Determination of some biochemical parameters in erythrocytes and serum of patients with prostate cancer related to lipid peroxidation

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Abstract:

The aim of this study is to assess some biochemical parameters related to oxidative stress in erythrocytes and serum of patients with prostate cancer. Glutathione - S – transferase (GST) , glutathione peroxidase (GPx) and Xanthine oxidase (XO) activities was measured in erythrocytes , malondialdehyde( MDA), High density lipoprotein cholesterol (HDLc) , Low density lipoproteins (LDLc) and selenium (Se) levels was measured in serum . Patients divided into two groups of men suffering from prostate cancer:- group A included (25) men suffering from this disease a period (1-2) years. Group B consisted of 25 patients suffering from this disease a period (2-4) years. Patients groups were compared with (25) healthy individual as a control group. Results have suggested that (GST) , (GPx) and (XO) activities were elevated in group (A) when compared to normal control. [ P<0.001 for (GST) , (GPx) and , (XO) ] , but then were decreased for group B [ P<0.01 for (GST) , (GPx) and , (XO) ]. Malondialdehyde ( MDA) level was increased in group A P<0.01 and this increasing was continued in group B P<0.001 .High density lipoproteins (HDLc) level was decreased in group A P<0.01 and this decreasing was continued in group B P<0.001 while low density lipoproteins (LDLc) level was increased in group (A) P<0.01 and this elevation was continued in group (B) P<0.001. Selenium (Se) was decreased in group (A) P<0.01 compared to healthy control, this decreasing was also continued in group (B) P<0.001.

Keywords: prostate cancer . lipid profile . GPx . GST . malondialdehyde.

Introduction:

Prostate cancer is the most prevalent cancer found in men above the age of fifty years. Although the specific etiological factors of cancer are not yet known, considerable evidence indicate that both genetic and environment play a role in the evolution of prostate cancer. Diet may not initiate prostate cancer but rather may promote its progression. Diets high in vegetables have been reported to
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Fat consumption has long been suspected to be a risk factor for prostate cancer. As a result, prostate cancer is high probably among atherosclerosis patients. Prostate cancer which is the third most common cancer in men is associated with lipid peroxidation. [1,2,3,4]

Reactive oxygen species are known to be mutagenic and therefore playing an important role in cancer formation. The mutagenic capacity of free radicals is due to the direct interaction of hydroxyl radicals with DNA. Reactive oxygen species induce membrane damage by peroxidizing lipid moiety with a chain reaction known as lipid peroxidation. The initial reaction generates a second radical, which in turn can react with a second macromolecule to continue the chain reaction. Among the most susceptible targets are polyunsaturated fatty acids. A newly formed free radical reacts with next lipid molecule and destroying thereby, propagation the lipid peroxidation process with the continuous formation of new free radical. The process is also terminated by free radical scavengers such as enzymatic and non-enzymatic antioxidant systems.[5,6]

On the other hand, the chemotherapeutic agents such as phpsphocycloamide (cytoxan) and deoxorubicin (adriamycin) now commonly used for treatment of cancer, have all been shown to increase lipid peroxidation and generation of reactive oxygen species. [7]

Glutathione peroxidase (GPx) is an antioxidant enzyme. It has been reported as the first and best characterized mammalian selenoprotein, capable of reducing equivalents form glutathione to detoxify hydrogen and lipid peroxides.[8]

Glutathione - S – transferase (GST), also antioxidant enzyme, plays a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens. [9]

In contrast, Xanthine oxidase (XO) promotes lipid peroxidations. It converts Xanthine and hypoxanthine to uric acid. The last reaction is associated with transferring electrons from xanthine and hypoxanthine to uric acid and in the same time oxygen molecule is reduced super oxide anions that initiate lipid peroxidation. [10]

Selenium (Se) is a very important component of antioxidant protect mechanism and anticancer properties. It has a protective effect against oxidative damage by decreasing the amount of free radicals and increasing the synthesis of glutathione peroxidase (GPx). [8,11]

Malodialdehyde( MDA), the end product of lipid peroxidation owing to its high cytotoxicity has been suggested to act as tumor promoter and co-carcinogenic agents. Malodialdehyde( MDA) levels have been found to be useful as tumor marker for diseases related to lipid peroxidation. [12,13]
Lipids specially cholesterol are important constituents of the cell. They play key roles in many vital physiological functions. Cholesterol is vital in the maintains of the structure and functional integrity of all biological membranes. Lipids (except HDL) might be associated with cancers as they have an integral role in the maintenance of cell integrity. Despite raised lipids are strongly associated with pathogenesis of atherosclerosis, researchers have also reported an association between plasma / serum lipids and lipoproteins (except HDL) with different types of cancers. [14,15]

**Materials and Methods :-**

Blood samples were collected from 50 men with cancer of the prostate, with age range of 60-70 years. The work was divided into two groups: group A included 25 men suffering from prostate cancer a period 1-2 years and group B consisted of 25 men suffering from the disease for 2-4 years. Two groups were selected to study the correlations between the determined parameters and period of time suffered from prostate cancer. Data resulting from group A and group B were compared to 25 healthy men with age 60-70 control group. Blood samples were collected from patients from (Al – benok ) laboratory in Baghdad and the work was perfected by cooperation with the center of chemical researches in Ministry of science and technology. Patients were treated with chemotherapeutic drugs (cyclophosphamide and doxorubicin). No treatment was given to control group patients. Seven parameters were measured in this study: glutathione - S - transferase (GST), glutathione peroxidase (GPx), Xanthine oxidase (XO), malodialdehyde (MDA), High density lipoprotein cholesterol (HDLc), Low density lipoprotein cholesterol (LDLc) and selenium (Se). All patients were suffering from atherosclerosis.

**Methods of assay :-**

Glutathione - S - transferase (GST) activity was determined in erythrocytes using glutathione as a substrate, the absorption was recorded at (340 nm). Glutathione peroxidase (GPx) activity was measured in erythrocytes using glutathione and hydrogen peroxide (H₂O₂), the detection was recorded at (340 nm). [18]

Xanthine oxidase (XO) activity was determined in erythrocytes depending on the enzymatic oxidation of xanthine to uric acid, the detection was recorded at (293 nm). [19]

Malodialdehyde (MDA) level was measured in serum on the basis of formation of colored complex upon the reaction with thiobarbutyric acid this complex was absorbed at (500 nm). [16]

High density lipoprotein cholesterol (HDLc) was determined in serum according to phosphotungestic acid in the presence of magnesium ions, the detection was recorded at (510 nm). Low density lipoprotein cholesterol (LDLc)
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was also determined in serum depending on [17] selenium (Se) level was determined by atomic absorption flameless. [20]

Statistical analysis :-

Students t.test was used as a statistical analysis. Results have been expressed in tables as Mean ± S.D. Values of (p<0.01) were considered to be high significant while values of (p<0.001) were considered to be very high significant

Results :-

Table (1):- Mean ± S.D values of serum GST activity in patients with prostate cancer compared to control group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>number</th>
<th>GST activity (U/gm Hb)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25</td>
<td>1.95±0.40</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>2.50±1.51</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>2.26±1.84</td>
<td>(P&lt;0.01)</td>
</tr>
</tbody>
</table>

Table (2):- Mean ± S.D values of serum GPx activity in patients with prostate cancer compared to control group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>number</th>
<th>GPx activity (U/gm Hb)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25</td>
<td>27.2±4.6</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>35.4±6.3</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>31.3±5.2</td>
<td>(P&lt;0.01)</td>
</tr>
</tbody>
</table>

Table (3):- Mean ± S.D values of serum XO activity in patients with prostate cancer compared to control group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>number</th>
<th>XO activity (U/gm Hb)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25</td>
<td>0.025±0.01</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>0.057±0.02</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>0.045±0.025</td>
<td>(P&lt;0.01)</td>
</tr>
</tbody>
</table>

Table (4) :- Mean ±S.D malondialdehyde of value serum patients with prostate cancer compared with healthy control group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number</th>
<th>MDA nmole / mL ±S.D</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25</td>
<td>3.5±0.15</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>15.4±5.2</td>
<td>(P&lt;0.01)</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>19.2±6.1</td>
<td>(P&lt;0.001)</td>
</tr>
</tbody>
</table>

Table (5):- Mean ± S.D values of serum HDLc level in patients with prostate cancer control group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>number</th>
<th>HDLc (mg/dL)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25</td>
<td>46.2±5.3</td>
<td></td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>Subject</th>
<th>Number</th>
<th>LDLc (mg/dL)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>control</td>
<td>25</td>
<td>114.5±12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>25</td>
<td>125.1±15.2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>25</td>
<td>147.1±2.02</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6: Mean ± S.D values of serum LDL level in patients with prostate cancer compared to control group.

<table>
<thead>
<tr>
<th></th>
<th>Subject</th>
<th>Number</th>
<th>(Se) level (µgm / dL)</th>
<th>t.test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>25</td>
<td>14.5±1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>25</td>
<td>12.4±1.5</td>
<td>(P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>25</td>
<td>9.54±2.6</td>
<td>(P&lt;0.001)</td>
</tr>
</tbody>
</table>

Table 7: Mean ± S.D values of serum Selenium (Se) level in prostate cancer patients compared to healthy control.

Discussion:

1. Determination of GST activity.

Table (1) illustrated that GST activity in group A was increased (P<0.001) compared to control group. This increasing may be explained by that GST which is a group of multifunctional protein, plays a central role in the detoxification of electrophilic chemicals and hepatic removal of potential harmful hydrophobic compounds resulted from chemotherapy drugs. But with the progression of disease (group B), GST activity was decreased (P<0.001). After a long time suffering from prostate cancer, toxic compounds resulted from chemotherapy treatment play an important role in decreasing of antioxidant enzymatic system. [1,2]

Chemotherapeutic drugs are hydrophilic and can not pentrate into the inner membranes of cells where they would be reduced by NADH located on the inner membrane surface. Chemotherapy drugs used in treatment of prostate cancer are able to enter the outer mitochondrial membrane and enter the cytosol. Intracellular rearrangements result in formation of a lipophilic dehydrogenase that can penetrate the inner membrane of the mitochondria.

These doxorubicin competes with the coenzyme Q10 as an electron acceptor and diverts electrons to molecular oxygen resulting in formation of superoxide radicals. Doxorubicin intercalates DNA coils and interferes with normal cellular metabolism through a diverse set of biochemical mechanisms that may explain its toxicity. It causes an increase in peroxidation of unsaturated
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Fatty acids of membrane phospholipids which leads to a decrease in the level of antioxidant enzymes like (GST) and generates a high level of oxidative stress.[1,2]

In other words, the low activity of GST might be due to depletion of antioxidant defense system. [1,2]

2- Determination of GPx activity.

Data in table (6) and figure(6) have shown that GPx activity was elevated in group A (P<0.001) compared to control group. Under conditions of chemotherapeutic treatment, lipid peroxidation level is generally increased, increasing of reactive oxygen species concentration may cause damage to many biomolecules like lipids, proteins and carbohydrates. GPx, which is an antioxidant, selenium dependent enzyme catalysis the reaction between glutathione and H2O2 that is confirmed a highly toxic oxygen species. GPx was increased in group A because it is one of the reactive antioxidant enzymes responsible for detoxification by transforming of H2O2 to water. [1]

Oppositively, data have proved that GPx activity was declined for group B (P<0.01). This decreasing due to increasing the level of lipid peroxidation in blood resulting from a long period of time after treatment with chemotherapy. [3,4]

A study have demonstrated that oxidative stress plays an important role for the intiation of DNA damage and decreasing activity of antioxidant system. [4]

Another study have investigated that the low activity of antioxidant enzymes (like GPx) might be due to depletion of antioxidant defense system. [20]

3- Determination of Xanthine oxidase (XO) activity.

Xanthine oxidase activity was significant increased in group A (P<0.001) compared in comparison with control group. Xanthine oxidase (XO) has been considered to play a crucial role in the pathogenesis of lipid peroxidation in prostate cancer and atherosclerosis patients because the enzyme reaction that transfers electrons from hypoxanthine to uric acid is coupled with the reduction of molecular oxygen into super oxide anions. [19]

Reversely in group B, Xanthine oxidase (XO) activity was declined (P<0.01). This decreasing due to elevation of Allupurinol concentration in blood after a long time of giving chemotherapy drugs. Allupurinol is orally taken by cancer patients about (24-28) hours before treatment with chemotherapy drugs. Allupurinol which is an inhibitor of xanthine oxidase is given with some cancer chemotherapy treatment to prevent damage to the kidneys the result is low activity of Xanthine oxidase (XO). [21,22]

4- Determination of malondialdehyde( MDA) level:-

Data in table (4) demonstrated that malondialdehyde( MDA) level in group A was elevated (P<0.01) compared to healthy control. This elevation was
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continued for group B (P<0.001). In other words, there is a positive relationship between malondialdehyde (MDA) level and the progression of prostate cancer. This elevation in MDA level could be explained by generation by free radicals and reactive oxygen species (ROS) that related to chemotherapy drugs. [12,13]

The prime targets of free radicals reactions are unsaturated bonds in membrane phospholipids.[21]

The consequence of these reactions termed, lipid peroxidation is the loss of membrane fluidity, receptor alignment and potential cellular lysis. Also free radical damage to sulfur containing enzyme and other proteins result in inactivation, cross linking and denaturation. Furthermore, nucleic acids can be attacked and subsequent to DNA can cause mutation that may be carcinogenic. [23,24]

Damage of cells resulted from LPO produces secondary products. The most important products indicates LPO is malondialdehyde (MDA), therefore MDA level was increased with the progression of any disease related to lipid peroxidation. [25,26]

5- Determination of HDLc level.

Data in table (5) have shown that HDLc level was elevated positively with the progression of prostate cancer. HDLc is able to counter balance and prevent LPO. It has been suggested that HDL-cholesteol prevents both enzymatic and non-enzymatic generation of reactive oxygen species like H2O2 and thus acts as an anticarcinogen and powerfull antioxidant. [25,26]

Since precuser particles of HDLc are thought to drive from lipolysis of TG, and the lipoprotein lipase (LPL) activity which responsible for TG lipolysis is decreased in cancer and atherosclerosis patients, increased TG may be an important factor that results in lower HDLc concentration. [26]

6- Determination of LDLc level.

Table (6) represented that LDLc level was increased with the propagation of disease. (P<0.01) for group A and (P<0.001) for group B. LDL-cholesterol is more susceptible to oxidation in various pathological conditions, which result in higher lipid peroxidation associated with prostate cancer and atherosclerosis. [14]

Many studies have supported a relationship between cholesterol in prostate tissues or secretions and benign or malignant prostate growth. [25,27]

7- Determination of selenium (Se) level.

Data in table (7) have demonstrated that Se level was positively declined in prostate cancer patients compared to control group. (P<0.01) for group A and (P<0.001) for group B. Selenium is a trace element that is known to be essential for the activation of GPx, which is a key enzyme in the defense against
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oxidative stress. For this reason, Se supplementation alone and in combination with other micronutrients has been extensively studied. [29]

A study has shown that dietary constituents have been reported to play vital roles in the development or prevention of cancer. Selenium, an essential trace nutrient, has been reported to improve immune function in animals, enhance neuropsychological function in humans and ameliorate specific disease conditions in humans and animals. Selenium deficiency has been associated with initiation of events leading to the development of tumors. Low levels of selenium have been associated with a higher risk of atherosclerosis and cancer in humans. [30,31]

The ability of selenium compounds to inhibit growth and induce tumor cell apoptosis has been suggested to be a potential mechanism for cancer chemoprevention. [32]

Conclusions :-

1. MDA and LDLc levels were increased positively with the progression of prostate cancer.
2. HDL level was decreased positively with the progression of prostate cancer.
3. GST, GPx and XO activities were increased in prostate cancer patients in its first two years but in the third and fourth years, they decreased relatively.
4. Se level was decreased positively with the progression of prostate cancer.

References :-

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تقدير بعض البارامترات (الأنزيمية و غير الأنزيمية) المرتبطة بالأكسدة الفوقية للدهون لرجال مصابين بسرطان البروستات.

د. عمار مولا حمود
وزارة العلوم والتكنولوجيا

الخلاصة:

إن الغرض من هذه الدراسة هو تقدير بعض المتغيرات المرتبطة بشدة الأكسدة في كريات الدم الحمراء ومصل الدم في المرضى المصابين بسرطان البروستات عن طريق قياسها ومقارنتها مع مجموعة السيطرة. تم قياس فعاليات أنزيمات الكولوتاشايبون - أس - ترانسسفيريز و الكولوتاشايبون بروكسيداز والزناتين أوكسيديز في كريات الدم الحمراء وكذلك تم قياس مستويات المالونداي ألدهياديد والبروتينات الدهنية عالية الكثافة والبروتينات الدهنية قليلة الكثافة والسيلانيوم في مصل الدم في مجموعتين من المرضى المصابين بسرطان البروستات.

الجمعية A تتضمن 25 من الرجال المصابين بسرطان البروستات ولمدة تتراوح ما بين سنتين إلى ستين. إن الغرض من هذا الدراسة هو تقديم بعض المتغيرات المرتبطة بسرطان البروستات لتحديد مدى التأثير الفعلي للمتغيرات. إن المستويات المقارنة في الجماعة أ كانت أقل من مستويات المارض ومستوى السيطرة بمستوى الاحتمال (p<0.001) والنتائج فعاليات الكولوتاشايبون - أس-ترانسسفيريز والكولوتاشايبون بروكسيداز والزناتين أوكسيديز إرتفعت في مقارنات مع مجموعة السيطرة (A). المجموعة B أيضاً تتضمن (25) من الرجال المصابين بنفس المرض ولمدة تتراوح ما بين سنتين إلى أربع سنوات. A والمجموعة B مجتمع المرضى تمت مقارنتهما ب (25) من الرجال الأصحاء كمجموعة السيطرة. أثبتت النتائج فعاليات الكولوتاشايبون-أس-ترانسسفيراز والكولوتاشايبون بروكسيداز والزناتين أوكسيديز إرتفعت في مقارنات مع مجموعة السيطرة (A) المجموعة B ولكنها انخفضت في المجموعة (B) ومستوى إحتمالية قدره (p<0.001) وبمستوى إحتمالية قدره (A) A. إن مستوى المالونداي ألدهياديد أرتفع لدى المجموعة A كما أن هذا الإرتفاع سيستمر (B) A. إن مستوى البروتينات الدهنية عالية الكثافة انخفضت في المجموعة A مقارنة بقبيمة السيطرة كما أن هذا الانخفاض سيستمر لدى أفراد المجموعة A. بسنتين إلى أربع سنوات في حالات المرضية بالمقارنة مع الفي، وعلى العكس فإن مستوى البروتينات الدهنية قليلة الكثافة (B) انخفضت في المجموعتين مرئية مقارنة مع مجموعة السيطرة كما أن هذا الانخفاض يتسبب طردياً مع الفترة الزمنية للإصابة بالمرض.

مجلة كلية كليوبترة
ملحق العدد الخامس والسبعون 2012
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