Role of ACE and IL-1β Gene Polymorphisms in Erythropoeitin Hyporesponsive Patients with Chronic Kidney Disease with Anemia

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Abstract

Background: Hyporesponsiveness to erythropoietin is a common problem seen in around 5-10% of patients. Recently the focus from these remediable factors has been shifted to the non-modifiable innate factors i.e., polymorphism of ACE and IL-1B gene and studies have shown that DD genotype and IL-1B CC genotype have lower erythropoietin requirement. The aim of our study was to evaluate the role of ACE and IL-1B gene polymorphisms in erythropoietin hyporesponse in CKD patients with anemia.

Methods: A total of 50 patients were selected. After taking pre-informed written consent, they were segregated into two groups, group A and B with 25 patients in each group. Group A included CKD stage III-IV patients and Group B included CKD stage V patients who were on regular maintenance. All patients were given erythropoietin and response was monitored using erythropoietin resistance index (ERI). Genotyping of ACE and IL-1B genes were done and serum levels of ACE and IL-1B were measured. Mean values of ERI were compared between different genotype subgroups and analysed using binary regression analysis.

Results: The study group included 6 patients with diabetic nephropathy and out of these 4 (66.6%) had DD genotype. On comparing the effect of ACE polymorphism on ERI levels it was seen that the mean ERI values in DD subgroup were significantly lower (16.97±5.35, 22.69±8.35 at 1, 3 and 5th month) as compared to ID (18.16±3.39, 24.7±3.66, 32.7±9.95 and II (20.7±5.17, 27.7±7.30, 41.08±13.83 U/Kg/g/dL). In the case of IL-1B the mean ERI values were lowest in the TT subgroup (16.46±4.45, 21.96±5.77, 23.39±8.48) as compared to CC (19.49±5.62, 25.46±7.07, 33.59±12.61) and CT (18.12±4.27, 24.14±5.70, 31.89±13.83 U/Kg/g/dL). The mean serum values of ACE were in a decreasing trend i.e DD> ID> II (238.05±52.46, 194.73±50.28 and 162.99±39.71 ng/ml, (p < 0.05). The mean serum values of IL1B in CC, CT and TT were 23.24±28.77, 18.32±16.25, 23.34±13.83 pg/ml (p>0.05).

Conclusion: D allele positively affected the serum ACE level but there was no association between IL-1B genotype and its levels. ACE gene polymorphism has an important role in determining the response to EPO and progression of CKD. Pre-treatment screening for genotype may help in predicting the patients at risk and poor responders.

Editorial Viewpoint

• Hyporesponsiveness to erythropoietin is seen in 5-10% of patients. In this study genotyping of ACE and IL-1B genes was done and serum levels of ACE and IL-1B were measured.
• ACE gene polymorphism was found to have a role in the response to EPO.

Introduction

Hyporesponsiveness to erythropoietin is a common problem seen in around 5-10% of patients. This hyporesponsiveness has been attributed to many factors like Vit B12 and folic acid deficiency, hyperparathyroidism, inadequate dialysis and inflammation with iron deficiency being the most common cause. Recently the focus from these remediable factors has been shifted to the non-modifiable innate factors i.e., polymorphism of ACE and IL-1B gene. The D allele has been linked to failure of the renoprotective action of ACE inhibitors, increasing CKD in hypertensive and diabetic patients. But Varagunam et al 2003 and Sharples et al 2006 studied CKD patients undergoing CAPD and saw that there was clear trend in EPO response among the different

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Subjects and Methods

A total of 60 patients were recruited out of which 7 patients did not come for follow up and 3 patients died before the study period ended. A total of 50 patients finished the study. The study was approved by the ethical committee of Pt. B.D Sharma PGIMS, Rohtak. After taking pre-informed written consent from them, these patients were segregated into two groups, group A and B with 25 patients in each group. Group A included newly diagnosed patients of Chronic kidney disease stage III-IV with anemia and Group B included known cases of Chronic kidney disease stage V who were on regular maintenance dialysis and were receiving EPO therapy and were showing inadequate hematological response (<1 gm/dl of Hb rise in one month) to EPO therapy. Inclusion criteria were age ≥18 years, newly diagnosed CKD stage III-IV with anemia (Hb <10 g%), CKD stage V on regular hemodialysis and on EPO once/twice a week with Hb < 10 g%. Exclusion criteria were chronic bleeding, hypothyroidism, malignant disease, hematologic disease, acute infectious diseases, hyperparathyroidism. Patient’s detailed history and systemic examination were taken. Routine haematological and biochemical investigations were done along with serum ferritin, iron, TIBC, Vit B12, Folic acid, serum iPTH, hsCRP.

Genotyping

Extraction of DNA of the collected blood samples was done according to Miller et al (1988) protocol. Extracted DNA samples were quantified using spectrophotometer (NanoDrop). All the samples were subjected to Polymerase Chain Reaction (PCR) to amplify the particular genomic regions of interest. In case of ACE, primer sequences for the PCR amplifications were Forward: 5’CTG GAG ACC ACT CCC ATC CTT TCT 3’ and Reverse: 5’ GAT GTG GCC ATC ACA TTC GTC AGAT 3’. The PCR product was checked on a 2% agarose gel. The PCR product corresponded to a 190-base pair fragment (D) and 490 bp (I). Primer Sequences for IL-1B were Forward: 5’ TGGCATTGATCTGGTTCATC 3’ Reverse: 5’GTTTAGGAAT CTTCCCACTT 3’. Quantikine human immunoassay was used to measure the serum ACE and IL-1B levels. The SPSS statistical package version 16.0 (SPSS; Chicago, IL), was used to compare all clinical and laboratory parameters. Differences between group means were tested using paired t-test and one-way ANOVA. All values were expressed as mean ±SD, with statistical significance defined as P <0.05. Binary logistic regression was used to see the effect of age, BMI, HTN, hs-CRP, ESR, ACE and IL-1B gene polymorphism on ERI.

Results

Total 60 patients were included in the study, of which 3 patients in group B died and patients in group B did not come for follow up. Of the total 50 patients who completed the study, 54% (n=27) were males and 46% (n=23) were females. The mean age, weight and BMI were 43.98 ± 15.63 years, 52.12 ± 9.19 kg, and 21.30 ± 3.79 kg/m² respectively. Hypertensive nephropathy was the commonest cause of CKD in group A, 44% (n=11) whereas chronic glomerulonephritis was commonest in group B, 36% (n=9). But, on the whole hypertensive nephropathy was the commonest, 36% (n=18). The study group included 6 patients with diabetic nephropathy and out of these 4(66.6%) had DD genotype and 1(16%) patient had II genotype. Out of total 50 patients, 18 had hypertensive nephropathy and among these 18, 9 (50%) patients
showed the minimum risk (7 fold).

The lowest risk was found to be in the recessive model (4 fold, CI(1.31-17.40)\(^\text{P}<0.05\)), where mutant homozygote (II) was considered to be the risk genotype as compared to ID+DD. On using binary regression analysis to determine the effect of IL1-B gene polymorphism on ERI levels it was seen that presence of T allele in homozygous or heterozygous form had no effect on the ERI values (Figure 4). Binary logistic regression analysis was used to see the effect of age, fluid overload, haemoglobin, PCV, ESR, hypertension, hsCRP and BMI. Only haemoglobin, PCV and fluid overload showed significant effect on ERI (Figure 5).

ACE and IL1B Levels

The mean serum values of ACE were in a decreasing trend i.e DD> ID> II. TT group showed the highest levels and CT showed the lowest levels with CC group having intermediate levels. There was no clear association between C or T allele and serum IL1B levels (Table 2).

Discussion

This study showed that on the whole, I allele was more prevalent in the study group than D allele. The higher prevalence of D allele in diabetic patients with nephropathy shows that D allele increases the risk of CKD in diabetics. These observations agree with previous studies.\(^1\)\(^-\)\(^3\) But, do not match with study in Eastern Indian population,\(^4\) where no such association was seen. Out of total 50 patients, 18 had hypertensive nephropathy and among these 18, 9 (50%) patients had ID genotype. This observation is in contrast to the results of previous metanalysis\(^5\) which showed higher prevalence of DD genotype in East Asian males with hypertensive nephropathy. The contrast could be due to the difference in ethnicity and may be such relationships are limited to Asian populations of China.

### Table 1: Genotype and allele frequencies (pooled)

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes</th>
<th>N (%)</th>
<th>Allele frequency</th>
<th>HWE chi sq, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE I/D (n=49)</td>
<td>DD</td>
<td>15 (30.61)</td>
<td>I=0.47</td>
<td>3.34, 0.063</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>18 (36.73)</td>
<td>D= 0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>16 (32.65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1B 511 C/T (n=50)</td>
<td>CC</td>
<td>19 (38.00)</td>
<td>C=0.63</td>
<td>4.52, 0.03</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>28 (56.0)</td>
<td>T=0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>3 (6.0)</td>
<td></td>
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</tr>
</tbody>
</table>

**Fig. 1: Comparison of ERI among ACE genotypes. (Pooled)**

**Fig. 2: Comparison of ERI among IL1B genotypes. (Pooled)**

had ID 5 (27.7%) had DD and 4 (22.2%) had II genotype.

Genotype and Allele Frequencies

The genotype and allele frequencies for ACE and IL1B genes were done. In case of ACE, II and I were the most frequent genotype and allele whereas in case of IL-1B CT and C were the most frequent (Table 1).

Genotype and ERI

On comparing the effect of ACE polymorphism on ERI levels it was seen that the mean ERI values in DD subgroup were significantly lower (16.97±5.35, 21.88±6.25, 22.69±8.35 at 1, 3 and 5\(^\text{th}\) month) as compared to ID (18.16±3.39, 24.17±3.66, 32.74±9.95) and II (20.73±5.17, 27.74±7.30, 41.08±13.83 U/Kg/g/dL) (Figure 1). In the case of IL-1B, the mean ERI values were lowest in the TT subgroup (16.46±4.45, 21.96±5.77, 23.98±8.48) as compared to CC (19.49 ±5.62, 25.46±7.07, 33.59±12.61) and CT (18.12±4.27, 24.14±5.70, 31.89±13.83 U/Kg/g/dL). Though there was a clear trend in the mean ERI values with TT having lowest and CC having highest values, the difference did not reach statistical significance (\(p>0.05\)) (Figure 2).

Binary Regression Analysis

To understand the role of I-allele in the causation of abnormal ERI, odds ratio analysis was performed using four different models, i.e, dominant (ID+II/DD), co-dominant (ID/DD), co-dominant (II/DD), and recessive (DD+ID/II) model. In all the models, DD was considered the wild type except in the recessive model and the risk was calculated (Figure 3). The presence of II showed maximum risk (18 fold) whereas ID showed the minimum risk (7 fold).
previous studies conducted in CKD patients undergoing CAPD, but were in contrast with an earlier study conducted by Hatano et al 2000 which showed no such correlation. Initially the difference in findings was thought to be due to the overriding effects of cytokines in the HD population as cytokines have been shown to inhibit erythropoiesis. But studies conducted later on showed findings similar to this study where they showed that patients on maintenance hemodialysis with DD genotype had lower erythropoietin requirements. Recently, researchers have observed that among patients receiving ACE inhibitors therapy, patients with DD genotype showed higher EPO requirement. It was hypothesized, that the presumably relative greater fall in AT-II levels and endogenous EPO production as a response to ACEi therapy in patients with DD genotype resulted in lower haemoglobin levels and higher exogenous rHuEpo requirement compared to patients with II genotype. Although they also observed a trend towards higher haemoglobin levels and lower erythropoietin resistance in matched patients with DD genotype compared to II genotype not receiving ACE inhibitors, this was statistically not significant.

It was observed that in anemia in AA amyloidosis was associated with the allele 2(T) of the IL-1B-511 promoter gene and was associated with increased levels of IL-1B. In this study we found that the ERI values were comparatively lower in TT genotype subgroup and there was clear increasing trend in ERI values from TT to CC though these findings were not statistically significant (p<0.05). Our findings were completely opposite to the previous studies which showed lower ERI values in CC genotype and highest levels of IL-1B in patients with CC genotype. The small sample size and gross underrepresentation of TT genotype and Japan and may not hold true for the Indian population which formed our study group. It was seen that TT genotype was highly underrepresented in the study population. This may be due to the protective effect of T allele against development of chronic kidney disease. We found that the mean ERI values were significantly lower in DD genotype subgroup as compared to ID and II. A clear trend was noticed as the ERI values progressively increased from DD to II genotype clearly showing that the presence of D allele positively influenced the response to erythropoietin. From observation made in binary regression analysis, it was evident that I-allele in the form of heterozygote or homozygote is disadvantageous and can lead to elevated ERI. As expected, the disadvantage was much higher for individuals with II as compared to ID. It has been shown that patients with DD genotype have higher levels of ACE which in turn leads to higher levels of angiotensin II levels and this activation of AT1 with angiotensin II enhances erythropoietin-stimulated erythroid proliferation in vitro. This sequence of findings gives us a possible explanation for the positive effects of DD genotype over erythropoiesis. Our findings were in accordance with
Conclusion

This study found that patients with DD genotype reduced the exogenous EPO requirement as compared to II genotype. Further, D allele frequency was higher in patients with diabetic nephropathy as compared to I allele which implies that D allele is an independent risk factor for progression to CKD especially in diabetic patients. Therefore, we infer that ACE genotyping and probably IL-1B genotyping can be used as a pre-treatment work up for identifying good and poor responders to EPO therapy so that appropriate doses of EPO can be prescribed. Moreover, determination of ACE genotype can help identify patients at increased risk of progression to CKD.

References


