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Review Article

Liver injury and hepatocellular carcinoma: A review

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ABSTRACT

Liver the largest gland of the body is at continuous risk of injury due to drugs, chemicals or foreign substances. Often the injuries depending upon the severity try to take a turn towards hepatocellular carcinoma. Cirrhosis is one of the common patterns of liver injury which is preceded by hepatitis and steatosis. F141L mutation of the HBsAg is held responsible for cirrhosis related injury. Mediators likeTGF-β_1, TIMP 1 and 2 play an important role in cirrhosis with specific histological changes. Irreversible type of hypoxic injury is the other type of injury characterized by an elevated serum transaminase level with the formation of bleb on the plasma membrane and increase in MCP-1and MIP-2 proteins. Injury due to free radical can be characterized by the accumulation of amyloid protein and lipofuscin. Where as injury due to chemical is confirmed by their cytotoxic and cholestatic pattern of changes. Sometimes an increase in iron overload, as can be seen with of hemochromatosis, leads to severe injury of liver with its specific histological pattern. Thus study of histological changes by biopsy along with other non-invasive techniques act as a confirmatory tool for the diagnosis of a particular disease and is of prognostic importance.

Keywords: Liver injury, Cirrhosis, Hypoxia, Free radical, Iron overload, Lipofuscin, Hepatic carcinoma, Hemochromatosis.

INTRODUCTION

Liver apart from being the largest gland of the body also serves as the primary site of biotransformation reaction, which helps in the process of detoxification of drugs and foreign substances. Thus it protects the body from the accumulative toxicity of drugs but many times it self suffers from crippling effect of the drug and chemicals, which brings about liver injury. As a result of which many important biological processes like glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification get affected[1]. Injury like cirrhosis frequently gives invitation to hepatic carcinoma.[2] Some of the pathological conditions like presence of infection with hepatitis B or hepatitis C also play an important role in the development of liver cancer[3]. Irrespective of the etiology of the cancer it kills the patient and claims to be the sixth most prevalent cancer[4], it consists the fourth cause of death related to cancer worldwide.[5] Histological studies help to reveal the type of liver injury associated and helps in the prognosis, enabling correct treatment strategy to be adopted.

Normal human liver and its histological importance

Adult Human liver weighs about 1500g and is reddish brown in colour. The liver is enclosed in a fibrous capsule. The liver is made of both parenchymal tissue and connective tissue fibre. It is mainly divided into right and left lobes[6]. It has dual blood supply, portal vein supplying 70% and hepatic artery with 30% of its required oxygen. Histologocally a healthy liver shows the presence of lobules which are the collection of 3-4 hepatocytes[7]. These lobules have their own blood supply and drainage system. The hepatocytes are arranged around the central vein, radiating away from it. A liver sinusoid is a type of blood vessel (with fenestrated, discontinuous endothelium) that serves as a location for the

oxygen-rich blood from the hepatic artery and the nutrient-rich blood from the portal vein Hepatocytes are separated from the sinusoids by the space of disse. Kupffer cells are located inside the sinusoids and can take up and destroy foreign material such as bacteria. The space of disse contains cells of Ito which store fat and fat-soluble vitamins. The space of disse and the cells of Ito play a major role as the determinant of liver injury.[8] Histology plays a major role in the following (1) for diagnosis (2) for assessment of prognosis (disease staging) (3) to assist in making therapeutic management decisions. All though noninvasive techniques (imaging, blood tests) are gaining popularity in the diagnosis of many liver diseases still liver histology is typically and most appropriately considered in conjunction with the clinical and laboratory data. Acute and chronic hepatitis, cholestatic disorders, fatty liver disease, vascular diseases, infiltrative or storage diseases, some infectious and granulomatous diseases, and other disorders may be associated with characteristic histological abnormalities that are helpful in diagnosis. Histological study through biopsy becomes the ultimate diagnostic marker when all other tests for example a thorough history, physical examination, biochemical, serological, and imaging investigation have failed to elucidate a diagnosis, that means in case of abnormal liver tests with unclear etiology.[9]

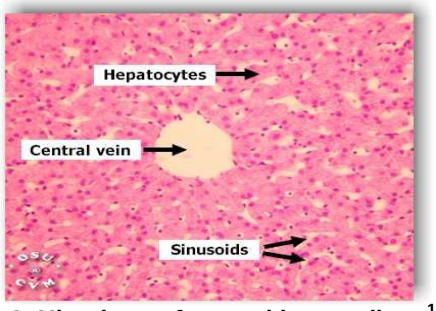

Fig. 1: Histology of normal human liver.[10]

Histological changes in liver injury and hepatic carcinoma and the pathways involved.

Liver injury due to cirrhosis

A histological change of an organ from the normal one to the other type is the indication of a particular disease, as we can see in the case of “cells of Ito”. Enlargement of these cells take place under certain pathological condition and is of prognostic importance for fibrosis and cirrhosis of liver[7].Cirrhosis is often preceded by hepatitis and fatty liver (steatosis), independent of the cause. Hepatitis B is the leading cause of liver cirrhosis and hepatocellular carcinoma. Mutation in the surface covering of the virus called HBsAg is suspected to play a role in liver cancer. Mutation called F141L was present in a significant number of liver cancer cases. To prove the relationship between F141L and hepatocellular carcinoma molecular epidemiology study using MboII PCR restriction analysis were performed. Even the colony forming assay proved that the colony forming rates of the cell lines, expressing the lerge surface hepatitis B protein with the F141L mutation, is about twice as high as those of the wild type.[11,12,13] Non alcoholic fatty liver disease , the other cause of cirrhosis, is associated with insulin resistance and increased body mass index of 25kg/m^2 or more. Disorders associated with non alcoholic steatohepatitis and steatosis are diabetes mellitus,hyperlipidaemia,Jejunoileal bypass, extensive small bowel resection, partial lipodystrophy, inflammatory bowel disease, obesity, biliopancreatic diversion ctc. Degree of steatosis is directly proportional to the level on insulin resistance [14,15] The pathological hallmark of cirrhosis is the development of scar tissue that replaces normal parenchyma, which blocks the portal flow of blood through the organ and disturbing normal function. Endogenous stellate cells are found as the culprit for the development of fibrosis which ultimately turns into cirrhosis. A common feature of liver cirrhosis is the damage to the hepatic parenchymal cells, this leads to the activation of the stellate cells leading to obstruction of blood flow. In addition it secretes mediators like TGF-β_1, TIMP 1 and 2, which brings about the process of proliferation of connective tissue and prevents break down of connective tissue in the extra cellular matrix .Thus increasing the production of extra cellular matrix and subsequent loss of liver function due to fibrosis.[12,16]

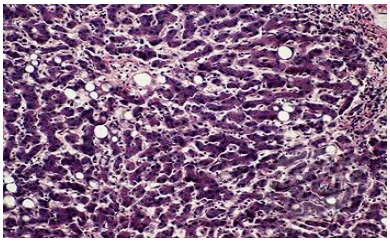

Fig. 2: Liver cirrhosis stage 1[17]

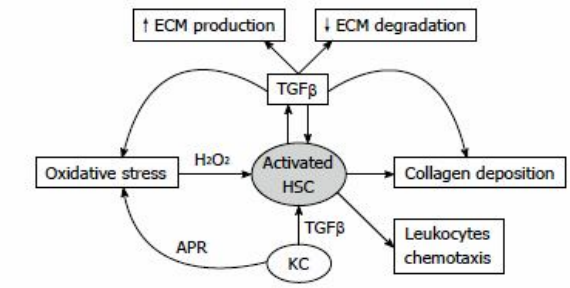

Fig. 3: The pathway of activated hepatic stellate cell actions and interactions in liver fibrosis process (HSC: Hepatic stellate cell; KC: Kupffer cell; APR: Acute phase response; ECM: Extracellular matrix; TGFβ: Transforming growth factor-β).[18]

Liver injury due to hypoxia

Right sided congestive heart failures leads to hypoxic liver injury also known as hypoxic hepatitis or ischemic liver or shock liver. Hypoxic liver injury may occur with obstruction of either the portal vein or the hepatic artery. A massive (greater than 20 times) and transient increase in serum transaminase level is the hallmark of hypoxic liver injury.[19] Histological changes include formation of blebs of the plasma membrane.[20,21]Along with bleb formation there will be dilation of cisternae of the endoplasmic reticulum, rounding and swelling of mitochondria and increase in total cell volume by 30-50%. All these are the reversible changes; the irreversible changes includes rupture of the bleb of the hepatocyte and sinusoid leading to a change in membrane permeability and imbalance in ionic gradient.[22,23] Hypoxic liver injury has been described in patients who are markedly hypoxemic due to chronic respiratory failure or sleep apnea syndrome where there is an increase in MCP-1and MIP-2 type of protein .[19,24] The pathway of hypoxic injury involves the vulnerability of four cell components (a) cell membrane integrity (b) ATP generation (c) protein synthesis (d) integrity of the genetic apparatus. If the stimulus of injury persists for a longer period of time then the injury turns into irreversible type. One earliest ultra structural marker in hypoxic injury is the accumulation of amorphous, calcium rich densities in the mitochondrial matrix. This is followed by continuous loss of proteins, co enzymes and ribonucleic acid from the hyper permeable plasma membrane. Leakage of essential metabolite from plasma membrane leads to the depletion of ATP level which leads to the loss of membrane bound phospholipids. Increase in intracellular calcium leads to the activation of phospholipase and proteases which leads to phospholipid breakdown and damage to the cytoskeleton eventually leading to cell death. During gut hypo perfusion induced hypoxia large amount of lipopolysaccharides pass into the liver through portal vein which activates the resident macrophages (i.e kupffer cells), activation of which leads to the release of mediators of inflammation including nitric oxide, which leads to liver injury.[25,26]

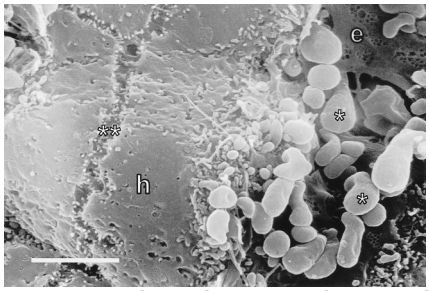

Fig. 4: Scanning electron micrograph of early cell surface bleb formation during hypoxia. Hepatocellular blebs (single asterisks) protrude through fenestrations of sinusoidal endothelial cells (e) after 15 minutes of low-flow hypoxia in a perfused rat liver. Blebbing occurs on the subsinusoidal surface of the hepatocytes. Intercellular surfaces of the hepatocytes (h) are not yet involved, and bile canaliculi (double asterisks) are normal. Bar is 5 µm.[20]

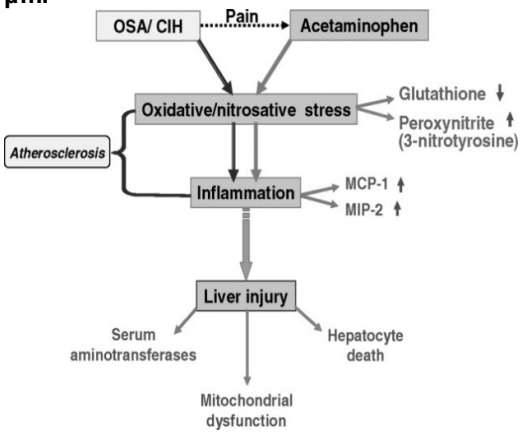

Fig. 5: A putative scheme describing liver injury in rodents exposed to chronic intermittent hypoxia or in patients with OSA using acetaminophen. Abbreviations: CIH= Chronic intermittent hypoxia; MIP-2 =Macrophage inflammatory protein-2; MCP-1 =Monocyte chemoattractant protein-1 and OSA= Obstructive sleep apnoea.[24]

Liver injury due to free radicals

Free radicals are the chemical species with a single unpaired electron in an outer orbital, extremely unstable and have the tendency to react with the inorganic and organic chemicals. They are otherwise called as reactive oxygen species (ROS). Any molecule that comes in contact with such a radical itself gets converted into free radical and thus continues the chain of auto catalytic reaction. [25] Examples of such free radicals are super oxide radical, hydrogen peroxide, nitric oxide. Histological changes of liver in free radical injury involves extensive areas of necrosis, vascular degeneration and inflammatory cell infiltration.[27] Presence of lipofuscin, the end product of free radical injury of cells, is the hallmark of free radical mediated injury of liver. Grossly an excess of lipofuscin produces a brown discoloration. The pathological effects of free radical are seen in three main cellular components-fatty acids, proteins and DNA. ROS brings about lipid peroxidation and damage to plasma membrane; oxidation of proteins leads to loss of enzymatic activity and abnormal folding of proteins. Abnormal folding of proteins or the misfolded proteins deposit in the form of fibrillar protein, leading to inflammatory condition as found with amyloidosis. Such a damage to the DNA leads to mutation.[25] Primary systemic amyloidosis can produce further complication in the form of cholestatic liver disease.[28] Both amyloid protein and lipofuscin bring about the process of aging and injury of the liver.

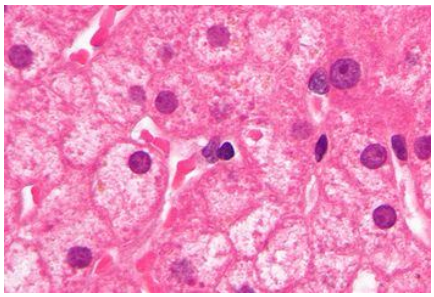

Fig. 6: The brown pigment noted in the hepatocytes represents lipofuscin.[29]

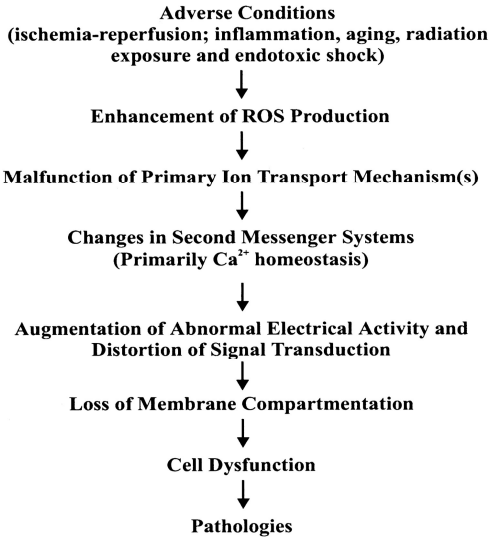

Fig. 7: Pathway of major processes of reactive oxygen species (ROS) pathologies [30]

Liver injury due to drugs and chemicals

Drug induced hepatic syndrome can be classified into hepatocellular, hepatocanalicular, mixed and canalicular. Chemical agents can produce cytotoxic or cholestatic type of injury; cytotoxic type of drug injury includes necrosis (zonal, diffuse and massive), steatosis or both and increased serum enzyme activity associated with necrosis, where as in cholestatic type there will be stasis of bile and inflammatory changes. [31] Drugs if not eliminated from the body after their therapeutic effect may produce toxicity, which is prevented by biotransformation reaction. Biotransformation reaction in the liver renders the drugs polar and helps their easy elimination in urine. During biotransformation reaction many harmful products like alkylating, arylating and free radical intermediates are formed; covalent binding of such metabolite with liver proteins is used as an index of their formation, such binding is related with the severity of lesion of the liver. As can be seen in case of liver necrosis produced by very higher dose of acetaminophen.[32] Enzyme like p450 which

plays a major role in the metabolism of many drugs, produces accumulative toxicity upon its inhibition, which can be seen during the metabolism of ritonavir versus other drugs.[33] Drug-induced injury mechanisms include covalent binding of the drug to cellular proteins which results in immune injury, inhibition of cell metabolic pathways, blockage of cellular transport pumps, induction of apoptosis and interference with mitochondrial function.[34] Drug induced liver injuries are manifested as symptoms resembling acute viral hepatitis, fatty infiltration of liver, cholestatic jaundice, liver granulomas, liver tumor both benign and malignant type , cirrhosis and vascular damage etc.[35] The histological pattern of drug induced liver injury involves acute hepatitis with or without cholestasis.[36] The pathology and path way of drug induced hepatotoxicity includes disruption of hepatocyte with decrease in ATP level as a result of destruction of mitochondria, disruption of transport protein at the canalicular membrane interrupting bile flow producing cholestasis and bile duct injury, activation of cytotoxic T-cells and cytokines following interaction of drug and p450 leading to apoptosis and cell death.[37]

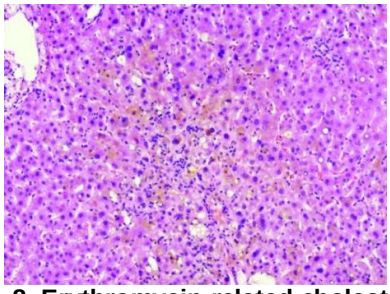

Fig. 8: Erythromycin-related cholestatic hepatitis.[36]

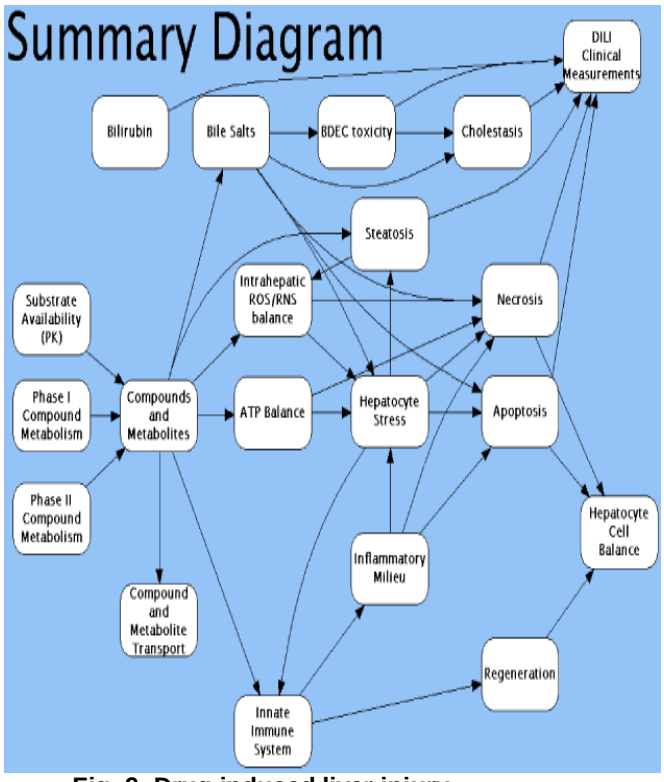

Fig. 9: Drug induced liver injury. Abbreviations: DILI= Drug induced liver injury; BDEC=Bile duct epithelial cells.[38]

Liver injury due to iron overload

Dietary iron is essential for the maintenance of blood picture and other beneficial activities. When taken within the normal range it is converted into safe forms like haemoglobin, myoglobin and haem-containing enzymes; and helps in the development of brain, cell differentiation, protein synthesis, synthesis and catabolism of many neurotransmitters.[39] Otherwise pathologic expansion of body iron stores takes place as a result of increased absorption of excess dietary iron. Iron accumulation can take place in the liver under a variety of circumstances which may be congenital, hereditary hemochromatosis, hemolytic conditions, anemia, hepatitis C, cirrhosis etc. Chronic iron overload targets cellular components like lysosome, mitochondria, microsome and collagen by its pro oxidative and direct effect leading to fibrosis, cirrhosis and hepatocellular carcinoma.[40,41,42]Iron deposits are found in hepatocytes, sinusoidal and portal macrophages, sinusoidal and portal endothelial cells and billiary cells; Parenchymal iron overload is due to intestinal hyper absorption

of iron where iron deposits within hepatocytes as fine granules at the biliary pole; mesenchymal iron overload is observed at a latter stage when the amount of hepatocytic iron is high and responsible for sideronecrosis ,here iron deposits within kupffer cells and portal macrophages; mixed iron overload represents the histological characteristics of both parenchymal and mesenchymal types and corresponds usually to complex conditions or to massive iron loading. The concentration of iron present in normal liver is less than 20 µmol/g of dry weight.[43]The extent of injury and histological changes depends on the stage of overload. In mild overload, characterized by hepatic iron concentration 93.5±23.3 µmol/g and hepatic iron index 2.3±0.7, cytosolic ferritin and scarce pericanalicular lysosomes (siderosomes) were seen in periportal hepatocytes (acinar zone 1), without signs of organelle damage and in the absence of sinusoidal cell siderosis. In moderate overload, characterized by hepatic iron concentration 190.8± 41.5 µmol/g and hepatic iron index 4.3±1.9, ferritin was identified in hepatocytes of all acinar zones and the pericanalicular siderosomes were abundant, especially in acinar zone 1. Single hepatocytes showed organelle damage and occasional sinusoidal cells showed siderosis. Severe overload characterized by hepatic iron concentration 308±49.0 µmol/g and hepatic iron index 7.5±1.7, hepatocytes of all acinar zones were-filled with large, hemosiderin-containing siderosomes, and changes in mitochondria, smooth and rough endoplasmic reticulum and nuclei were conspicuous. Marked sinusoidal cell siderosis and collagen deposition were observed predominantly in this stage.[44] If not treated early fibrosis turns into cirrhosis followed by hepatocellular carcinoma and death.

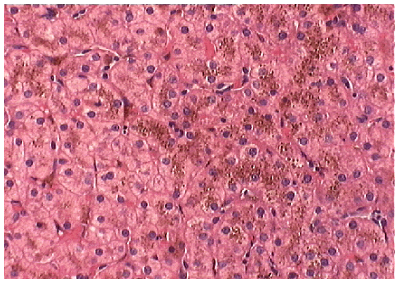

Fig. 10: Hemochromatosis of liver showing presence of coarse brown granule.[45]

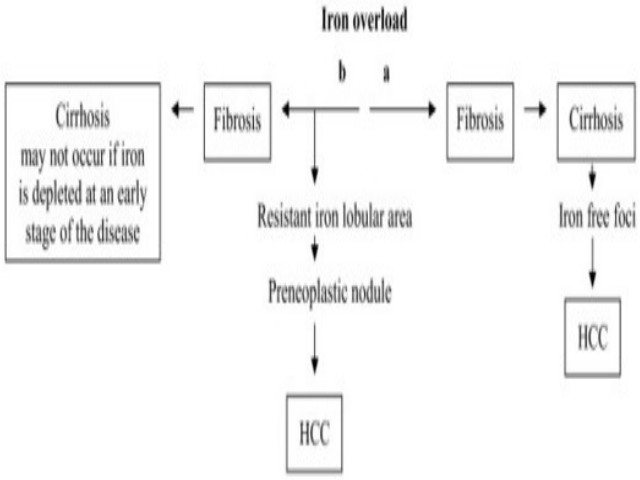

Fig. 11: Pathways leading to Hepatocellular carcinoma (HCC) in iron overload: (a) classical pathway; (b) alternate pathway (rarely observed) [46]

Hepatic Carcinoma

One of the most frequent primary malignant tumors in the world is hepatocellular carcinoma, which is a hyper vascular tumor having a pseudo-capsule composed of collagenous fibres and a layer of compressed liver tissue .[4] Injuries like cirrhosis and chronic hepatitis B plays an important role in the development of hepatocellular carcinoma, predictors for the development of hepatocellular carcinoma in patients with chronic hepatitis B virus (HBV)infection are (i) HBV DNA concentration greater than 10^4 copies/mL in individuals aged 30 years or older, independent of the level of serum alanine aminotransferase (ii) HBV genotype (iii) serum alanine aminotransferase level at least two times upper limit of normal.[47]Histological pattern in early stage of hepatocellular carcinoma includes stromal invasion with the following three types-crossing type, longitudinal type and irregular type; which intrudes into fibrotic tissue and portal tracts, portal or hepatic veins, and tumor thrombus. In the crossing type, HCC invades across fibrous septa of tumor nodules; In the longitudinal type, tumor cells grow

longitudinally within fibrous septa; In the irregular type, portal areas are irregularly invaded by tumor cells. The crossing type is usually observed in moderately or poorly differentiated HCC whereas the longitudinal and irregular types are usually found in well-differentiated HCC, also sometimes in moderately or poorly differentiated HCC.[48] Over activation of pathways like mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) play the crucial role to the escape of human hepatocellular carcinoma cells from the proapoptotic effects of Transforming growth factor-β (TGF-β) leading to hepato cellular carcinoma and death.[49]

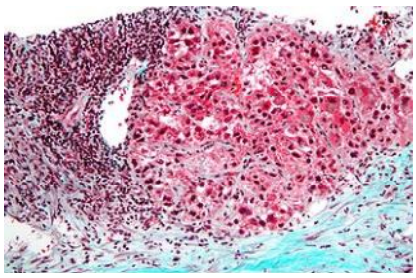

Fig. 12: Hepatocellular carcinoma as seen with trichrome stain .[50]

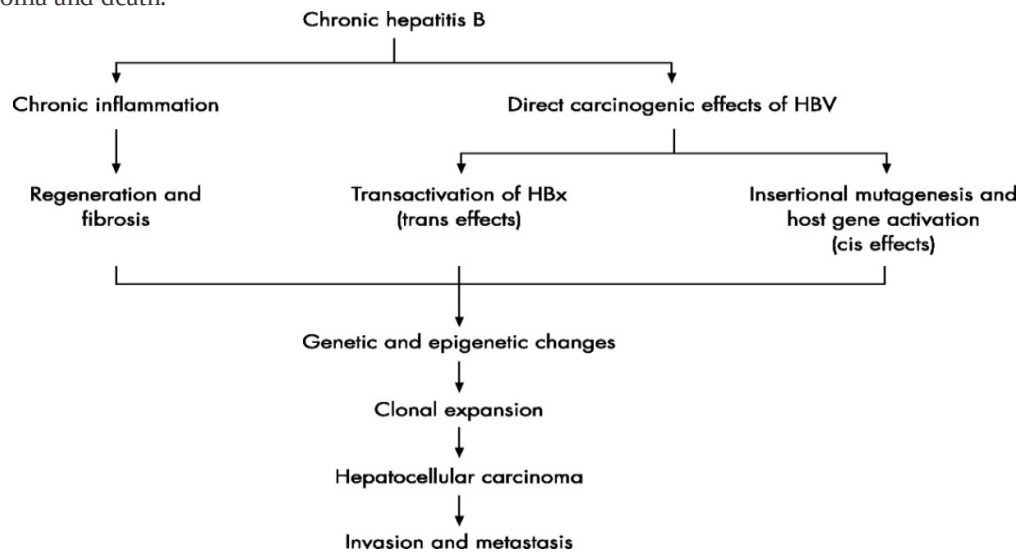

Fig. 13: Pathway of hepatic carcinoma following chronic hepatitis.[51]

CONCLUSION

Hepatocellular carcinoma a global morbid condition takes place under a variety of circumstances. Many of the injuries irrespective of their types try to take a turn towards hepatocellular carcinoma involving more or less the same pathway except some specific signs and symptoms, which acts as the identifying markers. Histological study and other non invasive studies confirms the type of injury associated, where histological study takes the upper hand in case of unclear etiology. Histological study based results are of prognostic importance and helps to identify a particular disorder at its specific stage hence to adapt the correct treatment procedure.

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