ROLE OF OXYGEN FREE RADICALS AS OXIDATIVE STRESS ON URINARY BLADDER CANCER DEVELOPMENT

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Abstract

This study conformed on patients having urinary bladder cancer fifty patients were choosen for this study complaining of haematuria, and proofed to have bladder cancer by histopathological study to the biopsy taken from the bladder by cystoscopy performed under general anesthesia.

In this study the type of cancer was transitional cell carcinoma of urinary bladder of different stages.

Full histories about patients were taken including age, sex, occupation, address, cigarette smoking, and family history of cancer.

In this work we study the changes of oxidant and antioxidant state in bladder cancer patients and compared the result with control groups.

In this study changes in malondieldehyde as biomarker of lipid peroxidation, the result revealed significant elevation in patient groups was compared with control, while the level of catalase activity and glutathione level (GSH) which considered as antioxidant defenses mechanism are significantly reduced in patients groups in comparism to control groups.

The changes in level of trace element copper and zinc are studied in this work , the result revealedare increased level of copper and reduced level of zinc in patients groups in comparism with control groups .

In this study ,it was concluded that there is oxidative stress in urinary bladder cancer due to depletion of antioxidant defense mechanism .

Abbreviations

Abbreviation	Details
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
a. a	Amino acid
BCG	Bacillus calmette-Guerin
BBN	N-butyl-n-4-hydroxy butylnitros amines
CIS	Carcinoma in situ
CT	Computerized tomography
CAT	Catalase
CuZn/SOD	Cupric zinc super oxide dismutase
DTNB	5,5-Dithiobis nitrobenzoite
DNA	Deoxyribo nucleic acid
EDTA-Na_2	Ethylene diamine tetra acetic acid disodium
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione- S- transferase
HPLC	High performance liquid chromatography
4-HHE	4-hydroxy-2-hexenal
IVU	Intravenous urography
IL-1	Interleukin-1
MRI	Magnetic Resonance Image
MDA	malondialedehyde
MSA	Microsatellite DNA Analysis
NADPH	Nicotinamide Adenine Dinucleotide Phosphate reduced
NMP	Nuclear matrix protien
NAT2	N- Acetyl transferase
PUFA	Poly unsaturated Fatty acid
Q_{10}	Quinine 10
RNS	Reactive nitrogen species
ROS	Reactive oxygen species

Abbreviation	Details
TCC	Transitional Cell Carcinoma
TUR	Transurethral resection
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid
UICC	International union against Cancer

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الخلاصة

هذه الدراسة تمت على مرضى سرطان المثانة . خمسون مريضا تم اختيارهم لهذه الدراسة ,يعانون من التبول الدموي وثبت إصابتهم بسرطان المثانة من خلال الدراسة النسيجية للعينات التي تم أخذها من بطانة المثانة من خلال ناظور المثانة الذي تم إجراءه تحت التخدير العام .
من هذه الدراسة كان نوع السرطان هو سرطان النسيج الطلائي الانتقالي للمثانة في مراحل مختلفة.
معلومات طبية كاملة أخذت عن المرضى تتضمن العمر, الجنس ,العنوان , التدخين تاريخ المرض في العائلة .
في هذا العمل تم دراسة التغيرات في عوامل الأكسدة, و مقاومات الأكسدة ومقارنة النتائج مع أشخاص أصحاء تم اختيارهم كمجموعة سيطرة.
وكذلك تم قياس مستوى المالونديهايد كدالة لأكسدة الدهون ,وكانت النتيجة ارتفاع ملحوظ في مستوى المالودايالديهايد عند مرضى سرطان المثانة مقارنة مع مجموعة السيطرة .
أما مستوى الكلوتاثايون المختزل الذي يعتبر كمضادات دفاعية ضد الأكسدة وكذلك مستوى فعالية إنزيم الكاتليز الذي يعتبر أيضا إنزيم ضد الأكسدة فقد كانت مستوياتهم منخفضة عند مرضى سرطان المثانة مقارنة عند مجموعة السيطرة .
وتم دراسة التغيرات في مستوى بعض العناصر حصرا النحاس و الخارصين و كانت النتيجة ارتفاع في مستوى النحاس و انخفاض في مستوى الخارصين عند مرضى سرطان المثانة مقارنة بمجموعة السيطرة .
من خلال ما توصلنا إليه من نتائج تم الاستنتاج بأنه ممكن توجد حالة إجهاد أكسدة في مرضى سرطان المثانة مع استنزاف مضادات الأكسدة الدفاعية .

دور حالة الإجهاد التاكسدي بواسطة جذور الأوكسجين الحرة في تطور مرض سرطان المثانة

رسالة مقدمة إلى
كلية الطب-جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في
علم الكيمياء الحياتية السريرية

من قبل الطالبة
ثناء محمد جودة
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1430 هجري 2009ميلادي

1.2. Reactive species:-

1.2.1. Reactive oxygen species

Reactive oxygen species (ROS) are molecules or atoms that have one or more unpaired electron in there's outer shells. They are in consequence have tendency to accept an electron from other substances make them highly reactive.

These ROS characterized by:_

.Short half life time

.Unstable

.React with other molecules to achieve stability (44).

The ROS including:-

$O_2^{\cdot -}$	Super oxide anion radical
$HO^{\cdot}$	Hydroxyl radical
H_2O_2	Hydrogen peroxide
$Roo^{\cdot}$	Lipid peroxide radical
$^1O_2$	Singlet oxygen
HOCL	Hypochlorous
O3	Ozone

ROS are formed through following reaction:

$O_2 + e \longrightarrow O_2^{\cdot -}$ reaction(1)

$2\ O_2^{\cdot -} + 2H^+ \longrightarrow 2H_2O_2 + O_2$ reaction (2)

$H^+ + CL^- + 2H_2O_2 \longrightarrow HOCL + H_2O$ reaction (3)

ROS vary in their reactivity and toxicity, H_2O_2 is less reactive but more toxic than $O_2^{\cdot -}$ because it has ability for penetration biological membrane .Singlet oxygen and Hydrogen peroxide are not free radical but because of their extreme reactivity they are included in the ROS (45).

1.2.2. Reactive nitrogen species

Reactive nitrogen species (RNS) these species are reactive and produced by oxidation of one of the terminal amino-nitrogen atoms of L-arginine which catalyzed by enzyme nitric oxide synthase.

RNS including mainly:

$NO^{\cdot}$ nitric oxide

$ONOO^{\cdot}$ Peroxy nitrite

$NO^{\cdot}$ Reacts with $O_2^{\cdot -}$ (super oxide) to form strong oxidant Peroxy nitrite which is reactive nitrogen radical, have oxidizing propriety (46).

1.2.3. Sources of ROS

The first sources for ROS are produced inside the cell by the mitochondria, where inside the mitochondria production of ATP (adenosine tri phosphate) take place in the process of oxidative phosphorylation, which involves transport of proton (hydrogen ion) across the inner mitochondrial membrane by electron transport chain and electron passes through several proteins until reach the last accepter which is oxygen and reduced to water.

Through this respiratory chain some of these oxygen molecules are incompletely reduced to give rise to reactive species generation (47).

The second sources for these ROS are enzymatic action as Diamine oxidase,treptophan dioxygynase, xanthenes oxidase ,cytochrome P_{450} (48). Nitric oxide synthase which produces $NO^{\cdot}$ form L-arginine a.a. via 5- electron redox reaction that leads to generation of ROS especially under low level of arginine these ROS react with $NO^{\cdot}$ to form potent cytotoxic Peroxy nitrite ($OONO^{\cdot}$)(49).

ROS are also produced by cyclooxygenase, which has implicated in ROS production in the cell, stimulated by tumor necrosis factor (TNF) and Interleukine-1(50).

ROS are generated by oxidation of dopamine and has been implicated in the aging related process, destruction of dopaminergic neuron receptor occurs in Parkinson's disease (51).

Third source for ROS are xenobiotics which can enhance the production of ROS within cell through inhibition mitochondrial respiratory chain and cause accumulation of free radicals, or through inactivation of antioxidant enzyme as catalase and depletion of radical scavenger .

These xenobiotics include Quinones,some dyes, drugs ,bipyridyl,hebicides,transition metal, aromatic nitro compound pollutants and food additive (52-53).

Other sources for ROS during the respiratory burst formation by leukocyte, in immune mediated host devence mechanism of monocyte, neutrophil and macrophage which produce super oxide anion and Nitric oxide and Peroxy Nitrite these ROS act as cytotoxic agent against pathogenic organism (54).

This response is triggered by stimulation of enzyme NADPH-oxidase (nucleotideadeninephosphate reduce)(55).

So some ROS has a role in biological system as in immune response of phagocytosis process, and $NO^{\cdot}$ act as neurotransmitter and muscle relaxant (56).while other radical play a role in the control of the transcription factors, nuclear factors and activator protein. Some of ROS has a role in cell signaling in biological system, ROS induce programmed cell death or necrosis, induce or suppress the expression of many genes , and activate cell signaling , such as those involving mitogen-activated protein kinase (57).

1.2.4 Antioxidant defense mechanism

1. Enzymatic antioxidant

These enzymatic antioxidant including scavenger enzymes of ROS mainly;

- Super oxide dismutase
- Catalase
- Glutathione peroxidase (58).

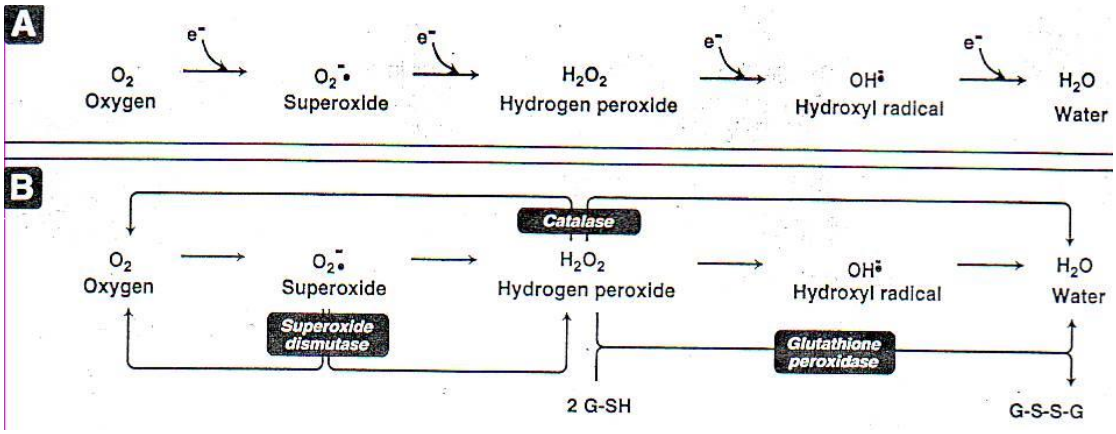

Figure (1) Antioxidant enzymes

The generation of reactive species and interaction of these species with antioxidant mechanism are run in net work of reaction between prooxidant and antioxidant and this relation ship can represent in the following figure:-

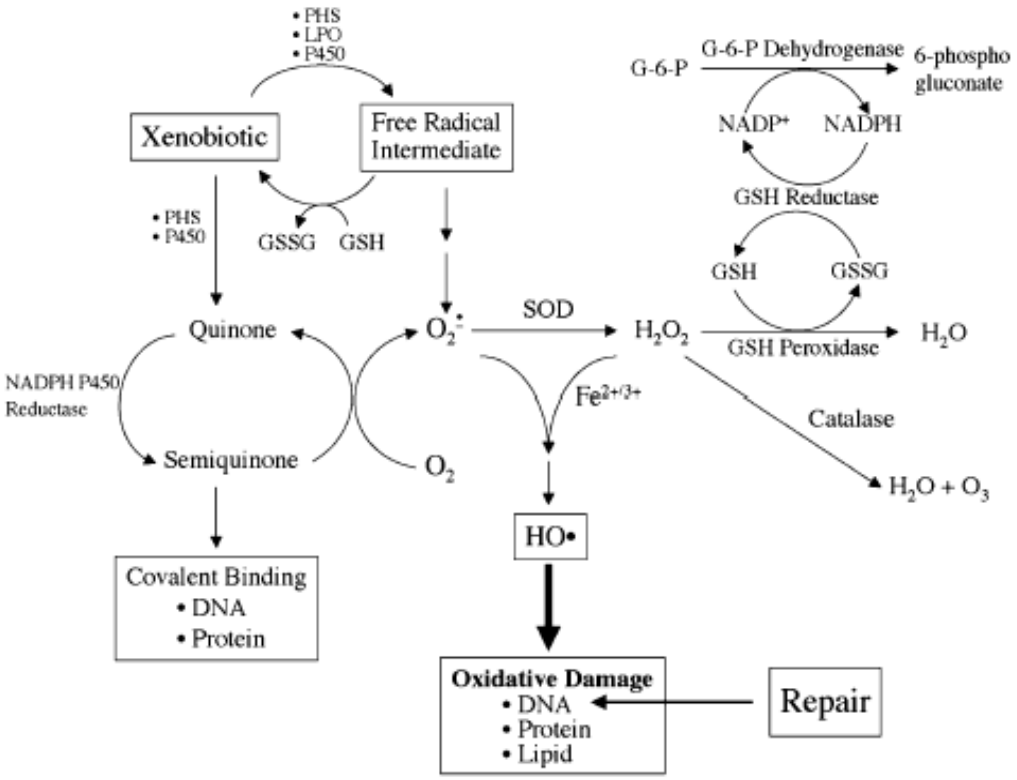

Figure (2)Prooxidant and Antioxidant net work (59).

2. Non enzymatic antioxidant

These antioxidants including lipid soluble membrane –bound antioxidant substances which has a power in preventing oxidative damage by ROS as vitamin E derivative of tocopherol(60).and ubiquinone (coenzyme Q_{10}) are redox active quinine derivative with a hydrophobic isoprenyl tail, it is play a role in mitochondrial electron chain and act as potent antioxidant in lipoprotein and other lipid membrane (61).

Vitamin C or ascorbate has a powerful antioxidant (62).and carotenoid which act as provitamine A (63).And glutathione (GSH) a powerful anti oxidant in biological system (64).

1.2.5. Oxidative stress:-

The disturbance between prooxidant and antioxidant balance result in oxidative stress which is defined as imbalance between the production of various ROS and the ability of organism natural protective mechanism to cope with these ROS component and prevent its adverse effect on biological system (65).

The effect of oxidative stress caused by ROS

- Chronic inflammatory disease as rheumatoid arthritis
- Disease of eyes as cataract
- Reperfusion injury as cardio vascular disease injury and atherosclerosis .
- Peptic ulcer its show that super oxide anion are involved in formation of ulcer.
- DNA damage mainly by ROS
- Degenerative brain disease mainly Parkinson's and Alzheimer and dementia
- ROS are involved in alcoholic liver cirrhosis, malignant hypertension and toxemia of pregnancy (66).

Effect of ROS in aging theory:-

According to the free radical theory ,oxidative damage initiated by ROS are major contributor to the functional decline which is characters of aging process, deleting and decreasing of anti-oxidant yields shorter life span the studies show that life span can be increased by over expression of super oxide dismutase and glutathione biosynthesized enzyme(67).

. *oxidative stress and telomere shortening*:-

Telomere are the specialized end of Eukaryotic chromosomes and consist of tandemly repeated DNA sequence ,telomeres are shorten with each cell division and it is well know that telomere shortening rate is increasing by oxidative state (68).A cohort study for telomeres length comparism between a cases of patients with urinary bladder cancer and control groups are free of disease show that telomere length are shorter in case of bladder cancer compared with control indicate that shortening of telomeres have a role in developing of malignancy (69).

1.3. Lipid peroxidation

Lipid peroxidation is oxidative degradation of lipid ,by which free radicals steal electron from lipid in cell membrane and resulting in cell damage (70).

Poly unsaturated fatty acid (PUFA) serve as excellent substrate for lipid peroxidation (71).

PUFA contain two or more double bonds, are particularly susceptible to peroxidation,because of presence of active bis-allylic methylene groups, the carbon-hydrogen bond on these active methylen unit have lower bond dissociation energy, making there hydrogen atom more easily abstracted by radical reaction ,peroxidation of PUFA increase with increase in number of unsaturated sites in lipid chain (72).

1.3.1. Mechanism of lipid peroxidation

The general process of lipid peroxidation consists of three stages;

- Initiation
- Propagation
- Termination.

Initiation phase of lipid peroxidation includes hydrogen atom abstracted by several species which can abstracted first hydrogen atom including hydroxyl ($OH^{\cdot}$), alkoxy, peroxy, but not H_2O_2 or $O_2^{\cdot-}$

The initial reaction of hydroxyl radical with PUFA produce lipid radical ($L^{\cdot}$).

Propagation phase, in this phase the lipid radical ($L^{\cdot}$) in turn react with molecules of oxygen to form a lipid peroxyl radical ($LOO^{\cdot}$), this radical are unstable species and can abstract hydrogen from neighboring fatty acid to produce a lipid hydro peroxide (LOOH) or a cyclic peroxide if had react with it self, through this phase propagation of radical chain developed (73).

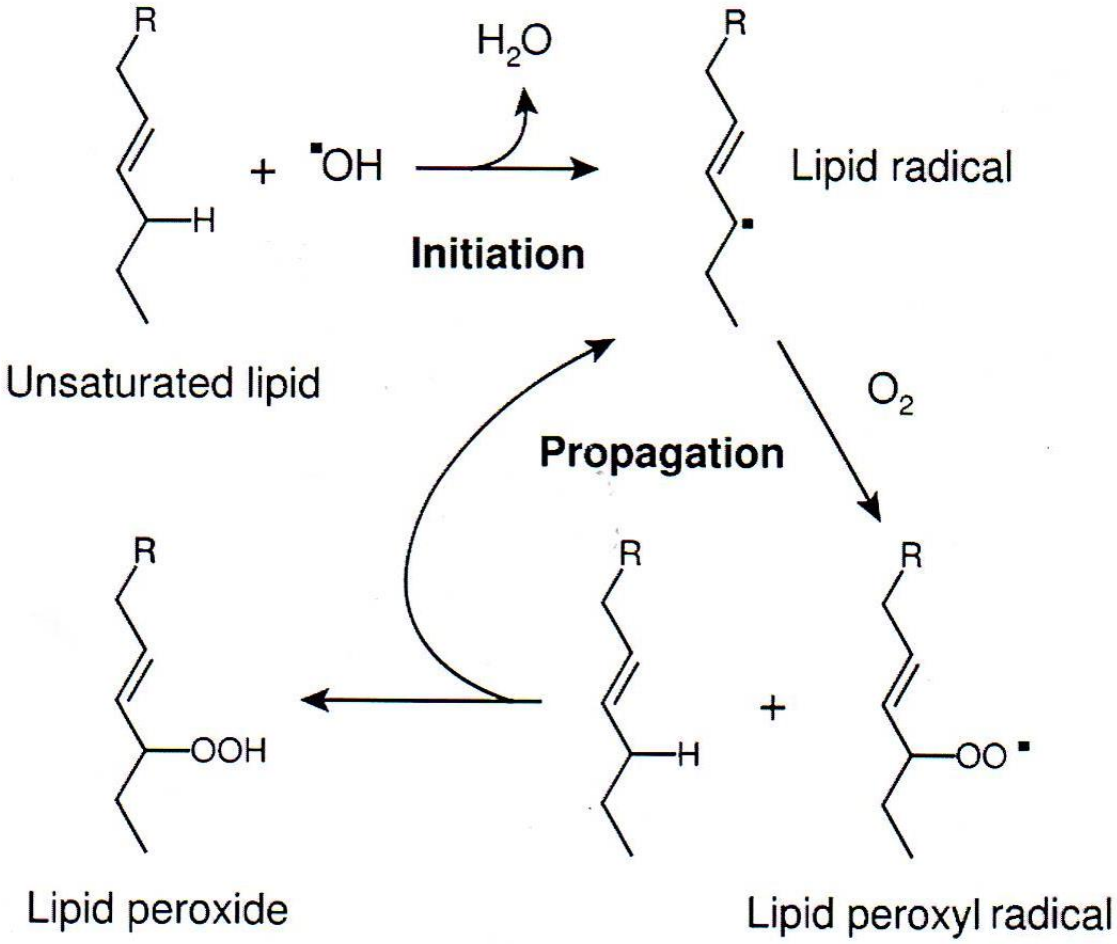

Figure (3) Mechanism of lipid peroxidation

Termination of lipid peroxidation occurs via the combination of any two lipid radicals to form non radical product ,which is stable and unable to propagate lipid peroxidation chain (74).

Peroxidation of lipid can disturb the assembly of membrane, causing changes in the fluidity and permeability, alteration of ion transport and inhibition of metabolic process (75). Injury to mitochondria induced by lipid peroxide radical can directed to further ROS generation (76).

1.3.2. End product of lipid peroxidation

Lipid peroxidation process yield several cytotoxic product including saturated aldehydes e.g. Malondialdehyde (MDA) and unsaturated aldehydes e.g. 4-hydroxy –trans-2-nonenal and

4- hydroxy-2-hexenal (4-HHE) and acrolein (77).These lipid derived aldehyde diffuse greater distances compared with their precursor ROS and behave as secondary toxic messenger that propagate and amplify oxidative injury .These aldehyde react with cellular nucleophiles such as glutathione (GSH)and cystiene,histidine,lysine residues of protein ,causes destructive functional modification and its effect on DNA resulting in genotoxic effect that alter cellular function (78).

1.3.3. Repair of lipid peroxidation

Lipid peroxidation product modify the physical characteristic of biological membrane, and incorporation of LOOH radicals change the physical structure of the membrane by decreasing the fluidity and increase the permeability so removal of lipid peroxidation product from membrane is important to repair membrane damage ,this done by two separated enzymetic system:-

- PhospholipaseA_2 with glutathione peroxidase
- Phospholipids hydro peroxide glutathione peroxidase (79).

Antioxidant such as ά-tocopherol(α-TOH) can act as excellent hydrogen atom donors generating LOOH and relatively inert tocopherol phenoxyl radical (α-TO), in absent of antioxidant the lipid radical can abstract hydrogen atom from another lipid molecules producing highly reactive carbon centered radicals.(80).

1.4. Glutathione (GSH)

Glutathione (GSH) reduced form is a linear tripeptide of L.glutamate,L-cysteine and glycine.GSH has sulfhydryl (SH) group on cysteinyl portion, which account for its strong electron donating character. As electron are lost from the GSH the molecule become oxidized and two such molecules become linked by disulfide bond to form glutathione disulfide or oxidized glutathione (GSSG) .This linkage is reversible upon rereduction, so GSH is under tight haemostatic control both intra cellular and extra cellular .So a dynamic balance is maintained between GSH synthesis, its recycling from GSSG and metabolism (81).

1.4.1. GSH Biosynthesis

The synthesis of GSH involves two closely linked enzymatic controlled reactions, GSH synthesized from L- glutamate cysteine and glycine. One molecules of adenosine tri phosphate ATP) is broken to ADP and phosphate for each peptide bond generation. Two main reactions in process of synthesis GSH:-

.The first reaction catalyzed by action of gamma- glutamyl cysteine synthetase as follow:-

1. Glutamate +cysteine +ATP ⟶ gamma-glutamyl cysteine +ADP+P

.The second reaction catalyzed by glutathione synthetase

2. Gamma glutamyl cysteine +glycine +ATP ⟶ glutathione + ADP+P

Reaction one is considered rate limiting step and inhibited by GSH suggestion a physiologically significant feed back control of GSH synthesis (82).

GSH which characterized by reactive thiol group and the gamma glutamyl bond which is unusual peptide bond (α which is standard peptide bond of protein) (83).

GSH recycling is catalyzed by glutathione disulfide reductase which uses reducing equivalent from NADPH to reconvert GSSG to 2 GSH, the source of NADPH mainly from pentose phosphate path way (58).

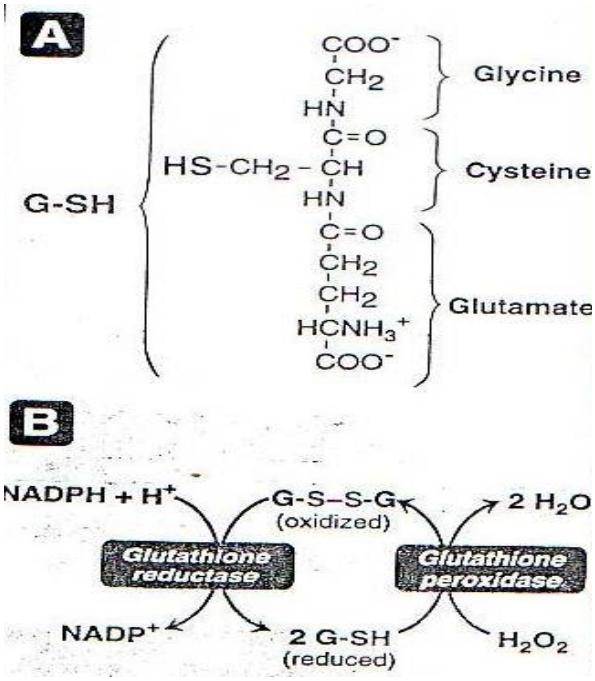

Figure (4) Recycle of GSH and GSSG

1.4.2. Transport mechanism of amino acid through glutathione

Although GSH is essential for normal cell function ,most organ and tissue can not synthesized GSH by themselves, instead GSH bas to be taken up from extra cellular sources and imported into the cell ,this occurs across cell membrane ,mainly by gamma glutamyl cycle ,which involved a sequences of six enzyme ,one of which is membrane bond and the remainder are present in the cytosol.

This cycle is important for transport GSH and a.a., the key enzyme of this cycle is membrane-bond enzyme gamma glutamyl transpherase as follow

Amino acid + GSH ⟶ γ-glutamyl a.a. +cysteinyl glycine

which result in transport of glutamyl residue of GSH to the incoming a.a.,the next step gamma glutamyl a.a. under goes cleavage to free a.a. and 5-oxyproline in reaction catalyzed by gamma glutamyl cyclo transferase.The cysteinyl glycine under goes hydrolysis by peptidase thus one a.a. molecules has been transported into cell on expense of the energy of hydrolysis the peptide bond of GSH.To regenerate GSH ,glutamate is reformed from oxy-proline in ATP- requiring reaction and GSH is re-synthesized from its 3 component part ,3 ATP molecules are used in the regeneration of GSH,one for forming glutamate from oxy-proline and two for formation of peptide bond(84).

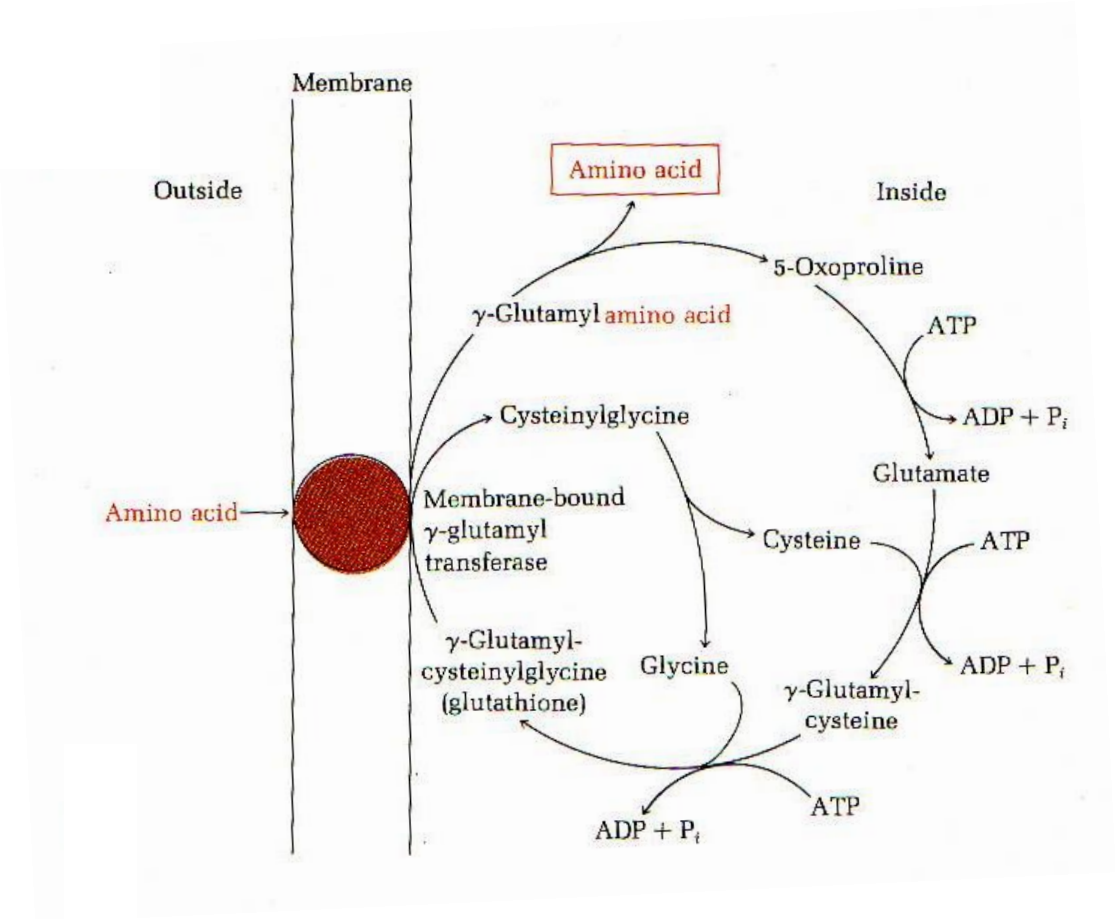

Figure (5) γ-Glutamyl cycle for transporting amino acid

1.4.3. Biological role of GSH:-

GSH used as co factor for many enzymes as

- Multiple peroxidase enzyme to detoxify peroxide generating from ROS and attack biological system and cause cell injury .
- Trans hydrogenase to reduce oxidizing centers on DNA,protein and other molecules.
- Glutathione –S- tansferase (GST) to conjugate GSH with endogenous substance as estrogen ,or to exogenous electrophils e.g. arene oxide, unsaturated carbonyl, organic halides and diverse xenobiotics .
- GSH is powerful antioxidants and called master of anti oxidant because all anti oxidant depend on it for functioning properly ,it is act as scavenger for ROS also has ability to reduce oxidized vitamin C and E to their non oxidized form. the anti oxidant of GSH is important for protection nucleic acid, and DNA repair.
- GSH play a role in biosynthesis of neurotransmitter and maintaining brain function so deficiency of glutathione are associated with degenerative brain disease as Alzheimer and parkinsonism.
- GSH are important for protective the integrity of red blood cell ,and play a role in cellular redux potential (85).
- GSH is an essential component of the human immune response proposed mechanism of immune enhancement which include:-
  1-Optimizing macrophage function
  2-Offseting oxidative damage associated with lymphatic monoclonal expansion.
  3- Stabilizing mitochondrial membrane there by reducing a apoptosis in lymphocyte.
- GSH play a central role in the proper function of the white blood cell and proper activity of lymphocyte is linked to availility of glutathione (86).

- GSH is the most free radical scavenger in the air way which cause damage to respiratory system and it is deficiency linked with lung disease (87).
- GSH play a role in detoxifies certain drug as acetaminophen and detoxifies substance in cigarette smoking, autoexhaust, and detoxifies pollutant including heavy metal, and pesticides, and protect tissue from lipid peroxidation and detoxifies many known carcinogen, so GSH protect from cancer developing (88).

1.4.4. Causes of GSH depletion:-

1.4.4.1. Exogenoussoured of GSH depletion

Many factors lead to a build up of acidic toxin cause depletion of GSH as following factors:-

- Acidic life style and diet
- Air and water pollution
- Prescription and recreational drugs
- Ultraviolet and radiation from cells phone ,computers, electrical cars power lines, hair dryer.
- Emotional and physical stress
- Injure trauma, or burn injury
- Heavy metals
- Cigarette smoke
- Household chemicals
- Acetaminophen poisoning
- Exhaust from motor vehicles
- Septic shock
- HIV infection has systemic manifestation of GSH depletion.
- GSH low in patient with inflammatory liver cirrhosis (89).

1.4.4.2. Endogenous depletion of GSH:-

This depletion of GSH is due to inherited deficiency of the enzyme gamma glutamyl cysteine synthetase first enzyme necessary for GSH syntheses ,it is associated with generalized

GSH deficiency ,the persons with this deficiency has hemolytic anemia, spino cerebelar degeneration, peripheral neurophathy myopathy and amino acid urea. The other causes for depletion GSH is deficiency of enzyme GSH synthetase, also associated with hemolytic anemia, low erythrocyte GSH also manifests in hereditary nonspherocytic lymphocytic leukemia, and glucose-6 phosphate dehydrogenase (G6PD) deficiency (90).

1-5. Catalase as anti oxidant enzyme:-

1.5.1. Catalase action

Catalase is enzyme found in blood, bone marrow, mucous membrane, kidney and liver. Its function assumed to be the destruction of hydrogen peroxide formed by the action of oxidative stress .Mainly catalase are found in peroxisomes (cytoplasmic organelles involved in oxidation reaction by using oxygen molecules), peroxisomes are rich in oxidase and catalase which suggest that there may be a biological advantage in grouping the enzyme that produce H_2O_2 with enzyme that destroy it .Other sources of H2O2 which is substrate for catalase is mitochondrial and microsomal electron transport system, as well as xanthene oxidase must be conceders as source of H_2O_2.

$$2H_2O_2 \longrightarrow 2H_2O+O_2$$

Other enzyme act on hydrogen peroxide is peroxidase which is found mainly in leucocyte, platelet,and other tissues involved in eicosanoid metabolism .prosthetic groups is proto-heme .the reaction catalyzed by peroxidase ,hydrogen peroxide is reduced at the expense of several substance that will act as electron accepters .The reaction catalyzed by peroxidase is complex ,but the over all reaction is as follow:-

$$H_2O_2+2H \longrightarrow 2\ H_2O$$

Another enzyme act on hydrogen peroxide is found in erythrocyte is glutathione peroxidase ,containing selenium as prosthetics groups, catalyzed the destruction of hydrogen peroxide ,using reduced glutathione (GSH) and this reaction lead to formation of water and oxidized glutathione(GSSG) (91).

Hydrogen peroxide which is substrate of catalase is reactive species but production of H_2O_2 in the body are used for synthesis and detoxification process ,as well as for immune defense , H_2O_2 are produced in thyroid gland as substrate for thyroperoxidase which catalyses the attachment of iodine to thymoglobulin which is important part in synthesis of thyroid hormones .

Hydrogen peroxide is generating in the peroxisomes to aid in the degradation of fatty acid and other molecules , H_2O_2 use for detoxification reaction involved in liver by cytochrome p_{450} also H_2O_2 use in the phagocytic process of leukocytes in defense process against bacterial infection in this inflammatory cells NADPH oxidase associated with plasma membrane reduce O_2 to generate super oxide radicals and this produce H_2O_2 by action of super oxide dismutase , and this H_2O_2 is substrate of catalase and when accumulate to toxic level cause oxidative stress(92).

1.5.2. Catalase composition and mechanism of action:-

Catalase is tetra mere of four polypeptide chains, each over 500 a. a. long and it contain four porphyrin heme groups that allow the enzyme to react with hydrogen peroxide. The complete action of catalase is occurs in two stages:-

$$H_2O_2+Fe(lll)\text{-}E \longrightarrow H_2O+O=Fe(lV)\text{-}E$$

$$H_2O_2+O=Fe(lV)\text{-}E \longrightarrow H_2O+Fe(lll)\text{-}E+O_2$$

(where Fe-E represent the iron center of the heme groups attached to the enzyme).

As hydrogen peroxide enter the active site it is forced to interact with amino acids(aspargine at position 147 Asn.147)and (histidine at position 74 His.74)causing a proton(hydrogen ion) to transfer between the oxygen atoms.

The free oxygen atom coordinates, freeing the newly formed water molecules, and Fe (lV)=O reacts with a second hydrogen peroxide molecule to reform Fe(lll)-E and produce water and oxygen.

Catalase cans also oxidizing different toxins such as formaldehyde, formic acid and alcohol, in doing so it uses hydrogen peroxide according to the following reaction,

$$H_2O_2+H_2R \longrightarrow 2H_2O+R$$

The optimum pH for catalase is approximately pH7,and optimum temperature is $37C^{o}$ which is temperature of human body

***Inhibition of catalase*:-**

Any heavy metal ions such as cupper cat ion which is act as non competitive inhibitor for catalase ,while the poison cyanide is competitive inhibitor of catalase ,it is strongly binding to the heme of catalase and stopping the enzyme action.

***Application of catalase in industry*:-**

. Catalase is used industrially for removing hydrogen peroxide from milk prior to cheese production. And use in the wrappers where it prevent food from oxidizing.

. Catalase use in the textile industry, removing hydrogen peroxide from fabrics to make sure that material is peroxide free.

. Catalase use in contact lens hygiene .Few lens-cleaning products disinfect the lens using a hydrogen peroxide solution Which contains Catalase, which is used to decompose the hydrogen peroxide before the lens is used again.

. Catalase recently use in aesthetics industry.

.Several mask treatment combine the enzyme with hydrogen peroxide on the face with intent of increasing cellular oxygenation in the upper layers of the epidermis.(93).

. *Acatalasemia* is a rare inherited condition in which there is a little or no catalase production; it is commonly in Asia population, although European have been reported. Acatalasemic have been demonstration impaired of preventing methemoglubin form in erythrocytes.(94).

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1.6. Zinc and copper trace elements and its role in biological system

1.6.1. Zinc

Zinc is essential trace element for all form of life. It is absorbed from duodenum, zinc is mostly transported bound to albumen, alpha-2-macroglobulin and transferene, zinc sequestration in enterocytes with metalothionein, some of this transfer to the plasma, and the rest is lost when the enterocytes are sloughed. So the body does not store zinc to any appreciable extent in any organ. Urinary excretion is fairly constant at 10μmol/24h. With re-excretion into the gut being the main route for adjusting the amount excreted. (95).

1.6.1.1. Function of Zinc:

Numerous aspects of cellular metabolism are zinc – dependent. Zinc plays important roles in growth and development, The immune response, neurological function, and reproduction. The function of zinc can be divided into three categories

1.Catalytic
2.Structural
3.Regulatory

1.catalytic role:-

Nearly 100 different enzymes depending on zinc for their ability to catalyze vital chemical reactions, as carboxy-peptidase,carbonic anhydrase ,alkaline phosphatase,lactate dehydrogenase,RNA polymerase and so many zinc dependent enzyme.

2. structural role:-

Zinc plays an important role in the structure of proteins and cell membranes. A finger- like structure, known as a zinc finger motif, stabilizes the structure of a number of proteins, for example ,copper provides the catalytic activity for the anti oxidant enzyme copper-zinc super oxide dismutase (cuznSOD),while zinc plays a critical structural role. The

structure and function of cell membranes are affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function.

3.Regulatory role :-

Zinc finger proteins have been found to regulate gene expression by acting as transcription factors(binding to DNA and has been influencing the transcription of specific genes),zinc play a role in cell signaling and influence hormone release and nerve impulse transmition. Also zinc has role in apoptosis(gene- directed cell death). Zinc has implication for growth and development.(96).

1.6.1.2. Antioxidant activity of zinc

Under in vitro conditions in biological systems, zinc can act as antioxidant by interacting with sulfhydryl groups of macromolecules, thereby inhibiting their oxidation and competing for binding sites on membranes with metal such as copper and iron, thereby decreasing the electron transfer capabilities of the latter ,in vivo at least one mechanism of zinc –induced antioxidant effect is the induction of metallothionein which is an effective free radical quencher.

Zinc administration can inhibit the toxicity of agent such as carbon tetrachloride, ethanol and ionizing radiation, which act in part through oxidative injury(97).

1.6.1.3. Zinc and vitamin A

Zinc and vitamin A interact in several ways. Zinc is a component of retinol binding protein, which is necessary for transporting vitamin A in the blood .Zinc is also required for the enzyme that converts retinol(vitamin A)to retinal, which is necessary for the synthesis of rhodopsin,a protein in the eye that absorbs light and thus is involved in dark adaptation, zinc deficiency is associated with decrease release of vitamin A from the liver, which contribute to symptoms of night blindness that are seen with zinc deficiency(98).

1.6.1.4. Zinc and cancer:-

Poor zinc nutrition may be an important risk factor in oxidant release and the development of DNA damage and cancer ,zinc is co-factor in proteins involved in antioxidant defense ,electron transport, DNA repair and P_{53} protein expression(99).

1.6.2. Copper:-

Copper is an essential trace element for humans. in the body copper shifts between the cuprous (cu^{+1}) and cupric (cu^{+2}), the ability of copper to easily accept and donate electron explains it is important role in oxidation- reduction (redox) effect about 90% of copper bound to ceruloplasmin which is copper contains protein the copper containing enzyme, while when copper first absorbed by intestine it is transported to liver bound to albumen. Ceruloplasmin (ferroxidasel) and (ferroxidasell), which have the capacity to oxidize ferrous ion (Fe^{+2}) to ferric ion(Fe^{+3})the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation.

The other copper –dependent enzyme ,cytochrome oxidase which involved mitochondria for reduction of O_2 to water and produce energy in form of ATP.

Another copper contains enzyme as lysyl oxidase, is required for cross-linking of collagen and elastin, which important for formation of strong and flexible connective tissue.

The enzyme superoxide dismutase is copper containing enzyme.

The copproenzyme, tyrosinase, is required for formation of pigment melanin.

Coppproenzyme as dopamine-b-monooxygenase catalyze the conversion of the dopamine to the neurotransmitter norepinephrine.(100).

1.6.3. Zinc and Copper interaction:-

The interaction between zinc and copper may be considered to be mutually antagonistig,imbalances between zinc and copper may occur because of either deficient or excessive copper intake, or excessive intake of zinc relative to copper. this may be due to that zinc and copper inhibit each others intestinal absorption under certain conditions. The competition between these metals demonstrated in intestinal mucosal cells is likely to explain the reciprocal relation ship ,other interaction sites should be determined for example ,ceruloplasmin, the major copper-binding protein in plasma which is synthesized in liver, also exhibits an inverse relation ship to zinc status. The factors that decrease zinc such as stress and acute infection, mediated in part by inter leukine-1(IL-1) cause's increase in plasma ceruloplasmin and decrease in zinc.

The other explanation for this reciprocal relation between zinc and copper is through metallothionein which is directly related to zinc status, zinc induce synthesis of metathionin in intestinal cells this enzyme has higher affinity for copper than zinc, copper absorbed by the intestinal mucosal cells is bound to metallothionein, does not enter the body and is returned to the intestinal lumen with turn over the intestinal mucosal cells (101).

Although the copper is element important in biological system, it is should be treated as if they are toxic, a significant portion of the toxicity of copper comes from it ability to accept and donate single electrons as it changes oxidation state. This catalyzed the production of very reactive free radical such as hydroxyl ($HO^{\cdot}$) radical and the effect of this free radicals become toxic when unsequestered and when increase to toxic level that cause cell damage(102).

Aims of the study

1. Study the role of oxidative stress on developing urinary bladder cancer.
2. Study the enzymatic antioxidant defense mechanism as catalase enzyme, and the level of reduced glutathione(GSH) as main non enzymatic antioxidant in patient with urinary bladder cancer and compared with control groups.
3. Study the role of lipid peroxidation through study the level of MDA as a biomarker of lipid peroxidation in patient with bladder cancer.
4. Study the changes occurs in serum level of trace element zinc and copper and study the changes in Zn/Cu ,zinc as antioxidant and copper as contributor to oxidative stress.