Original article:

Interplay between Nitric oxide, mitochondrial thiols and thioredoxin system in cancers of ovary and cervix.

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Abstract:

Introduction: Nitrosative stress in mitochondria which is mainly created by nitric oxide, nitrothiol and nitrotyrosine may alter thiol status in cancers of ovary and cervix. As an antioxidant defence Thioredoxin system may be playing role in restoring the functions.

Methods: Mitochondria were lysed and lysate was used for estimation of Trx, TR, nitrothiols, total and membrane thiol concentrations. The cell lysate was used for estimation of nitrotyrosine. NOx (nitrate and nitrite) levels were estimated in plasma samples.

Observation: We found that the levels of total mitochondrial and membrane protein thiols were decreased significantly (p<0.05). Levels of NO were not much affected and nitrothiols were not detected. However nitrotyrosine was detected which may be contributing to thiol modification. The levels of thioredoxin and thioredoxin reductase were elevated significantly (p<0.05).

Result and Conclusion: NO, nitrothiols and nitrotyrosine might be responsible for reduction in mitochondrial thiols. This modification of thiols may be counteracted by thioredoxin system.

Keywords: Nitric oxide, nitrotyrosine, nitrothiols, Thioredoxin system

Introduction:

Cancer is fundamentally a disease of failure of regulation of tissue growth and is characterised by inflammation and release of cytokines. Inflammation induced carcinogenesis is caused by many factors; and reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major contributors. The ROS/RNS damage bio-molecules like lipids, DNA and proteins in their close vicinity.

Nitric oxide (NO) is mainly responsible for generation of RNS. This molecule is synthesized from L-Arginine in the reaction catalysed by nitric oxide synthase (NOS). At low concentration it has protective role. At higher concentrations it causes damages to biomolecules. At equimolar concentration, NO forms reacts with superoxide to form peroxynitrite. Peroxynitrite is also responsible for nitration of free and protein bound tyrosine residue forming nitrotyrosine. It is thought that nitrotyrosine is footprint of increased NO production. In addition to this NO can react rapidly in the intracellular environment to form nitrite and nitrate, S-nitrosothiols etc. Thus thiol containing proteins more vulnerable to the action of NO and peroxynitrite. Mitochondrial proteins can be thus modified by nitration, S-nitrosylation, S-glutathionylation, or
carbonylation. NO° is oxidizing as well as nitrating agent which can traverse biological membranes. Protein oxidation and nitration results in altered function of many enzymes\(^5,6\).

Oxidized proteins are reduced back by various systems in the body. Thioredoxin system is one of them. It consists of thioredoxin reductase (TR), thioredoxin (Trx) and NADPH. Trx in its active form (reduced) provides reducing equivalents to target molecules including ribonucleotide reductase (involved in DNA synthesis), peroxiredoxins (cellular antioxidants) and various transcription factors. During these reactions Trx gets oxidised. This oxidised Trx is reduced back to its active form in presence of TR. Trx exists in two isoforms: Trx1 (cytosolic) and Trx2 (mitochondrial). Similarly, the TrxR, occurs both as cytosolic form (TrxR1) and mitochondrial form (TrxR2) that act upon Trx1 and Trx2, respectively \(^7,8\). Constitutive Trx and TrxR expression has been observed in several cell types of the mammalian body, including keratinocytes of the skin, placental cells, liver cells, secretory cells, and leukocytes \(^9,10\). Physiological stimuli, including UV light, hydrogen peroxide and mitogens can induce the expression of Trx and TrxR pointing at an important role in protection against oxidative stress and in regulating cell growth and cell death \(^11\).

Urbanization leading to changes in lifestyle made life more stressful and this may be one of the contributory factors for various disorders; Cancer is one of them. Prevalence of cancers of ovary and cervix is increasing in India. Keeping these facts in mind, the present work was designed to study the effects of NO° and Trx system on mitochondrial thiol levels in ovarian and cervix cancer. The present study was planned to know the levels of mitochondrial total and membrane protein thiols in cancers of ovary and cervix and to find the levels of plasma NOx levels and nitrothiols and nitrotyrosine from the cell lysate.

**Material and Method:**
Mitochondria were isolated\(^12\) from 35 tissues of ovarian cancer, 31 tissues of cervix cancer and 38 non-malignant tissues. Mitochondria were lysed and lysate was used for estimation of Trx, TR, nitrothiols, total and membrane thiol concentrations. The cell lysate was used for estimation of nitrotyrosine. NOx levels were estimated in plasma samples.

Table 1: Shows parameters estimated in the present study and the methods used for the estimation \(^13\text{-}19\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NOx concentration</td>
<td>Moshage et al and Granger et al.</td>
</tr>
<tr>
<td>Nitrotyrosine</td>
<td>Crow and Ischiropoulos</td>
</tr>
<tr>
<td>Nitrothiols</td>
<td>Cook et al</td>
</tr>
<tr>
<td>Thioredoxin (Trx)</td>
<td>Holmgren and Bjornstedt</td>
</tr>
<tr>
<td>Thioredoxin Reductase TR</td>
<td>Luthman and Holmgren</td>
</tr>
<tr>
<td>Mitochondrial total thiol</td>
<td>Modified Habeeb</td>
</tr>
<tr>
<td>Mitochondrial membrane protein thiol</td>
<td>Kowaltowski et al</td>
</tr>
</tbody>
</table>
The samples were run in duplicate and for each sample; the mean of the two values was taken. The statistical significance was calculated by Mann–Whitney U test by using NCSS-PASS statistical software. Statistical significance was chosen as p<0.005.

**Observation and Results:**

<table>
<thead>
<tr>
<th></th>
<th>Plasma NOx (µmol/L) Mean ± SD</th>
<th>Nitrotyrosine (µmol/L) Mean ± SD</th>
<th>Total thiol level (nmol/mg) Mean ± SD</th>
<th>Membrane protein thiol level(nmol/mg) Mean ± SD</th>
<th>Trx level (pmol/mg) Mean ± SD</th>
<th>TR level (pmol/mg) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=38)</td>
<td>35.61±5.94</td>
<td>Not detected</td>
<td>111.35±11.6</td>
<td>56.35±5.9</td>
<td>367.5±103</td>
<td>61.66±5.9</td>
</tr>
<tr>
<td>Ovarian cancer (n=35)</td>
<td>34.61±7.44</td>
<td>11.34*±3.67</td>
<td>62.3*±15.2</td>
<td>31.64*±6.4</td>
<td>3054*±1134</td>
<td>695.77*±6.5</td>
</tr>
<tr>
<td>Cervix cancer (n=31)</td>
<td>35.71±8.09</td>
<td>10.74*±3.93</td>
<td>62.4*±13.2</td>
<td>33.86*±7.1</td>
<td>3443*±1125</td>
<td>696.74*±7.1</td>
</tr>
</tbody>
</table>

[Table 2 showing results of plasma NOx, Nitrotyrosine, Nitrothiols, Trx, TR, Total and membrane thiol levels in control and experimental groups , *= p<0.05 statistically significant. (Nitrothiol was not detected in control and experimental group)]

**Discussion:** In the present study the levels of mitochondrial total and membrane protein thiols were found to be decreased significantly (p<0.05). No significant increase or decrease in the levels of NO\(^-\) was observed. Nitrothiols were not detected. However nitrotyrosine was detected. The levels of thioredoxin and thioredoxin reductase were elevated significantly (p<0.05).
superoxide to form peroxynitrite which further formed nitrotyrosine and this was detected in patients with ovarian and cervix cancer. This could be one of the factors modifying mitochondrial thiols. 

NO$^-$ might also react with thiols to form nitrothiol. However nitrothiol was not detected in experimental as well as control group. The fact can be explained by the role of TR in degrading nitrothiols mainly GSNO. This means that nitrothiols, the modified proteins as and when formed were degraded by TR. Also the levels Trx and TR were found to be elevated significantly (p<0.05). The increase in the Trx and TR, might be to restore the function of modified thiol groups. As a major intracellular reducing agent, these are upregulated in certain tumours, to protect cancer cells from oxidative stress. Since cancer cells are often under high oxidative and hypoxic stress it is not surprising that they also express high levels of antioxidant proteins. However these effects are no longer solely beneficial for the patient once the tumour is established. This probably could be the reason why levels of mitochondrial total thiols and membrane protein thiols were found to be decreased in presence of elevated levels of Trx and TR. The increased levels of Trx and TR as observed in the present study might be playing role in tumour progression by various mechanisms like increasing supply of reducing equivalents to ribonucleotide reductase for DNA synthesis, activation of transcription factors that regulate cell growth and an increase in the sensitivity of cells to other cytokines and growth factors or inhibiting apoptosis.

**Conclusion:** It can be concluded from the present study, that the RNS generated in the mitochondria of tissues of cervix and ovarian cancer have modified thiol groups of mitochondria forming nitrothiols and nitrotyrosine which may further affect mitochondrial function. Antioxidant defence system responds to it by elevating the levels of TR and Trx initially. However once the tumour is established elevated levels of these two proteins help in tumour progression and might not play restoring mitochondrial thiols. Further research using inhibitors of NOS to control production and effects of NO$^-$ will help in treating cancers.

**Acknowledgement:** To clinical staff of department of gynaecology and surgery for providing samples and laboratory staff of biochemistry department of BJMC.

**References:**

   The British Journal of Radiology. 81, S57–S68
24. Garth Powis, Debbie Mustacich, Amy Coon (2000). The role of the redox protein thioredoxin in cell growth and

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