Abstract
Infection with hepatitis C virus (HCV) imposes a global challenge with over 180 million cases worldwide. Only few patients spontaneously had their virus neutralized, while most patients develop chronic HCV infection. This implies a key role of genetic factors in viral clearance or persistence. The current study aimed at clarifying the effect of certain single nucleotide polymorphisms (SNPs) on individual's susceptibility to HCV infection. A total of 60 patients with confirmed HCV infection and 35 apparently healthy individuals were enrolled in this study. Blood sample was obtained from each participant, from which DNA was extracted. The JAK1 gene was amplified with conventional PCR technique using three sets of primers targeting three SNPs in this gene: rs2780895, rs4244165 and rs17127024. Restriction fragment length polymorphism (RFLP) was used for genotyping of PCR products. Each of rs2780895 and rs17127024 had two genotypes in both patients and controls, however, only the heterozygous genotype of the SNP rs2780895 (CT) significantly associated with the susceptibility to HCV. The SNP rs4244165 appeared in only with homozygous wild genotype (GG) in both patients and controls. It can be concluded that allele T of the SNP rs2780895 could be considered as a risk factor for infection with HCV.

Key words: HCV, JAK1 gene, polymorphism, RFLP

Genetic Variants in JAK1 Gene and Susceptibility to Hepatitis C Viral Infection in Iraq

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Introduction
Hepatitis C virus is one of the leading causes of morbidity and mortality due to viral infection [1]. The last 17 years witnesses an increase in seroprevalence of the disease burden to 2.8% with an estimated 185 million infections world-wide [2].

Host genetic factors play a major role in the resistance to this infection. Single nucleotide polymorphisms in different genes were found to affect the susceptibility to HCV infection. Ge et al. [3] found that certain SNPs in IL-28B gene could interfere with the outcome of HCV treatment. Afzal et al. [4] reported a significant association of IL-10-1082GG with susceptibility to HCV infection in Pakistani population; however, this result did not reproduced by Pasha et al. [5] who found such susceptibility to be associated with TNF-α-308 among Egyptian population. This may be attributed to the role of race in such susceptibility.

Janus kinase 1 is a member of protein-tyrosine kinases (PTK) identified as signaling molecules in the interferon pathway. It is essential transducers of the signaling from cytokine receptors [6]. Previous studies
have indicated that polymorphisms in \textit{JAK1} gene may be functional and could contribute in many human cancer and auto immune diseases \cite{7}. More recent evidence \cite{8} revealed that certain SNPs in this gene were associated with the outcomes of HBV infection. Despite these and other evidences, no one to the best of our knowledge investigate the association in the SNPs in the \textit{JAK1} gene with the incidence of HCV. Accordingly, we hypothesized that some SNPs in this gene can influence the susceptibility to HCV and we conducted this study which aimed to clarify the effect of certain single nucleotide polymorphisms (SNPs) on individual’s susceptibility to HCV infection.

\textbf{Materials and Methods}

\textbf{1- The study population}

This study included 60 patients (41 male and 19 female, age range 12-57 years old, mean= 39.17 years) who were attending Al-Imamain Al-Kadimain Medical City during the period from September 2013 to March 2014. Criteria of enrolling were patients with positive HCV antibodies by enzyme linked immunosorbent assay (ELISA) or HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Another 35 apparently age-matched individuals from those who were attending the same city for routine medical check were recruited as a control group. Exclusion criteria for control group were evidence of past or current infection by hepatitis viruses and/or cirrhosis or hepatocellular carcinoma. A consent form was obtain from each participant which included, age, sex, family history of HCV, alcohol drinking, residence, socioeconomic status and smoking.

\textbf{2- Sample}

Three ml of venous blood samples was taken from each participant in an EDTA-tubes. These tubes were kept at -20 C until be used for DNA extraction.

\textbf{3- DNA Extraction and Genotyping}

DNA extraction and genotyping was done in the laboratoried of medical research unit/ College of Medicine/ Al-Nahrain University. Genomic DNA was extracted from peripheral blood samples using ready kit (\textit{gSYNC} DNA Mini Kit Whole Blood Protocol/ Geneaid/ Korea) following the manufacturer's protocol. Three SNPs in the \textit{JAK1} gene were selected to be genotyped. These were rs2780895, rs4244165 and rs17127024. The Set of primers used for gene amplification corresponding these SNPs are shown in Table (1). Template DNA (10 ng) from each sample and primers (5 ng from each) were added to 0.5 ml ependorf tube contains 25µl of master mix. Nineteen µl of deionized water was added to reach a final volume of 50µl. The mixture then put in shaker and spinner for 10 cycles for better mixing. The master mix tubes were then transferred to the the rmoycler (Hybaid/UK). PCR conditions were almost identical for the three SNPs. These included an initial denaturation step at 95 °C for 5 min followed by35 cycles of 94 °C for 30 sec, 61 °C for 30 sec and 72 °C, and a final extension of 72 °C for 10 min. The only exception was that the annealing temperature for the SNP rs17127024 was 63 °C.

\textbf{4- Endonuclease Digestion}

One µg of DNA from rs2780895, rs4244165 and rs17127024 PCR products was mixed with a 5µl 10X NEB buffer (50mM NaCl, 10mM Tris-HCl, 10mM MgCl2, 1mM dithiothreitol, pH 7.9), and 1µl of \textit{NdeI}, \textit{HaeIII}, and \textit{HaeII} (10U) restriction enzyme ((New England Bio labs Inc./USA) respectively. Deionized sterile H2O was used to adjust the volume to 50 µl. The mixture was then incubated at 37 °C for 60 min.

\textbf{5- Agarose Gel Electrophoresis}

A 2% gel was prepared, and 10 µL aliquot of digested PCR product from each SNP was mixed with 2 µL loading dye and loaded into the wells. After 1 hour electrophoresis, the gel was stained with ethidium bromide (Biocase/Canada) (0.5 µg/ml) for 20 min and examined using U. V. transilluminator with camera. The size of digested product was determined depending on a commercial 100bp ladder (Kappa Biosystem/ USA) \cite{9}.

Table (1): Primer sets and endonucleases used for genotyping of the three SNPs

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
SNP & Primer sequence (5'-3') & Fragment length & Endonuclease \\
\hline
rs2780895 & F: CACAGGTAGATTGGAGGAG & 676 bp & \textit{NdeI} \\
 & R: AAGACGCTGATTGGAGGTAG & & \\
rs4244165 & F: GTGACTGACATAGTGAGGAGGT & 415 bp & \textit{HaeIII} \\
 & R: CTITAGAAAGCCCTATTGCC & & \\
rS17127024 & F: GTGAGCGATGGAGAGAATC & 395 bp & \textit{HaeIII} \\
 & R: TACTGGCACAAGCAGGACC & & \\
\hline
\end{tabular}
\end{center}
6- Statistical Analysis
The Statistical Package for the Social sciences (SPSS, version 14.0) was used for statistical analysis. Risk association between different genotypes and HCV susceptibility was estimated by the calculation of odds ratio and 95% confidence intervals. Differences in genotype and allele frequencies among the groups, and deviation of genotype distribution from Hardy-Weinberg equilibrium were tested using Chi-square test with one degree of freedom. The acceptable level of significant was p-value ≤0.05 [10].

Results and Discussion
The SNP rs2780895 appeared in three genotypes in both patients and controls. These genotypes were CC, CT and TT which represented 28(46.7%), 26 (43.3%) and 6 (10%) respectively among patients compared to 29 (82.85%), 5(14.29%) and 1 (2.86%) respectively among controls with significant (P≤0.05) difference in heterozygous genotype (OR=5.785, 95%CI=1.621-20.65, P=0.007) as shown in Table (2) and figure (1).

Table (2): Genotypes and allele frequencies in HCV patients and controls

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotype</th>
<th>Cases n=60</th>
<th>Control n=35</th>
<th>P-value</th>
<th>OR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2780895</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>CC</td>
<td>28(46.7%)</td>
<td>29 (82.85%)</td>
<td>0.004</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>26(43.3%)</td>
<td>5(14.29%)</td>
<td>0.007</td>
<td>5.785(1.621-20.65)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>6(10%)</td>
<td>1 (2.86%)</td>
<td>0.904</td>
<td>1.154(0.113-11.78)</td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>82(68.33%)</td>
<td>63 (90%)</td>
<td>0.001</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>38(31.67%)</td>
<td>7(10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs17127024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>GG</td>
<td>49(81.7%)</td>
<td>33 (94.28%)</td>
<td>0.017</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>9(15%)</td>
<td>1 (2.86%)</td>
<td>0.225</td>
<td>3.676(0.391-34.53)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>2(3.3%)</td>
<td>1(2.86%)</td>
<td>0.322</td>
<td>0.22(0.011-4.358)</td>
</tr>
<tr>
<td>Allele</td>
<td>G</td>
<td>107(89.17%)</td>
<td>67 (95.71%)</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13(10.83%)</td>
<td>3 (4.29%)</td>
<td>0.175</td>
<td>2.713(0.745-9.877)</td>
</tr>
</tbody>
</table>

Fig. (1): The 2% agarose gel electrophoresis showing the restriction digestion patterns of rs2780895 SNP in the JAK1 gene using NdeI enzyme. Lane 1: DNA marker. Lanes 2, 4, 5, 8, 9, 11, 12: homozygous wild type (CC). Lane 3, 10: homozygous mutant genotype (TT). Lanes 6, 7: heterozygous genotype (CT). At allele level, the mutant allele (T) represented 31.67% in HCV patients compared to only 10% in controls with highly significant (P≤0.05) difference (OR=4.171, 95%CI=1.747-9.96, P=0.001) Table (2).

On the other hand, the SNP rs4244165 appeared in one genotype (GG) in both patients and controls, Table (2) Figure (2).
Fig. (2): The 2% agarose gel electrophoresis showing the restriction digestion patterns of rs1244165 SNP in the JAK1 gene using HaellII enzyme. Lane 8: DNA marker. All the samples from HCV patients and control appeared only in homozygous wild type (TT).

Similar to the first SNP, rs17127024 appeared in three genotypes which were GG, GT, and TT. These genotypes represented 81.7%, 15%, and 3.3% respectively in HCV patients compared with 94.28%, 2.86%, and 2.86% respectively in controls, however, the differences were non-significant (P≥0.05) Table (2). Figure (3). At allele level, the mutant allele (T) accounted for 10.83% in HCV patients compared to 4.29% in controls with non-significant (P≥0.05) difference Table (2).

Fig. (3): The 2% agarose gel electrophoresis showing the restriction digestion patterns of rs17127024 SNP in the JAK1 gene using HaellII enzyme. Lane 1: DNA marker. Lanes 3, 4, 6,8: homozygous wild type (TT). Lane 2, 7: heterozygous genotype (GT). Lanes 5: homozygous mutant genotype (GG).

Results of this study revealed significant association of the SNP rs2780895 with the incidence of HCV among Iraqi patients. This result is partially in agreement with that obtained by [8] who found significant association of all the three studied SNPs with the outcomes of hepatitis B infections among Chinese population. In fact, JAK1 gene polymorphisms were reported to influence various kinds of diseases such as different kinds of malignancies [11] and infectious diseases [12].

Single nucleotide polymorphisms could occur in a very wide range among different genes. However, the influence of an SNP depends on many factors among which the gene hosts the SNP and the site of the SNP in that gene. The JAK1 gene is located on chromosome 1p31.1 and encodes for JAK1 protein which is part of JAK-STAT signal transducer. Disruption of this pathway may predispose for various diseases as a result of deregulation of cell proliferation, differentiation, and apoptosis [13]. The occurrence of an SNP in protein-coding region (exons) of the gene is a justifiable cause of aberrant in the function of the protein encoded by this gene. That is because many of these SNPs are non-synonymous and change the amino acid sequence of the protein [14]. Another effective location of the SNPs is the promoter region of the gene. In this case, the SNP could reduce or increase gene expression and eventually the activity of the gene product [15]. However, the SNP rs2780895 is located in non-coding regions of genes (intron). In fact, there are many explanations for the association of this SNP with increased incidence of HCV.

First, introns contain a multiplicity of functional elements including intron splice enhancers and silencers that regulate alternative splicing [16], trans-splicing elements [17], and other regulatory elements [18]. Polymorphisms in these sites un-doubtly would influence the mRNA and the translated protein. Second, some SNPs may influence the expression of remote genes at distance rather than the expression of those genes actually hosts them. Finally, the SNP rs2780895 could act as a tag SNP that associates with other NPs (not involved in this study) and collectively forming a haplo type that influence the activity of JAK1 [19].
In any case, the result would be disruption of JAK-STAT signaling pathway. This influences a wide variety of cytokine, may be the most important of which are interferon. Knowing the pivotal role of interferon in immunity against HCV, it is not surprising to find the association between the SNPs in JAK1 gene and the increased susceptibility to HCV.

In summary, the present study revealed an association of mutant allele of the SNP rs2780895 with the susceptibility to HCV among Iraqi population. However, a study with a larger sample size is required to draw a solid conclusion for such association.

References