A3872G Polymorphism of "C-Reactive Protein Gene (CRP)" in Patients with Breast Cancer

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Abstract — "C-reactive protein (CRP)" is the phenotype intense stage protein initiated by hepatocytes, known as an inflammatory biomarker. CRP is related with an extensive variety of infections, including atherosclerosis and diabetes mellitus. Breast malignancy remains a noteworthy issue of women' wellbeing and its rate is expanding worldwide. Due to importance of CRP polymorphism, we aimed to determine the frequency of "SNP rs1205 of CRP gene" and investigate this polymorphism in a group 20 breast cancer patients. G 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. The patients with breast cancer are more likely to own GG genotypes. These preliminary data can be considered as pilot ones. These data give reason to assume that G is a predisposing allele to breast cancer.

Keywords — C-Reactive Protein; Polymorphism; Breast Cancer; Genotype.

I. INTRODUCTION

"C-reactive protein (CRP)" is the phenotype intense stage protein initiated by hepatocytes, known as an inflammatory biomarker. "The CRP gene is situated at chromosome 1q21–1q23 comprising of two exons and spans 1.9 kb in length, including 29 single nucleotide polymorphisms (SNPs)". CRP is related with an extensive variety of infections, including atherosclerosis and diabetes mellitus" [1, 2]. Breast cancer is the second most basic malignancy around the world and, by a wide margin, somewhat continuous disease among females with an expected more than "1.67million" new malignancy cases analyzed in 2012 (25% of all tumors). Albeit former conclusion added to the achievement of treatment, this disease prevail a noteworthy issue of feminine wholeness and its rate is expanding in growing countries [3,4]." Several works indicated to the association of levels of CRP and chronic diseases for instance overall cancer risk and risks of lung, colorectal, endometrium, and ovarian cancers [5, 6]."

Though, information assessing the relationship amongst this biomarker and risk of breast malignancy is uncommon as well as conflicting. Recently, a great deal of studies investigated the connection between CRP quality rs1205 polymorphism and colorectum malignancy risk [7–11]. However, the results of these studies were conflicting and inconclusive because of the clinical heterogeneity, different ethnic populations, and small sample sizes. 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" [12]. 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 rs1205 of CRP gene and determine this polymorphism in 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 a new 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, Red 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).

**TABLE I. The Specific Primer CRP3872 of Gene**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Tm (ºC)</th>
<th>GC (%)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;CRP3872F&quot;</td>
<td>&quot;TGCTGGATTTCCAAGCTGAG&quot;</td>
<td>55.3</td>
<td>50</td>
<td>100 base pair</td>
</tr>
<tr>
<td>&quot;CRP3872R&quot;</td>
<td>&quot;TTGGCTCCTCCACTTCCAGTT&quot;</td>
<td>58.6</td>
<td>52.4</td>
<td></td>
</tr>
</tbody>
</table>

D. "Maxime PCR PreMix Kit (I-Taq) 20µLRxN (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.

The optimal condition to detect CRP3872 gene included: Initial denaturation at 95ºC for 3 minutes, then second denaturation at the same temperature for 45 seconds. Annealing was performed at58ºC for 45 seconds. The first extension was at72ºC for 50 seconds and the second extension at 72ºC for 10 minutes. The number of cycles for each reaction was40 cycles.

**FIGURE 2. Electrophoregram of "CRP1205gene" the molecular size 100 bp. "Electrophoresis on 2% agarose at 5 volt/cm2". 1X TBE buffer for 1:30 hours.**

III. RESULTS AND DISCUSSION

G allele is a major one in the studied population. This allele is more common in breast cancer patents (1.00). The minor allele in the patients with is T. Patients of this study
are possible to own GG genotypes. The individuals with genotype GA or AA have not been detected in the studied groups (Table 2). These preliminary data can be considered as pilot ones. These data give reason to assume that G is a predisposing allele to breast cancer.

**TABLE 2. THE GENOTYPES AND ALLELE FREQUENCY DISTRIBUTION OF BREAST CANCER PATIENTS BY CRP3872 G/A POLYMORPHISM.**

<table>
<thead>
<tr>
<th>CRP3872 G/A polymorphism</th>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Breast cancer patients</td>
<td>n=20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>%:100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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 [13].

Among the fifteen previous researches, two displayed an non-significant negative correlation between one factor shift in "ln(CRP)" and disease, and the other works uncovered clear correlation, statistical signification was exhibited in four of these studies. Increments in this reactive protein levels was connected with expanded risk of breast malignancy, particularly among the Asian population. In spite of the fact that causality proves 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 "affect the extent, poor prognosis or the high grade of many kinds of cancers in humans [14]. This relationship was clearly confirmed in cervical cancer [15]. However, the connection of CRP polymorphism and its protein level in serum with cancer prognosis is still debatable.

**REFERENCES**


