, individuals with the Lys allele experience side effects include nausea,flushing and vomiting. Due to negative drinking-related effects,

individuals with this gene may have a lower chance of excessive consumption [4]. Very few studies are done to find out the presence of this allele in ALD subjects.

OBJECTIVE: To ascertain the relationship between the Glu504Lys polymorphism in Alcohol Dehydrogenase 2 and Alcoholic Liver Disease (ALD) and the 504Lys protective effect against ALD.

MATERIALS AND METHODS:

Our study was a hospital-based cross-sectional study conducted on 64 patients in which 32 are cases diagnosed with alcoholic liver disease and 32 are controls, who are admitted in BLDEDU Shri B M Patil Medical College and Research Centre, Vijayapura, after getting approval from institutional ethical committee.

RESULTS:

The study was designed to identify single nucleotide polymorphisms (SNP), in the ALDH2 gene at position 504 in all subjects.

We found that prevalence of the common form of 504glu(glu/glu) is seen in 50% of ALD patients and 46.9% in controls ($p = 0.802$). In the control group, 53.7% had a heterozygous (glu/lys) genotype, compared to 50% in the ALD group.

We also found mutations in exon 13 of ALDH2 gene in 2 subjects who have lesser amount and shorter duration of alcohol consumption.

CONCLUSION:

Depending on the quantity and frequency of alcohol use, alcohol use is one of risk factors for health issues and injuries like liver diseases and liver cancer.

There was no statistically significant correlation between the incidence of ALDH2 Glu504lys variations and alcoholic liver disease (p=0.802).

However,we found out mutations in exon 13 of ALDH2 gene, in which patients had less amount and shorter duration of alcohol consumption and had alcoholic liver disease which is statistically significant.

We can conclude that mutations in those regions are responsible for predisposition of early disease.Early Genetic analysis in selected population for finding these mutations may prevent the occurrence of the disease by motivating for abstinence from alcohol.

INTRODUCTION

One of risk factors for health issues like illnesses, and cancer of liver is excessive alcohol drinking. The relationship of alcohol consumption and liver diseases is well established, and alcohol has been shown to have an ability to cause hepatocellular damage through ethanol metabolism-associated mechanisms [(1)].

According to WHO about 3 million deaths were attributed to alcohol consumption. Alcoholic liver disease (ALD) has been estimated to account for 48% of all deaths from cirrhosis.

The threshold for developing alcoholic liver disease is higher in men ≥14 drinks per week, while women are at increased risk for liver injury by consuming ≥7 drinks per week [(2)].

Alcoholic steatosis, which develops in more than 90% of drinkers, can be reversed by abstinence [(4)]. 10-15% of heavy drinkers may develop alcoholic cirrhosis, severe fibrosis, or alcoholic hepatitis if they continue to use alcohol excessively [(3)].

Alcohol is removed from the body by metabolically degrading into acetaldehyde in the liver, primarily through alcohol dehydrogenase (ADH). Aldehyde dehydrogenase (ALDH) then transforms acetaldehyde into acetate (fig 1).

Because the intermediate, acetaldehyde, has the potential to be toxic, the rate at which alcohol is metabolised by ADH, ALDH is crucial in determining its toxicity [(5)].

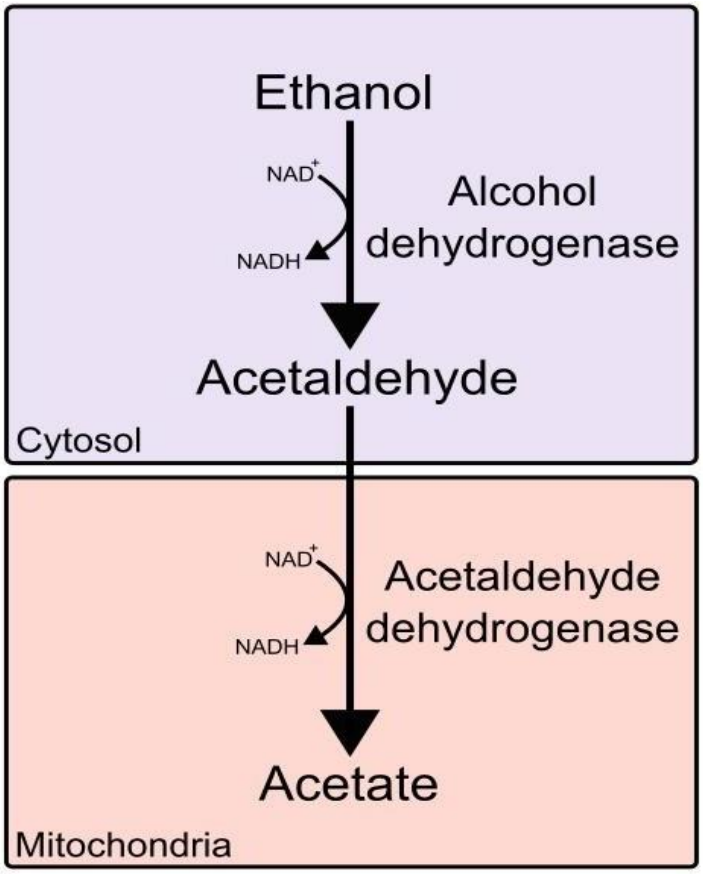


Fig 1-Ethanol metabolism

The two main ALDH isoforms are cytosolic ALDH1 and mitochondrial ALDH2.

Due to acetaldehyde's low molecular weight, mitochondrial ALDH2 among ALDH1 and ALDH2 is crucial for human acetaldehyde metabolism [(5)].

Alcohol dependency and alcohol-induced liver disease were thought to be hereditary conditions that might be affected by the ALDH2 gene.

The ALDH2 gene's exon 12 single nucleotide polymorphism (SNP) predicts LYSINE rather than GLUTAMIC ACID at position 504.. The acetaldehyde metabolism of an isoenzyme is restricted by the 504LYS allele, which creates a catalytically inactive isoenzyme [(6)].

Because of this, individuals who carry LYS gene have decreased ability to excrete acetaldehyde, and frequently experience side effects including NAUSEA, FLUSHING and VOMITING after consuming alcohol. People who carry this gene may be less likely to use alcohol excessively because of negative effects [(5)].

In study conducted by Meera Vaswani et al, concluded that high frequency of the ALDH2 Lys/Lys genotype (among alcohol-dependent subjects) being a risk-conferring factor for AD (alcoholic dependence) [(7)].

keeping in the view of present perspective and there are very few data on genes/polymorphisms that confer susceptibility to ALD in Indian population, this study will be conducted to find the association between ALDH 2 Glu(504)Lys polymorphism and alcoholic liver disease.

AIMS AND OBJECTIVES

To establish relationship between the 504Lys polymorphism in Alcohol Dehydrogenase 2 and Alcoholic Liver Disease and the 504Lys' protective effect.

REVIEW OF LITERATURE

ALCOHOL AND ITS METABOLISM

INRODUCTION [(1)]

Alcohol has psychotropic and poisonous effects, as well as the ability to cause dependence. Many people in the population regularly use alcoholic beverages in many modern societies. This is especially true for those who frequently engage in social interaction in high-profile social settings with societal significance on a global and national scale. The social, and health harm that drinking contributes to in this situation is simple to ignore or discount.

Worldwide, alcohol usage causes 3 million deaths a year in addition to millions more experiencing health problems and disabilities.Globally, hazardous alcohol use is responsible for 5.1% of all illnesses.

For men and women, respectively, the burden of disease worldwide, caused by harmful alcohol use in the proportions of 7.1% and 2.2%. Alcohol use is the leading cause of early mortality and disability among people between the ages of 15 and 49, accounting for 10% of all fatalities in this age group.

Early in life, drinking alcohol results in mortality and disability.In the 20- to 39-year-old demographic, alcohol is responsible for about 13.5% of all fatalities. Disadvantaged and especially vulnerable populations have higher rates of alcohol-related death and hospitalization.

PHARMACOLOGY AND NUTRITIONAL IMPACT OF ETHANOL [8]

Blood levels of ethanol are measured in milligrams or grammes per decilitre (100 mg/dL = 0.10 g/dL, for example), with readings of 0.02 g/dL coming from the consumption of one average drink.

A standard drink weighs 10 to 12 grams overall, as demonstrated by 115 mL (4 oz) of unfortified wine, 340 mL (12 oz) of beer, and 43 mL (1.5 oz) (a shot) of an 80-proof beverage (such as whisky); 0.5 L (1 pint) of an 80-proof beverage contains roughly 160 g of ethanol (about 16 standard drinks), and 750 mL of wine contains roughly 60 g. (fig.2)

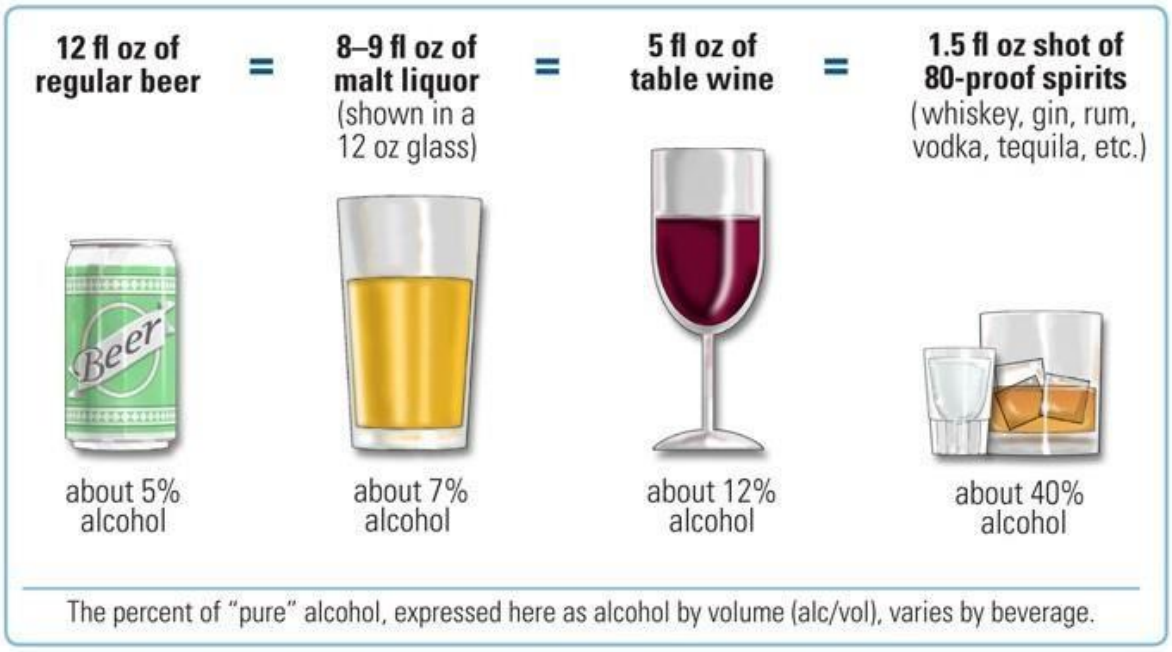


Fig.2: percentage of alcohol in various drinks

These drinks also contain other ingredients (congeners), which change the flavour and may have harmful effects in the body. Methanol,acetaldehyde, histamine, tannins,and lead are examples of congeners.

Alcohol abruptly lowers neuronal activity and shares behavioural effects and cross-tolerance with other depressants, such as benzodiazepines and barbiturates.

Distribution of Alcohol in the Body

The relative water content of tissue affects equilibrium alcohol concentration in that tissue. Alcohol equilibration in a tissue is influenced by its bulk, water content, and blood flow. Although it can pass through biological membranes like water, ethanol is essentially insoluble in fats and oils.

According to how much water each tissue and fluid has, ethanol is distributed from the blood into each one. The relative water content of a tissue affects the concentration of ethanol in that tissue, which soon approaches equilibrium with the plasma concentration of ethanol. Alcohol does not interact to proteins in plasma.

The same amount of alcohol per unit of body weight can result in very varying blood alcohol concentrations in various persons because of the huge variability in the amounts of water and fat that are found in different people's bodies, as well as the low ethanol lipid: water partition coefficient.

Women often have a lesser volume of distribution for alcohol than men do because women generally have a higher percentage of body fat than men do. Women will have higher peak blood alcohol levels than men if they are given the same quantity of alcohol as g per kg of body weight, but there is no difference if they are given the same amount as g per litre of body water.

First pass alcohol metabolism by the stomach, which may be higher in men, may also be a factor in why women's blood alcohol levels are higher than men's [9,10].

Factors Affecting Alcohol Absorption[11,12,13]

The pace of gastric emptying is a crucial element in determining the rate of absorption of ingested alcohol since the duodenum and jejunum absorb alcohol more rapidly than the stomach.

1. Alcohol diffuses passively across cellular membranes as its concentration gradient decreases. As a result, the bigger the alcohol content, the greater the concentration gradient that results, and the faster the absorption.

2. An effective blood flow will quickly remove alcohol from the absorption site, maintaining concentration gradient, and facilitating absorption.

3. Alcohol is known to be irritating, and drinking large amounts of it can lead to surface erosions, haemorrhages, and paralysis of the smooth muscle in the stomach. This will lessen the absorption of alcohol.

4. When ethanol is consumed in a single dose as opposed to multiple smaller doses, the peak blood alcohol levels are higher. This is likely because the alcohol concentration gradient is stronger in the former scenario.

5. In general, there is little variation in the rate of absorption of the same quantity of alcohol supplied in the form of different alcoholic beverages; therefore, the kind of alcoholic beverage consumed has minimal impact on the blood ethanol concentration.

6. The "don't drink on an empty stomach" maxim is based on the idea that food in the stomach delays gastric emptying, which in turn reduces alcohol absorption. Meals heavy in fat, carbohydrates, or protein all work well to delay stomach emptying.

Drinking alcohol on an empty stomach, with food, or after eating has a significant impact on how quickly it is absorbed.

Alcohol Metabolism-General Principles [14,15,16,17]

The liver contains the most of the major ethanol-oxidizing enzyme systems, including alcohol dehydrogenase and, to a lesser extent, the cytochrome P450-dependent ethanol-oxidizing system. Damage to the liver slows down the body's ability to oxidise alcohol and hence eliminate it.

Ethanol is a nutrient with caloric value (about 7 kcal per gram; carbohydrates and protein produce 4 kcal per gram, while fat produces 9 kcal).

Unlike carbs (found in the form of glycogen in the liver and muscle) and fat (found in the form of triglycerides in adipose tissues and the liver), alcohol is not stored and remains in body water until eliminated.

In contrast to how hormones like insulin/glucagon, leptin, catecholamines, and thyroid hormones govern the metabolism of the major nutrients, there is usually little hormonal regulation to pace the rate of alcohol removal.

Given these circumstances, the liver bears a tremendous burden in oxidising alcohol in order to remove it from the body.

Smaller species metabolise alcohol more quickly than larger animals do; for example, mice eliminate alcohol at a rate that is five times faster than that of humans. In other words, a species' ability to oxidise ethanol and common nutrients is similar since the rates of alcohol metabolism are connected with the basal metabolic rate for that species.

Alcohol will be oxidised preferentially over other nutrients, thus it's vital to remember that calories from alcohol are created at the expense of regular food metabolism [18–22].

Alcohol metabolism involves several different pathways.The most common of these pathways involves the enzymes aldehyde dehydrogenase (ADH) and alcohol dehydrogenase (ADH) (ALDH).These enzymes help break down the alcohol molecule so that it can be flushed out of the system..

First, the metabolism of alcohol by ADH results in the production of acetaldehyde, a molecule that is known to cause cancer and poses a severe risk to human health.

In the succeeding stage, acetaldehyde undergoes still another transformation, this time into the less reactive byproduct acetate.

After then, acetate can be easily removed (24) from the system by decomposing into water and carbon dioxide.. (fig.3)

Other enzymes

The enzymes catalase and cytochrome P450 2E1 (CYP2E1) also convert alcohol to acetaldehyde. However, catalase only partially metabolises alcohol in the body, and CYP2E1 only becomes active when a person has consumed a lot of alcohol [23]. By combining with fatty acids to create substances known as fatty acid ethyl esters, small amounts of alcohol are also eliminated (FAEEs). It has been established that these substances play a role in liver and pancreatic damage [25].

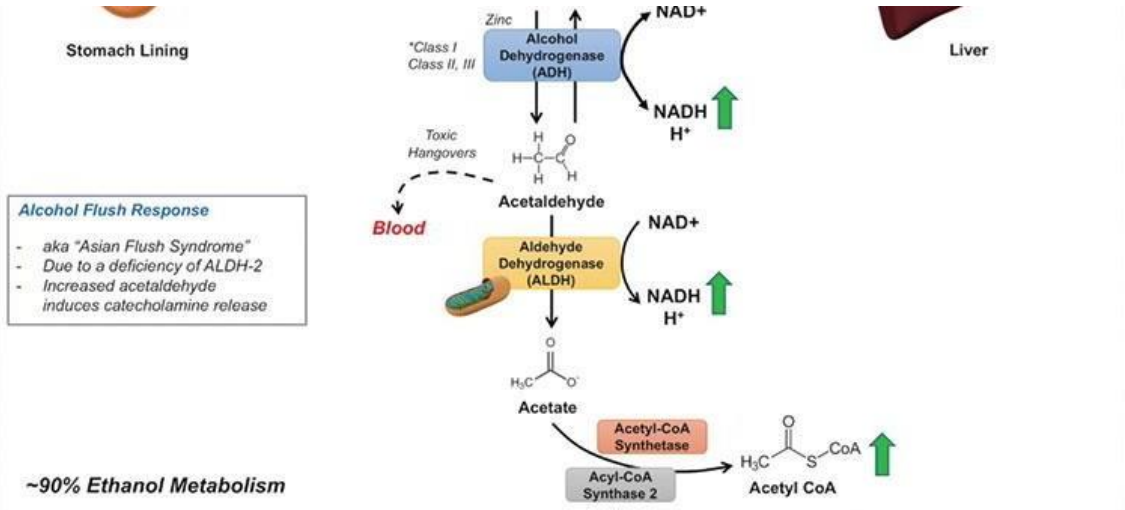


fig.3

THE EFFECTS OF ETHANOL ON THE ORGANS[2]

One or two drinks per day, which are relatively low doses of alcohol, may increase high-density lipoprotein cholesterol and decrease platelet aggregation, potentially lowering risk of embolic stroke, and occlusive coronary disease. Red wine contains flavinols and other chemicals that, at low concentrations, may have health benefits. Such moderate drinking may also reduce the risk of Alzheimer's disease and vascular dementia. Any potential health benefits, however, vanish if three or more drinks are regularly consumed each day. Fig. 4 shows how alcohol can affect other organs.

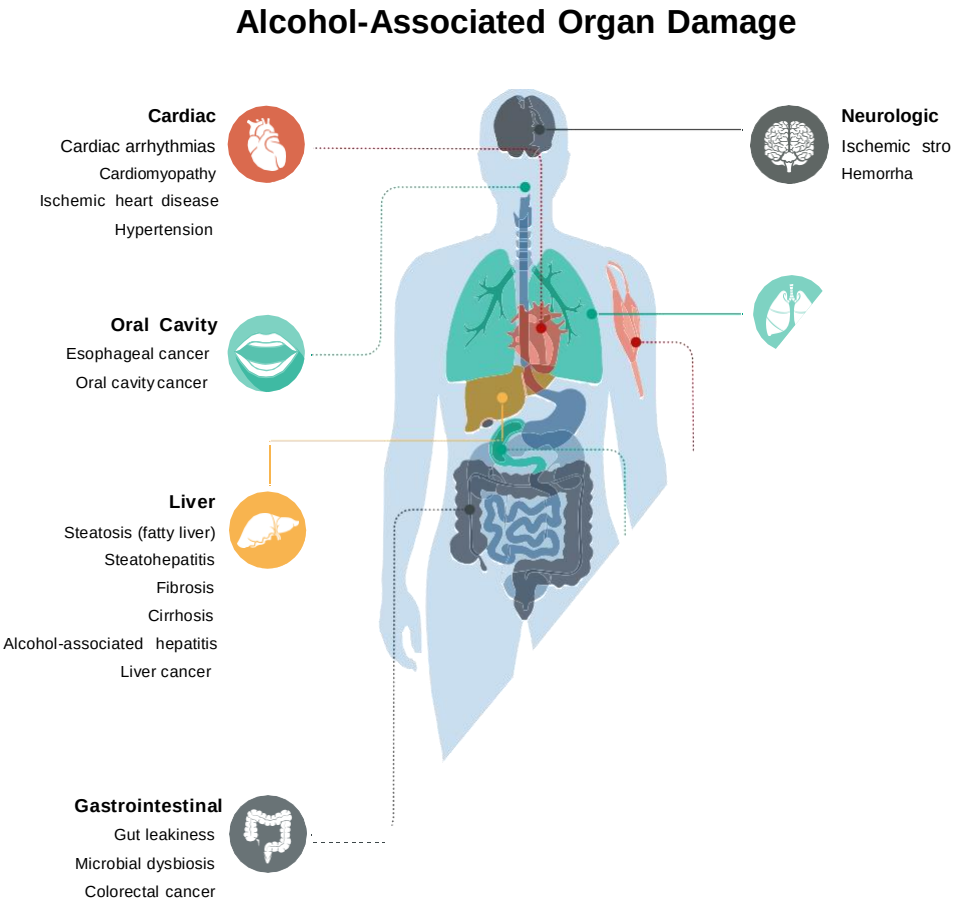


Fig.4: ILLUSTRATION OF TOXIC EFFECTS OF ALCOHOL

ALDH2 GENE

INTRODUCTION:

An step in the metabolism of ethanol, acetaldehyde, is oxidised by the mitochondrial enzyme aldehyde dehydrogenase (ALDH) 2 [26]. It is crucial for the metabolism of other harmful aldehydes, such acrolein and 4-hydroxy-2-nonenal (4-HNE) [27].

The Glu504Lys single nucleotide polymorphism (SNP) of the ALDH2 gene causes a deficiency in the enzyme activity of ALDH2, altering acetaldehyde metabolism and significantly lowering alcohol tolerance [28, 29]. It occurs with an incidence of 35–57% in various East Asian subpopulations.

Numerous human disorders, including late-onset Alzheimer's disease (AD), cancer, and cardiovascular disease (CVD), have been associated by epidemiological studies to the ALDH2 Glu504Lys SNP [30–33].

The impact of the SNP on lifestyle elements like alcohol intake and its interaction with other genetic variations are also factors in the connection between the ALDH2 Glu504Lys SNP and the onset of these disorders.

ALDH2 in Ethanol Metabolism and Beyond

The oxidation of endogenous and exogenous aldehydes to their corresponding carboxylic acids is catalysed by ALDH2, a member of the NAD(P)+-dependent ALDH supergene family [34]. Retinoic acid, betaine, and gamma aminobutyric acid are a few examples of compounds with significant biophysiological roles that are formed by the enzymatic activities of ALDHs [35–37].

Aldehydes, on the other hand, are extremely reactive substances that can form adducts with proteins, DNA, and lipids, impairing their functionality and resulting in cell toxicity. Endogenous

aldehydes are produced during the biotransformation of numerous medications and ambient chemicals, as well as during the metabolism of amino acids, carbohydrates, lipids, and vitamins [35, 38–40].

Additionally, the detoxification of reactive aldehydes such 4-HNE and acrolein depends on ALDH2 [27]. 4-HNE is an in vivo lipid peroxidation product that is an,-unsaturated aldehyde [41].

Small amounts of acrolein are present in many meals, including cheese, salmon, bread, and alcoholic beverages. However, acrolein is also present in significant concentrations in cigarette smoke and heated oils [42]. One of the crucial systems for eliminating these reactive aldehydes and safeguarding cells and organs from these harmful aldehydes may be ALDH2. Additionally, it has been hypothesised that ALDH2 may perform many catalytic tasks. (fig.5)

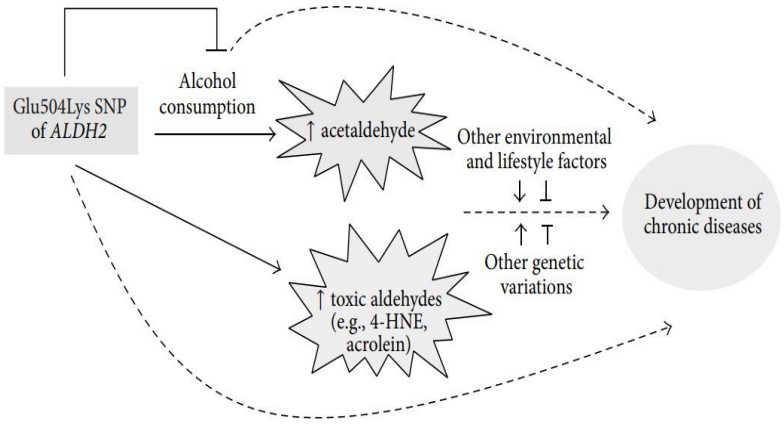


Fig.5: Other genetic variations, environmental circumstances, and lifestyle choices, in addition to alcohol consumption, confound the connection of the glu504lys SNP of the ALDH2 gene with human disorders.

SNP IN ALDH2 GENE

A protein specific to the mitochondrial matrix is encoded by the human ALDH2 gene, which is located on chromosome 12 (12q24.2). Heart, kidney, muscle, and the brain all contain the enzyme, which is particularly highly expressed in the liver [43].

The catalytic domain, the NAD+-binding coenzyme domain, and the oligomerization domain are the three primary domains that each of the four identical subunits of the enzyme ALDH2 is made up of. This information was obtained from analyses of the protein structure of ALDH2. Alcohol tolerance is significantly decreased by a dominant-negative ALDH2 SNP, which has a prevalence of 35–57% in several East Asian subpopulations [28,29].

People with the mutant ALDH2 genotype exhibit recognisable acetaldehyde-mediated acute consequences of alcohol use, such as flushing of the face and an elevated heart rate [44] .

A single nucleotide change in exon 12 from G to A is what causes the mutation, which changes glutamate to lysine at position 504 of the protein [28] .

The tetrameric enzyme's oligomerization domain contains Glu504, which is necessary for the production of both dimers and tetramers [45] .

Glu504 makes hydrogen bonds with Arg281 from the same subunit as well as Arg492 from the neighbouring dimer partner in wild-type ALDH2 (ALDH21). The mutation-carrying subunit's structure is altered, as is the structure of its dimer partner, by the breakdown of these connections caused by Lys504 in the mutant (ALDH22) [46] .

The mutant ALDH22 enzyme has an elevated K m for NAD+ and a lowered k cat, which results in very low enzymatic activity in vivo and impairs the coenzyme NAD+'s ability to bind to ALDH22. As a result, ALDH22 has a dominant-negative effect (47) .

Following a mild to moderate alcohol intake, persons with the phenotypic loss of ALDH2 activity had blood acetaldehyde concentrations that are roughly 6 and 19 times higher than those with active ALDH2 (ALDH2 1/ 2) and homozygous (ALDH 2/ 2) genotypes, respectively [48] .

MECHANISM OF LIVER DAMAGE AMELIORATION INDUCED BY LACK OF ALDH2

The observed associations between the ALDH2 genotype and alcohol consumption on serum transaminase levels have been explained by a few processes (Fig. 6).

Through suppression of reduced nicotinamide adenine dinucleotide (NADH) production in the mitochondria during the conversion of acetaldehyde to acetate, cyanamide, an ALDH inhibitor, inhibited the increase in oxidative stress induced by ethanol [49,50] .

According to this notion, the ALDH2*2 allele can lessen the abrupt rise in NADH that leads to mitochondrial malfunction, oxidative stress, and hepatocyte damage brought on by alcohol use. After receiving a single dosage of ethanol, Aldh2 mutant animals displayed decreased MDA and increased glutathione (GSH) levels, providing evidence in favour of this theory [51] .

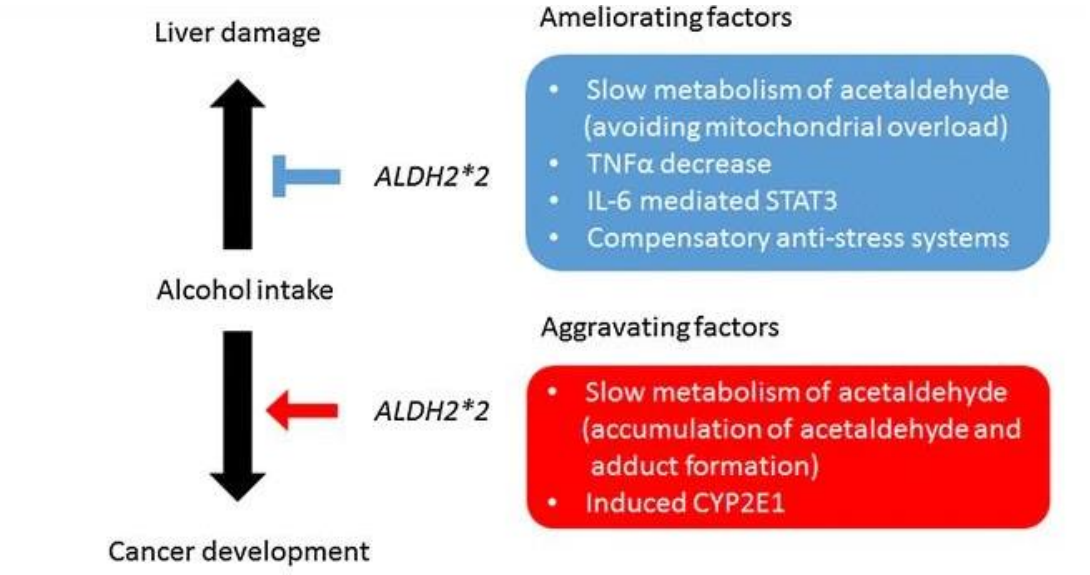


Fig 6; Alcohol consumption and the presence of a faulty ALDH2 polymorphism (ALDH2*2) are proposed as a method for ameliorating liver damage and accelerating the development of cancer. TNF (tumour necrosis factor-alpha), ALDH2 (aldehyde dehydrogenase 2), and STAT3 (signal transducer and activator of transcription 3)

Involvement of aldh2 enzyme in the regulation of multiple organ functions (fig.7)

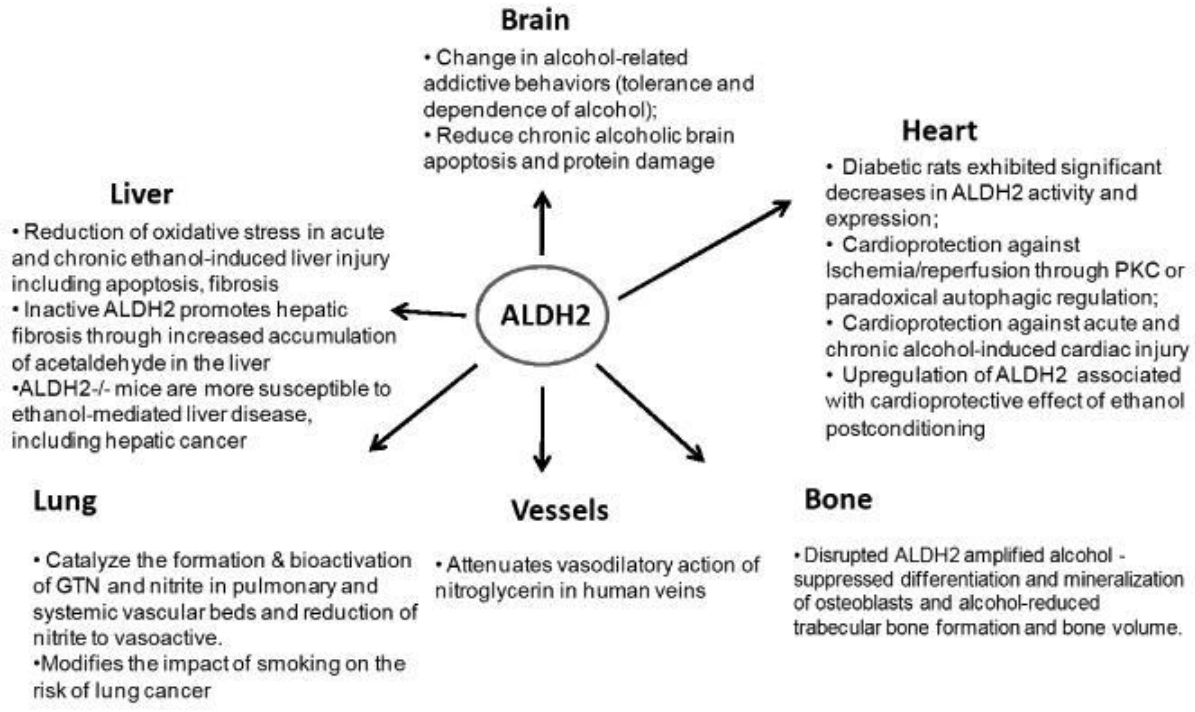


Fig.7

CIRRHOSIS OF LIVER:

CIRRHOSIS

In 1819, Rene-Theophile-Hyacinthe Laennec used the term "Cirrhosis" that is derived from the Greek "kirrhos" meaning tawny (light brown/yellowish color) but before his time, it was synonymous with 'hardening of the liver' [52].

Vesalius and Morgagni described it as shrunken liver though it is not just that[4].

Mallory said: "To the clinician the term cirrhosis means a chronic, progressive destructive lesion of the liver combined with reparative activity and contraction on the part of the connective tissue. The contraction of the connective tissue may lead to obstruction of the bile ducts, causing more or less jaundice, and it can interfere with the flow of blood through the blood vessels resulting in portal congestion and ascites. The pathologist uses the term cirrhosis in a broader sense. He applies it to all sclerosed conditions of the liver, whether progressive or not, in which destruction of liver cells is associated with real or apparent increase in connective tissue"[52].

Harvey in 1616, described 12 cases of which few are mentioned as "russet, hard or contracted, almost bloodless" and few as "large, hard liver like a scirrhous tumor, almost bloodless and with a rough surface"[52].

Payne in 1626, described liver as "scirrhosum et induratum, and juiceless, like rotten wood" in a person with intemperate habits with jaundice and ascites[52].

Matthew Baillie in 1793 said that forming tubercles in liver is one of the most common forms of its diseases and also observed that it is associated with drinking habits and more commonly observed in men more than females and described present day "nodule" in cirrhosis, as "common tubercule of the liver"[52] .

Best and his colleagues' experiments point that alcohol supplies only calories without proteins, aminoacids like choline and methionine whereas Klatskin said that alcohol increases the requirement of choline[52].

Presently, "cirrhosis is defined as a diffuse process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules".

It is characterised by[53] :

1. Involving most of the liver,

2. Fibrous septa which may be reversible or irreversible based on reversibility of disease process (zone of hypoperfusion),

3. Parenchymal nodules which are either micro (<3mm) or macro (>1cm) composed of both senescent and novel hepatocytes which are usually derived from hematopoietic stem cell niche (zone of hyperperfusion).

Clinically cirrhosis is described as uncomplicated/compensated which is detected by routine examination/other investigations or complicated/decompensated in which patients present usually with ascites, jaundice or gastrointestinal bleeding[54].

Once decompensation develops, cirrhosis-related mortality and morbidity dramatically rise, and the 1-year case-fatality rate might reach as high as 80%, depending on the source of decompensation [55].

GLOBAL BURDEN AND EPIDEMIOLOGY :

Prevalence of cirrhosis from autopsy studies arrays from 4.5% to 9.5% (50 million adult population) of the general population globally which is often underestimated owing to its asymptomatic patients. Cirrhotic deaths have increased from being ranked 14th leading cause of death in world in 2001 to 12th in 2020.

In Asia, the incidence of cirrhosis ranges from 16.5 per 100,000 in East Asia to 23.6 per 100,000 in Southeast Asia[56].

Globally alcohol consumption and hepatitis being the major causes of cirrhosis and being addressed with screening tools, immunization, antiviral therapies there is a trend change towards increasing incidence of cirrhosis secondary to non alcoholic fatty liver disease secondary to obesity and the metabolic syndrome[56,57].

Between 1990 and 2017, the number of compensated and decompensated cases of cirrhosis roughly doubled globally (55).

Worldwide, females had lower prevalence, age-standardized death rates, and disability adjusted life years than males (55).

The age group of 50 to 74 years has the greatest death rate, and greater rates are seen as people get older.

The top 6 regions with age-standardized death rates from cirrhosis in 2017 are Central Asia, Southeast Asia, Eastern Europe, Western, Eastern, and Central Sub-Saharan Africa. The bulk of cirrhotic deaths are caused by alcohol in Central Asia and Eastern Europe, whereas hepatitis is to blame in other places (55).

The six regions with the lowest age-standardized death rates from cirrhosis in 2017 are Australasia, East Asia, high-income Asia Pacific, Western Europe, Southern Sub-Saharan Africa, and high-income North America. Alcohol is the primary cause of cirrhosis in Western Europe, while hepatitis is the cause in the other regions (55).

Globally, 2.3 billion people drink alcohol, and the prevalence of alcoholic liver disease is increasing along with per-capita alcohol consumption, which increased from 5.5 litres in 2005 to 6.4 litres in 2016; alcohol-related cirrhotic fatalities were 27.3% in 2017. (55,56).

Despite the availability of screening methods and therapy, hepatitis caused 50.7% of cirrhotic deaths in women and 57% of cirrhotic deaths in men globally in 2017. (55).

77% of cirrhotic fatalities in 2017 were caused by NASH, and between 1990 and 2017, the incidence of compensated cirrhotics caused by NASH doubled, while the frequency of decompensated cirrhotics caused by NASH tripled (55)

When compared to age-matched controls, people with chronic liver disease have higher rates of unemployment (65.3% vs. 31.4%), incapacity to work owing to disability (30.5% vs. 6.6%), and reported days of disability per year (10.2 vs. 3.4), and the disability rises with cirrhotic consequences (56).

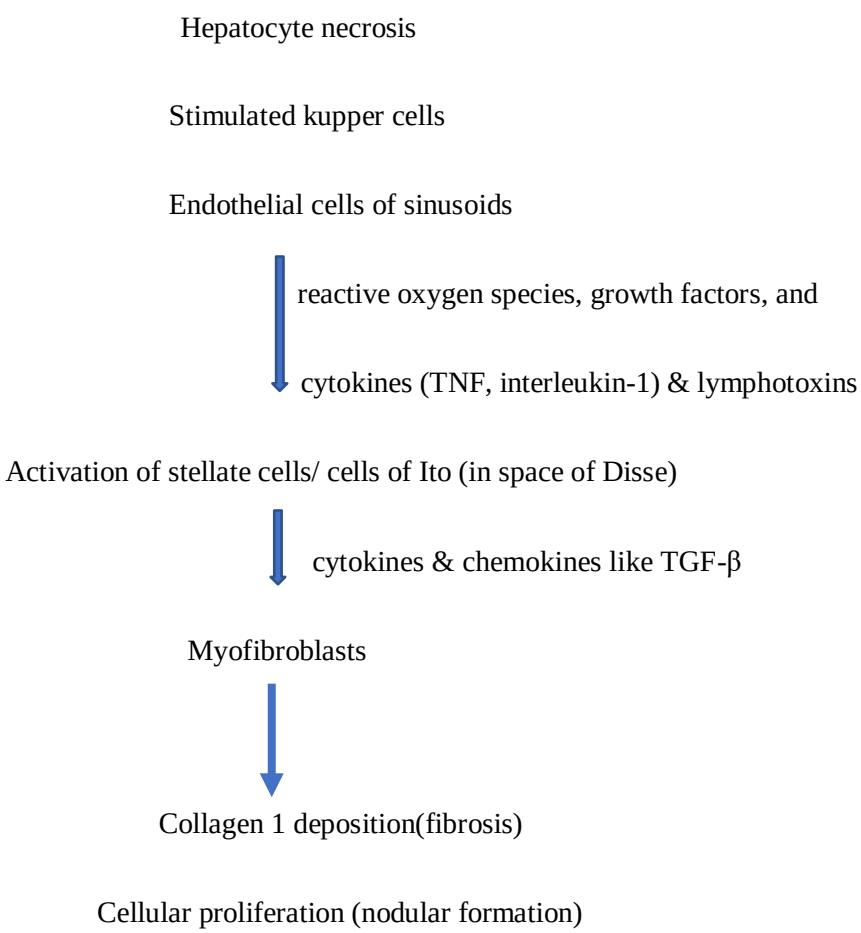
PATHOGENESIS :

Pathogenesis of cirrhosis is majorly explained by[53]:

1. Hepatocyte necrosis,

2. Dynamic process of extracellular matrix synthesis, deposition, and resorption controlled by altering balances between metalloproteases and its tissue inhibitors elucidating the possibility of cirrhotic regression even in late-stage disease, if the disease process is terminated.

FIGURE 8 : PATHOGENESIS OF CIRRHOSIS



3. Vascular injuries, changes, shunts[53]. Normally even with such a high blood flow of about 1050 ml/min from the portal vein and 300 ml/min from the hepatic artery, liver still maintains low vascular resistance

which is shown by smaller pressure gradient between 9 mmHg average pressure in the portal vein leading into the liver and 0 mmHg average pressure in the hepatic vein leading from the liver into the vena cava.

a) Loss of sinusoidal endothelial cell fenestrations,

b) Development of portal vein-hepatic vein and hepatic artery-portal vein vascular shunts,

c) Increased basement membrane formation

convert thin-walled sinusoids into higher pressure, fast-flowing vascular channels reducing the solute (particularly protein like albumin, lipoprotein, clotting factors) exchange between hepatocytes and the plasma resulting in portal hypertension[53].

The transport deficit is further aggravated by the loss of hepatic microvilli and thus cause liver cell dysfunction[53].

TABLE 1 : ETIOLOGY AND HISTOPATHOLOGY OF CIRRHOSIS :

Cirrhosis is a multifactorial disease.

Its etiological agents, specific histopathological changes and specific treatment are a follows[54]:

ETIOLOGY	HISTOPATHOLOGY	SPECIFIC TREATMENT
Alcohol related cirrhosis	Micro/macro nodular changes with acidophilic bodies and mallory's hyalin	Abstinence
Hepatits B & C	Micro/macro nodular changes with acidophilic bodies and ground glass hepatocytes	Antivirals
NASH	Steatosis with reversible cirrhosis	Reduction of weight
Hemochromatosis	Micronodular pattern with deposition of iron	Venesection

Wilson's disease	Macronodular pattern with deposition of copper, acidophilic bodies and mallory's hyalin.	Chelation of copper
α 1-antitrypsin deficiency	Micro/macro nodular pattern with acidophilic bodies, mallory's hyalin and PAS positive globules.	?Transplant
Indian childhood cirrhosis	Macronodular changes with cholestatis, acidophilic bodies and mallory's hyalin.	?Transplant
Primary biliary cirrhosis & Primary sclerosing cholangitis	Cholestasis, acidophilic bodies and mallory's hyalin.	?Transplant
Venous outflow obstruction	Reversible cirrhosis	Relieve the vein Block/ ? Transplant. Treat the cardiac cause if heart failure is the etiology.
Galactosemia	Reversible cirrhosis	Withdraw milk and its products
Tyrosinemia	Reversible cirrhosis	Withdraw dietary tyrosine
Intestinal bypass surgery	Micronodular changes with steatosis, acidophilic bodies and mallory's hyalin.	? Transplant
Toxins and drugs(Methotrexate, Amiadarone, Enalapril)	Micro/macro nodular changes with steatosis, mallory bodies, periportal and pericellular fibrosis	Recognise and withdraw

CLINICAL FEATURES :

SKIN :

ARTERIAL SPIDERS :

Potent steroid, oestrogen not only acts on the endometrial spiral arterioles in pregnancy but also on the cutaneous vessels making them appear in upper part of body with a body and legs, structure analogous to spiders. They are either end arteries in skin or coiled artery in subcutis with aggregates of glomus cells or the pericytes of Zimmermann which break into many feeding vessels. These are pulsatile and blanch on pressure with a glass slide[58].

PALMAR ERYTHEMA :

In 1899, Chambers noticed red colour of palms in habitants of the Gold Coast. Lane described the red colour of palms in few patients as hereditary/familial. Association of palmar erythema and arterial spiders was described by Walsh and Becker in pregnant females but first, Forman described palmar erythema in pregnant females in 1934. Liver palms or palmar erythema appearing in chronic liver disease was described in 1942 and 1943 by Ratnoff and Patek, Perera and William Bean.

“An older Miss Muffett

Decided to rough it

And lived upon whisky and gin.

Red hands and a spider

Developed outside her

Such are the wages of sin” mentioned Dr.William Bean[58].

2 types of palmar erythema[54,58] :

1. Familial/simple exaggeration of speckled mottling without true accentuation of blood flow regionally.

2. Liver palms with warmth and intense red in hypothenar, thenar eminences and finger pulps which may extend onto dorsum of hands in horse shoe pattern at base of nails.

Arterial spiders are twice more common in liver disease than palmar erythema.

LEUCONYCHIA :

White finger nails are due to hypoalbuminaemia[54].

CLUBBING :

FIGURE 9 : MECHANISM OF CLUBBING

Aggregated platelets passing through pulmonary arteriovenous shunts

↓

Plugging of capillaries and release of platelet derived growth factors

↓

Digital clubbing and hypertrophic osteoarthropathy[54]

Other nail changes like Onychorrhexis, pitting, longitudinal melanonychia, brittle nails, dystrophic nails occur due to[59] :

1. Decreased cell-mediated immunity,
2. Immunosuppression,
3. Anemia (Iron deficiency).

DUPUYTREN ' S CONTRACTURE :

Due to thickened palmar fascia, may be due to vitamin E deficiency. It is named after Aron Guillaume Dupuytren who observed this deformity as a result of retraction of palmar aponeurosis[60].

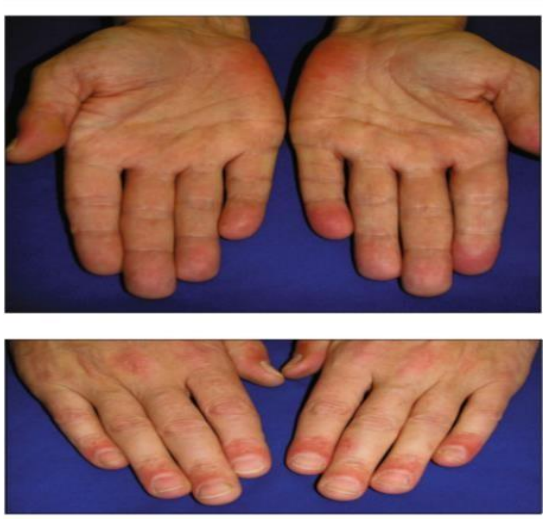


FIGURE 10 : Palmar erythema in a hepatitis patient involving both palmar and dorsal surfaces[61]

Figure 11 : Leukonychia

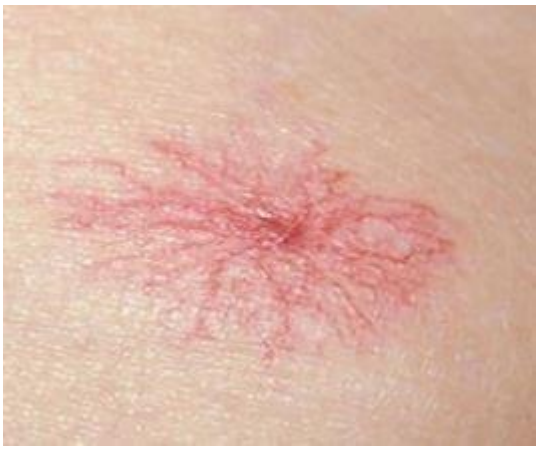
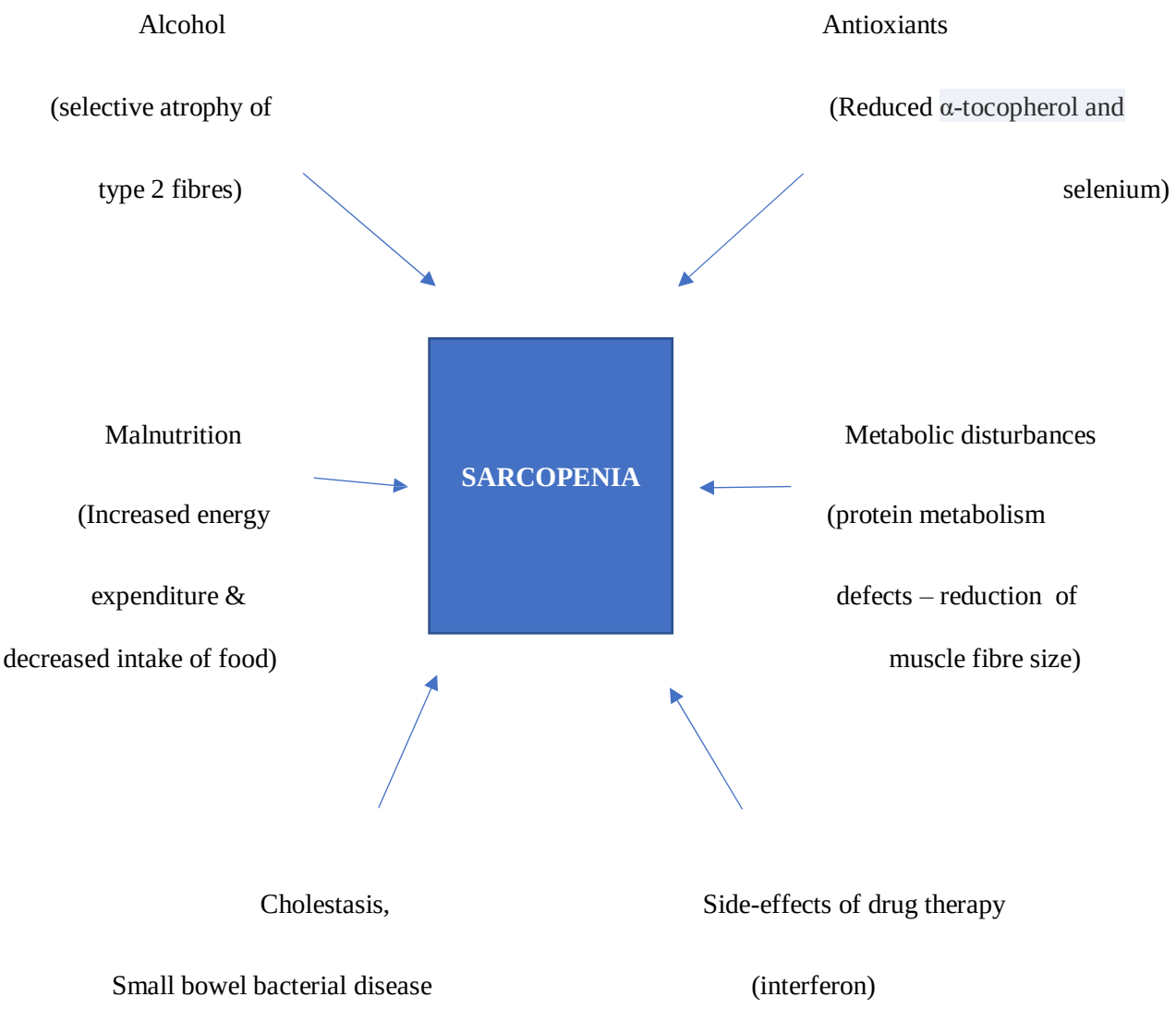


Figure 12 : Arterial spider

MYOPATHY / SARCOPENIA :

It is caused by many factors[62].

FIGURE 13 : FACTORS CAUSING SARCOPENIA



Proximal myopathy is seen usually.

Apparent diffusion coefficient on MRI of skeletal muscles is recently reported to be high in liver disease[62].

FOETOR HEPATICUS :

Sweetish, slightly faecal smell of the breath due to dimethyl sulphide and ketones in alveolar air. Wide spectrum antibiotics altering the gut flora and post defaecation, smell is observed to be less intense thus, the thought of its origin from intestine. In patients of coma, this smell gives a diagnostic clue[54].

FIGURE 14 : HYPOGONADISM[54]

Hepatocellular dysfunction,

Cholestasis

↓

Reduced conjugation of steroid hormones,

Reduced excretion of oestrogen,

Upturning of hepatic oestrogen receptors,

Downturning of hepatic testosterone receptors

Males	Females
Soft and small testis, Prostatic hypertrophy,	Ovulatory failure,
Diminished libido and potency	Loss of breast and pelvic fat,
Loss of secondary sexual hair,	Erratic menstruation, Infertility.
Gynaecomastia.	

HAEMATOLOGICAL MANIFESTATIONS :

These include combination of cytopenias like thrombocytopenia, anemia, leukopenia as well as coagulopathies[65].

Factors predisposing to hemorrhage[65] :

Thrombocytopenia

Decreased levels of factors II, V, VII, IX, X, XI

Dysfibrinogenemia

Decreased alpha-2-antiplasmin

Factors predisposing to thrombosis[65] :

Increase in factor VIII and Von Willebrand factor

Decreased protein C and protein S

Decreased antithrombin

Reduced plasminogen

In early stages of cirrhosis, prothrombotic condition is observed whereas in later stages, hemorrhagic manifestations are seen.

GASTROINTESTINAL FEATURES :

Parotid gland enlargement,

Splenomegaly,

Abdominal wall collaterals,

Ascites,

Peptic ulceration (duodenal ulcers > gastric ulcers),

Small bowel bacterial overgrowth (more common in those with ascites than without),

Abdominal herniae,

Pigment type gall stones,

Chronic relapsing pancreatitis and,

Pancreatic calcification are the gastrointestinal manifestations in cirrhosis of liver.

RENAL MANIFESTATIONS :

Redistribution of blood flow away from cortex results in hepatorenal syndrome which further may lead to intrinsic renal failure that inturn result in hypotension and shock.

Cirrhotic glomerular sclerosis is seen with thickening of the mesangial stalk and capillary walls.

IgA deposition in basement membrane of glomerulus is seen in alcoholic cirrhosis.

Chronic hepatitis C infection is associated with cryoglobulinaemia and membrano proliferative glomerulonephritis.

NEUROLOGICAL MANIFESTATIONS[54] :

Range from tremors, confusion, stupor to coma.

History of Occupation, age, sex, domicile, fatigue and weight loss, anorexia and flatulent dyspepsia, abdominal pain/distention, jaundice, itching (deposition of bile salts in cutis), colour of urine and faeces, bleeding manifestations like haemorrhage-nose, gums, skin, alimentary tract,loss of libido, menstrual irregularities should be enquired.

Past history of jaundice, hepatitis, drugs ingested, blood transfusion, history of alcohol consumption, family history of liver disease/ autoimmune disease should also be enquired and taken into consideration.

TABLE 2 : INCIDENCE OF VARIOUS SIGNS IN CIRRHOSIS[66]

SIGNS	REPORTED PERCENTAGE
Jaundice	30-70
Telangiectasia	17-45

Spider nevi	15-62
Collateral circulation	19-63
Cyanosis or clubbing	5-18
Edema	35-69
Ascites	48-93
Hepatomegaly	70-79
Splenomegaly	24-55
Hemorrhoids	9-34
Sexual changes	5-50

DIAGNOSIS OF CIRRHOSIS :

Liver biopsy, an invasive procedure is the gold standard for diagnosis and is associated with risk of organ injury.

Transient elastography (fibroscan) is a rapid non invasive method of evaluating liver fibrosis/ cirrhosis. Liver stiffness ranges from 12.5 to 75.5 kilo Pascals in cirrhotic patients. Alcohol induced liver cirrhotics have higher liver stiffness values compared to other causes. To diagnose histologically alcoholic cirrhosis takes longer time than compared to hepatitis induced cirrhosis[67].

In most of the cases of cirrhosis, diagnosis is made with combination of clinical and radiological features.

COMPLICATIONS OF CIRRHOSIS :

Many complications are due to decreased liver cell function, arterial dilatation & hyperdynamic circulation resulting in portal hypertension, and a high risk of hepatocellular carcinoma[53].

Jaundice, ascites, bleeding varices, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome, hyponatraemia, high output cardiac failure and hepatocellular carcinoma are the complications of cirrhosis[54].

JAUNDICE :

Jaundice is yellowish discoloration of skin and mucus membrane when systemic retention of bilirubin is above 3.0 mg/dL. The term 'Icterus', derived from the Greek word 'ikteros' which actually refers to a plant disease in which the leaves turn into yellow or yellow-green is used as a synonym to jaundice. Hepatic or cholestatic types of jaundice occur with cirrhosis[68].

Jaundice is milder and not persistent in alcoholic cirrhosis compared to post necrotic / post hepatitic cirrhosis. It is severe in 3 conditions in alcoholic cirrhosis[66] :

1. Early stage of cirrhosis with acute illness,
2. When cirrhosis is well established with onset of complications/decompensation,
3. Hepatic precoma.

In case of biliary cirrhosis, jaundice deepens as the disease process progresses.

ASCITES :

Ascites occurs in more than 50% of patients within 10 years of the diagnosis of cirrhosis[69]. It is the accumulation of fluid in peritoneum which when 500ml is present, becomes clinically detectable. Ascites due to cirrhosis is a serous fluid with high protein of around 3 g/dL of protein (albumin) and the serum to ascites albumin gradient(SAAG) is more than or equal to 1.1 g/dL.

3 mechanisms of ascites formation[53,70] :

1. Initiation of ascites formation by increased sinusoidal pressure (minimal portal pressure gradient of 12 mmHg).
2. Maintainence of ascites formation by vascular changes (Underfill/Overfill theories) leading to sodium and water retention.
3. Leakage of fluid from hepatic lymph into peritoneum as a result of excessive lymph flow of around 20 litre per day (normal thoracic duct lymph flow is around 1 litre/day). Hepatic lymph has high protein content and low level of triglycerides, thus the protein-rich ascitic fluid.

Most widely accepted theory is underfill / vasodilatory theory which is explained as below[70].

FIGURE 15 : UNDERFILL / VASODILATORY THEORY[70]

Peripheral vasodilatation (production of vasodilators like nitic oxide, adrenomedullin, carbon monoxide, endocannabinoids, prostacyclin, tumour necrosis factor alpha and urotensin & inhibition of nitric oxide synthase)

↓

Reduction of effective arterial blood volume

Reduction of systemic blood pressure

↓ Carotid sinus baroreceptors

Rise in renin, aldosterone, vasopressin

Sympathetic system stimulated

↓ Increased luminal uptake and basolateral passage of sodium

Sodium and water retention

Renal vasoconstriction

↓

Plasma volume expansion

+

portal hypertension

↓

Ascites

FIGURE 16: OVERFILL THEORY[70]

Hepatic signal (baroreceptor, reduced hepatic synthesis of a natriuretic agent, reduced hepatic clearance of a sodium retaining hormone / a ‘ hepatorenal reflex ’ of unknown etiology)

Renal Na+ and H2O retention

Increased plasma volume

Increased cardiac output

Reduced systemic vascular resistance

+

Portal hypertension

Overflow into peritoneal cavity

GRADES OF ASCITES[69]

Grade 1 : Mild ascites with no clinical detection, only detectable by ultrasonography.

Grade 2 : Moderate ascites presenting as umiform distension of abdomen.

Grade 3 : Gross ascites with marked abdominal distension.

UNCOMPLICATED ASCITES : Uncomplicated ascites is defined as “ascites that is not infected and that is not associated with the development of the hepatorenal syndrome (HRS)”[69].

COMPLICATIONS OF ASCITES :

1. REFRACTORY ASCITES : International Ascites Club(1996) defined refractory ascites as "ascites that cannot be mobilized or the early recurrence of which cannot be satisfactorily prevented by medical therapy". It constitutes 5% to 10% of all the ascitic patients[(69)].

It is further of 2 types :

a. Diuretic-resistant ascites - Fluid overload that is unresponsive to dietary salt restriction and to high doses of diuretics.

b. Diuretic-intractable ascites - Ascites wherein there is inability to treat ascites with maximum doses of diuretics because of their side effects[(71,72)].

Refractory ascites has significantly been associated with hepato renal syndrome type-2, ascitic infection, dilutional hyponatraemia, myopathy and hepatic hydrothorax. It has an omnious prognosis with 2-year probability of survival among patients with refractory ascites of 30% compared to a minimum of 40% patients with uncomplicated ascites being alive at 5 years[(72)].

"Revised diagnostic criteria of refractory ascites

1. Treatment duration: Patients must be on intensive diuretic therapy (spironolactone 400 mg/day and furosemide 160 mg/day) for at least 1 week and on a salt-restricted diet of less than 90 mmoles/ day or 5.2 g of salt/day.

2. Lack of response: Mean weight loss of o 0.8 kg over 4 days and urinary sodium output less than the sodium intake.

3. Early ascites recurrence: Reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization.

4. Diuretic-induced complications:

a. Diuretic-induced hepatic encephalopathy: development of encephalopathy in the absence of any other precipitating factor.

b. Diuretic-induced renal impairment: increase of serum creatinine by 4100% to a value 42 mg/dl in patients with ascites responding to treatment.

c. Diuretic-induced hyponatraemia: decrease of serum sodium by 410 mmol/L to a serum sodium of o125 mmol/L.

d. Diuretic induced hypo or hyperkalaemia: change in serum potassium to o 3 mmol/L or 46 mmol/L despite appropriate measures"[72].

FIGURE 17 : PATHOGENESIS OF DIURETIC-RESISTANT ASCITES[72]

Systemic and portal haemodynamic abnormalities,

Activation of neurohumoural vasoactive systems,

Renal re-absorption of sodium and water

+

Progressive decline in renal perfusion and glomerular filtration rate

↓

Sodium re-absorption at the proximal convoluted tubule markedly increases

Delivery of sodium to distal segments of the nephron is markedly decreases

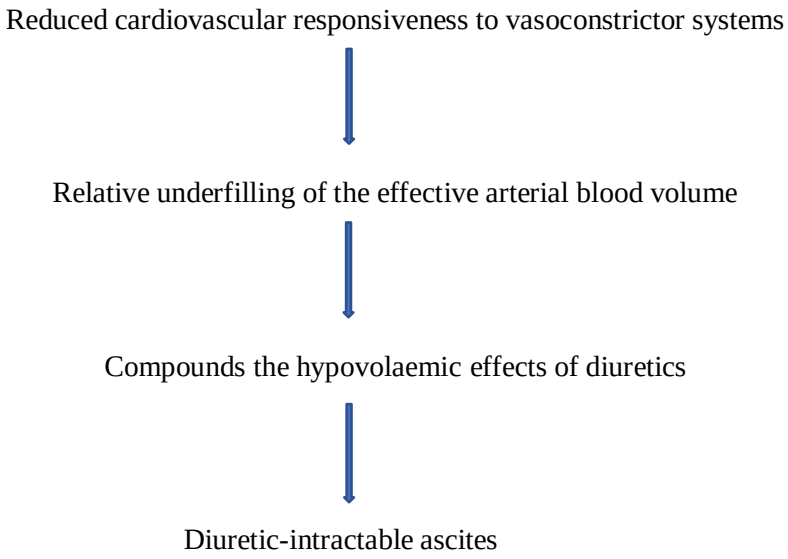
↓

Renal sodium retention mainly occurs proximal to the site of action of both anti mineralocorticoid as well as loop diuretics

↓

Diuretic-resistant ascites

FIGURE 18 : PATHOGENESIS OF DIURETIC INTRACTABLE ASCITES[72]



NON REFRACTORY ASCITES :

- **RECIDIVANT / RECURRENT ASCITES :** Defined as the "Peritoneal effusion that recurs on at least three occasions within a 12-month period inspite of reduction in dietary salt and adequate diuretic doses"[72].
- Massive/tense ascites is also not considered as refractory unless the failure to respond to treatment has been demonstrated[72].

2. ASCITIC INFECTION :

In 1975, Correia and Conn coined the term spontaneous bacterial peritonitis because it is without any inflammatory focus intra abdominally and without any continguous source of infection[70,73].

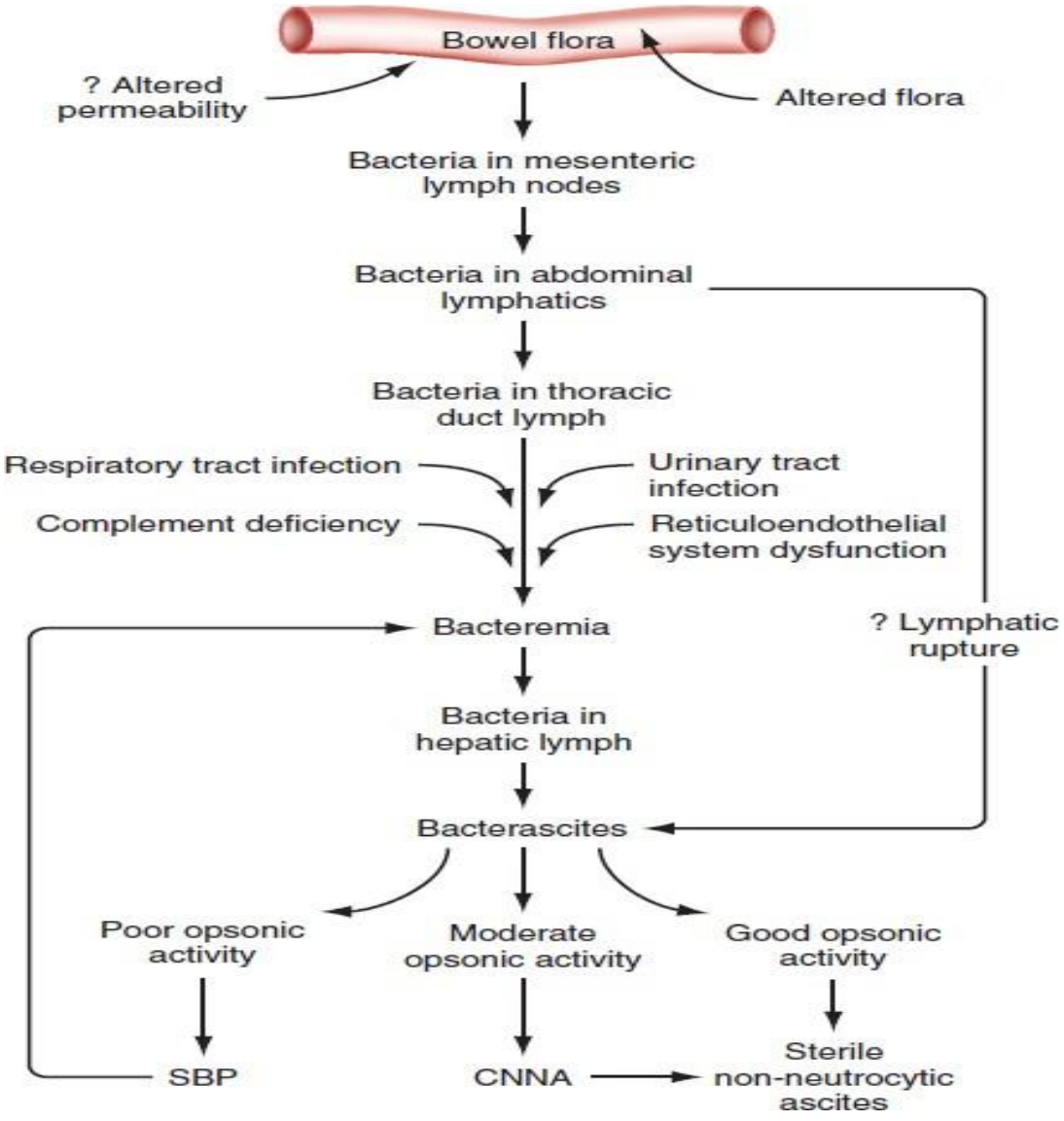
5 types of ascitic infection based on ascitic culture results, neutrophil count, and presence or absence of a surgical source of infection[73] :

TABLE 3 : DIFFERENT TYPES OF ASCITIC INFECTION AND THEIR DIAGNOSTIC CRITERIA

ENTITY	DIAGNOSIS
SBP(Spontaneous bacterial peritonitis)	1. Positive ascitic fluid culture 2. High ascitic absolute PMN count (at least 250 neutrophils/mm3)
MNB(Monomicrobial non-neutrocytic bacterascites)	1. Positive ascitic fluid culture for a single organism, 2. Ascitic fluid PMN count 250/mm3 3. No evidence of an intra abdominal surgically treatable source of infection
CNNA(Culture-negative neutrocytic ascites)	1. Ascitic fluid culture grows no bacteria 2. Ascitic fluid PMN count $\geq$ 250/mm3 3. Not even a single dose of antibiotic has been given 4. No other explanation for an elevated ascitic PMN count (hemorrhage into ascites/ peritoneal carcinomatosis/TB/pancreatitis)
Secondary bacterial peritonitis	1. Ascitic fluid culture is positive (usually for multiple organisms) 2. PMN count $\geq$ 250/mm3 3. Intra-abdominal surgically treatable primary source of infection (perforated intestine, perinephric abscess) has been identified.
Polymicrobial bacterascites (needle perforation of the bowel)	1. Multiple organisms seen on Gram stain or cultured from the ascetic fluid 2. PMN count $\leq$ 250/mm3

Most common organism causing SBP is Escherichia coli. Streptococcus viridans, Staphylococcus aureus, Enterococci are other organisms responsible for ascitic infection[74]

FIGURE 19 : PATHOGENESIS OF ASCITIC INFECTION[73]



3. UMBILICAL HERNIAE :

Diastasis of recti or herniae occur in the umbilical, femoral or inguinal regions or through old incisions as a result of increased intra abdominal pressure. 20% of the cirrhotics with ascites develop herniae and may be

in upto 70% in long standing ascites/tense ascites. These herniae are prone to rupture or incarceration. Thus, after effective treatment of ascites, definitive mesh repair of these herniae is to be planned[70].

4. HEPATIC HYDROTHORAX :

Trans-diaphragmatic movement of fluid from peritoneal to plueral cavity results in right sided pleural effusion in 85%, left sided in 13% and bilateral in 2%. Detection of radiotracer (intraperitoneal injection of Tc - 99m - labelled sulphur colloid or macroaggregated serum albumin) in the pleural space is demonstrated generally within 2 hours of injection gives the diagnosis. Ascites should be effectively treated and TIPS is proven more successful than drainage and pleurodesis[70].

5. PERIPHERAL OEDEMA :

Due to a functional inferior vena caval block due to high intrabdominal pressure due to the ascitic fluid[70].

TABLE 4 : TREATMENT OF ASCITES AND ITS INFECTION[74]

Grade 1	Salt restriction(to <2g/day)
Grade 2	Spirinolactone (max dose: 400mg/day), Furosemide (max dose : 160mg/day).
Grade 3/ Refractory ascites	Large volume paracentesis, Lee Van shunt (TIPS), Liver transplant.
Ascitic infection	Prophylaxis : Oral norfloxacin (400 mg/day every 12 hrs for a minimum of 7 days)[70] Treatment : Third generation cephalosporins like cefotaxime 2 g iv every 8 hours usually for 5 days but duration of antibiotic is determined based on clinical response and serial ascitic fluid PMN counts and cultures[73,74].

	• In case of secondary bacterial peritonitis & polymicrobial bacterascites, anti anaerobic drug such as metronidazole is added. • In case of secondary bacterial peritonitis, emergency surgical intervention is indicated.

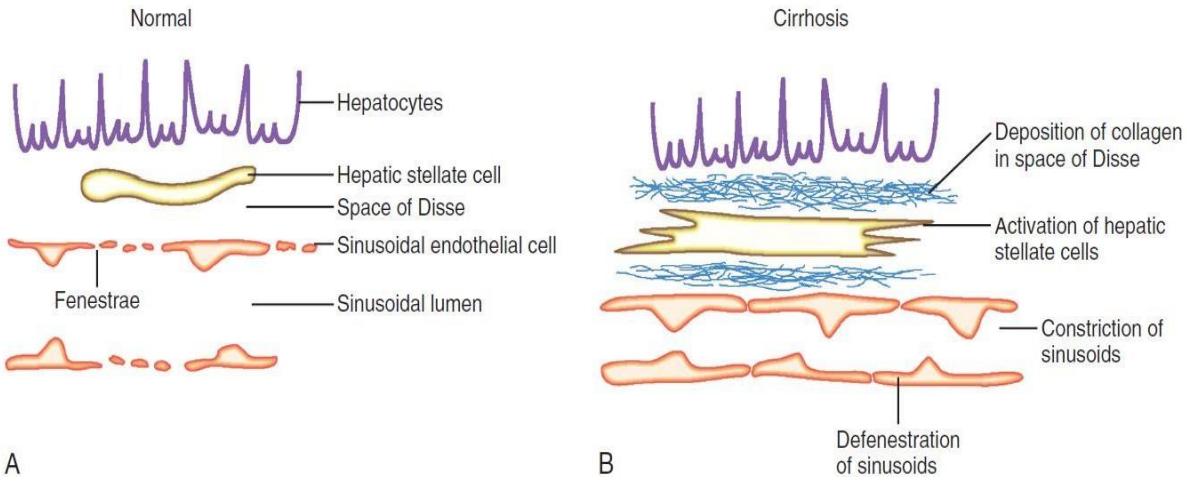
PROGNOSIS OF CIRRHOTICS WITH ASCITES :

Cirrhotics with ascites indicates a poor prognosis, especially with refractory ascites and those who develop SBP. At 2 years, the probability of mortality in hospitalized cirrhotics with ascites is 40%[(69)]. 10-20% of the patients with SBP die at hospital admission and the median survival at 1 year is 9 months. Immediate survival and resolution of SBP is 100% in uncomplicated community acquired SBP[(70)].

PORTAL HYPERTENSION AND BLEEDING VARICES :

The superior mesenteric vein and the splenic vein just behind the head of the pancreas at about the level of the second lumbar vertebra form the portal vein. It has segmental intrahepatic distribution along with the hepatic artery. Portal blood flow is about 1- 1.2L/min and portal pressure is about 7mmHg. When the portal circulation is obstructed, collateral circulation develops to carry the portal blood into the systemic veins[(75)].

FIGURE 20 : PATHOGENESIS OF PORTAL HYPERTENSION



According to Ohm's law, the portal pressure gradient(ΔP) is given by portal flow(F) multiplied by the resistance to its flow(R)[76].

$$\Delta P = F*R$$

In cirrhosis, both portal flow and resistance offered to it are increased, thus the pressure gradient leading to portal hypertension[76].

New vascular structures sprout (angiogenesis) and the collaterals dilate(collateralization) occur connecting the high-pressure portal venous system with lower-pressure systemic veins, but even these 2 processes are not enough for normalizing portal pressure and inturn causes complications of portal hypertension, such as esophageal varices, hemorrhoids[76].

COLLATERAL CIRCULATION AND VARICES :

Normally blood flow is towards the portal circulation through the collaterals but as the portal pressure increases in cirhhosis, the blood flow is reversed and flows away from the portal circulation[76].

Portal pressure should be a minimum of 10mmHg for the varices to develop and 12mmHg for the varices to bleed[76].

The major sites of porto-systemic or porto-caval shunts are lower part of esophagus, umbilicus, rectum and have been depicted in the following picture[75].

FIGURE 21 : PORTO-CAVAL SHUNTS

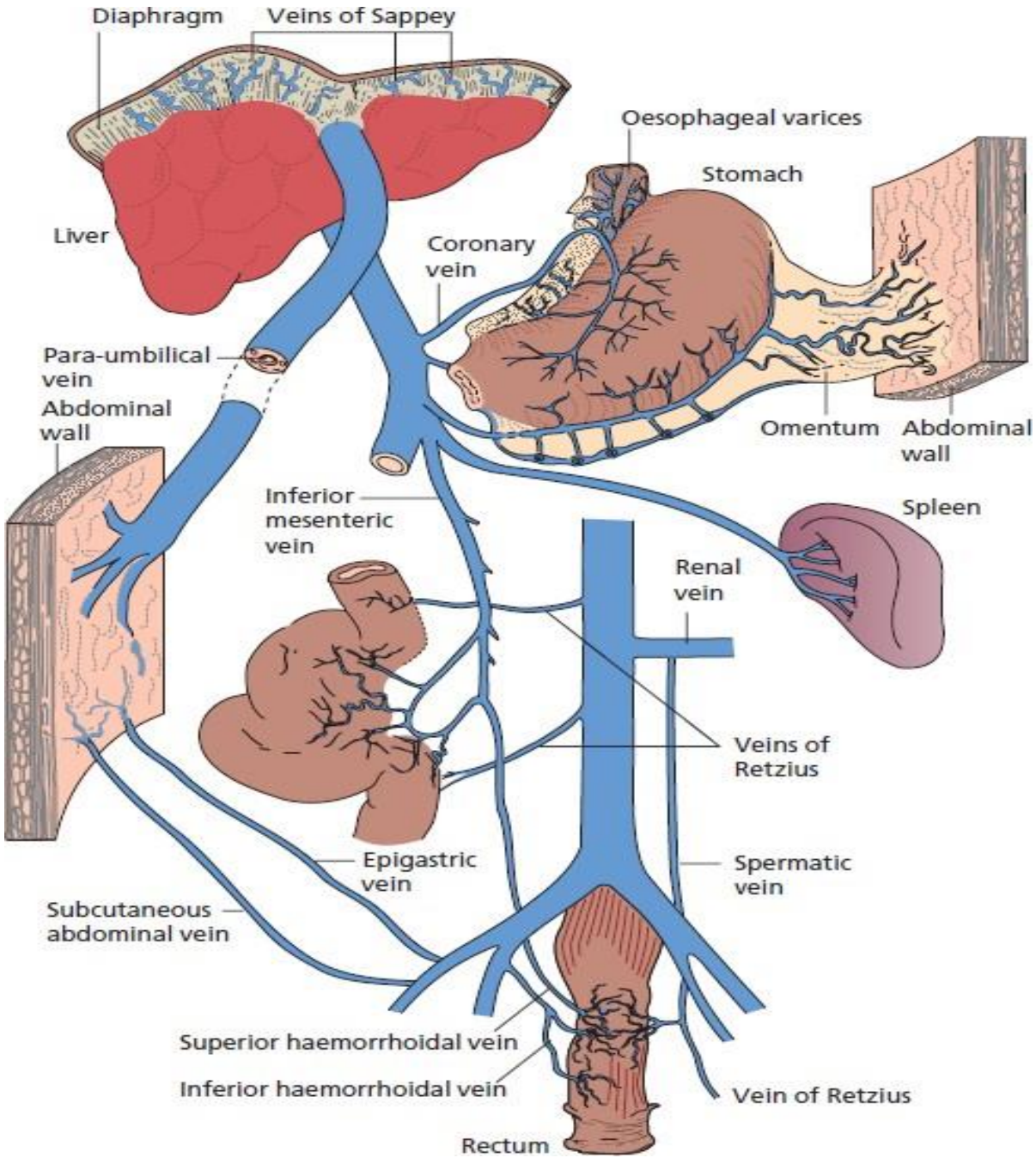
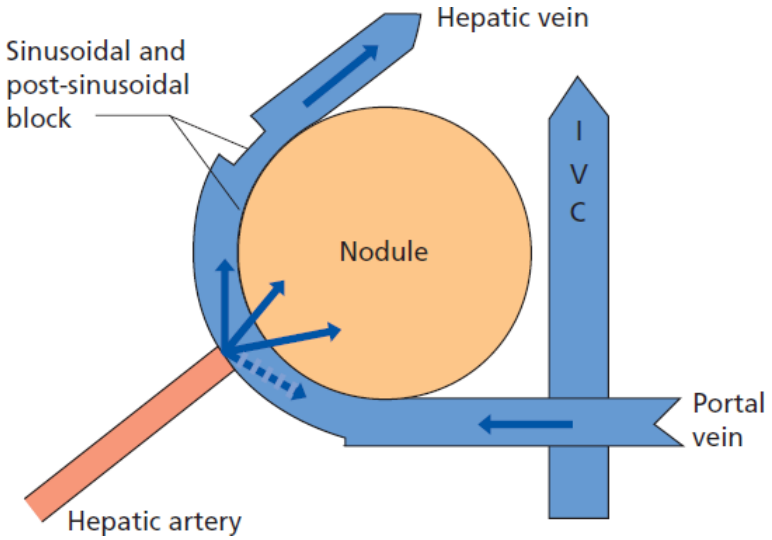


TABLE 5 : CLASSIFICATION OF PORTAL HYPERTENSION[76]

POST HEPATIC	INTRA HEPATIC	PREHEPATIC
Budd-Chiari syndrome	**PRE SINUSOIDAL**	Portal vein thrombosis
Constrictive pericarditis	Idiopathic portal hypertension	Splenic vein thrombosis
Inferior vena caval obstruction	PBC	
Right-sided heart failure	Sarcoidosis	
Severe tricuspid regurgitation	Schistosomiasis	
	SINUSOIDAL	
	Alcoholic cirrhosis	
	Alcoholic hepatitis	
	Cryptogenic cirrhosis	
	Postnecrotic cirrhosis	
	POST SINUSOIDAL	
	Sinusoidal obstruction syndrome	

FIGURE 22 : CIRRHOTIC NODULE OBSTRUCTING THE HEPATIC VESSELS



Nodules in cirrhosis mainly supplied by the hepatic artery obstructs the sinusoids and hepatic veins[75]

CLINICAL FEATURES OF PORTAL HYPERTENSION[75] :

Most common cause of portal hypertension is cirrhosis.

Most common presentation is hematemesis.

ABDOMINAL WALL VEINS : Dilated veins radiating from the umbilicus termed as caput Medusae. Cruveilhier-Baumgarten syndrome is the association of dilated abdominal wall collaterals and a loud venous murmur at the umbilicus.

SPLENOMEGALY : Fibrocongestive splenomegaly is more due to reticuloendothelial hyperplasia rather than the portal hypertension. It is larger in younger cirrhotics & in macronodular than in micronodular cirrhosis.

ASCITES : The portal hypertension increases the capillary filtration pressure and thus causes ascites.

GASTRIC & INTESTINAL VASCULOPATHY : Portal hypertensive gastropathy, Gastric antral vascular ectasia. Congestive jejunopathy and colonopathy are seen.

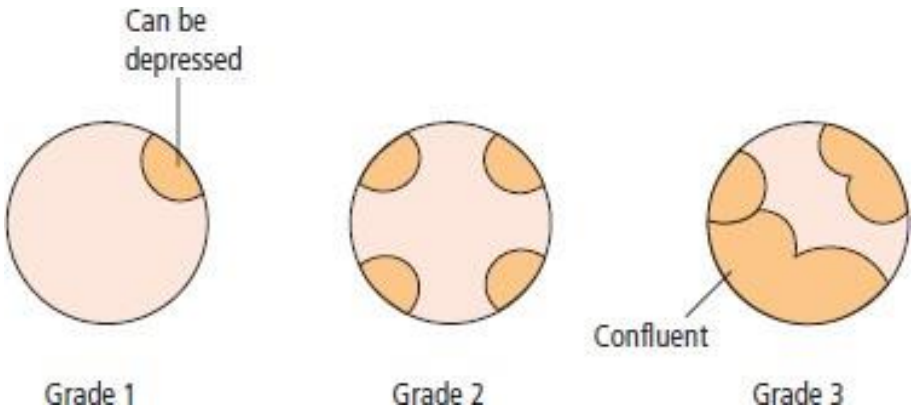
ANORECTAL VARICES : Present in 44% of patients with cirrhosis. They must be differentiated from simple haemorrhoids which are prolapsed vascular cushions which do not communicate with the portal system.

DIAGNOSIS OF PORTAL HYPERTENSION :

Ultrasound, Doppler studies, MRI, Venography, Portal pressure measurement help in diagnosis of portal hypertension[75].

For varices, barium studies & endoscopy are helpful. The following picture shows grades of varices on endoscopy[75].

FIGURE 23 : GRADES OF VARICES ON ENDOSCOPY



Grade 1: Small and straight

Grade 2 : Tortuous and occupying less than one third of the esophageal lumen

Grade 3 : Large and occupying more than one third of the esophageal lumen[76].

MATERIALS AND METHODS

Study title:

"ASSOCIATION BETWEEN ALDEHYDE DEHYDROGENASE 2 (GLU504LYS) POLYMORPHISM AND ALCOHOLIC LIVER DISEASE"

Aims and objectives:

To determine the association between Alcohol dehydrogenase 2 Glu504Lys polymorphism and alcoholic liver disease and the protective effect of 504Lys against ALD.

Study centre:

Department of general medicine, BLDE DU Shri B M Patil medical college hospital and research centre.

Study design: Cross sectional study.

Study period: From January 2021 to June 2022.

Selection of Patients:

The study included a total of 64 cases, 32 cases diagnosed as Alcoholic liver disease patients and 32 are controls reporting to the in our hospital. All the subjects were clinically examined and necessary laboratory investigations are done. Ethical clearance was obtained from Institutional Review Board. Informed written consent was obtained from all the participants before blood samples were drawn.

Inclusion criteria

1) Patients who had a history of excessive alcohol consumption.

2) Patients with Established diagnosis of Alcoholic Cirrhosis.

3) Patients of both genders are included.

Exclusion criteria

1) Patients with existing or past co-infection with Hepatitis B or C.

2) other causes of chronic liver disease

For control group

Subjects with:

1) no history of excessive alcohol consumption.

2) no liver diseases.

3) LFT (Normal).

Laboratory investigations:

Samples were collected from all the participants to estimate

- Liver function tests
- Haemoglobin levels
- Platelet counts
- Mcv(mean corpuscular values)
- Usg abdomen & pelvis for all cases
- Upper GI endoscopy if indicated.
- 1ml blood sample in EDTA vacuatiners for gene analysis.

Clinical Sample (Blood) Collection:

Consent was obtained from the subjects, who were enrolled in the study. After taking consent, 1 ml peripheral blood samples were collected in the EDTA-coated vacutainers (BD367863) and stored at 4°C.

Isolation of Genomic DNA and Quantification

From 300µl of peripheral blood genomic DNA was isolated, with the help of a commercial DNA isolation kit (Bangalore Genei, India).

Genomic DNA Isolation Protocol:

1. In a 1.5 ml EDTA-coated vial 300 µl of peripheral blood was collected.
2. By adding1 ml of 1 X solution A (provided by the kit) RBC cells were lysed.
3. At room temperature the vials were centrifuged for 5 min at 8000 RPM.
4. Until a clear white WBC pellet was obtained the above step was repeated.
5. 600µl of solution B was added (provided by the kit) to the WBC and mixed gently for clear lysis.
6. It was centrifuged at room temperature for 10 min at 10,000 RPM.
7. The Supernatant was collected and 0.9 ml absolute cold ethanol was added to it and mixed.
8. Centrifuged at 4oCfor 20 min, at 10,000 RPM.
9. Precipitate DNA was washed with 0.5 ml of 75% ethanol.
10. Centrifuged for 5 min at 10,000 RPM.
11. 100 µl of solution C was added (provided by the kit) after air drying the DNA pellet.
12. The vial was incubated at 55oC for 10 min.
13. To remove any insoluble materials it was centrifuged at 10,000 RPM for 2 min.

14. The DNA thus obtained was stored at -20oC until further use.

The quality of the isolated DNA was checked under gel electrophoresis. 100 ml of 1% agarose gel was prepared (1 gm of Agarose + 100 ml of 1X TAE buffer). The same isolated DNA was quantified under "Nanodrop" (Quawell) and the quantity and quality of the DNA were reported.

Exon-specific intronic primer designing:

The web-based freely available program "Primer3" which is widely accepted was used, (http://frodo.wi.mit.edu/ primer3/ input. Html) for designing PCR primers. Primer 3 is a Bioinformatics tool that helps in designing the primers for the target region in the given nucleotide sequence as per the requirement of the user or applications.

The designed primers using Primer 3 were reconfirmed for the specificity of its binding site using the web-based bioinformatics tool "Genome Build 36" (https://genome.ucsc.edu/FAQ/FAQreleases.html), and for its Insilco amplification on "Insilco PCR" (http://insilico.ehu.es/PCR/). All the designed primers for our target genes or region are tabulated in table No. 1 along with the annealing temperature and amplicon size. Primers were got synthesized by a commercial oligo synthesizer (MWG Biotech, India).

Table 6. Details of the primer sequences and annealing temperatures used for the amplification of exon 12,13 of ALDH2 gene.

Sl.No	Primer ID	Sequence	Product Size	Annealing Temperature
1	ALDH2 12F	5′-TTTGGTGGCTAGAAGATGTC-3′	187	58.5^0C

2	ALDH212R	5′ - CACACTCACAGTTTTCTCTT-3′		
3	ALDH2-EX-13-F	5’-ATCATGCAAGCTTCCTCCCT-3’	213	60.0^0C
4	ALDH2-EX-13- R	5’-ACTCTTACCCTCAGCCAACC-3’		

Polymerase Chain Reaction (PCR):

PCR amplification was carried out in a 20µl reaction volume containing 0.5 µl of genomic DNA (75ng/µl to 150 ng/µl), 0.5µl of each primer (5pmol), 0.4µl of dNTP (10pmol), 0.2µl Taq DNA polymerases (3units/ µl), 4 µlTaq Buffer (5X) (BioRad, USA) and the total volume was adjusted to 20µl using molecular biology grade water. Amplification was carried out in Master cycler gradient (Eppendorf, Germany) under the following conditions: an initial denaturation at 980C for 10sec, followed by 35 cycles at 980C for 10sec (cycle denaturation). The primer annealing temperature was set depending on the annealing temperature of the primer (Table-1) for 10sec 720C for 15sec (primer extension) and a final extension at 720C for 5 min. PCR products were confirmed for their respective amplicon size by gel electrophoresis with a standard 100bp ladder.

The PCR cycling conditions were as follows Initial Denaturation is 980C for 10 sec, Denaturation is 980C for 10 sec, Annealing is primer dependent for 10 sec, Elongation 720C for 5min & Hold at 4 0C.

Agarose Gel Electrophoresis

Gel electrophoresis is one of the molecular biology techniques used to separate DNA and RNA depending on the length of fragments. It is a widely used and accepted method, to estimate the size of DNA and RNA fragments or to separate proteins by charge. Nucleic acid molecules are separated based on an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and migrate farther than longer ones

because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving

.

DNA Sequencing (Capillary Based)

PCR products were subjected for capillary based Big-Dye terminator sequencing. Prior to sequencing, the PCR products were subjected to cycle sequencing and plate processing.

Cycle Sequencing

As per the Sanger Sequencing protocol, Big-Dye labeling and chain termination were carried out by the cycle sequencing method. To label each base, the PCR amplicon was subjected to a cycle sequencing reaction with a single primer. Big-Dye TM terminator
v3.1was used for cycle sequencing (Applied Biosystems, USA) following the manufacturer's guidelines. Cycle sequencing of the PCR products was carried out according to the annealing temperature of the primers.

Table 7. Cycle sequencing PCR mixture constituents

SL. No.	Constituents	Quantity
1	Molecular Biology grade water	6.3 µL
2	Big Dye Buffer (5X)	1.3 µL
3	Big Dye	1.0 µL
4	Template (PCR product)	1.0 µL

5	Forward Primer	0.2 µL
6	Reverse Primer	0.2 µL
Total		**10 µL**

Note: Only one of the primers i.e either forward or reverse primer was used during cycle sequencing

Table 8. The cycle sequencing conditions

process	**Temperature**	**Time**
Initial denaturation	98	10sec
Denaturation	98	10sec
Annealing	Primer dependent	10sec
Elongation	72	5min
Hold	4	

Note: The annealing temperature is primer dependant and varies for each primer.

Sequencing Clean-up (Plate Processing)

To remove the unbounded florescent DNTPs from the terminator sequencing reaction, 2µl of 3M sodium acetate, and 50µl of 100% ethyl alcohol were added to each sample and incubated at room temperature for 15 minutes to precipitate the DNA. The samples were centrifuged at 4000 rpm for 30 minutes at 4°C.

The supernatant was discarded and the reaction plate was centrifuged in a reverse manner at 300 rpm for 20 seconds. 100µl of 75% alcohol was added to each sample and centrifuged at 4000rpm for15 minutes at 25°C. The supernatant was discarded and the plate was centrifuged in a reverse manner at 300 rpm for 20 seconds to remove the alcohol completely.

The plate was dried at room temperature until the last drop of alcohol dripped off. 10µl of Hi-Di Formamide was added to each well of the sample plate.

The samples were heated to 96°C for 5 minutes and immediately cooled to 4°C to denature and linearise the cycle sequencing products. The processed products were loaded in the sequencer for sequencing.

Sequencing Run

Sample information sheets which contain analysis protocols along with the sample details were prepared and imported into the data collection software. Prepared samples were analyzed on ABI 3730 genetic analyzer (Applied Biosystems, USA) to generate DNA sequences or electropherograms. After completion of the sequencing reaction, the quality of generated sequence was checked by using Sequencing Analysis v5.4software (Applied Biosystems, USA).

Sequence Alignment

The generated sequences were aligned to their respective reference sequences with the use of Variant reporter software (ABI v1.1). The variant reporter is one of the compatible software of Applied Biosystems designed for automated sequence data analysis. It performs sequence comparisons for novel mutations, known variants, insertions, and deletions. It allows analysis of the resequenced data, comparing the consensus sequences to a known reference sequence.

The results of the variant reporter were tabulated in PDF format as the default program of the software.

Observations and Results

- Here, we used this technique to check the isolated genomic DNA from whole blood.
- EtBr stain was used to stain the DNA fragments. In all the 64 subject samples as shown in

figure 1. Which confirmed the presence of genomic DNA and the same samples were taken for quantification based on Nanodrop.

Quantification of Genomic DNA

- We used the Q3000 UV Spectrophotometer (Nanodrop) for the quantification of genomic DNA. Quawell 3000 is a micro-volume UV spectrophotometer specifically designed for the measurement of nucleic acids and purified proteins.
- Its unique technology holds 0.5-2.5 ul samples between upper and lower measurement surfaces without the use of a cuvette. The Q3000 measures the samples in less than 2 seconds with a high degree of accuracy and reproducibility.
- The Quawell 3000 works on the principle, “Nucleic acids absorb light at a wavelength of 260 nm and when 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and the amount of light absorbed by the sample can be inferred. For doublestranded DNA, an Optical Density (OD) of 1 at 260 nm correlates to a DNA concentration of 50 ng/µl, so that DNA concentration can be easily calculated from OD measurements”.

Polymerase Chain Reaction (PCR)

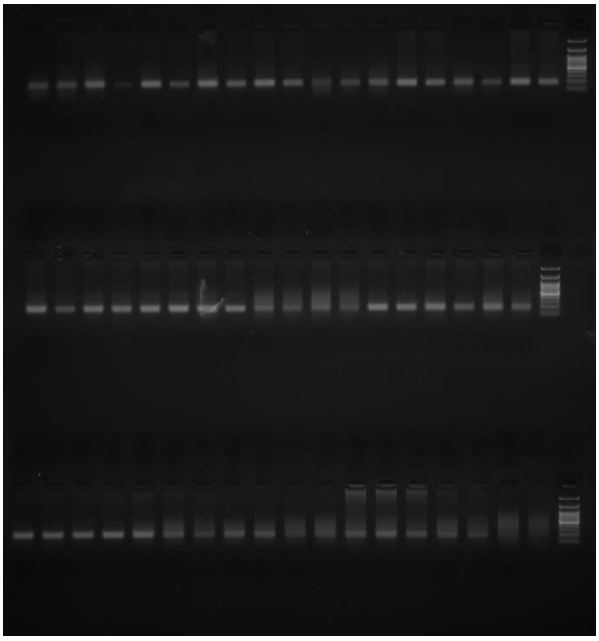
- PCR (Polymerase Chain Reaction) is a revolutionary method developed in the 1980s by Kary Mullis. PCR is based on using the ability of DNA polymerase to synthesize new strands of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a pre-existing 3'-OH group, it needs a primer to which it can add the first nucleotide.
- This requirement makes it possible to delineate a specific region of the template sequence that the researcher wants to amplify. At the end of the PCR reaction, the specific sequence

will be accumulated in billions of copies (amplicons).

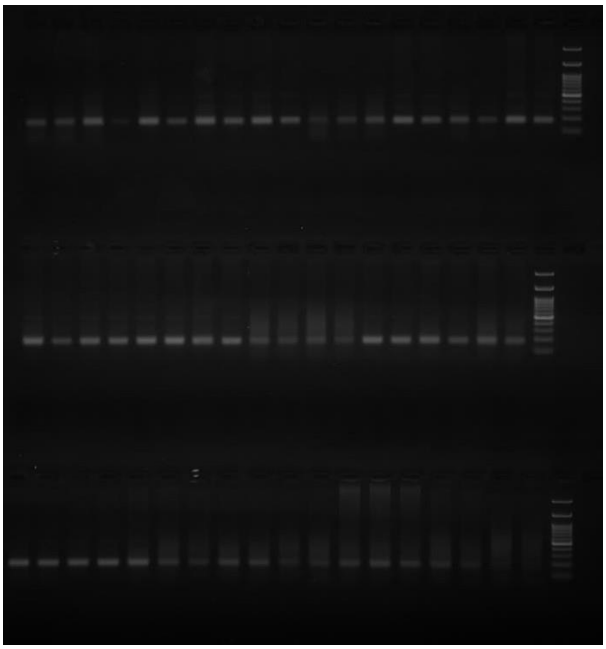
- We used ALDH2 gene exon 12,13 specific primers as given in table 1 and carried out the PCR reactions. After PCR, the products were subjected to Gel electrophoresis, and results were documented for subject samples.

- In all the products we observed bright specific amplicons in comparison with the 100bp ladder, which confirmed the primer-specific amplification in the PCR reaction. Primers (ALDH2) specific amplification results are shown in figure 2. After the PCR amplification, the amplicons were run through 1% agarose gel electrophoresis and the DNA bands were observed in gel documentation . The PCR product of 100bp.

Figure 24: Agarose gel electrophoresis image of amplified products of exon13 of ALDH2 gene.



PCR PRODUCTS with 1-32 SAMPLES with 190bp size of the primer aldh2 13 exon



PCR PRODUCTS WITH 1-32 SAMPLES with 190bp size of the primer aldh2 13 exon

Sequencing Results (Capillary Sequencing):

- After sequencing, all the electropherograms of cases and control samples were subjected to analysis in comparison with reference sequences. No mutations were recorded in exon 12 of Aldh2vgene in all the 64 samples of present study cohort. However in studying exon 13 of ALDH2 gene we found out 2 mutations in cases.

Statistical analysis:

- The data obtained will be entered in a Microsoft Excel sheet, and statistical analysis will be performed using statistical package for the social sciences (Verson 20). Results will be presented as Mean (Median)±SD, counts and percentages and diagrams.
- For normally distributed continuous variables between two groups will be compared using Independent t-test For not normally distributed variables Mann Whitney U test will be used. Categorical variables between the two groups will be compared using Chi-square test.
- Correlation coefficient will be used to find the correlation between quantitative variables.

$p<0.05$ will be considered statistically significant. All statistical tests will be performed two-tailed.

RESULTS

1. Gender distribution:

All subjects(64) included are male.

2. Demographic and clinical characteristics of the cases cohort.

The detailed characteristics of subjects with ALD are summarised in table. The mean age of the patients is 48.8,which is comparable to that controls . Average amount of alcohol per day is 173g (Table 9)

	Mean	Std. Deviation
age	48.08	11.894
amount of alcohol per day (gms)	173.88	60.441
bmi (kg/m2)	26.526562	4.4749122
total bilirubin (mg/dl)	5.454688	11.4783015
AST (U/L)	71.05	91.092
ALT (U/L)	34.59	27.803
ALP (g/dL)	114.59	77.654
Hb (g/dL)	11.306250	2.4151555
mcv (fl)	92.420	6.8765
platelets (thousands)	226.61	116.713

3.The prevalence of ALDH2 variants in healthy controls and patients with ALD(table 10)

- We found that the prevalence of the common form of 504glu(glu/glu) is seen in 50% of ALD patients and 46.9% in controls. Among controls 53.7% had heterozygous (glu/lys) genotype compared to 50% in those with ALD

	Controls (N=32)		**Cases (N=32)**				
Genotype distribution	n	%	n	%	p-value	OR	95%CI
Glu/Glu	15	46.9	16	50	0.802	1.133	0.4249 to 3.0226
Glu/Lys	17	53.1	16	50			

- The prevalence of ALDH2 Glu504lys variants in patients with alcoholic liver disease did not show statistically positive correaltion(p=0.802) with OR(odds ratio=1.133), 95% CI - 0.4249 to 3.0226.

4.clinical characteristics of ALD patients stratified by ALDH2 variants.(table 11)

	ALDH2 glu504lys variants			
Clinical characteristics	**Glu/Glu(n=16)**	**Glu/Lys(n=16)**	**P-value**	**Mann-whitney u test**
Age (years)	44.375 14.075	43.750 8.226	0.734	118.500
duration of alcohol intake (years)	13.500 ± 5.680	12.438 ± 2.874	0.909	131.500
amount of alcohol per day (gms)	161.000 ± 53.277	186.750 ± 66.014	0.233	97.500
bmi (kg/m2)	30.256 ± 2.2121	30.163 ±3.266	0.955	130.00
total bilirubin (mg/dl)	5.725 ±8.444	14.213 ± 18.877	0.050	75.500
AST (U/L)	81.938 ± 41.115	138.37 ± 155.390	0.484	109.000
ALT (U/L)	42.563 ± 39.998	43.938 ± 23.499	0.375	104.000
ALP (g/Dl)	170.438 ± 61.193	161.625 ±91.052	0.429	149.5000
Hb (g/dL)	9.075 ±1.649	10.031 ±1.764	0.136	88.000
mcv (fl)	95.200 ±4.290	95.756 ± 4.289	0.850	133.5000
Platelets(thousands)	146.250 ±48.427	155.375 ±71.478	1.000	128.500

We determined the alcohol consumption history as well as clinical characteristics of ALD patients stratified by ALDH2 variants (table 11).

ALD patients with heterozygous(Glu/Lys) genotype had shorter duration of drinking before the enrolment (12.438 ± 2.874) than those with Glu/Glu genotype (13.500 ± 5.680), though the difference was not statistically significant (p=). Daily alcohol consumption was higher in patients with heterozygous genotype (186.750 ± 66.014 vs 161.000 ± 53.277). Most of the laboratory tests were comparable between both groups, ALD patients with heterozygous (Glu/Glu) genotype had higher bilirubin (14.213 ± 18.877 vs 5.725 ±8.444) , higher AST levels (138.37 ± 155.390 vs 81.938 ± 41.115) , lower ALP levels (161.625 ±91.052 vs 170.438 ± 61.193), and higher platelet levels (155.375 ±71.478 vs 146.250 ±48.427) than those with Glu/Glu genotype.

5.EXON 13 of ALDH2 sequence analysis (table 12)

Total No of Cases studied for ALDH2 mutation -32

Total No of Cases studied for ALDH2 mutation -32

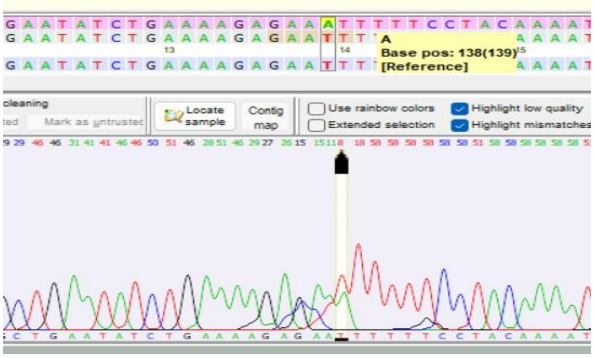
List of mutations recorded in cases: 02

SL. NO.	VARIANT type	SAMPLE NO.	gDNA (NG_012250.2)	Exon	MUTATION – NOVEL/REPORTED
1	3'UTR	ALDH2-5	g.47794 A>T (heterozygous)	13	rs1273980328SNP
2	3' UTR	ALDH2-7	g.47854 T>G (heterozygous)	13	Not recorded

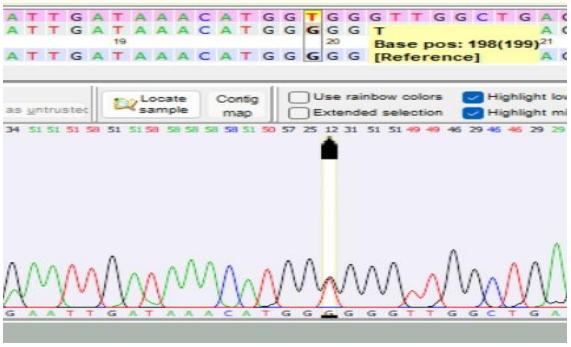
List of Mutation recorded in controls: 00

IMAGES: (fig-25)

SL NO-1 -g.4b 7794 A>T



SL NO 2 - g.47854 T>G



Variant 3 UTR means:

The three prime untranslated region [**3'UTR**] is the part of messenger RNA that is next to the translation termination codon. The 3'UTR of mRNA is transcribed from DNA, but is not translated into protein. **3'UTR** are best known to regulate mRNA-based processes, such as mRNA localization, mRNA stability, and translation

Present study records mutation in 3'UTR region of the targeted gene that might disrupts the ability of miRNAs to target genes resulting in differential mRNA and protein expression.

DISCUSSION

- Alcohol is a toxic and psychoactive substance with dependence producing properties. In many of today's societies, alcoholic beverages are a routine part of the social landscape for many in the population. Alcohol consumption contributes to 3 million deaths each year globally as well as to the disabilities and poor health of millions of people. Overall, harmful use of alcohol is responsible for 5.1% of the global burden of disease.
- Aldehyde dehydrogenase (ALDH) 2 is a mitochondrial enzyme that catalyzes the oxidation of acetaldehyde, an intermediate of ethanol metabolism [1] . The Glu504Lys single nucleotide polymorphism (SNP) of ALDH2 gene, which occurs with an incidence of 35–57% in different East Asian subpopulations, causes defect in the enzyme activity of ALDH2, leading to alterations in acetaldehyde metabolism and markedly reduced alcohol tolerance [3, 4].
- Epidemiological studies have linked ALDH2 Glu504Lys SNP with increased risk for human diseases including cardiovascular disease (CVD), cancer.
- The association between ALDH2 Glu504Lys SNP and the development of these diseases is also related to the effect of the SNP on lifestyle factors such as alcohol consumption and its interaction with other genetic variations.
- A single nucleotide polymorphism (SNP) at exon 12 predicts LYSINE at residue 504 instead of GLUTAMIC ACID of the ALDH2 gene. The 504LYS allele produces a inactive isoenzyme and limits its activity to metabolize acetaldehyde .
- As a result, subjects with Lys allele have unpleasant side effects such as flushing, nausea, vomiting due to reduced elimination of acetaldehyde. People with this allele could have a decreased use due to adverse reactions from drinking .
- Dawei Li, Hongyu Zhao, et al. studied the protective effect of aldehyde dehydrogenase

gene ALDH2 504 Lys allele against alcoholism and alcohol-induced diseases concluded that ALDH2 504 Lys allele could significantly lower the risk for alcohol dependence [9].

- In the view of the present perspective, there were multiple stuidies conducted in east Asian population including china and Japan and there are very few data on genes/polymorphisms that confer susceptibility to ALD in the Indian population, this study is conducted to find the association between ALDH 2 Glu504Lys polymorphism and alcoholic liver disease**.**
- The finding in our study is that patients with ALDH2 504lys variant are equally associated with ALD as compared to ALDH2 504glu variant using genotypic analyses.
- The Lys allele plays an important role in regulating the ALDH2 activity, for instance reduction in ALDH2 activity in patients with heterozygous(glu/lys) genotype is more than 100-fold compared with that of glu/glu homozygotes.
- Several studies demonstrated a reduced frequency of the lys allele in alcoholics compared ti the non alcoholics. Individuals with lys allele have 10 fold reduction in the risk of alcohol dependence, drink less alcohol and have lower prevalence of binge drinking. In our study which is done in Indian population we could not find this relation between lys allele and ALD.
- When we carefully analysed alcohol drinking history, we found out that lys allele cases had shorter duration and more daily quantity of alcohol intake when compared to ALDH2 504glu, which is again a negative correaltion between ALDH2 504lys SNP and ALD.
- HOWERVER there was a breakthough finding while conduction sequence analysis of exon 13 of ALDH2 gene. Out of 32 cases we found out there are exon 13 mutations in 2 of the samples g.47794 A>T (heterozygous), g.47854 T>G (heterozygous). These mutations occurred in patients who are younger with less amount of alcohol consumption when compared with remaining cases.

CONCLUSION

- Alcohol consumption is one of the risk factors for health problems such as injuries, diseases, and liver cancers, depending upon the amount and duration of alcohol consumption.
- The prevalence of ALDH2 Glu504lys variants in patients with alcoholic liver disease did not show statistically positive correaltion($p=0.802$).
- However,we found out mutations in exon 13 of ALDH2 gene, in which patients had less amount and shorter duration of alcohol consumption which is statistically significant.
- We can conclude that mutations in those regions are responsible for predisposition of early disease.
- Early Genetic analysis in selected population for finding these mutations may prevent the occurrence of the disease.

LIMITATIONS

- The sample size of the study was small.
- The study should ideally done in selected population before the occurrence of the disease.
- There is no female population in the study. Variation of single nucleotide polymorphisms based on sex could not be assessed.

SUMMARY

Alcoholic Liver Disease (ALD) is one of the major problems affecting the world and is the primary cause of at least 60 major types of systemic diseases, according to the World Health Organization on alcohol .

A single nucleotide polymorphism (SNP) at exon 12 predicts LYSINE at residue 504 instead of GLUTAMIC ACID of the ALDH2 gene. The 504LYS allele produces a catalytically inactive isoenzyme and limits its activity to metabolize acetaldehyde (.

subjects with Lys allele have unpleasant side effects such as flushing, nausea, vomiting due to reduced elimination of acetaldehyde. People with this allele could have a decreased risk of excessive use due to adverse reactions from drinking .

Early Genetic analysis in selected population for finding these mutations may prevent the occurrence of the disease.

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ANNEXURE I

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

ANNEXURE II

SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA-586 103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: " ASSOCIATION BETWEEN ALDEHYDE DEHYDROGENASE 2 (Glu504Lys) POLYMORPHISM AND ALCOHOLIC LIVER DISEASE "

PG GUIDE : DR. S M BIRADAR

PG STUDENT : DR. SETHU REDDY

PURPOSE OF RESEARCH: I have been informed about this study. I have also been given a free choice of participation in this study.

BENEFITS:-

I understand that my participation in this study will help the investigator to diagnose the disease better and will help in the management of the disease.

PROCEDURE:-

I understand that relevant history will be taken and I will undergo detailed clinical examination after which necessary investigations will be done and accordingly treatment will be given.

RISK AND DISCOMFORTS:-

I understand there is no risk involved and I will experience no pain during the procedures performed.

CONFIDENTIALITY:-

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION: -

I understand that I may ask more questions about the study at any time Concerned. The researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION: -

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that the researcher may terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician, if this is appropriate.

INJURY STATEMENT: -

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's / relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Investigator / P. G. Guide Date

I confirm that …….. (Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____

_____

Participant / guardian Date

_____ _____

Witness to signature Date

ANNEXURE-III: SCHEME OF CASE TAKING PROFORMA

B.L.D.E (DEEMED TO BE UNIVERSITY)

SHRI B M PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,VIJAYAPURA, KARNATAKA

Name of the patient : IP NO:

Age and Sex : Religion:

Address: Occupation:

Annual income:

Presenting Complaints :

Past history:

Personal history:

1 . Diet- Veg/Mixed

2. Sleep

3. Appetite

4. Bowel and Bladder Habits

5. Habits: Alcoholic history- amount of alcohol/day:

Type of alcohol :

Duration of consumption:

Family history:

GENERAL PHYSICAL EXAMINATION :

Built :

Nourishment :

Ht(Cm) : Wt(kg): BMI:

Pallor: Lymphadenopathy:

Cyanosis: Edema:

Clubbing:

GENERAL EXAMINATION (HEAD TO TOE) :

1. scalp and hair:
2. eyes:
3. ears:

4. nose:

5. oral cavity:

6. neck:

7. chest:

8. upperlimbs:

9. abdomen:

10. lower limbs:

Vital parameters a) Pulse : b) BP:

c) temperature: d) Respiratory rate:

SYSTEMIC EXAMINATION:

ABDOMEN EXAMINATION:

INSPECTION:

PALPATION:

PERCUSSION:

AUSCULTATION:

CARDIOVASCULAR SYSTEM

RESPIRATORY SYSTEM

CENTRAL NERVOUS SYSTEM

INVESTIGATIONS:	
1) liver function test	
Bilirubin, total	
Bilirubin conjugated (direct)	
Bilirubin (indirect)	
Alanine aminotransferase (ALT/SGPT)	
Aspartate aminotransferase (AST/SGOT)	
Alkaline phosphatase	
Protein total	
Albumin	
Globulin	
A/G ratio	
2) URINE ROUTINE:	
3) HBsAG	
4) HCV	
5) PT/INR	
6) complete blood count	
Hb	gm/dl
Total count	Cells/cumm
Differential count Neutrophils	%
Lymphocytes	%
Eosinophils	%

Basophils	%
Monocytes	%
ESR	
Platelets	

PERIPHERAL SMEAR STUDY:

STOOL 1) ROUTINE

2) FOR OCCULT BLOOD:

ULTRASOUND DIAGNOSIS:

UPPER GI ENDOSCOPY(IF REQUIRED):

SAMPLE COLLECTION FOR GENE ANALYSIS:

CONCLUSION :

Diagnosis -

Stage of disease

FOLLOW UP OF PATIENT DURING HOSPITAL STAY:

SIGNATURE:

DATE:

ANNEXURE IV

MASTER CHART

KEY TO MASTER CHART
ALP: ALKALINE PHOSPHATASE
ALT: ALANINE TRANSAMINASE
AST: ASPARTATE TRANSAMINASE
BMI: BODY MASS INDEX
HB: HEAMOGLOBIN
SNP: SINGLE NUCELOTIDE POLYMORPHISM
TB: TOTAL BILIRUBIN

	patient name	age	sex	ipno	doa	dod	chief complaints	duration of alcohol intake (ye	amount of alcohol per day (gi	bmi (kg/m2)	total bilirubin (mg/dl)	AST (U/L	ALT (U/L	ALP (g/dL)	Hb (g/dL)	mcv (fl)	platelets (thousands	snp polymorphism	other mutations	
2	Ningappa gaur	40	M	294606	13/12/2021		abdominal distension,abdominal pain and bfl lowerlimb swe	12	144	32.3	1.1	44	30	153	12.1	98.4	132			
3	yamanappa hanamantapp	80	M	313066	27/12/2021		abdominal distension,yellowish discolouration of eyes	10	216	32.2	1.7	83	26	131	11.4	98.7	109	gluglu		
4	Dharmanna ningappa	70	M	103383	24/03/2022		abdominal distension,abdominal pain and bfl lowerlimb swe	30	144	30.9	0.8	28	15	67	6.4	88.5	196	gluglu		
5	Ameerhamaja sajedbash	33	M	278712	02/12/21		abdominal distension,abdominal pain,yellowish discolourati	12	288	33.9	24.7	163	74	54	9.5	95.2	263	glufiys		
6	Sudhakar ankoli	43	M	107303	28/03/2022		abdominal distension, breathlessness	10	144	33.1	3.8	112	44	144	7.9	97.3	164	gluglu		
7	Shankar sanakanavar	34	M	252452	15/11/2021		abdominal pain,abdominal distension,yellowish discolourat	15	144	35.6	9.2	660	56	94	8.7	92.3	49	glufiys		
8	Hanmanth guraddi	50	M	104356	25/03/2022		abdominal distension,abdominal pain,black coloured stool	15	216	28.1	7.5	93	51	169	9.2	105.5	94	glufiys		
9	Yamanappa beleppa pujar	41	M	170534	07/09/21		abdominal distension,abdominal pain,yellowish discolourati	14	216	31.2	21.5	156	63	84	10.6	98.2	89	glufiys		
10	Sidappa malkarji kawalagi	37	M	187647	22/09/2022		abdominal distension,abdominal pain	10	216	29.7	1.8	43	20	180	7.4	99.3	104	gluglu		
11	Yamanappa sidappa	45	M	110048	30/03/2022		abdominal distension,abdominal pain,bfl lower limb swelling	15	288	32.2	3.5	112	57	234	10.7	96.8	120	gluglu		
12	basappa channappa haiya	60	m	73892	27/02/2022		abdominal distension,yellowish discolouration of eyes	18	216	30.4	2.7	92	38	92	6.7	94.6	109	glufiys		
13	basavaraj shivalingappa b	45	M	70803	24/02/2022		abdominal distension,vomitings	12	144	29.8	3.6	19	16	164	11.9	90.2	257	glufiys		
14	shivangoda patil	39	m	65472	25/05/2021		abdominal distension,breathlessness,blood in vomitings	8	288	30.1	76.9	222	104	211	7.7	101	219	glufiys		
15	parashuram goudappa bir	43	m	69504	23/02/2022		abdominal distension,lower limb swelling,yellowish discolou	20	72	27.6	6.4	119	27	288	9.8	97.3	159	gluglu		
16	hanmant laxman	38	m	140589	11/08/21		abdominal distension,pedal odema,breathlessness	10	144	21.1	5.6	33	20	435	11.6	92.4	136	glufiys		
17	mahantesh paramanna	30	m	137544	24/04/2022		abdominal distension,bfl lower limb swelling,reduced apetite	12	144	27	36.1	178	118	98	7.5	89.4	150	glufiys		
18	rajshekar lingdalli	42	m	154269	07/05/22		abdominal distension,yellowish discolouration of eyes,bfl lo	14	144	29.1	25	91	35	160	11.1	95.5	156	glufiys		
19	parashuaram basappa	32	m	30515	23/01/2022		abdominal distension,yellowish discolouration of eyes	12	216	31.9	6	116	12	175	9.5	91.9	149	glufiys		
20	shivanand kavatagi	47	m	152870	06/05/22		abdominal distension,altered sensorium,yellowish discolou	10	288	31.1	2.8	44	18	111	8.9	92	167	glufiys		
21	shivanand kalappa jeer	48	m	39082	31/01/2022		abdominal distension,blood in vomitings	14	108	34.2	1.8	54	41	146	8.9	97.5	264	glufiys		
22	shivaning pawar	40	m	82786	07/03/22		abdominal distension,yellowish discolouration of eyes	18	200	30.5	9.9	52	51	268	10.4	86.5	129	glufiys		
23	yamanappa sidappa	25	m	110048	03/30/22		abdominal distension,yellowish discolouration of eyes	4	144	26.8	3.6	112	57	234	10.7	96.8	120	glufiys	exon 13	
24	mohan dashvant	50	m	128888	04/16/22		abdominal distension,bfl lower limb swelling	13	144	28.2	1	40	15	125	7.5	96.8	301	gluglu		
25	ramangouda	61	m	127635	04/15/22		abdominal distension,bfl lower limb swelling,reduced apetite	14	216	29.4	0.9	23	14	80	9.8	93.1	235	glufiys		
26	bhimrao	36	m	172567	05/21/22		abdominal distension,yellowish discolouration of eyes	12	108	27.8	4.4	67	18	185	7.9	96.2	144	gluglu		
27	muragesh	45	m	164309	05/16/22		abdominal distension,bfl lower limb swelling	12	144	31.1	3	42	18	184	8.2	94.9	139	gluglu		
28	vittal pawar	41	m	120035	04/08/22		abdominal distension,yellowish discolouration of eyes	14	216	28.5	6.4	65	49	255	9.2	102.8	111	glufiys		
29	rajashekhar	42	m	154269	05/07/22		abdominal distension,yellowish discolouration of eyes,bfl lo	10	144	29.6	25	91	35	160	11.1	95.5	156	glufiys		
30	suresh mali	45	m	138183	04/25/22		abdominal distension,yellowish discolouration of eyes	12	144	32.1	7.5	155	50	205	13.1	91.7	91	glufiys		
31	madivalappa	40	m	196218	06/09/22		abdominal distension,reduced apetite	11	108	30.7	3.1	65	19	115	8.9	92.4	132	gluglu		
32	sadashiva kamble	34	m	204575	06/15/22		abdominal distension,yellowish discolouration of eyes	7	72	28.4	6.3	253	39	166	12.5	94.6	90	glufiys	exon 13	
33	bhimangouda	54	m	152870	09/14/21		abdominal distension,yellowish discolouration of eyes	15	144	32.1	5.4	98	154	146	8.9	102	92	gluglu		
34	gurunath	60	m	56467	02/12/22		giddiness,breathlessness on exertion	occassional		23.2	0.8	22	15	17	12.6	85.4	562	glufiys		
35	raghavendra	38	m	47967	02/06/22		breathlessness on exertion,easy fatiguability	occassional		24	1.5	86	66	48	11.4	109	350	gluglu		
36	kuntappa	49	m	84394	03/08/22		easy fatiguability	occassional		22.4	1.3	34	23	44	12.3	87	254	gluglu		
37	laxman	52	m	89762	03/12/22		fever,breathlessness on exertion	occassional		24.5	0.7	31	24	50	10.4	76	489	gluglu		
38	jahangeer	57	m	126398	04/13/22		retrosternal chest pain,giddiness	occassional		29	0.6	19	11	67	12.8	82	269	gluglu		
39	sangondappa	48	m	78120	06/12/22		fever,rt sided chest pain	occassional		27.2	0.8	22	34	52	13.2	88	329	gluglu		
40	shivangouda biradar	55	m	116862	21-Jul		breathlessness on exertion, palpitations	occassional		22	0.4	21	14	58	12.7	86.2	289	gluglu		
41	vinod shankar	35	m	271529	27-Nov		burning micturition	occassional		21.6	1.8	66	40	78	13.4	96.5	212	glufiys		
42	gurappa	50	m	34166	01/26/22		breathlessness on exertion	occassional		23	0.5	26	19	101	14	84.7	283	gluglu		
43	shivanand	42	m	162156	05/13/22		easy fatiguability	occassional		22.5	0.4	19	12	51	13.2	89	339	gluglu		
44	laxman	65	m	165742	05/16/22		left sided chest pain	occassional		26	0.6	15	13	65	12.9	87.6	238	glufiys		
45	siddanagouda	60	m	186803	06/01/22		fever,burning micturition	occassional		24.2	1.1	26	35	41	13.7	82.5	211	glufiys		
46	basavaraj	52	m	195500	06/08/22		fever with chills	occassional		20.6	0.3	18	9	42	13.1	88.9	361	gluglu		
47	basavaraj	55	m	94091	07/01/21		giddiness,breathlessness on exertion	occassional		27.4	0.9	39	13	57	12.4	107	372	glufiys		
48	satappa	65	m	91224	03/14/22		vomitings,loose stools	occassional		21.6	0.6	18	11	66	14.7	87.3	469	glufiys		
49	basappa	51	m	142911	03/16/21		fever	occassional		19.8	0.5	22	37	28	11.2	88	271	gluglu		
50	chandru laxman	38	m	148998	07/13/21		vomitings,loose stools	occassional		22.7	1.4	22	36	48	11.7	92.7	328	gluglu		
51	shreeshail	55	m	197596	10/02/21		loose stools	occassional		23.3	1.4	21	42	36	11.8	84.8	278	glufiys		
52	surjakanth	70	m	157907	05/10/22		fever,joint pains	occassional		21.3	1.2	11	23	41	12	85	221	glufiys		
53	hanmant	62	m	363938	10/15/22		breathless on exertion,fever	occassional		20.3	0.9	43	23	87	15	96.3	160	glufiys		
54	ramesh	25	m	268474	08/03/21		fever,loose stools	occassional		19.4	0.8	22	15	18	12.4	94.2	235	glufiys		
55	sidappa	48	m	372108	10/23/21		chest pain	occassional		23.7	1.6	39	33	153	12.6	88.6	347	glufiys		
56	subhas	50	m	197139	03/15/22		exertional breathlessness	occassional		22.4	1.4	39	19	121	12.2	97.7	156	glufiys		
57	manjunath	45	m	200643	10/04/21		abdominal pain	occassional		20.7	1	28	17	45	12.6	86.5	416	glufiys		
58	chandappa	65	m	261997	11/21/21		abdominal pain,loose stools	occassional		23.5	1.5	38	14	67	16.9	88.6	176	glufiys		
59	bapugowda	64	m	169018	09/10/21		uncontrolled type 2 diabetes	occassional		21.5	0.8	31	23	52	10.4	88.3	392	glufiys		
60	pintu rathod	32	m	382562	11/02/22		abdominal pain,vomitings	occassional		20.6	0.6	95	105	124	15.3	72	543	glufiys		
61	haleppa	65	m	221304	10/20/21		breathlessness,vomitings	occassional		23.3	0.7	21	13	98	16.6	93.9	357	glufiys		
62	ashwatappa	52	m	172165	09/09/21		fever,cough with espectoration	occassional		22.1	1.3	26	22	75	15.2	103	165	glufiys		
63	rachappa	60	m	382660	11/02/21		bilateral lower limb weakness	occassional		23.7	1.3	60	44	93	13.7	84.8	160	gluglu		
64	shivangouda	65	m	101968	03/23/22		easy fatiguability	occassional		24.6	0.7	19	14	45	13.9	87.9	178	glufiys		
65	sangamesh	37	m	120201	04/08/22		breathlessness on exertion	occassional		18.9	0.7	23	11	53	11.6	90.2	267	glufiys		