
# تقدير مستويي الكلوتاثايون المختزل

# و أل- ألفا -L- فيوكوز

# كمؤشرين حيويين محتملين

# لسرطان البروستات

**رسالة مقدمة إلى مجلس كلية الطب - جامعة بابل**

**كجزء من متطلبات نيل درجة الماجستير في**

**علم الكيمياء الحياتية السريرية**

**من قبل**

**أسامة فخري مزاحم**

**بكلوريوس طب وجراحة عامة**

**جامعة بغداد (١٩٩٢)**

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# *THE EVALUATION OF REDUCED GLUTATHIONE AND ALPHA- L-FUCOSE, A POSSIBLE BIOMARKER FOR PROSTATE CANCER*

**A THESIS SUBMITTED TO THE COUNCIL OF THE COLLEGE OF MEDICINE-BABYLON UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN**

**CLINICAL BIOCHEMISTRY**

***BY***

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١٩٩٢ ***A.D.***

**١٤٢٧ A.H.** **٢٠٠٦ A.D.**

# *DEDICATION*

*TO:*

*Holy IMAM*

*AL-HUJJA AL-MAHDI*

*MY FAMILY,*

*MY PARENTS,*

*MY SUPERVISOR S ,*

*My FRIENDS,*

*MY WIFE,*

*&*

*MY SONS ALI & HUSSAIN,*

*MY DAUGHTER FATIMA*

*Osama*

# *The Evaluation of Reduced Glutathione and Alpha -L- Fucose A Possible Biomarker for Prostate Cancer*

We , the examining committee , after reading this thesis and examining the student in its contents , find it adequate as a thesis for the degree of master of science in clinical biochemistry

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بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي
وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا (٨٥)

سورة الإسراء ﷺ

الآية (٨٥)

# *SUMMARY*

Reduced glutathione (GSH) and Alpha-L-total fucose (TF) in patients with proved prostate cancer have been estimated to find the possibility of using such parameters as a biomarker in the diagnosis of prostate cancer patients compared to control of the same age group.

Sera of (٤٠) prostate cancer patients has been taken to estimate the levels of GSH and TF compared to those levels of the prostate specific antigen (PSA) of the same patients .

All the studied patient samples were already male with mean age of (٦١.٥٣) years old ; ٧٧.٥ % were exposed to chemicals pollutants (those used in insecticides and pesticides industries, dye industry, tobacco industry) also physical pollutants (depleted uranium which is proved to be present at a very high levels especially at the regions were the battles of the last wars occurred especially the wars of ١٩٩١ and ٢٠٠٣) ,٩٢.٥٪ of patients had a past history of either prostate cancer or other type of malignancy,٨٥٪ were smokers , ٨٢.٥٪ had a previous history of recurrent prostatitis, ٥٠ % of patients were either farmers or lived at rural areas, the highest percentage of the patients (٤٥٪) were from the countryside of Babylon Governorate.

The results of the study revealed that serum (GSH) decrease in prostate cancer , while serum (TF) increase in the same patients exposing an inverse relationship between the two parameters ,the sensitivity of serum (GSH) in prostate cancer ٧٢.٥٪ while the specificity was ٧٧.٥٪.

The sensitivity of serum (TF) in prostate cancer was ٧٠٪ while the specificity was ٧٢.٥٪ .

Plotting PSA against GSH in prostate cancer patients resulted in a negative correlation between the two parameters, while plotting PSA against

TF in prostate cancer patients resulted in a positive correlation between the two parameters.

The tumor cell opposition response to apoptosis (programmed cell death occurs in normal cell) affected both TF and GSH serum levels ; in which the study proved presence of a significant decrease in serum GSH opposed by a significant increase in serum TF in relation with serum PSA in both prostate cancer and benign prostatic hyperplasia (BPH) patients therefore the study reveled that its possible to use serum TF either alone or in combination with PSA as a possible trend biomarker of prostate cancer ; concerning serum GSH cannot be used in combination with PSA and can be used alone only as a possible biomarker for prostate cancer .

On the basis of these results, the inverse relationship between serum (TF) and serum (GSH) either with or without serum (PSA) could be considered as possible new biomarker for prostate cancer.

It is concluded that prostate cancer affects (TF) and (GSH) levels in the patients serum also the two parameters affected in a noticeable degree by ; smoking, exposure to chemicals had a significant effect on blood levels of (TF) and (GSH).

aging considered one of the most important risk factors for PC , therefore the study reveled that the majority of the patients affected by prostate cancer are those whom related to the age group range from ٦٠-٦٩ years which is identical to the age group distribution of the patients affected by benign prostatic hyperplasia (BPH).

## الخلاصة

قد تم قياس الكلوتاثايون المختزل وألألفا-ل- فيوكوز في مصل المرضى المثبت اصابتهم بسرطان البروستات وذلك لأيجاد احتمالية استعمالهما كمؤشرين حيويين محتملين في تشخيص مرضى سرطان البروستات مقارنة بالأصحاء ومن الفئة العمرية المماثلة للمرضى.

تم أخذ مصل أربعون مريظا مصاب بسرطان البروستات لقياس مستويي كل من الكلوتاثايون المختزل وألألفا ل- فيوكوز بالمقارنة مع مستوى المستضد المعين البروستاتي.

كل العينات المرضية التي تمت دراستها هم رجال بمعدل عمر ٦١.٥٣ سنة , ٧٧.٥ % قد تعرضوا لمختلف أنواع الملوثات الكيميائية( ألمواد ألكيميائية المستعملة في صناعة المبيدات الحشرية والأسمدة الكيميائية وصناعة الأصباغ وصناعة السكائر)والملوثات الفيزيائية كاليورانيم المستنفذ المثبت وجوده وبنسب عالية جدا في تقارير منظمة الصحة العالمية وبالأخص في المناطق التي كانت ساحة للمعارك في الحروب الأخيرة مع القوات ألأمريكية وخصوصا حرب ١٩٩١و٢٠٠٣ , ٩٢.٥ % من المرضى لديهم تأريخ مرضي أما لسرطان ألبروستات أو أي نوع آخر من السرطان, ٨٥% منهم كانوا مدخنين, ٨٢.٥% لديهم تأريخ مرضي سابق لألتهاب البروستات المتكرر المزمن, ٥٠% منهم هم من الفلاحين أو من القاطنين في المناطق الريفية أو ما يجاورها , أعلى نسبة من المرضى ٤٥% تقريبا هم من الساكنين في المناطق الريفية المجاورة لمحافظة بابل.

نتيجة الدراسة كانت اثبات نقص الكلوتاثايون المختزل في مصل مرضى سرطان البروستات بينما يزداد الألفا ل- فيوكوز عند نفس المرضى موضحا بذلك علاقة عكسية بين العاملين, حيث كانت الحساسية للكلوتاثايون المختزل المصلي في مرضى سرطان البروستات ٧٢.٥% بينما الخصوصية كانت ٧٧.٥%, بينما الحساسية لل-ألفا-ل فيوكوز الكلي المصلي لدى مرضى سرطان البروستات هي ٧٠% بينما الخصوصية كانت ٧٢.٥%.

ولمعرفة العلاقة بين المستضد المعين البروستاتي وألكلوتاثايون المختزل المصلي تم رسمهما احصائيا وأظهرت النتيجة ترابطا سلبيا بين العاملين, بينما عند رسم العلاقة بين المستضد المعين البروستاتي مقابل أل-ألفا-ل فيوكوز الكلي المصلي في مرضى سرطان البروستات أظهرت النتيجة ترابطا أيجابيا بين العاملين.

ولوحظ أيضا بأن عدم استجابة الخلية السرطانية للموت المبرمج في الخلية الطبيعية

(apoptosis, programmed Cell death)

يؤثر على مستوى كل من ألكلوتاثايون المختزل المصلي وأل-ألفا-ل فيوكوز الكلي المصلي حيث أثبتت الدراسة وجود نقص معنوي في مستوى ألكلوتاثايون المختزل المصلي يقابله زيادة معنوية في مستوى أل-ألفا-ل فيوكوز الكلي المصلي بالمقارنة مع المستضد المعين البروستاتي لمرضى سرطان البروستات وتضخم البروستات الحميد .

لذلك أوضحت الدراسة أنه من الممكن استخدام أل-ألفا-ل فيوكوز الكلي المصلي أما بصورة منفردة أو بمصاحبة المستضد المعين البروستاتي كدليل حيوي جديد محتمل لسرطان البروستات , أما فيما يخص ألكلوتاثايون المختزل المصلي فلا يمكن استخدامه بمصاحبة المستضد المعين البروستاتي لكن من الممكن استخدامه منفردا كدليل حيوي جديد محتمل لسرطان البروستات, أيضا أن النتائج أثبتت بأنه من الممكن اعتماد العلاقة العكسية بين أل-ألفا-ل فيوكوز الكلي المصلي وألكلوتاثايون المختزل المصلي أما مع أو بدون المستضد المعين البروستاتي المصلي كدليل حيوي جديد محتمل اخر لسرطان البروستات.

استنتج ومن خلال البحث بأن سرطان البروستات يؤثر وبشكل واضح على مستويات أل-ألفا-ل فيوكوز الكلي المصلي وألكلوتاثايون المختزل المصلي في مصل الدم وكذلك يتأثر وبشكل ملحوظ كل من العاملين في مصل الدم نتيجة الى التدخين , التعرض للمواد الكيميائية حيث أظهرت النتائج وجود تأثير واضح وملموس على مستوى كل من أل-ألفا-ل فيوكوز الكلي المصلي وألكلوتاثايون المختزل المصلي .

وحيث أن التقدم في العمر يعتبر أحد أهم عوامل الخطورة المساعدة على حدوث الأصابة بالمرض , لذلك أثبتت الدراسة بأن غالبية المرضى ألمصابين بسرطان البروستات هم من الفئة العمرية المحصورة بين (٦٠- ٦٩ ) وهي مماثلة للفئة العمرية للمرضى المصابين بتضخم البروستات الحميد.

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# *Abbreviations*

| *Abbreviation* | *Details* |
|---|---|
| **AIDS** | Acquired immune deficiency syndrom |
| **ADP** | Adenosine di phosphate |
| **ATP** | Adenosine tri phosphate |
| **AFP** | Alpha-fetoproteins |
| **BPH** | Benign prostatic hyperplasia |
| **BRCA١** | Breast cancer ١ |
| **BRCA ٢** | Breast cancer ٢ |
| **CDBP** | Calcium dependent binding proteins |
| **CEA** | Carcino embryonic antigen |
| **CXR** | Chest X ray |
| **GRN-A** | Chromogranin |
| **DNA** | Deoxy ribonucleic acid |
| **DRE** | Digital rectal examination |
| **DHT** | Dihydro testosterone |
| **DW** | Distilled water |
| **ELISA** | Enzyme –linked immunosorbent assay |
| **ESR** | Erythrocytes sedimentation rate |
| **FDA** | Food and drug adminstration |
| **FDA** | Food and drug association |
| **FBC** | Full blood count |
| **Gal-١-P** | Galactose-١-phosphate |
| **GGT** | Gamma glutamyl- transpeptidase |

| | |
|---|---|
| **GGT** | Gamma glutamyl traspeptidase |
| **Glc-١-P** | Glucose-١-phosphate |
| **GSH-PX** | Glutathione peroxidase |
| **GR** | Glutathione Reductase |
| **GST** | Glutathione s-transferase |
| **GST P١** | Glutathione s-transferase- P١ isoenzyme |
| **Hb** | Hemoglobin |
| **HPLC** | High performance liquid chromatography |
| **HCG** | Human chorionic gonadotrophins |
| **Pi** | Inorganic phosphate |
| **K d** | Kilo dalton |
| **LDH_٥** | Lactate dehydrogenase isoenzyme ٥ |
| **LH** | Luteinizing hormone |
| **LHRH** | Luteinizing hormone releasing hormone |
| **µ M** | Micro molar |
| **M** | Molar |
| **MW** | Molecular weight |
| **Nm** | Nano meter |
| **G-S-S-G** | Oxidized glutathione |
| **PABA / NO** | Para amino benzoic acid / nitric oxide |
| **PCR** | Polymerase chain reacti |
| **PPV** | Positive predictive value |
| **PAP** | Prostate acid phosphatase |
| **PC** | Prostate cancer |
| **PSA** | Prostate specific antigen |
| **PSMA** | Prostate specific membrane antigen |
| **PSCA** | Prostate stem cell antigen |
| **PIN** | Prostatic intraepithelial neoplasia |
| **RNS** | Reactive nitrogen species |
| **ROS** | Reactive oxygen species |
| **GSH** | Reduced Glutathione |
| **NADPH** | Reduced nicotinamide adenine di nucleotide phosphate |
| **RNA** | Ribo nucleic acid |
| **SOD** | Superoxide dismutase |
| **TERT** | Telomerase reverse transcriptase |
| **- SH** | Thiol group |
| **TF** | Total fucose |
| **TURP** | Trans Urethral Radical Prostatectomy |
| **TRUS** | Transrectal ultrasonography |
| **TNM** | Tumor, Node,Metastasis |
| **US** | Ultrasound |
| **U&Es** | Urea and electrolytes |
| **UDP-Gal** | Uridine diphosphate galactose |
| **UDP-Glc** | Uridine diphosphate glucose |
| **UMP** | Uridine mono phosphate |
| **WBC** | White blood cell |

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# Introduction

## ١.١ The Prostate Cancer

Carcinoma of the prostate is one of the commonest cancers of the internal organs of Males in the developed countries, usually being exceeded only by carcinomas of bronchus, stomach and large intestine. The increase is probably entirely due to the increased number of old men in the population, for this tumor has its more principal incidence later in life than most common cancers , because so many cases are geriatric patients already suffering from other disabilities ; the high frequency tends to be disregarded. The tumor arises anywhere in the prostate but often in the periphery of the gland (outside the area chiefly affected by benign prostatic hyperplasia BPH) and especially on the posterior surface , histologically the lesion is an adenocarcinoma .fig [١] (١).

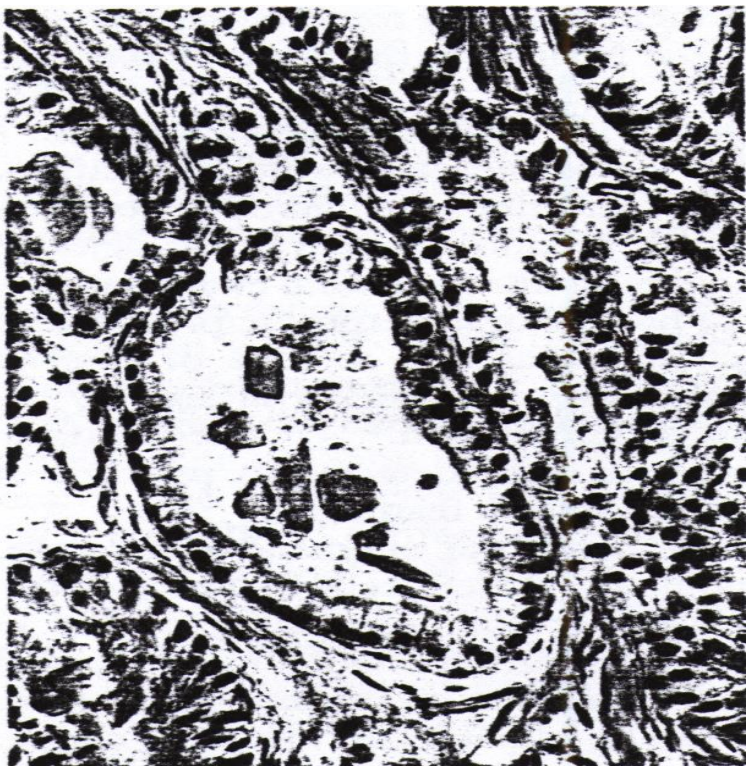


**Fig [١]** The histological appearance of well differentiated prostatic adenocarcinoma. (١)

Prostate cancer rates among black males are nearly double those of white males, thus hormonal factors appears to play a role in the development of the prostate cancer since the disease does not occur in eunuchs castrated before puberty and its incidence is low in patients with hyperestrogenism resulting from liver cirrhosis (١,٢) . Most cases of prostate cancer are diagnosed after the age of ٥٠ years but they can be seen in younger adults and even in children and adolescents (٢).

## ١.١.١.Prostate Cancer Epidemiology

Carcinoma of the prostate is the second leading cause of male cancer – related death in United States , and it is estimated that in ٢٠٠٣ there were approximately ٢٢٠,٩٠٠ new cases and ٢٨,٩٠٠deaths from this disease. (٣)

Moreover it is also the most common malignant tumor in men over the age of ٦٥ years. In England and Wales in ٢٠٠٠, ٩٠٠٠ men were die of ٢١٠٠٠ case registered (٤) . However , calculating the percentage of the prostate cancer patients to the total number of all cancer patients recorded and taken from

Babylon cancer control center at the Marjan specialized hospital for the last year ٢٠٠٥ was ٣.٦٦٪ that represents only ١٤ cases from the whole total number of cancer cases recorded which was ٢٨٣ cases (٤).

While the percentage of prostate cancer that is recorded by the same center for the year ٢٠٠٤ was ٢.٨٧٪ that represents ١٧ cases from the total number ٥٩٣ cases , knowing that these records are not for Babylon governorate but also for the surrounding and nearby governorates (٤).

These records revealed that the reduced number of cases in ٢٠٠٥ in comparison with ٢٠٠٤ but still there was an increase in percentages in ٢٠٠٥ by about ٠.٧٩٪ as shown in fig [١-٢].

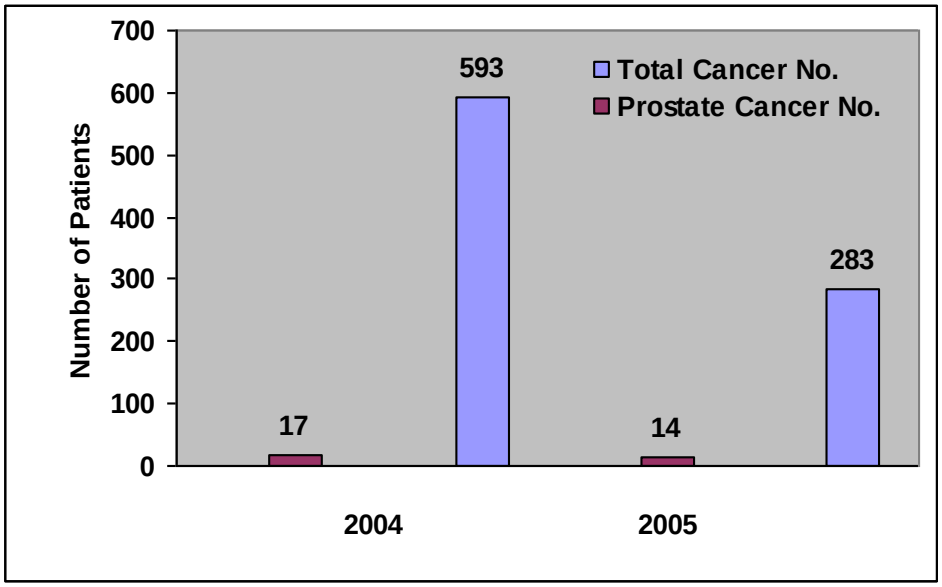
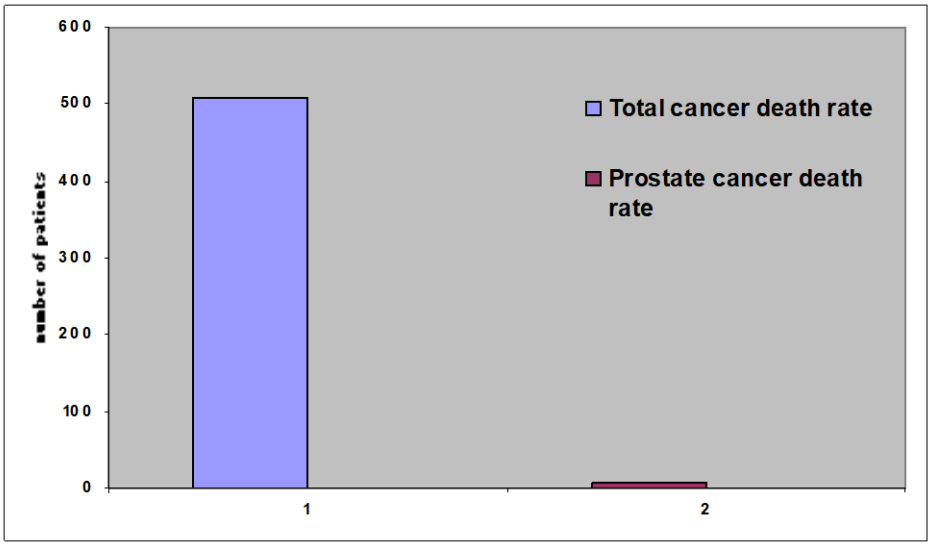



**Fig [١-٢]** the number of prostate cancer cases in comparison to the total number of cancer cases recorded by the cancer control center in Marjan specialist hospital for the years ٢٠٠٥ and ٢٠٠٤

Moreover, the prostate cancer death rates for the year ٢٠٠٥ is equal to ١.٩٦٩ = ٢ % representing ١٠ cases from ٥٠٨ of the total cancer deaths, as shown in fig [١-٣].




**Fig[١-٣]** the death rates of prostate cancer in comparison with the total number of cancer death rates in ٢٠٠٥

It can be concluded that even with increase incidence rates still prostate cancer had the lowest death rates among all other types of cancers (٤).

The chance of a man developing invasive prostate cancer during his life time is ١ in ٦ or ١٥.٤٪,the risk increase with age as shown in table **[١]**.

table **[١]** shows the chance of a man developing invasive prostate cancer during his life time. (٦)

| Age range (years) | Prostate cancer risk |
|---|---|
| From birth – ٣٩ | ١ in ١٠,٠٠٠ |
| ٤٠-٥٩ | ١ in ١٠٣ |
| ٦٠-٧٩ | ١ in ٨ |

At the age of ٥٠ years , a man has a ٤٢٪ chance of developing prostate cancer and ٢.٩٪ chance of dying of the disease ; that mean age is the most important risk factor with estimates of ٧٠٪ of men over the age of ٨٠years old having some histological evidence of cancer in their prostates(٦).While men in their ٣٠s and ٤٠s have a high incidence of small foci of cancer, whereas older men have larger lesions , implying a stepwise progression (٧). Studies also reveal that the frequency of histological cancers in men of equivalent age is similar around the world. (٨), but still the clinical incidence of the disease widely varies geographically, in Switzerland ,Sweden and Norway all experienced a clinical prostate cancer , incidence of ٢٠ or more per ١٠٠,٠٠٠ in comparison to ١٦.٨ per ١٠٠,٠٠٠ in the United States , ٢.٦per ١٠٠,٠٠٠ in Hong Kong , ٣.٨ per ١٠٠,٠٠٠ in Japan and ٤.٢ per ١٠٠,٠٠٠ in Singapore. This would suggest that there is either an inherent genetic difference in the two populations or that the specific environmental factors can contribute to prostate cancer progression in different parts of the world (٩).

Diet could be another risk factor for the development of prostate cancer i.e. there is a significant association of prostate cancer risk with total fat intake for all ethnic groups ,after adjusting for saturated fat risk was weakly associated with mono- unsaturated fat , and was unrelated to protein, carbohydrates , poly unsaturated fat and total food energy (١٠). Saturated fats may have a causal role in prostate cancer incidence (٦). High consumption of dietary fats , and in particular the fatty acid alpha- linoleic acid in red meat and butter is believed to increase risk two-three fold (١١). The isoflavinoid genistein, a component of soy is believed to be protective by inhibiting the

function of ٥-alpha – reductase enzyme inhibiting angiogenesis and glucose transport (١٢-١٤). Race is another risk factor that may contribute to the increased incidence of prostate cancer at a lower age. Studies show that African males are more affected because they have higher serum testosterone levels , the same for the Caucasian male (١٥). whereas Asian males are at a lower risk to develop prostate cancer since they have a reduced ٥-alpha-reductase activity (١٦). A genetic predisposition for the prostate cancer shows that the development was proposed on the basis of epidemiologic studies showing that if the disease was diagnosed in one first degree relative , the risk factor increased by a factor of ٢ ;this became a factor of ٤ if two or more first degree relative were affected. Moreover about ٤٠٪ of early onset prostate cancers are hereditary and follow a Mendel Ian inheritance pattern i.e. the genetic predisposition is defined as the diagnosis of prostate cancer in three generation , more than three affected first degree relatives , or two individuals in the same generation who are diagnosed below the age of ٥٥ years. (١٧-١٨) . The role of vasectomy ,if any ;is still controversial and vasectomy should not be considered a risk factor of prostate cancer (١٩-٢٠).

## ١.١.٢. Prostate Gland Pathophysiology

The prostate gland has a sexual function , but it is unclear how important its secretions are , to human fertility. The growth of the prostate is controlled by many local and systemic hormones whose exact functions are not yet known (٥). The main hormone acting on the prostate is the testosterone, which is secreted by the leydig cells of the testes under the

control of the luteinizing hormone L.H., which is secreted from the anterior pituitary gland under the control of the hypothalamic luteinizing hormone releasing hormone LHRH , LHRH has a short half life and is released in a pulsatile manner; this pulsatile release is important as receptors for LHRH will become desensitized if permanently occupied. So the administration of LHRH analogues in a continuous, non pulsatile manner exploits the concept of receptor desensitization and this forms the basis for the androgen deprivation therapy in prostate cancer. Testosterone is converted to ١,٥ dihydrotestosterone DHT by the enzyme ٥ alpha reductase , which is found in high concentration in prostate , while estrogenic steroids are secreted by the adrenal cortex and in the aging male may play an important role in disrupting the delicate balance between DHT and the local peptide growth factors , increasing the risk of benign prostatic hyperplasia BPH (٥).Thus Increased level of serum estrogens by acting on the hypothalamus , decreases the secretion of LHRH and decreases LH pituitary secretion by there decreasing serum testosterone levels , thus the pharmacological levels of estrogens causes atrophy of the testes and prostate by means of reduction in the testosterone (٥).

However the main function of the prostate gland is the elaboration and secretion:

١. Prostate specific antigen PSA.

٢. Prostatic acid phosphatase PAP.

Also other locally acting peptides are secreted by the prostatic epithelium and mesenchymal stromal cells in response to steroid hormones include:-

١. Epidermal growth factor.

٢. Insulin-like growth factor.

٣. Basic fibroblast growth factor.

٤. Transforming growth factor alpha and beta (٥). Pathologically the prostate gland is affected by various types of pathological conditions ranging from prostatits [acute and chronic], prostatic abscesses, prostate infarct, prostate calculi and tuberculous granulomas of the prostate and prostatic tumors which is either benign [nodular hyperplasia or BPH] or malignant [prostate cancer] ; over ٩٩٪ of the cancer that develop in the prostate are adenocarcinomas derived from the epithelial cells and Rarely, tumors develop in the germ cells or the mesenchymal cells of the gland (٢١)

## ١.١.٣ Prostate Cancer Histological Appearance

The prostate is a glandular structure consisting of ducts and acini; thus, the histological pattern is one of an adenocarcinomas, ٩٥ percent of all prostatic carcinomas are adenocarcinomas (٢٢). the prostate glands are surrounded by a layer of myoepithelial cells. The first change associated with carcinoma is the loss of basement membrane as the cell type becomes less differentiated ,more solid sheets of carcinoma cells are seen , a classification of the histological pattern based on the degree of glandular de –differentiation and its relation to stroma has been devised by Gleason .(٢١)

Usually prostate cancer exhibits heterogeneity within tissue , and so two histological areas of prostate are each scored between ١ and ٥ then the scores are added to give an overall Gleason score of between ٢ and ١٠ ; this [ and the volume of the cancer ] appears to correlate well with the likelihood of prostate cancer spread and prognosis, so the Gleason system is the most

widely-utilized among several histological grading systems which have been proposed to determine the biologic potential of the prostatic tumor .(٢١)

## ١.١.٤ Prostate Cancer Staging

١. **Tumor١a**,**Tumor١b** and **Tumor١c** are incidentally found tumors in a clinically benign gland after histological examination of prostatectomy specimen. **Tumor١a** is a tumor involving less than ٥٪ of the resected specimen; those tumors are usually well or moderately well-differentiated.

**Tumor١b** , is a tumor involving more than ٥٪ of the resected specimen .

**Tumor١c**, tumors are impalpable found following investigation of a raised PSA.

٢. **Tumor٢a** disease presents as a suspicious nodule on rectal examination confined within the prostate gland capsule and involving one lobe

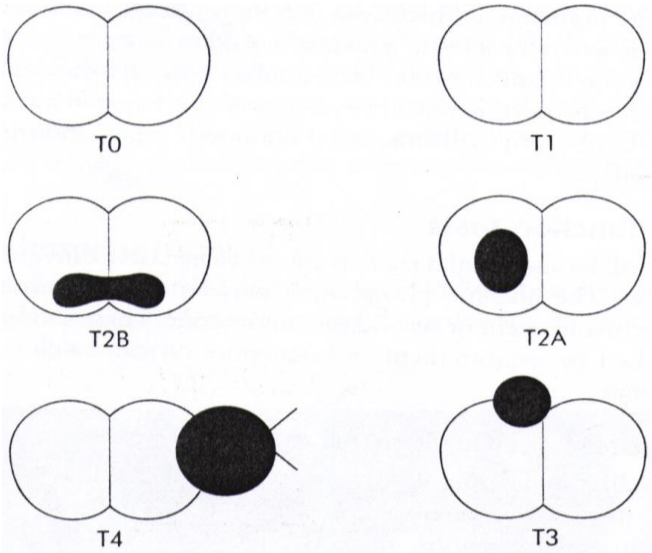
**Tumor٢b** disease involving both lobes.

٣. **Tumor٣** tumor extends through the capsule

**Tumor٣a** uni or bilateral extension.

**Tumor٣b** seminal vesicle extension

٤. **Tumor٤** is a tumor which is fixed or invading adjacent structures other than seminal vesicle-rectum or pelvic side wall . Fig [٤] (٥)



**Fig[٣]T**umor **N**ode **M**etastasis (**TNM**) staging system for prostate cancer(٥)

## ١.١.٥ Prostate Cancer Symptoms and Signs

Prostate cancer symptoms and signs may be asymptomatic or presented as a hard craggy mass on routine rectal examination. On the other hand it may be presented with incontinence, dysuria ,haematuria , hesitancy , dribbling and retention (٢٢). However they may sometimes be presented as a bone pain, pathological fractures and sciatica from distant metastasis to the bone or even nerves , and as it is considered as chronic disease so it is also associated with anemia and weight loss , while on examination, especially the rectal examination may reveal nodule in prostate or hard craggy mass involving whole prostate; the median sulcus between the lobes may be obliterated .While metastatic prostate cancer may revealed a palpable bladder , tenderness over bones and hepatomegaly.(٢٣)

## ١.١.٦ Prostate Cancer Diagnostics History

Concerning the history of prostate cancer diagnostics , the first documented case of prostate cancer was reported by Lang staff in ١٨١٧; one hundred and eighteen years later , in ١٩٣٥, prostatic acid phosphates (PAP) levels were identified in the ejaculate of men , thus linking this enzyme to the prostate(٢٤,٢٥). Subsequent studies showed high (PAP) concentrations in primary and metastatic prostate cancer tissues and in human serum , making it the first candidate marker for the diagnosis of prostate cancer (٢٦,٢٧). Reduction in serum (PAP) levels was found to occur in response to antiandrogen therapy whereas increasing serum levels was associated with treatment failure and relapse (٢٨,٢٩). However , whereas serum (PAP) levels

were elevated in a significant number of men with metastatic diseases (٢٨), fewer than ٢٠٪ of men with localized prostate cancer exhibited abnormal (PAP) enzyme levels (٣٠,٣١). Thorough sample collections and preparation were required because both platelets and leukocytes are contaminating sources of acid phosphates, and because PAP activity is rapidly lost at room temperature, development of radio immunoassay for PAP in ١٩٧٥ provided some improvement in test sensitivity levels which still inadequate for detection of early stage disease , therefore ;it was clear that a more sensitive and specific indicator of disease presence would be required to detect prostate cancer in its earlier stages, when cure is more likely (٣٢,٣٣) . Between ١٩٦٦ and ١٩٧٨ , three independent laboratories identified proteins in seminal fluid that were named gamma semino protein , E١ antigen and P٣٠ (٣٤) . Wang and colleagues in ١٩٧٩ purified a protein from benign and malignant prostatic epithelial cells and named it prostate specific antigen PSA (٣٥) . In ١٩٧١ PSA was originally identified in seminal plasma ,purified in ١٩٧٢ (٣٥) . And in ١٩٧٩ was isolated independently from human prostate tissue (٣٤) . PSA immuno staining is useful in establishing the prostate as the site of origin of a tumor i.e. it is useful for the early detection , staging and follow up of prostate cancer patients (٣٦) . PSA is prostate specific , not prostate cancer specific , as such ; PSA Levels can be elevated by any prostatic diseases e.g. prostatitis , benign prostatic hyperplasia (BPH) or even prostatic manipulation during prostatic massage or digital rectal examination (DRE) or prostatic biopsy in men with prostate cancer . PSA levels are dependent on the volume of the cancer present . Serum PSA result showed an elevated results during (DRE) by about ٠.٤ng/ml (٣٧) . The same may occur during prostatic massage and transrectal ultrasonography (TRUS), while PSA results did not affect following flexible or rigid cystoscopy and following ejaculations (٣٨,٣٩) . However prostatic biopsy

caused an immediate median elevations of ٧.٩ ng/ml which required a median of ١٥ days to return to base line , also TURP (transurethral resection of prostate causes a median elevation of ٥.٩ng/ml for a median of ١٧ days , because all these interactions concerning PSA determination , clinicians are advised to delay obtaining PSA levels for at least ٤-٦ weeks after such procedures to avoid spurious False positive results (٤٠). The medical therapies for BPH also affect the serum PSA results , finasteride a synthetic ٤-azasteroid that competitively inhibits ٥-alpha reductase, and produce a median ٢٠٪ decrease in prostate size after ٦ months of treatment (٤١,٤٢) .

## ١.١.٧ Prostate Cancer Detection

Generally , to detect prostate cancer we have to perform the following investigations hemoglobin (Hb),full blood count (FBC), erythrocyte sedmentation rate (ESR), uera and electrolytes (U&Es), Creatinine, while PSA is performed to facilitate early detection and evaluate response to treatment , and chest x-ray (CXR) is used to detect metastasis in lungs and ribs , bone radiograph is used to find any sclerotic deposit in pelvis , spine ,or skull . However, bone scan is a sensitive indicator of early metastasis. Abdominal ultrasound (USS) is used to detect residual urine, upper urinary tract obstruction, finally transrectal prostatic biopsy is used if urinary obstruction is found ; specimens may be obtained at TURP for histology(٢٣).The development in PSA regarding its complex forms and its use as a diagnostic marker have led to diagnostic algorithms to aid clinicians in providing the most thorough, yet cost effective evaluation of the prostate gland (٤٣) . Prostate cancer detection should be restricted to men of ٥٠ years of age or older with a life expectancy of ١٠ years or more ; elderly or debilitated patients with a life

expectancy of less than ١٠ years should not be subjected to either a serum PSA determination or a DRE for the sole purpose of detecting early disease (٣٦). The age specific reference ranges for the serum prostate specific antigen is shown in table (٣) (٣٦).

**Table [٢]** The age specific reference ranges for the serum prostate specific antigen.(٣٦)

| Age (year) | Serum levels (ng/ml) |
|---|---|
| ٤٠-٤٩ | ٠- ٢.٥ |
| ٥٠-٥٩ | ٠ -٣.٥ |
| ٦٠-٦٩ | ٠ -٤.٥ |
| ٧٠-٧٩ | ٠ -٦.٥ |
| > ٨٠ | > ٦.٥ |

The evaluation should begin at the age of ٤٠ years for individuals at high risk for the development of prostate cancer , including men with a positive family history (٤٤). If the serum PSA is less than or equal to the age specific range and the patient has unremarkable DRE , the patient should be followed with yearly PSA levels and DRE ;if the PSA level is greater than the age specific range and the DRE is unremarkable TURS should be performed along with accompanying biopsy of visible lesions , while if the DRE is remarkable , the patient should undergo TURS , regardless of the PSA level ; the sensitivity

of the PSA can be correlated with the disease staging ; ١٠ % sensitivity for stage A ; ٢٤ % sensitivity for stage B ; ٥٣٪ sensitivity for stage C and ٩٢ % sensitivity for stage D (٤٥,٤٦). However PSA lacks sufficient sensitivity and specificity to be used alone as a screening test for prostate cancer, because PSA is only organ specific and not tumor specific (٤٧) . PSA levels increase with advancing age, thus ruckle and associates advocate the use of age specific reference range in evaluating men at high risk of developing prostate cancer (٤٨). Age specific ranges are designed to increase PSA sensitivity in younger men by reducing the false negative rate and to improve specificity in older men by decreasing the number of the false positive results (٤٩). There are no markers or tests that can differentiate clinically relevant from clinically benign disease so the advance search for better indicators of prostate cancer presence and progression are still needed to avoid unnecessary treatment , to predict disease course and to develop more effective therapy , a variety of prostate cancer markers has been described in human serum, urine , seminal fluid and histological specimens these markers having different capacities to detect prostate cancer and to predict disease course ; however , the recent screening of a symptomatic populations , shows that prostate cancer incidence rates have increased dramatically since the introduction of serum prostate specific antigen PSA as a tumor marker (٥٠-٥١)

## ١.٢ Tumor Marker

Warburg in ١٩٧٧ was the first to note that malignant tumors usually exhibit a high rate of glycolytic activity in the presence of oxygen. Since then glycolytic enzymes have been monitored during the management of certain cancer patients (٥٢). Even in recent years , several enzymes and isoenzymes

were still being used extensively as tumor markers , which can defined as a substances of different chemical nature synthesized by tumors or produced by the host in response to the presence of tumor cells (٥٣) . Many cancers are associated with abnormal production of enzymes , proteins , and hormones which can be measured in plasma or serum (٥٤). However , the characteristics of an ideal tumor marker ,from analytical requirements point of view should follow the following requirements:

- High. Analytical Sensitivity.
- High analytical specificity.
- Accuracy.
- Precision.
- Rapid
- Turn around time.
- Easy to measure at a low cost.

And from clinical requirements point of view should follow the followings :

- High sensitivity for the disease , no false negative results and ability to detect micro Metastasis.
- High specificity for the disease, no false positive results and negative in Disease free individuals.
- Levels should reflect tumor burden.
- Levels should remain constant and not fluctuate in patients with stable disease.
- Should be undetectable or low in patients in complete remission.
- Should predict outcome in patients with stable disease.

The potential applications of tumor markers in cancers is to :

- Screen for disease in the general population.
- Diagnosis in patients with symptoms.
- Adjunct in clinical staging.
- Indicator of tumor volume.
- Aid for selecting appropriate therapy.
- Monitor response to therapy.
- Prognostic indicator.
- early detection of disease recurrence (٥٥) .

Measurements of some tumor markers are now essential features of management of some types of cancers ,e.g. carcino embryonic antigen (CEA) a tumor marker associated with cancer of colon ,lung, breast, and pancreas, alpha-fetoproteins(AFP) a tumor marker specific for liver and germ cell cancer, human chorionic gonadotrophins (HCG) is specific for trophoplastic and germ cell cancers, calcitonine is specific for thyroid cancer(medullary carcinoma),while prostate cancer has many clinically useful tumor markers such as prostatic acid phosphatase (PAP) prostate specific antigen (PSA) carcino-embryonic antigen(CEA),lactate dehydrogenase isoenzyme ٥ (LDH-٥) (٥٦) .

However three major conclusions have emerged from the study of tumor markers:

١. No single marker is useful for all types of cancers or for all patients with a given type of cancer.

٢. Markers are most often detected in advanced stages of cancer rather than early stages, when they would be more helpful.

٣. Of the uses of markers, the most successful have been the monitoring of the response to therapy and the detection of early recurrence (٥٤) .

## ١.٢.١ Prostate Cancer Tumor Marker:

Concerning prostate cancer and along with the recent revolutionary scientific studies and researches that deal with the hope to discover a true ideal tumor marker by passing through other fields than the use of enzymes or proteins or even hormones related to prostate glands as prostate cancer tumor markers (٥٥). Many new studies and researches show that the possibility of using the glutathione (GSH) system and its associated enzymes , catalase ,reductase, peroxidase and S-trasferase and the use of fucose or even sialic acid as a new potential , useful tumor markers for prostate cancer, depending on their effective crucial biochemical and biological activities that affect cancer cell homeostasis and apoptosis(programmed cell death) (٥٥). Glutathione, (GSH) is a small molecule found in almost every cell ; it can not enter most cells directly and therefore must be made inside the cell, from its three constituent amino acids: glycine, glutamate and cysteine molecule that give the glutathione molecule its biochemical activity. (٥٥). Glutathione is the major antioxidant produced by the cells , protecting it from the harmful effect of free radicals(oxygen radicals or oxy radical) ; these highly reactive substances if left unchecked, will damage or destroy key cell components i.e. membranes , DNA in microseconds. Also, it is a very important detoxifying agent enabling the body to get rid of undesirable toxins and pollutants. It forms a soluble compound with the toxins that can then be excreted through the urine or the gut . The liver and the kidneys which contain high levels of glutathione as they have the greatest exposure to toxins .The lungs are also rich in glutathione

partly for the same reason (٥٧). Many cancers producing chemicals, heavy metals , drug metabolites are disposed in this way ; glutathione plays a crucial role in maintaining a normal balance between oxidation and anti-oxidation ; this in turn regulates many of the cells vital functions such as the synthesis and repair of DNA, the synthesis of proteins and the activations and regulations of enzymes (٥٨) . Glutathione is needed to carry out an immune response, it is needed for the lymphocytes to multiply in order to develop a strong immune response, and for the killer lymphocytes to be able to kill undesirable cells such as virally infected cells or Cancer cells(٥٩) . Glutathione values decline with age and higher values in older people are seen to Correlate with better health, under scoring the importance of this remarkable substance for maintaining a healthy , well functioning body (٥٦) .Thus Several authors have suggested that increasing GSH content and GST activity might be significant in human cancer as indicators of resistance to chemotherapy because they increase the formations of drug GSH conjugates (٦٠,٦١) . So GSH as well as GST content and other glutathione metabolizing enzymes were significantly higher in malignant than in benign breast tissues (٦٢) . The GSH concentrations and glutathione reductase (GR) activity in ovarian tissues were significantly higher in malignant than benign tumors while GST activity was greater in early than in late ovarian tumors (٦٣) . Biopsies of human liver tissues from patients with hepatoma showed a non-significant decrease in GSH, GSH-PX, GR and GST activities compared to benign tissues (٦٤) . The significant elevations of GSH and its related enzymes in blood of patients with benign liver diseases suggest that they are not specific to malignant disease , it may also indicate the possible contribution of the GSH system in the detoxification of toxins associating benign hepatic disorders (٦٥) .Therefore A correlation analysis showed that the tissue levels of GSH were significantly correlated with GST and GSH-PX in

breast cancer and with GST only in prostate cancer, also a reported GST expression in benign prostatic tumors and their complete absence in malignant tumors (٦٦) . Apparently measurable GST activity in malignant prostatic tissues i.e. most but not all prostate cancer fail to express GST-P١ despite an abundant presence in benign prostatic tissue , suggesting a common genetic alterations(٦٧) . However, there is insignificant elevation of GSH-PX activity in malignant over benign prostatic tissues while there is a non significant increase in blood levels of GSH and GR in benign prostatic disorder (٦٨) . Moreover, GSH system expression in several human tumors, reported significant decrease of all and / or some components of the GSH system in patients with malignant breast,ovarian and prostatic tumors as well as metastatic liver diseases and may be of clinical value ,also the GSH concentrations was the best discriminative parameter in ovarian and breast malignant tumors , whereas GSH-PX isoenzyme was the best in hepatic and prostatic tumors . Moreover, GSH system expression in several human tumors, reported making The level of GSH is a significant marker or indicator for predicting ,diagnosing, follow up and responding to treatment of patients with prostatic carcinoma. thus it appears that GSH was significantly decreased in sera of patients with different types of cancers as a response to oxidative stress(٦٩,٧٠) . Prostate cancer is a disease associated with aging, in which GSH level is already depleted resulting in shift in the pro-oxidant - anti-oxidant balance of many tissues toward a more oxidative state , i.e. increased in the oxidative stress(٧٠) . Androgen exposure ,which has long been associated with the development of prostate cancer , may be a means by which the pro-oxidants-antioxidants balance of the prostate cell is altered, and physiological levels of androgens are capable of increasing the oxidative stress in the androgen- responsive LN cap prostate carcinoma cells ; the evidence suggests that this result is due in part to

increased mitochondrial activity .androgens also alter the intracellular GSH levels and the activity of certain detoxification enzyme gamma glutamyl transpeptidase ( GGT) that is important for the maintenance of the cellular pro-oxidant _ anti-oxidant balance (٧١) .

Fucose , a ٦- carbon deoxy hexose that is commonly incorporated into human glycoproteins and glycolipids is found at the terminal or the pre-terminal positions of many cell- surface oligosaccharide ligands that mediate cell-recognition ,adhesions and signaling pathways ; these include such normal events as early embryologic development and blood group recognition and pathologic processes including inflammation ,infectious diseases recognitions and neoplastic progression .Fucosylated oligosaccharide ligands mediate cell-cell adhesions through binding to cell surface selectins (a calcium dependent binding proteins CDBP ) and calcium dependent interactions with other cell – surface carbohydrates counterligands and thereby alter cellular homeostasis ,i.e. alpha-L-fucose is critically important for cell –cell and cell- Matrix adhesions in a variety of normal and pathologic processes ,particularly neoplasia (٧٢) . Fucosylated glycans have been implicated in the pathogenesis of several human diseases ; two prominent examples of altered glycosylation in cancer involve fucose –containing oligosaccharides , first expression of A and B blood group antigens is lost in many tumor changes that correlate with poor clinical prognosis. Second, up-regulation of sialyl groups has been demonstrated in numerous cancers and this increase is also associated with advanced tumor grade and poor prognosis (٧٢) . Moreover increased alpha(١,٦)-fucosylation of alpha-fetoprotein is observed in hepato-cellular carcinoma patients and can be used clinically as a marker for distinguishing hepato-cellular carcinoma from chronic liver disease , Increased expression of fucosylated glycans has also been reported on serum immunoglobulins in both

juvenile and adult rheumatoid arthritis patients. Similarly ,fucosylation of mucins has been observed to be increased in cystic fibrosis with concomitant decrease in sialylation . As a result of their role in leukocytes selectin –selectin ligand interactions contribute to the development of numerous pathological processes including atherosclerosis ,reperfusion injury following ischemic heart disease , inflammatory skin diseases and asthma (٧٢) . Cell-surface fucoligands have been demonstrated in most common human malignant neoplasms including carcinoma of the colon, breast , ovary ,lung ,stomach, pancreas, endometrium , kidney , bladder, thyroid, Hodgkin's disease , selected cases of melanomas ,neuroblastomas ,hepatocellular carcinoma ,carcinoma of the skin leukaemias and carcinoma of the prostate (٧٢) . However , in developed countries , most prostate cancer patients present as a result of screening for the disease by measurement of prostate specific antigen (PSA) (٧٣).

## ١.٢.٢ Prostate Specific Antigen

The prostate specific antigen (PSA) ,the optimal tumor marker for prostate cancer would be effective for early detection ,staging and monitoring patients after definitive treatment ,the PSA as a tumor marker would have a high sensitivity ,specificity and positive predictive value for distinguishing men with BPH from men with prostate cancer (٥) . The PSA is a kallekrein – like serine protease that was first described in ١٩٧١. PSA a single chain glycoprotein with a M.W. of ٣٤kd (٣٤,٣٥) . It consists of ٢٣٧ amino acids and ٤ carbohydrates side chains (٧٤) . High levels of PSA are found in the seminal fluid ,a very little PSA is found in the circulation of healthy men in addition to prostate . PSA is also produced by periurethral glands and perirectal glands (٧٥). PSA is secreted from prostate epithelial cells and encoded by an androgen

responsive gene located on chromosome ١٩ q ١٣.٣-١٣.٤(٧٦). The main function of PSA is to liquefy human semen through its proteolysis action (٧٧). PSA was initially thought to be a prostate specific protein ; subsequently investigations demonstrated that PSA is secreted in small quantities from a number of other normal male tissues and even some female tissues . PSA was first detected in the serum of prostate cancer patients in ١٩٨٠ ,and a normal PSA serum concentrations limit of ٤ ng\ml for men was subsequently established , a serum level of above ٤ ng \ml was taken as an indicator of possible presence of prostate cancer and served as the trigger for further clinical evaluation (٧٨,٧٩). Eventually, a number of studies enrolling large numbers of men over the age of ٥٠ years suggested that quantization of serum PSA was a useful diagnostic tool for detecting the presence of prostate cancer, particularly when combined with digital rectal examination (DRE) (٨٠,٨١) . However, other studies have called into question the sensitivity and specificity of the PSA test (٨٢, ٨٣) . One problem is that serum PSA levels can be elevated as a result of conditions other than prostate cancer, such as BPH and prostatitis, as a result of false positive tests, a significant problem for the PSA test that can lead to unnecessary biopsies and other interventions of great concern, ٢٠-٣٠٪ of men with prostate cancer have serum PSA levels in the normal range, resulting in undiagnosed disease (٨٤,٨١) . Astudy by Stamey et al. , has concluded that pre-operative serum PSA levels do not correlate with cancer volume or the gleason grade of radical prostatectomy specimens ; this study also showed a poor correlation between pre-operative serum PSA levels in the ٢-٩ ng \ml range and prostate cancer cure rates (٨٣). However, PSA is still currently the best clinical marker available for prostate cancer and the only one approved by the United States food and drug administration for both post

treatment monitoring of disease recurrence and, when combined with digital rectal examination, evaluation of asymptomatic men (٨٥, ٨٦) .

## ١.٢.٣ Prostate Cancer PSA Based Detection

The prostate cancer PSA based detection , find that the interpretating reports of screening or case finding must also consider what the primary stimulus for the elevation was and whether the need for the additional diagnostic studies was based on the findings of the DRE alone ,PSA alone, TRUS or a combination of the three ; in most reports ,further diagnostic testing is advised if the PSA value exceeds ٤ng/ml (٨٧) .

**Table [٣]** The sensitivity ,specificity and positive predictive value for DRE ,PSA(using a ٤ng/ml cut off) and TRUS. (٨٧)

| method | Sensitivity % | Specificity % | Positive predictive value % | Detection rate % |
|---|---|---|---|---|
| Digital rectal examination (DRE) | ٦٩-٨٩ | ٨٤-٩٨ | ٢٦-٣٥ | ١.٣-١.٧ |
| Prostate specific antigen (PSA) | ٥٧-٧٩ | ٥٩-٦٨ | ٤٠-٤٩ | ٢.٢-٢.٦ |
| TRUS | ٣٦-٨٥ | ٤١-٧٩ | ٢٧-٣٦ | ٢.٦ |

The representation of the sensitivity,specificity, positive predictive value and detection rates of digital rectal examination, serum prostate specific antigen and TURS are still of great value in P.C. diagnosis (٨٧) . Patient underwent quadrant prostatic biopsies if the PSA was more than ٤ng/ml , the digital rectal examination was abnormal or suspicious lesion was seen on TURS while using DRE alone, the cancer detection rate was ٣.٢٪ while PSA alone ٤.٦٪ and ٥.٨٪ for the two modalities combined ,the positive predictive value (PPV) was ٣٢٪ for PSA and ٢١٪ for the DRE (٨٨) .So, the need for serial examinations was shown in the American cancer society national prostate cancer detection project , in which the overall rate of detection was ٢.٨٪ in the first year , ١.٩٪ in the second year and ١٪ in the third year (٨٩) .

The cancer detection rate using measurement of PSA is between ٢٪ and ٤٪ and approximately ٣٠٪ of men with an elevated PSA will have prostate cancer confirmed by biopsy while unfortunately , ٢٠٪ of men with clinically significant prostate cancer will have PSA values within normal range .There is therefore some controversy over the usefulness of PSA alone as a screening procedure ,currently ;a number of prospective trails aimed at determining whether or not PSA testing reduces the disease specific mortality of prostate cancer are underway(٤) . However the use of the PSA for diagnostic testing was approved by the FDA in ١٩٩٤; this affirms that in some patients PSA screening detects clinically important and potentially life threatening disease .(٩٠)

## ١.٢.٤ Genes and Proteins Correlating with Prostate Cancer Presence and Progression :

The direction of the United States congress and super headed by the national cancer institute, support for basic and translational research in prostate cancer has expanded dramatically since ١٩٩٢.This has resulted in a snow slide data, much of it attempting to correlate various gene and protein markers with the prostate cancer presence, progression, or disease free survival some of these markers have also been proposed as a potential therapeutic targets for the prostate cancer treatment, however to date, none of these candidate markers has been adequately validated for clinical use, and no replacement for PSA is visible on the scientific horizon (٩١) . Researches provide information on ٩١ genes and their encoded proteins , all of which have a potential role in prostate carcinogenesis and progression and all display some level of correlation with one or more of the following factors :

١. Presence of the prostate cancer.

٢. Disease progression.

٣. Cancer recurrence.

٤. Prediction of response to therapy.

٥. Disease free survival.

Of these ٩١ new prostate cancer potential markers, ٨٩ are proteins and ٢ transcripts gene and only five potential prostate cancer diagnostic markers are the most important ones and these are:

١. Chromogranin A (GRN-A).

٢. Prostate stem cell antigen (PSCA).

٣. Prostate specific membrane antigen (PSMA).

٤. Telomerase reverse transcriptase (TERT).

٥. Glutathione (GSH) system , which is one of the antioxidants could be one of these markers , although its enzyme glutathione-S-transferase P١ (GSTP١), which is the one extensively studied and has the highest correlation with prostate cancer and carries the most promising outcome for the detection as well as treatment modalities for prostatic carcinoma (٩١). GST P١, glutathione s-transferase- P١ is a member of a large family of glutathione s-transferaseses that function to protect cells from oxidative insults (٩٢). The biological principle for selecting this marker is its role in preventing damage to cells by neutralizing free radicals; this marker is also unique in its capacity to provide a simple methylation based – detection method for an important epigenetic phenomenon (٩٢). Studies have shown high sensitivity for this marker to detect the presence of both prostatic intraepithelial neoplasia (PIN) and prostate cancer , an ability to distinguish these from BPH , and a prevalence of methylation in the range of ٦٠-٨٠٪ in prostate cancer (٩٣). There is no correlation between GST P١ methylation status and the PSA levels , making GST P١ a potential early and independent marker for prostate cancer ; the ability of GST P١ hypermethylation to distinguish between BPH and prostate cancer is well documented (٩٤).

Validating, successfully GST P١ methylation testing of cells derived from serum and urine samples may have clinical usefulness for both early detection of prostate cancer and post treatment monitoring of disease; however, there is one drawback for the use of GST P١ expression and hyper methylation is the need for advance technology combined with PCR, so studying the level of glutathione GSH in its reduced form in serum as tumor marker for prostate

cancer as an alternative for GST P١ despite of its being less sensitive and for achieving good results (٩٤) .

## ١.٣ Glutathione(GSH)

Glutathione (GSH) is the most important nonprotein thiol present in human and animal cells as well as in plant and bacteria .

It was discovered by F.G.Hopkins in ١٩٢١ and identified as the tripeptide gammaL-glutamyl-L-cysteinyl-glycine (٩٦,٩٧) as shown in fig. [٥]

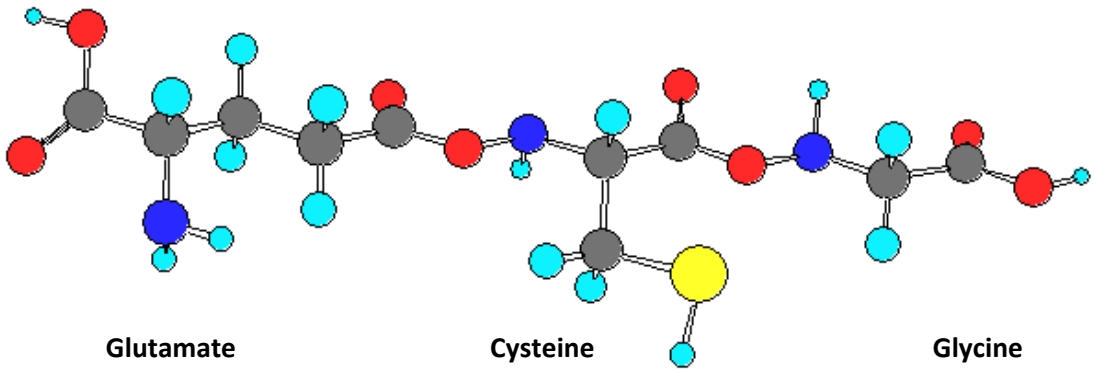
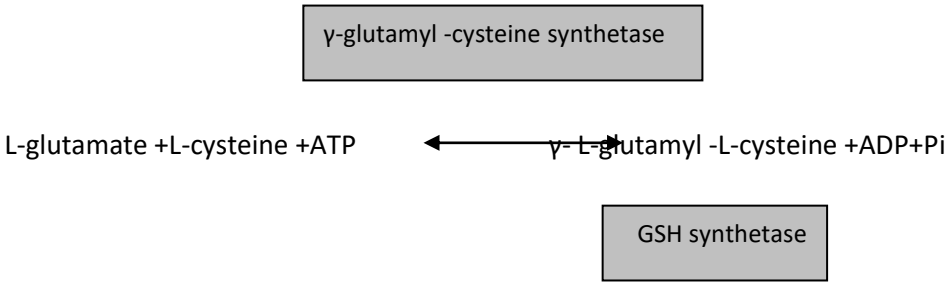



**Fig. [٥]** Shows the structure of glutathione (٩٥)

GSH is synthesized in two steps catalyzed by gamma – glutamyl –cysteine synthetase and GSH synthetase respectively, as shown in fig. [٦]



γ-L-glutamyl- L-cysteine + glycine +ATP ⟵ GSH+ADP+Pi

**Fig. [٦]** GSH synthesis pathway

The first reaction is considered the rate limiting and inhibited by GSH , suggesting a physiologically significant feedback control of GSH synthesis, (٩٨,٩٩) . Glutathione is a naturally occurring tripeptide whose nucleophilic and reducing properties play a central role in metabolic pathways as well as in the antioxidant system of most aerobic cells ; GSH plays a critical role as a coenzyme with a variety of enzymes including , glutathione peroxidase ,glutathione-s-transferase and thiol traneferase ;GSH also plays major roles in drug metabolism , calcium metabolism (٩٩) . GSH is crucial to a variety of life processes including the detoxification of xenobiotics, maintenance of the –SH level of proteins , thiol-disulfide exchange ,removal of hydro peroxides and free radicals , and amino acids transport across membranes (٩٨) . Physiological values of the intracellular GSH generally range from

١ to ١٠ µM. Glutathione can not enter all cells directly and therefore must be made inside the cells ,from its three constituent amino acids , glycine ,glutamate and cysteine ,the rate at which glutathione can be made depends on the availability of intracellular cysteine ,which is relatively scarce in food stuffs. (٩٩) . GSH usually mediate the intracellular reduction of hydrogen peroxide by the intracellular NADPH. Thus the GSH as an antioxidant inactivate the $H_2O_2$ free radical risk effect on the cell function and viability (٩٥).

NADPH + $H^+$ G-S-S-G $_2H_2O$

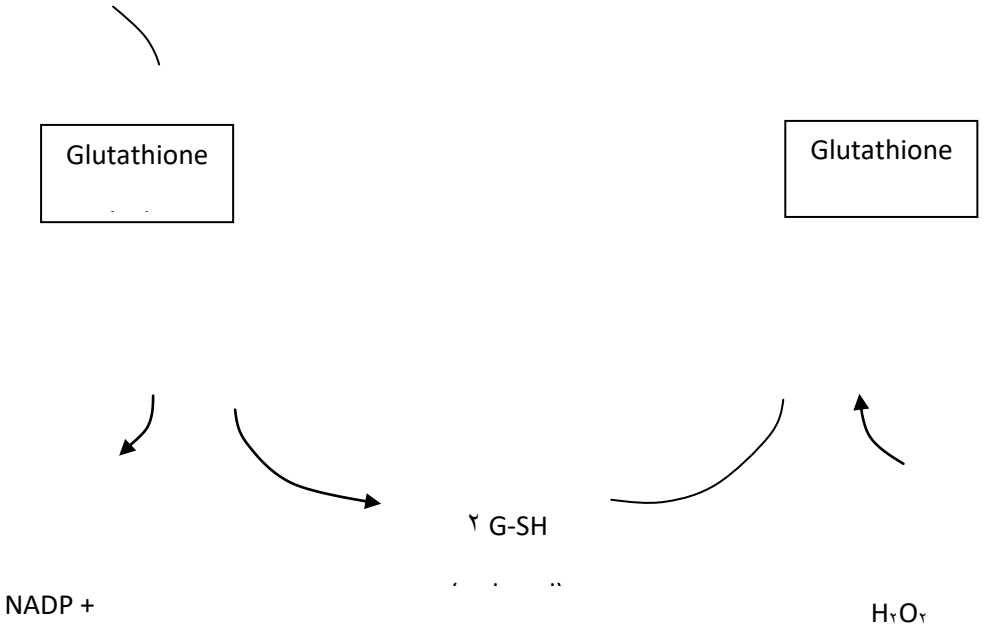



**Fig. [٧]** Shows the reduction reaction of the hydrogen peroxide by the NADPH mediated by glutathione (٩٥)

Cysteine molecule has a sulfer –containing portion , which gives the whole G-SH molecule its biochemical activity ,i.e. its ability to carry out the following vitally important functions. Firstly ,GSH is the major antioxidant produced by the cell, protecting it from free radicals (oxygen radicals , oxy radicals ) ,which is if left unchecked will destroy the key cell component (membrane and DNA) (٩٩) (٩٦) . Oxy radicals are generated in many thousand mitochondria located inside each cell ,where nutrients like glucose are burnt using oxygen to make energy .(mitochondria can be thought of as the batteries that provide the power for the cell to operate ). In addition , GSH recycles other wellknown antioxidants such as vitamin C and vitamin E keeping them in their active state (٩٨) (١٠٠) . Secondly, GSH is a very important detoxifying agent enabling the body to get rid of undesirable toxins and pollutants ; it forms a soluble

compound with the toxins that can then be excreted through the kidney or the gut (١٠٠,١٠١) . Thirdly , GSH plays a vital role in maintaining a normal balance between oxidation and anti-oxidation . This, in turn , regulates many of the cells vital functions such as the synthesis and repair of DNA, the synthesis of proteins and the activation and regulation of enzymes (٥٨). Fourthly , GSH is required in many of the complex steps needed to carry out an immune response (e.g. it is needed for the lymphocytes to multiply in order to develop a strong immune response and for killer lymphocytes to be able to kill undesirable cells such as virally infected cells and cancer cells (٥٩) . The importance of the glutathione can not be overstated; it has multiple roles as indicated and indeed, as one examines each system or organ more closely , the necessity for glutathione becomes increasingly evident , in spite of the fact that glutathione values decline with age but higher values in older people are seen to correlate with better health ; low concentration of GSH has been implicated in numerous pathological conditions , including diabetes, alcoholic liver disease , AIDS, acute hemorrhagic gastric erosions , cataracts , xenobiotecs induced oxidative stress and toxicity and aging(١٠٢). However ,GSH concentration in the blood may act as a beneficial indicator of disease risk in humans (١٠٢). It has been reported that the level of the oxidized GSH (GSSG)in serum may serve as an index of several diseases and aging (١٠٣) . Prostate cancer is a disease associated with aging , also commonly associated with increasing age because there is a shift in the pro-oxidant -antioxidant balance of many tissues toward a more oxidative state , i.e., increase oxidative stress ; therefore, it is hypothesized that androgen exposure , which has long been associated with the development of prostate cancer , may be a means by which the pro oxidant – antioxidant balance of prostate cells is altered , since the physiologic levels of androgens are capable of increasing oxidative stress in androgen

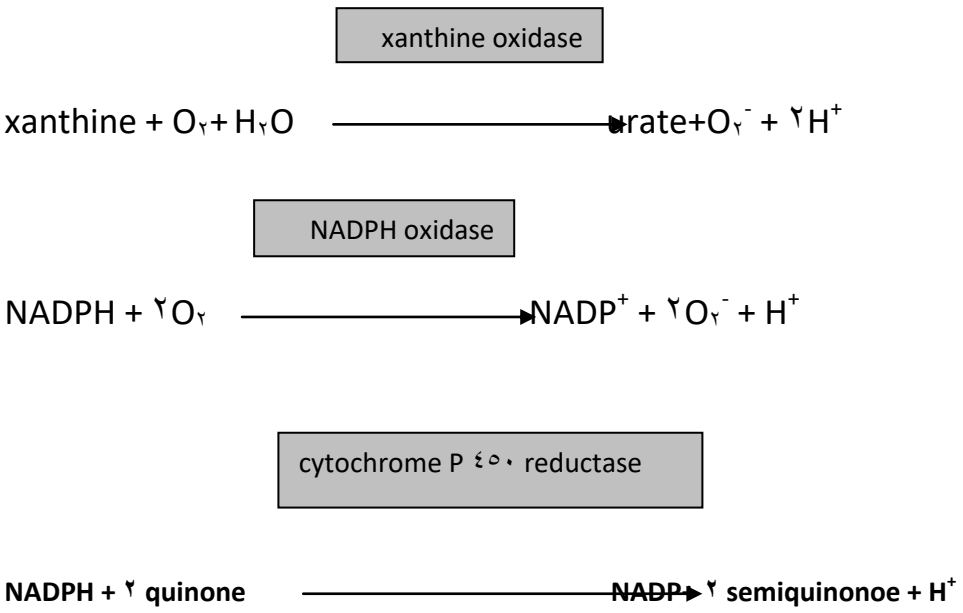
responsive prostate carcinoma cells . The evidence suggests that this result is due to , in part , to increased mitochondrial activity . androgens also alter intracellular glutathione levels and the activity of certain detoxification enzymes such as gamma- glutamyl- transpeptidase (GGT) that are important for the maintenance of cellular pro oxidant – antioxidant balance (١٠٤) .

## ١.٣.١ Oxidants (Free Radicals) in Human Body:

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to x-ray , ozone , cigarette smoking , air pollutants and industrial chemicals (١٠٥) .

Free radicals (oxidants) formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions as shown

Fig [٨] (١٠٥) .




[enzymatic free radical formation]

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^-$

$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^-$

[non enzymatic free radical formation ]

Fig [٨] Examples of free radicals productions (١٠٥) .

Enzymatic reactions which serve as sources of free radicals (oxidants) include those involved in the respiratory chain , in phagocytosis , in prostaglandin synthesis and in the cytochrome P ٤٥٠ system (١٠٥) . Free radicals also arise in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations ; if free radicals are not inactivated , their chemical reactivity can damage all cellular macromolecules including proteins , carbohydrates , lipids and nucleic acids as shown in fig [٩] (١٠٥)

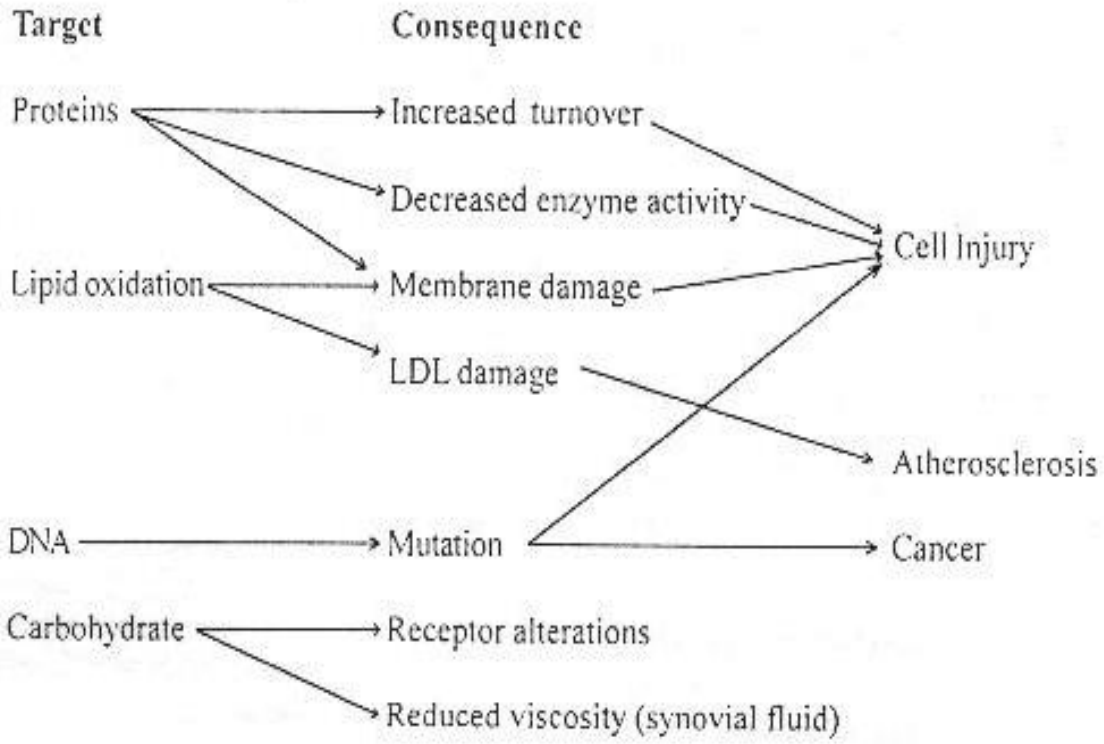


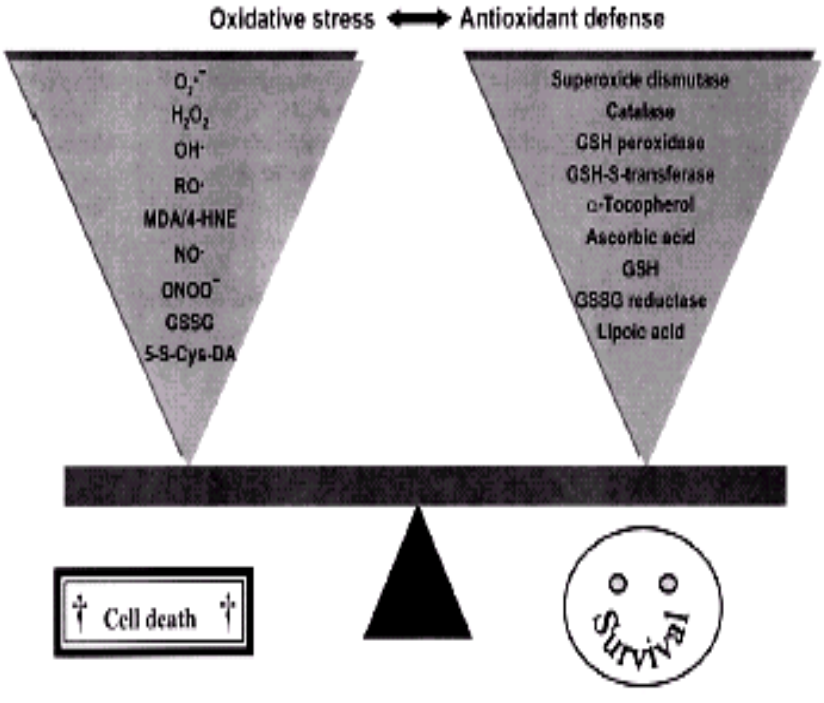
Fig [٩] [Free radical damage] free radicals destructive effects on proteins and DNA are implicated in the causation of cancer (١٠٥).

The theory associating free radicals with the aging process has also gained widespread acceptance i.e. there are increased levels of oxidants with increasing age , thus affecting the normal oxidant–antioxidant balance , so a well-accepted fact is the increasing incidence of disease with advancing age ; this gives a reasonable explanation for the association of age and disease is based on the implication of free radical reaction in the pathogenesis of several diseases and disorders ; therefore, free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body ; however, such normal changes with age are relatively common to all, which is superimposed on this common pattern that is influenced by genetic and environmental differences that modulate free radical damage ; these are manifested as diseases at certain ages determined by genetic and environmental factors and cancer is the most important one of them which is the major cause of death ; considered as a silent free radical disease , cancer initiation and promotion is associated with chromosomal defects and oncogene activation ,it is possible that the endogenous free radical reactions like those initiated by ionizing radiation , may result in tumor formation (١٠٥). Free radicals , however, are not always harmful ; they also serve useful purposes in human body , several observations indicate that the oxygen radicals in living system are probably necessary compounds in the maturation processes of cellular structures , also white blood cells as a part of the immune system , release free radicals to destroy invading pathogenic microbes as part of the body defense mechanism against disease , hence , the complete elimination of these radicals would not only be impossible , but also harmful (١٠٥). Free radicals have important regulatory functions in physiological

processes and they constitute an important part of the defense against invading microorganisms ; when free radicals are produced in excess , they may attack and injure several organs and may cause several diseases, including cancer (١٠٦).

A system of enzymes including glutathione (GSH) peroxidase , super oxide dismutase (SOD) and catalase ,which decrease the concentrations of most harmful oxidants in the tissues , anti oxidants such as Glutathione (GSH) , Vit.C , Vit.E constitute another line of defense against free radicals damage (١٠٥,١٠٦,١٠٧,١٠٨). Halliwell introduced a border definition, an anti-oxidant is any substance that , when present at a low concentration compared to those of an oxidizable substance ,significantly delays or prevents the oxidation of that substrate ; the term [oxidizable substrate ] includes almost every thing found in living cells including proteins , lipids, carbohydrates and DNA(١٠٨).

Bagchi and Puri introduced another definition , an antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and to neutralize it , thus reducing its capacity to damage cells, tissues and organs



(١٠٥).

Fig [ ١٠ ] balance of oxidants and antioxidants active systems ( ١٠٦) .

## ١.٣.٢ Glutathione Protects Cells from Oxidative Stress

Several powerful free radicals (oxidants) are produced during the course of metabolism, in both blood cells and most of other cells of the body. These include super oxide ($O_2^{\bullet}$) ; hydrogen peroxide ($H_2O_2$), peroxyl radicals ($ROO^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$) , the last is a particularly reactive molecule and can react with proteins , nucleic acids , lipids and other molecules to alter their structure and produce tissue damage ; super oxide is formed in the red blood cells by the auto-oxidation of the hemoglobin to met-hemoglobin (٥٤).

Reaction (١) $O_2 + \bar{e} \rightarrow O_2\bar{\bullet}$

In other tissues , it is formed by the action of enzymes such as cytochrome P ٤٥٠ reductase and the xanthine oxidase ; when stimulated by contact with bacteria , neutrophils exhibit a respiratory burst and produces super oxide in a reaction catalyzed by NADPH oxidase (٥٤).

$$2O_2 + NADPH \xrightarrow[\text{oxidase}]{NADPH} 2O_2^{\cdot} + \bar{N}ADP + H^{+} \quad \text{…….. reaction (٢)}$$

super oxide spontaneously dismutates to form $H_2O_2$ and $O^{2}$ ; however , the rate of this same reaction is speeded up tremendously by the action of enzyme super oxide dismutase reaction (٣).

Hydrogen peroxide $H_2O_2$ is subjected to a number of fates the enzyme catalase present in many types of cells , converts it to $H_2O$ and $O^2$ reaction (٤).

Neutrophils possess a unique enzyme myeloperoxidase that uses $H_2O_2$ and halides to produce hypohalous acids reaction (٥) .

The selenium – containing enzyme glutathione peroxidase will also act on reduced glutathione (GSH) and $H_2O_2$ to produce oxidized glutathione (GSSG) and $H_2O$ this reaction (٦) .

This enzyme can also use other peroxides as substrates , OH and OH can be formed , from $H_2O_2$ in a non enzymatic reaction catalyzed by $Fe^{+2}$ the Fenton reaction, reaction (٧) .

$O^{2-}$ and $H_2O_2$ are the substrates in the iron catalyzed Haber –Weiss reaction , reaction (٨).

reaction (٨)., which also produces OH and $OH^-$ , super oxide can release iron ions from ferritin . Thus ,production of OH may be one of the mechanisms involved in tissue injury due to iron overload (hemochromatosis) (٥٤).

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as Pro oxidants. On the other hand , compounds and reactions disposing of these species , scavenging them , suppressing their formation ,or opposing their actions are anti-oxidants, which include compounds such as NADPH ,GSH, ascorbic acid and Vit.E ; in normal cells there is an appropriate pro-oxidants : anti oxidants balance ; however, this balance can be shifted toward the pro-oxidants when the production of oxygen species is increased greatly (e.g. following ingestion of certain chemicals or drugs ) or when levels of anti- oxidants are diminished (e.g. by inactivation of enzyme involved in disposal of oxygen species and by conditions

that cause low levels of the anti-oxidants; this state is called [oxidative stress] and can result in serious cell damage if the stress is massive or prolonged ; oxygen species are now thought to play an important role in many types of cellular injury and cell death. (٥٤).

## ١.٣.٣ The Role of Glutathione in Tumor Cell

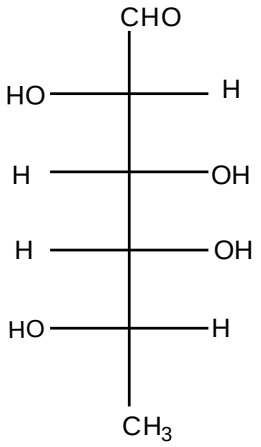
Cancer cells and normal cells are known to respond differently to nutrients and drugs that affect glutathione status . Numerous studies have shown that tumor cells have elevated levels of glutathione, which confers resistance to chemotherapy drugs , one of the challenges of cancer therapy is how to deplete tumor cells of glutathione , so as to make them more vulnerable to the effect of chemotherapy drugs , while at the same time allowing normal cells to remain relatively unaffected by chemotherapeutic drugs ; a number of new findings have emerged that reaction (٨). take into consideration the role of glutathione in pathways that promote programmed cell death [apoptosis] (٥٤). In cancer cells ,a German study has reported that glutathione (GSH) plays a critical role in cellular mechanisms that result in cell death or apoptosis . The study found that cancer cells resistant to apoptosis had higher intracellular GSH levels ; depletion of glutathione in these tumor cells made them more vulnerable to the effect of anticancer drugs or the gene that promote apoptosis[ CD٩٥ or APO-١/ FAS ] . The researchers concluded that apoptosis resistance in tumor cells depends at least , in part , on intracellular GSH levels (١٠٩). In another study conducted in Spain , researchers found that lowering GSH concentration may be convenient not only for the efficiency of chemotherapy but also to induce a rather fast and direct apoptosis mechanisms in tumor cell (١١٠). Based on that premise

that the glutathione s-transferase enzyme is expressed at high levels in many tumors , researchers at the fox chase cancer center in Pennsylvania,went on to design a novel pro-drug [PABA/NO].The glutathione s- transferase enzyme , in tumor cells converts PABA/NO to lethal nitric oxide , resulting in death of tumor cells ; the pro-drug was shown to have anti-tumor effect in animal model for human ovarian cancer (١١١).

## ١.٤ Alpha-L-Fucose:

A recent data suggest that the sugar , alpha-L-fucose is essential for the expression of the fully transformed phenotype in many human cell populations ; evidence for such a role comes from studies of common Adenocarciomas and Hodgkins disease as well as certain melanomas , Neuroblastomas and Leukemias ; reaction (٨). Alpha-L-fucose is one of the eight essential sugars the body requires for optimal function of cell communication , the L-form is the only common form of the sugar while the D-form is a galactose analogue (١١٢).

Fucose [ ٦- carbon deoxy hexose ]or ٦-deoxy-L-galactose or L-methyl pentose a monosaccharide present in low concentrations in normal circulation (١١٢) .



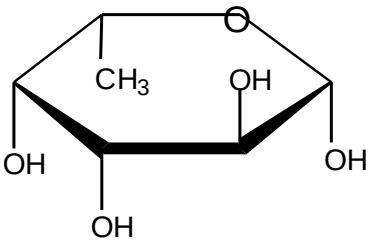



Fig [١١] the structure of alpha-L-fucose (١١٣)

## ١.٤.١ Alpha –L- Focuse Occurrence

Fucose is found in a wide variety of natural substances from many different sources and commonly incorporated into human glycoproteins and glycolipids ; it is found at the terminal or periterminal positions of many cells – surface oligosaccharide ligands that mediate cell recognitions and adhesions signaling pathways , although much remains to be learned regarding its specific roles in biological function (١١٢). An increasing number of studies of its absorption ,distributions ,metabolisms and excretion have been published ,and the biological activities of fucose and its glycoconjugates are being characterized (١١٤,١١٥). Fucose containing glycoproteins and glycolipids are now known to be important in cell communications involved in both normal function and disease ,functioning as receptors on cell surfaces , fucose glycoconjugates become an essential part of disease processes , such as cancer , inflammation and immune disease (١١٤) .

## ١.٤.٢ Alpha-L-Fucose Absorption

Fucose can be readily absorbed when given orally , absorbed from the small intestine and incorporated directly or after metabolism into glycoproteins and glycolipids, unabsorbed fucose is metabolized by intestinal bacteria ,although animal studies show that fucose is absorbed from small intestine in vitro by non- active diffusion transport process (١١٦).

Many cells also possess a specific facilitative transport for fucose (١١٧).And fucose can inhibit the transport of some other actively transported sugars ; thus, it appears that there is a potential for regulation of fucose entry into certain cells based on fucose concentration and the presence of other sugars (١١٨).

## ١.٤.٣ Alpha-L-Fucose Distribution

Fucose is widely distributed throughout the body in glycoproteins and glycolipids consistent with a cell – cell communication role for fucose glycoconjugates (١١٤) (١١٩,١٢٠) .

Although , endogenous fucose produced from other sugar precursors is utilized in those processes, exogenous ingested or injected fucose is also incorporated into glycoproteins and glycolipids (١١٥),for example fucose is incorporated in vitro into photoreceptor layer of human eye retina , where it may be involved in biosynthesis of rod cell glycoproteins (١٢١), fucose is also incorporated into human skin epidermal cells in vitro , where it may be involved in synthesis of membranes of cells involved in maintaining skin hydration ; fucose is also distributed in various normal skin structures , such as glands and vascular endothelium (١٢٢-١٢٣).

Fucose glycoconjugates have been identified in various other tissues as well as , for example , fucose glycoproteins are found in animal and human brain cells (١٢٤,١٢٥). They are present in synaptic junction areas where nerve cells meet , implying a role in synaptic membrane involvement in nerve impulse transmissions , fucose glycoconjugates are also very rich in human tests germ cells , which are altered during germ cells differentiation

(١٢٥,١٢٦). Fucose glycoconjugates have been localized to the proximal tubules of human kidney implying diverse functional roles for these complex carbohydrates in this important organ , fucose is also distributed in macrophages , which are critically important cells in the immune system (١٢٧,١٢٨). Additionally , fucose is found in glycoproteins and glycolipids red blood cells antigens , which are involved in determining blood type ; fucose distribution is altered in certain disease states ,e.g., there is a higher fucose content in the serum glycoproteins of cancer paiteints (١١٤),(١٢٩,١٣٠). Serum levels of free non- glycoconjugates fucose are increased in patients with diabetes (١٣١,١٣٢) . Cancer(١١٤). And also in various types of malignant tumors , breast cancer , pancreas cancer , multiple myloma , stomach cancer , colon cancer , rectum cancer , hodgkins disease ,chronic myeloblastic leukemia (١٣٣). And even in thalassemia (١٣٤). Fucose content of mucins from colon cancer patients is reduced (١٣٥). Fucose studies are also showing that it plays a significant role in many diseases including pathogenesis and its spread ; research is still on-going but showing promise in the areas of inhibiting leukemia and breast cancer , including the suppression of tumor growth ; some studies conclude that fucose and mannose appeared to be the most effective of the essential sugars it came to slowing the growth of cancer cells ; levels of fucose are low in those with rheumatoid arthritis and supplementations is safe and harmless and surprisingly effective treatment (١٣٦).A nuclear magnetic resonance spectra from malignant cells and tissues suggest that fucose was detectable in those cells but was limited or undetectable in non-malignant cells from which they were believed to be derived (١٣٦).

### ١.٤.٤ Alpha-L-Fucose Metabolism

The biosynthesis of L-fucose include conversion of guanosine -٥-D-mannosyl diphosphate [ G-DP-man] (A) into [GDP-Fuc.] (D), an oxidation – reduction process used NADP+ as a cofactor , the hydroxyl group at position ٤ or carbon atom no. ٤ of (A) oxidized to keton group and the primary alcohol is converted into methyl group , epimerization of (B) at C-٣ and C-٥ to afford (C) followed by reduction of ٤-ketone to hydroxyl by NADPH leads to GDP Fucose production (١٣٧,١٣٨).

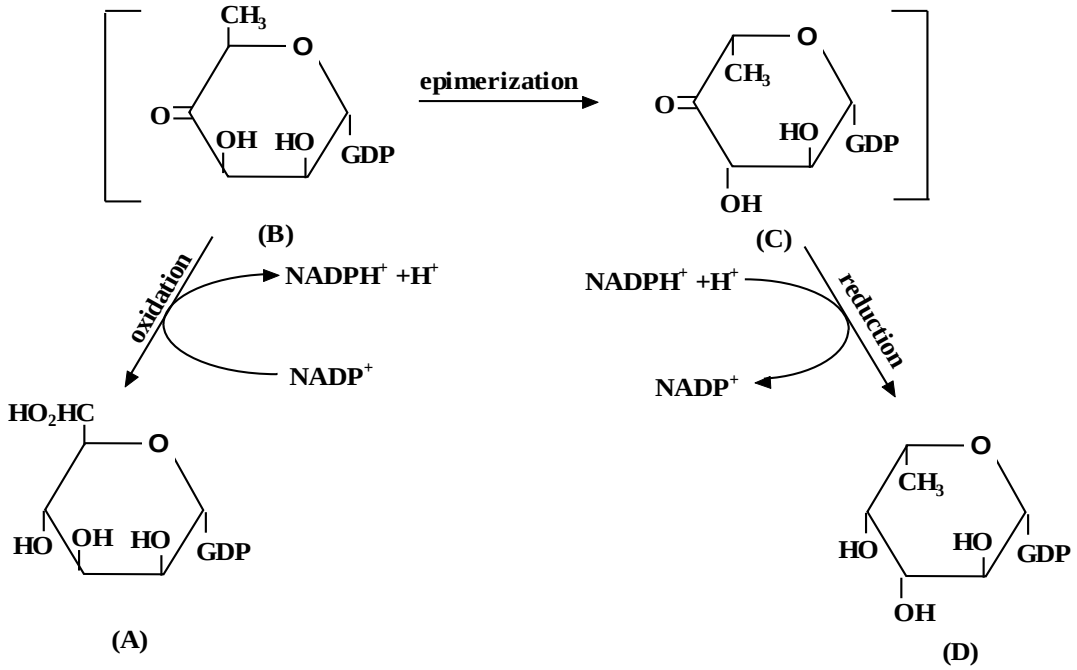



**Fig** [١٢] the biosynthesis of alpha-L-fucose[Christopher, et.al, ١٩٩٨] (١٣٨).

Fucose metabolism is important for the formation of glycoproteins and glycolipids [١٣٩]; endogenous fucose is produced in sugar – nucleotide form [GDP- fucose ] from [GDP-mannose] via a dehydratase and an epimerase – reductase enzyme [١٤٠].

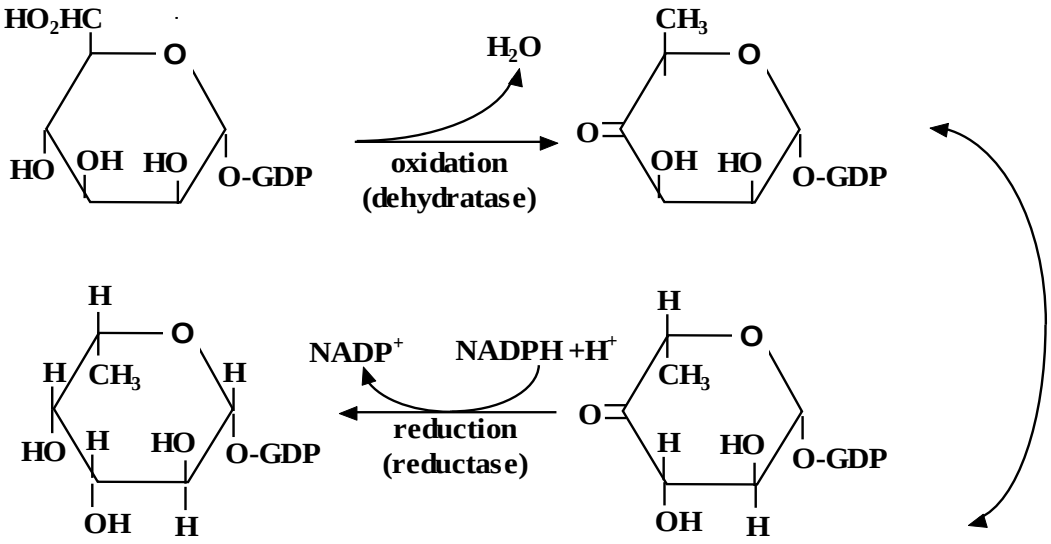



**Fig** [١٣] GDP-L-Fucose [biosynthesis of endogenous alpha l-fucose ] (١٤٠).

Exogenous [i.e. dietary] fucose is converted to fucose -١-phosphate by fucokinase and then to GDP- Fucose by a pyrophosphorylase enzyme , exogenous fucose can be incorporated directly into fucose –containing proteins , other macromolecules with little or no metabolism to other sugars (١٤١,١٤٢).

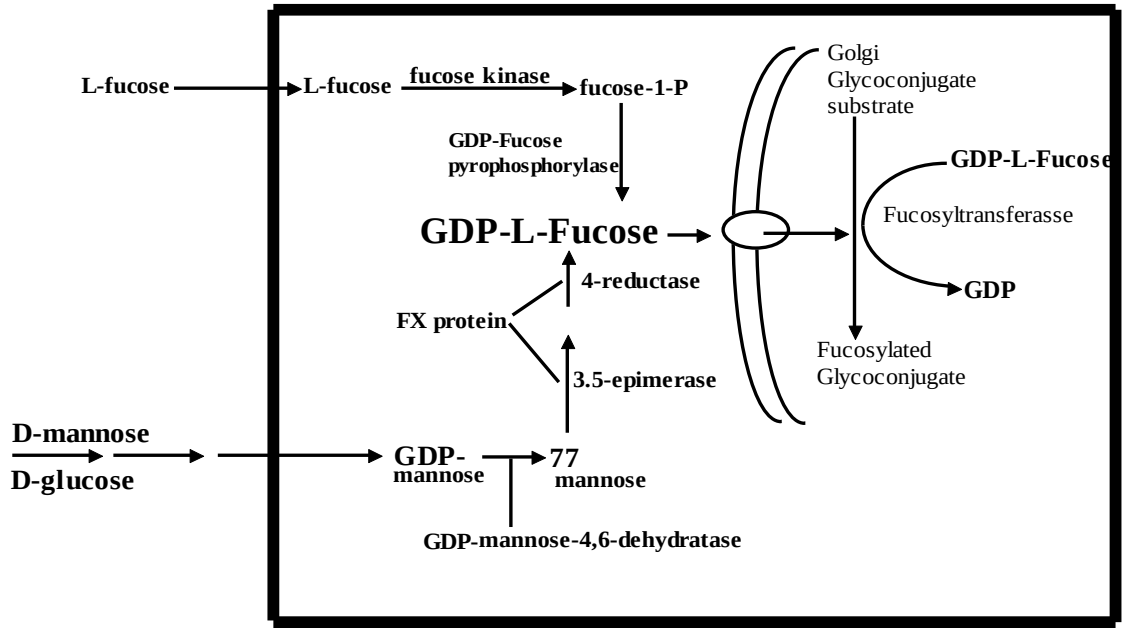



**Fig**. [١٤] Fucose metabolic pathways [pastuszak,et.al.,١٩٩٨] (١٤٣).

## ١.٤.٥ Alpha-L-Fucose Biological Function And Activities

Fucose appears to be an important immune modulator which is active in inflammatory diseases (١٤٤).

Fucose stimulated rabbit macrophage migration suggests that it might be part of macrophage cellular receptor site for migration enhancement factor , an essential component of the immune system (١١٦)(١٤٥).

Also it is now known that fucose glycoconjugates are part of eliminating or reversing such disease processes as cancer , inflammation and immunity.

Fucose concentrations are found in such areas as :- at the junctions between nerves , implying that deficiency could affect transmission , in the proximal tubules of human kidney , indicating the vital need for proper kidney function . In the testes ,suggesting that it plays an important role in reproduction. Also in the outer layer of skin , where it may be involved in maintaining skin integrity. (١٤٥)

Fucose is profoundly important for efficient neuron transmission in the brain , fucose is known to influence brain development and may also help in improving brains ability to create long term memories , several studies have shown that , decreased fucose containing proteins leads to the development of amnesia. Also, Fucose is a powerful immune modulator; it is distributed in macrophage , which is important to immune function . Fucose is also particularly active in inflammatory disease and has the ability to suppress skin reactions as contact dermatitis.(١٤٥)

Also injected fucose into lab animals is found to be a possible treatment of cancer . U- Fucoidan , a complex polysaccharide found in brown seaweed , suggest that the sugars were able to break down the DNA within each cancer cell by enzyme action and the destruction was self-induced;thus ,

Fucose appears to inhibit cancer growth and metastasis (١٤٦,١٤٧); fucose also has therapeutic implications in treating or preventing respiratory tract infections (١٤٨),

Although fucose specific lectins act as a tool for laboratory diagnosis of major human malignancies , including leukemias and carcinoma of the colon , stomach and breast ,also fucose specific lectins in cancer research and diagnosis shows that the sugar alpha-L-fucose is over expressed in many

human malignancies , especially on specific glycoproteins and glycolipids , found on cancer cell surfaces; many of these molecules are known as suspected mediators of cell-cell adhesion or cell signaling motility , or invasion .(٧٢)

Cancer cell surface alpha –L– fucose is a logical target for selective therapeutic ablation ; reduction of fucose content on the surfaces of malignant cells should effectively cripple the cells physiologic functions by altering or dysregulating cell-cell or cell- matrix interactions i.e. fucose is critical for maintaining the malignant phenotype (٧٢) (١٤٩).

## ١.٥ The correlation between pentose phosphate pathway and glutathione , L-Fucose as tumor biomarker

Tumor biomarkers are either intracellular proteins or cell surface glycoproteins and glycolipids released into the circulation and detected by immuno assays(١٥٠)

The pentose phosphate pathway handles ٥٪ to ١٠٪ of metabolized glucose in normal red cells in the process generating ٢mol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for each ١ mol of glucose metabolized ; NADPH is an essential cofactor for the enzyme glutathione reductase , which maintains glutathione in the reduced state necessary for the detoxification of toxic oxygen products such as superoxide anion ($O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals(OH) as shown in fig [١٥]

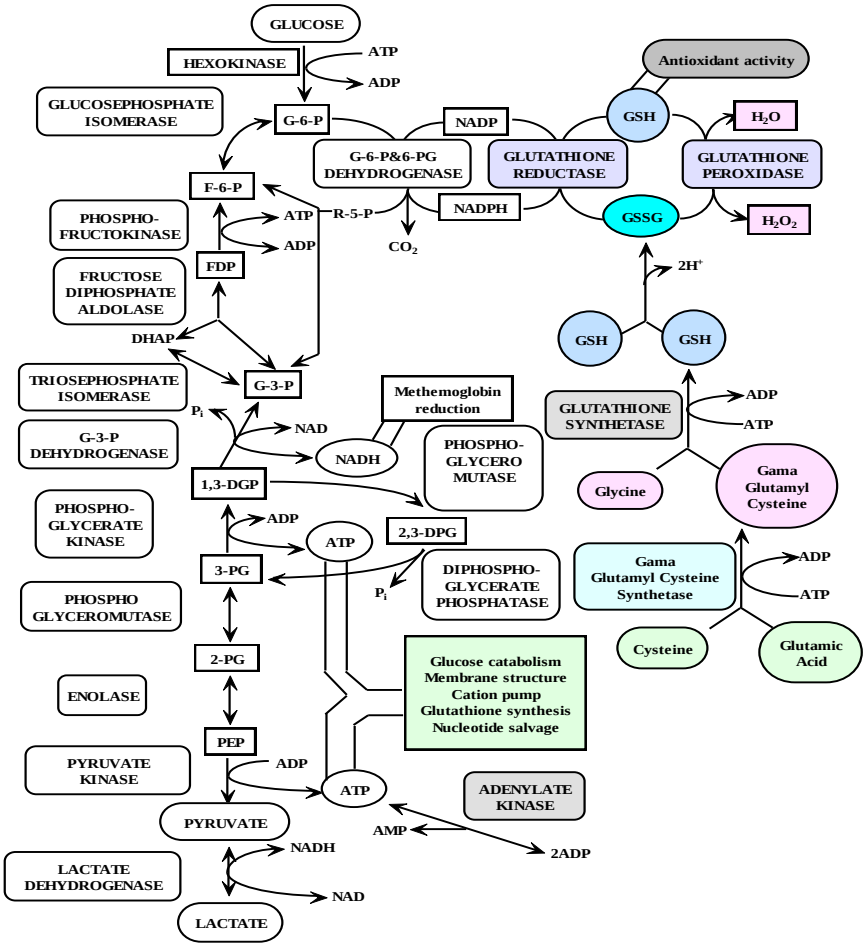



Fig [١٥] The pentose phosphate pathway and glutathione in red blood cells (٢٢).

The enzymatic free radical formation resulting in consuming more (NADPH) in a reaction being catalyzed by NADPH oxidase enzyme and the cytochrome P٤٥٠ reductase lead to increase the level of toxic oxygen products especially , superoxide ($O_٢$) ; moreover , reduction of NADPH concentration , as a cofactor for the glutathione reductase enzyme which is also reduced causing finally a noticeable reduction of the reduced glutathione (GSH), and in the absence of reduced glutathione toxic oxygen products (oxyradicals) can damage red cells lipids , proteins and results in hemolysis and anaemia.

However , normal red cells are continually subjected to the oxy radicals as a result of intracellular hemeoxidation being strictly and obviously concerned

with the non-enzymatic free radical formation in which the oxidation of ferrous iron $Fe^{+2}$ to ferric iron $Fe^{+3}$ that will rise the intracellular levels of free radicals superoxide and hydroxyl group, resulting in an imbalance between pro-oxidant and anti-oxidant levels, so the fate of reduced glutathione level (GSH) inside the cells opposed by rising levels of free radicals that if not inactivated their chemical reactivity can damage all the cellular macromolecules including proteins , carbohydrates , lipids and nucleic acids , as shown in fig [١٦ ]. (١٥١)

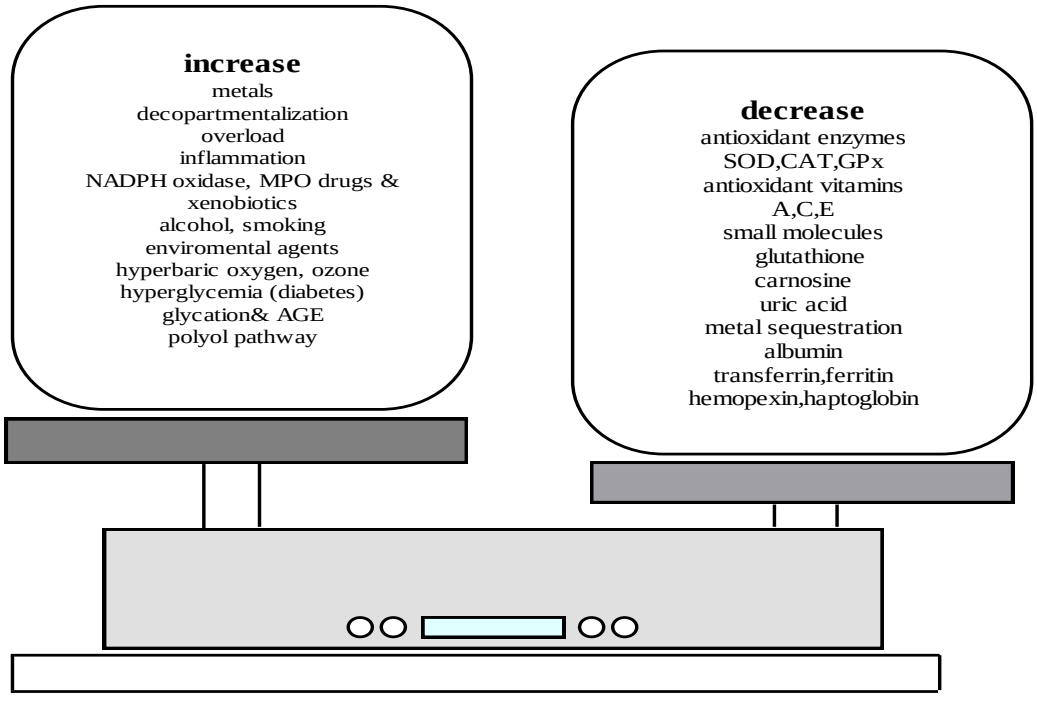



Fig. [١٦] Oxidative stress an imbalance between pro-oxidant and anti-oxidant system (١٥١)

Under conditions of oxidative stress the pentose phosphate pathway can increase in activity to use ٥٠٪ or more of the available glucose ; this increase in activity is stimulated by NADP and inhibited by NADPH , thereby tightly coupling intracellular antioxidant supply and demand (٢٢).

L-Fucose (٦-deoxy-L-galactose), is a monosaccharide that is a common component of many glycoprotein's and glycolipids produced by mammalian ; it gains two structural features that distinguish it from other six carbon sugars present in mammals:

١. lack of OH group on the carbon at the $٦^{th}$ position(C٦).

٢. L-configuration. (١٥٢)

glycoprotein's and glycolipids are usually located mainly in the outer leaflet of the plasma membrane as one of its major composition , embedded in its fluid phospholipids bilayer as shown in fig [١٧]

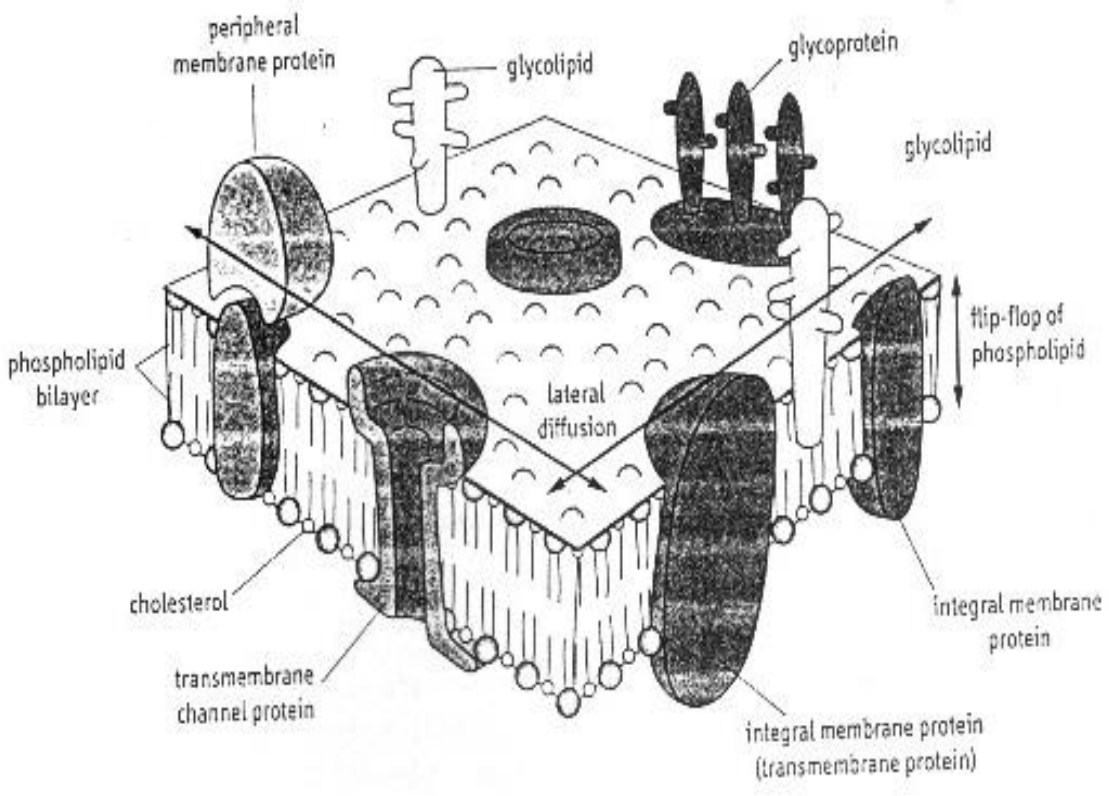



Fig.[١٧] shows glycoprotein's and glycolipids as a major composition of the biomembrane.(١٥١)

Thus , destruction or damage of the cell membrane under the oxidative stress effect of free radicals may result in liberation of more fucosylated glycoproteins and glycolipids(١٥١)

L-fucose as an L-galactose analogue and knowing that one of the physiological importance of galactose is that it can be changed to glucose in the liver and then metabolized ; also , it is a major constituent of the glycoproteins and glycolipids, concerning galactose metabolic pathway and its conversion to glucose , galactose is first phosphorylated by specific hepatic kinase (galactokinase) to form galactose-١-phosphate(Gal-١-P) , the conversion of (Gal-١-P) to glucose-١-phosphate

(Glc-١-P) involves the nucleoside diphosphate sugar intermediate uridine diphosphate glucose (UDP-Glc) ; the (Glc-١-P)arising from the galactose metabolism can be converted to glucose-٦-phosphate (Glc-٦-P) by phosphoglucomutase and thus can enter glycolysis. (١٥١)

(UDP-Glc) is present at only micromolar concentration in cells , so that the availability for galactose metabolism would be quickly exhausted; were it is not for the presence of the UDP-Gal ٤-epimerase the enzyme catalyzes the equilibrium between (UDP-Glc) and (UDP-Gal) provide a constant source of (UDP-Glc) during galactose metabolism, furthermore according to the oxidative stress effect of intracellular free radicals on the cell itself that is formed continuously inside the cells as a consequence of both enzymatic and non-enzymatic reaction resulting and associating with reduced level of GSH as major anti-oxidants to these free radicals that rise to the point expecting to show their chemical reactivity damaging all cellular macromolecules including proteins , carbohydrates , lipids and nucleic acids; thus nucleic acids damage results in an increase of the level of uridine as a pyrimidine base as a uridine monophosphate (UMP) and through pyrimidine –salvage pathway (UMP) converted to uridine diphosphate.

(UDP) in a reaction catalyzed by the (UMP) kinase enzyme, thus increasing level of (UDP) resulting in an increase of (UDP)- ٤-epimerase enzyme synthesis and availability causing an expected accumulation of UDP-Gal causing an increase in its level and concentration intracellularly

that may be under a reversible change to UDP-Glc and enter Glycolysis.

On the other hand , all sugars in Glycoconjugates (glycoproteins and glycolipids) can be synthesized from D-Glucose epimerization and interconversion as shown in fig (١٨) , fig (١٩).

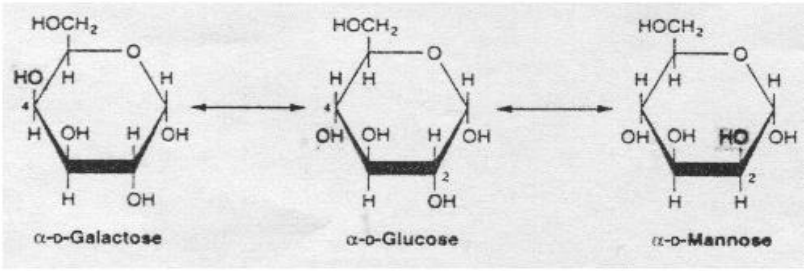



Fig. (١٨)

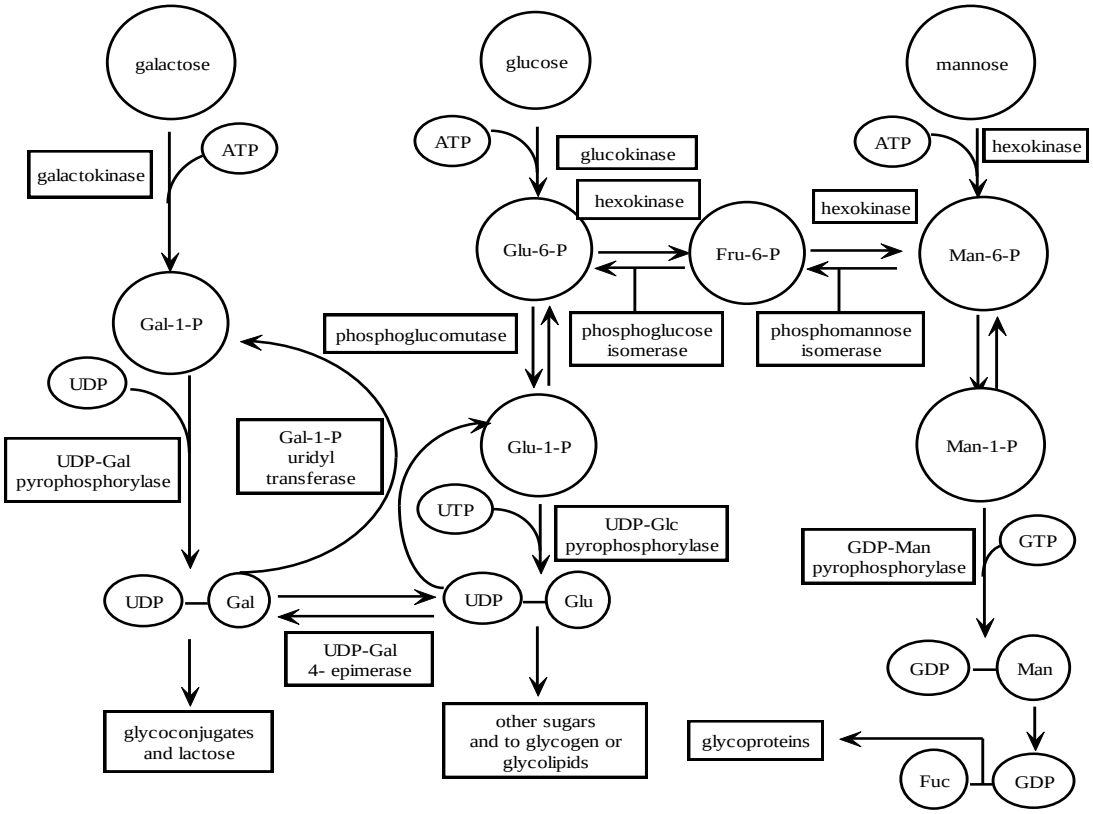



Fig. (١٩)

The reaction that occurs in animal cells to convert glucose to mannose or galactose can be achieved by the epimerization of nucleoside diphosphate sugar UDP-glucose (UDP-Glc.) to UDP-galactose (UDP-Gal.) by UDP-Gal٤-

epimerase enzyme , providing a source of UDP-Gal for glycoconjugates biosynthesis.(١٥١)

However , knowing that one of the biochemical changes often found in fast growing tumor cells is increased rates of aerobic and anaerobic glycolysis, providing metabolites for branch points to numerous other metabolic pathways , including the pentose phosphate pathway , this pathway provides pentoses for the synthesis of DNA and RNA in nucleated cells and NADPH for biosynthetic reactions ; NADPH is also required to maintain reduced glutathione (GSH) which is an essential cofactor for the antioxidant defense system that protects the cell against oxidative stress , in which the rate of generation of reactive oxygen species ROS exceeds our ability to protect ourselves against them , resulting in an increase in oxidative damage of the main biomolecules of plasma membrane (١٥٢).

Also human glutathione peroxidase ١ (h GPX١) , a selenium – dependent enzyme which participates in the detoxification of activated oxygen species by catalyzing reduction of these genotoxic compounds (١٥٣).

Suggesting an association between low levels of selenium and risk of prostate cancer (١٥٤), knowing that the hydroxyl radicals is the most damaging ROS that reacts with the biomolecules primarily by hydrogen abstraction and addition reactions making the cell membrane as the most sensitive site for the free radical damage ; however , the per-oxidative damage to the cell membranes affects the integrity and function of the membrane compromising the cell ability to maintain ion gradients and membrane phospholipids asymmetry, so reactive oxygen species ROS considered as the sparks of the oxidative metabolism and the oxidative stress is the price we pay for using oxygen.

Therefore , reactive oxygen species ROS and reactive nitrogen species RNS cause oxidative damage to all major classes of principal biomolecules of the plasma membrane , in which the body reacts to these oxidative effects by a number of protective antioxidants mechanisms , including sequestration of redox-active metal ions , enzymatic inactivation of major ROS , inactivation of organic radicals by small molecules such as GSH and vitamins A,E and C which can be used as a biomarker of the oxidative stress associated with other major biomolecules that may be liberated as a result of oxidative stress cellular damage as glycoproteins and glycolipids of the cell membrane major outer costituent that can be readily detected in tissues inflammation , which improve the fact that oxidative cellular metabolic stress is increasingly implicated in the pathogenesis of chronic diseases including cancer.(١٥١,١٥٢)

## ١.٦ Prostate Cancer Treatment

Transurethral radical prostatectomy TURP is for obstruction while a localized prostatic carcinoma may be completely removed by radical prostatectomy .

Most tumors are usually discovered when some spread has occurred , hormonal manipulation for metastatic disease , sub capsular orchidectomy slows tumor growth ; other techniques of hormonal manipulation include . LHRH agonists which produce a fall of LH from the ant. pituitary with consequent reduction of testicular secretion of testosterone , e.g. cyproterone acetate or bicalutamide . Stilboesterol is rarely used nowadays as it causes gynaecomastia ,fluid retention , and possible thrembophelic complications finally, local radiotherapy especially for bony metastetic pain (٢٣)

However , the frequency with which a finding of prostate cancer in the elderly is irrelevant to their health , has limited usefulness of screening tests for the early detection of prostate cancer using the serum marker (PSA), so the decision to treat early –stage prostate cancer can only be taken in the full context of the patients life expectations and co-morbid conditions ; therefore , the surgical cure can be achieved only by radical prostatectomy with nerve sparing techniques , reducing the risk of impotence and incontinence. Moreover radical radiotherapy may also offer long term disease control , though with similar risk although , adjuvant treatment with androgen deprivation therapy by monthly depot injections of a gonadotrophin releasing hormone analogue such as goserelin , buserelin or leuprorelin all are (LHRH analogues) or by orchidectomy which improves the survival of patients given local therapy , but at the cost of impotence, concerning metastatic prostate cancer with either local or often skeletal spinal spread is rapidly and effectively palliated in ٧٠٪ of patients by androgen deprivation, the medium duration response is ٢ years and alternative treatments e.g. chemotherapy , have yet to find a role outside clinical trials . On the other hand radiotherapy provides a very effective palliation of painful skeletal metastases and can be delivered systematically by intravenous bone-seeking strontium-labeled bisphosphate for paitients with multiple affected sites .(١٥٠)

## ١.٧ Prostate Cancer Prognosis

prostate cancer prognosis is so variable and dependent on the stage of presentation . patients with clinically localized tumors treated radically may expect a normal life expectancy . those with metastatic disease at presentation have a median ٣- year survival.(٢٣)

many parameters have been evaluated for their ability to predict outcome in patients with prostatic carcinoma such as:

١ clinical stage . This is a very important prognostic determinator , and it is likely to become even more so with the incorporation of newer technology

٢. pathologic stage. This represents the ultimate indicator of tumor extent and , as such , the most accurate predictor of prognosis currently available. So in case with nodal metastases , the prognosis is worse when they are multiple rather than solitary and when they are detectable grossly rather than only microscopically .

٣. microscopic grading . A direct correlation exists between clinical or pathological staging and microscopic grading regardless of the grading system used.

In addition , there is convincing evidence that microscopic grading using Gleason's score system is an independent prognostic variable.

Gleason 's score was by far the best predictor of progression.

٤. surgical margins .A retropubic prostatectomy specimens for prostate cancer patients with clinical stages A and B , showed that positive surgical margins were strongly correlated with progression.

٥. tumor volume . It has been shown that tumor volume as measured in whole sections with morphometric techniques , correlates with Gleason' s grade , capsular penetration, capsular margins of resection , seminal vesicle invasion , and lymph node metastases .

However , measurement of tumor volume does not provide additional prognostic information beyond that given by the parameters particularly

Gleason’ s score , and it is therefore difficult to justify the performance of this costly and time consuming determination in routine practice.

٦. age . On the whole , the patients’ age is not an important prognostic determinator ; it is true that the few reported cases of prostatic carcinoma in men under ٣٥ years of age are usually characterized by poor differentiation and very aggressive behavior .

However , a statistical analysis of prostatic carcinomas occurring after the of ٤٠ years has not shown a definite relationship between age and survival.

٧. Race . Black males have a higher mortality from prostatic carcinoma than white males ; this is due to the fact that they are more likely to have a more advanced stage at presentation . when the disease is stratified for grade and stage , survival is similar in both races.

٨. Method of initial diagnosis. Those patients in whom prostatic carcinomas was diagnosed by TUR have a higher incidence of tumor dissemination than those diagnosed by needle biopsy .

It is not yet clear whether this is the result of the TUR procedure itself or a reflection of the fact that TUR-diagnosable tumors are usually more advanced.

٩. PSA serum levels. The serum level of PSA is related to prognosis in prostatic carcinoma , as an indirect indicator of tumor volume , tumor extension , and response to therapy.

١٠. Androgen-receptor status . Tumors in which the androgen receptor cannot be detected immunohistochemically have a worse prognosis than those in which this marker is present .

Therefore mutations of the androgen-receptor gene have been detected in metastatic prostatic carcinomas and postulated to be the reason for the androgen independence of such tumors.

١١.DNA ploidy. Tumor aneuploidy, as determined by image or flow cytometry , correlates both with a higher Gleason' s score and local and distant spread .

However , there is still no agreement as whether this technique provides independent prognostic information.

The Karolinska Institute authors , who have obtained the most impressive results with this technique, believed that the controversies related to its use are largely related to methodologic inadequacies.

١٢. Chromosomal abnormalities . Patients with clonal karyotypic abnormalities may have shorter survival rates than those with normal karyotypes.

١٣. p ٥٣ expression. The p٥٣ expression tumor suppressor gene has been found to be mutated in a subset of advanced stage prostatic carcinomas

it remains to be seen whether this finding is of value independent of stage and grade.

١٤. ras oncogene . expression of the ras oncogene p٢١ has also been found to correlate with the degree of nuclear anaplasia and therefore with microscopic grading , a feature closely related to prognosis .

However , there is no indication that the expression of this oncogene has independent prognostic value.(١)

# Chapter Two

## ٢. Materials and Methods

### ٢.١ Materials.

### ٢.١.١ Chemicals.

All common laboratory chemicals were obtained from the Firms, Fluka , Hopkins and Williams, Sigma chemicals , and Merck.

All chemicals were used as supplied without further purification. **Table(٢-١)**

| Chemicals | Purity% | Supplied Company |
|---|---|---|
| Ethylenediaminetetra cetic aciddehydrate (EDTA).$_{٢}H_{٢}O$ | ٩٩.٥ | Fluka Switezerland |
| methanol | ٩٩.٨ | Fluka Switezerland |
| Alpha -L- Fucose(standard) | ٩٩.٨ | Fluka Switezerland |
| Cystien hydrochloride | ٩٩ | Merck USA |
| Concentrated $H_{٢}SO_{٤}$ | ٩٩.٥ | Merck USA |
| Tris(hydroxy methylene) aminomethane | ٩٩.٥ | Merck USA |
| ٥,٥-dithiobis(٢-nitrobenzoicacid) | ٩٩.٥ | SigmaChemicals Switezerland |

| | | |
|---|---|---|
| DTNB | | |
| Trichloracetic acid (TCA) | ٩٩ | Hopkins&Williams USA |
| Glutathione | ٩٩.٥ | Biochemicals USA |

**٢.١.٢ Instrumental Analysis and Equipment.**

**Table(٢-٢)**

| Instrument | Supplied Company |
|---|---|
| pH meter microprocessor PH meter HI ٩٣٢١ | HANNA instruments HI- ٩٣٢١ ,(Portugal) |
| Sensitive balance | Sartorius AG GOTTINGEN BL ٢١٠٥ (Germany) |
| Vortex mixer (Electronic) | VIOBROFIX,VF-١ JANKE and KUNKEL IKA- labrotechnik (Germany) |
| Water bath | Schutzart DIN ٤٠٠٥٠-IP ٢٠ Memmert Gmbh, Schwabach FRG (Germany) |
| Magnetic stirrer with Hot plate | Jlassco (India) |
| Spectrophotometer Type ٢١(Digital ultraviolet and visible) | Spectronic (٢١) MILTON ROY COMPANY,Bouch and Lamp (USA) |
| ELISA Reader and washer | Bikman Keouldeir (USA) |
| centrifuge | Griffin and George BS ٤٤٠٢-D(UK) |
| Centrifuge tube | AFMA (Jordan) |
| Plane tube | AFMA- Dispo (Jordan) |
| Micropipette ١٠- ٥٠ ml | SLAMED (Germany) |
| Micropipette ١٠٠- ٢٠٠ ml | SLAMED (Germany) |
| Micropipette ١٠٠ – ١٠٠٠ ml | SLAMED (Germany) |

| | |
|---|---|
| Disposable syringe | Bulim medical (South Korea) |
| Incubator (isotemp) | Fisher scientific company,model ٥٣٧٠,CAT.١١-٦٩٠-٥٣٨D, (USA) |

## ٢.١.٣. Patients and Controls

Study samples were obtained from, AL-Hilla teaching hospital , Hilla and from the surgical specialty hospital ,medical city , Baghdad , from may ٢٠٠٤ to July ٢٠٠٥ .

Individuals with adenocarcinoma of the prostate (cases) were obtained from the urology units of these hospitals ; controls were obtained primarily from these hospitals .

The cases being choosen according to suitable eligible criteria were:-

Patients of age ٤٥ years or more, histologically proved adenocarcenoma of the prostate, or benign prostatic hyperplasia (BPH) .

Controls were of age ٤٥ years or more, male gender, and completely healthy i.e. not suffering from any chronic disease such as hypertension , diabetes and not affected by any other types of malignancy with normal liver function and renal function tests .

After preparing a questioner list and collecting the required information that is important for the study , the studied group (patients and controls) were asked about , occupation , social history ,past medical and past surgical histories , and family history of cancer , especially for the prostate cancer .

٥ mL of peripheral blood were taken from each subject, transferred from the disposable syringe to plain tube without anticoagulant and then after ١٥

minutes blood is allowed to clot ; the clot shrinks and serum can be obtained by centrifuging ,the obtained serum ٣mL is divided on to two aboundroff tubes ١.٥ mL and then labeled by number specific for each separated sample and the samples obtained from the surgical specialty hospital , medical city ,Baghdad; after centrifuging and separation in tubes , should be freezed down to -١٠ to -٢٠ °C to be transferred by a especial and completely sealed iced container to be analyzed in our research biochemical unit.

The cases are divided into three distinct categories :

**Category (١)**

(٤٠ cases) of proved p. adenocarcinoma by true cut biopsy , PSA more than ١٥ , highly suspected DRE.

**Category (٢)**

(٤٠ cases) of BPH without any evidence of any malignancy , normal or slightly raised PSA , positive DRE.

**Category (٣)**

(٤٠ cases) healthy control subjects.

## ٢.٢.Methods

### ٢.٢.١.Determination of serum reduced glutathione (GSH)

Serum GSH was determined by using a modified procedure using Elmans reagent (DTNB),which is depend on the action of the sulfhydryl groups.of the GSH [١٥٥ ,١٥٦]

**Principle.**

٥,٥-dithiobis(٢-nitrobenzoic acid ) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound . The absorbance of the reduced chromogen is measured at ٤١٢ nm and directly proportional to the GSH concentration[١٥٣] .

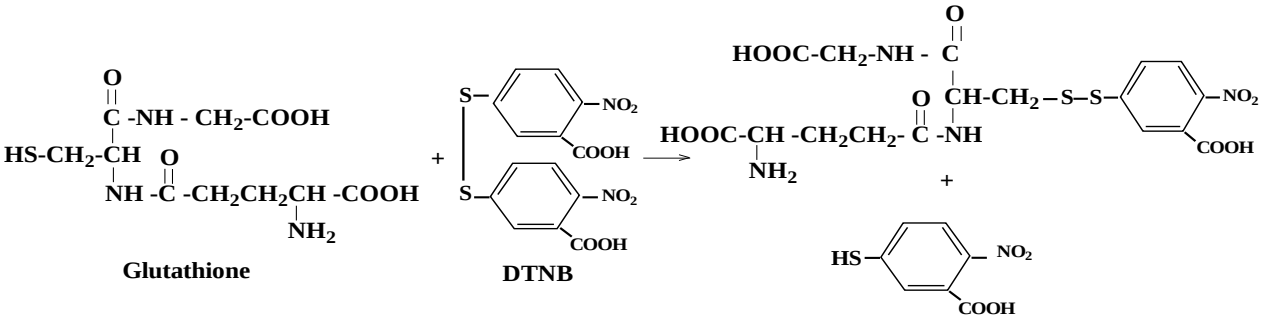



**Fig (٢-١)** Reaction between GSH and DTNB fig taken from [١٥٥ ,١٥٦]

## Preparation of Reagents

### ١. Precipitating Solution [Trichloro Acetic Acid (TCA) ٥٠٪]

٥٠ gm of TCA are dissolved in a final volume of ١٠٠ ml of distilled water (D.W.)

### ٢. Ethylenediamine Tetra Acetic Acid– Di Sodium ( EDTA $Na_٢$) (٠.٢M)

٧.٤٤٤٨ gm of EDTA Na٢ dissolved in a final volume of ١٠٠ml DDW or ٣.٧٢٢٤ gm of EDTA Na٢ dissolved in a final volume of ٥٠ ml DDW.

### ٣.Tris – EDTA Buffer (٠.٢M ) pH ٨.٩

٠.٤٨٤٥gm of Tris are dissolved in ٨ ml of DDW .

١ ml of (٠.٢M) EDTA Na٢ solution are added and brought to a final volume of ١٠ ml with DDW.

The PH was adjusted to ٨.٩ by the addition of ١ M of HCL.

[ this solution is stable for at least ١٠ days ]

**٤. DTNB Reagent ( ٠.٠١ M)**

٠.٠٣٩٦ gm of DTNB is dissolved in absolute methanol , and brought to a final volume of ١٠ ml .

[this solution is stable for at least ١٣ weeks . at ٤$C^{o}$]

**٥. GSH Standards Solution (٠.٠٠١M)**

Stock standards solution (٠.٠٠١ Molar M) is prepared by dissolving ٠.٠٠٧٦٨ gm of GSH in a final volume of ٢٥ml of (٠.٢M) EDTA solution ,dilutions are made in EDTA solution to ٥,١٠,١٥,٢٠,٣٠,٤٠,٥ micro M .

[this working standard solution should be prepared daily ]

**Procedure**

Serum GSH was determined by using a modified procedure using Elmans reagent (DTNB),which is summarized as follows:

Duplicates of each standard and sample test tube are prepared then pipetted into test tubes.

| Reagents | Sample µL | Reagent blank µL | Standard µL |
|---|---|---|---|
| Serum | ١٠٠ | | |
| Standard | | | ١٠٠ |
| DDW | ٨٠٠ | ٩٠٠ | ٨٠٠ |
| TCA | ١٠٠ | ١٠٠ | ١٠٠ |

Tubes are mixed in a vortex mixture intermittently for ١٠-١٥ min , and centrifuged for ١٥ min at ٣٠٠٠ xg , then pipetted into test tubes .

| Reagents | Sample mL | Reagent blank mL | Standard mL |
|---|---|---|---|
| Supernatant | ٤٠٠ | ٤٠٠ | ٤٠٠ |
| Tris –EDTA buffer | ٨٠٠ | ٨٠٠ | ٨٠٠ |
| DTNB reagent | ٢٠ | ٢٠ | ٢٠ |

Tubes are mixed in vortex mixture , the spectrophotometer is adjusted with reagent blank to read zero absorbance (A)at ٤١٢ nm and the absorbance of standards and sample is read within ٣ minutes of the addition of the DTNB reagent.

**Calculation of serum GSH**

The concentration of serum GSH is obtained from the calibration curve in Mm. **Fig [٢-٢].**

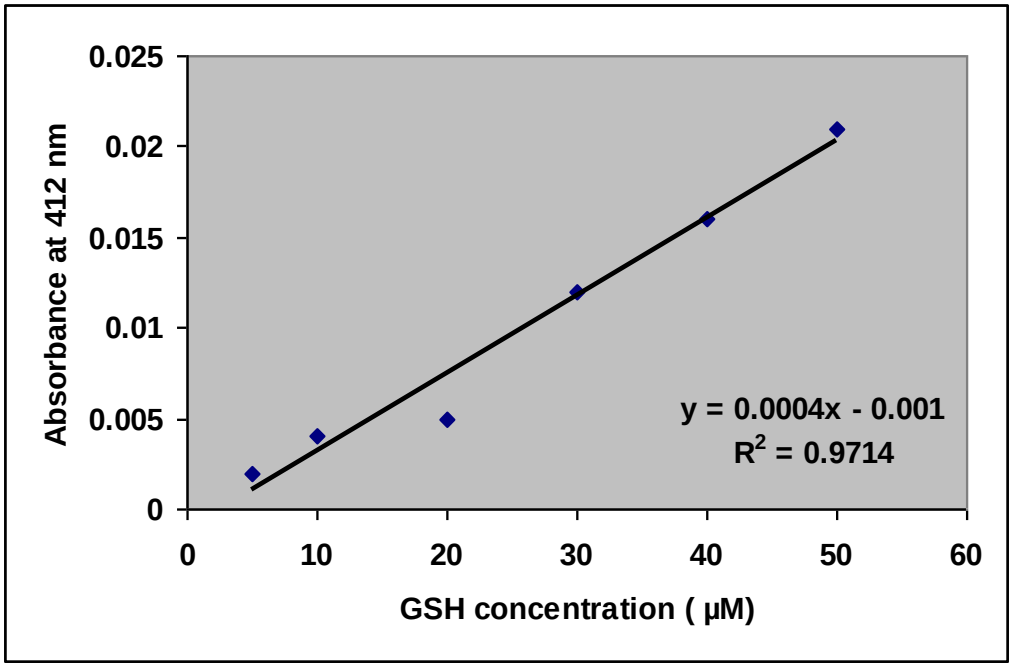



**Fig ( ٢-٢)**Standard curve of glutathione (GSH) concentration.

**٢.٢.٢.Determination of Total Fucose (TF)**

**According to Dische and Sheetels Methods (Dische, ١٩٤٨)** (١٥٧)

**Principle**

This method depends on a direct reaction of concentrated sulfuric acid with serum components ; the reactants combine with cysteine , and the colour product measured at (٣٩٦ and ٤٣٠ nm).

The differences in absorbance were directly proportional to alpha-L- fucose content of the solutions.(١٥٧)

**Reagents**

**Reagent (١)**. Six parts concentrated $H_2SO_4$ + one part distilled water.

٦ ml $H_2SO_4$ ——————→ ١ml DW.

٥٠ ml $H_2SO_4$ mixed slowly with ٨.٣ ml DW.

**Reagent (٢)**.٣٪ cysteine hydrochloride solution was prepared weekly and stored in the refrigerator .

٣٪ i.e. ٣gm in ١٠٠ ml DDW.

To avoid loss of chemicals , we prepared ٠.٣ gm and dissolved it in ١٠ ml of DDW., since it is weekly prepared.

**Reagent (٣)**. Standard alpha-L-fucose .

١٠ mic gm of methyl pentose dissolved in ١ ml DDW.

We prepared ٠.٠١ gm of methyl pentose to be dissolved in ١٠٠٠ ml DDW. to avoid loss of chemicals.

**Reagent (٤)**. Distilled water.

**Procedure**

١. To (٠.١ml) or ١٠٠ mic L . ice cold serum , add (٤.٥ ml) of chilled solution of reagent (١) was added slowly with constant shaking in ice bath [ to prevent rise in temperature ].

٢. The tubes were transferred to a water bath at room temperature for few minutes exactly ١٠ min. , and then tubes are transferred to a vigorously boiling water bath for exactly ٣ min ; the tubes are placed in a water bath at room temperature for ١٥ minutes.

٣. Add (٠.١ ml) or ١٠٠ mic L of reagent (٢) to each tube and then mix and after two hours the absorbance was measured at٣٩٦ nm and at ٤٣٠ nm .

٤. The same procedure was applied for standard fucose solution.

**Calculations**

$$\text{Total fucose (mg/dl)} = \frac{A_T\,396 - A_T\,430}{A_S\,396 - A_S\,430} * 12$$

Where:

A = absorbance.

$A_T$= the absorbance of the test with cysteine.

$A_S$ =the absorbance of the standard with cysteine.

١٢ = dilution factor.

## ٢.٢.٣.Determination of serum PSA using PSA enzyme immunoassay test kit

The PSA ELISA test is based on the principle of a solid phase enzyme – linked immunosorbent assay. The assay system utilizes a rabbit anti- PSA antibody directed against intact PSA for solid phase immobilization (on the microtiter wells).

A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody – enzyme conjugate solution , the test sample is allowed to react first with the immobilized rabbit antibody at room temperature for ٦٠ minutes , the wells are washed to remove any unbound antigen , the monoclonal anti-PSA-HRP conjugate is then reacted with the immobilized antigen for ٦٠ minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies and the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for ٢٠ minutes , resulting in development of a blue color ; the color development is stopped with the addition of stop solution changing the color to yellow . The concentration of PSA is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at ٤٥٠nm. (١٥٨)

## Aims and Objectives of the Study

١- To detect the reference of serum reduced glutathione [GSH] and serum total fucose [TF] as valuable biomarkers in transformation particularly of male malignant prostate.

٢-To investigate and measure the values of the reduced glutathione [GSH] in serum of patients with prostate cancer and correlate this with the levels of serum total fucose [TF] as possible useful trend biological tumor markers in the early diagnosis of prostate cancer correlated with the result of serum PSA and DRE .

٣-To make a comparison between the results of the serum reduced [GSH] and serum [TF] in patients with prostate cancer and BPH , (benign prostatic hyperplasia) , to find the possibility of using the test in differentiating between the two conditions.

٤-To determine the sensitivity and specificity of the proposed test and its value in the early detection of prostatic tumors compared with prostatic specific antigen [PSA] and digital rectal examination [DRE] alone or in combination with it.

٥- Presenting the effects of age, smoking, family history of tumors, exposure to chemicals[organophosphorous in pestisides , insectisides] on both reduced GSH and TF , reflecting the rate of incidence of prostate cancer.

## Conclusions

١. The prostate cancer incidence rate increased with the decreasing level of GSH associated with an increasing level of TF during tumor development process , thus reflecting an inverse relationship between serum GSH and serum TF as a trend biomarker in prostate cancer .

٢. The sensitivity of serum GSH in prostate cancer was ٧٢.٥٪ while its specificity was ٧٧.٥٪ ; on the other hand , the sensitivity of serum TF in prostate cancer was ٧٠% while its specificity was ٧٢.٥٪ ; both the sensitivity and the specificity of GSH and TF in BPH patients are much lower than those in prostate cancer patients . In conclusion , serum GSH is more sensitive and more specific than serum TF as a considerable biomarker in prostate cancer but less considered in BPH.

٣. The study shows non significant correlations between each of GSH , TF and PSA that can be used , from practical point of view , as a separate beneficial biomarker assisting PSA and DRE in diagnosing , staging , grading and monitoring prostate cancer patients.

٤. Both serum GSH and serum TF in BPH were changed in the same manner as for prostate cancer patients but at a less degree of affection , since serum GSH in BPH is less decreased and serum TF is less increased

the results revealed that serum GSH in BPH is less sensitive but more specific than serum TF , while serum TF in BPH is more sensitive but less specific than serum GSH.

٥. Exposure to chemicals in prostate cancer patients caused a decrease in GSH and an increase in TF.

٦. Smoking had a significant effect on both GSH and TF in prostate cancer patients.

٧. PSA and TF are more positively correlated with age while GSH is weak and negatively correlated with age in prostate cancer patients .

Both GSH and TF are negatively correlated with age in BPH patients while PSA is positively correlated with age ; this reflects a wide and variable range of affection of age on GSH , TF and PSA in both prostate cancer and BPH patients.

٨. Family history of tumor affects both serum GSH and serum TF .

٩. Prostate cancer is more incident in those with positive history of recurrent prostatitis.

١٠. Geriatrics who previously receive medication for BPH or baldness are at low risk to develop prostate cancer.

١١. the age group distribution for PC and BPH is identical as the present study confirms.

## Recommendations

١. Further studies dealing with the level of antioxidants , V it. E ,GSH and GST in semen and compare it with the blood levels in prostate cancer patients may be carried out .

٢. Further studies dealing with the wars hazardous effect on the environment and the reflection of the effects of environmental pollution of air, water and

soil and its harmful effect on antioxidant level , leading to oxidative stress that may eventually counteract in cancer development in general may be carried out .

٣. Further studies dealing with the determination of GDP-mannose , the precursor of endogenous GDP-fucose and estimation of dehydratase and epimerase – reductase enzymes may be carried out .

Also for exogenous fucose the determination of fucokinase enzyme that converts fucose to fucose-١-phosphate and pyrophosphorylase enzyme that converts fucose-١- P to GDP- fucose.

٤. Further studies dealing with the determination of the effect of oxidative stress from one side and the cancer from the other side on the levels the two enzymes that catalyze the two steps GSH synthesis, gamma-glutamyl-cysteine synthetase and GSH synthetase.

٥. The use of electrophoresis and atomic absorption to detect the enzymes and the isoenzymes of both GSH and TF metabolic pathways and their close association to the tumor grade and size, so the estimation of $NADP^+$ and NADPH as a cofactors for the oxidation-reduction process in the biosynthesis of L-fucose may be studied.

also the estimation of NADPH as an essential cofactor for the enzyme glutathione reductase and the enzyme itself that maintains the glutathione in its reduced state necessary to perform its action in detoxifying the free radicals to find the effect of oxidative stress on these parameters.

٦. A high specialized center supplied with advanced lab equipments like HPLC and PCR is needed in order to determine the GSTP١ isoenzyme and other GSTP

isoenzymes in blood ,serum and semen and evaluate their relation to carcinoma of the prostate.

٧. Detection of androgen receptors status using immunohisto - chemistry , to estimate the pathogenesis of the disease and determine its prognosis.

٨. The use of the polymerase chain reaction (PCR) amplifications , using genomic DNA extracted from the blood samples of proved prostate cancer cases for the genotyping of the GPX١ GCG, (a selenium dependent enzyme) to find the association between the GPX١ and dietary selenium levels in the development of prostate cancer.

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