Are we over estimating serum oxidative stress/lipid peroxidation in alcoholic liver diseases? A review

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ABSTRACT

Almost 95% of alcohol is metabolized by the liver but a small fraction is also metabolized in other organs like brain, heart, kidney, pancreas, skeletal muscle etc. Alcohol is metabolized by oxidative and non-oxidative pathways. Alcohol metabolism results in the generation of reactive molecule acetaldehyde and reactive oxygen species (ROS), which in turn leads to oxidative stress. The reactive metabolic products of alcohol and ROS cause lipid peroxidation, the lipid peroxidation products (LPP) like malondialdehyde (MDA) enter the circulation. In most of the studies on alcoholic liver disease the serum levels of ROS/LPP were estimated, but these products are also contributed by other tissues. Even though the maximum amount of ROS/LPP is produced by the liver, after reaching other organs through circulation, ROS/LPP further multiply and again reach the circulation. At this point by measuring the ROS/LPP in blood and claiming it's of liver, are we estimating it correctly?

Keywords: Alcohol, oxidative stress, lipid peroxidation.

INTRODUCTION

Alcohol is both water and lipid soluble; thereby permeates all the tissues of the body and affects vital functions. Excessive alcohol ingestion leads to various gastrointestinal, neurological, cardiovascular and malignant diseases.[1]

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There is an increased risk of cirrhosis with ingestion of more than 60–80 g/day of alcohol in men and more than 20 g/day in women. However, only 6–41% of these will develop cirrhosis. In extremely high daily alcohol intake more than 120 g/day, only 13.5% develop alcohol induced liver damage, other risk factors being genetic predisposition, dietary habits etc.[1,2] Over 90–98% of alcohol consumed is metabolized by oxidative and non-oxidative pathways, liberating chemically active compounds like acetaldehyde, acetate, fatty acid ethyl ester etc.[3–10] These compounds generate highly reactive molecular fragments called reactive oxygen species (ROS). The ROS can diffuse from the site of generation and damage the

structural and functional integrity of cells causing tissue damage.[11]

The peroxidation of lipid components of the cells by ROS generate lipid peroxidation products (LPP) like malondialdehyde (MDA), and 4-hydroxynonenal (HNE).[12] The MDA is measured in the form of thiobarbituric acid-reactive substances (TBARS) assay. Increased ROS production lowers the cellular antioxidant levels and enhances the oxidative stress in many tissues.[13] Oxidative stress is a disturbance in the oxidant-antioxidant balance leading to potential cellular damage. Alcohol is metabolized by many tissues and ROS produced by alcohol metabolism, reach various tissues through blood, causes increase in serum oxidative stress and lipid peroxidation. But many a times, the liver is held responsible for increase in serum oxidative stress and lipid peroxidation. In this context, the present review was undertaken to highlight the role of liver and other organs in increasing the oxidative stress and LPP in the serum.

METABOLISM OF ALCOHOL

Alcohol absorption begins in the stomach within 5–10 minutes of ingestion, is readily absorbed by duodenum (80%) and in small amounts by the stomach (8%) and esophagus,[3,4] which takes place by passive diffusion.[7,14] Absorption and rise in blood alcohol concentration is rapid when taken on empty stomach. Food particularly fatty food delays the gastric emptying and delays the absorption of ethanol.[3,4,15] Other factors which influence absorption include concentration of ethanol consumed, carbonation of alcoholic beverage and the duration of ingestion.[16] Blood transports alcohol to all parts of the body, so most of the tissues are exposed to the same concentration of alcohol as in blood.[1]

Elimination of absorbed ethanol occurs primarily through the biotransformation via oxidative and non-oxidative metabolism (95–98%), with the small fractions of non-metabolized alcohol being excreted in the lungs (1.7%), brain (0.7%), sweat (0.1%) and urine (0.3%) (7,10). Metabolism of alcohol follows zero order kinetics and volume of distribution is 0.6–0.8 L/Kg^3.[4,14,17,18] Maximum amount of alcohol metabolism occurs in the liver; about 10 mL/hr. is oxidized by liver.[14]

Alcohol is metabolized by many organs including the stomach, small intestine, liver, brain, pancreas etc.[5–10,14] The relative organ specificity and high energy content of alcohol along with the lack of effective feedback control of hepatic metabolism may result in displacement of 90% metabolic substrates in the liver which probably explains the striking metabolic imbalances produced by ethanol disposal.

Alcohol metabolism decreases the total body fat oxidation by 79% (NADH formed via the ADH pathway inhibits β-oxidation of fatty acids), protein oxidation by 39% and almost completely abolishes the 249% rise in carbohydrate oxidation following glucose infusion.[19]

Oxidative pathways

Alcohol dehydrogenase (ADH) pathway (Figure 1)[5,8]

Human liver ADH is a zinc metalloenzyme with five classes of multiple molecular forms that arise from the association of eight different types of subunits, α, β_1, β_2, β_3, γ_1, γ_2, π, and χ into active dimeric molecules. ADH_1 (Class-I): has low Km value and there are three types of subunit α, β, and γ in class I. ADH_2 (Class-II): has relatively high Km value. ADH_3 (Class-III): does not participate in the oxidation of ethanol in liver because of its very low affinity for ethanol. ADH_4 (Class-IV): New isoenzyme of ADH has been found in human stomach (vide infra), the so-called σ- or μ-ADH and another new form of ADH_5 (class V) in liver and stomach was reported.[19] ADH activity was found in granular cells, and purkinje cells of cerebellum. ADH_1, ADH_3 and ADH_4 subtypes were expressed in the brain.[9] It has been demonstrated that contribution of ADH_3 to alcohol metabolism in vivo increases linearly and is dose dependent. Mammalian liver contains ADH isoenzymes ADH_1, ADH_2 and ADH_3. Both ADH_2 and ADH_3 have higher km for ethanol than ADH_1.By CM-Chromatography of ADH isoenzymes of mouse liver, it was found that, ADH_1 accounts more than 62% of total liver ADH activity, ADH_2-2% and ADH_3-13%.[19]

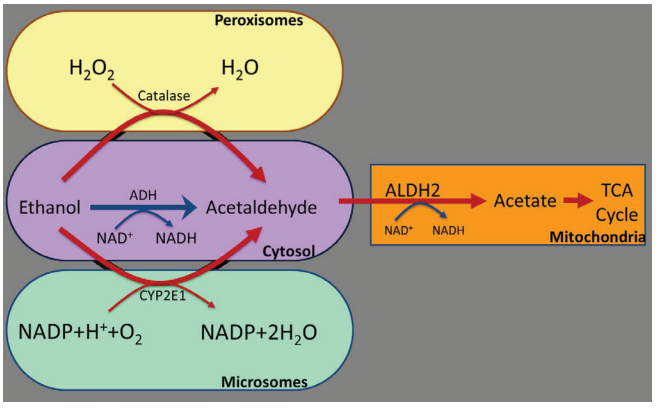

Figure 1. Schematic representation of oxidative metabolism of ethanol.

As the concentration of ethanol increases, the percentage of ADH_3 fraction increases while ADH_1 fraction decreases. With increased alcohol dose, ADH_1 and ADH_3 together account for most of the alcohol metabolism. The data suggests that chronic binge drinking and the resulting liver disease shifts the key enzyme in alcohol metabolism from low Km ADH_1 to high Km ADH_3 and thereby reduces the rate of alcohol metabolism.[19]

The reaction in the liver cells results in a state that is vulnerable to damage from the byproducts of ethanol metabolism such as free radicals, NADH and acetaldehyde and it was postulated that the latter may be involved in the hepatotoxicity.[6] Acetaldehyde is oxidized to acetate by aldehyde dehydrogenase 2 ($ALDH_2$) in mitochondria. Several isoenzymes of ALDH have been identified, of which $ALDH_1$ (cytosolic) and $ALDH_2$ (mitochondrial) are involved in the acetaldehyde metabolism. Genetic polymorphism of the $ALDH_2$ gene results in allelic variants $ALDH_{2-1}$ and $ALDH_{2-2}$ which is virtually in-active.[9,19]

Acetaldehyde is produced and hydrogen is transferred from ethanol to the cofactor nicotinamide adenine dinucleotide (NAD^+), which is converted to its reduced form (NADH). The acetaldehyde so formed again loses hydrogen and is metabolized to acetate, most of which is released into the bloodstream.[5,6,19,20]

Most of the acetate resulting from alcohol metabolism escapes into blood stream and is eventually metabolized to carbon dioxide in heart, skeletal muscles and brain cells.[6] The acetate formed enters the Kreb's cycle producing 7 kcal of energy per gram of ethanol.[14]

Each metabolic step requires NAD^+; thus oxidation of 1 mol of ethanol (46 g) to 1 mol acetic acid requires 2 mol NAD^+ (approximately 1.3 Kg). This greatly exceeds the supply of NAD^+ in the liver, hence when blood alcohol concentration is more than 9 mg/100 mL, elimination occurs by zero order kinetics, limiting the elimination capacity to 8 g or 10 mL in a 70 Kg adult, or approximately 120 mg/Kg/hr.[3,4] Ethanol metabolism causes fall in NAD^+/NADH ratio leading to metabolic consequences like increased lactate and slowing down of the Kreb's cycle.[15]

Cytochrome P450 (pathway CYP 450)

The microsomal ethanol oxidizing system (MEOS) is located in the smooth endoplasmic reticulum of hepatocytes (nonparenchymal cells, Kupffer cells), brain (cerebellum, cerebral cortex, thalamus and hippocampus), pancreas and other extrahepatic tissues which metabolizes alcohol through cytochrome P450 2E1.[7,9,14,19,21,22]

In humans there are some indications of the involvement of P-450s other than CYP2E1 such as CYP1A2 (in B-lymphoblastoid cells) and CYP3A4 that can oxidize ethanol. CYP2E1-dependent ethanol oxidation is twice that of CYP1A2 and CYP3A4. Thus although CYP2E1 plays the major role in the ethanol oxidation by the liver microsomes, the combined activity of CYP1A2 and CYP3A4 is comparable to that of CYP2E1.[19]

The CYP2E1-dependent metabolism of alcohol produces an initial product, the gem-diol, which is chemically unstable and decomposes to acetaldehyde. In alcohol oxidation by CYP2E1, there is sequential reduction of oxygen in four single-electron steps. The addition of one electron to oxygen produces superoxide. If superoxide is reduced by another electron, the two-electron–reduced form of oxygen, a peroxide, is produced. This will accept two hydrogens to produce hydrogen peroxide. Hydrogen peroxide can accept another single electron, and this usually occurs as a result of the transfer from a reduced metal ion (e.g. reduced iron or copper). When it does accept the electron, hydrogen peroxide is converted to a hydroxyl radical ($HO^•$) and a hydroxide anion (HO^-) which on combining with hydrogen produces water.[20]

Ethanol can also be reduced by liver microsomes to acetaldehyde through a non-enzymatic pathway involving the presence of hydroxyl radicals originating from iron-catalyzed degradation of hydrogen peroxide.[19]

Catalase (CAT)

It is located in the peroxisomes and capable of oxidizing the alcohol in vitro in the presence of hydrogen peroxide generating system which is a minor pathway of alcohol oxidation, except in fasting state.[6] Catalase along with hydrogen peroxide will oxidize alcohol in the brain. In the intestine ethanol oxidation is comparatively low but may be much higher in specific structures and cell's known for their increased catalase activity. RBC causes the non-enzymatic conversion of ethanol to acetaldehyde.[9]

Non-oxidative pathways

Fatty acid ethylesters (FAEE)

FAEE are synthesized with the participation of specific synthases, whose activity have been revealed in the heart,

brain, liver and the pancreas.[6,10] These esters may also contribute to long term toxicity.[15]

Phosphatidylethanol (PE)

Phospholipase-D catalyzes conversion of phospholipids to phosphatidic acid and also the phosphorylation of alcohol with phosphatidylcholine to form PE.[10] Phospholipase-D has high Km value for alcohol; hence phosphotidylethanol is formed in high alcohol concentration. PE is poorly metabolized and may get accumulated in the body; which can be detected in chronic alcoholics.[6] Phospholipase D is detected in mammals in microsomal fractions of the liver, lungs and brain.[10]

The formation of PE in brain and renal tissue of mammalians after the consumption of ethanol has been proved. PE has also been detected in neutrophils, erythrocytes and platelets in chronic alcoholics. PE can be used as a potential laboratory indicator of alcohol consumption over the preceeding 2 weeks period. Aradottir et al., showed that feeding rats with ethanol for a period of 4.6 weeks caused an accumulation of PE in the brain, the muscular layer of the stomach, lungs, kidney, liver and spleen. The high concentration of PE in spleen leads to splenic degradation of erythrocytes.[23]

Ethyl glucuronide (EG)

Ethyl glucuronide is formed exclusively by alcohol consumption. The 0.5–1.6% of the total alcohol dose becomes conjugated with glucuronic acid, catalyzed by UDP glucuronyltransferase, greatest activity occuring in the liver. Such activity is also detected in kidney, intestine, brain, myocardium, suprarenal gland, spleen and the lungs. EG acts as a link between markers with short and long half-lives and is a prospective marker for the consumption of alcohol, which has high sensitivity and specificity.[10]

OXIDATIVE STRESS IN ALCOHOLISM

Acetaldehyde inhibits the repair mechanism of alkylated nucleoproteins, leading to decrease in the activity of several enzymes and mitochondrial damage (megamitochondria);[13] and decreased function of several organs including liver and heart; decreased respiratory rate and ATP levels; and increased production of ROS.[7] Mitochondrial respiratory chain in various cells generates most of the ROS produced in the body.[24] Enzymatic systems including the MEOS and cytosolic enzymes like xanthine oxidase system and aldehyde oxidase have been implicated as source of superoxide anion (O_2) and hydrogen peroxide in parenchymal cells during ethanol intoxication.[7,19] Induction of CYP2E1 by ethanol is associated with proliferation of the endoplasmic reticulum, which is accompanied by increased oxidation of NADPH with resulting hydrogen peroxide generation.[19]

Increased production of NADH after alcohol metabolism provides more starting material and enhances activities of the respiratory chain including oxygen (O_2) consumption and ROS formation.[24]

In alcohol oxidation by CYP2E1, the delivery of electrons to oxygen must be very carefully controlled to prevent the generation of the intermediate ROS. A small, but significant, portion of the oxygen that is reduced in the mitochondria is released as the one-electron–reduced form, superoxide. Thus, increased mitochondrial activity and NADH use will result in greater superoxide production.

During conversion of alcohol to gem-diol and to acetaldehyde, oxygen utilization can lead to the generation of ROS. As a result CYP2E1 will indirectly catalyze the formation of ethanol derived free radical (1hydroxyethyl radical) which also contributes to oxidative damage. The production of ROS by CYP2E1 is referred to as an "uncoupled reaction" because the oxygen does not end up in the substrate. CYP2E1 generates ROS and oxidative stress more readily than other P450s. A very important feature of P450s is that when the enzyme uses oxygen in the reaction ultimately adding one atom of oxygen to the substrate molecule, sometimes the reaction does not proceed as planned and the enzyme itself can generate the ROS.[20]

There is a high rate of NADPH oxidase activity which leads to the production of large quantities of O_2 and hydrogen peroxide.[7]

Xanthine oxidase (XO)–Under normal conditions it acts as xanthine dehydrogenase. Alcohol consumption promotes conversion of xanthine dehydrogenase to XO which generates ROS.[24] In hypoxia, increased NADH may inhibit the activity of NAD+ dependent xanthine dehydrogenase, thereby favoring oxygen dependent XO. The XO metabolizes purine leading to the production of ROS and lipid peroxidation.[19]

A. Kasdallah – Grissa et al., found that chronic alcohol treatment provoked a clear toxicity in rats, which induced oxidative stress as monitored by lipid peroxidation products in several organs like liver, heart, brain and testis

and also showed that resveratrol, an antioxidant, supplementation strongly decreased the alcohol induced lipid peroxidation in heart and testis.[25] Acetaldehyde interaction with enzymes, microsomal proteins, microtubules and lipids can also lead to radical formation.[6,24]

FAEE accumulate in organs like brain, pancreas, myocardium, adipose tissue and in hair. In in-vitro research, FAEE reveal particular toxicity in relation to cellular membranes, especially mitochondrial and lysosomal membranes. FAEE also disturbs the cellular anti-oxidative potential, causing oxidative stress.[10] It has been ascertained that FAEE increased the activity of glutathione S-transferase and cause an increase in the concentration of lipid hydroxyl peroxides.[10,26]

In non-enzymatic reduction of alcohol in liver, there is production of ethanol-free radicals which may be due to an oxidizing species bound to cytochrome P-450 and abstracting a proton from the alcohol α-carbon in catalyzing the free radical activation of aliphatic alcohols.[19] Apoptosis is presumed to represent the last common pathway of ROS-mediated cell injury.[27]

Alcohol induced oxidative stress in different organs

Chronic alcohol consumption leads to ROS generation and lipid peroxidation in liver, heart, testis, salivary glands, gastrointestinal tract, skeletal muscles, pancreas and brain leading to cellular damage.[25]

Heart

Chronic alcoholism has direct relationship with alcohol induced heart muscle disease which may result in arrhythmias, cardiomegaly, cardiomyopathy, cardiac dysfunction and congestive heart failure.[1,28]

Various mechanisms have been proposed in alcoholism such as induction of autoantibodies to heart muscle due to the formation of acetaldehyde-protein adducts.[29] Lipid peroxidation and free radical mediated damage has also been hypothesized in the pathogenesis of alcoholic cardiomyopathy (ACM). Histological features of lipid peroxidation are seen in damaged organelles.[29,30]

Animal studies have shown more compelling evidence of ROS-induced damage and cardiac lipid peroxidation. A decrease in cardiac creatine kinase (CK) activity after an acute dose of alcohol has been demonstrated, which is due to damage by ROS. The implication of these findings is that XO generates superoxide radicals (O_2^-) hydroxyl radical (OH.) and also inactivates CK.[29] There is no clear demonstration that ROS injury causes alcohol induced contractile dysfunction.[30] The "acetaldehyde toxicity" may also contribute to the pathogenesis of ACM.[28]

Kidney

Oxidation of alcohol also occurs in the kidney.[31] Acetaldehyde exerts nephrotoxic effects on the kidney[32] ROS generation may contribute to the oxidative stress in the kidneys.[31,32] ROS and oxidative stress mediated injury have been considered as primary cause of alcohol induced, swelling of glomerula and tubules, proliferation of mesangial cells and hyaline drops in tubular epithelial cells are seen in the kidney.[1,31,32] Ethanol metabolites – protein adducts and hyaline in the tubular epithelial cells are observed which precipitates alcohol related diseases.[1,32] The role of free radicals in alcoholic renal disease is well established. Shanmugam et al., in their animal study showed that activities of superoxide dismutase (SOD), reduced glutathione(GSH), ascorbic acid and uric acid were decreased in liver, heart, brain, kidney and muscle treated with alcohol as compared to rats dosed with ginger which contains antioxidants. XO, glutathione, and serum transferase activities were increased in rats treated with alcohol as compared to those dosed with ginger.[32]

The chronic ethanol consumption resulted an increased ADH activity in rat kidney. The studies have shown a significant increase in MDA levels in the kidney after chronic ethanol ingestion. Diana Dinu et al., in their study observed the increase in kidney CAT activity after ethanol treatment. The increase in CAT/SOD ratio may suggest an increase in kidney resistance to oxidative damage. They also demonstrated that chronic exposure to ethanol exerts an oxidative stress and alters the GSH homeostasis. The long-term ethanol exposure increases enzyme activities related to the recycling and utilization of glutathione in the kidney.[31]

Testis

Chronic alcohol consumption results in oxidative stress followed by lipid peroxidation resulting in both endocrine and reproductive failure which in-turn alters the mechanism of testosterone production by inhibiting protein kinase C, a key enzyme in testosterone synthesis.[27] Testicular membranes are rich in polyenoic fatty acids that are prone for peroxidative decomposition, which may result in membrane injury leading to necrotic cell death and gonadal dysfunction that occurs in acute and chronic alcoholism.[1,27,33] Thus oxidants generated by

alcohol metabolism can contribute to cell damage and may play a role in the pathogenesis of alcohol-induced gonadal injury.[27] It also appears that the oxidative stress created in the Leydig cells as a consequence of chronic alcohol exposure diminishes the steroidogenic capacity of the testis, lowering circulating testosterone levels.[34] In chronic alcoholism increase in the endogenous and the H_2O_2 induced DNA damage was also observed in lymphocytes and testis.[33]

Skeletal muscles

The fundamental cause of alcohol-related myopathy is likely multifactorial.[35] Alcohol induced muscle diseases (AIMD) are more common than hereditary muscle diseases making AIMD possibly the most prevalent skeletal muscle disorder.[29]

Alcoholic myopathy is seen in 50–60% of alcoholics.[36] Increased lipid peroxidation is related to type II skeletal muscle fiber atrophy, loss of muscle mass (loss up to 30%, muscle strength may occur), difficulties in gait, and myalgia.[29,36–38] The antioxidants like serum selenium, alpha tocopherol and carnosinase activity are lower in myopathic alcoholics compared to nonmyopathic alcoholics. Carnosinase hydrolyses carnosine, an important skeletal muscle antioxidant. The combination of both alpha-tocopherol and selenium deficiency raises muscle TBARS.[29]

Calmodulin on exposure to oxidative stress leads to altered intracellular calcium homeostasis and muscle contractility. Reimers EG et al., in their study showed that increased peroxidation may impair calmodulin function and muscle structure and contractility due to vitamin D deficiency an antioxidant, resulting in alcoholic myopathy.[36] Interestingly, in their study there was a significant relationship between serum vitamin D levels and muscle glutathione peroxidase (GPX) and SOD activities, the antioxidant defence. They suggested that oxidative damage may be involved in alcoholic myopathy.[35,36,38]

The generation of ROS and/or enhanced lipid peroxidation leads to reduced tissue protein synthesis. Increased skeletal muscle production of ROS has been suggested to arise from intramuscular XO activities causing raised lipid peroxidation of fatty acid moieties within the cellular and subcellular membranes.[39]

Acetaldehyde may be a primary factor underlying alcohol-induced muscle dysfunction.[40] Immunohistochemical studies have shown that treatment of rats with alcohol and cyanamide increased the amount of MDA and acetaldehyde derived protein adducts in skeletal muscle.[41] The formation of MDA-protein adduct species suggests that increased lipid peroxidation occurs in alcohol exposed muscle. If MDA-acetaldehyde protein adducts were found it would suggest that alcohol oxidation and lipid peroxidation occurred simultaneously within the same microenvironment for a prolonged time.[41,42]

Transforming growth factor-β (TGF-β) is a superfamily of cytokines that can be induced by ROS. Jeffrey et al., showed that alcohol-induced oxidative stress increases expression of TGF-β1 and results in severe lung dysfunction and skeletal muscle catabolic conditions. They concluded that chronic alcohol ingestion alters redox balance in skeletal muscle. Type II fiber–rich muscles are particularly sensitive to alcohol-induced redox state, because of lower GSH levels. They also provided evidence that skeletal muscles from alcohol-fed rats have increased atrogin-1 mRNA expression, which appears to be sensitive to oxidative stress.[35]

Brain

Alcohol affects the CNS in dose dependant fashion, producing sedation that progresses to sleep, unconsciousness, coma, surgical anesthesia, respiratory depression and cardiovascular collapse.[14] Alcohol metabolism generates ROS and nitric oxide (NO) via induction of NADPH/XO and nitric oxide synthase in human neurons contributing to oxidative and nitro-stress.[7]

Increased permeability of blood brain barrier as a result of the toxic effect of ethanol, increased levels of ROS and lipid peroxidation products in the neurons affect the number, size and shape of mitochondria, increases micropinocytic vesicles, proliferation of smooth endoplasmic reticulum and affects golgi apparatus, causes edema of astrocytic processes and a steady increase in the levels of DNA lesions inhibiting gene expression and leading to neuronal death.[1]

Pancreas

Oxidative stress has been implicated as a possible mechanism of pancreatitis. Acute alcohol administration increase levels of lipid peroxidation products in rat pancreas thus providing direct evidence that alcohol causes oxidant stress within the pancreas.[22] There is growing evidence that exocrine pancreas is vulnerable to damage from ROS generated by ethanol. It has been shown that ethanol leads to ROS production in mouse pancreatic acinar cells.[43] Non oxidative metabolism of ethanol resulting

in the formation of FAEE in the pancreas appears to be another major cause of pancreatitis.[1]

Liver

Ethanol is a hydroxyl radical scavenger, ethanol interacts with hydroxyl radical to produce 1 hydroxyl ethyl radical (HER). Liver microsomes can oxidize ethanol to HER in an NADPH or NADH dependent manner by iron catalyzed process.[44,45] Acetaldehyde affects the immune system, alters cytokine production, antioxidant enzymes and chemicals, particularly mitochondrial and cytosolic glutathione. Acetaldehyde, MDA, HNE, and HER can bind to proteins to produce protein adducts. MDA acetaldehyde adduct can bind to other proteins to produce hybrid adducts. Such adducts can produce toxicity because the adducted proteins may lose function and provoke immune response.[45]

Alcohol induced hypoxia is seen in the pericentral region of the liver where extra O_2 is required to metabolize alcohol.[24] Hypoxia leads to ROS production and lipid peroxidation via XO in liver. Lipid peroxidation associated alcoholic liver injury,[19] serum markers of lipid peroxidation such as MDA, 4-hydroxynonenal and F2-iso prostanes are increased.[7]

Endotoxin is one of the components of the outer cell wall of gram negative bacteria. High alcohol concentration facilitates the endotoxin absorption, alteration of gut permeability to endotoxin, modification of gut flora or changes in rates of endotoxin clearance[46] and direct damage to the cells lining the interior of the intestine. Endotoxin physically attaches to the surface of Kupffer cells by a receptor CD14, a co-receptor toll-like receptor 4 (TLR4) together with another protein called LPS-binding protein and generates the signal to the cell's interior. TLR4 activates inter-leukin–1 receptor-associated kinase[47] where it triggers the numerous biochemical reactions through which an activated Kupffer cell performs its functions.[24,47] Kupffer cell activation leads to the production of ROS, particularly superoxide by NADPH oxidase, which in large amounts can lead to oxidative stress.[45,47] The free radicals activate nuclear factor – kappa B, leading to an increase in production of tumor necrosis factor alfa (TNFα), followed eventually by tissue damage.[45] Results of several studies suggest that the oxidative stress associated with chronic alcohol consumption is largely attributable to endotoxin-induced activation of Kupffer cells (Figure 2).[47]

Lipid peroxides stimulate stellate cells, which are major source of extracellular matrix thus stimulating collagen synthesis, which may be important in many forms of liver fibrosis.[48]

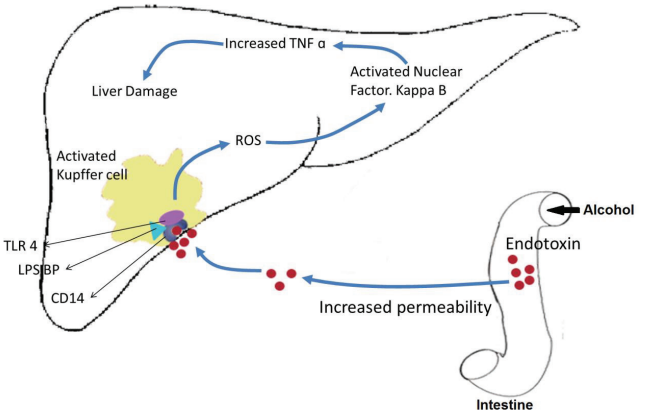

Figure 2. Endotoxin-ROS-Liver damage axis.

DISCUSSION

From the above, it is clear that alcohol metabolism is not solely confined to liver but also occurs in several other organs namely heart, brain, kidney, GIT, pancreas, skeletal muscles, testis, lungs, RBC, lymphocytes etc. Even though most of the studies on alcoholic liver disease blame liver alone for increase in serum oxidative stress and lipid peroxidation, it is clear that although major contribution may be by the liver but not the whole. This means that other organs do contribute in the formation and release of acetaldehyde, ROS and LPP, which will ultimately enter into the circulation (Figure 3). At times, the oxidative stress of other organs may be more than that of the liver. Even a minimal contribution by skeletal muscles to the serum concentration may, for instance, become considerably significant as the total mass of skeletal muscle (31 kg) is about 10 times more than that of the liver (2.6 kg).[49] Similarly the contribution of all the organs towards oxidative stress, together, may at times outweigh hepatic contribution towards serum oxidative stress.

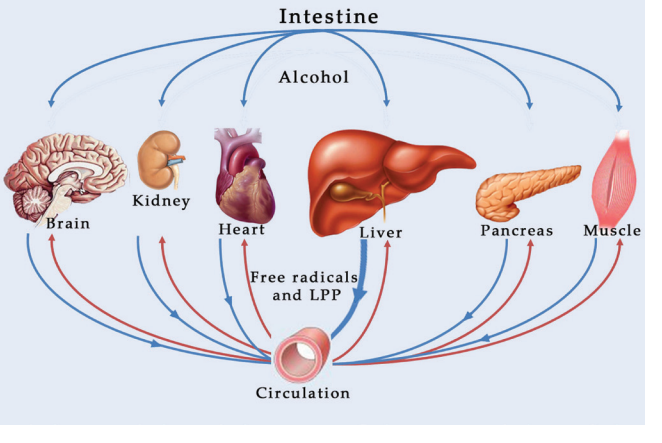

Figure 3. Overview of ethanol absorption, formation and circulation of free radicals and lipid peroxidation products (LPP).

Most of the human studies done have estimated the oxidant, antioxidant levels in serum in alcoholic liver diseases but not correlated these parameters to the hepatic tissue level.[50–53] Bogdanska J et al., in their study found that there is statistically significant increase in erythrotic SOD (ESOD) in alcoholics than in non-alcoholics, also there was no significant difference in ESOD in alcoholics with and without palpable liver, which seemed to be not related to liver damage,[54] which indicated a role of other organs too. Circulating phospholipid hydroperoxides have been proposed as new markers of oxidative stress in alcoholic patients, Yang L et al., in their rat experiment data showed that measurement of oxidative stress does not directly correlate with the hepatic oxidative stress imposed by liver catabolism of ethanol. Instead, these lipids reflect an unappreciated, temporally distinct, hepatic and non-hepatic response to chronic ethanol exposure.[55] Virgina and co-workers in their rat experiment found that there was no significant change in CAT and vitamin E levels in hepatic tissue of ethanol fed group and control group.[56] Similar results were also found even in non-alcoholic liver disease, Rousselot et al., in their study on non-alcoholic liver disease concluded that routine blood oxidative stress markers probably do not accurately reflect hepatic oxidative stress.[57] Therefore, the amount of ROS and LPP produced by each organ needs to be estimated in order to claim a particular organ as culprit of oxidative stress in alcoholic liver disease.

Hence further detailed animal and human studies are required to correlate the ROS/LPP in the serum and other organs including liver, in order to determine extent of liver's role in serum ROS/LPP in alcoholic liver diseases. In conclusion, a question that needs a thought is -Are we over estimating the contribution of liver towards serum oxidative stress in alcoholic liver disease? Since there is significant contribution of oxidative stress by other organs but the whole brunt is borne by liver. Is it fair to blame only the liver?

CONFLICT OF INTEREST

None of the authors have an actual or potential conflict of interest concerning this study and its publication.

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