Association between interleukin 4 (IL-4) VNTR, gene polymorphism, and breast cancer susceptibility in Iranian population: experimental and web base analysis

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ABSTRACT

BACKGROUND: Breast cancer (BC) is one of the most common types of cancer and the second leading cause of cancer death among women. Epidemiological studies showed that BC is linked to genetic and environmental factors, and inheritance plays a key role in the pathobiology of this disease. Interleukin 4 (IL-4) is a key differentiation cytokine and is produced by Th2 and activates Th2 development. Hence the current study aimed to assess the possible association between interleukin 4 (IL-4) VNTR polymorphism, and BC susceptibility in a sample of Iranian population.

MATERIAL AND METHODS: IL-4 VNTR polymorphism was evaluated in 150 women with BC and 150 age-matched healthy women by polymerase chain reaction method.

RESULT: Among 3 possible alleles for IL-4 gene, we only observed 2 alleles. Current findings indicate that RP2/RP2 genotypes can be regarded as potent protective factors against breast cancer (OR = 0.929 [95%CI, 0.929–0.995]).

CONCLUSION: Our result showed that the RP2/RP2 genotype of the IL-4 VNTR polymorphism could be a protective factor for BC susceptibility (Tab. 2, Fig. 1, Ref. 46).

KEY WORDS: IL-4, breast cancer, VNTR polymorphism.

Introduction

Breast cancer with 252710 estimated new cases in 2017 in United States is the most common type of cancer worldwide (1, 2). Although the incidence rate is decreasing, in developed countries it is still the leading cause of cancer mortality. There are legion risk factors related to breast cancer such as: age, genetic background, hormonal condition, obesity, exposure to radiation and chemical carcinogens. Histopathological markers, including the expression of sex hormone receptors and human epidermal growth factor receptor (HER2) are commonly used as diagnostic means to contribute to choosing an effective approach in clinical treatment (3–6). Almost 60 to 70 % of breast cancer cases are ER/PR positive and so tumor growth is dependent on estrogen. And also, 20 to 25 % over-express Her2, which are more aggressive (7, 8). About 30 % of ER positive tumor and more than 70 % of tumors, which overexpress Her2, respond poorly to specific therapies related to these biomarkers. Moreover, 10 to 20 % of breast cancer patients are classified as triple negative, which are more aggressive and lack target for specific treatment (9). Consequently, these markers are not sufficient to cover the heterogeneity of breast cancer and in turn resort to accurate prediction of treatment outcome (10). In order to dwindle any over or under treatment and ultimately determine an optimal therapy, introducing new biomarkers is considered to be of paramount importance. Numerous studies are claiming to identify factors or profiles that predict response to treatment or influence patient survival whereas, only a small portion, of which has been reported, are validated by organizations in authority such as American society of clinical oncology. However, a substantial set of newly introduced markers are shown to have a promising potential to be used in future (11). Information provided from molecular studies have also brought novel and deeper view of breast cancer complexity and lead to improvement of breast cancer molecular classification and more comprehensive understanding of the clinical respect of the disease (12).

Therefore, genetic variation associated with risk of breast cancer has been studied widely, hoping that polymorphism genotyping will stratify breast cancer risk more accurately (13–15).
Interleukin 4 (IL-4) is an important differentiation cytokine produced by Th2 and activates Th2 development. Th2 subset of lymphocytes have the responsibility of tumor cell clearance by the means of granulocytes and Eosinophils activation and also inhibition of angiogenesis (16–18). As a further matter, Th2 subset antagonize IFN-γ, inhibit macrophage activation and its anti-tumor function on variety of cancers such as: colon, breast cancer and renal cancer were reported by some studies (19, 20). However, it has been reported that IL-4 can act as a double edge sword in controlling tumor growth. It has been reported that IL-4 contribute to initiation, progression and metastasis of head and neck carcinoma (20) and higher levels of IL-4 mRNA were found in more advanced stages of gastric cancer (20). Besides, IL-4 interfere with anti-tumor immunity by down-regulating of TH1 cytokines (19, 21). Legion of surveys were conducted on several IL-4 polymorphism in order to determine its contribution on human cancer risk. Among which, one paramount polymorphism is a variable number of tandem repeats (VNTR) of a 70-bp sequence located in intron 3 (22, 23). In the present study, we aimed to assess the probable association between 70-bp VNTR polymorphism of the IL4 gene and the risk of breast cancer in Iranian population.

**Material and methods**

**Study population**

In this study a total number of 300 subjects were recruited, 150 cases with breast cancer and 150 healthy control samples. Breast cancer patient’s sample was provided by cancer research center of Shahid Beheshti University of Medical science. This study was approved by ethical committee of Shahid Beheshti University of Tehran, Iran (Ethical code: IR.SBMU.REC.1396.133). Control women were assessed and confirmed of having no detectable breast cancer at the time of sampling and had no personal or family history of cancer.

**IL-4 Genotyping**

Genomic DNA was extracted from peripheral blood mononuclear cells using salting-out method. Polymerase chain reaction (PCR) was conducted to analyze the 70 bp VNTR of IL-4 gene intron 3. Oligonucleotide primers were as followed: forward: 5´AG-GCTGAAAGGGGGAAAGC-3´, reverse: 5´CTGTTCACCT-

**Statistical analysis**

Data analysis was performed using SPSS V.22 software. Independent sample t-test $\chi^2$ test or Fisher’s exact test was used to evaluate the differences. Allele frequency was determined by using a direct gen counting method. Allele frequency between breast cancer patients and the control group was compared by $\chi^2$ test and Fisher’s exact test. The odd ratio (OR) and 95% confidence intervals (95%CI) had also been calculated. $p < 0.05$ was considered statistically significant.

**Results**

Demographic data of the breast cancer patients and the control group is shown in the Table 1.

### Tab. 1. Demographic and clinical characteristics of the study population.

| Age (year) | Normal (n=150) | Malignant (n=150) |
|------------|----------------|-------------------|
| <40        | 30 (20%)       | 26 (17%)          |
| 40–60      | 90 (60%)       | 84 (56%)          |
| >60        | 30 (20%)       | 40 (27%)          |
| Grade      |                |                   |
| 1          | 18 (12%)       |                   |
| 2          | 101 (67%)      |                   |
| 3          | 31 (21%)       |                   |
| Stage      |                |                   |
| I          | 31 (21%)       |                   |
| II         | 66 (44%)       |                   |
| III        | 63 (35%)       |                   |
| ER/PR      |                |                   |
| Positive   | 104 (69%)      |                   |
| Negative   | 46 (29%)       |                   |
| c-erbB2    |                |                   |
| Positive   | 46 (29%)       |                   |
| Negative   | 104 (69%)      |                   |

### Tab. 2. Genotypes and Alleles Frequency of IL4 VNTR Polymorphism.

| Genotype      | Control (n=150) | Case (n=150) | P     | OR (95% CI) |
|---------------|-----------------|--------------|-------|------------|
| RP1/RP1       | 124             | 119          | Ref=1 |            |
| RP1/RP2       | 21              | 31           | 0.107 | 1.53 (0.837–2.826) |
| RP2/RP2       | 5               | 0            | 0.037 |            |
| Dominant      |                 |              |       |            |
| RP1/RP1       | 124             | 119          | Ref=1 |            |
| RP1/RP2+RP2/RP2 | 26            | 31           | 0.27  | 1.242 ( 0.697–2.26 ) |
| Recesive      |                 |              |       |            |
| RP2/RP2       | 5               | 0            |       |            |
| RP1/RP1+RP1/RP1 | 145         | 150          | 0.03  |            |
| Allele        |                 |              |       |            |
| RP1           | 269             | 269          | Ref=1 |            |
| RP2           | 31              | 31           | 0.553 | 1 (0.59–1.69) |
Using web analysis, no significant difference in survival was found between low expression of IL-4 and high expression of IL-4 (p = 0.615). However, data collected from this study indicates that low expression of IL-4 is associated with better survival in breast cancer patients (Fig. 1).

Among 3 possible alleles for IL-4 gene, we only observed 2 alleles. Allelic and genotypic frequency distributions of the IL-4 VNTR are shown in the Table 2. Current findings indicate that RP2/RP2 genotypes can be regarded as potent protective factors against breast cancer (OR = 0.929 [95%CI, 0.929–0.995]).

Discussion

Cancer, as a heterogeneous disease, is caused by complex interaction of environment and genetic influence, which results in a poor clinical outcome predictability (26–28). Inflammation as an inherent characteristic of cancer is associated with development, invasion, and metastasis, however, many types of cancer cells evade immune system. Cancer inflammation is exerted and controlled by chemokines, prostaglandins, and cytokines (29). Among which, as the key inflammatory cytokine, IL-4 has been linked with various types of cancer. It is shown that elevated plasma levels of IL-4 were linked with the risk of melanoma, head and neck squamous carcinoma, prostate, colon, renal cell, small cell lung cancer, acute myeloid leukemia and breast cancer (30–33). Moreover, several polymorphism related to IL-4 encoding gene were shown to be a cancer risk factor (34–38). Among which, intron 3 VNTR polymorphism has a paramount role in controlling IL-4 production. Our results indicated that frequency of RP2/RP2 genotype was significantly higher in healthy, control group. Furthermore, several studies indicated that Th1/Th2 balance was altered in cancer (39, 40). IL-4 VNTR polymorphism is located in intron 3 of IL-4 gene and could change messenger ribonucleic acid splicing, which subsequently leads to different splice variants, which can effect gene expression (18). It is also reported that RP2/RP2 genotype is associated with a low expression of this cytokine (41). We have also found a higher frequency of RP2/RP2 in the control group. This finding supports the fact that serum levels of interleukine 4 in breast cancer are decreased (42). Contradictory, in a meta-analysis study performed by Li et al, no association was found between IL-4 polymorphism and cancer risk (43). In another meta-analysis, Duan et al. reported that RP2 allele was associated with a decreased cancer risk (44). On the other hand, Konwar et al. indicated a lack of association between VNTR polymorphism of IL-4 and breast cancer. However, RP2/RP2 genotype in patients affected with breast cancer was significantly less frequent than in normal individuals (45). Besides, according to Shekari et al findings, RP1/RP2 genotype frequencies in cervical cancer patient were significantly higher than in healthy women (46). These observations supported the theory that immunological, inflammatory, and anti-inflammatory processes could play key roles in breast cancer development. At the end it is worth mentioning that this study faced some difficulties such as: low sample size, different ethnic groups and environmental conditions. Ergo, in order to minimize the confounding variables, more extensive studies in sample size are recommended for a better understanding of the pathogenetic role of IL-4 VNTR polymorphism in breast cancer.

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