

N-nitrosodimethylamine (NDMA), Liver Function Enzymes, Renal Function Parameters and Oxidative Stress Parameters: A Review

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Abstract: The aim of this study is to review a procarcinogen, the N-Nitrosodimethylamine (NDMA), liver and kidney functional enzymes (in assessing action of toxicants such as NDMA) as well as oxidative stress parameters (in assessing the extent of free radical damage and scavenging). Catalase and hydro peroxidase enzymes convert hydrogen peroxide and hydro peroxides to non-radical forms and functions as natural antioxidant in human body. Enzymes like Superoxide Dismutase (SOD) and Catalase (CAT) and compounds such as tocopherol and ascorbic acid can protect organisms against free radical damage. Lipid peroxidation is a mechanism generally recognized as being the most important in the pathogenesis of liver injury by a number of toxic compounds including NDMA.

Keywords: Carcinogenesis, CYP2E1, liver, N-nitrosodimethylamine, renal, ROS

INTRODUCTION

Nitrosamines, one of the most important environmental carcinogen, has been suggested to cause the generation of Reactive Oxygen Species (ROS) resulting in oxidative stress, alter the antioxidant defense system in tissues and cellular injury, which may be one of the factors in the etiology of cancer (Bansal *et al.*, 2005; Mittal *et al.*, 2006).

Scientific evidence has suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions (O_2^-), hydroxyl radical (OH) and peroxy radicals (H_2O_2) are produced in biological system. These reactive oxygen species can damage DNA which causes mutation and chromosomal damage. It also oxidizes cellular thiols and extracts hydrogen atoms from unsaturated fatty acids to initiate the per oxidation of membranes lipids (Halliwell and Gutteridge, 1998). Moreover, the production of excessive free radicals stimulates the oxidative damage and such situation contribute to more than one hundred disorder in humans including atherosclerosis, liver disease, diabetics, coronary heart disease, neurodegenerative disorder, cancer and they play major role in the aging process (Pong, 2003; Sandhya *et al.*, 2010). Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules.

However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein and lipid membrane. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by a variety of antioxidant enzymes which includes Superoxide Dismutase (SOD), Catalase (CAT), Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx), Glutathione Reductase (GR) etc.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals induce oxidative stress (Ozsoy *et al.*, 2008). Antioxidants prevent diseases by various mechanisms including, scavenging free radicals, against oxidative stress and inhibiting lipid per oxidation (Miller and Rice-Evans, 1997). The balance between phase I carcinogen activating enzymes and phase II carcinogen detoxifying enzymes is important in determining the risk of developing chemically-induced cancer (Maliakal *et al.*, 2001). The present study reviews N-nitrosodimethylamine (NDMA), a procarcinogen, liver and kidney functional parameters and oxidative stress parameters.

N-NITROSODIMETHYLAMINE (NDMA)

N-nitrosodimethylamine (NDMA) is a member of a family of extremely potent carcinogens, the N-

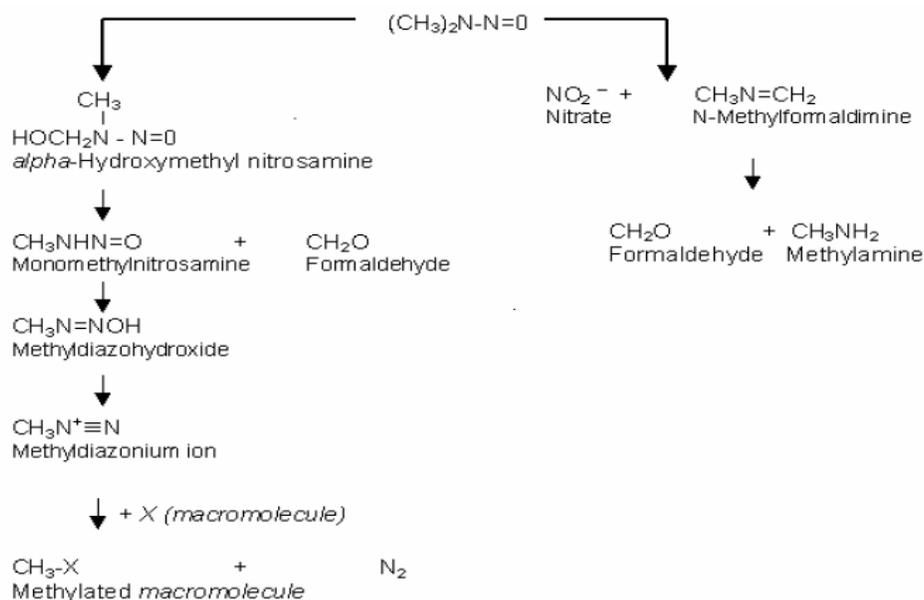


Fig. 1: Metabolic pathway for NDMA (Liteplo *et al.*, 2002)

nitrosamines (U.S. EPA, 2002), also known as Dimethylnitrosamine (DMN), Dimethylnitrosoamine (DMNA), *N, N*-dimethylnitrosamine, *N*-methyl-*N*-nitrosomethanamine, *N*-nitroso-*N, N*-dimethylamine. NDMA has no commercial use and no industrial production and is generated from the *in situ* reaction of Dimethylamine (DMA) with monochloroamine in the disinfection process or the nitrosation of DMA by nitrite (Mitch and Sedlak, 2002; Gerecke and Sedlak, 2003; Choi and Valentine, 2002).

DMN is a potent hepatotoxin, carcinogen and mutagen (George *et al.*, 2001) which exerts carcinogenic effects and induces hepatic necrosis in experimental animals through metabolic activation by CYP2E1 (Guengerich *et al.*, 1991) in experimental animals. NDMA is a potent hepatotoxin that can cause fibrosis and tumors in the liver of rats through an activation of CYP450 enzymes (George *et al.*, 2001; Kasprzyk-Hordern *et al.*, 2005). NDMA is activated by CYP2E1, which hydroxylates one methyl group. The resulting hydroxymethylnitrosamine is unstable and decomposes to formaldehyde, which is also used to quantify the metabolic rate and methane-diazonium-ion, which methylates DNA and protein or reacts with water to methanol (Frei *et al.*, 2001). The electrophilic intermediates produced by metabolic activation of nitrosamines, react rapidly with cellular nucleophiles and attention has been focused on reactions with DNA because this is generally considered to be the critical cellular target for carcinogens during tumor initiation. Although 7-methylguanine (66.8%) is the most abundant modified base in DNA produced by NDMA, a variety of other products have been identified. These include alkylphosphate triesters (12%), 1-, 3- and 7-methyladenine (0.9, 2.3, 0.7%); and 0⁶-methylguanine

(6.1%) and unidentified products (Sheweita, 2000) (Fig. 1). The formation of Reactive Oxygen Species (ROS) like H₂O₂, superoxide anion (O₂⁻) and Hydroxyl radicals (OH) has been demonstrated during the metabolism of nitrosamines resulting in oxidative stress, which may be one of the key factors in the induction of pathological conditions such as hepatocellular necrosis, carcinogenicity, neoplastic changes and tumor formation (Pradeep *et al.*, 2007; Wills *et al.*, 2006). Teufelhofer *et al.* (2005) reported that metabolism of the nitrosamine by CYP2E1 in mouse liver stimulated kupffer cells leading to generation of superoxide and other ROS capable of damaging liver cells. Enhancement of oxidative stress has been implicated in DMN-induced fibrosis and possibly hepatocarcinogenesis in rats (Vendemiale *et al.*, 2001). The hepatic Cytochromes P450s (CYP) are a multigene family of enzymes that play a critical role in the metabolism of many drugs and carcinogens (Sheweita, 2000). Cytochrome P450 activates Polycyclic Aromatic Hydrocarbons (PAHs) into more reactive intermediates that covalently bind to DNA, a key event in the initiation of carcinogenesis. The induction of carcinogen-metabolizing enzymes plays a critical step in initiation of carcinogenesis caused by chemical carcinogens such as Polycyclic Aromatic Hydrocarbons (PAHs) and *N*-nitrosamines (Sheweita, 2000; Sheweita *et al.*, 2007). Human CYP2E1 gene is located on the 10th chromosome, consists of 9 exons and 8 introns, contains a typical TATA-box and occupies 11413 B.P. of genomic DNA. CYP2E1 is constitutively expressed primarily in the liver (Danko and Chaschin, 2005). CYP2E1 expression level is significantly lower in other organs and tissues, in particular, kidneys, pancreas, brain, lung, nasal and

intestinal mucosa (Nakajima and Aoyama, 2000; Danko and Chaschin, 2005). Cytochrome P450 2E1 is a membrane-bound protein with molecular weight of ~57 kDa. It consists of 493 amino acid residues and is primarily associated with endoplasmic reticulum membranes (Danko and Chaschin, 2005). CYP2E1 is the main enzyme component of microsomal ethanol oxidation system. It is involved in metabolism of more than 80 low-molecular hydrophobic toxicologically dangerous compounds and contributes to activation of many procarcinogens and several drugs to highly reactive metabolites (Nakajima and Aoyama, 2000; Danko and Chaschin, 2005). Specifically, CYP2E1 activates N-nitrosamines, contained in tobacco smoke and foodstuffs (Wang *et al.*, 2002; Danko and Chaschin, 2005) and several industrial (Nakajima and Aoyama, 2000) and endogenous carcinogens (Bartsch *et al.*, 2000). CYP2E1 possesses a unique ability to reduce molecular oxygen to highly reactive compounds-superoxide anion radical (O_2^-), single oxygen, hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$) even in the absence of substrate. Enhanced synthesis of active oxygen forms leads to intensified lipid and protein per oxidation, DNA damage and carcinogenesis (Danko and Chaschin, 2005). The occurrence of dietary and environmental chemicals such as N-nitrosamines, their precursors (nitrate and nitrite) and malondialdehyde in our foods and drinks including fish, meat and food drinks as well as fresh supermarket products is well established (Okafor and Nwogbo, 2005; Okafor *et al.*, 2007). Non-dietary N-nitrosamine sources include tobacco products, cosmetics and occupational exposures in rubber or rocket fuel factories and leather tanneries (Stepanov and Hecht, 2005). Griesenbeck *et al.* (2009) reported that nitrites and nitrosamines are most commonly associated with the consumption of meats, processed meats and fish with beer having the largest estimated amount of nitrosamine per serving. In a food survey conducted in Germany, N-nitrosodimethylamine (NDMA) was detected in 31.5% of analyzed samples. The major dietary sources of this compound included cooked meat products (0.2-2.5 $\mu\text{g}/\text{kg}$), cooked fish (0.5-8.0 $\mu\text{g}/\text{kg}$) and Spices (0.1-1.4 $\mu\text{g}/\text{kg}$). Analysis of 280 samples of tinned food revealed that 46.1% contained NDMA and/or N-nitrosopiperidine (NPIP) in the range of 0.07-4.5 $\mu\text{g}/\text{kg}$ (Domanska and Kowalski, 2002). Volatile N-nitrosamines including NDMA have been found in rubber products including gloves, balloons, toys, baby bottle teats, soothers and condoms (Proksch, 2001). NDMA, NDEA and NPYR were found in Chinese Rugao ham during ripening and post-ripening but could be degraded with γ -irradiation suggesting that γ -irradiation may be a potential decontamination measure for certain chemical compounds found in dry-cured meat.

Nitrates, nitrites and nitrosamines have been shown to have etiologic role in adverse pregnancy outcomes and chronic diseases such as cancer (Griesenbeck *et al.*, 2009). Eighty six percent of 232 nitrosamines including N-nitrosodimethylamine (NDMA) studied showed carcinogenic activity in experimental animals (Domanska and Kowalski, 2002). Subjects with high-risks of developing stomach, esophageal, colon and urinary bladder cancers were found to excrete higher levels of N-nitrosamines in their urine compared to their relevant low-risk control groups (Sheweita *et al.*, 2007). It has been demonstrated that tea reduces formation of NDMA in human stomach (Krul *et al.*, 2004) with similar results obtained on experimental animals (Choi *et al.*, 2002). Farombi *et al.* (2009) reported that Kolaviron inhibited dimethylnitrosamine-induced liver injury by suppressing Cyclooxygenase (COX-2) and inducible Nitric Oxide Synthase (iNOS) expression via Nuclear Factor kappa B (NF- κ B) and Activator Protein-1 (AP-1). Varsha *et al.* (2006) showed that garlic powder ingestion caused significant reduction of DNA damage in the liver and colon of rats treated by NDMA via depressed formation of O^6 -methylguanine in liver DNA of rats injected with NDMA.

LIVER FUNCTION PARAMETERS

Alpha-Fetoprotein (AFP): Alpha-Fetoprotein (AFP) is a single polypeptide chain glycoprotein, produced by the liver and its level decreases below 10 ng/mL after birth. Elevated maternal serum AFP is associated with neural tube defect, placenta abnormalities whereas decreased AFP is related to fetal chromosomal abnormalities (Down syndrome). AFP can act as a carrier protein binding several types of molecules including steroids, bilirubin, fatty acids, flavanoids etc. AFP is frequently re-expressed at high levels in patients with HCC or embryonal malignancies (Di Bisceglie *et al.*, 1998), depending on the degree of differentiation (Abelev and Eraiser, 1999). AFP synthesis can be up-regulated in normal mature hepatocytes under non-malignant conditions and may be expressed at high levels during the course of fulminant hepatic failure and other forms of hepatic injury including viral hepatitis and cirrhosis. In addition, high level AFP synthesis can be observed after partial hepatectomy followed by rapid liver regeneration (Spear, 1999). AFP levels are abnormal in 80% of patients with Hepatocellular Carcinoma (HCC) (Johnson, 2001).

5'-Nucleotidase (5'-NT): 5'-Nucleotidase (5'-NT) is a test specific for cholestasis or damage to intra or extrahepatic biliary system and in some laboratories, is used as a substitute for γ -glutamyltransferase for ascertaining whether an elevated Alkaline phosphatase is of biliary or extra-biliary origin. 5'-NT is an enzyme

catalyzing the hydrolysis of nucleoside-5'-monophosphates to nucleosides and inorganic phosphate. The enzyme is widely distributed in human and animal tissues. The activity present in sera is released from the membrane of liver cells by bile salts and has been used as a marker for liver disease (Goldberg, 1973). Increased enzyme levels in sera are associated with certain forms of liver disease, such as intra- or extra-hepatic obstruction and particularly in cases of hepatic carcinoma as well as in mastectomy patients with recurrent metastases. The diagnostic value of 5'-NT has been shown to be superior to other liver enzymes, especially in cases of liver metastasis (Bertrand and Buret, 1982; Goldberg, 1973).

5'-NT activity is found not only in soluble but also in membrane-bound, surface-located form. In addition to a broad spectrum of 5'-purine and pyrimidine mononucleotides, 5'-dinucleotides and 5'-trinucleotides, or even complex nucleotides like UDP-glucose or FAD, can also be hydrolysed by the various 5'-nucleotidases. Whereas cytosolic 5'-nucleotidase activity controls intracellular levels of nucleoside 5'-monophosphates, surface-located 5'-nucleotidase is a major contributor to the cascade that completely hydrolyses extracellular ATP to adenosine and thus of major pharmacological interest. The physiological function of the enzyme probably differs in various organisms and tissues and possibly extends beyond its catalytic activity. Thus for example surface-located 5'-nucleotidase anchored to the plasma membrane by glycosyl-phosphatidylinositol has been implicated in cell-matrix or cell-cell interactions and even in transmembrane signalling. 5'-nucleotidase was found to be elevated in the animals with solid tumors (Ip and Daw, 1978). The increased activity of this enzyme seems to have originated from the proliferating tumor cells (Dao *et al.*, 1980). Elevated activities of 5'-nucleotidase in carcinoma of liver and leukemia has been reported (Thirunavukkarasu and Sakthisekaran, 2003).

Beta-glucuronidase: β -Glucuronidase hydrolyzes glucuronide moieties from steroids and xenobiotics so that circulating glucuronyl conjugates can interact with target tissues. In animal models, dietary constituents can alter β -glucuronidase activity. In humans, serum β -glucuronidase activity reflects liver enzyme loss during cell turnover and thus is a surrogate for hepatic β -glucuronidase (Lampe *et al.*, 2002). Metabolite of N-nitrosodimethylamine (NDMA) activation generates ROS which reacts with the lipid bilayer of intracellular organelles including lysosomes, which destabilizes lysosomal membrane and results in rupture of lysosomes. Increased rate of release of β -glucuronidase from the lysosomes provides evidence for increased lysosomal fragility and decreased lysosomal stability during pathogenesis of NDMA-induced hepatic fibrosis (George, 2008). Lysosomal membrane plays a vital role

in the regulation of lysosomal enzyme secretion in pathophysiology (Pillay *et al.*, 2002) and in various inflammatory processes. A compromise of lysosomal membrane integrity may lead to an undesirable elevation of lysosomal enzymes in both intra- and extra cellular space, which could pave the way for cellular and tissue disorders including apoptosis.

Alanine Transaminase (ALT): Alanine Transaminase (ALT) also called Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Aminotransferase (ALAT) is an enzyme present in hepatocytes. When a cell is damaged, it leaks this enzyme into the blood, where it is measured. ALT rises dramatically in acute liver damage. Alanine aminotransferase is a more reliable marker of liver integrity than aspartate aminotransferase (Ojiako and Nwanjo, 2006). The liver enzymes are normally found in circulation in small amounts because of hepatic growth and repair. As a liver specific enzyme, ALT only significantly elevates in hepatobiliary disease. Increase in AST levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma (Moss and Handerson, 1999; Vozarova *et al.*, 2002).

Aspartate Transaminase (AST): Aspartate Transaminase (AST) also called Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore not specific to the liver. Increase in AST levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma (Moss and Handerson, 1999; Vozarova *et al.*, 2002). In medicine, the presence of elevated values of alanine and Aspartate Aminotransferases (ALT and AST) is indicative of liver damage (Giboney, 2005).

Alkaline Phosphatase (ALP): Alkaline Phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue. Alkaline phosphatase belongs to a group of enzymes that catalyze the hydrolysis of phosphomonoesters at alkaline pH. ALP is present in cell surface in most human tissues. The highest concentrations are found in the intestine, liver, bone, spleen and kidney (Moss and Handerson, 1999; Gitnick *et al.*, 1992). The specific location of the enzyme within both sinusoidal and bile canalicular membranes accounts for the more predominant elevations in certain disorders (Bishop *et al.*, 2005). Elevation of alkaline phosphatase is one of the signs, suggesting space-

occupying lesions in the liver. Development of tumor results in tissue damage that lead to the release of ALP into circulation (Iqbal *et al.*, 2004). Increased level of alkaline phosphatase has been attributed to the damaged structural integrity of hepatic cells because the enzyme alkaline phosphatase is located in the cytoplasm and is released into the circulation after cellular damage (Sallie *et al.*, 1991).

Gamma Glutamyl Transpeptidase (γ -GT): Gamma Glutamyl Transpeptidase (γ -GT) is reasonably specific to the liver and a more sensitive marker for cholestatic damage than ALP. γ -GT may be elevated with even minor, sub-clinical levels of liver dysfunction. γ -GT is raised in chronic alcohol toxicity. γ -GT has been shown to play an important role in the metabolism of foreign substances and also during cell growth and differentiation (Thusu *et al.*, 1991) and is over expressed in tumor cells resistant to therapeutic drugs (Bailey *et al.*, 2001). Experimental studies have shown that γ -GT was strikingly activated during the course of hepato-carcinogenesis induced by several hepatocarcinogens in animals (Fiala and Fiala, 1973); chemical carcinogens may initiate some systematic effects that induce γ -GT synthesis (Vanisree and Shyamaladevi, 1998). This elevation reflects the progress of carcinogenesis, since its activity correlates with tumor growth rate, differentiation and survival of the host (Rajagopal *et al.*, 2003).

Lactate Dehydrogenase (LDH): Lactate Dehydrogenase (LDH) is a cytoplasmic enzyme that catalyzes the oxidation of lactate to pyruvate and vice versa. LDH is found in many body tissues, including the liver. Elevated levels of LDH may indicate liver damage. LDH is a fairly sensitive marker of solid neoplasm (Lippert *et al.*, 1981) and very high LDH levels correlate with treatment failure (Pui *et al.*, 1985); numerous reports revealed increased LDH activity in various types of tumors (Thangaraju *et al.*, 1998). The elevated levels of LDH may be due to its overproduction by tumor cells. Proliferating malignant cells exhibit very high rates of glycolysis, which subsequently lead to elevated LDH activity. LDH has five forms. They are LD1, LD2, LD3, LD4, LD5. Out of them, LD1 is the fastest in migration towards anode and LD2-LD5 are slow successively.

Is enzyme	Source
LD1:	Cardiac muscle (Rich source)
LD2:	Kidneys (Rich source)
LD3:	Cardiac muscle, liver, spleen, pancreas
LD4:	leukocytes
LD5:	Liver (Rich source)

Elevated serum total LDH activity was reported in human liver cirrhosis with the increased LDH activity in serum due to the extreme necrosis of the liver tissue

during fibrosis and simultaneous leakage of the enzyme into the blood stream (George and Chandrakasan, 1997).

Bilirubin: Bilirubin is a breakdown product of haem (a part of haemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. Heme total bilirubin causes jaundice and can signal a number of problems such as hemolytic anemias, internal hemorrhage, cirrhosis and viral hepatitis. Bilirubin, an endogenous organic anion binds reversibly to albumin and it is transported to the liver and then conjugated with glucuronic acid and excreted in the bile. Hepatobiliary disease is indicated when bilirubin fraction exceeds normal (Rosen and Keefe, 1998). Increased bilirubin content in serum reflects the path physiology of the liver. Therefore, hyperbilirubinaemia is one of the most sensitive and useful test to substantiate the functional integrity of the liver and severity of necrosis. It measures the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocytes degradation rate (Singh *et al.*, 2005). An increased level of total bilirubin reflects depth of jaundice (Dahiru and Obidoa, 2008)

Albumin: Albumin is a protein made specifically by the liver and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including the immunoglobulin's). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephritic syndrome, where it is lost through the urine. The half-life of albumin is approximately 20 days.

Glucose 6-Phosphate Dehydrogenase (G6PDH): In oxidative stress, up regulation of Glucose 6-Phosphate Dehydrogenase (G6PDH) has been reported (Cramer *et al.*, 1995). G6PDH is the first and rate-limiting enzyme in the pentose phosphate pathway. Its activity involves generation of NADPH and this is required for maintaining glutathione in its reduced state (for the detoxification of free radical and lipid hydroperoxides) (Halliwell and Gutteridge, 1998). Besides, NADPH maintains the catalytic activity of catalase and thus contributes to the reduction of H₂O₂ (Kirkman *et al.*, 1999). Thus, G6PDH level of a tissue may suggest the antioxidant status of that tissue. G6PD is elevated in response to external stimuli like toxic agents and oxidative stress. Frederiks *et al.* (2003) reported that the activity of G6PD is up regulated by carcinogens and oxidative stress.

Renal function parameters:

Urea/uric acid: The elevation of blood Urea is a good indicator for kidney disorders. Urea is the principal end product of protein catabolism. The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely *et al.*, 1987). Enhanced

protein catabolism and accelerated amino acid deamination for gluconeogenesis is possibly an acceptable postulate to interpret the elevated levels of urea (Bishop *et al.*, 2005). The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely *et al.*, 1987). Uric acid is the end product of the catabolism of tissue nucleic acid, i.e., purine bases metabolism. The increments in uric acid concentrations might be due to degradation of purines or to an increase of Uric acid levels by either overproduction or inability of excretion.

Creatinine: Elevated serum creatinine is indicative of renal injury (Bennett, 1996). Creatinine is the last variable of non-protein nitrogenous blood constituents. It appears in the serum in amounts proportional to the body's muscle mass and is more readily excreted by the kidneys than urea and uric acid (Stryer, 1995). Elevated creatinine concentration is associated with abnormal renal function, especially as it relates to glomerular function (Bishop *et al.*, 2005).

OXIDATIVE STRESS PARAMETERS

Superoxide Dismutase (SOD) and Catalase (CAT): Superoxide Dismutase (SOD) catalyses the dismutation of superoxide anion radicals ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2), whereas catalase degrades H_2O_2 into a molecule of oxygen and water (McCord, 2000; Saravanan *et al.*, 2003). SOD is a ubiquitous chain breaking antioxidant, plays an important role in protection against deleterious effects of lipid peroxidation. It converts the highly reactive superoxide radical to hydrogen peroxide, which in turn is either metabolized by CAT or by glutathione peroxidase (Sankaran *et al.*, 2010). Superoxide ion ($O_2^{\cdot-}$) and hydroxyl radicals are known to cause marked oxidative injuries to surrounding tissues and organs. Therefore removing superoxide ion and hydroxyl radicals is probably one of the most effective defense mechanisms against a variety of diseases. Lowered activities of SOD, CAT and glutathione peroxidase will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function (Dahiru and Obidoa, 2008). The primary roles of Catalase (CAT) is to scavenge H_2O_2 and convert it into H_2O . It plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells (Sankaran *et al.*, 2010). Oxidative stress dependent upon superoxide radical can account for a number of acute and chronic disease states, which include inflammation and ischemia-reperfusion (1995). SOD protects murine peritoneal macrophages from apoptosis induced by adriamycin (Domínguez-Rodríguez *et al.*, 2001). Furthermore over expression of SOD in fibrosarcome

cells protect against apoptosis and promote cell differentiation (Zhao *et al.*, 2001).

The SOD family has 3 members which are Copper-Zinc Superoxide Dismutase (Cu/ZnSOD), Manganese Superoxide Dismutase (MnSOD) and Extracellular Superoxide Dismutase (EC-SOD) and they all play critical roles in scavenging $O_2^{\cdot-}$. Decreased SOD activity results in elevated level of superoxide which in turn leads to decreased Nitric Oxide (NO) but increased peroxynitrite concentrations. The major intracellular SOD is a 32-kD Copper and Zinc containing homodimer (Cu/Zn SOD). The Mitochondrial SOD (MnSOD) is a manganese-containing 93-kD homotetramer that is synthesized in the cytoplasm and translocated to the inner matrix of mitochondria. EC-SOD is the primary extracellular SOD enzyme and is highly expressed in many organs. Increased SOD activity levels are seen in Down's syndrome while decreased activity is seen in diabetes, Alzheimer's disease, rheumatoid arthritis, Parkinson's disease, uremic anemia, atherosclerosis, some cancers and thyroid dysfunction (Giacco and Brownlee, 2010)

Glutathione Peroxidase (GPx): Glutathione Peroxidase (GPx), a selenium-dependent enzyme, is found in cytoplasmic and mitochondrial fractions of cells. GPx acts on Lipid Hydroperoxide (LHP) substrates that are released from membrane phospholipids by phospholipase A_2 (Van Kuijk *et al.*, 1987). It can utilize cholesterol hydroperoxide (Thomas *et al.*, 1990) and hydrolyzes H_2O_2 at low concentrations (Grisham, 1992). The antioxidant enzyme catalyzes the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids, thereby effectively removing toxic peroxides from living cells. It plays the important role of protecting cells from potential damage by free radicals, formed by peroxide decomposition (Ursini *et al.*, 1995). The activity of GPx is coupled to Glutathione Reductase (GSSG-R), which maintains reduced Glutathione (GSH) levels (Bompart *et al.*, 1990). Using Glutathione (GSH) as a reducing reagent, the GPx enzymes catalyze the reduction of H_2O_2 and organic peroxides (R-O-O-H) to water and the corresponding stable alcohol thus inhibiting the formation of free radicals. Enzyme activity can be decreased by negative feedback from excess substrate or from damage by oxidative modification (Tabatabaie and Floyd, 1994). Glutathione peroxidase detoxifies endogenous metabolic peroxides and hydroperoxides that lead to the oxidation of GSH. It has a high potency in scavenging reactive free radicals in response to oxidative stress (Sankaran *et al.*, 2010). Various diseases show different levels of the universally present GPx in all tissues. A reduction in enzyme level is associated with Parkinson's disease (Kunikowska and Jenner, 2003) and in Chronic Glomerulonephritis patients (Zhou *et al.*, 2002). In patients with End-Stage

Renal Disease (ESRD), GPx activity in adult patients was comparable to that in the control groups (children and adults); the GPx in children with ESRD was almost twice as high than in the other groups (Sommerburg *et al.*, 2002). GPx activity decreased significantly in liver and increased in kidney in 3-week-old diabetic rats that showed a reversal with a change in diet (Gotia *et al.*, 2001).

Glutathione reductase: Glutathione reductase is a flavoprotein that is required for the conversion of oxidized Glutathione (GSSG) to reduced Glutathione (GSH). At the same time, it oxidizes Nicotinamide Adenine Dinucleotide Phosphate (NADPH). This universally present enzyme is essential for the maintenance of reduced Glutathione (GSH) levels *in vivo* (Mannervik and Carlberg, 1985). Reduced glutathione plays an important role in oxidoreduction processes and detoxification of H₂O₂ and organic peroxides, which are substances produced in large quantities during inflammatory processes in living cells (Armstrong, 1998). Glutathione reductase therefore plays a major role in Glutathione Peroxidase (GPx) and Glutathione S-Transferase (GST) reactions as an adjunct in the control of peroxides and free radicals (Bompart *et al.*, 1990). When levels of catalase, another universally present antioxidant enzyme, are decreased, the glutathione dependant enzymes become activated (Gastani *et al.*, 1994). A deficiency of glutathione reductase is characterized by hemolysis owing to the increased sensitivity of erythrocyte (RBC) membranes to H₂O₂ that lead to osmotic fragility (Harmening, 1992). This reaction is thus required for the stability and integrity of red cells.

Glutathione-S-Transferase (GST) and reduced Glutathione (GSH): The cytosolic Glutathione-S-Transferase (GST) and GSH plays an important role in the detoxification of many environmental chemicals including mutagens and carcinogens (Sheweita and Tilmisany, 2003). Previous studies have demonstrated that GST reduced the covalent binding of epoxides of well known chemical carcinogens with DNA (Gopalan *et al.*, 1992). In addition, GSH can directly react with and inactivate toxic electrophiles in the diet (Waxman, 1990). Previous studies have shown that consumption of food high in GSH content is associated with about 50% reduction in the risk of oral and pharyngeal cancer (Flagg *et al.*, 1994). Induction of GST has been found to be effective in decreasing the hepato-carcinogenesis caused by carcinogenic compounds (Gopalan *et al.*, 1992). Inducers of GSTs are generally considered as protective compounds against cancer. GST was induced in the stomach by coumarin and α -angelicalactone and in the pancreas by flavone. Several dietary compounds have been demonstrated to reduce gastrointestinal cancer rates in both human and animals. For example,

sulforaphane, indole-3-carbinol, D-limonene and Relafen induced GST levels in small intestine, livers and stomach (Van Lieshout *et al.*, 1998). Aqueous extracts of either green or black tea induced GST and UDP-Glucuronyl transferase after administration to rats as the sole drinking fluid for 4 weeks (Cao *et al.*, 1996; Yang *et al.*, 2002).

Non-enzymatic antioxidants such as reduced Glutathione (GSH) normally counteract damaging effects of intracellular Reactive Oxygen Species (ROS) by either repairing the oxidative damage or directly scavenging oxygen radicals (Dorval and Hontela, 2003). The balance between phase I carcinogen activating enzymes and phase II carcinogen detoxifying enzymes is important in determining the risk of developing chemically-induced cancer (Maliakal *et al.*, 2001)

Malondialdehyde (A lipid peroxidation product):

Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by free radicals. Free radicals react with lipids causing peroxidation, resulting in the release of products such as Malondialdehyde (MDA), Hydrogen Peroxide (H₂O₂) and hydroxyl radicals. An increase in lipid peroxides indicates serious damage to cell membranes, inhibition of several important enzymes, reduced cellular function and cell death (Pompella *et al.*, 1991). Lipid peroxidation generates a complex variety of products, many of which are reactive electrophiles some of which react with protein and DNA and as a result are toxic and mutagenic (Marnett, 1999). Malondialdehyde (MDA) is one of the products of lipid peroxidation that reacts with DNA to produce MDA-DNA adducts, which have been implicated in the induction of G→T transversions and A→G transitions (Benamira *et al.*, 1995). The ability of MDA-DNA adducts to induce frame shift mutations in sequences for genetic instability is emerging as a possible direct link between oxidative stress and human cancers (Valko *et al.*, 2004; Zienolddiny *et al.*, 2000). The level of MDA-DNA adducts is found to be increased in several cancers (Munnia *et al.*, 2006; Bartsch *et al.*, 2002). Malondialdehyde (a by-product of lipid peroxidation) can react with deoxyguanosine in DNA resulting in the formation of cyclic pyrimidopurine N¹ N² malondialdehyde-de-oxyguanosine (M₁dG) adduct. The adduct has the potential to cause mutations that may lead to liver carcinogenesis (Rajinder *et al.*, 2001). MDA, owing to its high cytotoxicity and inhibitory action on protective enzymes is suggested to act as a tumor promoter and a co-carcinogen (Sundaresan and Subramanian, 2003). Although many risk factors have been reported, lipid peroxidation plays an important role in hepatic carcinogenesis. Further, enhanced lipid peroxidation associated with depletion of antioxidants is a characteristic finding in a variety of malignancies (Sundaresan and Subramanian, 2003).

CONCLUSION

Oxidative stress which has been implicated in the induction of cancers by N-nitrosodimethylamine (NDMA) can be monitored looking at some antioxidants status as well as lipid peroxidation products such as Malondialdehyde (MDA). Induction of hepatocellular carcinoma by NDMA can be studied looking at the activity of alpha-fetoprotein (AFP), 5'-Nucleotidase (5'-NT), beta-glucuronidase etc. Also, there should be more awareness by government agencies, NGOs and other other agencies on the various sources of NDMA and the dangers that can arise and also the possible ways of scavenging and detoxifying products of NDMA metabolism.

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