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THE EVALUATION OF ALPHA-L-FUCOSE AND REDUCED GLUTATHIONE, A POSSIBLE BIOMARKER FOR PROSTATE CANCER IN IRAQ MALES

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Summary

Alpha-1- fucose and reduced glutathione (GSH) 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 .Sera of (40) prostate cancer patients has been taken to estimate the levels of alpha-1-fucose and GSH compared to those levels of the prostate specific antigen (PSA) of the same patients .The results of the study reveled that serum (GSH) decrease in prostate cancer, while serum Total fucose(TF) increase in the same patients exposing an inverse relationship between the two parameters, the sensitivity of serum (GSH) in prostate cancer 72.5% while the specificity were 77.5% . The sensitivity of serum (TF) in prostate cancer 70% while the specificity were 72.5% .It is concluded that prostate cancer affects (TF) and (GSH) levels in the patients' serum , smoking , exposure to chemicals , age had a significant effect on blood levels of (TF) and (GSH) .

الخلاصة

تم قياس الألفا- ل- فيوكوز والكلوتاثايون المختزل في المرضى المثبت اصابتهم بسرطان البروستات وذلك لأيجاد احتمالية استعمالهما كدلائل حيوية في تشخيص مرضى سرطان البروستات مقارنة بالأصحاء ومن الفئة العمرية المماثلة للمرضى.
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 72.5% بينما الخصوصية كانت 77.5%، بينما الحساسية لل-ألفا-ل فيوكوز الكلي المصلي لدى مرضى سرطان البروستات هي 70% بينما الخصوصية كانت 72.5%.
استنتج ومن خلال البحث بأن سرطان البروستات يؤثر وبشكل واضح على مستويات أل- ألفا - ل فيوكوز الكلي المصلي وألكلوتاثايون المختزل المصلي في مصل الدم.

Introduction

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 principle 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 any where 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 [1] [1].

Fig [1] The histological appearance of well differentiated prostatic adenocarcinoma. [1]

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 2003 there were approximately 220,900 new cases and 28,900deaths from this disease. [2]

Moreover it's also the most common malignant tumor in men over the age of 65 years in England and Wales in 2000. 21,000 men were registered and 9000 died from it [3] .

Prostate Cancer Histological Appearance

The prostate is a glandular structure consisting of ducts and acini; thus, the histological pattern is one of an adeno carcinoma, ninety five percent of all prostatic carcinomas are adenocarcinomas [4], 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 .[5]

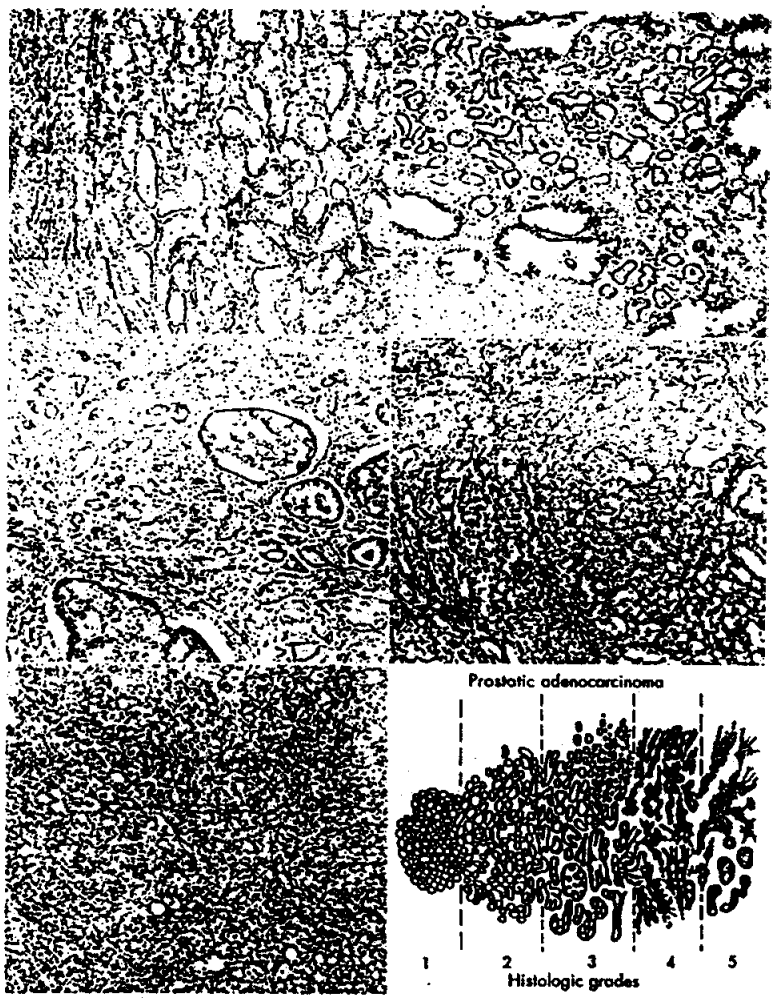


Fig [2] The histological grading of adenocarcinoma of prostate (Fig. taken from [5]

Usually prostate cancer exhibit heterogeneity within tissue , and so two histological areas of prostate are each scored between 1 and 5 .
The scores are added to give an over all Gleason score of between 2 and 10 ; 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 have been proposed to determine the biologic potential of the prostatic tumor .[5]

Tumor Marker

Warburg 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. [6]
Even in recent years , several enzymes and isoenzymes were still being used extensively as a 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.[7]
Many cancers are associated with abnormal production of enzymes , proteins , and hormones which can be measured in plasma or serum. [8]

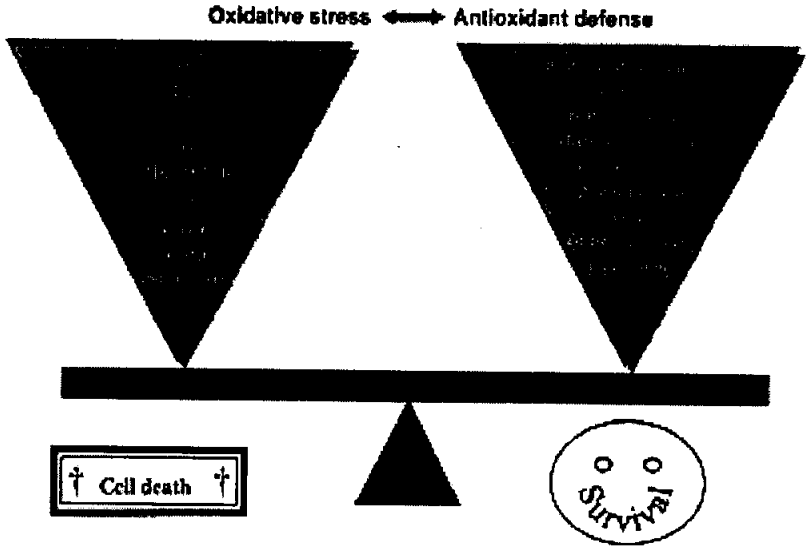
Prostate Cancer Tumor Marker:

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.(9)

Many new studies and researches showing 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).(9)

Correlation analysis showed that the tissue levels of GSH was 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. (66). Fucose , a 6- carbon deoxy hexose that is commonly incorporated into human glycoproteins and glycolipids ,it 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 there by 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 .

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.

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 , 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 .

Fucose [6- carbon deoxy hexose]or 6-deoxy-l-galactose or L-methyl pentose a monosaccharide present in a low concentrations in normal circulation [10]

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

Tumor biomarker are either intracellular proteins or cell surface glycoproteins and glycolipids released into the circulation and detected by immuno assays[11].The pentose phosphate pathway handles 5% to 10% of metabolized glucose in normal red cells in the process generating 2mol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for each 1 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 (O2), hydrogen peroxide (H2O2), and hydroxyl radicals(OH) .

The aim of the present work is :

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

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

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

Materials and Method

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

Study samples were obtained from, AL-Hilla teaching hospital Hilla-Iraq and from the surgical specialty hospital ,medical city Baghdad-Iraq, during may 2004and July, 2005 .

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:-

ents age 45 or more, histologically proven adenocarcenoma of the prostate, or benign prostatic hyperplasia BPH .

Controls was age 45 or more, male gender, and completely healthy i.e. not suffering any chronic disease such as hypertension , diabetes and not affected by any other types of malignancy with normal liver function and renal function .5 mL of peripheral blood were taken from each subject, transferred from the disposable syringe to plain tube without anticoagulant then after 15 minutes blood allowed to clot , the clot shrinks and serum can be obtained by centrifuging ,the obtained serum 3mL divided on to two aboundroff tubes 1.5 mL then labeled by number specific for each separated samples and the samples that obtained from the surgical specility hospital , medical city ,Baghdad; after centrifuging and separation in tubes , should be freezed down to -10 to -20 °C to be transferred by a special and completely sealed iced container to be analyzed in our research biochemical unit.

The cases are divided into three distinct categories :

Category (1)

(40 cases) of proved prostate adenocarcinoma by true cut biopsy , PSA more than 15 , highly suspected DRE.

Category (2)

(40 cases) of BPH without any evidence of any malignancy , normal or slightly raised PSA , +ve DRE.

Category (3)

(40 cases) healthy control subjects.

Determination of serum reduced glutathione (GSH)

The concentration of serum GSH is obtained from the calibration curve in Mm. **Fig (3)** .

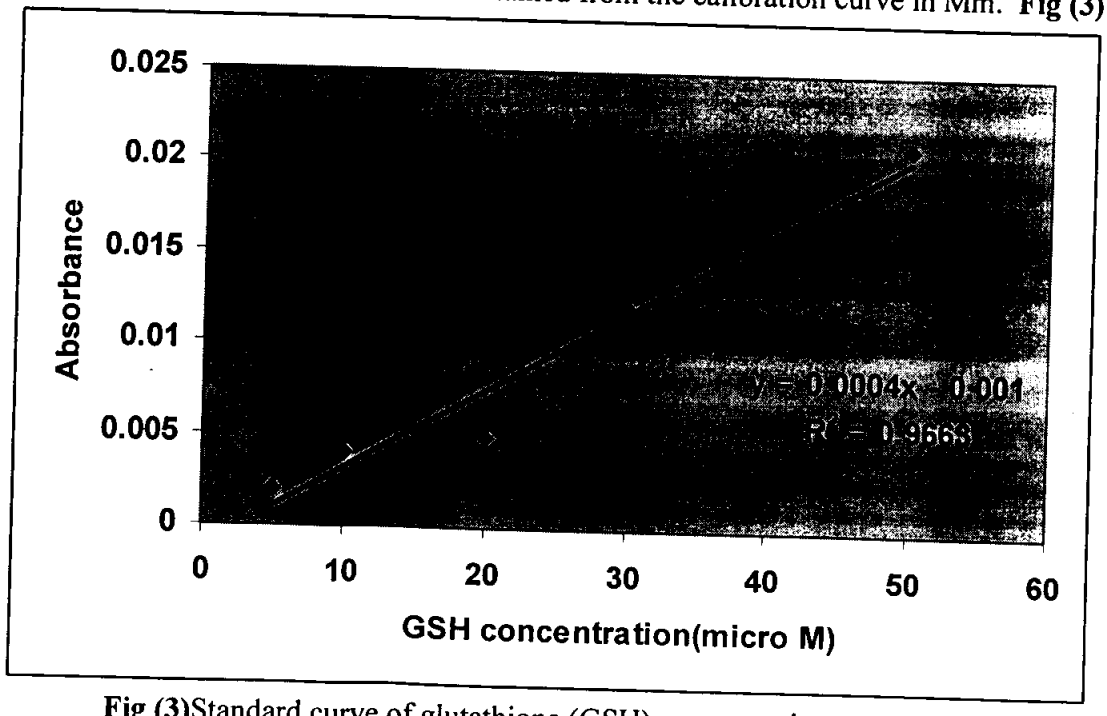


Fig (3) Standard curve of glutathione (GSH) concentration.

Determination of Total Fucose (TF) According to Dische and Sheetels Methods (Dische, 1948)

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 (396 and 430 nm).The differences in absorbance were directly proportional to alpha-L- fucose content of the solutions.[12]

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). [13]

Results and Discussion

Glutathione concentrations [μM] , total fucose [mg/dl] concentrations and PSA[ng/ml] in sera of prostate cancer, BPH patients and healthy controls were determined as follows:
From 100 samples being collected very hardly ;15 samples had been lost during transport , and 5 other samples were spoiled during analysis , and only 80 samples yielded sufficient readings and the following results being obtained:

Category 1

We have 40 cases with a proved CA. prostate by true cut biopsy as adenocarcenoma , PSA more than 15 , DRE positive, with a mean age 61.53 year.[15]cases result in a reduced level of GSH i.e. 72.5 % of this category result a positive test. While (28) of cases of this category result in a high level of TF i.e. 70% result in a positive test for TF.Only (11) cases for GSH and (12) cases for TF for the same category show negative test result i.e. 27.5% result in a normal level of GSH and 30% result in a normal level of TF.

Category 2

We have other (40) cases with a BPH proved by true cut biopsy , PSA normal or slightly raised , DRE negative.With a mean age 60.55 year.
Only (14) cases results in a reduced GSH i.e. 35% consider as a positive test result, while (27) cases results in a high TF i.e. 67.5% consider as a positive test result for TF.However (26) cases of the same category shows a normal GSH level i.e. 65% consider as a negative test result while only (13) cases shows a normal TF level i.e. 32.5% consider as a negative test results.

Category 3

We have (40) healthy, non smoker control cases , with a mean age 58.73 year.For all (40) cases studied parameter results in a normal level of both GSH and TF i.e. 100% of this category results in a negative test for both parameters.

GSH and TF in Prostate Cancer Patients

The mean serum level of reduced glutathione (GSH) had shown a decreased in its level in patients with carcinoma of the prostate in contrast to that of the control group and it revealed a positive significant difference with serum GSH in control group ($p< 0.01$). (table.1)

Table (1): The mean serum GSH in prostate cancer patients in contrast to control group.

Group	Mean GSH mg/dl	S.D.(mg/dl)	P value
patients	5.47	5.17	0.001
Control	12.49	6.5	

Serum GSH values normally decline with advancing age and therefore most of the adult male subjected to prostate cancer are those > 50 year [15,16,17,18]

Also GSH is required to carry out an immune response since its needed by the lymphocytes to multiply in order to develop a strong immune response for killer lymphocytes to be able for attacking and killing cancer cells.[16]

GSH directly reduces the radicals that are critical to anti tumor activity on the other hand GST catalyzes the reaction between GSH and either hydrophobic or electrophilic compounds that consume more GSH. [19]

GSH plays an important role in maintaining normal balance between oxidation and anti-oxidation, in prostate cancer that balance being shifted towards the oxidation side because the GSH as an intracellular anti oxidant consumed by the cells trying to regulate the cells vital functions such as the synthesis and repair of DNA, the synthesis of proteins, the activation and regulation of enzymes [16,20].

The sensitivity of GSH in prostate cancer was 72.5% while the specificity was 77.5%.

On the other hand the serum total fucose level in prostate cancer patients had shown a significant increase in contrast to the serum level of total fucose in control group, also there was a significant difference between the serum TF level in prostate cancer patients with the TF level of the control group ($p <0.01$). **(table. 2)**

This significant increase because the TF level was found to be at higher values in carcinomas tissues in comparison with tissues undergoes normal proliferation [21].

Although fucose is widely distributed through out the body in glycoprotein's and glycolipids dependable with a cell-cell communication and farther subsequent division which is expected to occur widely and in uncontrolled manner during oncogenesis where a rapid division of immature cells occur that's why found a high fucose content in the serum glycoprotein of cancer patients.[22-23]

Fucose is distributed in macrophages which expected to be highly increased in cancer state since it's the critically important cells in the immune system [24] Also the nuclear magnetic resonance spectra from malignant cells and tissues suggesting that fucose was detectable in these cells but was limited or un detectable in non malignant cells from which they were believed to be derived [25].

The sensitivity of TF in prostate cancer was 70% while the specificity was 72.5%.

Table)(2) The mean serum TF level of prostate cancer patients in contrast to control group.

Group	Mean TF mg/dl	S.D. mg/dl	P value
patients	47.5	26.03	0.007
control	13.68	1.38	

using percentage find that 72.5% positive test for GSH and 27.5 % negative test for GSH while the results for TF are 70% and 30 % respectively as shown in **Fig (4)** .

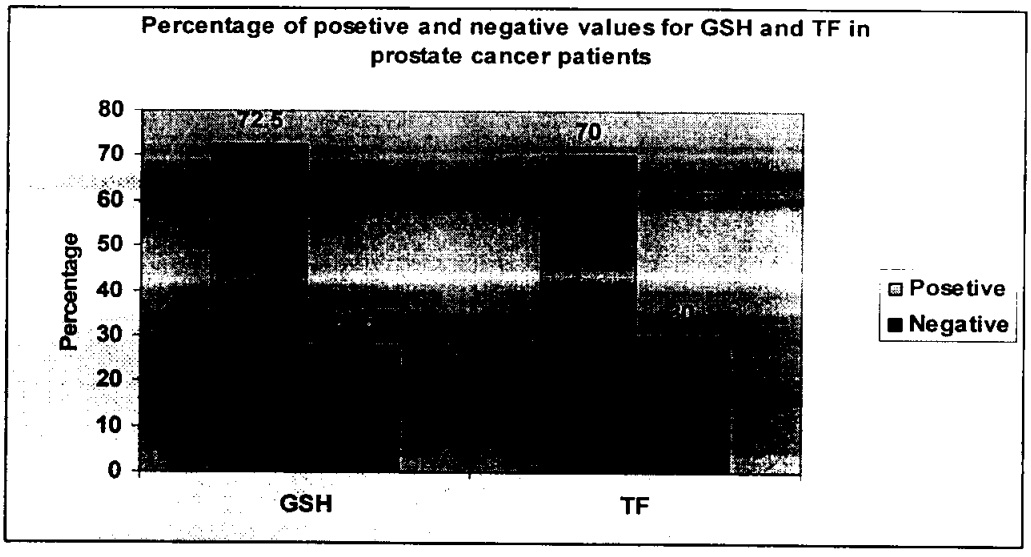


Fig (4) The percentage of positive and negative values for GSH and TF in prostate cancer patients .

By estimating the final results of the GSH and the TF in prostate cancer patients show an inverse relationship since the GSH is significantly decreased while TF is significantly increased for the same the reasons mentioned previously as show in fig (5) and fig (6)

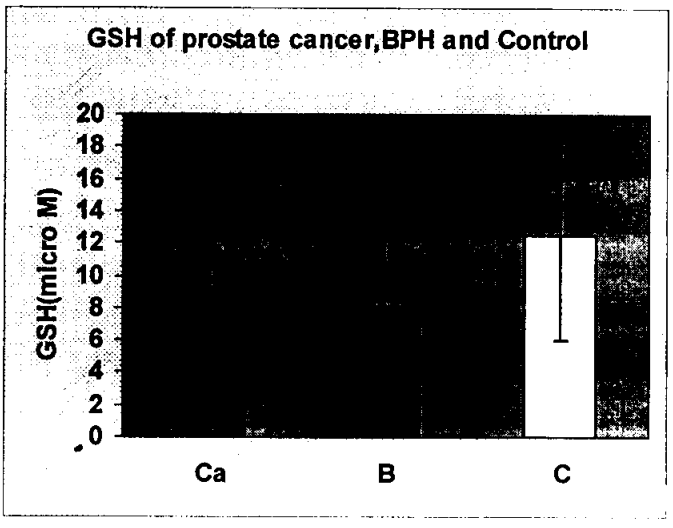
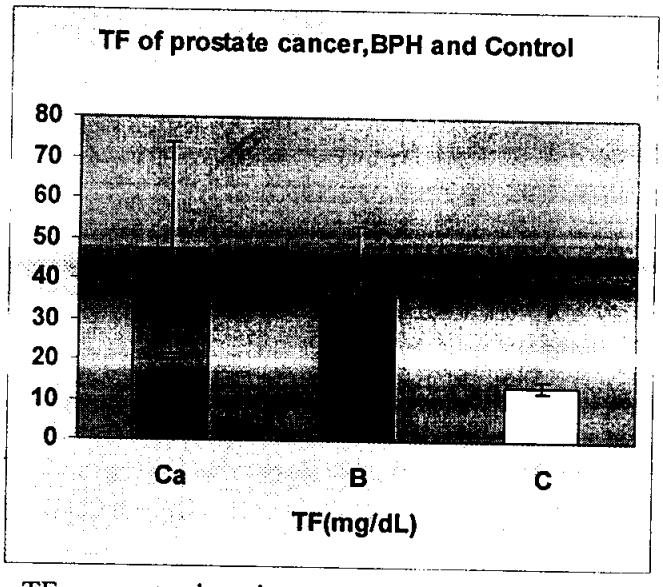


Fig (5) The GSH concentrations in prostate cancer and BPH patients in relation to control.



Fig(6) The TF concentrations in prostate cancer and BPH patients in relation to control.

Although when plotting the GSH and TF in prostate cancer patients find that they are significantly negatively correlated with P value 0.0001 and correlation coefficient(r) value (0.8243) as shown in fig. (7)

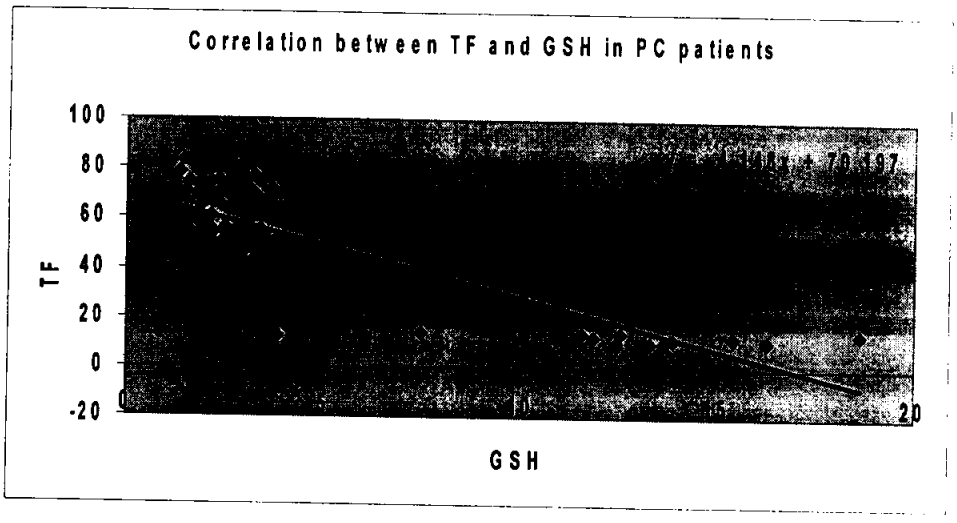


Fig (7) The correlation between TF and GSH in PC patients.

Plotting PSA against GSH in prostate cancer patients find that it significantly Negatively correlated with P value 0.001 and correlation coefficient (r) value (0.1587) as show in fig (8) .

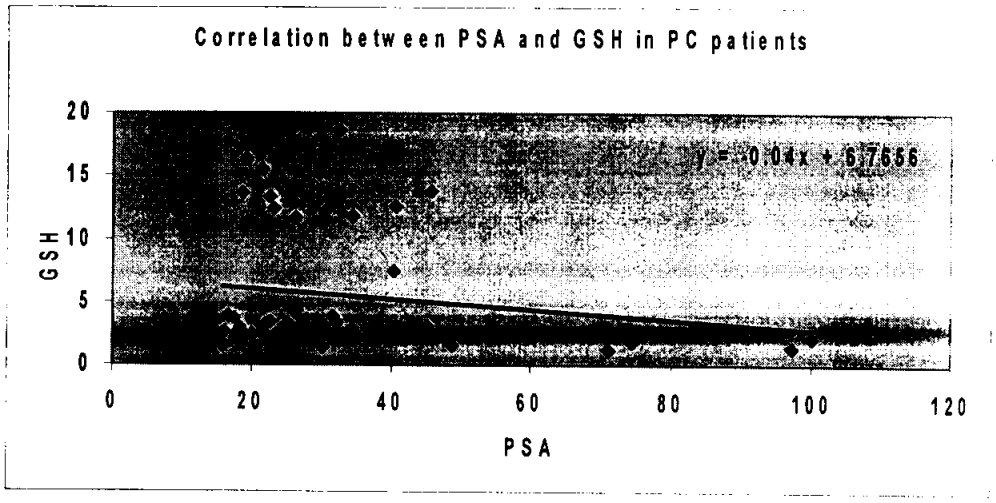


Fig (8) The correlation between PSA and GSH in PC patients

While plotting PSA against TF in prostate cancer patients resulting in a significant positive correlation with P value 0.001 and correlation coefficient (r) value (0.1628) as show in fig (9) .

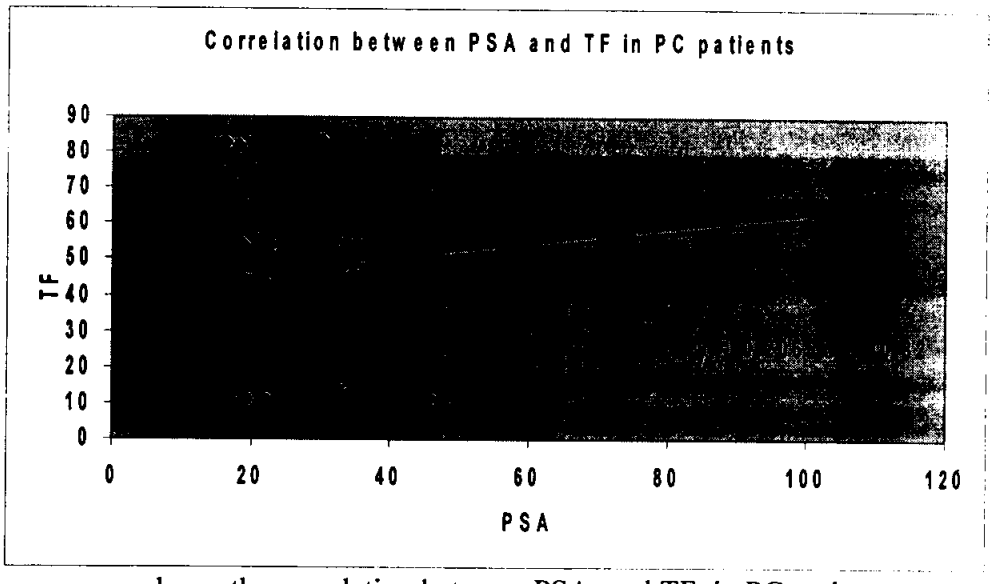


Fig (9) shows the correlation between PSA and TF in PC patients

Conclusions

1. The prostate cancer incidence rate was increased with the deceasing level of GSH associating 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 .
2. The sensitivity of serum GSH in prostate cancer was 72.5% while its specificity was 77.5% on the other hand the sensitivity of serum TF in prostate cancer was 70% while its specificity was 72.5%

3. The study shows a significant correlations between each of GSH , TF and PSA that can be used from practical point of view as a beneficial biomarker assisting PSA and DRE in diagnosing , staging , grading and monitoring prostate cancer patients.
4. Both serum GSH and serum TF in BPH was changed in the same manner as for prostate cancer patients but at a less degree of affection , since serum GSH in BPH less decreased and serum TF is less increased .
5. Smoking had a significant effect on both GSH and TF in prostate cancer patients.
6. PSA and TF are more positively correlated with age while GSH is weak negatively correlated with age in prostate cancer patients .
7. Family history of tumor affect both serum GSH and serum TF .
8. Prostate cancer is more incident in those with positive history of recurrent prostatitis.
9. Prostate cancer is more incident in those patients depends on animal source of diet mainly animal fat .
10. Geriatrics who talking aspirin infant and or cholesterol lowering agent daily and frequently, are at less risk to develop prostate cancer .

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