

Investigating the Expression Levels of Glutathione Peroxidase and Glutathione Reductase Genes in Mastectomies Women

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Abstract

Introduction: Important genes that may be expressed in women candidates for mastectomy due to breast cancer are important. The expression of these genes in these women can play an important role in the treatment of these patients. The aim of this study was to investigate the expression level of glutathione peroxidase and glutathione reductase genes in mastectomies women.

Procedure: After cell culture, the cell suspension was planted in 96-well microplates and treated for 24 hours in a CO₂ incubator at 37 °C. To synthesize cDNA, extracted RNA molecules were used with the help of Fermentaz Revert Aid TM kit, produced in the United States.

Results: The concentration of 500 micrograms of nanoparticles caused the death of more than 60% of MCF 7 cells (P> 0.001) while there was no significant difference in all concentrations of nanoparticles on the normal HEK293 cell line.

Conclusion: The anti-tumor effects of zinc oxide nanoparticles were shown in increasing the expression of glutathione peroxidase and glutathione reductase genes in MCF-7 breast cancer cell line of mastectomy candidates.

Keywords: Glutathione peroxidase, Glutathione, Mastectomy, Breast cancer, Gene expression.

Introduction

Breast cancer is one of the most common non-skin malignant cancers diagnosed among women. Surgery is a preventive method for the development of breast cancer in women. Different strategies such as targeted treatment, hormone therapy, radiation therapy, surgery, and chemotherapy are used in patients who are diagnosed with breast tumor [1-3]. Undesirable side effects of

breast cancer treatment are one of the effective factors in finding alternative methods. Concerning the high number of women with breast cancer and the increase in the rate of this cancer in the last decade, the importance of studying this cancer was clear. In addition, the treatment method and effective drugs for breast cancer are very important because many patients develop resistance to it after treatment with a specific drug or method and the disease recurs [4-6].

The most synthesis instructions can be zinc oxide nanoparticles have received special attention in recent years due to their effects in cancer treatment. This compound exerts its therapeutic effects by taking more zinc ions by cancer cells [7].

The glutathione system plays a vital role in protecting the body from oxidative stress. This system converts hydrogen peroxides resulting from the activity of superoxide dismutase enzyme on superoxide ion, which is a dangerous reactive oxygen species (ROS), into water. Glutathione peroxidase and glutathione reductase are enzymes that play a vital role in this cycle and their gene expression maximizes when ROS increase and the body is in a state of oxidative stress. Oxidative stress induced by reactive oxygen species causes apoptosis in cancerous and non-

cancerous cells and their destruction [8-10].

Anatomy and Physiology of the Female Reproductive System

In this research, concerning the possibility of adverse effects of zinc nano oxide on non-cancerous cells, and also the necessity of determining the apoptosis-inducing factor caused by zinc nano oxide, the expression level of glutathione peroxidase and glutathione reductase genes at the mRNA level, which are indicators from above removal of ROS and oxidative stress is an inducer of apoptosis (Figure 1). Important genes that may be expressed in women candidates for mastectomy due to breast cancer are important. The expression of these genes in these women can play an important role in the treatment of these patients [11-13].

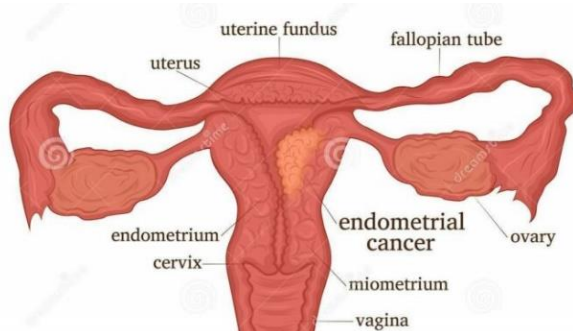


Figure 1 Uterine Cancer: What are the best treatments available?

Blood Supply and Nerve

Most external genital blood is supplied by the external iliac (hypogastric) artery (Figure 2).

The main arteries that supply internal genital blood are the uterine arteries (originating from the iliac artery) and the ovarian arteries (branching from the main aortic branch).

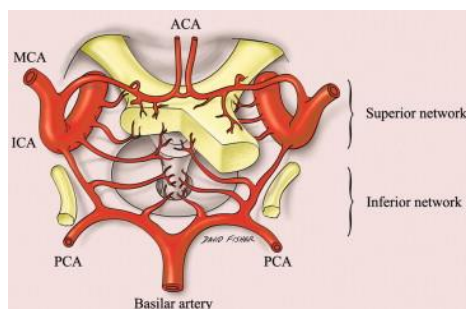


Figure 2 Blood supply of the cranial nerves

The autonomic nervous system is involved in innervating the internal structures of the pelvis. Sexual arousal is controlled by parasympathetic vasodilation in the vestibular and clitoral protrusions. The uterine myometrium is

innervated only by sympathetic nerve fibers, and the perineum is innervated by the pondal nerve (Figure 3). Cervix also responds to these changes, with the most important change being the mucus secretion as a clear fluid that increases to receive sperm before ovulation.

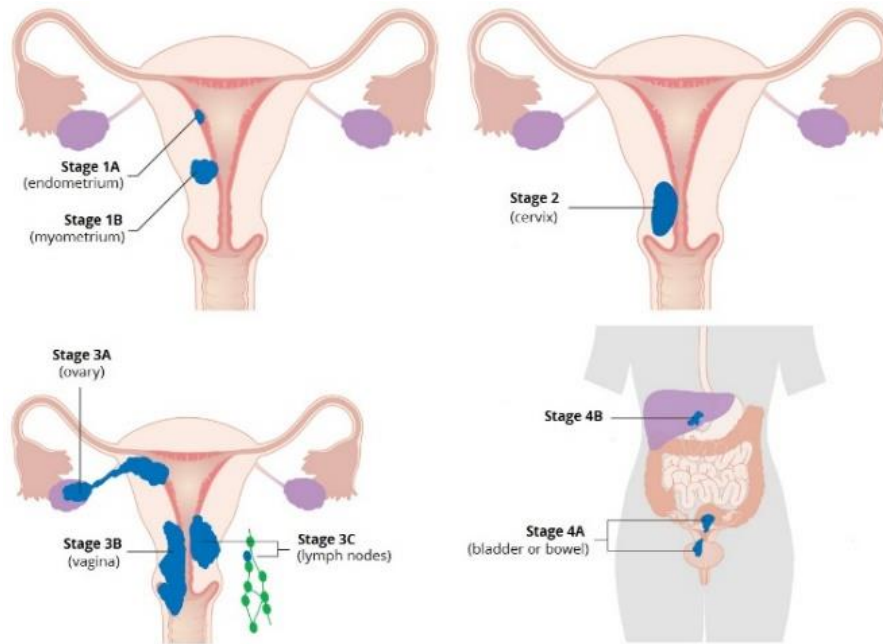


Figure 3 Uterine disorders

This stage is called the amplification phase in the secretory or luteal phase (day 14 of 28 days).

years old and older and those who are sexually active (regardless of the age) (Figures 4 and 5).

Physical Examination

Annual pelvic and breast examinations should be performed for all women of 18

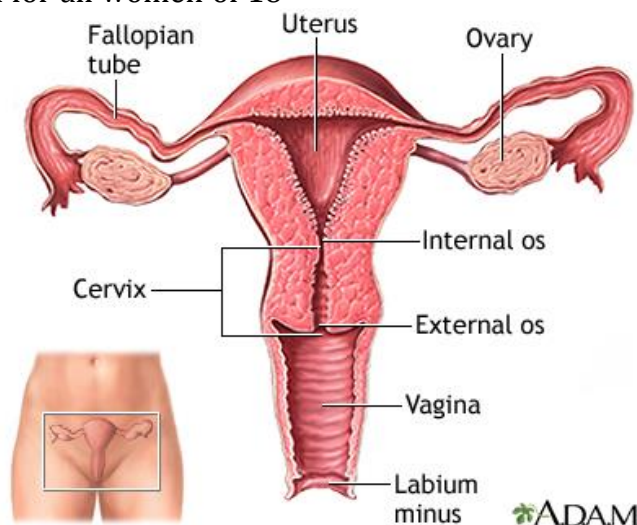


Figure 4 Uterus

ADAM.

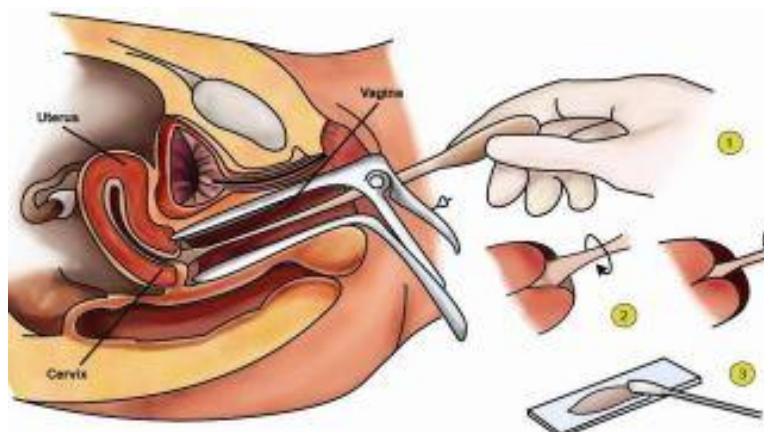


Figure 5 Pap smear

Menopause is partly associated with atrophy of breast and genital tissue, decreased bone mass and vascular changes (Figure 6).

6 SIGNS & SYMPTOMS OF MENOPAUSE

<p>YOU'RE REALLY HOT (LITERALLY) Flashes can come out of nowhere and pair up with sweating and chills.</p> <p>YOU'VE GOT NIGHT(MARE) SWEATS The flashes that strike in the night can completely soak your bed.</p> <p>YOU'RE DRY DOWN THERE Low estrogen can make vaginal tissue feel dry and irritated.</p>	<p>YOU'RE PEEING ALL THE TIME You gotta go constantly, and you have to do it like right now.</p> <p>YOU'RE MOODY x10 Menopause emotions are like period mood swings on steroids.</p> <p>YOU'RE...WAIT, WHAT? Brain fog is the norm for two-thirds of women in menopause.</p>
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Figure 6 What is menopause?

Premenstrual Syndrome

It is a set of symptoms that develop in the premenstrual phase and disappear after the onset of menstrual bleeding. The cause of this syndrome is unknown,

but the theory of serotonin regulation is the most important accepted theory (Figure 7).

PMS Symptoms Checklist







 Fatigue	 Period Pain	 Nausea / Vomiting	 Constipation / Diarrhea	 Headaches
 Backaches	 Appetite Changes / Food Cravings	 Mood swing	 Bloating	 Breast Tenderness

Figure 7 Are you struggling with premenstrual syndrome?

Nutritional factors are also important because carbohydrates affect serotonin. It is thought to be caused by excessive secretion of prostaglandins, which causes painful uterine contractions and vascular spasm.

Method

In this research, ZnONPs were purchased from Pishgaman Nanomaterials Iranian Company with a size between 10 and 30 nm and 99% purity from the American Company of US Research Nanomaterials. Evaluation of morphology and size of nanoparticles was investigated with the help of TEM model Zeiss-EM10C-100 KV. Two breast cancer cell lines, MCF-7 and normal HEK293 was used, which was obtained from Pasteur Institute of Iran. Then, the optical absorption of the samples was measured at a wavelength of 570 nm by the Graylyze reading. The percentage of cell viability was also calculated from the following formula:

ODT: Optical absorption of cells treated with zinc oxide nanoparticles, and

ODC: Optical absorbance of control treated cells.

A concentration of the tested compounds that reduces the percentage of cell viability by half was considered as IC50.

Evaluating the Expression of Glutathione Peroxidase and Glutathione Reductase Genes with the Help of Real Time PCR Technique

Whole cell RNA extraction was done by culturing 1×10^6 cells. After the treatment with oxide nanoparticles on the plates, it was done in a CO₂ incubator at 37 °C. The RNA extraction with appropriate quality and quantity from cells was done with the help of Trans RNA extraction kit, according to the

manufacturer's instructions. The concentration of all the extracted RNAs was checked using the bio-photometer of Eppendorf Company with wavelength A260/A230.

To synthesize cDNA, extracted RNA molecules were used with the help of Fermentaz Revert Aid™ First Strand cDNA Synthesis kit, produced in the United States.

The website www.ncbi.nlm.nih.gov was used to design the primers. The sequence of the designed primers was searched using BLAST software in the human genome sequence to ensure the sequence specificity and the uniqueness of their binding site. In this study, GAPDH gene was used as an internal control.

Genes quantification was done with the help of ABI 7300 Real Time PCR machine (Applied Bio systems, Foster City, CA, USA). The reaction solution included 5 microliters of cDNA, 12.5 microliters of PCR reaction mixture containing Cyber green (SYBER-Green PCR Master Mix), 1 microliter of each forward and reverse primer, and 5.5 microliters of deionized distilled water without nuclease. Likewise, thermal time schedule of the device for gene amplification includes the initial opening of DNA at 95 °C for 30 seconds and annealing at 95 °C for 20 seconds during the repetition of 40 cycles and binding of primers to template DNA at 62 °C. Grading was done for 60 seconds and the pattern elongation was done for 30 seconds at 72 °C.

The PCR reaction of the difference in cycle threshold (Ct) of treated and untreated cells with nanoparticles was obtained. In addition, using the DDCT formula, the ratio of target gene to the reference gene was calculated through the DDCT-2 formula. The data of this research were statistically analyzed with the help of SPSS version 22 software.

Results

Morphological investigation of the effect of zinc oxide nanoparticles on the MCF-7 breast cancer cell line was investigated with the help of a microscope. Morphological examination of the effect of nanoparticles on MCF-7 cell line showed that the number and size

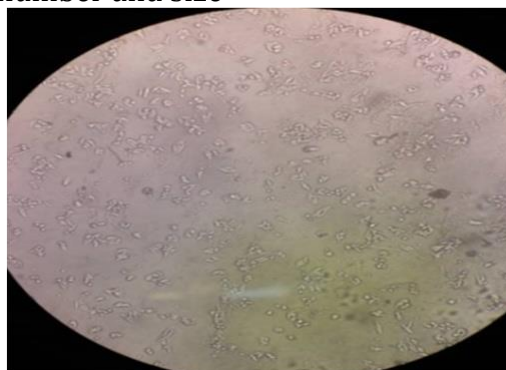


Figure 8 Investigating the morphology of toxicity effect of zinc oxide nanoparticles with the help of a microscope after 24 hours' treatment with zinc oxide nanoparticles.

After 24 hours, cell viability was evaluated with the help of MTT test. The toxicity results obtained for the MCF-7 cell line indicate that zinc oxide nanoparticles after a period of 24 hours at concentrations of 125, 250, and 500 $\mu\text{g/ml}$ have a reducing effect on cell survival in the MCF cell line they have 7. The concentration of 500 micrograms of nanoparticles caused the death of more than 60% of MCF-7 cells and it was statistically significant ($p > 0.001$) while it showed no significant difference in all concentrations of nanoparticles on the normal HEK293 cell line compared to the control group. Examining the cell survival rate using the MTT method showed that zinc oxide nanoparticles reduce the growth of MCF-7 cells. Melting curve analysis was used to confirm whether the gene fragments were specifically amplified without contamination and primer dimer.

Changes in the expression of glutathione peroxidase and glutathione reductase genes in MCF-7 cells treated with zinc oxide nanoparticles were

of treated cells decreased compared to the control group, which indicated the toxic effects of nanoparticles. Figure 1 shows that with the effect of nanoparticles, in addition to reduce the number of cells, some changes such as the reduction of cell cytoplasm is also observed.

evaluated using the real time PCR method after 24 hours. Thus, the expression ratio of glutathione peroxidase and glutathione reductase genes to the internal control gene in the cell line was (2.13 ± 0.07 ($p > 0.001$)) and (1.22 ± 0.05 ($p > 0.05$)) that increased twice.

Discussion

Today, chemotherapy is one of the most common treatment methods used to prevent cancer progression, which has side effects that threaten the patient. On the one hand, it leads to drug resistance, treatment failure, and low effectiveness [14-16]. Cancer cells become resistant to the spread of drugs. On the other hand, the anticancer effects of zinc oxide nanoparticles compounds have been identified in recent years due to their effects in treatment [17-19].

The results of the MTT test of MCF-7 cells in this research showed that zinc oxide nanoparticles are toxic and lethal against these cells, and the concentration of this compound increases when the cell

survival rate decreases [20]. On the other hand, this compound is not toxic or lethal for normal cells. This difference in toxicity in cancer and normal cells indicates the difference in penetration of zinc nano oxide by these cells [21-23]. This issue has been mentioned and even proven in similar researches. For example, we can refer to the research similar to the present study in which the uptake of zinc oxide nanoparticles was investigated by cells and at different stages of the cell cycle and found that the uptake of zinc oxide nanoparticles by cancer cells is significantly higher than that of normal cells. In addition, it was found that zinc oxide nanoparticles induce apoptosis in HepG2 human liver cancer cells. They showed that zinc oxide nanoparticles increase the expression of P53 and Bax genes. Moreover, they showed that ROS leads to these effects [24-26].

Some researchers studied the effects of treating carp cells with zinc oxide nanoparticles and showed that the expression of genes and glutathione peroxidase increases with maximizing the concentration of nanoparticles [27]. These effects are the result of ROS production, which in high concentrations can cause apoptosis and cell death. Also, some other researchers suggested that zinc nanoparticles cause the ROS production, increase the ratio of bax to Bcl-2, and increase apoptosis [28-30]. Various studies have been presented in relation to the evaluation of antioxidant properties of metal nanoparticles [31]. In research, the antioxidant activity of silver nanoparticles synthesized by biological method was evaluated through DPPH and ABTS test and it was shown that in concentrations of 7.12 and 16.17 $\mu\text{g/ml}$, they are able to inhibit 50% of radicals were released [32-34].

Antioxidant property of zinc oxide nanoparticles synthesized by biological method with the help of assessing

absorption of DPPH radicals and superoxide anion, which had 50% inhibition of free radicals at a concentration of 200 $\mu\text{g/ml}$ [35]. It was research on the anticancer effects of zinc nanoparticles synthesized by chemical pyrolysis method. They showed that zinc oxide nanoparticles lead to an increase in ROS, lipid peroxidation, glutathione reduction, the expression of antioxidant enzyme genes, and the activity of its enzymes in cells [36].

In addition, during another research on the effects of zinc oxide nanoparticles on the activity of antioxidant enzymes and the mRNAs expression in C2C12 and 3T3-L1 cells, it was found that treatment of cells with zinc oxide nanoparticles decreased the level of glutathione, increased ROS, and lipid peroxidation. And almost all cells die. Studies have shown that treatment of zinc nano oxide causes the ROS creation, and these ROS are the ones that cause apoptosis in the cell by destroying DNA and other vital compounds of the cell. ROS resulting from the effects of nano zinc oxide intensify the process of cell death by reducing the charge potential of the mitochondrial organelle membrane and increasing the ratio of Bax to Bcl-2 [37-39].

The production of reactive oxygen species by various methods such as the activation of caspase cascade and sphingomyelinase cause the release of cytochrome C from the inner membrane of mitochondria and the activation of internal pathway of apoptosis. In the present study, the toxicity effects of different concentrations of zinc oxide nanoparticles against MCF-7 breast cancer cell line and normal HEK293 cells were evaluated [40-42]. The cell line viability study showed that after 24 hours of treatment, zinc oxide nanoparticles caused a significant decrease in the viability of cancer cells under the influence of nanoparticles

while in normal cells treated with different concentrations of nanoparticles, no significant difference was observed [43].

According to most researches, metal oxide nanoparticles have been used in cancer treatment. The results of this finding could further show the same action. In this research, besides the toxicity of zinc oxide nanoparticles, the expression of glutathione peroxidase and glutathione reductase genes was evaluated in MCF-7 cell line, which showed a significant increase [44]. The activity of glutathione system increases with the increase in the amount of ROS to prevent further damage to the cells by removing them. Therefore, increasing the activity of glutathione system dependent on increasing the activity of glutathione peroxidase and glutathione reductase enzymes can be considered as a marker for increasing ROS production [45-47].

Conclusion

The antitumor effects of zinc oxide nanoparticles were shown in increasing the expression of glutathione peroxidase and glutathione reductase genes in MCF-7 breast cancer cell line. This increase is a marker for increasing ROS production and subsequent apoptosis. Therefore, the potential medicinal approach of nanoparticles requires more attention in animal model research and pharmaceuticals, which can be used as a therapeutic aid in various fields of treatment, including cancer.

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