Review Article

Role of Oxidative Stress in Liver Cancer

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Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_176_16

Quick Response Code:

Abstract

The present article provides an overview of the role of oxidative stress in the development and progression of liver cancer (LC). Oxidative stress ensues when the balance between the production of reactive oxygen species (ROS) and reactive nitrogen species overrides the antioxidant defense of the target cell and body fails in detoxifying their harmful effects. Therefore, the interaction of these reactive species with critical cellular macromolecules may cause oxidative damage. Moreover, ROS may interact with cellular components including proteins, lipids, and DNAs, which results in altered target cell function. The accumulation of oxidative damage products has been implicated in both acute and chronic cell injury suggesting a possible role in the pathogenesis of Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, and cancers. Alcoholism, viral agents, obesity, and smoking increase the occurrence of oxidative stress and consequently the risk of LC.

Keywords: *Cancer, carcinogenesis, free radical, liver, oxidative stress*

Introduction

Oxidative stress occurs through overproduction of several types of reactive species in the body or as a result of a decrease in their detoxification mechanisms. These species are called prooxidants including reactive oxygen species (ROS) and reactive nitrogen species (RNS). There are many natural sources of oxidative stress, for example, exposure to environmental oxidants, toxins such as heavy metals, ionizing and ultraviolet irradiation, heat shock, and inflammation.[1] High levels of ROS and RNS exert a toxic effect on intracellular and extracellular macromolecules (e.g., DNA, proteins, and lipid membrane), thus leading to the oxidative damage in different parts of cell.[2]

ROS are chemically reactive components containing oxygen which are a natural by-product of aerobic metabolism cycle.[3] ROS can be supplied through endogenous and/or exogenous resources.[4] Exogenous ROS can be produced either by direct or indirect mechanisms in confronting with drugs, hormones, and other xenobiotic chemicals.[5,6]

ROS include a number of species such as superoxide anion (O_2^-), hydroxyl, and peroxyl radicals and certain nonradicals such as singlet oxygen and hydrogen peroxide (H_2O_2) that can be easily converted into radicals. Some species including O_2^- and H_2O_2 are constantly produced during metabolic processes in all living cells. ROS can be regarded as a trigger of genetic mutations as well as chromosomal alterations, thus contributing to cancer development through various steps of carcinogenesis.[7] In physiological conditions, cellular ROS production is counterbalanced by the action of antioxidant enzymes and other redox molecules. The balance between O_2^- production and elimination is important for maintaining proper cellular redox state. A moderate increase in ROS can stimulate cell growth and proliferation.[1,2]

Besides their harmful effects in clinical conditions, the importance of ROS and RNS as mediators in different cellular processes and cell signaling pathways is apparent.[8,9] Similarly, RNS include reactive species such as peroxynitrite, nitrogen dioxide, and nitric oxide (•NO). Like ROS, RNS are derived from the interactions of biologically generated free reactive species to form more persistent species resulting in multiple biological effects.[10-12] Because of their potential harmful effects, excessive ROS and RNS must be eliminated quickly from the cell's environment. Antioxidants are the first line of defense against free radical damage

How to cite this article: Forouzandeh H, Kalantari H, Saki N, Foruozandeh Z, Arefian E, Farahani A, *et al.* Role of oxidative stress in liver cancer. Clin Cancer Investig J 2017;6:1-9.

and are critical for maintaining optimum health. The body has developed several antioxidant systems to deal with the overproduction of pro-oxidants. These systems can be divided into enzymatic and nonenzymatic. The enzymatic system includes O_2^- dismutase, catalase, glutathione (GSH) peroxidase, and GSH reductase. None-enzymatic system is being provided by nutrient-derived antioxidants including minerals (Se, Mn, Cu, and Zn), vitamins (A, C, and E), and other compounds (GSH, flavonoids, bilirubin, uric acid, etc.).[13,14] Alternatively, oxidative stress occurs in concurrent with the shortage of antioxidant reservoir of the cell. Antioxidant levels provided by either enzymatic or none-enzymatic systems can be decreased through several mechanisms including modification in gene expression, a decrease in their uptake through nutrition, or overproduction of ROS in the cells.[15,16]

Acute oxidative injury as a result of above-mentioned events may produce selective cell death and eventually a compensatory increase in cell proliferation. This stimulus may result in the formation of new preneoplastic cells. Similarly, fatal acute oxidative injury may produce unrepaired DNA damage, formation of new mutations and potentially, newly engendered cells. In contrast, the sustained chronic oxidative injury may lead to normal cellular growth under the control of nonfatal modification mechanisms.[17] Moreover, the role of reactive species in the etiology of cancer is supported by epidemiologic studies. These epidemiologic studies specifically illustrated the protective role for antioxidants against cancer development.[18,19]

Numerous studies on the liver carcinogens showed a dose-dependent decrease in liver antioxidant concentrations along with an increase in ROS formation and oxidative damage. This increase in oxidative stress correlated with an increase in hepatocytes DNA synthesis.

The liver is one of the largest organs in the human body and the major site for metabolism and excretion. It has a wide range of functions, including detoxification, protein synthesis, and production of pivotal biochemicals necessary for digestion cycle.[20] Liver diseases have become one of the major causes of morbidity and mortality of human beings worldwide, and liver cancers (LCs) seem to be the worst.[21] To an extent, LC ranks fifth in frequency worldwide.[22]

Liver can be affected by primary LC, which primarily arises in the liver, or can emerge following metastasis of cancer cells from other parts of the body to the liver. Because the liver is made up of several different types of cells, several types of tumors can form there. Hepatocellular carcinoma (HCC) is among the most common primary LCs, which is characterized by hepatocytes involvement. Other types of cancers formed within the other structures of the liver include hepatoblastoma (formed by immature liver cells), cholangiocarcinoma (bile duct involvement), angiosarcoma (characterized by blood vessel cells involvement), and fibrosarcoma (connective tissue involvement).[23,24]

The Most Important Organelles Attributed to Reactive Oxygen Species Production

Peroxisomes and reactive oxygen species

Oxygen is consumed in various metabolic cycles in different parts of cell, where mitochondria, endoplasmic reticulum, and peroxisomes are on the top of these sites.[25] Peroxisomes are involved in a variety of important cellular functions, and its major role is considered to be in the decomposition of H_2O_2.[26]

Peroxisomes play a key role in both the production and scavenging of ROS within the cells.[27] To maintain the equilibrium equivalence between production and scavenging of ROS, peroxisomes harbor several powerful defense mechanisms and antioxidant enzymes.[28] Such conditions are considered to generate peroxisome-induced oxidative stress, which may overwhelm the antioxidant capacity leading to cancer. Furthermore, transition metal ions such as iron and copper are abundant in peroxisomes, and under certain conditions, these metal ions can be released and catalyze the formation of •OH in the Fenton reaction, thus leading to lipid peroxidation, damage of the peroxisomal membrane, and loss of peroxisomal functions.[29,30]

Peroxisomal enzymes responsible for reactive oxygen species generation

As shown in Table 1, peroxisomal enzymes attributed to ROS generation.

Peroxisomal enzymes scavenging reactive oxygen species

Peroxisomal enzymes that scavenge ROS are discussed in Table 2.

Peroxisome proliferation and induction of oxidative stress

The disproportionate increase of H_2O_2-generating oxidases is suggested to be responsible for oxidative stress leading to the development of hepatic tumors in rodents treated

Table 1: Peroxisomal enzymes attributed to reactive oxygen species generation

Acyl-CoA oxidases[31]
Urate oxidase[32]
Xanthine oxidase[33]
D-amino acid oxidase[34]
Polyamine oxidase[35]
D-aspartate oxidase[36]
Pipecolic acid oxidase[37]
Sarcosine oxidase[38]
L-alpha-hydroxy acid oxidase[39,40]
Nitric oxide synthase[41]

Table 2: Peroxisomal enzymes that scavenge reactive oxygen species

Enzymes	Description
Catalase	Metabolizes H_2O_2 and a variety of substrates such as ethanol, methanol, phenol, and nitrites by peroxidatic activity[42]
	Has an important protective function against the toxic effects of peroxides generated within peroxisomes and removes them efficiently[43]
	Its activity within the peroxisomes significantly reduces in cancerous cells of the liver[44]
GPx	Along with catalase plays important roles in cellular antioxidant defense by reducing the levels of hydroperoxides, which can otherwise be converted to highly reactive hydroxyl radicals through the metal mediated Fenton reaction[45]
MnSOD	Major antioxidant enzymes that play critical roles in scavenging the superoxide radical, thus protecting cells against damages from free radicals
Cu, Zn SOD	
	High levels of MnSOD expression have been detected in various primary human cancer tissues, that can be regarded as a consequence of ROS stress,[46,47] suggestive of a tumor suppressor role for MnSOD[48]
	MnSOD with high expression in cancer tissues reduces cancer cell growth indirectly through elimination of superoxide.[49] The loss of such an antioxidant mechanism would lead to accumulation of superoxide and stimulation of cell proliferation and tumor growth[14]
Epoxide hydrolase	Metabolizes compounds containing an epoxide residue; by converting this residue to two hydroxyl residues through a dihydroxylation reaction producing diols[50]
Peroxiredoxin I	Plays an antioxidant protective role in cells through reducing H_2O_2 and alkyl hydroperoxides
PMP 20	This protein has thiol specific antioxidant activity in human and mice
	Capable of removing H_2O_2 via its thiol-peroxidase activity, thus protecting peroxisomal proteins against oxidative stress[51]

ROS: Reactive oxygen species, H_2O_2: Hydrogen peroxide, GPx: Glutathione peroxidase, MnSOD: Manganese superoxide dismutase, Cu, Zn SOD: Copper zinc superoxide dismutase, PMP 20: Peroxisomal membrane protein 20

with peroxisome proliferating compounds.[52] Some compounds have been regarded as peroxisome proliferation stimulants including hypolipidemic drugs, industrial chemicals (e.g., plasticizers, lubricants, and agrochemicals), and other toxic environmental pollutants.[53,54] However, the oxidative stress does not seem to be exclusively responsible for the development of tumors in rodents exposed to peroxisome proliferators.[55] indeed, other mechanisms such as suppression of apoptosis,[56] perturbation of cell proliferation, and release of O_2^- radicals from Kupffer cells[57] have also been suggested to play critical roles in the pathogenesis of tumors associated with peroxisome proliferation.[58]

Mitochondria

More than 90% of the oxygen received by aerobic cells is consumed in mitochondria, and only 1%–2% of this oxygen in mammalian mitochondria is used for the production of reactive oxygen intermediates. Thus, oxygen-free radicals and hydroperoxides are being generated continuously as a product of mitochondrial respiratory chain,[59] causing oxidative damage (particularly the hydroxyl radical). The mitochondrial respiratory chain generates O_2^- anions, which are converted to H_2O_2 within mitochondria, which will be released outside of the mitochondria.[60] It may cause damage to surrounding structures, especially mitochondrial DNA (mtDNA).

mtDNA is more prone to oxidative damage and mutation, since it lacks protective histones.[61-63] Increased ROS generation in the liver may lead to premature oxidative damage of hepatic mtDNA leading to the development of HCC.[64,65] The amounts of oxidative stress's damaging effects on mtDNA are several times greater than those of nuclear DNA, since mtDNA's structural properties makes it several times more sensitive to mutations than nuclear DNA.[66] Mitochondrial reduced GSH plays a key role in protecting mtDNA against oxidative damage. Indeed, the oxidative damage to mtDNA is directly related to oxidation of mitochondrial GSH.[67] The respiratory enzymes containing the defective mtDNA-encoded protein subunits may thus increase the ROS production, which in turn aggravates the oxidative damage to mitochondria.[68] O_2^- radicals produced during mitochondrial respiratory chain's activity also react with NO inside the mitochondria to produce destructive agent "peroxynitrite."[69] Furthermore, mitochondria are themselves a source of NO, which may increase the formation of O_2^- radicals and H_2O_2 by mitochondria.[70] An increase in the production of ROS is responsible for the decline in the activity of mitochondrial membrane proteins thus inhibiting mitochondrial respiratory chain's activity.[71] The activation of stimulatory receptors causing enhanced production of NO and O_2^- will provide another source of peroxynitrite production.[72] NO inhibits reversibly the activity of respiratory chain at the site of cytochrome C oxidase,[73] on the contrary, peroxynitrite inhibits the activity of respiratory chain irreversibly via inhibition of cytochrome oxidase and complexes I–III.[74]

Effect of Oxidative Damage on Intracellular DNA, Lipids, and Proteins: Potential Adverse Consequences of Oxidative Stress

Oxidative stress may result in damages of critical cellular macromolecules including DNA, lipids, and proteins. Oxidative DNA injury may participate in ROS-induced

carcinogenesis.[75] DNA damage has been observed in a wide range of mammalian cell types exposed to oxidative stress.[30] These damages can occur in different ways including single- and double-stranded DNA breakages, deletions and insertions of single nucleotides, and even chromosomal aberrations. Major molecular mechanisms involved in DNA injuries can occur following the direct reaction of hydroxyl radicals and carbonyl compounds with DNA resulting in the activation of nucleases.[30] Superoxide and H_2O_2 just can react with DNA in the presence of transitional metal ions which cause hydroxyl radicals formation. The hydroxyl radical may attack to deoxyribose, purines, and pyrimidines, giving rise to numerous products, such as 8-hydroxydeoxyguanosin, thymidine glycol, and 8-hydroxyadenosine.[76] One of the most common forms of DNA injury is the formation of hydroxylated bases in DNA structure, which is considered to be an important event in the chemical carcinogenesis cycle.[77] Formation of such by-products interferes with normal cell growth through genetic mutations and alterations in the ordinary transcription processes of genes. Oxidative DNA injury causes mutations through different pathways, including chemical modification of nucleotide moieties in DNA leading to alterations in their hydrogen bonding, exacerbation of polymerase-specific hot spots, conformational changes in the DNA templates, and the induction of an error-prone DNA polymerase conformation.[78]

Cellular fatty acids may be another target of oxidative stress products and can be readily oxidized by ROS producing lipid peroxyl radicals and lipid hydroperoxides.[7] Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA). Moreover, these lipid radicals can diffuse through membranes modifying the structure and the function of the membrane, thus resulting in disruption of cell homeostasis. In addition, lipid peroxides may interact with cellular DNA triggering the formation of DNA-MDA compounds.[77] Lipid damage through lipid peroxidation may result in several possible processes in which the most important is protein oxidation.[79] Proteins are also easily attacked by ROS through lipid peroxidation. Protein-derived radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modifications of enzymatic activities.[80] In addition to enzymes, damages to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intercellular calcium and potassium triggering a series of changes in target cells.[81] Alterations in receptor and gap junction proteins may also modify signaling in cells. In some cases, structural changes of proteins may allow the target proteins to be under the further attacks of proteinases.[82]

Other Targets of Oxidative Stress

Activation of transcription factors is an important signaling pathway for the regulation of gene transcription by ROS.[83] Transcription factors are proteins that can bind to the promoter region of a gene, thus regulating the transcription of genes involved in the development, growth, and aging of cells.[84] Regulation of subcellular localization from cytoplasm to cell nucleus is the first step for transcription factor's activity, which is believed to be involved in this process. Considered to be the target of oxidative stress.[85] Nuclear factor kappa B and activator protein-1 (AP-1) are considered to be amongst the most important targets of oxidative stress.[86] The AP-1 transcription factor controls genes required for cell growth and its activity is increased by compounds with a major role in inducing the cellular proliferation. ROS can cause activation of AP-1 as well as inducing the synthesis of it.[18] Oxidative stress can also increase AP-1 transcription factor's activity, concluding that ROS may play a central role in intracellular signal transduction. High levels of ROS may alter signal pathways through oxidative injury induced in cell membrane, changes in enzymatic activity, and/or the activation of transcription factors. These alterations may create important links between oxidative stress and tumorigenesis.[83] Consequences of ROS production on gene transcription may also inhibit normal cell apoptosis and result in an increase in the number of cells.

Oxidative Stress Indifferent Stages of Cancer

Induction of cancers through chemicals is a multistage process which can be defined by at least three steps or stages: initiation, promotion, and progression. Initiation stage contains a nonlethal and inheritable mutation in cells by the interaction of a chemical with DNA, conferring an additional growth to target cells. Activation of the carcinogen to an electrophilic DNA-damaging moiety is critical for initiatory stage of DNA injury. ROS compounds are believed to mediate the activation of such carcinogens through hydroperoxide-dependent oxidation that can be mediated by peroxyl radicals.[6] ROS compounds or their derivatives from lipid peroxidation, MDA, can also directly react with DNA to form oxidative DNA adducts.[77] The presence of carcinogen-DNA and oxidative DNA adducts generated through chemical carcinogen's activities suggest an interactive role for ROS in initiation stage. Therefore, ROS can have multiple effects on the initiation stage of carcinogenesis by mediating carcinogen activation, causing DNA injury, and interfering with the repair of the damaged DNA. The second stage (promotion) consists of the selective clonal expansion of the initiatory cell populations through either increased cellular proliferation and/or inhibition of cell death (apoptosis). Promotion stage of tumors will be accompanied by the involvement of selective clonal expansion of the initiatory cell populations through either increased cell division and/or decrease in the occurrence of cell death (apoptosis).[87,88] The final stage of tumorigenesis (progression) comprises the development of irreversible cancer growth from the preneoplastic cells of lesions.[89] This results in the formation of the preneoplastic

lesions (foci from) as a pathologic consequence of above-mentioned processes. ROS agents are specifically generated in initiatory cell populations such as preneoplastic foci in the liver. Since ROS generation is related to P450 enzyme's activity, oxidative stress may have an important role in the clonal expansion of these initiatory cells. In fact, higher levels of ROS have been found in neoplastic nodules of rat liver in comparison with surrounding normal cells of liver's tissues.[90] Another source of ROS can result from the oxidation of GSH by y-glutamyl transpeptidase in preneoplastic foci.[90] Moreover, extracellular sources of ROS may come from inflammatory cells.[91]

These multiple sources of ROS may contribute to the formation of a persistent oxidative stress environment resulting in pathophysiologic changes and consequently allows for the selective growth of preneoplastic initiatory cells. Tumor progression results in the development of malignant benign lesions. At this point of the progression stage, oxidative stress may directly be effective on the emersion of cancerous lesions characteristics such as uncontrolled growth, genomic instability, chemotherapy resistance, invasion, and metastasis of cancerous cells. Tumor cells continually undergo high and persistent oxidative stress.[92] This persistent oxidative stress does not seem to be effective enough to induce cell death, since tumor cell's sensitivity to oxidative stress is decreased.[93]

Oxidative Stress and Hepatocarcinogenesis: Causes and Triggers

Viral infections

Viral infection with either hepatitis C virus (HCV) or hepatitis B virus (HBV) is the most common and main cause of LC.[94,95] One possible mechanism of hepatocarcinogenesis of HCV is the involvement of oxidative stress, triggering genetic mutations as well as chromosomal alterations thus contributing to cancer development.[96]

Viruses cause HCC as a consequence of massive inflammation, fibrosis, and eventual cirrhosis within the liver.[97] numerous genetic and epigenetic alterations occur in liver cells during HCV and HBV infection, considering to be the major factor in the induction of the liver tumors. Viruses induce malignancy inducing changes in cells by altering gene methylation, affecting gene expression and promoting or repressing cellular signal transduction pathways. Thereupon, viruses can prevent apoptosis and promote viral replication and persistency.[97] The presence of HCV may induce the production of ROS itself in human liver and render hepatocytes susceptible to DNA damage, the accumulation of which may lead to malignant transformation.[96] Some mechanisms attributed to the generation of free radicals and increased oxidative stress in HCV-infected individuals include: activation of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase within Kupffer cells and polymorphonuclear neutrophil cells during inflammation, iron overload and lipid peroxidation, activation of NADPH oxidase by NS3 protein of HCV, increased production of mitochondrial ROS/RNS by the electron transport chain of core and NS5A proteins of HCV, reduction in GSH output as a consequence of liver injury, decreased antioxidants and related genes expression, enhancement of pro-inflammatory cytokines, increased expression/activity of cyclooxygenase2, amplification in the expression of CYP2E1.[98-100]

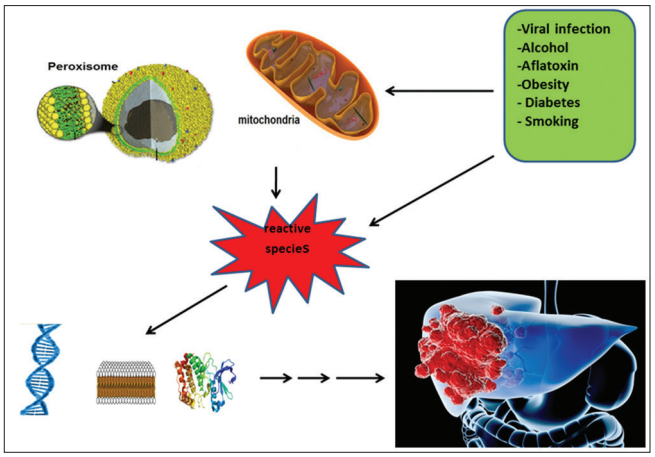

Figure 1: Viral infection, obesity, smoking, diabetes, alcohol consumption directly or indirectly affects mitochondrial or peroxisome enzymes to produce reactive species, resulting in the gradual formation of hepatocellular carcinoma

Alcohol

Liver is the major site of ethanol metabolism, thus chronic alcohol consumption is associated with progressive liver diseases.[101] In alcohol-related liver disorders, free radicals play a role in the pathogenesis of liver damage. Ethanol consumption increases ROS production, reduces cellular antioxidant levels, and enhances the oxidative stress in many tissues, especially the liver.[102] Acetaldehyde produced through the oxidation of alcohol has got the ability to inhibit the repair of alkylated nucleoproteins, decrease the activity of several enzymes, and causing damages to mitochondria. It also promotes cell death by depleting the levels of reduced GSH through inducing lipid peroxidation, and increasing the toxic effects of free radicals. Finally, acetaldehyde has been shown to enhance collagen synthesis.[103]

Chronic ethanol treatment suppresses mitochondrial function.[104] Alcohol-induced inflammatory and innate immune-mediated responses of Kupffer cells increase ROS-induced injury and fibrinogenesis-inducing factors (e.g., acetaldehyde or lipid peroxidation products).[105]

Aflatoxin

Aflatoxins are a group of chemicals produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*.

Food contamination by such fungi leads to ingestion of the chemicals. Aflatoxin is a potent hepatotoxic and hepatocarcinogenic agent, that exposure to it can lead to the development of HCC.[106] The mechanism by which aflatoxins cause cancer is through genetic mutations of gene involved in the prevention of cancer called p53.[107] In aflatoxicosis, oxidative stress would be a common mechanism that contributes to the initiation and progression of hepatic damage. It is metabolized in the liver cells and activated by hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, which subsequently binds to nucleophilic sites of DNA. Moreover, its genotoxic proprieties can induce oxidative stress more than ever.[108]

Obesity, Diabetes, and Smoking

Several studies have been established a link between smoking, diabetes, and obesity with a state of excess oxidative stress [Figure 1].[109-111]

Obesity has been implicated in the genesis of noncancerous liver diseases, such as nonalcoholic fatty liver disease (NAFLD). However, without proper management, NAFLD may cause severe liver inflammation, termed as nonalcoholic steatohepatitis, which can cause liver fibrosis and cirrhosis with serious complications, including liver failure, and HCC.[112] Epidemiological studies indicated that HCC shows the most strong straight correlation with obesity amongst all other cancers.[113] Fat accumulation and elevated levels of fatty acids correlate with systemic oxidative stress in human beings.[114] Remarkably, obese people display elevated levels of systemic oxidative stress, thus enhancing ROS which occurs in concurrent with lipid accumulation. Thus, adipose tissue represents an important source of ROS and oxidative stress may be a linking factor between obesity and cancer.[115] Epidemiological studies indicated that diabetes mellitus is another risk factor for chronic liver disorders and HCC.[116] Several mechanisms may explain the association between diabetes and primary LC. Patients with insulin-independent form of diabetes (insulin resistant diabetes) showed compensatory hyperinsulinemia, which may stimulate hepatic cell proliferation.[117] Moreover, patients suffering from diabetes may undergo liver alterations, including fatty degeneration and cirrhosis, which favor the process of liver carcinogenesis through the stimulation of cell proliferation.[118] Furthermore, lipid peroxidation is considered as a source of mutagens triggered by ROS. Such condition has been shown to encourage the development of cancer-promoting mutations in diabetic patients.[119]

In addition to the critical role of obesity and diabetes in LCs, many studies have shown a strong association of LCs with smoking as it significantly elevated the risk of HCC. The effect of cigarette smoking on individuals may promote the progression from hepatitis to cirrhosis, or from cirrhosis to HCC.[120] The presence of several compounds in tobacco and the role of liver in the metabolism of these compounds, makes the liver prone to HCC.[121]

Discussion and Future Perspective

According to recent studies, oxidative stress appears to be an important factor in a number of human diseases including the induction of LC. Several agents seem to induce oxidative stress either directly or indirectly through alterations of cellular antioxidant defense mechanisms. In conclusion, formation of ROS triggered by toxic agents, specifically chemical carcinogens, may be considered as an important mechanism in evaluating LC.

Acknowledgment

The authors would like to thank Dr. Mehdi Goudarzi for scientific assistance.

Financial support and sponsorship

The study was supported by the Ahvaz Jundishapur University of Medical Sciences for grant 93023.

Conflicts of interest

There are no conflicts of interest.

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