

# *Human "C-Reactive Protein (CRP) 1919 A/T" Polymorphism in Patients with Breast Cancer*

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**Abstract** — "C-reactive protein (CRP)" demonstrates essential prognosis in many diseases due to its vital role in inflammation response. Breast cancer is the most common cancer in the world and is the second largest among the various types of cancer among women. In this study we aimed to determine the frequency of "SNP rs1417938 of CRP gene" and investigate this polymorphism in a group of breast cancer patients. The most common "CRP 1919 (rs1417938)" was evaluated in 20 patients with breast cancer. A allele is a major one in the studied population. This allele is more common in breast cancer patients (1.00). The patients with breast cancer are more likely to own AA genotypes. The individuals with genotype AT or TT have not been detected in the studied groups. These preliminary data can be considered as pilot ones. These data give reason to assume that A is a predisposing allele to breast cancer.

**Keywords** — C - reactive protein; Polymorphism; Breast Cancer; Genotype.

## I. INTRODUCTION

"C-reactive protein (CRP)" demonstrates essential prognosis in many diseases due to its vital role in inflammation response [1]. Breast cancer is the most common cancer in the world and is the second largest among the various types of cancer among women. [2] "CRP" is created by liver cells in response to inflammation, and tissue damage. In addition, a mild increase in protein levels was observed in chronic inflammatory diseases.

The structure of the "CRP" molecule is a cyclic metamer. Around the central pore there are five non-covalent promoters and each promoter consists of 206 amino acids with ligand-binding site having a pocket containing two sites of calcium ions that are necessary for molecular stability [3]. One of the important keys to follow the progression of carcinogenesis and the development of cancer is the chronic inflammatory reactions. Gene for CRP is present on "the long arm of chromosome 1 (1q21-q23)". Gene expression control of interleukins and transcriptional proteins play an important role in "CRP" synthesis. Interleukin 6 is the primary catalyst of the "CRP gene".

Blood levels of CRP are associated with polymorphism in and out of CRP sites.

"Outside loci are leptin receptors (LEPR), IL-6 receptors, hepatocytes nuclear factor 1 A, and apolipoprotein E locus (APOE) [4]".

Many evidences confirm the importance of interleukins and cells that mediate inflammation in the process of malignant cancer metastasis and immune suppression [5]. In recent works, the CRP rs1417938 A/T single nucleotide polymorphism (SNP) was studied for its relationship with cancer risk. Since 40% heritability has been recommended for CRP levels, hereditary polymorphisms of CRP have turned into a matter of enthusiasm for forecast of cardiovascular events [6]. Considering the importance of genetic polymorphism of "CRP" among breast cancer patients and the lack of studies on this subject in Iraq, we propose to investigate the frequency of SNP rs1417938 of CRP gene and determine this polymorphism in a group of patients with breast cancer.

## II. METHODS

## A. SUBJECTS

Twenty women with breast cancer from Wasit, Najaf and Baghdad-Iraq were enrolled in this investigation.

## B. DNA EXTRACTION

"DNA was isolated from blood samples by G- spin dna extraction kit , intron biotechnology , cat.no. 17045".

## C. RED SAFE NUCLEIC ACID STAINING SOLUTION

RedSafe Nucleic Acid Staining Solution (20,000x) is anew and safe nucleic acid stain, an alternative to the traditional ethidium bromide (EtBr) stain for detecting nucleic acid in agarose gels. It emits green fluorescence when bound to DNA or RNA. This new stain has two fluorescence excitation maxima when bound to nucleic

acid, one centered at 309nm and another at 419nm. In addition, it has one visible excitation at 514nm. The fluorescence emission of Red Safe bound to DNA is centered at 537nm. RedSafe Nucleic Acid Staining Solution (20,000x) is as sensitive as EtBr. The staining protocol for Red Safe Nucleic Acid Staining Solution (20,000x) is similar to that for EtBr. Compared to EtBr, known as a strong mutagen, Red Safe Nucleic Acid Staining Solution (20,000x) causes much fewer mutations in the Ames test. In addition, Res Safe Nucleic Acid Staining Solution (20,000x) has a negative result in mouse marrow chromophilous erythrocyte micronucleus test and mouse spermary spermatocyte chromosomal aberration test. So it is wise to choose RedSafe Nucleic acid Staining Solution (20,000x) instead of EtBr for detecting nucleic acid in agarose gels. (Cat. No. 21141).

TABLE1. THE SPECIFIC PRIMER CRP1919 OF GENE

| Primer    | Sequence                        | Tm (°C) | GC (%) | Product size  |
|-----------|---------------------------------|---------|--------|---------------|
| CRP1919SE | 5'- TCTCTCATGCTTTTGGCC AGAC- 3' | 57.5    | 50     | 150 base pair |
| CRP1919AS | 5'- ACCATGAAGGATGCTCCACTGT- 3'  | 58.5    | 50     |               |

The optimal state of PCR reactions was determined after several experiments. The temperature was changed by working (PCR gradient) of all samples to determine the optimal state. The concentrations of the DNA template were also changed between 1.5-2 µl. The PCR reactions are performed using the following Kit:

**"MAXIME PCR PREMIX KIT (I-TAQ) 20MLRXN (CAT. NO. 25025)"**

"iNtRON's Maxime PCR PreMix Kit" has not just different sorts of PreMix Kit as indicated by encounter reason, yet in addition a 2X Master blend arrangement." Maxime PCR Pre Mix Kit (I-Taq)" is the item what is blended each part:" I-Taq DNA Polymerase, dNTP blend, response support, et cetera in one tube for 1 rxn PCR". This is the item that can get the best outcome with the most comfort framework. The main reason is that it has each segment for PCR, so we can do "PCR" simply include a layout DNA, groundwork set, and D.W.. The second reason is that it has Gel stacking support to do electrophoresis, so we can do gel stacking with no treatment.

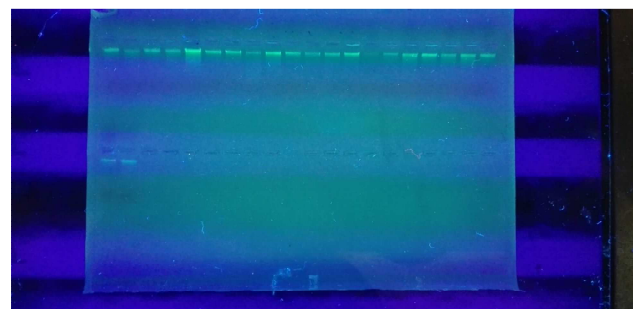


FIGURE 1. ELECTROPHOREGRAM OF DNA EXTRACTION FROM SPECIMENS , 1% "AGAROSE GEL AT 5 VOL /CM FOR 1:15 HOUR".

The optimal condition to detect CRP1919 gene included: Initial denaturation at 95°C for 3minutes, then second denaturation at the same temperature for 45 seconds. Annealing was performed at 58°C for 45 seconds. The first extension was at 72°C for 50 seconds and the second extension at 72°C for 10 minutes. The number of cycles for each reaction was 40 cycles.

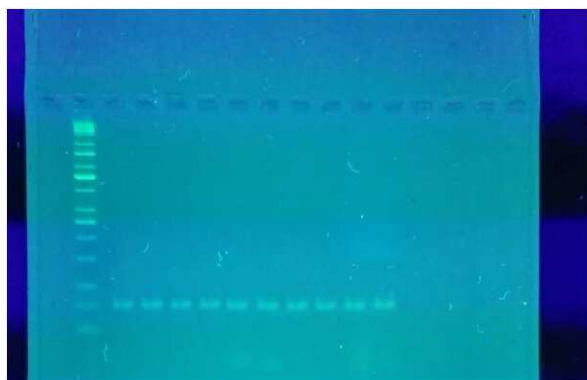


FIGURE 2. ELECTROPHOREGRAM OF " CRP1919 GENE " THE MOLECULAR SIZE 100 BP." ELECTROPHORESIS ON 2% AGAROSE AT 5 VOLT/CM<sup>2</sup>". 1X TBE BUFFER FOR 1:30 HOURS.

### III. RESULTS AND DISCUSSION

A allele is a major one in the studied population. This allele is more common in breast cancer patients (1.00) the minor allele in patients with breast cancer is T. Patients of this study are more likely to own AA genotypes. The individuals with genotype AT or TT have not been detected in the studied groups (Table 3). These preliminary data can be considered as pilot ones. These data give reason to assume that A is a predisposing allele to breast cancer.

TABLE 2. THE GENOTYPES AND ALLELE FREQUENCY DISTRIBUTION OF BREAST CANCER PATIENTS BY CRP1919 A/T POLYMORPHISM.

| CRP1919 A/T polymorphism |   |       | Genotype |     |     | Allele frequency |     |
|--------------------------|---|-------|----------|-----|-----|------------------|-----|
|                          |   |       | AA       | AT  | TT  | A                | T   |
| Breast cancer patients   | n | 20    | 20       | 0.0 | 0.0 | 1.00             | 0.0 |
|                          | % | 100.0 | 100.0    | 0.0 | 0.0 |                  |     |

The importance of the prognosis of rs1417938 practical polymorphism inside the "CRP" quality has been confirmed based on the primary role of chronic inflammation in cervical cancer and on the basis of the previous information on CRP genotyping in other female cancers [8]. Among the fifteen previous researches, two displayed a non-significant negative correlation between one factor shift in "ln(CRP)" and disease, and the other works uncovered clear correlation, statistical significance was exhibited in four of these studies. Increments in this reactive protein levels were connected with expanded risk of breast malignancy, particularly among the Asian population. In spite of the fact that causality proof was deficient, these discoveries seemed to help a part of interminable aggravation in breast carcinogenesis. Much attention has been paid to the "CRP an inflammation-related gene", based on the importance of inflammation in cancer prognosis. Many studies have linked functional polymorphism at "CRP locus" with cancer. A few professions have associated the useful polymorphisms at CRP locus with tumor. Hardly any investigations have analyzed these CRP variations with disease. The study of the genetic polymorphism of "CRP gene" affected the extent, poor prognosis or the high grade of many kinds of cancers in humans [9]. The presence of "CRP1919T>A", known to be related with higher CRP levels in normal women, was independently associated with impaired overall survival [10]. The correlation of the CRP polymorphisms

and levels of its protein with cancer prognosis still controversy.

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