INVITED REVIEW

Role of free radicals and antioxidants in gynecological cancers: current status and future prospects

Lokanatha Valluru[1], Subramanyam Dasari[1], Rajendra Wudayagiri[2]

[1]Department of Biotechnology, Dravidian University, Kuppam; Andhra Pradesh, ***India***
[2]Department of Zoology, Sri Venkateswara University, Tirupati; Andhra Pradesh, ***India***

Received July 15, 2013
Accepted November 10, 2013

Published Online December 18, 2013

DOI 10.5455/oams.201113.rv.011

Corresponding Author
Lokanatha Valluru
Department of Biotechnology,
Dravidian University,
Kuppam-517 426,
Andhra Pradesh, India.
lokanath.valluru@gmail.com

Key Words
Cervical cancer; Endometrial cancer; Free radicals; Reactive species

Abstract
The potential role of free radicals and associated oxidative stress has been well documented in the development of many diseases. Free radicals are mainly derived from oxygen (reactive oxygen species, ROS) and nitrogen (reactive nitrogen species, RNS), under various physicochemical or pathological conditions. Excessive amount of free radicals eventually attack biomolecules including proteins, lipids and DNA; thus result in increased oxidative damage, lead to alter the physiological functions of the cell, play role in the activation of transcription factors, and trigger a number of human diseases including carcinogenesis. Apart from many dietary components, mammalian cells have endowed with protective antioxidant defense system, which includes enzymatic (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and non-enzymatic (glutathione, vitamin E (tocotrienols and tocopherols), vitamin C) antioxidants. The present review describes the role of the free radicals in the development of gynecological cancers and their key factors of the non-specific immune defense mechanism (antioxidants). The review also emphasizes the different potential applications of antioxidant/free radical manipulations in prevention and/or control of cancer. The novel and future approaches for better control of diseases are including gene therapy to produce more antioxidants, genetically engineered plant products with higher level of antioxidants, artificial antioxidant enzymes, novel biomolecules and the use of foods enriched with antioxidants.

INTRODUCTION

In the last decade there has been a growing interest in understanding the role of free radicals in biomedicine. Free radicals are atoms with unpaired electrons such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). In popular scientific/biomedical literature the term 'free radical' is used in a broad sense and also includes related reactive species such as 'excited states' that lead to free radical generation or those species that results from free radical reactions. In general, free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds. Some of the biologically important reactive species are described in Table 1. ROS are molecules that contain oxygen and have higher reactivity than ground state molecular oxygen [1]. The reactive oxygen species includes: the hydroxyl radical (•OH), the most damaging of this chemical species; which also include superoxide anions ($O_2^{\bullet -}$); singlet oxygen ($^1O_2$); and hydrogen peroxide (H_2O_2). ROS can be formed under the aerobic condition not only during oxidative phosphorylation, through the action of mixed function oxidases, and as by-products of normal metabolism by enzymes such as superoxide dismutase (SOD), NADPH oxidase, and xanthine oxidase (XO) in neutrophils, but can also be generated from redox cycling of certain drugs and by radiation [2]. A well known example for RNS is nitric oxide (NO), a short-lived endogenous gas that acts as a signaling molecule in the body. NO was synthesized by nitric oxide synthase (NOS) and produced by almost all mammalian cells. Excessive or unregulated NO synthesis has been implicated as causal or contributor to some pathophysiological conditions including cancer. Expression of NOS has been distinguished in various cancers including cervical, breast, central nervous system, laryngeal, and head and neck cancers [3-7].

Providentially, the mammalian cells have endowed by an antioxidant defense mechanism that allows equilibrium between the generation of oxidants and antioxidants. The interrupted condition in between these two factors develops an oxidative stress, and despite the antioxidant defense mechanism to counteract the reactive species-related deleterious effects, damage to macromolecules occurs as a result of these reactions. Oxidative damage accumulates during the life cycle and lead to different pathological progressions like myocardial infection, atherosclerosis, neurodegenerative disorders, rheumatoid arthritis, and cancer [8].

Globally, among several diseases, cancer has become a big threat to human beings. As per Indian census data, the rate of mortality due to cancer was high and

alarming with about 806,000 existing cases by the end of the last century. Cancer is the second most public disease in India responsible for high mortality with about 0.3 million deaths per year. This is owing to the poor availability of prevention, diagnosis and therapy of the disease. All cancers have been reported in Indian population including the cancers of skin, breast, lungs, rectum, prostate, stomach, cervix, liver, esophagus, bladder, mouth and blood, *etc*. [9].

Gynecological (ovarian, endometrial and cervical) cancers are the most common malignancies among females in many developing countries. It has been noticed that 90,000 new cases of gynecological cancer are reported every year in India [10]. Gynecological cancers represent a great clinical challenge in oncology. Since most cases are asymptomatic until the disease has metastasized, two-thirds are diagnosed with advanced stage. Hence, most of the gynecological cancers have the highest fatality-to-case ratio of all women malignancies [11].

Ovarian carcinoma often is lethal gynecologic malignancy with epithelial neoplasms in adult women. In India, approximately 15% of all gynecological cancers are ovarian malignancy [12] and it represents the greatest clinical challenge. Riley and Behrman [13] stated that the role of ROS and antioxidant enzymes, *i.e.* copper/zinc SOD (Cu/ZnSOD), manganese SOD (MnSOD) and glutathione peroxidase (GSH-Px), in oocyte maturation. It is documented that, oxidative stress play a major role in ovarian function through the intensified lipid peroxidation in the pre-ovulatory Graafian follicle [14]. Paszkowski *et al* [15] also demonstrated that GSH-Px may help in maintaining low levels of hydroperoxides inside follicle, suggesting a significant role of oxidative stress in ovarian function. Oxidative stress and inflammatory process have roles in the pathophysiology of polycystic ovarian disease and drugs such as Rosiglitazone may be effective by decreasing the levels of oxidative stress [16].

Endometrial carcinoma is the next most common subtype of gynecological malignancies representing 15% of cases [17]. Augmented generation of ROS by peritoneal fluid macrophages, with improved lipid peroxidation in patients with endometriosis, has been demonstrated, whereas other researchers have reported contrary findings [18]. It is well established that markers of lipid peroxidation such as diminished peritoneal fluid antioxidants, elevated oxidized lipoproteins and lysophosphatidyl choline provide further evidence of oxidative stress in the peritoneal microenvironment of patients with endometriosis [19]. Increased production of autoantibodies to oxidatively modified lipoproteins that are antigenic has been reported in patients with endometriosis. The investigations of various biomarkers have then revealed presence of oxidative stress locally and systemically in patients with endometriosis [20].

Cervical cancer refers to the epithelial malignancy that arises from the cervix. Cervical cancer is the most common cancer among women worldwide after breast cancer. According to the World Health Organization (WHO) report, globally, cervical cancer comprises 12% of all cancers in women and it is the leading gynecological malignancy in the world. Approximately 20,000 new cases were detected in India [21]. Particularly, in Southern India, carcinoma of the uterine cervix is the most common form of cancer in females [22]. Recently a report says that there are an estimated 132,000 new cases and 74,000 deaths annually in India [23].

Carcinogenesis is a multi-step process leading a cell from normal to pre-cancerous stage and finally to an early stage of cancer [2]. Cancer development is characterized by cumulative action of multiple events occurring in a single cell and can be described as initiation, promotion and progression. Free radicals can acts in all the stages of carcinogenesis [24] (Fig.1). In view of these facts, the present review describes the role of various types of free radicals or reactive species and their defense mechanisms in gynecological cancers. In addition to this, efforts have also been made to predict new-fangled approaches include gene therapy to produce more antioxidants, genetically engineered plant products with increased levels of antioxidants, artificial antioxidant enzymes, biomolecules with antioxidant and the use of functional foods enriched with antioxidants.

FREE RADICAL MEDIATED CARCINO-GENESIS

Development of cancer is a multistage process requiring the cumulative action of multiple events that occur in one cell clone. The role of free radicals in carcinogenesis and their contribution to the initiation and progression of the cancer process is well established [25]. The overall process includes a three stage model:

-first (**initiation**), a permanent change occurs in the genetic material (one somatic cell);

-secondly (**promotion**), the mutated cell clone expants;

-and finally (**progression**), malignant conversion into cancer develops (Fig.1).

ROS can stimulate carcinogenesis by acting at all three stages [26]. There is a complex interplay of cytokines, hormones and other stressors that affects cellular generation of free radicals; these molecules act further through the modulation of many transcription factors and gene expression [19].

 DOI 10.5455/oams.201113.rv.011

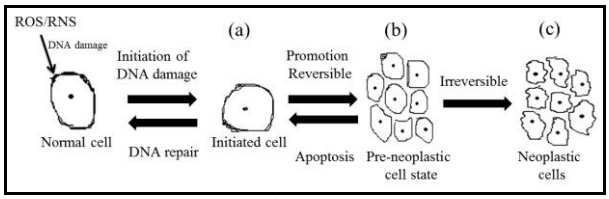

Figure 1. Three-stage model of carcinogenesis: **(a) initiation** (attack by ROS/RNS as carcinogens), accumulation of carcinogenic mutations; **(b)** progresses through pre-neoplastic (reversible) stages by the acquisition of more mutations and up regulated cell signaling (**promotion**); **(c) progression** by tumor promoters and development of angiogenic potential leading to the expression of neoplastic stage which is an irreversible condition.

Initiation step

In the initial step of the cancer development, a permanent change in genetic material of one cell is achieved by DNA mutation. Oxidative DNA damage can occur through hydroxyl radical generated from H_2O_2. Increased oxidative stress, which may in turn deplete the endogenous antioxidant reserves, is an important signal leading to Ca^{2+} mobilization. ROS-mediated Ca^{2+} changes lead to the activation of endonucleases which can cause DNA fragmentation during apoptosis [2].

There is a steady formation of DNA lesions in living cells. Hydroxyl radical and other free radicals attack upon DNA and generate a series of DNA damage by a variety of mechanisms. These include sugars and base modifications, strand breaks and DNA protein cross-links. Modified DNA base (pyrimidine and purine) constitute one of the most lesions which has mutagenic properties being potentially able to damage the genome integrity [27]. One of the most studied lesions generated by the modified DNA bases is the guanine-cytosine → thymine-adenine (G-C → T-A) transversion mutagenesis [28]. Several modified bases which have also been shown to possess miscoding potentials and thus perhaps premutagenic properties. Most of these DNA damages are thought to be prepared mainly by base excision repair [29]. A large body of evidences indicated that a direct correlation between 8-OH-Gua (8-hydroxyguanine) generation and carcinogenesis *in vivo* [30]. Furthermore, the G-C → T-A transversion have been frequently deleted in the tumor suppressor p53 gene and proto-oncogene *ras* either through the inactivation of tumor suppressor gene or the activation of oncogene; thus, ROS-related mutations may lead to the initial step in the development of cancer.

Tumor promotion

The effect of oxidative stress is strongly involved in the promotion of carcinogenesis. A number of tumor promoters are thought to stimulate endogenous oxygen radical production by altering cellular metabolic processes [2]. ROS/RNS can stimulate the expression of mutated cell clones by temporarily modulating the gene related to proliferation or cell death. While an overload from high levels of oxidative stress halts proliferation by cytotoxic effects, low levels can stimulate cell division and promote the growth of tumor cells [31]. Hence, the stimulation of intracellular production of reactive species is considered the main way to promote the free radical mediated tumors [32]. In *in vitro* experiments of Toyokuni et al [33] reported that, the DNA bindings of p53, activator protein (AP)-1 and nuclear factor (NF)-κB are activated in a reductive condition and repressed in an oxidative condition. However certain transcription factors are activated by oxidation while others are repressed by oxidation [34].

Tumor progression

The progression of carcinogenesis comprises the acquisition of malignant properties to the tumor cell. Progression is characterized by accelerated cell proliferation, escape from immune surveillance, tissue invasion and metastasis [34]. The generation of large amounts of free radicals, together with the increase in the level of oxidatively modified DNA bases, may attribute to the ability of some tumors to transform (mutate), inhibit anti-proteases and damage local tissues [35]. Conversely, the increased levels of modified DNA bases may contribute to the genetic instability and metastatic potential of the tumor cells in fully developed cancers cells [36]. However, another study reported that, an intense oxidative stress may kill cells; on the other hand, cancer development does not ensue in response to increased levels of oxidative DNA damage [37]. Hence, it has been suggested that oxidative DNA base damage alone may be insufficient to cause cancer development, or damage over only a certain range is active and excessive damage may have an anti-cancer effect by promoting apoptosis [38].

It is a fact that, the healthy individuals, under the normal conditions produce ROS through their aerobic metabolism. Hence, cells have developed a wide range of antioxidant mechanisms to prevent and inactivate the activity of ROS and then repair cell damage [39]. Under normal health conditions, the unbalance between ROS and antioxidants leads to the development of oxidative stress. This oxidative stress plays a key role in female reproductive tract. Agarwal and Said [39] reported that, ROS have important roles in the normal functioning of female reproductive system and in the pathogenesis of female infertility. ROS can regulate cellular functions and can impair the intracellular environment resulting in diseased cells. The excessive levels of ROS can result in the pathogenesis of female reproduction leading to development carcinogenesis through modifying gene expression. ROS such as $O_2^{\bullet -}$ are able to diffuse through cell membranes and alter cellular molecules such as lipids, proteins and nucleic acids. As a result, multiple consequences like embryo cell block, mitochondrial modifications, ATP

Table 1. Reactive oxygen and nitrogen species involved in Carcinogenesis and their interactions

Reactive Species	Half-life (seconds)	Production	Interaction	Associated cancer
ROS				
Hydroxyl radical (•OH)	10^{-9}	Produced by increased iron concentration in body	Cellular compounds like carbohydrates, nucleic acids, lipids and proteins	Bronchogenic and colorectal carcinoma
Superoxide (O_2•$^-$)	10^{-6}	Generated in mitochondria and cardiovascular system	Inactivates enzymes containing iron-sulfur clusters	Colorectal carcinoma
Hydrogen peroxide (H_2O_2)	Stable	Produced during large number of metabolic reactions	Intercats with lipids, protiens and nucleic acids	*****Hepatocellular carcinoma
Organic hydroperoxide (ROOH)	Stable	Reacts with transient metal ions to yield reactive species	Intercats with lipid peroxidation of poly-unsaturated fatty acids	Melanoma and other skin cancers
Singlet oxygen ($^1O_2$)	10^{-6}	Generated during photosensitization and chemical reaction	Cellular proteins and lipids	*****Ovarian cancer
RNS				
Nitric oxide (NO•)	5	Neurotransmitter and blood pressure regulator	Breakdown and deamination of nucleic acids	Breast cancer, *****Brain cancer
Peroxynitrite ($ONOO^-$)	10^{-3}	Formed from NO• and O_2•$^-$	Activation of cyclooxygenase gene	Breast cancer, Cervical cancer
Peroxynitrous acid (ONOOH)	Fairly stable	Protonated form of $ONOO^-$	Intercats with neurotransmitters	*****Gastric cancer

*****Indicates the indirect role of reactive species in the development of cancer.

diminution and apoptosis take place [40]. ROS also induces lipid peroxidation with related effects in cell division, metabolic transport and mitochondrial dysfunction. Table 1 shows the involvement of ROS and RNS in carcinogenesis and their interactions with different target cells.

FREE RADICALS IN GYNECOLOGICAL CANCERS

Free radicals in ovarian and endometrial cancers

Recent epidemiological studies described that oxidative stress has a causal role in the carcinogenesis of two histological subtypes of ovarian cancer, namely clear cell carcinoma (CCC) and endometrioid adeno-carcinoma (EAC). Because of recurrent hemorrhage in endometrial cysts, excess of ROS are produced due to increased iron ions, which results in direct genome mutation of epithelial cells and exaggeration of oxidative stress by stromal cells. In endometrial associated ovarian cancer, genomic changes in specific genes such as p53, ARID1A, K-ras, PTEN and PI3CA have been reported [41].

Endometrial cysts are well known lesions in endometriosis that contain fluid with excess of ferric iron (Fe^{+3}) because of recurrent hemorrhage in cyst. In 1925, Sampson [42] mentioned the first time on endometriosis associated cancer. The deposition of hemosiderin, heme or iron in endometriotic lesions has been assumed to trigger oxidative damage and chronic inflammation [43]. In particular, intracellular iron activates the NF-kB pathway and exaggerates chronic inflammation [44]. As a result, prominent oxidative stress or an excess of ROS, is consistently produced. This process leads to have a causative role in endometriosis development and progression leading to carcinogenesis [45]. The high concentration of free iron in endometrial cysts may directly provide oxidative stress that induces genomic mutation in epithelial cells [41]. The excess of iron in experimental animals enhances the epithelial cell proliferation [46] and causes malignant tumors with genomic abnormalities [47], which suggest a similar mechanism leading to carcinogenesis in human endometriosis. Hence, there is scientific evidence in the literature that minimal levels of ROS may be necessary for the development of gynecological cancers (Fig.2). However, further studies are awaited to elucidate the precise role of iron-deposition induced oxidative stress in carcinogenesis of endometriosis-associated cancer.

Free radicals and oxidative stress have been inconsistently associated with ovarian cancer risk. The women's health initiative (WHI) study performed on post-menopausal ovarian cancer patients demonstrated that the intake of dietary antioxidants, carotenoids, and vitamin A are not associated with a reduction in ovarian cancer risk [48]. Several risk factors including usage of oral contraceptives, tobacco products may influence disease risk [49]. However, these factors are not modifiable. Experimental evidence suggested that ovarian cancer patients exhibit significantly elevated levels of oxidative stress [50]. Patients diagnosed with ovarian cancers have been shown to have significantly reduced plasma antioxidants [51] and vitamin A concentrations. On the other hand these patients show

 DOI 10.5455/oams.201113.rv.011

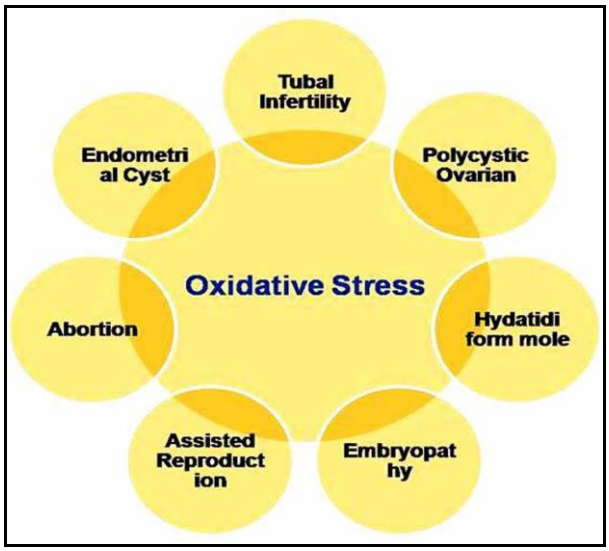

Figure 2. Oxidative stress mediated gynecological disorders in female reproduction. Oxidative stress causes most of the gynecological disorders namely tubal fertility, polycystic ovarian, endometrial cyst and embryopathies which finally lead to the neoplastic condition through the increased risk factors.

increased levels of oxidative stress and induce the free radical mediated carcinogenesis.

A great effort has been made to understand the effects of ROS on DNA damage, induction of mutations and the role of ROS on epigenetics [52]. Most of the signal transduction factors are highly prone to free radical damage resulting in altered function and have been implicated in the activation of transcription factors. Through their ability to stimulate cell proliferation and either positive or negative control of apoptosis, transcription factors can arbitrate many of the physiological and pathological exposures.

Hypoxia-inducible factor (HIF)-1 is a heterodimeric transcription factor that plays an important role in signaling and cellular oxygen levels. HIF-1 has been implicated in ROS-induced carcinogenesis of breast, bladder, colon, ovarian, hepatocellular, pancreatic, prostate and renal cancers [53]. Rankin and Giaccia [54] reported that, elevated HIF-1 expression correlates with poor outcome in patients with nasopharyngeal, colorectal, pancreatic, breast, cervical, endometrial, ovarian, bladder, gastric carcinoma and glioblastoma. Some of the findings highlighted that HIF-1 activation is a common event in cancer. Emerging evidence indicates that ROS produced by mitochondrial complex-III are required for hypoxic activation of HIF-1 [55]. Hence, ROS are considered to be activators of HIF-I in hypoxic tumors.

Free radicals in cervical cancer

A large number of evidence indicates that infection with the sexually acquired human papillomavirus (HPV) is the primary risk factor for cervical cancer and plays a key role in cervical carcinogenesis [56]. Few prospective studies suggests that HPV infection alone may not be sufficient to promote cervical carcinogenesis and a number of co-factors such as smoking, oral contraceptives, deficiency in antioxidants and inflammation are involved in the development of cancer [57].

Risk factors like cigarette smoking [58] co-infection with bacteria (*Chlamydia trachomatis*) [57] and cervical inflammation [59] are associated with elevated load of oxidative stress and have been shown to be independently associated with either HPV persistence or grade 2/3 cervical intraepithelial neoplasia (CIN II/III) and cancer. Oxidative stress is implicated in the pathogenesis of cancer; generally two mechanisms contribute to an increase in oxidant load, either excessive generation of ROS or inadequate antioxidant defense [60]. ROS appear to have a key role in cell signaling by activating AP-1 and NF-kB (transcription factors), cell proliferation and apoptosis [61] (Fig.3). These findings are particularly relevant to cervical carcinogenesis when viral replication, expression of HPV-16 E6 and E7 proteins, cell proliferation and apoptosis are important events in cervical carcinogenesis. Using *in vitro* and *in vivo* models, scientists have demonstrated that ROS increases the viral titer [62] and infectivity of the influenza virus. In *in vitro* studies, increases in the cellular oxidative load have been shown to increase the human immunodeficiency virus (HIV) replication [63].

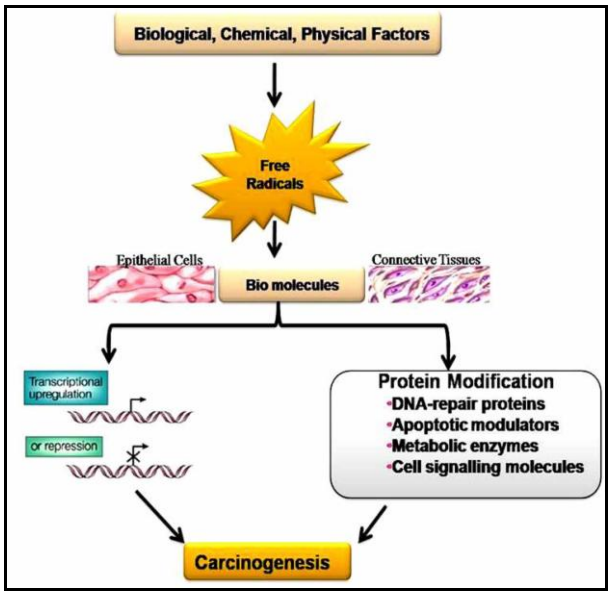

Figure 3: Oxidative stress mediated cancer development in gynecological tissues. Biological, chemical and physical factors mediated free radicals, damages the biomolecules of gynecological tissues/cells (epithelial and connective tissues). The damaged biomolecules initiates the neoplastic cells through the up-regulation of transcriptional factors and inactivation of tumor suppressor genes. The damaged biomolecules also alters the functions of DNA repair proteins, apoptic modulators, metabolic enzymes and signaling pathways induces the neoplastic condition.

AP-1 is a central transcription factor for the expression of oncoproteins E6 and E7 of the oncogenic HPV which can be activated by ROS. It is believed that this effect is to be due to the ROS-activated NF-kB, as a nuclear transcriptional factor that is required for the replication of HIV [63]. It is well documented that the NF-kB has a functional binding site in the HPV upstream regulatory region (URR) and its effect on HPV gene expression is currently being investigated. Previous findings suggest that inhibition of NF-kB will up-regulate HPV gene expression and, *vice versa*, activation of NF-kB will down-regulate expression [64]. One of the recent study also reported that the levels of ROS and expression of NF-κB and p53 were higher in nasopharyngeal carcinoma tissue than those in normal nasopharyngeal tissue [65]. Wang *et al* [66] reported that 25.4% of cervical cancers, 48.4% of endometrial cancers, 21.9% of ovarian cancers and 29.4% of breast cancers shows that one or more mitochondrial microsatellite instability (mtMSI), which was frequently detected in the D-loop region but rarely occurred in the coding region [67] reported that the mutation in the D-loop takes part in carcinogenesis and progression of cervical cancer through the effect of increased ROS. Wei *et al* [68] reported that, NO acts as a molecular co-factor with HPV infection in cervical carcinogenesis. In another study, Wei *et al* [69] also found that the presence of HR-HPV is associated with an increased release of NO in the human uterine cervix and that physiological dose of NO could endorse malignant progression of HPV-infected cells *in vivo*. They also stated that, various epidemiologically defined co-factors for cervical cancer increase NO levels in the cervical microenvironment of cervix [68]. This increase in NO induces earlier mRNA expression, declined retinoblastoma protein (pRb) and p53 levels, low p53 activity, and apoptotic indices in HPV-infected cells in the cervix, consequently resulting in increased survival of mutant cells and leading to carcinogenesis.

Epidemiological studies have revealed a number of risk factors like smoking, multiparity, long-time usage of oral contraceptives pills, chronic inflammation and other sexually transmitted infections (*e.g.*, *Chlamydia trachomatis* and herpes simplex virus (HSV) type 2) [70]. Interestingly, all these co-factors increase the NO levels in the microenvironment of uterine cervix [71]. Significantly increased levels of NO were observed in serum of patients with cervical cancer as compared to healthy controls [72]. Increased NO levels and markers of NO-mediated mutagenesis have been observed in the cervixes of women with CIN [73]. All these findings suggested that NO has potential mutagenic and carcinogenic activity in cervical cancer.

Antioxidants against to free radicals

Nature has endowed each biological cell with adequate protective mechanism against any harmful free radicals. In the 19th and early 20th century the term antioxidant has been referred specifically to a chemical that prevents the consumption of molecular oxygen. Literally the term antioxidant defines the 'against to the oxygen' or so called antioxygen. A well-established research has been developed on the antioxidants which protect cells against the damaging effects of ROS, such as singlet oxygen, peroxyl radicals, superoxide and hydroxyl radicals, and RNS, such as peroxynitrite and nitric oxide [74]. There are two types of antioxidants: enzymatic and non-enzymatic: enzymatic antioxidants are also known as natural antioxidants, act by neutralizing excessive reactive oxygen species, and prevent it from damaging the cellular structure. They are composed of catalase (CAT), glutathione redutase (GR), GSH-Px and SOD, which causes reduction of hydrogen peroxide to water and alcohol [39]. Superoxide dismutase and glutathione peroxidase are natural antioxidants present in organisms which eliminate some ROS and glutathione peroxidase catalyzes the reduction of peroxide by oxidizing glutathione (GSH) to oxidized glutathione [74]. Non-enzymatic antioxidants are synthetic antioxidants or dietary supplements [39].

The body's antioxidant system is influenced by dietary intake of antioxidant, vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurin, hypotaurin, glutathione (GSH), beta carotene and carotene [75] Vitamins C and E are not produced in the body but must be obtained through diet. GSH is produced by the body, but levels of this antioxidant decline with age [76]. Vitamin E has been considered as a natural antioxidant that reacts with soluble free radicals in lipids membranes which prevents the process of lipid peroxidation, as antioxidants reduce agents that disrupt the oxidative chain reactions, often by scavenging ROS before they can cause damage to the cells [77]. This vitamin protects lipids from peroxidation, while being oxidized to tocopheryl quinone or into tocopheroxyl free radical. In both cases, it is reduced by ascorbate (vitamin C), which is afterwards oxidized into dehydroascorbate or α-ascorbate free radical.

Either enzymatic or non-enzymatic antioxidant mechanisms are rapidly attacking the radical and thereby terminate its damaging pathways. This mechanism presumes that the resulting antioxidant derived radical is a 'harmless' one, *i.e.* the reactivity of the antioxidant radical toward typical biomolecules must be low [78].

ANTIOXIDANT ENZYME LEVELS IN CANCER

There is large epidemiological evidence that have shown inverse correlation between the levels of established antioxidants/phytonutrients present in

 DOI 10.5455/oams.201113.rv.011

tissue/blood samples and occurrence of cancer. However, some recent meta-analysis shows that supplementation with single antioxidants may not be that effective [79]. Based on the majority of epidemiological and case control studies recommendations were made for the daily dietary intake of some established antioxidants like vitamin E and C. Antioxidants may act *in vivo* to decrease oxidative damage to DNA, protein and lipids; thus, drop the cancer risk [80]. These include SOD that catalyses the dismutation of superoxide to H_2O_2, and CAT that breaks H_2O_2 down to water [81].

How antioxidant mediated treatment could protect normal cells against damage from treatment, while often increasing their cytotoxic effect against cancer cells?

There are two concepts which explain this question; one is the recent evidence that radiation and chemotherapy often harm DNA to a relatively small extent, which causes the cells to undergo the process of apoptosis, rather than necrosis [82]. Hence, many antioxidant treatments induce apoptotic pathways [83]; the potential exists for a synergistic effect with radiation or chemotherapy with antioxidants.

A second concept is that the defensive mechanisms of many cancer cells are known to be decreased. This apparently makes tumor cells unable to use the extra antioxidants in a repair capacity; this has been illustrated *in vitro* [83]. The cellular changes would ideally, enhance tumor cell killing, largely by apoptosis, and reduce the probability of normal cell death. Antioxidant enzymes and detoxifiers have the ability to inhibit tumor initiation and promotion *in vivo* and *in vitro* [1]. Other studies in association with antioxidant status in human cervical carcinoma showed a significant reduction in the content of GSH, vitamin E and C, GSH-Px and SOD when compared to normal controls. The reduction was more marked in late stages (II, IV) than in early stages (I, II) [26]. However, at early stages, the activities of antioxidant defense enzymes SOD and CAT in Jamaican women with cervical cancer, showed no substantial changes in the GSH and SOD levels in patients compared to that of controls (normal healthy women). On the contrary, CAT activity was significantly higher in patients than that of the controls [2]. Dasari *et al* [84] also reported similar results indicating elevated lipid peroxidation and impaired antioxidant status in cervical cancer patients.

The increased activity of SOD in some tumor cells is not a characteristic of all tumors [85]. Thus, MnSOD is reduced in a variety of tumor cells and the lowest activity of total SOD (Cu/ZnSOD and MnSOD) has been associated with fastest growing tumor [86]. MnSOD constitutes an enzyme with variable activity in tumors. A significant overexpression of MnSOD has been found in colorectal and gastric adenocarcinoma. Similarly, other studies have revealed a significant increase of MnSOD mRNA in both esophageal and gastric cancers, compared to normal tissue [87]. It is well established that the individual variability of SODs due to polymorphisms may predispose to carcinogenesis. MnSOD polymorphisms have been investigated in several types of malignancies namely lung cancer, mesothelioma, breast cancer and colon carcinoma. The primary MnSOD polymorphism studied is the presence of valine or alanine (Val/Ala) at position 16 in the MnSOD-targeting sequence for the mitochondria [88]. Therefore MnSOD polymorphism seems to be the main role in the abnormal expression of SOD in different cancers.

Several investigations have reported the preventive role of selenium against cancer in a variety of organs and species. In fact, regarding the association between low selenium level and advanced tumor disease, it needs yet to be decided whether this phenomenon is more likely to be a consequence or a causative factor for development and course of the disease [89]. Subramanyam *et al* [90] also reported that, the antioxidant activity was directly proportional to the levels of serum selenium, which indicates that selenium is one of the key components of the antioxidative mechanism. Selenium exerts its chemopreventive defense mechanism against oxidative damage by scavenging the ROS and improves the synthesis of enzymatic antioxidant GSH-Px [91]. Decreased levels of reduced/oxidized glutathione (GSH/GSSG) ratio in blood were observed in patients with breast and colon cancers. These decreased levels of GSSG were especially recorded in advanced stages of cancer progression. This is may be due to the increased peroxide generation, which leads to an affectation in the GSH-related enzymes, and an increased GSSG release from different tissues within the red blood cells [92]. In fact, these high GSH and peroxide levels in the cells have been reported when a substantial proliferative activity exists. On the other hand, this antioxidant content decreases when cell proliferation and the rate of protein synthesis in the tumor decreases [93].

Antioxidants in cancer therapy

Antioxidants enzymes prevent cellular damage by reacting with and eliminating oxidizing free radicals. Usage of antioxidant in cancer treatment is a rapidly developing area, because they have been extensively studied for their ability to prevent cancer in humans [94]. However, in cancer treatment, a mode of action of certain chemotherapeutic agents involves the generation of free radicals to cause cellular damage and necrosis of tumor cells. Hence, a concern has logically

developed as to whether exogenous antioxidant compounds taken concurrently during chemotherapy could reduce the beneficial effect of chemotherapy on tumor cells. The importance of this concern is underlined by a recent study which estimates 23% of cancer patients take antioxidants [95]. The primary focus of either chemo- or radiotherapy is to produce irreversible DNA damage in tumor cells that will prevent their replication and lead to their demise [96]. Cancer patients with antioxidant supplementation can alleviate the toxicity and improve long-term outcome. The modulating effects of antioxidants in treatment depend on a wide range of factors, including the metabolic state of the patient, the stage and site of the disease, and the modality being used. Another course of action is to alter cellular homeostasis and modify signal transduction pathways and disposition to apoptosis.

Antioxidants in chemotherapy

Anticancer or chemotherapeutic drugs work by affecting DNA synthesis; they do not kill resting cells unless those cells divide soon after exposure to the drug. Therefore, the efficacy of anticancer drugs used in chemotherapy is limited by the fraction of actively dividing cells. Most anticancer drugs do not rely on ROS, although a few produce free radicals that play a role in treatment; these include bleomycin, doxorubicin (adriamycin) and cisplatin. Although bleomycin is more toxic to oxygenated cells, similar to x- and γ-rays, doxorubicin is preferentially toxic to hypoxic cells [96]. Antioxidant protection of normal cells in all treatments, even when the mechanism of the chemotherapeutic drug is independent of free radical action, help to maintain the health of normal tissues and protect them from the toxic effects of free radical-producing cytokines that circulate in cancer patients and increase with the severity of the disease [97]. Gautam *et al* [98] reported that, decreased antioxidants were increased in ovarian cancer patients after 6 weeks of chemotherapy treatment.

Antioxidants in radiotherapy

Radiotherapy uses ionizing radiation (x- and γ-rays) to induce cancer cell death through free radical formation. There are two mechanisms; the first mechanism is apoptosis which results in cell death within a few hours of radiation, and the second one is radiation-induced failure of mitosis and the inhibition of cellular proliferation which kills cancer cells. The principal target of radiation is considered to be cellular DNA. However, experimental studies indicate the signals for apoptosis can be generated by the effect of radiation on cell membranes through lipid peroxidation. This indicates an alternate mechanism to the hypothesis that DNA damage is required for cell death [99]. About two thirds of x- and γ-ray damage is caused by free radicals that kill tumor cells but threaten the integrity and survival of surrounding normal cells. Radiation induces mitotic cell death in dividing cells and activates pathways that lead to death by apoptosis in interphase cells and differentiated cells. Response to radiation depends on the type, dosage and time intervals of radiation, inherent tissue sensitivity, and intracellular factors that include position in the cell cycle, concentration of oxygen, thiols and other antioxidants [100].

Combinations of antioxidants

Combinations of antioxidants have been shown synergistic anti-tumor effects *in vivo*. Combinations of antioxidants with chemotherapy and radiation have been shown to increase survival time and reduce toxicity in humans. Whelan *et al* [101] reported that co-administration of beta carotene and alpha-tocopherol led to much greater tumor regression than either agent alone. During the treatment of radiation, the cancer infected epithelial cells were shrinkage and tumor size was reduced. Hence, the radiotherapy along with chemotherapy kills and decreases the size of cancer cells which facilitate the significant alterations (increased) in the development of antioxidant system, which is not possible in case of chemotherapy alone. Dasari *et al* [84] reported that significant upsurge was observed in antioxidant levels between the patients treated with radiotherapy and chemotherapy than the patients treated with chemotherapy alone. Hence, the combinational treatment of radiation with chemotherapy in cervical cancer causes sensitization to antioxidants. An open trial of combination antioxidant treatment along with chemotherapy and radiation in patients with small-cell lung cancer had encouraging results with greater two-year survival rate than that of patients received normal treatment [99].

NOVEL APPROACHES TO REDUCE FREE RADICAL DAMAGE AND FUTURE PROSPECTS

Several new approaches were developed for the study of free radicals/antioxidants for the improvement of human health. A number of physiological and genetical changes occur in cancer patients, even in the absence of degenerative conditions. Recent studies have found associations between the decline of free radicals and lower status of antioxidants.

The experimental, clinical, and epidemiological studies support the notion that consumption of foods obtaining high levels of dietary antioxidants (Table 2), in addition to exerting several health benefits, may reduce the risk of free radical mediated cancer or other diseases. Different naturally derived SOD mimetics are well suited for a drug because of having much lower molecular weight, being more stable, and seeming not to elicit an immune response in the body. SOD

 DOI 10.5455/oams.201113.rv.011

Table 2. Dietary antioxidants and their interactions

	Antioxidants	Mode of action on cancers
Chemical drugs as antioxidant supplements		
1	Vitamin C	Regenerates active α-tocopherol (vitamin E) by reducing its radical form
2	Vitamin E	Transport and storage depend on selenium; absorption is reduced when vitamin A and β-carotene levels are high – gynecological cancers
3	β-carotene	Conversion to vitamin A requires vitamin E
4	Selenium	Synergistic with vitamin E – gynecological cancers
Dietary antioxidant supplements (vegetables and fruits)		
5	Carrots, green vegetables	Carotenoids - breast cancer
6	Cruciferous, vegetables, yellow vegetables and tomatoes	Rich in lycopene – prostate cancer
7	Allium vegetables (garlic, onions)	High levels of antioxidant organosulfur compounds – gastrointestinal cancer
8	Green tea	Rich in polyphenols – breast, ovarian and cervical cancers
9	Apples	Quercetin, an antioxidant abundant in apples (equals to vitamin C)
10	Apricots	The high β-carotene content
11	Banana	Vitamins B and C
12	Mustard apple	Antioxidants – reduce the side effects of chemotherapy

Extracted from Borek [96].

mimetics also have ability to increase antitumor effects of interleukins, besides being efficient radioprotectors [8]. Development of genetically engineered plants, to yield vegetables with higher level of compounds is another approach to increase antioxidant availability. Tomatoes with up to three times lycopene concentration as well as with longer shelf life were developed. 'Orange cauliflower' is found to be rich in carotene. Intake of fruits and vegetables with ORAC (oxygen radical absorbance capacity) values between 3000 and 5000 per day is recommended to have significant impact of the beneficial effect of antioxidants [102].

One of the major applications of nanotechnology in biomedicine was the production of bioactive nanoparticles. Nanoparticles can be engineered as nanoplatforms for effective and targeted delivery of drugs and imaging labels by overcoming the many biological, biophysical, and biomedical barriers [103]. To avoid problems of cancer chemotherapy and increase the absorption of drugs in the exact target sites, nanotechnological targeted cancer chemotherapy has been proposed. Nanotechnological based system includes nanoparticles, nanofibers, nanocapsules, nanorods, nanocrystals, nanotubes, stealth nanoparticles, liposomes, stealth liposomes, pH and temperature sensitive liposomes, *etc*. All such delivery materials implies selective and effective localization of pharmacological active moiety at pre-identified (*e.g.* over expressed receptors in cancer) targets in therapeutic concentration while restricting its access to non-target sites, thus, reduces toxicity, maximizes therapeutic index as well as improves the biodistribution of the drug, which is a major factor in success of cancer chemotherapy [104].

CONCLUSION

Free radicals have been implicated in the etiology of large number of cancers, especially gynecological cancers. They can adversely alter many crucial biological molecules leading to loss of structure and function. Such undesirable changes in the women reproductive system can lead to conditions through ROS or RNS, known as gynecological cancers. Antioxidants can protect against the damage induced by free radicals at various levels. Either enzymatic or non-enzymatic antioxidants act as scavengers against free radical induced damage. Excessive intake of foods with functional attributes including high level of antioxidants is one strategy that is gaining importance in advanced countries and is making its appearance in our country (India). It is important to note that the traditional Indian diet and medicinal plants are rich bases of natural antioxidants. Harmonized research involving scientists, nutritionists and physicians can make significant variance to human health in the coming decades. Investigation on free radicals and antioxidants is one such effort in the right direction.

ACKNOWLEDGEMENTS:

The second author (SD) is thankful to the University Grants Commission (UGC), New Delhi for providing the Basic Scientific Research (BRS)-Non-SAP (Special Assistance Programme) fellowship.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

1. Oberley TD, Oberley LW. Antioxidant enzyme levels in cancer. Histol Histopathol 1997; 12:525-35.

2. Trueba GP, Sanchez GM, Giuliani A. Oxygen free radical and antioxidant defense mechanism in cancer. Front Biosci 2004; 9:2029-44.

3. Taysi S, Uslu C, Akcay F, Sutbeyaz MY. MDA and nitric oxide in the plasma of patients with advanced laryngeal cancer. Surg Today 2003; 33:651-4.

4. Reveneau S, Arnould L, Jolimoy G, Hilpert S, Lejeune P, Saint-Giorgio V, Belichard C, Jeannin JF. Nitric oxide synthase in human breast cancer is associated with tumor grade, proliferation rate, and expression of progesterone receptor. Lab Invest 1999; 79:1215-25.

5. Cobbs CS, Brenman JE, Aldape KD, Bredt DS, Israel MA. Expression of nitric oxide synthase in human central nervous system tumors. Cancer Res 1995; 55:727-30.

6. Rosbe KW, Weissler MC, Prazma J, Pertrusz P, Mims JW, Ball SS. Immunohistochemical characterization of nitric oxide synthase activity in squamous cell carcinoma of the head and neck. Otolaryngol Head Neck Surg 1995; 113:P75.

7. Thomson LL, Lawton FG, Knowles RG, Beesley JE, Riveros-Moreno V, Moncada S. Nitric oxide synthase activity in human gynecological cancer. Cancer Res 1994; 54:1352-4.

8. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India 2004; 52:794-804.

9. Ali I, Wani WA, Saleem K. Cancer scenario in India with future perspectives. Cancer Ther 2011; 8:56-70.

10. Notani PN. Global variation in cancer incidence and mortality. Curr Sci 2001; 81:465-74.

11. Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol 2000; 19:3-10.

12. Chhabra S, Sonak M, Prem V, Sharma S. Gynaecological malignancies in a rural institute in India. J Obstet Gynaecol 2002; 22:426-9.

13. Riley JC, Behrman HR. Oxygen radicals and reactive oxygen species in reproduction. Proc Soc Exp Biol Med 1991; 198:781–791.

14. Jozwik M, Wolczynski S, Szamatowicz M. Oxidative stress markers in pre-ovulatory follicular fluid in humans. Mol Hum Reprod 1999; 5:409–413.

15. Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependent glutathione peroxidase activity in human follicular fluid. Clin Chim Acta 1995; 236:173-80.

16. Yilmaz M, Bukan N, Ayvaz G, Karakoç A, Toruner F, Cakir N, Arslan M. The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. Hum Reprod 2005; 20:3333-40.

17. Barda G, Menczer J, Chetrit A, Lubin F, Beck D, Piura B, Glezerman M, Modan B, Sadetzki S. Comparison between primary peritoneal and epithelial ovarian carcinoma A population-based study. Am J Obstet Gynecol 2004; 190:1039-45.

18. Halme J, Becker S, Hammond MG, Raj MH, Raj S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 1983; 145:333-7.

19. Ashok A, Sajal G, Suresh S. The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol 2006; 18:325-32.

20. Murphy AA, Palinski W, Morales AJ, Parthasarathy S. Evidence for oxidatively modified lipid-protein complexes in endometrium and endometriosis. Fertil Steril 1998; 69:1092-4.

21. Senapathy JG, Umadevi P, Kannika PS.. The present scenario of cervical cancer control and HPV epidemiology in India: an outline. Asian Pacific J Cancer Prev 2011; 12:1107-1115.

22. Shanta V, Krishnamurthi S, Gajalakshmi CK, Swaminathan R, Ravichandran K. Epidemiology of cancer of the cervix: global and national perspective. J Indian Med Assoc 2000; 98:49-52.

23. Peedicayil A, Abraham P, Sathish N, John S, Shah K, Sridharan G, Gravitt P. Human papillomavirus genotypes associated with cervical neoplasia in India. Int J Gynecol Cancer 2006; 16:1591-5.

24. Klauning JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004; 44:239-67.

25. Cook JA, Gius D, Wink DA, Krishna MC, Russo A, Mitchell JB. Oxidative stress, redox, and the tumor microenvironment. Semin Radiat Oncol 2004; 14:259-66.

26. Ahmed MI, Fayed ST, Hossein H, Tash FM. Lipid peroxidation and antioxidant status in human cervical carcinoma. Dis Markers 1999; 15:283-91.

27. Wallace SS. Enzymatic processing of radiation-induced free radical damage in DNA. Radiat Res 1998; 150:S60-79.

28. Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. Trends Genet 1993; 9:246-9.

29. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 2002; 32:1102-15.

30. Feig DI, Reid TM, Loeb LA. Reactive oxygen species in tumorigenesis. Cancer Res 1994; 54:1890-4s.

31. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 1995; 18:775-94.

32. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer 1996; 324:30-8.

33. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett 1995; 358:1-3.

34. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 2003; 17:1195-214

35. Malins DC, Polissar NL, Gunselman SJ. Progression of human breast cancer to the metastatic state is linked to hydroxyl radical-induced DNA damage. Proc Natl Acad Sci USA 1996; 93:2557-63.

36. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. Cancer Res 2001; 61:4756-60.

37. Thomas E, Brewster DH, Black RJ, Macfarlane GJ. Risk of malignancy among patients with rheumatoid conditions. Int J Cancer 2000; 88:497-502.

38. Halliwell B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radic Biol Med 2002; 32:968-74.

39. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. BJU Int 2005; 95:503-7.

40. Dennery PA. Role of redox in fetal development and neonatal diseases. Antioxid Redox Signal 2004; 6:147-53.

 DOI 10.5455/oams.201113.rv.011

41. Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, Fujii S. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Res 2008; 14:32-40.

42. Sampson JA. Endometrial carcinoma of the ovary, arising in endometrial tissue in that organ. Arch Surg 1925; 10:1-72.

43. Van Langendonckt A, Casanas-Roux F, Eggermont J, Donnez J. Characterization of iron deposition in endometriotic lesions induced in the nude mouse model. Hum Reprod 2004; 19:1265-71.

44. Lousse JC, Defrere S, Van Langendonckt A, Gras J, Gonzalez-Ramos R, Colette S. Donnez J. Iron storage is significantly increased in peritoneal macrophages of endometriosis patients and correlates with iron overload in peritoneal fluid. Fertil Steril 2009; 91:1668-75.

45. Ngo C, Chereau C, Nicco C, Weill B, Chapron C. Batteux F. Reactive oxygen species controls endometriosis progression. Am J Pathol 2009; 175:225-34.

46. Defrere S, Van Langendonckt A, Vaesen S, Jouret M, Ramos R G, Gonzalez D, Donnez J. Iron overload enhances epithelial cell proliferation in endometriotic lesions induced in a murine model. Hum Reprod 2006; 21:2810-6.

47. Hu Q, Akatsuka S, YamashitaY, Ohara H, Nagai H, Okazaki Y, Takahashi T, Toyokuni S. Homozygous deletion of CDKN2A/2B is a hallmark of iron-induced high-grade rat mesothelioma. Lab Invest 2010; 90:360-73.

48. Thomson CA, Neuhouser ML, Shikany JM, Caan BJ, Monk BJ, Mossavar-Rahmani Y, Sarto G, Parker LM, Modugno F, Anderson GL. The role of antioxidants and vitamin A in ovarian cancer: results from the women's health initiative. Nutr Cancer 2008; 60:710-9.

49. Riman T, Nilsson S, Persson IR. Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies. Acta Obstet Gynecol Scand 2004; 83:783-95.

50. Senthil K, Aranganathan S, Nalini N. Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta 2004; 339:27-32.

51. Schweigert FJ, Raila J, Sehouli J, Buscher U. Accumulation of selected carotenoids, alpha-tocopherol and retinol in human ovarian carcinoma ascitic fluid. Ann Nutr Metab 2004; 48:241-5.

52. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 2004; 567:1-61.

53. Talks KL, Turley H, Gatter, KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157:411-21.

54. Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ 2008; 15:678-85.

55. Klimova T, Chandel NS. Mitochondrial complex III regulates hypoxic activation of HIF. Cell Death Differ 2008; 15:660-6.

56. Giuliano A. Cervical carcinogenesis: the role of co-factors and generation of reactive oxygen species. Salud Publica Mex 2003; 45:S354-60.

57. Castel PE, Giuliano AR. Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients--assessing their roles as human papillomavirus cofactors. J Natl Cancer Inst Monogr 2003; 31:29-34.

58. Sedjo RL, Papenfuss MR, Craft NE, Giuliano AR. Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). Cancer Causes Control 2003; 14:319-26.

59. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, Sherman ME, Burk RD, Rodriguez AC, Alfaro M, Hutchinson ML, Morales J, Schiffman M. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomark Prev 2001; 10:1021-7.

60. Onorato JM, Thorpe SR, Baynes JW. Immunohistochemical and ELISA assays for biomarkers of oxidative stress in aging and disease. Ann NY Acad Sci 1998; 854:277-90.

61. Palmer HJ, Paulson KE. Reactive oxygen species and antioxidants in signal transduction and gene expression. Nutr Rev 1997; 55:353-61.

62. Peterhans E. Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation. J Nutr 1997; 127:962-5S.

63. Pace GW, Leaf CD. The role of oxidative stress in HIV disease. Free Radic Biol Med 1995; 19:523-8.

64. Hiscott J, Nguyen TL, Arguello M, Nakaei P, Paz S. Manipulation of the nuclear factor- kB pathway and the innate immune response by viruses. Ocogene 2006; 25:6844-67.

65. Rofi'i A, Fatchiyah F, Rahayu P, Muhyi R, Sumitro SB. Reactive oxygen species, NF-kB, and p53 levels in tissue of undifferentiated nasopharyngeal carcinoma. Oxid Antioxid Med Sci 2013; 2:143-7.

66. Wang Y, Liu VW, Tsang PC, Chiu PM, Cheung AN, Khoo US, Nagley P, Ngan HY. Microsatellite in stability in mitochondrial genome of common female cancers. Int J Gynecol Cancer 2006; 16:259-66.

67. Zhao Y, Wang J, Chen X, Wu Q. Significance of mutation in the D-loop region in cervical cancer. West Indian Med J 2010; 59:291-4.

68. Wei L, Gravitt PE, Song H, Maldonado AM, Ozbun MA. Nitric oxide induces early viral transcription coincident with increased DNA damage and mutation rates in human papillomavirus-infected cells. Cancer Res 2009; 69:4878-84.

69. Wei XM, Wang Q, Gao SJ, Sui L. Relationship between nitric oxide in cervical microenvironment and different HPV types and effect on cervical cancer cells. Zhonghua Fu Chan Ke Za Zhi 2011; 46:260-5.

70. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002; 2:342-50.

71. Chang K, Lubo Zhang. Review article: steroid hormones and uterine vascular adaptation to pregnancy. Reprod Sci 2008; 15:336-48.

72. Naidu MSK, Suryakar AN, Swami SC, Katkam RV, Kumbar KM. Oxidative stress and antioxidant status in cervical cancer patients. Indian J Clin Biochem 2007; 22:140-4.

73. Tavares-Murta BM, de Resende AD, Cunha FQ, Murta EF. Local profile of cytokines and nitric oxide in patients with bacterial vaginosis and cervical intraepithelial neoplasia. Eur J Obstet Gynecol Reprod Biol 2008; 138:93-9.

74. da Silva FM, Marques A, Chaveiro A. Reactive oxygen species: a double-edged sword in reproduction. Open Vet Sci J 2010; 4:127-33.

75. Pierce JD, Cackler AB, Arnett MG. Why should you care about free radicals? RN 2004; 67:38-42.

76. Packer L, Colman C. The Antioxidant Miracle. John Wiley & Sons, New York, NY, 1999.

77. Wolf G. The discovery of the antioxidant function of vitamin E: the contribution of Henry A. Mattill. J Nutr 2005; 135:363-6.

78. Kirsch M, De Groot H. NAD(P)H, a directly operating antioxidant? FASEB J 2001; 15:1569-74.

79. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. Lancet 2003; 361:2017-23.

80. Sierens J, Hartley JA, Campbell MJ, Leathem AJ, Woodside JV. Effect of phytoesterogen and antioxidant supplementation on oxidative DNA damage assessed using the commet assay. Mutat Res 2001; 485:169-76.

81. Sies H. Antioxidants in Disease, Mechanisms and Therapy, Academic Press, New York, NY, pp 165-170, 1996.

82. Schmitt CA, Lowe SW. Apoptosis and therapy. J Pathol 1999; 187:127-37.

83. Mediavilla MD, Cos S, Sanchez-Barcelo EJ. Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. Life Sci 1999; 65:415-20.

84. Dasari S, Wudayagiri R, Valluru L. Efficacy of treatment on antioxidant status in cervical cancer patients: a case control study. Free Radic Antioxid 2013; 3:87-92.

85. Kong Q, Lillehei KO. Antioxidant inhibitors for cancer therapy. Med Hypotheses; 1998; 51:405-9.

86. Bertrand JS. The molecular biology of cancer. Mol Aspects Med 2000; 21:167-223

87. Izutani R, Asano S, Imano M. Expression of manganese superoxide dismutase in esophageal and gastric cancers. J Gastroenterol 1998; 33:816-22.

88. Vuokko LK, James DC Superoxide dismutases in malignant cells and human tumors. Free Radic Biol Med 2004; 36:718-44.

89. El-Bayoumi K, Narayanan BA, Desi DH, Narayanan NK, Pittman B, Amin SG, Schwartz J, Nixon DW. Elucidation of molecular targets of mammary cancer chemoprevention in the rats by organoselenium compounds using cDNA microarray. Carcinogenesis 2003; 24:1505-14.

90. Subramanyam D, Subbaiah KV, Rajendra W, Lokanatha V. Serum selenium concentration and antioxidant activity in cervical cancer patients before and after treatment. Exp Oncol 2013; 35:97-100.

91. Patrick L. Selenium biochemistry and cancer: a review of the literature. Altern Med Rev 2004; 9:239-58.

92. Carretero J, Obrador E, Anasagasti MJ, Martin JJ, Vidal-Vanaclocha F, Estrela JM. Growth-associated changes in glutathione content correlate with liver metastatic activity of B 16 melanoma cells. Clin Exp Metastasis 1999; 17:567-74.

93. Navarro J, Obrador E, Carretero J, Petschen I, Avino J, Perez P, Estrela JM. Changes on glutathione status and the antioxidant system in blood and in cancer cells associate wiyh tumour growth in vivo. Free Radic Biol Med 1999; 26:410-8.

94. Singh DK, Lippman SM. Cancer chemoprevention part 1: retinoids and carotenoids and other classic antioxidants. Oncology 1998; 12:1643-60.

95. VandeCreek L, Rogers E, Lester J. Use of alternative therapies among breast cancer outpatients compared with the general population. Altern Ther Health Med 1999; 5:71-6.

96. Borek C. Dietary Antioxidants and human cancer. Integr Cancer Ther 2004; 3:333-41.

97. Sozen S, Coskun U, Sancak B, Bukan N, Gunel N, Tunc L, Bozkirli I. Serum levels of interleukin-18 and nitrite+nitrate in renal cell carcinoma patients with different tumor stage and grade. Neoplasma 2004; 51:25-9.

98. Gautam S, Bhatt MLB, Singh R, Mehrotra S, Singh U, Saxena JK, Singh RK. Impact of therapeutic intervention on oxidants and antioxidants status in patients with ovarian malignancy. Biomed Res 2011; 22:259-62.

99. Lamson DW, Brignall MS. Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. Altern Med Rev 1999; 4:304-29.

100. Coia LR, Moyland DJ. Introduction to Clinical Radiation Oncology. Medical Physics Publishing, Madison, WI, pp 15-19, 1998.

101. Whelan RL, Horvath KD, Gleason NR, Forde KA, Treat MD, Teitelbaum SL, Bertram A, Neugut AI. Vitamin and calcium supplement use is associated with decrease adenoma recurrence in patients with a previous history of neoplasia. Dis Colon Rectum 1999; 42:212-7.

102. Lachnicht D, Brevard PB, Wagner TL, DeMars CE. Dietary oxygen radical absorbance capacity as a predictor of bone mineral density. Nutr Res 2002; 22:1389-99.

103. Grodzinski P, Silver M, Molnar LK. Nanotechnology for cancer diagnostics: promises and challenges. Expert Rev Mol Diagn 2006; 6:307-18.

104. Kakde D, Jain D, Shrivastava V, Kakde R, Patil AT. Cancer therapeutics - opportunities, challenges and advances in drug delivery. J Appl Pharm Sci 2011; 1:1-10.

 DOI 10.5455/oams.201113.rv.011