ALTERATIONS IN SERUM 5’NUCLEOTIDASE AND MALONDIALDEHYDE IN BREAST TUMORS

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ABSTRACT
Background and objectives: the 5’nucleotidase (5’NT) is one of hydrolytic enzymes present in different organs which catalyze hydrolysis of 5’ nucleotides to ribonucleosides and phosphate. Malondialdehyde (MDA) is the end product of lipid peroxidation by oxidative stress (free radicals). The aim of present study was to measure the serum activity of 5’NT, and MDA concentration in breast tumors. Material and method: A prospective study was carried out from May to December 2013 by clinical biochemistry department in College of Medicine-Hawler Medical University on (30) healthy female individuals, (group 1) and (30) females with breast tumor (group 2). Results: The mean value of serum MDA was significantly higher in females with breast tumors (group 2), than that of healthy female individuals, (group 1) (p<0.01), and serum 5’NT activity was significantly higher in females with breast tumors (group 2), than that of healthy individuals (group 1) (p<0.01). Conclusion: Based on findings of the present study it can be concluded that breast tumors can cause release of the enzyme 5’NT from tumor cells, and lipid peroxidation by reactive oxygen species (ROS), which cause elevation of MDA. 
Key words
Serum 5’ nucleotidase (5’NT), serum malondialdehyde (MDA), oxidative stress, breast tumors.

I. INTRODUCTION
5’nucleotidase (5’NT) is an intrinsic membrane glycoprotein, present as an ectoenzyme in a wide variety of mammalian cells. 5’NT hydrolyzes 5’-nucleotides to their corresponding nucleosides, is belonging to hydrolase class of enzymes (EC 3.1.3.5)1. 5’nucleotidase is present in various tissues of human body. The enzyme was discovered by Reis2. The major functions of 5’nucleotidase are:
1. Production of adenosine from nucleotides of intra and extracellular, liberating which acts as a neurotransmitter and production of adenylate cyclase3.
2. It is important for scavenging of extracellular nucleotide and foreign RNA parts obtaining by cells4.
3. It is important for specific activation process of lymphatic cells in human body5.

In cancer patients, elevated serum 5’NT activity does not always indicate hepatobiliary involvement; in some cases, 5’NT may be released into serum from the primary tumor or local metastases6.

Oxidative stress is described as an imbalance in the production of pro oxidants such as reactive oxygen species (ROS); that are formed from the partial reduction of molecular oxygen8, such as super oxide, hydrogen peroxide, hydroxyl and radical hydroxide9 (ROS) can cause peroxidation of lipids leading to damage of membrane permeability, loss of enzyme activity involved in the decomposition of ROS, DNA damage leading to mutagenesis, and apoptosis of cell10.

Malondialdehyde (MDA) is one of many low molecular weight-end products of lipid peroxide decomposition and in the most often measured as an index of lipid peroxidation11. The plasma concentration of MDA is the most frequently used biomarker providing an indication of the overall lipid peroxidation level when produce caused by many diseases12.

Diseases associated with oxidative stress such as; diabetes mellitus, and cancer13. Reactive oxygen species can cause oxidative DNA and protein damage, damage to tumor suppression cells14.

Oxidative DNA damage may be involved in the development of breast cancer. Increased steady-state levels of DNA base damage with a pattern characteristic of OH, attack has been reported in inflammatory breast cancer15.

The reactive oxygen intermediates, produced in mitochondria, peroxisomes, and the cytosol, are scavenged by cellular defending systems, some are located in cell membranes, others in the cytosol, and others in the blood plasma16. An antioxidant is a substance which protects biological tissues from free radical damage17.

The cellular antioxidant systems can be divided into two major groups, enzymatic and nonenzymatic. Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase. Some non enzymatic low molecular weight antioxidant compounds such as (vitamins C, E, and, A)18.

Breast tumors it is estimated that 50% of women have breast symptoms at some times in their lives.

Benign breast tumors are most commonly seen in women who are of menstruating age. Risk factors for benign breast disease include irregular menses, small breast, family history of benign or malignant breast disease spontaneous abortion and late menopause19.

Breast cancer is the most common cancer in women who do not smoke, the screening program with mammography every three years from the age 55 and improvements in multi modality treatment have improved overall surgery has greatly ameliorated the psychosexual impact of the disease (kumar & klark)20.

The strongest associations for the development of breast cancer are personal and family histories of breast cancer.

Hormonal factors are important determinants of breast cancer risk. Oral contraceptive use beginning at a young age
and for longer than 10 years seems to increase breast cancer risk only slightly.\(^{18}\)

The present study was therefore undertaken to measure the 5'NT enzyme activity in breast tumors. Oxidative stress, antioxidants and their association with 5'NT were also investigated.

II. MATERIALS AND METHODS

A. Subjects

This study was conducted over a period of five months, from May to December 2013, and the subjects include (30) healthy female volunteers (group1), and (30) females with breast tumor (group 2), which is classified into two subgroups, benign breast mass (group 2A), and malignant breast cancer (group2B). All the cases in both groups (1&2), are non smoking and non drinking alcohol.

B. Serum Sampling

Four to six mls. of venous blood was withdrawn from each individual using disposable syringes. The samples were immediately centrifuged for [10] min at 3000 rpm, and the serum obtained was analyzed the same day.

C. Methods

1. Estimation of Serum 5' Nucleotidase activity:--

Incubate the serum containing enzyme activity with the substrate adenosine 5' monophosphate at optimum condition, (temperature 37, and pH 7.5), for half an hour, release phosphate and adenosine, serum proteins are precipitated by trichloroacetic acid, and the phosphate is converted to a phosphomolybdate. The addition of P-methyl aminophenol sulphate (metol compound) reduces the [Mo\(^{VI}\)] in the complex to yield an intensity blue-colored phosphomolybdate complex [Mo\(^{VI}\)]. The absorbance of the solution at 700nm is proportional to the serum phosphate concentration.\(^{1}\)

2. Malondialdehyde (MDA) measurement:--

Lipid peroxidation was estimated according to the method described by Rehncrona et al. Measurement of (MDA), a secondary product of lipid peroxidation, was based on the colorimetric reaction withthiobarbituric acid \(^{21}\). This is based on the reaction of (MDA) with thiobarbituric acid (TBA) forming red, fluorescent MDA-TBA2 adduct, that absorbs strongly at wavelength 532 nm.

\[
\text{Thiobarbituric acid + MDA} \rightarrow \text{MDA-TBA2 adduct} \]

Figure 2. Formation of the MDA-TBA2 adduct (Ge’rard-Mornier et al., 1997).

D. Statistical Analysis

All biochemical parameters were compared between groups using T-test. The statistical software SPSS Version


III. RESULTS

Table 1: provides the mean ± S.E.M. of serum 5NT activity and enzyme activity in breast tumors. The results obtained reveal that the mean ± S.E.M. enzyme activity in benign breast group (1.92 ± 0.25 IU/L) was significantly higher than that of females with breast tumors (p< 0.001).

Table (1): Details of the subject’s age

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Age(years) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy female individuals (group 1)</td>
<td>30</td>
<td>43.15±10.5</td>
</tr>
<tr>
<td>Females with breast tumor (group 2)</td>
<td>30</td>
<td>45.26±10.8</td>
</tr>
</tbody>
</table>

Females with breast cancer V Healthy female individuals

Z= 6.32          \( P < 0.001 \)

Table (2): The mean ± S.E.M. of S.5'NT activity in normal and breast tumors groups.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number</th>
<th>S.5'NT (IU/L) Mean ±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy female Individuals (group 1)</td>
<td>30</td>
<td>7.78 ±1.176</td>
</tr>
<tr>
<td>Females with Breast tumors (group 2)</td>
<td>30</td>
<td>21.92 ±3.295</td>
</tr>
</tbody>
</table>

Females with breast cancer V Healthy female individuals

Z= 4.32          \( P < 0.001 \)

Table (3): shows the mean values of serum MDA in healthy female individuals 1.08±0.176 µmol/L, and females with Breast tumor 1.92±0.25 µmol/L .The mean level of serum MDA in healthy female individuals was significantly higher than that of females with breast tumors (p< 0.001).

Table (3): Details of serum Malondialdehyde (µmol/L) [Mean ± S.E.M.] in healthy non athletes and healthy athletes.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number</th>
<th>Mean ±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy female Individuals (group 1)</td>
<td>30</td>
<td>1.08 ±0.176</td>
</tr>
<tr>
<td>Females with Breast tumors (group 2)</td>
<td>30</td>
<td>1.92 ±0.25</td>
</tr>
</tbody>
</table>

Table (4), provides the mean S.5'NT activity in two subgroups, benign breast cancer, and malignant breast cancer. The results obtained indicate that the mean S.5'NT activity was (29.93 ±4.5IU/L), in malignant breast cancer. This exceeds significantly than those obtained in benign breast tumor group (14.66 ±2.8) (P<0.001). The results obtained indicate that the mean S.MDA was (2.27 ±0.27 uMol/L), in malignant breast cancer. This exceeds significantly than those obtained in benign breast tumor group (1.66 ±0.198) (P<0.01).
Table (4): The mean ± S.E.M. of each S.5’NT activity and S.MDA in benign breast cancer and malignant breast cancer.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Numbers</th>
<th>S.5’NT(IU/L) Mean ±S.E.M</th>
<th>MDA(uMol/L) Mean ±S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign breast tumor (group2A)</td>
<td>12</td>
<td>14.66 ±2.8</td>
<td>1.66 ± 0.198</td>
</tr>
<tr>
<td>Malignant breast cancer (group2B)</td>
<td>18</td>
<td>29.93 ±4.5</td>
<td>2.27±0.27</td>
</tr>
</tbody>
</table>

A. S.5’NT(IU/L)

Females with malignant breast cancer V Females with benign breast tumor

Z= 5.32   \ P < 0.001

B. MDA (µMo/L)

Females with malignant breast cancer V Females with benign breast tumor

Z= 2.32   \ P < 0.01

IV. DISCUSSION

Table (2) shows the mean activities of serum 5’NT in healthy female individuals 7.78±1.176 IU/L, and female with breast tumors 21.92±3.295 IU/L. The mean activity of serum 5’NT in female breast tumors were significantly higher than that of healthy female individuals (p< 0.001). Our results are in agreement with those obtained by Emmon D. Ryan and Gloria Bilous33, Climent Ip and Thomas Dao 25, and Kruger K. H, Thompson L. F, Kaufmann. M and Miller P 26, but the results disagreed with Curt B. Dixon 19. Whose study indicates that moderate-intensity whole-body resistance exercise had no effect on serum MDA concentration.

5’-Nucleotidase (5’NT) that catalysis the hydrolysis of 5’-nucleotides to nucleosides and inorganic phosphate, is found to be elevated in the sera of 90% breast cancer patients before treatment. In many cases, a successful response to treatment was associated with a decrease of plasma 5’-NT levels. This has led to the conclusion that breast cancer cells are also a source of plasma 5-NT and sequential determinations in breast cancer patients may have a possible prognostic relevance 27. In other study by Wang Li et al 28, showed that ecto-5’-nucleotidase promotes invasion, migration of human breast cancer cells.

Serum lactate dehydrogenase, leucine aminopeptidase and 5’-nucleotidase activity observations in patients with carcinoma of the pancreas and hepatobiliary disease 29.

Table (3): Shows the mean values for serum MDA in both groups (healthy female individuals and female with breast tumors). The mean concentration of serum MDA in female breast tumors were significantly higher than that of healthy female individuals (p< 0.001). Our results are in agreement with those obtained by Ebubekir Bakan et al 30, Gonenc A31, and Bulent Actan et al 32.

Prime targets of oxygen free radicals are poly unsaturated fatty acids in lipid membranes. This attack causes lipid peroxidation. Increased peroxidation and decreased antioxidant protection generate epoxides that may spontaneously react with nucleophilic centers in the cell and thereby covalently bind to DNA, RNA, and proteins. Such reactions may lead to cytotoxicity, mutagenecity, and carcinogenicity 33.

It has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes 34. Hang et al 35 reported significantly increased lipid peroxidation, measured as MDA, in the serum of breast cancer patients.

Thomensen and co-workers 36 showed that nitric oxide and MDA were present in fresh human tumor tissue, where it was localized to tumor cells in gynecological cancers and to Struma of breast cancers.

Table (4) shows the mean levels for serum 5’NT activity, and serum concentration of MDA in both benign and malignant breast cancers, the mean serum 5’NT activity in females with malignant breast cancer was significantly higher than that of females with benign breast tumor P< 0.001, and serum MDA in females with malignant breast cancer were significantly higher than that of females with benign breast tumor P<0.01. The same results obtained by Vanu R R et al 37 and Shruby S et al38, the levels of 5’NT activity and MDA increased with increase the activity of the disease and all these factors were significantly brought back to near normal levels on drug treatment. Higher levels of MDA in breast tumor group were suggestive of higher oxidative stress in patients with breast tumors 39. This finding is consistent with the reports from several studies that confirmed the involvement of free radicals in the pathogenesis of breast tumors, the extent of oxidative stress is more profound in malignant breast than in benign breast cancer. 40, 41.

V. CONCLUSION

This study attempted to establish the extent of serum 5’NT and oxidative stress in breast tumors as compared with normal individuals (controls). Based on study results, it is concluded that the activity of serum 5’-NT is consistently higher in breast tumor. We can also conclude that there is an increase in oxidative stress in breast tumor whereby the extent of oxidative stress is more profound in malignant breast cancer than in benign breast cancer. The parameters of lipid peroxidation and antioxidant defense may be useful markers for monitoring patients with breast tumors.

REFERENCES


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