Pawan K. Maurya
Kamal Dua *Editors*

Role of Oxidative Stress in Pathophysiology of Diseases

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The publication of this book was finalised during the Coronavirus (COVID-19) pandemic. We would like to dedicate this book to all those who were affected by the pandemic, and in particular, to our health workforce around the world for their dedication and care during this difficult time

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About the Editors

Pawan K. Maurya is currently an Associate Professor at the Department of Biochemistry, Central University of Haryana. He is also Deputy Director of Deen Dayal Upadhyay Kaushal Kendra (DDUKK) at the same university. He holds a Ph. D. from the University of Allahabad and pursued research work as a Postdoctoral researcher at Taipei Medical University, Taiwan and the Federal University of Sao Paulo (UNIFESP), Brazil. His research focuses on oxidative stress, aging, telomere biology, biochemical diagnostics, nanomedicine, and clinical biochemistry. Dr. Maurya has received several national and international awards such as Science without Borders, Brazil (2014), and a Distinguished Service Award, Allahabad (2018). He has published 1 book, 10 book chapters, and over 60 research articles in respected international journals. He has also served on numerous technical program committees and organizing committees for various international conferences.

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His research experience spans 12 years which lies on the interdisciplinary boundary between the acquired extensive knowledge on the application of the principles of pharmacy (primarily formulation and pharmaceutics) to biomedical sciences (immunology and microbiology). He has published 90 peer-reviewed manuscripts in various respected pharmacy and pharmacology journals and has authored 2 books, 4 book chapters.

Oxidative Stress and Oral Diseases

1

Aravind Kumar Subramanian, Vivek Narayan, and R. Navaneethan

Abstract

Oral diseases such as dental caries, lichen planus, oral cancer, and most importantly chronic periodontitis are also believed to be linked to oxidative stress. In periodontitis, the incessant presence of inflammation releases free radicals and via various mechanisms such as DNA damage, lipid peroxidation, protein damage, oxidation of antiproteases, and release of pro-inflammatory cytokines causes free radical-induced damage. The role of free radicals in carcinogenesis has been studied for many decades. The free radicals cause DNA alterations such as mutations, DNA-based oxidation, mutation of tumor suppressor genes, and oxidative protein damage which facilitate the development of oral cancer. The inflammatory infiltrate present in oral lichen planus has CD4+ lymphocytes and is a source of reactive oxygen species which causes cellular damage. Moreover, the saliva itself has an antioxidant system which prevents free radical-mediated damage in certain oral diseases. Hence this chapter is intended to provide an insight about oxidative stress and its association with various oral diseases.

Keywords

Oxidative stress · Periodontitis · Free radicals · Oral diseases · Oral cancer

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1.1 Introduction

Oral diseases are numerous in number, and understanding their impact on general health is paramount. Diseases of the teeth and surrounding soft tissue are frequently the factors of bad health. General physicians often overlook this fact, thereby resulting in poor diagnosis and treatment. On the other hand, a dentist's judgment is insufficient on the grounds that by instruction and propensity he is basically worried about sparing teeth and his knowledge in pathology is limited to an exceptionally thin field. The teeth and the oral tissues are continuous with the rest of the body and is not a separate area. It is therefore essential that dentists and physicians collaborate with each other for the welfare of the patient in order to arrive at a proper diagnosis and initiate the appropriate treatment.

Oxidative stress happens as a condition of unsettling influence between free radical delivered and the ability of antioxidant system to balance [1]. Free radicals are chemicals with an unpaired electron, which are highly reactive and are potentially harmful [2]. The two basic types of free radicals produced are the reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS includes highly reactive oxygen-containing molecules such as hydroperoxyl radical, hydrogen peroxide, and singlet and triplet oxygen [2], while nitric oxide, nitric dioxide, and peroxynitrite comprise the RNS [1]. Biomarkers for oxidative stress can be studied and evaluated in various biological fluids such as blood, saliva, and urine, which is very helpful in understanding and diagnosis of various oral diseases and their association with oxidative stress.

1.2 The Role of Oxidative Stress in Oral Diseases

1.2.1 Chronic Periodontitis

Periodontitis is one of the most common oral diseases affecting the teeth and their supporting structures. Initially there is inflammation of the gingiva known as gingivitis (Fig. 1.1) which eventually progresses to loss of attachment and bone loss

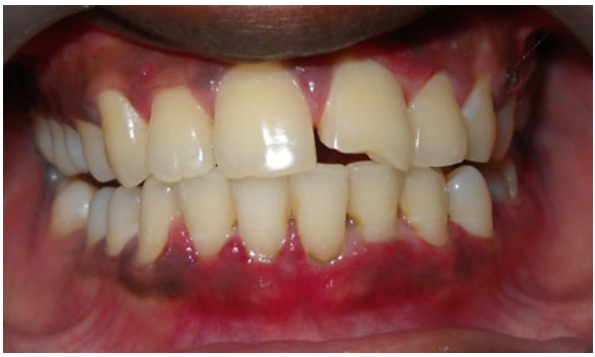

Fig. 1.1 Gingivitis of lower anterior gingiva

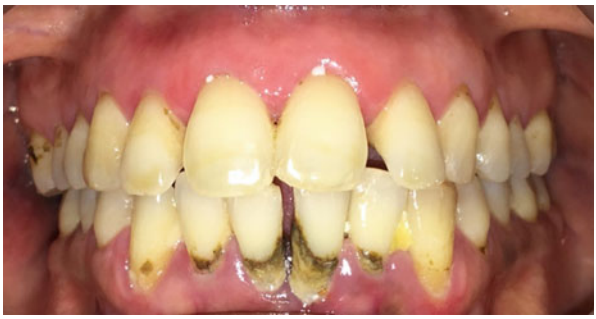

Fig. 1.2 Periodontitis of lower anterior gingiva

resulting in deepening of the gingival sulcus called as the periodontal pocket formation which is a common manifestation of periodontitis (Fig. 1.2). Exfoliation of the teeth occurs due to different types of periodontitis and can cause poor mastication resulting in malabsorption and malnourishment. Chronic periodontitis is also the cause for various systemic diseases of infective etiology by the mechanism of oral foci of infection. Periodontitis is a chronic inflammation affecting the supporting structures of the tooth, namely, gingiva, periodontal ligament, cementum, and the alveolar bone which are collectively called periodontium. About 800 species of bacteria are believed to be associated with periodontitis [3]. This bacterial spread also determines the efficiency of the host immune response. Bacteria, host immunity, and behavioral factors such as smoking are believed to play a vital role in causing periodontitis [3]. Patients suffering from severe periodontitis are 10.5–12% of the global population [4]. A systematic review, published before 2010, revealed that men were more prevalent compared to women (37.4% versus 28.1%); this might be due to the factor of smoking which is more common in men and can contribute to periodontitis [5]. This was supported by the National Health and Nutrition Examination Survey (NHANES) in 2011 who also found out the prevalence of the disease to be more in men than women [6]. The American Dental Association classifies periodontitis based on the periodontal attachment status as follows:

- Type I: Gingivitis
- Type II: Mild periodontitis
- Type III: Moderate periodontitis
- Type IV: Advanced periodontitis [7]

A systematic review in 2010 evaluated about the sexual dimorphism in destructive periodontal disease. In that review, it was found that periodontitis was more prevalent in males than females [5]. It also reports that periodontal surveys having more than 750 subjects describe a more occurrence of periodontitis in men. The world workshop for classifying periodontitis held in 2017 states that the factors smoking and diabetes have to be incorporated in the grading of periodontal disease, and a considerable amount of literature has found that the variables diabetes and smoking

have a significant weightage in causing periodontal disease [5]. The review concludes by stating that variations in sex might become useful in indicating the risk.

1.2.2 Pathogenesis

The disease commences by the accumulation of the bacteria at the dentogingival margin. Bacteria initiate the disease but the role of specific bacteria is obscure. The host immune response determines the composition of the biofilm, and inflammation precedes the overgrowth of the bacteria. The host immune mechanism responds by producing inflammatory cell infiltrate near the periodontal pocket. The usual clinical features include accumulation plaque which eventually leads to calculus formation in the supragingival and subgingival areas. This is accompanied by inflammation of the gingiva [8].

The initial stage is reaction of leukocytes and the endothelial cells to the biofilm formation. There are no obvious clinical signs observed at this stage yet histologic signs are evident. The junctional epithelium is triggered by the bacteria to produce cytokines and neuropeptides resulting in vasodilatation. Chemokines aid the neutrophils to be transferred to the inflammation site. Macrophages, lymphocytes, plasma cells, and mast cells appear after the neutrophils. Rete pegs formation from the epithelium occurs and activation of complement proteins takes place. This clinically appears as gingival inflammation and bleeding is present. The gingival crevicular fluid flow is increased [9].

The immune mechanism responsible so far was the innate immunity, and transition to acquired immunity takes place further. Accumulation of macrophages, plasma cells, T lymphocytes, and I_gG 1 and I_gG 3 subclasses of B lymphocytes is present. Impairment of flow of blood with increased collagenolytic activity occurs along with increased production of collagen by fibroblasts. Clinically this manifests as moderate to severe gingivitis characterized by gingival bleeding and color and contour changes. The final stage would be an advanced periodontitis characterized by irreversible attachment loss, bone loss, and the inflammation extending deeper into the alveolar bone [9].

1.2.3 The Role of Oxidative Stress

The course and severity of the disease is determined by the host immune response against the microorganisms [10]. The fundamental causative factor is the engagement of host and the bacterial enzymes in the destroying the periodontium [11]. Polymorphonuclear neutrophil (PMN) is the primary cell which is produced initially by the host immunity in response to the bacterial pathogens. PMNs arrive at the site of inflammation due to the pro-inflammatory cytokines secreted as a result of the immune mechanism. There is release of ROS and proteolytic enzymes catalyzed by the NADPH oxidase [12]. The presence of unpaired electron in the free radicals derived from oxygen makes it highly reactive in nature [13]. There is a rapid release

of oxygen from the PMN by the mechanism called the "respiratory burst," and this is catalyzed by NADPH oxidase enzyme during the process of phagocytosis [14]. The discharged free radicals are not target-specific, and there is complementary harm to the host tissues, and this can happen either by direct oxidation of the fundamental tissue parts or by actuation of transcription factors. ROS are also produced by osteoclasts in the bone and might have influence bone resorption [15]. Hence the PMN infiltration is considered to be the main event by the host immune system against the invasive microorganisms. PMNs thereby lead to ROS formation resulting in the destruction of periodontal tissues.

The role of oxygen tension appears to have a vital function in the production of ROS by the PMNs. This is because the PMNs require 1% of oxygen concentration and a pH of 7–7.5 approximately to produce the oxygen free radicals [16]. The function of transition metal ions is significant in producing highly reactive OH species. The presence of iron and copper in the gingival sulcus influences the growth and virulence of the periodontal pathogens. The OH free radicals are produced by Fenton and Haber-Weiss reaction. The extracellular matrix provides strength and support to the cells in the connective tissue. They have fibrous collagenous and non-collagenous network which surrounds the cell. The effect of ROS extends to the connective tissue constituents such as proteoglycans and glycosaminoglycans. Aggrecan is a type of proteoglycan having chondroitin sulfate/keratan sulfate glycosaminoglycan chains. Non-radical species such as hypochlorous acid and hydrogen peroxide and radical species such as OH have been implicated in aggrecan degradation by various in vitro studies. Reactive oxygen species play a vital function in degradation of periodontal tissues. The ROS degrades the connective tissue components and modifies the structures within the connective tissue, and this eventually results in the loss of function of the connective tissue of the periodontium.

1.3 Potentially Malignant Disorders (PMDs) and Oral Cancer

The term "potentially malignant disorders" was defined by the World Health Organization (WHO) as the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at a future date [17]. This terminology was a recent modification of older terminologies such as precancerous lesions and conditions. A precancerous lesion is a morphologically altered tissue which has a high capacity for transforming into malignancy, and precancerous condition is a state of the entire body which makes the host susceptible to acquiring malignancy. The potentially malignant disorders predispose the host to malignancies that affect the oral cavity. The development of the PMDs is due to a spectrum of etiological agents such as adverse habits which include tobacco in the form of smoking and chewing along with areca nut usage. Areca nut contains alkaloids such as arecoline and arecaidine which are believed to be the causative factor of a particular PMD. Some examples of PMDs include oral leukoplakia, oral lichen planus, and oral submucous fibrosis to name a few.

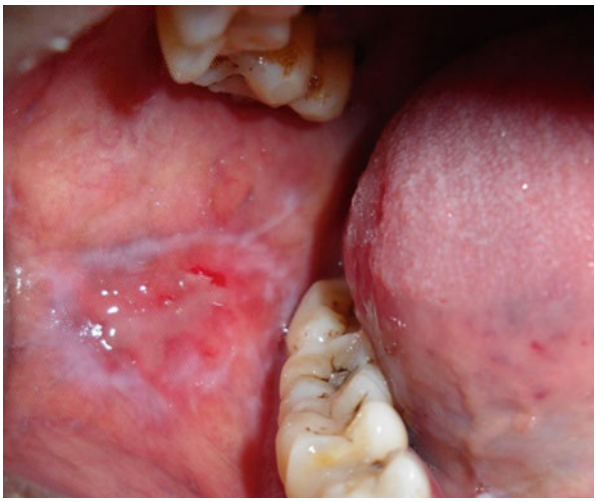

Fig. 1.3 Oral erosive lichen planus of the right buccal mucosa

1.3.1 Oral Lichen Planus

Oral lichen planus is defined as a chronic inflammatory mucocutaneous immune-mediated condition. Erosive-type oral lichen planus appears to have the malignant transformation potential, and the rate of it transforming into malignancy is sparse [18]. Clinically the lesion appears as having a mixed red and white appearance. The white component of the lesion appears as crisscross interlacing lines which are referred to as "Wickham's striae" and surrounds the erythematous eroded mucosa (Fig. 1.3). The lesion is often noticed in the buccal mucosa of the person. The person often has stomatodynia which gets intensified upon consuming spicy foods. The condition also affects the skin and has lesions appearing as purple, pruritic, polygonal papules. Oral lichen planus affects 1.27–2% of the population [17]. Patients with oral lichen planus have high levels of malondialdehyde and 4-hydroxy-2-neonenal which are products of lipid peroxidation [19]. The oxidative stress markers initiate a series of biological responses including initiation of apoptosis. This cellular apoptosis is brought about by B-cell lymphoma 2-associated X proteins, pro-inflammatory T-lymphocytes, nuclear localization, and activity of factor kappa B [20]. Reduction of antioxidants in saliva of patients with oral lichen planus is observed when compared with healthy controls in recent studies [21].

1.3.2 Oral Leukoplakia

Oral leukoplakia is the most common potentially malignant disorder of the oral mucosa. It is defined as a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer [22]. The common etiological agent is tobacco in the form of smoking. Smokeless forms available as chewing products also are responsible for causing the lesion. The

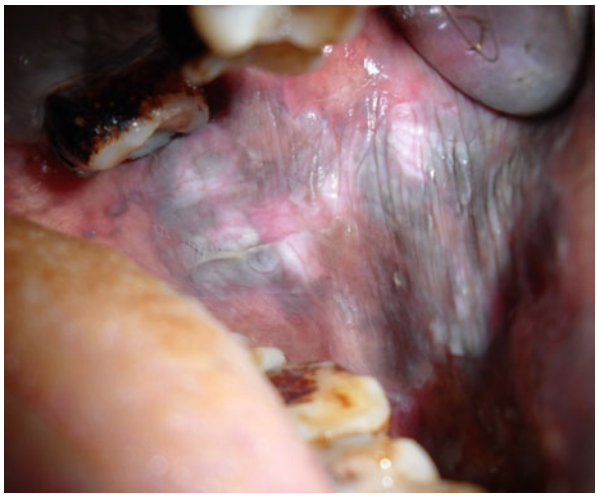

Fig. 1.4 Leukoplakia of the left buccal mucosa

prevalence of the lesion is 1.49–2.6% with a high occurrence among geriatrics especially in India due to excessive use of chewing varieties of tobacco along with areca nut [23]. Clinically the lesion commonly affects the buccal mucosa as the concentration of hot smoke in individuals with smoking habit is more concentrated in the commissures and the buccal mucosa (Fig. 1.4). The second likely place is the lateral borders of the tongue. The lesion is often bilateral in presentation and appears as thick non-scrapable white patch/plaque which is homogenous and having a characteristic "cracked mud" appearance. The lesion appears raised and the affected part of the mucosa is slightly roughened. Leukoplakia is of different types which are homogenous, speckled/nodular type, verrucous, erythroleukoplakia, and proliferative verrucous leukoplakia (PVL). Among these, the PVL and erythroleukoplakia have a greater propensity for malignant transformation. Salivary biomarkers of oxidative stress such as thiobarbituric acid and advanced glycation end products are significantly higher in individuals with leukoplakia than in those without the lesion, and the antioxidant levels were also found to be significantly lesser in individuals with leukoplakia [24]. Lipid peroxidation as a result of ROS has mutagenic effects. 8-Isoprostane (8-ISO) is an ROS and if present in high concentrations can make the individuals more susceptible to leukoplakia [25]. Lipid peroxidation can occur in cells with a cell membrane sensitive to ROS leading to production of mutagenic carbonyl compounds which can result in malignant transformation.

1.3.3 Oral Cancer

Oral cancer ranks the sixth leading type of cancer in Asia [26]. Almost 274,300 new cases occur every year [27]. Oral cancer is a wide term under which many forms are categorized. It can take origin from any type of tissue. The malignancies arising from

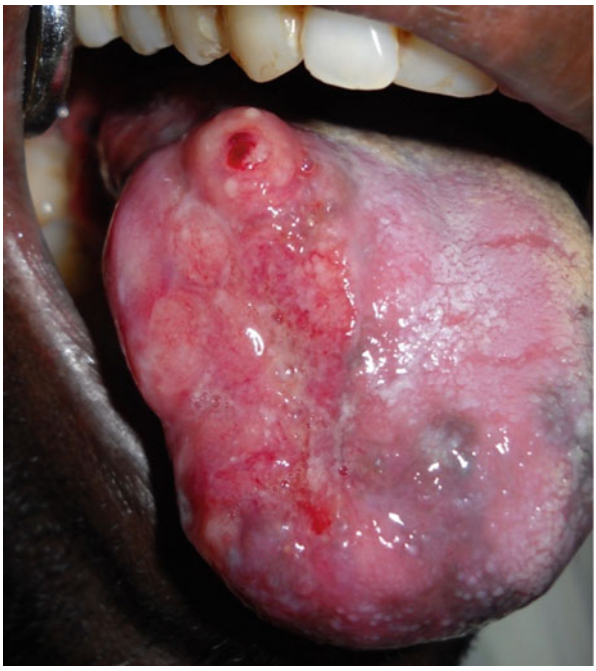

Fig. 1.5 Carcinoma of the right dorsolateral aspect of anterior two-thirds of tongue

an epithelial origin are called as carcinoma and those arising from the connective tissue are sarcoma. The treatment and prognosis for oral cancer often depend on the nature and more importantly the vehemency of the disease. It has a great influence on the day to day functions of the patient. Most of the patients visiting the hospital are often at the advanced stage of the disease. This unfortunate situation might be due to the nature of the disease and how the disease unfolds itself to the patient. Patients often do not notice the presence of the cancerous lesion in the early stages of the disease as it is asymptomatic and is hardly noticeable. Adverse habits such as smoking, chewing tobacco products along with areca nut and betel leaf, alcohol consumption along with low socioeconomic status can lead to oral malignancies. Most of the oral cancers are detected in individuals older than 40 years of age, and the average age of diagnosis of oral cancer is 60 years [27]. The most common type of oral cancer is oral squamous cell carcinoma. The lateral border of the tongue is usually involved (Fig. 1.5).

The imbalance between the oxidants and the antioxidants seems to be playing the etiological role in carcinogenesis [2]. Reactive oxygen species can cause tissue damage by the following mechanisms,

- Lipid peroxidation
- Protein damage
- DNA damage
- Stimulation of pro-inflammatory cytokine
- Oxidation of antiproteases [28]

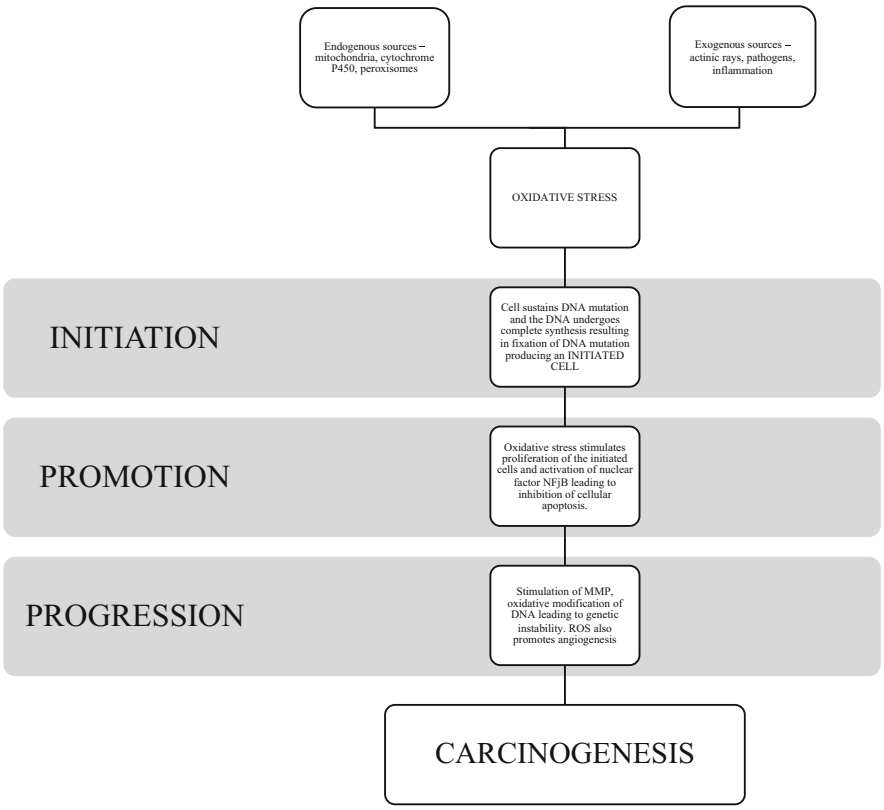

Fig. 1.6 A flowchart showing the process of carcinogenesis by the mechanism of oxidative stress. (*Courtesy Katakwar P. et al., oxidative stress marker in oral cancer 2016* [2])

ROS can be produced by endogenous and exogenous sources. Endogenous sources include mitochondria, peroxisomes, and cytochrome P450, and the exogenous sources include inflammation, actinic radiation, and pathogens. These sources trigger the oxidative stress and leads to damage to the genetic material, proteins, and lipids, gene mutations, chromosomal alterations, and expression of the mutated gene resulting in carcinogenesis (Fig. 1.6).

Carcinogenesis can be explained in three stages:

1. Initiation
2. Promotion
3. Progression

Initiation—the mutated DNA is present in a normal cell and the cell sustains it. Then the DNA undergoes the process of synthesis and this mutated DNA is now fixed. The mutated DNA in the cell now initiates the process of carcinogenesis.

Oxidative DNA changes happen in the cancer tissues due to the reactive oxygen species [29].

Promotion—this stage takes place by the proliferation and expansion of the initiated cells. This cancer development occurs by the combination of proliferation of the initiated cells and inhibition of apoptosis. ROS favors the expansion of the initiated cells by modulating the genes related to cellular proliferation and death [30]. It leads to activation of NFjB activation with induction of genes coding for proteins that inhibit apoptosis. Oxidative stress can stimulate cell division, thereby promoting tumor growth.

Progression—stimulation of matrix metalloproteinases, inhibition of proteases, and mutation are caused by the reactive oxygen species. DNA bases undergo oxidative modifications, and this makes the cell genetically instable and increases the chances of metastasis. ROS also influences angiogenesis in metastasis [31].

Tobacco in the form of smoking and chewing causes oxidative stress to increase, thereby disrupting the balance between the antioxidants and oxidative stress. There is also increased lipid peroxidation, oxidative damage to the DNA, and upsetting of the antioxidant defense which would favor malignant transformation. Chronic alcohol ingestion causes single nucleotide polymorphism of CYP2E1. This then leads to lipid peroxidation and mutation of the DNA [2].

1.4 Conclusion

The genes are often associated with oxidative stress, thereby predisposing individuals to a lot oral diseases. Some lifestyle diseases are multifactorial; hence other factors apart from oxidative stress also play a role in causing various diseases. Advancement in technology has aided us in identifying biomarkers for various oral diseases; hence, prompt diagnosis and initiating appropriate treatment are possible. Recent advancement in the field of molecular biology has enabled us to store a lot of genetic information about diseases and to aid in significant progress in the field. A lot of adverse habits are closely associated with oxidative stress and also cause elevation of the biomarkers for oxidative stress. Many environmental factors can release oxygen species which causes lipid peroxidation and DNA damage paving way for the development of carcinogenesis.

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The Role of Oxidative Stress in Chronic Liver Diseases

2

Devaraj Ezhilarasan, Shanmugam Rajeshkumar, and Thangavelu Lakshmi

Abstract

The liver plays a central role in the biotransformation of a variety of drugs and xenobiotics. During the biotransformation of drugs and chemicals, the liver generates various reactive intermediates and in turn attacks the hepatocyte membrane to generate free radicals such as superoxide, hydroxyl, peroxyl, hydrogen peroxide, peroxynitrite, peroxynitrous acid, etc. Hepatic stellate cells (HSCs), also known as perisinusoidal cells or Ito cells play a central role in the onset of various forms of chronic liver diseases. The free radicals liberated during biotransformation in the liver activate the quiescent HSCs into myofibroblast-like phenotype responsible for the excessive synthesis of extracellular matrix proteins that cause hepatic fibrosis, cirrhosis, portal hypertension, and hepatocellular carcinoma. On the other hand, hepatocytes also have several first-line intracellular antioxidant defenses such as superoxide dismutase, catalase, and glutathione and detoxifying enzymes to neutralize/protect the free radicals generated during oxidative stress. During chronic liver injury, the redox homeostasis is altered, and an enormous amount of free radicals are released with the concomitant decrease in the intracellular antioxidants causing oxidative stress. This chapter summarizes the molecular mechanisms of oxidative stress-induced chronic liver diseases.

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Keywords

Chronic liver injury · Hepatic fibrosis · Hepatic stellate cells · Free radicals · Oxidative stress

2.1 Introduction

Chronic liver diseases (CLDs)-associated complications are responsible for significant morbidity and mortality worldwide. Oxidative stress due to chronic liver injury with excessive inflammation and mitochondrial dysfunction-mediated energy metabolism often contributed to a variety of liver diseases [1]. The liver is one of the highly susceptible organs due to its unique anatomic location and function [2]. When the liver is injured by a variety of etiological factors including acute or chronic ethanol intoxication, hepatotoxic drugs, aflatoxin contaminated food, hepatitis B and C virus (HBV and HCV) infections, metabolic diseases, etc. [3, 4], it undergoes deviation from normal architecture into various hepatotoxic manifestations like inflammation, steatosis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma (HCC) [5]. Therefore, CLDs cannot be approached as a single manifestation. Various forms of CLDs are depicted in Fig. 2.1. Ample evidence has confirmed the predominant role of oxidant and antioxidant imbalance in the pathogenesis of a variety of drugs and chemical-induced CLDs. Hence, this chapter discusses the role of oxidative stress in the onset of various forms of CLDs.

2.2 Free Radicals and the Liver

Biotransformation of hepatotoxic drugs and chemicals in the liver produces a variety of highly reactive metabolites including reactive oxygen/nitrogen species (ROS/RNS) [6, 7]. Physiological ROS production is critical for redox homeostasis. However, in pathological conditions, oxidative stress is said to occur when the intracellular antioxidant defense system such as antioxidants is overwhelmed by an increased intracellular ROS and other free radicals. Free radical is classically referred to as an atom or molecule that contains one or more unpaired electrons in an outermost shell of their orbitals and is capable of independent existence. The presence of unpaired electrons in an outermost shell makes free radicals highly unstable and electrophilic [2, 8]. When a free radical reacts with a non-radical to stabilize the outermost shell, the latter generally becomes a radical and thus a series of a chain of reaction is initiated. Some important free radicals known in biological systems are singlet oxygen, superoxide ($O_2^{\bullet -}$), hydroxyl, peroxyl, hydrogen peroxide, peroxynitrite, peroxynitrous acid, and nitric oxide (NO_2), and they act as a source for ROS [9–11]. Hepatocyte intracellular organelles like endoplasmic reticulum, mitochondria, and peroxisomes are the main sites for ROS production. A wide array of enzymatic systems including cytochrome P450 enzymes, cyclo oxygenases, lipoxygenases, xanthine oxidase, nicotinamide adenine dinucleotide phosphate

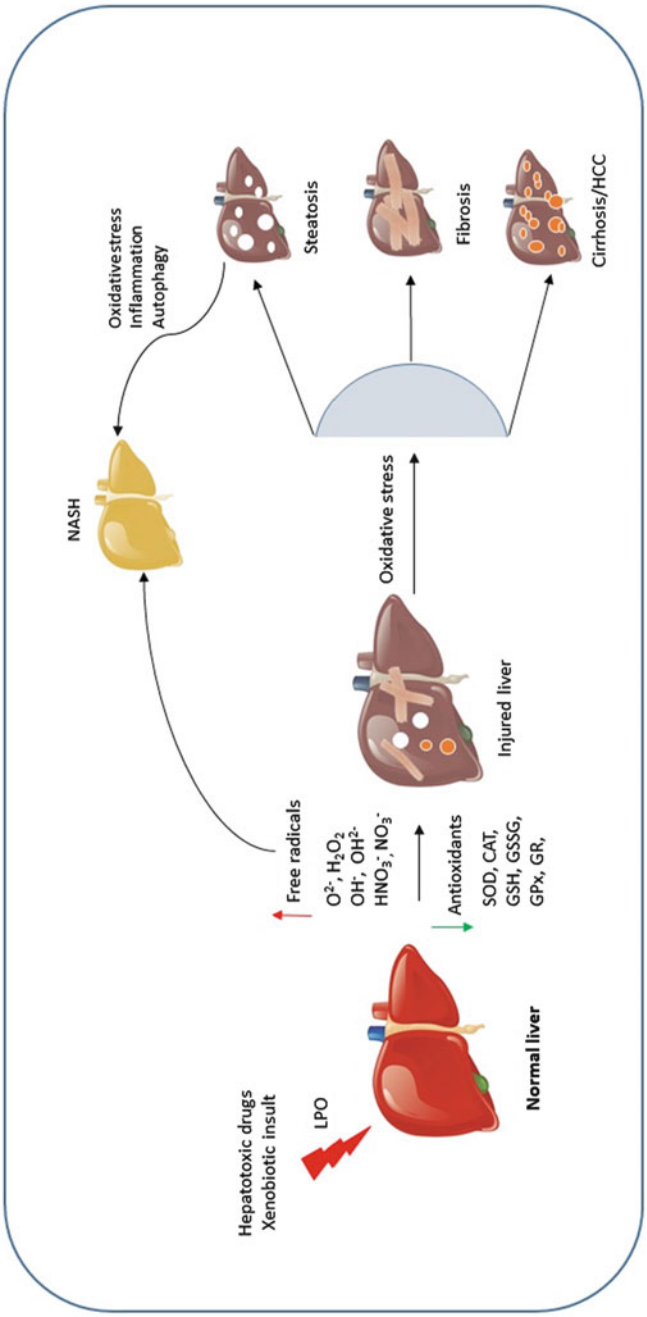

Fig. 2.1 Oxidative stress induced different forms of chronic liver diseases. *LPO* lipid peroxidation, superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxyl radicals (OH^{2-}), peroxynitrous acid (HNO_3^-), peroxynitrite (NO_3^-), *SOD* superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *GPx* glutathione peroxidase, *GR* glutathione reductase, *NASH* non-alcoholic steatohepatitis

oxidases, and NO_2 synthase from these organelles contribute to ROS production [12]. The free radicals primarily target polyunsaturated fatty acids of hepatocyte cell membrane as they are rich in double bonds and hence, hepatocyte membrane is more vulnerable to free radical attack during intracellular oxidative stress. Other cellular structures that are mainly affected by free radicals are macromolecules including structural and functional proteins, lipids, and DNA. On the other hand, overwhelmed ROS generation depletes the intracellular antioxidants that consequently fail to neutralize the excessive ROS leading to cellular injury. Undoubtedly, the excessive intracellular ROS and other free radicals with concomitant downregulation of antioxidants induce oxidative stress and have been associated with several forms of CLDs [13]. Therefore, natural and synthetic antioxidants are often employed to treat oxidative stress-induced CLDs.

2.3 The Role of Intracellular Antioxidants/Antioxidant Response Element (ARE) in CLD

The liver contains several intracellular defenses in the name of antioxidants to protect them from free radicals attack. The first-line intracellular enzymatic antioxidants are the primary defense against free radicals, and these include superoxide dismutase (SOD) to dismutate $O_2^{\bullet -}$ radicals, catalase (CAT) to neutralize hydrogen peroxide radicals, glutathione reductase (GR), and glutathione peroxidases (GPx), etc. The non-enzymatic antioxidants are reduced glutathione (GSH), vitamins C and E, and β-carotene [2, 14]. Under oxidative stress conditions, intracellular antioxidant levels are often reported to decrease due to their over-utilization towards the suppression of various free radicals generated during the biotransformation of various drugs and chemicals in the liver [6, 15]. However, studies are also reported the increased levels of antioxidants during oxidative stress conditions. This was correlated with the induction of oxidative stress contributing the cell to induce the synthesis of antioxidants [16]. Both these hypotheses are accepted and reported widely. Therefore, at this juncture, we do not know whether increased or decreased intracellular antioxidants are responsible for oxidative stress conditions.

At the molecular level, transcription factors such as erythroid 2-related factor 2 (Nrf2) play a serious role in the oxidative stress condition and was shown to upregulate several cytoprotective genes [17, 18]. Under normal physiological conditions, Nrf2 gets activated via binding into kelch-like ECH-associated protein-1 (Keap1) in the cytoplasm, and the unbound or inactivated Nrf2 is easily degraded. Intracellular oxidative stress caused dissociation of Nrf2 from Keap1 by Keap1 modification or Nrf2 phosphorylation and is thus activated [19]. This activated Nrf2 translocates into the nucleus, offers cytoprotective effect, and interacts with ARE, promoting the expression of enzymic antioxidants and phase II detoxifying enzymes such as heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) [14, 17, 20]. Thus, Nrf2 is considered as a potential therapeutic target to attenuate oxidative stress-induced liver injury [21]. Several studies showed that activation of Nrf2 signaling pathway can ameliorate liver injury [22–25].

2.4 The Role of Oxidative Stress in CLDs

2.4.1 Oxidative Stress in Hepatic Steatosis

Hepatic steatosis is a multifactorial disease that occurs due to non-alcoholic fatty liver disease (NAFLD), ethanol consumption, drugs used in chemotherapy, and metabolic diseases, xenobiotics, infectious causes, and so on [26]. NAFLD is commonly considered one of the most common forms of CLDs, and it refers to various manifestations of liver damage including non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [27]. Day and James [28] classically categorized NASH as two-hit model. The "first hit" is hepatic steatosis that is a mild condition and is considered less harmful. The "second hit" includes excess fat accumulation and mitochondrial dysfunction that leads to inflammation and its associated damage due to oxidative stress [29]. In view of the above report, it is clear that oxidative stress is responsible for "second hit" and aggravates the pathogenesis of NASH. NAFLD is often implicated with the presence of ROS-meadiated mitochondrial dysfunction [30]. The mitochondrial respiratory chain is one of the major sources of intracellular ROS, which in turn damage mitochondrial DNA, lipids, and proteins. Lipid accumulation in hepatocytes increases the mitochondrial β-oxidation and interferes with the electron transport chain that results in electron leakage. The cytochrome C oxidase (VI complex)-mediated oxygen reaction and a proton reaction are also impaired, and this, in turn, causes direct interaction of an electron with oxygen forms ROS [30].

In experimental models of NASH, decreased GPx activity due to GSH depletion and impaired GPx transport to mitochondrial matrix from cytosol were reported [31]. Cardiolipin, an inner mitochondrial membrane phospholipid is reportedly susceptible to ROS attack. Interestingly, cardiolipin abnormalities have been implicated with a mitochondrial abnormality in several liver disease conditions, including NAFLD [27]. In an experimentally induced NAFLD model, administration of a high fed diet caused downregulation of NRF-dependent transcription signaling pathway, and cytoprotective enzymes like HO-1 and NQO1 with a concomitant increase in lipid peroxidation indicate the possibility of oxidative stress via NRF signaling pathway in NAFLDs [32, 33]. Since oxidative stress is associated with the progression of NAFLDs, antioxidants have been tried as a therapeutic candidate against different NAFLD experiment models, and such studies concretely proved that antioxidant treatments could decrease CLD progression through attenuation of oxidative stress [33–36]. Clinically, oxidative stress has also been reported in NAFLD patients, and therefore, antioxidants have also been tested in patients with NAFLDs [37, 38].

Oxidative stress-mediated hepatic steatosis is also well established in alcohol injury models. Acute alcohol intake increases oxidative stress and induces hepatic lipid accumulation through cytochrome P450 2E1 (CYP2E1)-mediated reactions. Direct involvement of CYP2E1 from mitochondria in ROS generation is reported both in experimental and clinical models of NASH [30]. The c-Jun N-terminal kinase (JNK) activation and ROS generation are significant events in acute alcohol

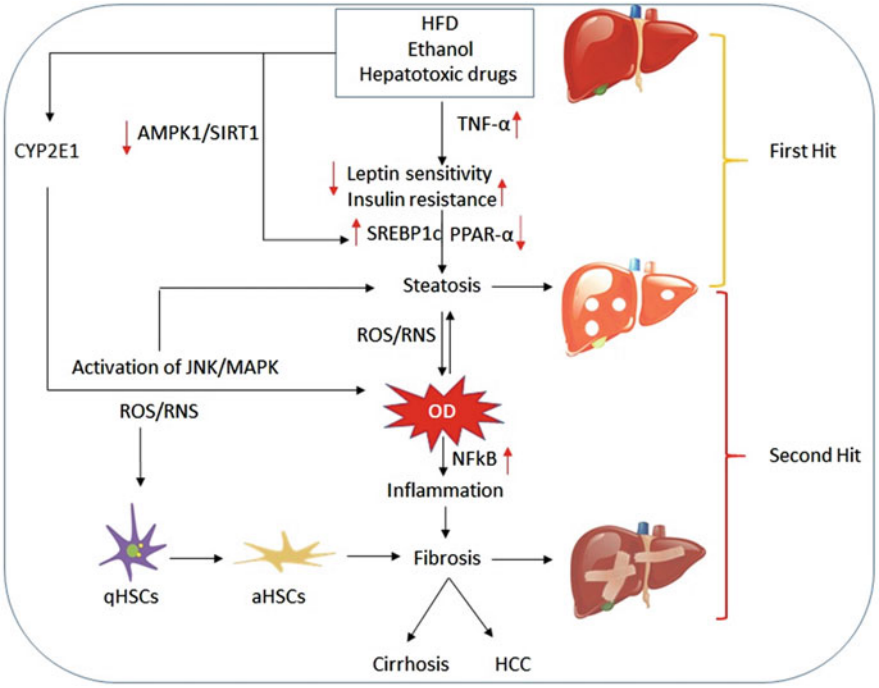

Fig. 2.2 Role of oxidative stress in the progression of steatotic liver injuries. *HFD* high fed diet, *CYP2El* cytochrome P450 2E1, *TNF-α* tumor necrosis factor alpha, *AMPK1* 5′ AMP-activated protein kinase 1, *SREBP1c* sterol regulatory element-binding protein 1c, *PPAR-α* peroxisome proliferator-activated receptor-alpha, *ROS* reactive oxygen species, *RNS* reactive nitrogen species, *JNK* c-Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *NF-KB* nuclear factor kappa B, *qHSC* quiescent hepatic stellate cells, *aHSCs* activated hepatic stellate cells, *HCC* hepatocellular carcinoma

intoxication-induced hepatic steatosis. Sterol regulatory element binding proteins (SREBP) is an important transcription factor which regulates the ROS-mediated inflammatory pathway and lipid synthesis via activation of hepatic nuclear factor kappa B [9, 10]. Acute alcohol intake also decreased autophagy and increased SREBP expression [39]. The mechanism of oxidative stress-induced fatty liver disease is depicted in Fig. 2.2.

2.4.2 Oxidative Stress in Hepatic Fibrosis

Liver fibrosis is a significant health problem that affects 100 million people worldwide with significant morbidity and mortality [40]. Liver fibrosis is a wound-healing response that is responsible for the deposition of an enormous amount of extracellular matrix (ECM) in the liver as a result of chronic liver injury [41]. Hepatic stellate cells (HSCs) are non-parenchymal cells that reside in the perisinusoidal space or

space of Disse is implicated in the hepatic fibrogenesis. In normal liver, quiescent HSCs (qHSCs) are responsible for the synthesis of normal ECM and retinoid storage. As a result of liver injury, qHSCs are activated by autocrine and/or paracrine signaling molecules from injured hepatocytes and acquired myofibroblast (MFB) like phenotype [2, 3, 42]. This phenotypic transdifferentiation is responsible for the synthesis of a variety of profibrogenic cytokines, i.e., transforming growth factor β receptor type II (TGFRIIβ), platelet-derived growth factor receptor β (PDGFRβ), and fibrosis markers such as fibril-forming collagens such as type I and III, vimentin, desmin, α-smooth muscle actin, and so on [43]. Fibrogenic signaling mediates the accumulation of excessive ECM in the perisinusoidal space which hinders liver metabolic functions and increases portal hypertension. It is a proven fact that oxidative stress is one of the main driven factors for the activation and phenotypic transdifferentiation of qHSCs into MFBs.

In vitro, experimental studies have shown that lipid peroxidation products can activate HSCs proliferation [44–46]. For instance, $O_2^{\bullet -}$ radical is involved in the progression of hepatic fibrosis, and entry of $O_2^{\bullet -}$ radical via chloride channels into HSCs is said to play a critical role in HSC activation [44]. In vivo, oxidative stress-induced fibrosis has been well reported in ethanol, carbon tetrachloride, thioacetamide, and dimethyl nitrosamine-induced experimental models [47]. For instance, during the biotransformation of ethanol, it first oxidizes into acetaldehyde by alcohol dehydrogenases, which subsequently oxidized to acetate by aldehyde dehydrogenases. Ethanol biotransformation in the liver produces an enormous amount of acetaldehyde and ROS, and these highly reactive intermediates, in turn, upregulate the TGF-β signaling and activate the HSCs to synthesize high amount of ECM [47, 48]. Accumulation of 4-hydroxynonenal (4-HNE), a lipid peroxidation product, has been reported experimentally as well as in patients with alcoholic liver diseases [49, 50]. Interestingly, unlike hepatocytes, HSCs are from mesenchymal origin do not contain strong intracellular antioxidant defense like hepatocytes, and hence they are more susceptible to get free radical attacks easily from injured hepatocytes as well as from Kupffer cells in the injured liver. Thus, studies have clearly shown that ROS accumulation could activate the HSCs and induce the fibrosis progression. Ironically, few studies have also reported that lipid peroxidation products are not responsible for HSCs activation [51, 52]. However, this concept is meagerly reported. Oxidative stress is now considered as a major contributor of HSCs activation and hepatic fibrosis progression. The mechanism of oxidative stress-induced HSCs activation and their consequence is presented in Fig. 2.3.

2.4.3 Oxidative Stress in Hepatocellular Carcinoma

HCC occurrence is increasing globally and ranks second in cancer-related mortality worldwide [53]. Multiple etiological factors including HBV and HCV infections, chronic ethanol consumption, NASH, obesity, diabetes, and aflatoxin-contaminated food are involved in the progression of HCC [54]. HCV infection induces excessive ROS and impairs the function of endogenous antioxidants, and therefore, HCV

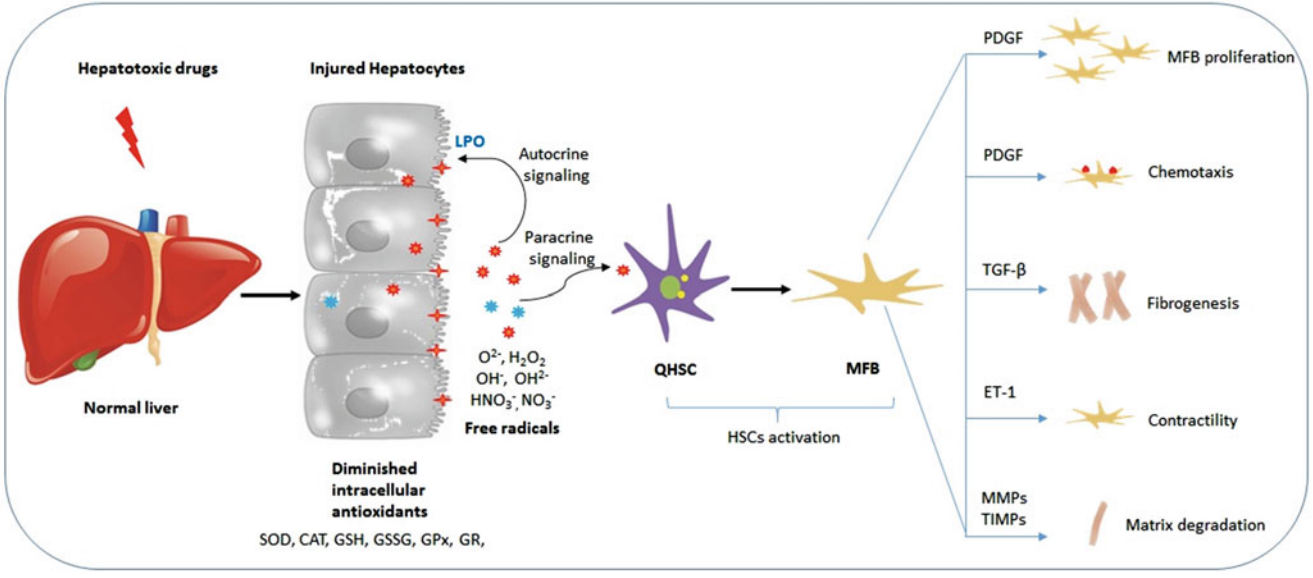

Fig. 2.3 Role of free radicals in oxidative stress induced hepatic fibrosis. *LPO* lipid peroxidation, superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxyl radicals (OH^{2-}), peroxynitrous acid (HNO_3^-), peroxynitrite (NO_3^-), *qHSCs* quiescent hepatic stellate cells, *MFB* myofibroblasts, *PDGF* platelet derived growth factors, *TGF-β* transforming growth factor β, *ET-1* endothelin-1, *MMPs* matrix metalloproteinases, *TIMPs* tissue inhibitor of metalloproteinases, *SOD* superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *GPx* glutathione peroxidase, *GR* glutathione reductase

infection is considered as the predominant cause of ROS mediated HCC progression [55]. ROS are capable of activating various signaling cascades responsible for angiogenesis, cancer cells invasion, metastasis, proliferation, and survival. In a recent preclinical study, thioredoxin reductase-1 (TrxR1), GR, and Nrf2 transcription factor null mouse have demonstrated to have a high susceptibility to HCC induced by diethylnitrosamine when compared to wild type indicating the possibility of antioxidant diminution in the progression of HCC [56]. Protein sequestosome 1/p62 (p62) acts as an intracellular defense against oxidative stress through Keap1/Nrf2 activation. Increased accumulation of p62 as inclusion bodies in HCC has been reported [57]. Oxidative stress-responsive miRNAs are recently identified in HCC cell lines. For instance, miR-34a-5p, miR-150-3p, miR-638, and miR-1915-3p are modulated in oxidative stress condition; therefore, the analysis of such miRNAs may provide a novel approach for the prognosis and diagnosis of HCC [58].

In clinical studies, oxidative stress was well correlated with the levels of NASH-HCC markers. The diminished antioxidant functions were observed in patients with NASH-HCC [59]. Interestingly, before tumor resection, HCC patients had increased oxidative stress and diminished GSH and antioxidant capacity [60]. Surgical resection is the main treatment of HCC; however, patients may develop oxidative stress-mediated liver inflammation after surgery, and coenzyme Q10 (ubiquinone), an antioxidant, level has significantly decreased in patients with HCC, thus confirming the oxidative stress. Therefore, coenzyme Q10 was used as an antioxidant therapy in HCC patients who underwent surgery [61]. Expression of Nrf2 and 8-hydroxydeoxyguanosine (8-OHdG) remarkably increased in the cancerous tissue of patients with HCC [62]. These studies are clearly indicating the concrete role of oxidative stress in the progression of HCC.

2.5 Conclusion

Undoubtedly, chronic toxic insult to the liver produces a variety of free radicals. When these free radical levels are increased in the intracellular milieu, it causes oxidative stress with a concomitant decrease in the intracellular antioxidant defense. The free radicals act as a signaling molecule and interfere with several cell signaling responsible for liver pathogenesis. More importantly, in the view of the "second hit" concept of steatosis, oxidative stress aggravates the simple fatty infiltration into complex diseases like hepatic inflammation, NASH, fibrosis, and HCC. In fibrosis conditions, free radicals from injured hepatocytes and Kupffer cells are responsible for the phenotypic transdifferentiation of the qHSCs into MFBs, thereby worsening the disease. Thus, oxidative stress injury plays a critical role in the onset of various forms of CLDs. Therefore, several experimental and clinical studies are now focusing on the verge of developing several antioxidants derived from synthetic and natural sources for CLDs.