Association between polymorphism of the CRP gene rs1130864 and hypertension among Iraqi hypertensive patients

Luay Qasim Abdulhameed*, Mohanad W. Mahdi Alzubaidy

Abstract

Background: C-reactive protein (CRP) a reactant of the acute phase and a measure of persistent low-grade inflammatory processes. Objectives of this paper to the role of rs1130864 polymorphism in CRP gene among Iraqi hypertensive patients.

Methods: Samples of blood were collected from patients with hypertension and controls, their age (37 to 75). Hardy-Weinberg equilibrium (HWE) was used to calculate the risk score for the disease of hypertension, Moreover, PCR/SNP (specific primers) was used to do genotyping.

Results: HWE analysis of hypertensive patients and controls showed that the rs1130864 genotypes were in agreement with the equilibrium, without differences clear between the observed and expected genotype frequencies (p >0.153, 0.238). The frequencies of genotype and alleles were compared in hypertensive patients with controls, with no significant differences. The common GG genotype of rs1130864 recorded a significant increase in hypertensive patients and controls and was regarded as a preventative fraction (RR = 0.76), whilst, GA, AA genotype were considered the etiological fraction (RR = 1.06, 2.59) and associated with hypertension. Compared to A allele, which might be the cause of the disease, G allele might be preventive.

Conclusion: The findings showed that the Iraqi population’s GA, AA genotype, and A allele are risk factors for hypertension. However, the need for additional findings utilizing bigger samples is needed are necessary to verify our findings.
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Introduction

The global prevalence of hypertension, a prevalent cardiovascular disease, is roughly 1 billion. Even though more than 70% of patients with hypertension are aware of their disease, only 25% to 49% of them receive treatment, and only fewer (20%) are adequately managed achieving control [1]. Because it commonly affects target organs (brain, heart, kidney, and eyes) before clinical symptoms appear, hypertension is known as the “silent killer”[2]. It is postulated that the cause of primary hypertension, which accounts for 95% of all cases of hypertension, is a mosaic network of environmental and genetic variables that interact with each other [3]. Moreover, there are factors (such as obesity, smoking, negative socio-economic conditions and various disease states) that increase C-reactive protein (CRP) levels, which in themselves affect blood pressure levels [4].

Several researchers have recently noted that those with hypertension have greater CRP concentrations. This suggests that one of the primary factors resulting in an elevated risk for myocardial infarction (MI) in hypertensive patients, Caused by high CRP levels [5]. In clinical practice, serum CRP levels exceeding 3.0 mg/l are commonly used as a cardiovascular prognostic marker, despite the fact that these values are known to vary depending on population genetics, sex, age, and other cardiovascular risk factors. However, the usefulness of CRP in predicting cardiovascular risk beyond traditional risk factors is still debatable [6]. CRP a reactant of the acute phase and a measure of persistent low-grade inflammatory processes. It can predict heart attacks and strokes; it was also mentioned as the only circulating biomarker associated with vascular wall biology [7].

The human CRP gene location is at 1q23.2 region on the proximal long arm of first chromosome, and the CRP gene sequence contains 1 intron separating 2 exons [8]. Many investigations have demonstrated the relationship between the CRP gene’s single-nucleotide polymorphisms (SNPs) and differences in blood serum CRP levels, or with diabetes, insulin resistance, microangiopathic stroke, coronary heart disease, hypertension, and metabolic syndrome [9]. Moreover, recent research has demonstrated that serum CRP levels could be affected by CRP SNPs (single nucleotide polymorphisms) and haplotypes in the various populations. Additionally, it was discovered that CRP genotypes were linked to cardiovascular event mortality [10]. For the recently identified polymorphisms in the CRP gene, several polymorphisms have already been reported including a role for the rs1130864 polymorphism in cardiovascular diseases [11]. However, there is conflicting data about the function of 1444G>A (rs1150864), although some researchers have found positive correlations with heart disease and blood vessels [12]. Our current research was carried in order to verify the role of rs1150864 polymorphism in CRP gene among Iraqi hypertensive patients.

Methods

Participants

The total number of adult hypertensive patients was 45 with 55 adult individuals consider as control group taken from different regions of Diyala Province, and their age range was 37-75 years. They were attending out laboratories in Baqubah city/ Diyala province during September 2020 – July 2021 for treatment and diagnosis. The samples of blood were stored in tubes containing EDTA and maintained kept at 20–25°C until they were utilized for DNA extraction from each isolation.

Preparation and DNA extraction

Genomic DNA has been extracted with the ReliaPrepTM gDNA Miniprep System from EDTA blood (Promega, USA), and exposed to PCR amplification after ensuring purity and concentration. Primers used for rs1130864 design by PRIMER3PLUS software, as shown in Table 1.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>(5’-3’ Seq)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1130864-F</td>
<td>5’-CTGCTCTGACCATGAGAT-3’</td>
<td>588 bp</td>
</tr>
<tr>
<td>rs1130864-R</td>
<td>5’-AAACGAGCTCCAGAGC-3’</td>
<td></td>
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</tbody>
</table>

Table 1: rs1130864 was amplified using a primer.

Preparation PCR mixture

The 25μl blend is made up of Green Master Mix (Bioneer company),template DNA, deionized water and primer solution with each of the following volumes (Table 2). PCR was used for detecting rs1130864 in hypertensive patients. The mixture-containing PCR tubes were placed inside a preheated thermocycler, with program was initiated as given in Table 3.

<table>
<thead>
<tr>
<th>Amount</th>
<th>Component</th>
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</thead>
<tbody>
<tr>
<td>12.5μl</td>
<td>Master Mix</td>
</tr>
<tr>
<td>3μl</td>
<td>DNA template</td>
</tr>
<tr>
<td>1.5 Forward + 1.5 Reverse</td>
<td>Primer</td>
</tr>
<tr>
<td>6.5μl</td>
<td>Deionized water</td>
</tr>
<tr>
<td>25μl</td>
<td>Total</td>
</tr>
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</table>

Table 2: PCR reaction mixture components.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94</td>
<td>min5</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>58</td>
<td>30 sec</td>
<td>35</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>10 min</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Program of PCR reaction conditions for rs1130864.

Exclusion criteria

Volunteers with lipid disturbance, heart failure, valvular heart disease, ischemic heart disease, chronic kidney illness, thyroid disease, and any other chronic systemic illnesses including cardiovascular disease or...
malignancies were not allowed to participate in the study.

**Statistical analysis**

The X2-test was used to identify the Hardy-Weinberg equilibrium in both controls and cases. The frequencies of genotype and alleles differences between the patients and controls were discovered using chi-square analysis. 95% confidence intervals (CIs) and Odds ratios (ORs) were extracted. P-values < 0.05 were regarded as significant.

**Results**

Electrophoresis of agarose gel for PCR amplified products (rs1130864 SNP) of the CRP gene revealed a single 388 bp molecular size band, as shown in the current study (Fig. 1). Three genotypes (AA, GA, GG) and two alleles (A and G) were presented for the rs1130864 (G/A; Chromosome1). There were no significant variations (p >0.153-0.238) in the expected and observed genotype frequencies in the genotypes of hypertension patients and healthy participants, indicating that the genotypes were in agreement with the equilibrium (Table 4).

**Discussion**

The CRP gene - rs1130864SNP, according to the findings of this study, gave rise to three genotypes (GG, GA, and AA), each of which corresponds to two alleles (Table 4), in accordance with the Hardy-Weinberg equation (HWE). These genetic patterns (G and A) were be compatible as in Table 5.

The rs1130864SNP polymorphism study revealed, using Fisher’s exact test, in the control group, all genotype frequencies were non-significantly higher than in the patient group, with probability Fisher (0.547 vs. 0.409), the genotype GG and G allele were shown to have a high ratio in both the patients (53.33 vs. 72.2%) and control group (60 vs. 78.2%, respectively). Consequently, it represents the genotype that is most prevalent in Iraqi population. Furthermore, GG genotype and the G allele were considered the Preventive fraction depending on their RR values (0.76, 0.73). However, patients had significantly higher frequencies of the A allele and the AA genotype (8.89 vs. 27.8%, respectively) compared to the control group (3.64 vs. 21.8%, respectively), according to Probability Fisher (0.404 vs. 0.409). The AA genotype and A allele were regarded as the etiological fraction and related with hypertension based on the values of RR (2.59, 1.38). While, the GA genotype frequency was significantly higher in patients compared to controls (37.78 vs. 36.56%, respectively), with values of RR (1.06). In addition, the GA genotype has been considered to be a contributing factor in the etiology of hypertension.

The study’s findings were in agreement with those reported in the results [14], that certain single-nucleotide polymorphisms (SNPs) of CRP are related to higher CRP levels and involved in the development of atherosclerosis with consider to be an indicator of ischemic stroke (IS) in older individuals. On the other hand, study [15] indicated that a single nucleotide polymorphism 1444GA SNP (rs1130864) located in the 5′-untranslated (UTR)region of CRP, which the A allele has been related with elevated levels of CRP than the G allele in several inflammation conditions, such as cardiovascular disease and gingivitis. This corresponds to our current study results. Another study suggested that rs-1150864 variant was strongly associated with microangiopathic stroke [16]. In IS patients, the T allele of rs1150864 was a significant associated with a poor functional outcome, and TT genotype variant increases susceptibility to coronary artery disease (CAD) [17]. SNPs rs1150864 has been linked to the incidence of IS and CAD in a number of previous studies [14, 18].

The results of our study agreed with another study that revealed genetic variations in the CRP gene were significantly related plasma CRP levels and help predict the onset of hypertension. rs1130864 genotypes have
been linked to adult hypertension and higher serum CRP levels, and patterns AA and GA have been identified as genetic markers for these conditions [3]. Besides, gene-gene and interactions between genes and the environment seem to affect CRP levels. However, our analyses focused on the impact of CRP gene variations, and this was an only limitation to our study. In conclusion, this is a first article to detail the genotypes distribution of the CRP gene for SNP rs1130864 in the adult population of Iraq and to demonstrate a link between the hypertension condition and genotypes for the CRP gene for SNP rs1130864.

**Competing Interest**
The authors declare that there is no conflict of interest.

**Author Contributions**
Luay Qasim Abdulhameed: the research article proposal, experiment design, explaining the findings, and article writing.

Mohand W. Mahdi Alzubaidy: Preparing materials, Funding acquisition, Data curation, Statistical analysis and review & editing.

**References**


