Original Research Article: Investigating the Relationship Between A/T 251 Polymorphism of IL-8 Gene and Cancer Recurrence After Lumpectomy

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ABSTRACT

Introduction: This research investigates the relationship between A/T251 polymorphism of IL-8 gene (as a genetic marker) with cancer recurrence after lumpectomy and instead of PCR-RFLP method which most common and expensive method for detecting polymorphisms is the more economical technique of PCR Tetra Arms.
Methodology: After DNA extraction, a nano drop device was used to evaluate the quantity and quality of DNA and to know its concentration and degree of purity. 1 microliter of the extracted DNA was placed in the device and its optical absorption was read at different wavelengths. For the correct performance of the method and in order to determine the sequence and confirm the PCR product, several samples of dominant homozygotes, recessive homozygotes and heterozygotes were selected and purified by Cena clone kit.
Results: Heterozygous individuals were identified with bp169 and bp228 long fragments along with bp439 control band. If mutant homozygous individuals with A allele showed bp 228 fragment and wild homozygous individuals with T allele showed bp 169 fragment on 2% electrophoresis gel.
Conclusion: Several cytokines, including IL-8, play an important role in causing laminogenic inflammation in tissue and as a result, causing and aggravating cancers. These studies have studied the role of IL-8 gene polymorphisms and its relationship with types of cancer in different populations. Therefore, this study was conducted with the aim of investigating the 251 A/T polymorphism of the IL-8 gene in the population of Iranian women with breast cancer and the results of this study showed.

Introduction

The importance of signaling pathways of interleukin 8 receptors and this chemokine itself in promoting the development of malignant cancers

expression in tumor cells, which are often induced in response to chemotherapy or environmental pressures such as hypoxia [1-3]. Increasing Interleukin-8 gene expression in tumor cells is of great importance in the survival of these types of tumors through the

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chemokines receptors 1, 2 genes) receptors in cancer cells. Based on these studies, it has been determined that cell lines with high metastasis have more Interleukin-8 gene expression than cells with low metastasis [4-6].
In order to specifically investigate the relationship between this polymorphism and breast cancer in the population of Iranian women, and considering the importance of the prevalence of breast cancer and its diagnosis and prognosis, this research investigates the relationship between the A/T251. Interleukin-8 gene (as a genetic marker) with cancer recurrence after lumpectomy and instead of the PCR-RFLP method, which is a common and expensive method for detecting polymorphisms. One of the more economical techniques of PCR tetra arms has been discussed [7-9].

Endometriosis

It is a benign lesion or lesions in which cells, like the lining of the uterus, grow abnormally in the pelvic cavity and outside the uterus, and the main cause of pelvic floor pain and infertility. Family history seems to be a contributing factor (Figure 1 & 2) [10].

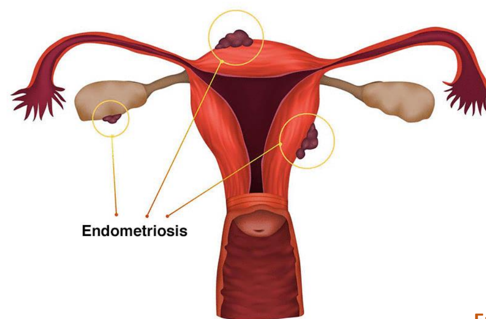

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Figure 1. Endometriosis illustration

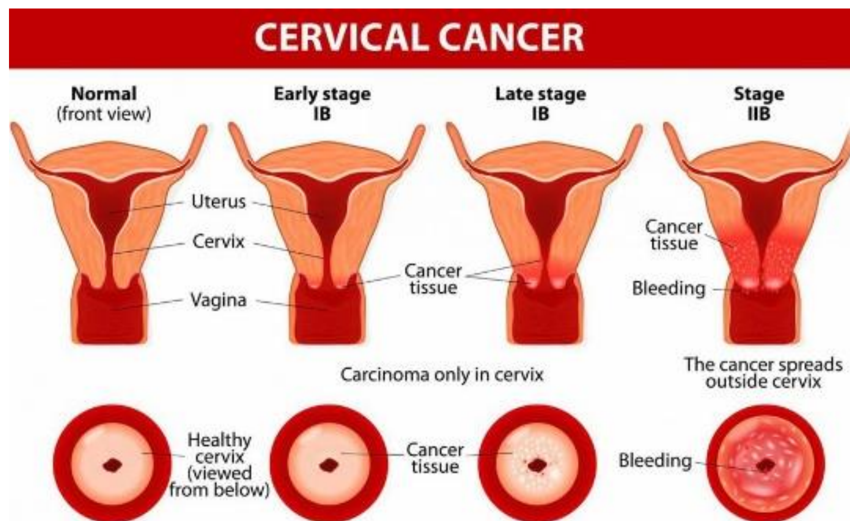

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Figure 2. Cervical cancer stages

Fistula formation, urethral obstruction, bladder dysfunction and pyelonephritis) [11-13].

Cervical cancer (endometrium)

Most uterine cancers are endometrionitis (originating in the lining of the uterus). Endometrium is the fourth most common cancer in women and the most common pelvic neoplasm (Figure 3) [14-16].

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Son rings (in case of improper intercourse) are risk factors for cancer are vaginal (Figure 4) [17].

Figure 3. Female reproduce Cancer small

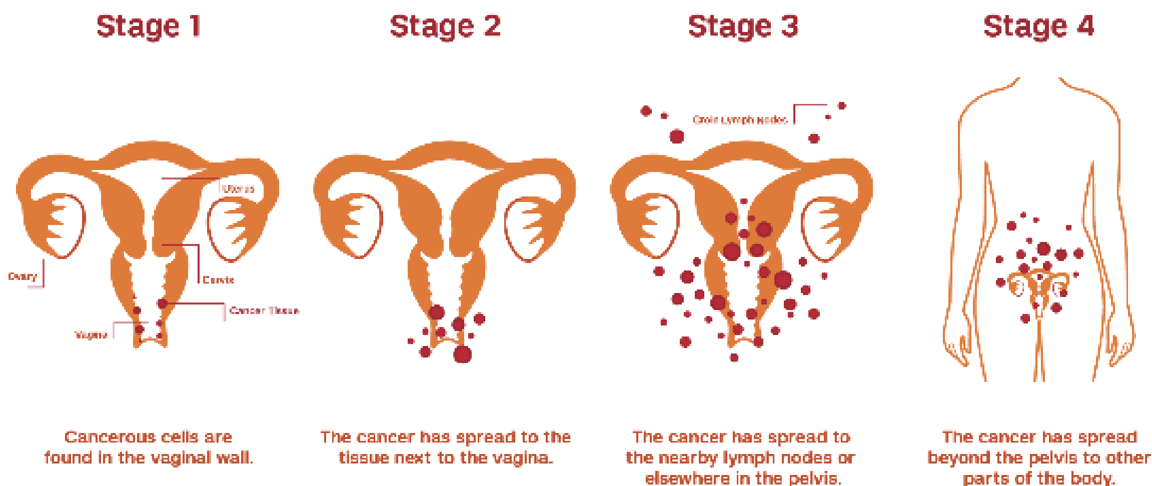

Figure 4. Vaginal cancer

The cervix is preserved. In the radical type, in addition to the uterus, the surrounding tissues, including the upper third of the vagina, lymph nodes, pelvis, and ovaries, are also removed (Figure 5, 6 and 7) [18-20].

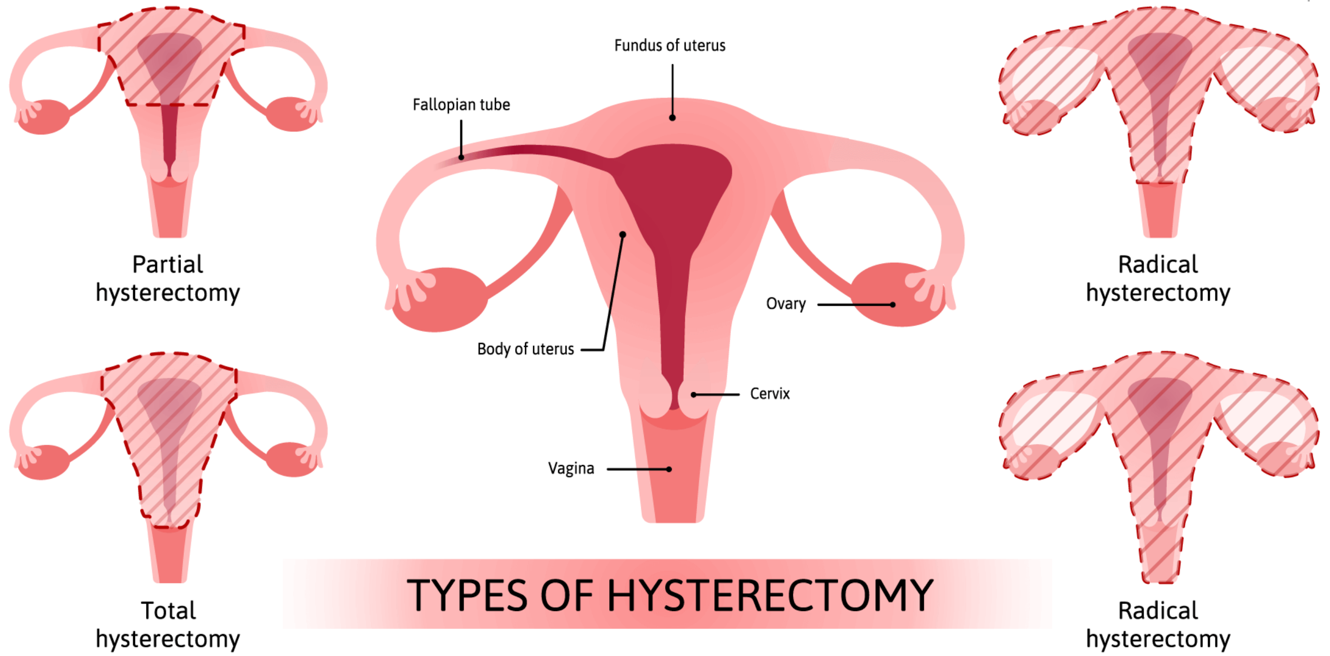

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Figure 5. Types of hysterectomy

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Figure 6. Radiotherapy

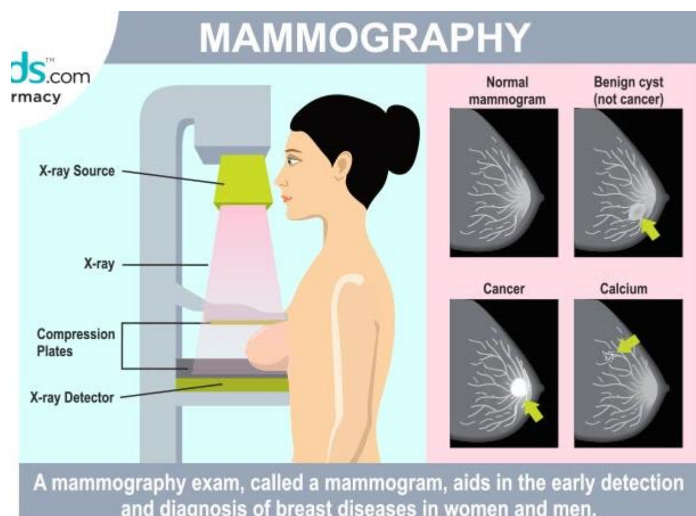

Figure 7. Mammography

Method

Type of study and statistical population

In this study, sampling was done in a case-control manner from two groups of patients and controls with written consent. The number of 50 women with breast cancer who were diagnosed by the examination of a gynecologist and ultrasound and mammography were selected, and the control group was 50 healthy women with the same age as the patients. In this calculation, 50 patient samples and 50 control group samples were estimated with an error level of 5%.

Sampling method

The amount of 5cc of blood from the patient group and healthy control subjects in prepared Venoject tubes with a volume of 5 ml (graner/UK company), which contains a specified amount of 1ml of sodium citrate anticoagulant (to it was used due to the combination with blood calcium and having the anticoagulant effect of blood, as well as the least effect on the biochemical factors of the blood) and was collected with caps. After sampling and until reaching the laboratory, the samples were kept in a box containing packed ice.

DNA extraction from blood samples

After transferring the blood containing sodium citrate to the laboratory, the genome of each sample was isolated using the DNA extraction method using the Cinnapure kit. It should be mentioned that after DNA extraction, the said samples were kept at -40 to -70 degrees Celsius.

Quality and quantification of extracted DNA

Electrophoresis on 2% agarose

To ensure the quality of purified DNA, 3µl of DNA was electrophoresed on 2% agarose gel. (2% gel was used to determine the band of the DNA extraction product according to the length of the genome in sufficient time with the appropriate voltage on the gel) [21-23].

Determining the concentration of extracted DNA using the nano drop device

After DNA extraction, the nano drop device was used to evaluate the quantity and quality of DNA and to know its concentration and degree of purity. 1 microliter of the extracted DNA was placed in the device and its optical absorption was read at different wavelengths [24-26].

Tetra Arms PCR reaction

To perform the tetra arms PCR reaction, specific primers including 1 pair of external primers and 2 internal primers for each of the dominant and recessive alleles of the A/T251 polymorphism of the IL-8 gene given in table 1 is used. Also, the materials required for the PCR reaction and the program of the device are listed in tables 2 and 3, respectively [27].

Table 1. The sequence and characteristics of the primers used to perform the PCR Tetra Arms reaction

Gene name	Primer sequence	PCR product length (bp)
T allele	TGTAATCCCAGCAGTTTGGGAGGT	169
A allele	CTCATCTTTTCATTATGTCAGAG	228
5’–3’	CATGATAGCATCTGTAATTAACTG	349
5’–3’	CACAATTTGGTGAATTATCAAA	349

Table 2. Materials needed to perform PCR (volume 20 microliters)

Density	Volume (microliters)	Reaction contents
1.5 mmol/ $MgCl_2$	10	Master Mix PCR 1X
10 pmol	1	Forward Primer
10 pmol	1	Reverse Primer
-	5	Sterile water
200 nano grams	3	DNA

Table 3. Temperature program of the PCR machine

Temperature (°C)	Time	Levels
95	10 minutes	Primary training
95	30 seconds	Training
57	40 seconds	Connection
72	60 seconds	Expansion
72	10 minutes	Final expansion

Distribution of Emergency human personnel resource

Evaluation of the PCR product

To evaluate the PCR product, 5 microliters of the PCR product was electrophoresed on a 2% agarose gel (according to the band length of the PCR products and the proper visibility of the bands on the gel) at the same time as the biological identifier (bp 50). For staining, instead of using ethidium bromide dye and preventing its toxic and harmful effects, DNA safe dye from Sina Clone Company was used (1 microliter for every 5 microliter of sample). Then, to see the bands, the gel was placed in the UV-Doc device and photographed. It should be mentioned that the bands related to outer amplification, bp 349 and two alleles T and A with bp 169 and bp 228 respectively were targeted. Also, for each series of samples, a positive control that was previously confirmed by sequencing and a negative control sample with a bp 50 marker were used.

Validation of genotyping results

To validate the performance of the method and to determine the sequence and confirm the PCR product, several samples of dominant homozygotes, recessive homozygotes, and heterozygotes were selected and purified using the Sina clone kit. Then they were sequenced by sending to Tekapozit company and finally blasting was done.

Results

About 50 blood samples of patients and 50 blood samples of healthy people were collected and DNA extraction was done from the samples.

The age range of people was between 30 and 60 years. The average age for the patient group was 44.16 and for the normal group was 40.23, which was almost the same in the two groups. According to the results, 44% of people were in stage two of the disease and only 10% of them were in the acute stage of the disease (stage four).

DNA extraction results

Results of optical absorption of DNA by nano drop device

The optical absorption results of the extracted DNAs were read by the nano drop device. The samples that had the optical absorption ratio between 260 and 280 nm between 1.8 and 2 were considered suitable for further work.

The results of electrophoresis of extracted DNA on agarose gel

In order to ensure the correctness of the extracted DNA and its quality, the resulting DNA was electrophoresed on a 2% agarose gel (Figure 8).

Figure 8. DNA extracted on 2% agarose gel, house 1: 100 bp molecular marker, house 2 and 3: Two DNA samples

Genotyping results by Tetra-Arms PCR method

Genotyping of 251T/A polymorphism of IL-8 gene, 50 patients with breast cancer and 50 healthy samples was done by Tetra Primer Arms PCR technique and then the genotype of people was determined based on PCR product electrophoresis on agarose gel. Heterozygous individuals were identified with bp169 and bp228 long fragments along with bp439 control band. If mutant homozygous individuals with A allele showed bp 228 fragment and wild homozygous individuals with T allele bp 169 fragment on 2% electrophoresis gel (Figure 9).

House 1: molecular marker bp 100, house 2: Positive control sample and house 3: Negative control sample, houses 4, 9, 14 and 15: Samples of heterozygous (AT) individuals (bp 349, bp 228, bp 169), houses 5, 6 and 7: Samples of homozygous (AA) subjects (bp 349, bp 228), houses 8, 10, 11, 12 and 13: samples of homozygous (TT) subjects (bp 349, bp 169).

Sequencing to confirm the Tetra-Arms PCR product

In the current study, in order to confirm the PCR product from each of the dominant and recessive homozygous and heterozygous conditions, together with the primers for sequencing, were sent to Tekapo Biot Company [29-31].

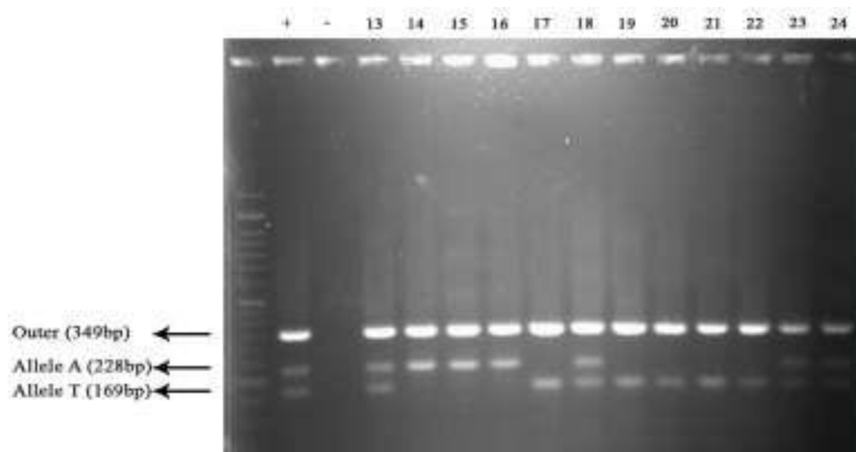

Figure 9. Tetra Primer Arms PCR product of 251 T/A polymorphism of IL-8 gene, on 2% agarose gel.

Discussion

This study was conducted with the aim of investigating the 251 A/T of the Interleukin-8 gene in the population of Iranian women [32]. Also, instead of expensive methods for detecting polymorphisms, the 3 arms PCR technique was used in this research. In the control group, the genotype distribution of the 251 A/T of the Interleukin-8 gene was 0%, 88%, and 12% for AA, AT, and TT genotypes, respectively. Also, the genotype distribution in the patient group was 12%, 54% and 34%, respectively [33-35]. Also, most of the examined people in stage 3 and 4 of the disease had TA and AA genotypes. Based on the results of recent studies conducted in the population of Chinese women, it has been determined that there is a relationship between the 251 A/T of the Interleukin-8 gene and breast cancer [36]. Especially interleukin 8, which seems to be one of the critical steps in creating metastasis in cells. Previous research indicates that interleukin-8 is not only a factor in activating the proliferative pathways of cancer cells, but also controls the apoptotic pathways [37-39].

Zhang et al. in a research study determined that interleukin 8, as a member of the chemokine family, can play an essential role in regulating inflammation and immune system processes. They investigated polymorphisms of interleukin 8 gene including 251T/A polymorphism by PCR-RFLP method [40]. Their results showed this polymorphism and the development of breast cancer in the population of Chinese women. Also, Hay and his colleagues conducted a study on interleukin 8 gene polymorphisms and their relationship with breast cancer in the Chinese female population. Their research determined that several polymorphisms, including the 251T/A polymorphism of the IL-8 gene, are effective in increasing the risk of breast cancer among Chinese women. Snooz et al. in a case-control study investigated genetic variation in the IL-8 gene with increased risk and poor prognosis of breast cancer [41-43].

Huang et al reported that the above polymorphism is related to recessive heterozygous and homozygous genotypes in people with breast cancer and in the acute stage of this disease. Therefore, a review of the studies conducted regarding the 251T/A polymorphism of the IL-8 gene, which can be effective in causing many cancers in the Asian female population [44], shows the need for the present study, which investigates the relationship between the aforementioned polymorphism with breast cancer in the Iranian women as a population of Asian women and also justifies the necessity of using the tetra arms PCR method compared to the PCR-RFLP method which is a more accurate and cost-effective method show [45].

Conclusion

Breast cancer is considered as one of the most frequent cancers and the main cause of death among women all over the world and Iranian women. Several factors, including environmental factors, body weight, lifestyle factors such as alcohol consumption, physical inactivity, and smoking, as well as the most important genetic factors of people, play an important role in the development of breast cancer tumors. The results of the studies show that several cytokines, including IL-8, play an important role in causing inflammation and angiogenesis in tissue, and as a result, causing and aggravating cancers.

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