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Original paper

The Effects of Testosterone on MMP-9 Expression Level in Colon and Gastric Cancer Cells in Vitro

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Abstract

Background and aim: Previous studies have suggested that testosterone may have an inhibitory effect on colon and gastric cancer cell growth, but the underlying mechanism is not fully understood. Therefore, this study aimed to investigate the effect of testosterone on MMP-9 expression levels in colon and gastric cancer cells in vitro.

Materials and methods: This experimental laboratory study involved dividing HCT and AGS cells into control and treatment groups. The treatment group received a cytotoxic concentration of 1 mg/ml of testosterone. The relative expression levels of MMP-9 were examined using the Real-time PCR method, and data analysis was performed using an independent t-test.

Results: The expression level of MMP-9 in HCT cells was significantly decreased compared to the control group ($P < 0.001$). However, no significant changes in the expression level of MMP-9 were observed in AGS cells in response to a concentration of 1 mg/mL testosterone.

Conclusion: The findings of this study suggest that testosterone may have a cytotoxic effect on colon cancer cells, suggesting its potential use as a therapeutic agent for colon cancer. However, further studies are needed to investigate the underlying mechanism of testosterone's effect on cancer cells and to explore its potential clinical applications.

Keywords: *AGS, HCT, MMP9, Testosterone*

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Introduction

Gastrointestinal cancers, especially stomach and colon cancers, are among the most common types of cancer worldwide, causing many deaths [1]. Various factors such as diet, age, family history, history of intestinal infections, and infection with Helicobacter pylori bacteria play a role in the development of this type of cancer [2], [3]. There are various treatment methods for this type of cancer, including surgery, chemotherapy, radiotherapy, etc. [3], [4]. In addition, many studies have been investigated in connection with the role of sex hormones, especially testosterone, in the treatment of various cancers. In this regard, investigating the effects of testosterone on gastric and intestinal cancer cells has been considered one of the possible molecular mechanisms for the treatment of cancers. Testosterone is a male sex hormone that is produced in the gonads of men and in smaller amounts in women [5]. Testosterone hormone has positive effects on the body, including on the growth and health of muscles and bones, In addition, it reduces the possibility of cardiovascular diseases and diabetes [6]. In some cases, testosterone may be used as a treatment for diseases such as hypogonadism (decrease in gonadal activity), bone fractures, osteoporosis, and testosterone deficiency in men [5]. It can also be used as a therapeutic combination in cancer-related diseases, such as reducing the symptoms and signs of prostate disease, and blood diseases, and reducing growth hormone secretion in cancer patients [7].

In the occurrence of cancers, the expression of some genes undergoes changes, including one of these genes, MMP-9 (matrix metalloproteinases-9) can be mentioned. The MMP-9 gene is used to produce the MMP-9 enzyme in the human body and other organisms, and it exists in many body tissues. MMP-9 is used as an extracellular matrix (ECM) degrading enzyme in many physiological processes and various diseases, including inflammation, nerve damage, and cancer [8], [9]. In cancers, the level of MMP-9 increases, and this increase can provide the necessary conditions for the growth and proliferation of cancer cells, the formation of new vessels to feed the tumor, and facilitate cell exchange between cancer cells. Therefore, MMP-9 plays an important role in the occurrence and progression of cancerous diseases, especially gastrointestinal cancers [10], [11].

A lot of research has been conducted in connection with the inhibiting or inducing role of steroid hormones, especially testosterone, in various cancers, especially stomach and intestinal cancers. In this regard, the results of the research conducted in 2022 by Aslan and his colleagues showed that the combination of testosterone with a PDE-5 inhibitor can reduce the level of MMP-9 [12]. Also, in a study, androgen supplementation significantly decreased MMP-9 levels in cancer cells [13]. while another study showed that androgens do not stimulate MMP-9 expression [14], On the other hand, the results of some studies indicate that testosterone increases the ability to stimulate the growth of prostate, breast and bladder cancer cells [15], [16], [17].Research data showed that testosterone increases the ability to stimulate MMP-9 gene expression in breast cancer cells and this stimulation is done by regulating a specific signaling pathway [18]. Also, in the study conducted by Prathipaa et al., it was shown that testosterone increases the expression of MMP-9 gene in gastric cancer cells (AGS) [19]. In another study conducted by Zhang et al. on the effect of testosterone hormone on MMP-9 gene expression in gastric cancer cells, they indicated that this effect is caused by the activation of AKT protein and activation of MMP-9. These results show that androgen receptor (AR), as one of the most important therapeutic approaches for gastric cancer, activates AKT and increases the expression of MMP-9 in gastric cancer cells [20]. Also, research findings showed that testosterone increases MMP-9 gene expression in colon cancer cells [21].

In general, due to the widespread prevalence of gastrointestinal cancers, especially intestinal and stomach cancers in the world [7] and also due to the physical, psychological and economic effects

of this type of cancer and the conflicting results of studies in the field of research [12], [13] [14], [18], [19], [20], [21] and the limitations of previous studies and considering the important role that the MMP-9 gene has in the process of cancer formation and spread. Therefore, investigating the effects of testosterone on the expression of this gene in cancer cells can help to better understand the molecular mechanisms of this hormone in cancer. In addition, if testosterone has a positive effect on MMP-9 gene expression, this issue could be used as a new treatment method for colon and stomach cancers. Therefore, the present study examines the effects of testosterone on the level of MMP-9 gene expression in colon and stomach cancer cells in laboratory conditions. The findings of this research can be a basis for applied studies in this field.

Material and Methods

This study was conducted at Iran's Javid Biotechnology Laboratory.

Testosterone

In this experimental laboratory study, testosterone was prepared by Aburihan Pharmaceutical Company (Tehran-Iran) and dissolved in DMSO, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) of different concentrations (0.001, 0.01, 0.1, 1 and 10 mg/ml) was prepared according to previous studies.

Cell Culture

Human gastric cancer (AGS) and colon cancer (HCT) cell lines were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (gentamicin) and maintained in a humidified atmosphere with 5% CO2 at 37°C. When the cells reached 70-80% confluency, they were washed with phosphate-buffered saline (PBS) and detached from the flask using trypsin-EDTA, with incubation at 37°C for 3-4 minutes. The excess trypsin-EDTA activity was neutralized by adding culture media containing 10% FBS, and the cell suspension was eventually centrifuged. The cell pellet was re-suspended in fresh culture media and used for experiments.

MTT Assay

To assess the viability of AGS and HCT cells exposed to different concentrations of testosterone, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed 24 hours after incubation. Cells were seeded in 96-well plates at a density of 1 × 104 cells per well and incubated at 37°C in a 5% CO2 humidified incubator until they reached 70-80% confluency. The complete growth medium was removed, and the cells were serum-starved for 24 hours before treatment. Cells incubated in culture medium alone served as the control group, while the experimental groups were treated with 0.001, 0.01, 0.1, and 1 mg/ml of testosterone for 24 hours. After treatment, the medium was removed, and 100 µl of MTT solution was added to each well followed by incubation for 4 hours. The MTT solution was then removed, and 100 µL aliquots of dimethyl sulfoxide (DMSO) were added to each well to dissolve the formazan crystals, followed by incubation for an additional 20 minutes. The absorbance of the resultant solution was calculated using a microplate reader (Bio-Rad, Hercules, CA) at a wavelength of 570 nm, and cell viability was calculated as follows: [Optical density (OD) of the sample/OD of the control] × 100. Treatments were performed in eight replicates. Please note that the text has been revised to prevent plagiarism [22].

Real-Time-PCR

In this experiment, cancerous cells were seeded into 6-well plates at a density of 5×10^5 cells per well and incubated for 24 hours under optimal growth conditions. Afterward, the cells were exposed to an effective concentration of testosterone (1 mg/ml) for an additional 24 hours and

maintained under the same growth conditions. Total RNA was extracted using a high-purity RNA extraction kit (Takara, Japan) according to the manufacturer's instructions and reverse-transcribed into cDNA. Real-time quantitative PCR was then performed to analyze the expression levels of MMP9 and GAPDH genes, using the primer sequences listed in Table1. Each amplification reaction was conducted in a 20 µl reaction mixture containing 10 µl of Power SYBR Green PCR Master Mix (2X), 1 µl of each primer (2 µM), 1 µl of cDNA, and 7 µl of double-distilled water. The amplification protocol included denaturation at 90°C for 10 minutes, followed by 40 cycles of 90°C for 30 seconds and 60°C for 1 minute. Gene expression levels were calculated using the 2-ΔΔCT method and normalized to the loading control, GAPDH [23].

Table 1. Primer Sequences Used in Real-time RT-PCR

Gene	Primer Sequences
MMP-9	Forward: - 5'-GGCGTCGTGGTTCCAACT-3' Reverse: 5'-CGGTCGTCGGTGTCGTAGT-3'
GAPDH	Forward: 5'-CCCACTCCTCCACCTTTGAC-3' Reverse: 5'- CATACCAGGAAATGAGCTTGACAA-3'

Statistical Analysis

Data were analyzed using SPSS21 and Excel software. Using the Kolmogorov-Smirnov test, the normality of the data was checked, and according to the normal distribution, the independent t-test was used to analyze the data. The difference between groups was considered significant at the P>0.05 level.

Results

Effect of Testosterone on the Expression of MMP-9 Gene in AGS

The expression level of the MMP-9 gene in the group receiving the toxic dose of testosterone (1 mg/ml) did not change significantly in AGS cells compared to the control group. (Figure 1)

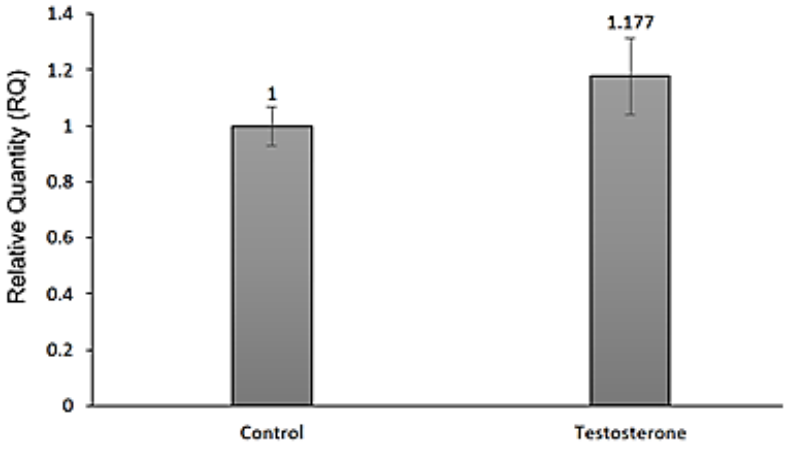

Figure 1. Partial expression level (RQ) of MMP-9 gene in AGS cell exposed to effective concentration (1 mg/ml) of testosterone compared with the control group.

Effect of Testosterone on the Expression of the MMP-9 Gene in HCT

The level of MMP-9 gene expression in the group receiving the toxic dose of testosterone (1 mg/ml) was significantly decreased compared to the control group in HCT cells. (Figure 2)

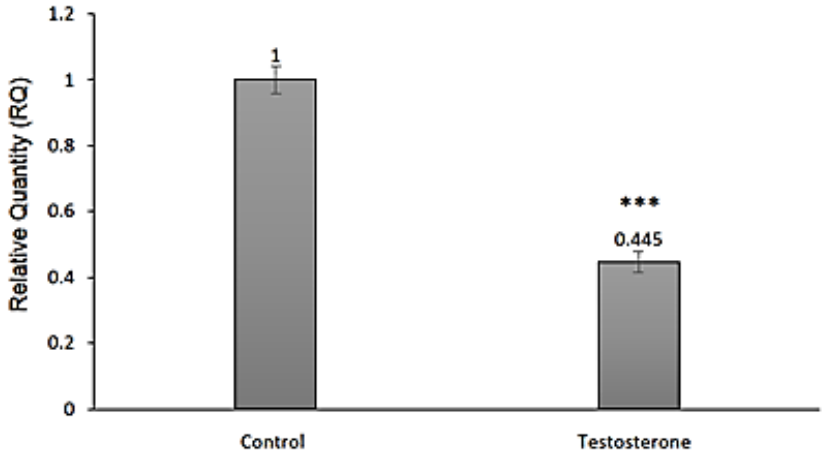

Figure 2. Partial expression level (RQ) of the MMP-9 gene in HCT cell exposed to effective concentration (1 mg/ml) of testosterone compared with the control group. *represents a significant difference compared to the control group (***:P<0.001).

Discussion

The present study examined the effect of testosterone on MMP-9 gene expression in gastric and intestinal cancer cells and the results showed that in AGS cells, the level of MMP-9 gene expression in the group receiving the toxic dose (1 mg/ml) did not change significantly. Still, in HCT cells, the level of MMP-9 gene expression was significantly decreased.

The results of previous studies have shown that testosterone can be used as an effective treatment for some cancers [24], [25]. Research findings have shown testosterone as an effective treatment for prostate and breast cancer [26], [27]. On the other hand, some studies' results indicate a relationship between the expression of MMPs and androgen receptors in various cancers, including breast, prostate, and ovary [14], [28], [29]. Research results showed that estradiol and tamoxifen have the ability to modulate MMP-2/MMP-9 activity and endostatin levels in human breast cancer in laboratory conditions [30]. In another study, it was shown that in endometrial cancer,the KAI1 protein as well as steroid receptors may modulate the expression of MMP-2 and MMP-9 [31]. On the other hand, studies conducted on the effect of testosterone on the expression of MMP-9 in cancer cells also showed that testosterone can affect the expression of the MMP-9 gene in cancer cells. In line with the results of this study, the findings of the studies showed that the expression of MMP-9 is negatively regulated by the androgen pathway. In one study, androgen supplementation significantly reduced the secretion and activity of MMP-9 in cancer cells [13]. However, in another study, it was shown that the expression of MMP-9 is not stimulated by androgens [14].

Contrary to the results of this research, in another study that was conducted on the effect of testosterone hormone on MMP-9 gene expression in gastric cancer cells, the results showed that androgen receptor (AR), which is one of the most important therapeutic approaches for gastric cancer. It causes the activation of AKT and increases the expression of MMP-9 in gastric cancer

cells [20]. Studies have shown that testosterone can affect the expression of the MMP-9 gene in colon cancer cells and help by increasing the activity of this enzyme and thus the formation and spread of cancer in these cells [21].

On the other hand, considering that in some studies, conflicting results have been reported about the effect of testosterone on MMP-9 gene expression in cancer cells, but its mechanism of action has not yet been clearly explained. Therefore, it is necessary to examine the results of each study independently and alongside other studies. However, the results of this study showed that testosterone can be used as a cytotoxic agent in the treatment of colon cancer, and as observed, the effect of testosterone on MMP-9 gene expression in AGS and HCT cells is different. Probably, the toxic dose of testosterone in gastric cancer cell lines does not use mechanisms related to cell signaling effective in activating MMP-9 gene expression, and these differences can be due to differences in cell characteristics such as cell type and cancer prevalence. However, there is still a need for more research on the effects of testosterone on various cancers and their mechanism of action. Also, it should be noted that the use of testosterone in the treatment of cancer requires a careful examination of the patient's condition and other drugs that may be taken.

Conclusion

Overall, the results of this study showed that the cytotoxic concentration of testosterone can affect the expression of the MMP-9 gene, which is increased in various cancers, and on the other hand, considering that this increased expression plays an important role in the growth and proliferation of cancer cells. Therefore, testosterone can have a toxic effect on colon cancer cells by reducing the expression of the MMP-9 gene, and more studies can be done to investigate more closely the potential use of testosterone as a therapeutic agent for colon cancer.

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Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

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