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# Vascular Endothelial Growth Factor Receptor 2(VEGFR2) Gene Polymorphism And Treatment Outcome Following Imatinib Therapy In Iraqi Patients With Chronic Myeloid Leukemia.

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***Abstract: Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative disorder derived by formation of Philadelphia chromosome (Ph chromosome). Formation of the Ph chromosome is caused by a reciprocal translocation between the chromosomes 9 and 22 t(9;22)(q34;q11), resulting in a fusion protein known as BCR-ABL which has constitutive tyrosine kinase activity and promotes the proliferation of leukemia cells via multiple mechanisms. Vascular endothelial growth factor receptor types 2 (VEGFR2) gene single nucleotide polymorphisms have been detected in CML and evidence suggests its influence on leukemia susceptibility and outcome. Aim: To evaluate the polymorphism of VEGFR 2 gene among Iraqi CML patients, and the relation of the gene receptor polymorphism in response to treatment and prognosis. Material and Methods: the study included 80 subjects, 40 CML and 40 healthy control age and sex matched. Routine investigations were collected including clinical signs and symptoms, physical examination, CBC, liver function tests and BCR-ABL1 &/or FISH. Real time PCR technique was used to detect VEGFR-2 gene polymorphism with SaCycler 96 using genomic DNA extracted from peripheral blood and TaqMan SNPs genotyping assay ((rs1531289, rs1870377 and rs2305948))for both patients and controls. Results: VEGFR-2 polymorphisms SNP1 (rs1531289 T>C) in CML patients presented with 82.5% showing the variant genotypes and 17.5% showing the wild homozygous genotype, while control showed 80% variant genotypes and 20 % wild genotype ,SNP2 (rs1870377 T>A) in CML patients presented with 80% showing the homozygous wild genotype and 20% showing the variant genotypes ,while control showed 62.5% the homozygous wild genotype and 37.5% showing the variant genotypes;SNP3 (rs2305948)in CML patients presented with showed 97.5% the homozygous wild genotype and 2.5% showing the variant genotypes while control showed 87.5% the homozygous wild genotype and 12.5% showing the variant genotypes P-values (0.4,0.004 and 0.09 respectively) .60% of CML patients who showed Molecular response were related to the heterozygous variant genotype of SNP (rs1531289 T>C) of VEGFR-2 Polymorphism, while 55% of patients with molecular response were related to the wild homozygous genotype of (rs1870377 T>A)and 70 % of patients with molecular response were related to the wild homozygous genotype of (rs2305948 C>T).Conclusion: VEGFR-2 polymorphism is common among Iraqi CML patients and might impose a high risk of CML development and influence treatment response.***

## Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder derived from a hematopoietic stem cell (HSC), harboring Philadelphia chromosome (Ph chromosome). Formation of the Ph chromosome is caused by a reciprocal translocation between the chromosomes 9 and 22 t(9;22)(q34;q11), resulting in a fusion protein known as BCR-ABL which has constitutive tyrosine kinase activity and promotes the proliferation of leukemia cells via multiple mechanisms(1). CML exists in three disease phases: chronic phase (CP), accelerated phase (AP), blast crisis phase (BC). The majority (90 %) of the patients are diagnosed in the CP even though 20–40 % of the patients are asymptomatic(2). Gene expression profiling has shown a close correlation of gene expression between accelerated phase CML (AP-CML) and blast phase CML (BPCML). The bulk of the genetic changes in progression occur in the transition from CP-CML to AP-CML(3). The patients with CML typically present with a hypercellular marrow with granulopoietic predominance, leukocytosis is the main feature and may reach levels greater than 200 × 109/L , a left shift, basophilia characteristic feature , thrombocytosis, Presence of the BCR-ABL1 gene fusion by RT-PCR analysis and in 98% of cases Ph chromosome on cytogenetic analysis , Serum uric acid is usually raised(4) After some time, anemia also develops. Constitutional symptoms are mild or absent in patients with (chronic phase) CML. (5). In Iraq CML presented in about different percentages among the other types of Leukaemias in both male as 6th commonest type of Leukemia (5.85 % from other leukaemias )and Female as the 5th commonest type of leukemia (7.73% from other leukaemias )(6). The three commercially available TKIs for the frontline treatment of CML include Imatinib, Dasatinib, and Nilotinib. Current guidelines endorse all three as options for the initial management of CML in the chronic phase (CML-CP)(7).

Imatinib mesylate is a selective inhibitor of this tyrosine kinase. It is the first-line treatment for CML-patients. However, it became clear that Philadelphia-positive (Ph+) cells could evolve to elude inhibition due to point mutations within the BCR-ABL kinase domain. To date more than 40 mutations have been identified and their early detection is important for clinical treatment(7). Many of these patients will eventually develop imatinib failure so they need to be closely observed. There is a general consensus that patients who fail imatinib therapy should switch without hesitation to either Nilotinib or Dasatinib. The choice should be guided by the mutation profile, if relevant, and the comorbidities of the patient(8).

Vascular endothelial growth factor receptor type 2 (VEGFR2), or kinase insert domain-containing receptor (KDR), consists of 1356 amino acids. VEGFR2 gene is located in 4q11–q12 and consisted of 26 exons. VEGFR2 plays a critical role in leukemia-associated angiogenesis and transduces the major signals for angiogenesis via its strong tyrosine kinase activity . VEGFR2 is expressed mostly on endothelial cells and in a fraction of hematopoietic stem cells. Autocrine or paracrine loop of VEGFA and VEGFR2 exists between tumor cells and vascular endothelial cells for the stimulation of angiogenesis. Another study investigated the impact of four VEGF (VEGFA) and three VEGFR (VEGFR2) gene single nucleotide polymorphisms (SNPs) on the treatment outcome of CML patients following IM therapy in CML patients response to imatinib therapy in terms of three parameters: (i) response to imatinib therapy [hematological response, major/complete cytogenetic response (MCyR/CCyR) and major/complete molecular response (MMoR/CMoR)]; (ii) treatment failure [loss of response (LOR) and primary resistance] and (iii) progression to accelerated phase (AP) or blast crisis (BC)(9).

## Aim of Study

To evaluate the relation of the gene receptor polymorphism in response to treatment and prognosis. In current study will investigate correlations between complete Molecular response (CMyR) and VEGFR2 genotypes (rs1531289/rs187 0377, rs2305948)between treatment failure and VEGFR2 genotypes of Iraqi CML patients following imatinib therapy.

## Materials and Methods

The study included 80 subjects; 40 cases were selected from the National Center of Hematology who were diagnosed as CML (CP ) and 40 subjects as control group who were sex and age matched. Written Informed consent was taken from every patient and approval of the Ethical committee was provided. Routine investigations were collected including clinical signs and symptoms, physical examination, CBC, liver function tests and BCR-ABL1 &/or FISH. A 5 ml peripheral blood was collected in EDTA tubes. DNA extraction was performed using **[Blood MiniPrep; Quick-gDNA™] [Zymo/USA]** (Cat No. 17046). Real time PCR technique was used to detect VEGFR-2 gene polymorphism with SaCycler 96 using TaqMan® SNP Genotyping Assay (Applied Biosystems/USA) {(rs1531289/C-T)(C___7439188_20)},{(rs1870377/T-A)(C__11895315_20)}and{(rs2305948/C-T)(C__22271999_20)}. CML cases were on Imatinib treatment for at least 12 months .

## Statistical methods

The data of studied group were analyzed by application of Microsoft excel program and Statistical Package for Social Sciences (SPSS) version 23. Outcomes of analysis were arranged in scales variables (means & standard deviation) and in categorical variables. Chi square test and Fishers exact test were used for categorical variables. P value of 0.05 or less was regarded as significant.

## Results

Mean age of CML patients was 43.4±11.2 (Mean±SD) years and the highest proportion of study subjects in case and control groups was found in age group [40 – 60] years(Figure 1), male to female ratio 1.2:1. Splenomegaly was the most presenting sign in CML patients (87.5%) followed by weakness/fatigue (82.5%), and fever (80%).The VEGFR-2 SNPs((rs1531289, rs1870377 and rs2305948)) variant genotypes(TC+CC),wild genotype(TT) and wild genotype(CC) respectively for each SNP were more frequent among CML patients (37.5%+45%) ,(80%)and (97.5%)compared to control as SNP rs1531289 variant genotypes(TC+CC)about(25%+55%), SNP rs1870377 wild genotype(TT)about (62.5%) and SNP rs2305948 wild genotype(CC)about 87.5% (p=0.4,**p=0.004** and p=0.09 respectively)) (Table 1). When we compared the variations of the SNPs in presence of homozygous wild types (TT),(TT)and (CC) respectively with variant(hetero/homozygous )genotypes ;the majority of patients had been presented with combination of the 3 SNPs TC+CC/TT/CC about (62.5%),see table 2 . Comparisons between the wild VEGFR-2 genotypes (TT,TT &CC) and combined variant (TC/CC,TA and CT) respectively for each SNP as regards several haematological parameters including: total WBC count, haemoglobin, platelet count, basophil and eosinophil %) was carried out as shown in( table 3). Generally

presenting symptoms had been presenting in majority of patients and where highly presented in with variant genotype of VEGFR-2 (rs1531289 T>C) and in wild genotypes of VEGFR-2 genotypes (rs1870377 T>A and rs2305948 C>T),as in( table 4) . the 1st SNP VEGFR-2 genotypes of (rs1531289 T>C) most of patients with major haematological response (MHR) had presented with variants TC+CC genotypes(86.7%+77.8%) ,all patient with minor haematological response (mHR) had presented only with variants TC+CC genotypes with no statistical significance as p value 0.3. The 2nd SNP VEGFR-2 genotypes of ( rs1870377 T>A) majority of patients who had MHR or mHR had been presented with wild TT genotype with no statistical significance as p value 0.5. The 3rd SNP VEGFR-2 genotypes of (rs2305948 C>T ) most of patients with major HR had presented with wild CC genotype (84.6%) while all patients with minor HR had presented with wild CC genotype with no statistical significance as p value 0.8( see table 5). As Like the presenting symptoms laboratory findings had been presenting in majority of patients and where highly presented in with variant genotype of VEGFR-2 (rs1531289 T>C) and in wild genotypes of VEGFR-2 genotypes (rs1870377 T>A and rs2305948 C>T),(see table 5) .

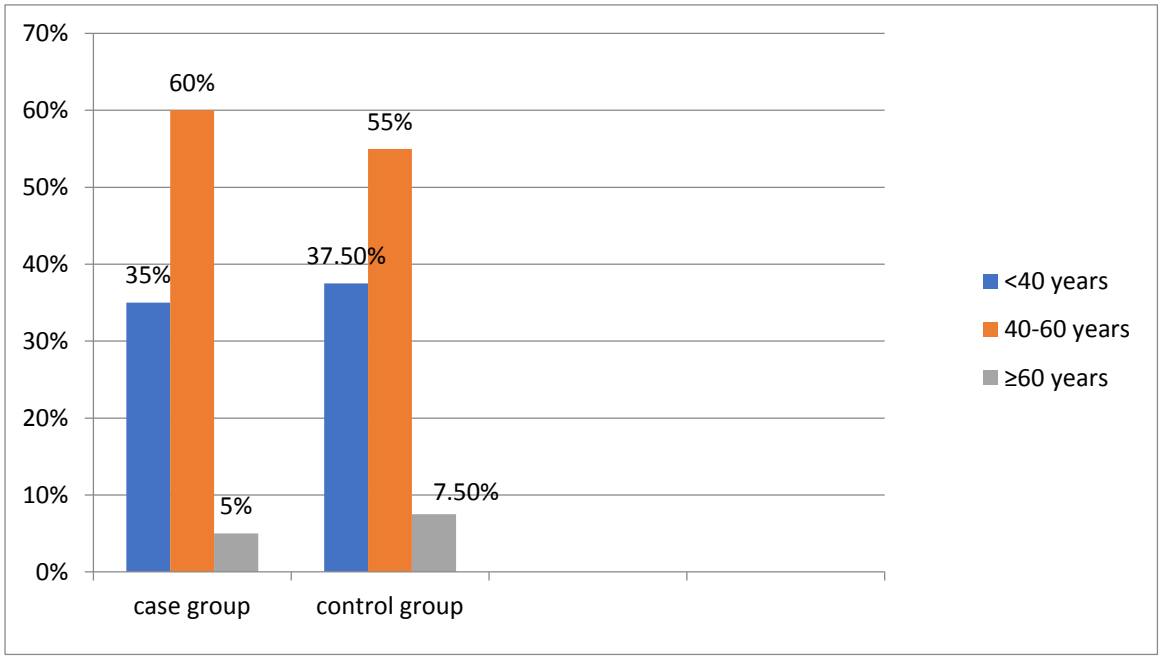


**Figure 1 : Distribution of study subjects' groups by age**

**Table 1: Genotype distributions of VEGFR2 genotypes [T>C, T>A and C>T respectively] polymorphisms among CML patients and controls**

| Study group VEGFR2(n=80) | Genotypes | | | P value |
|---|---|---|---|---|
| | WW | WM | MM | |
| SNP1 rs1531289 | TT No. / Percentage | TC No. / Percentage | CC No. / Percentage | |
| Case | 7/ 17.5% | 15/ 37.5% | 18/ 45.0% | 0.4* NS |
| Control | 8/ 20.0% | 10/ 25.0% | 22/ 55.0% | |
| SNP2 rs1870377 | TT No. / Percentage | TA No. / Percentage | AA No. / Percentage | |
| Case | 32/ 80% | 8/ 20% | 0/ 0% | 0.04** S |

| | | | | |
|---|---|---|---|---|
| **Control** | **25/ 62.5%** | **10/ 25%** | **5/ 12.5%** | |
| **SNP3 rs2305948** | **CC No. / Percentage** | **CT No. / Percentage** | **TT No. / Percentage** | |
| **Case** | **39/ 97.5%** | **1/ 2.5%** | **0/ 0%** | 0.09** NS |
| **Control** | 35/ 87.5% | **5/ 12.5%** | **0/ 0 %** | |

*WW wild, WM heterozygous, MM homozygous mutant, MAF minor/mutant allele frequency,*
Significant association at * p\0.05 *Significant, NS=not significant. p value*

**Table 2: Combined analysis to study the association between VEGFR-2 genotypes polymorphisms among the controls and cases**

| VEGFR-2 rs1531289 | VEGFR-2 rs1870377 | VEGFR-2 rs2305948 | Control n=40 % | CML cases n=40 |
|---|---|---|---|---|
| **TT** | **TT** | **CC** | **6 (15%)** | **6 (15%)** |
| **TT** | **TT** | **CT+TT** | **0** | **1 (2.5%)** |
| **TT** | **TA+AA** | **CT+TT** | **1 (2.5%)** | **0** |
| **TC+CC** | **TA+AA** | **CT+TT** | **3 (7.5 %)** | **0** |
| **TC+CC** | **TA+AA** | **CC** | **10 (25%)** | **8 (20%)** |
| **TC+CC** | **TT** | **CC** | **17(42.5%)** | **25(62.5%)** |
| **TC+CC** | **TT** | **CT+TT** | **3 (7.5 %)** | **0** |

**Table 3: VEGFR-2 polymorphic genotypes expression versus hematological parameters**

| Variable | rs1531289 | | P-Value | rs1870377 | | P-Value | rs2305948 | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Wild(N. 7) Mean ± Std. Dev | Variants(N. 33) Mean ± Std. Dev | | Wild(N. 8) Mean ± Std. Dev | Variants(N. 32) Mean ± Std. Dev | | Wild(N. 39) Mean ± Std. Dev | Variants( N.1) Mean ± Std. Dev | |
| **WBC** | 6.414 ± 2.042 | 8.282 ± 3.244 | 0.153* NS | 7.835 ± 3.413 | 8.437 ± 2.03 | 0.520* NS | 7.967 ± 3.219 | 7.5±0.0 | 0.8* NS |
| **Hb** | 12.2 ±4.373 | 13.106 ± 1.822 | 0.372* NS | 12.765 ±2.479 | 13.687 ± 2.06 | 0.283* NS | 12.948 ±2.437 | 12.9±0.0 | 0.9* NS |
| **PLT** | 222 ± 102.277 | 232.454 ± 83.836 | 0.774* NS | 241 ± 92.835 | 189.125 ± 53.99 | **0.043** | 231.846± 87.531 | 183±0.0 | 0.5* NS |
| **BAS** | 0.3 ± 0.0025 | 0.703 ± 0.009 | **0.0001** | 0.634 ± 0.008 | 0.625 ± 0.01 | **0.023** | 0.633 ± 0.008 | 0.6±0.0 | 0.9* NS |
| **EO** | 2.97 ± 0.028 | 3.614 ± 0.033 | **0.0001** | 3.055 ± 0.018 | 5.287 ± 0.04 | **0.0001** | 3.537 ± 0.032 | 2.1±0.0 | 0.6* NS |

Significant association at * p\0.05 *Significant, NS=not significant. p value*

**Table 4: Correlation of genotypes of VEGFR-2 genotypes {rs1531289, rs1870377 and rs2305948 [T>C, T>A and C>T respectively ] } polymorphism versus clinical presenting symptoms of CML patients**

| Characteristics | SNP 1 rs1531289 n (%) | | | SNP2 rs1870377 n (%) | | | SNP 3 rs2305948 n (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| CML patients N=40 | TT | TC | CC | TT | TA | AA | CC | CT | TT |
| Splenomegaly present n=35(87.5%) | 6(17.1%) | 13(37.1%) | 16(45.8%) | 28(80%) | 7(20%) | 0 | 34(97.1%) | 1(2.9%) | 0 |
| Splenomegaly | 1(20%) | 2(40%) | 2(40%) | 4(80%) | 1(20%) | 0 | 5(100%) | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| absent N=5 (12.5%) | | | | | | | | | |
| P value | 0.9* NS | | | 1.0* NS | | | 0.7* NS | | |
| Weakness/fatigue n=33 (82.5%) | 5(15.1%) | 12(36.4%) | 16(48.5%) | 25(75.8%) | 8(24.2%) | 0 | 32(97%) | 1(3%) | 0 |
| No weakness/fatigue n=7 (17.5%) | 2(28.6%) | 3(42.8%) | 2(28.6%) | 7(100%) | 0 | 0 | 7(100%) | 0 | 0 |
| P value | 0.5* NS | | | 0.1* NS | | | 0.6* NS | | |
| Fever n=32(80%) | 6(18.8%) | 13(40.6%) | 13(40.6%) | 25(78.1%) | 7(21.9%) | 0 | 31(96.9%) | 1(3.1%) | 0 |
| No fever n=8(20%) | 1(12.5%) | 2(25%) | 5(62.5%) | 7(87.5%) | 1(12.5%) | 0 | 8(100%) | 0 | 0 |
| P value | 0.5* NS | | | 0.5* NS | | | 0.6* NS | | |
| Bone pain n=28(70%) | 5(17.9%) | 10(35.7%) | 13(46.4%) | 22(78.6%) | 6(21.4%) | 0 | 27(96.4%) | 1(3.6%) | 0 |
| No bone pain n=12(30%) | 2(16.6%) | 5(41.7%) | 5(41.7%) | 10(83.3%) | 2(16.7%) | 0 | 12(100%) | 0 | 0 |
| P value | 0.9* NS | | | 0.7* NS | | | 0.5* NS | | |
| abdominal pain &night sweating n=21(52.5%) | 5(23.8%) | 9(42.9%) | 7(33.3%) | 16(71.2%) | 5(23.8%) | 0 | 21(100%) | 0 | 0 |
| Without abdominal pain &night sweating n=19(47.5%) | 2(10.5%) | 6(31.6%) | 11(57.9%) | 16(84.2%) | 3(10.5%) | 0 | 18(94.7%) | 1(5.3%) | 0 |
| P value | 0.3* NS | | | 0.1* NS | | | 0.3* NS | | |
| Weight loss n=30(75%) | 3(10%) | 10(33.3%) | 17(56.7%) | 23(76.7%) | 7(23.3%) | 0 | 29(96.7%) | 1(3.3%) | 0 |
| No weight loss n=10(25%) | 4(40%) | 5(50%) | 1(10%) | 9(90%) | 1(10%) | 0 | 10(100%) | 0 | 0 |
| P value | **0.01* S** | | | 0.3* NS | | | 0.5* NS | | |
| Other symptoms n=2 (5%) | 0 | 1(50%) | 1(50%) | 2(100%) | 0 | 0 | 2(100%) | 0 | 0 |
| No other symptoms n=38(95%) | 7(18.5%) | 14(36.8%) | 17(44.7%) | 30(78.9%) | 8(21.1%) | 0 | 37(97.4%) | 1(2.6%) | 0 |
| P value | 0.79* NS | | | 0.4* NS | | | 0.8* NS | | |

Significant association at * p\0.05 *Significant, NS=not significant. p value*

**Table 5 : Correlation of genotypes of VEGFR-2 genotypes {rs1531289, rs1870377 and rs2305948 [T>C, T>A and C>T respectively ] } polymorphism versus clinical presenting symptoms of CML patients**

| **Characteristics** | **SNP 1 rs1531289 n(%)** | | | **SNP2 rs1870377 n(%)** | | | **SNP 3 rs2305948 n(%)** | | |
|---|---|---|---|---|---|---|---|---|---|
| **CML patients N=40** | **TT** | **TC** | **CC** | **TT** | **TA** | **AA** | **CC** | **CT** | **TT** |
| **WBC count** mean ±SD (7.9±3.17 x$10^3$) n=40 | | | | | | | | | |
| **Low n=2(5 %)** | **1(50%)** | **1(50%)** | **0** | **2(100%)** | **0** | **0** | **2(100%)** | **0** | **0** |
| **Normal n=33 (82.5%)** | **6(18.2%)** | **13(39.4%)** | **14(42.4%)** | **27(81.8%)** | **6(18.2%)** | **0** | **32(97%)** | **1(3%)** | **0** |
| **High n=5(12.5%)** | **0** | **1(20%)** | **4(80%)** | **3(60%)** | **2(40%)** | **0** | **5(100%)** | **0** | **0** |
| **P value** | **0.3* NS** | | | **0.5* NS** | | | **0.9* NS** | | |
| **Platelets count** mean±SD (230.6±86.7) n=40 | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Thrombocytopenia n=4(10 %) | **1(25% )** | **1(25%)** | **2(50%)** | **2(50%)** | **2(50% )** | **0** | **4(100 %)** | **0** | **0** |
| Normal platelets n=35(87.5%) | **6(17.1 %)** | **14(40% )** | **15(42.9 %)** | **29(82.9 %)** | **6(17.1 %)** | **0** | **34(97.1 %)** | **1(2.9 %)** | **0** |
| Thrombocytosis n=1(2.5%) | **0** | **0** | **1(100 %)** | **1(100 %)** | **0** | **0** | **1(100 %)** | **0** | **0** |
| **P value** | **0.8* NS** | | | **0.2* NS** | | | **0.9* NS** | | |
| **Hemoglobin** mean±SD **(**12.9±2.4**) n=40** | | | | | | | | | |
| **Normal n= 26(65%)** | **4(15.4 %)** | **8(30.8 %)** | **14(53.8 %)** | **20(76.9 %)** | **6(23.1 %)** | **0** | **25(96.2 %)** | **1(3.8 %)** | **0** |
| **Anemic n=14(35%)** | **3(21.4 %)** | **7(50%)** | **4(28.6 %)** | **12(85.7 %)** | **2(14.3 %)** | **0** | **14(100 %)** | **0** | **0** |
| **P value** | **0.2* NS** | | | **0.3* NS** | | | **0.6* NS** | | |
| **Liver function n=40** | | | | | | | | | |
| Normal n=38(95%) | **7(18.4 %)** | **14(36.8 %)** | **17(44.8 %)** | **30(78.9 %)** | **8(21.1 %)** | **0** | **37(97.4 %)** | **1(2.6 %)** | **0** |
| Abnormal n=2(5 %) | **0** | **1(50%)** | **1(50%)** | **2(100 %)** | **0** | **0** | **2(100 %)** | **0** | **0** |
| **P value** | **0.7* NS** | | | **0.4* NS** | | | **0.8* NS** | | |
| **Hematological response n=40** | | | | | | | | | |
| MHR(Major) n= 34(85%) | **7(20.6 %)** | **13(38..2 %)** | **14(41.2 %)** | **28(82.4 %)** | **6(17.6 %)** | **0** | **33(97.1 %)** | **1(2.9 %)** | **0** |
| mHR(Minor) n= 6(15%) | **0** | **2(33.3 %)** | **4(66.7 %)** | **4(66.7 %)** | **2(33.3 %)** | **0** | **6(100 %)** | **0** | **0** |
| **P value** | **0.3* NS** | | | **0.5* NS** | | | **0.8* NS** | | |
| Molecular Response n=40 | | | | | | | | | |
| Major MoR (MMoR) n= 22 (55%) | **4 (18.2 %)** | **11(50% )** | **7(31.8)** | **16(72.7 %)** | **6(27.3 %)** | **0** | **22(100 %)** | **0** | **0** |
| Poor MoR (pMoR) n=6(15% ) | **0** | **3(50%)** | **3(50%)** | **6(100 %)** | **0** | **0** | **6(100 %)** | **0** | **0** |
| Failure n= 12(30%) | **3(25% )** | **1(8.3%)** | **8(66.7 %)** | **10(83.3 %)** | **2(16.7 %)** | **0** | **11(91.7 %)** | **1(8.3 %)** | **0** |
| **P value** | **0.07* NS** | | | **0.3* NS** | | | **0.3* NS** | | |

Significant association at * p\0.05 *Significant, NS=not significant. p value*

So if we considered that 70% of patients showed molecular response(optimal &suboptimal ) to treatment and 30% who had failed to response depending on - BCR-ABL1 (IS) 0.1% or 3-log reduction in BCR-ABL1 mRNA from the standardized baseline. For the 1st SNP of VEGFR-2 genotype (rs1531289 T>C) most of patients who showed molecular response presented with variants TC+CC genotypes (93.3%+55.6%) and most of patients who failed to treatment presented with variant CC genotype (44.4%) with statistical significance as P value 0.04. While the 2nd SNP of VEGFR-2 genotype (rs1870377 T>A) most patients who had response to treatment presented with wild TT genotype (68.7%) while all patients who failed to treatment had presented only with wild TT genotype with no statistical significance as P value 1.0. In the 3rd SNP of VEGFR-2 genotype (rs2305948 C>T) all patients who had response to treatment presented with wild CC genotype (71.8%) while only one patient of those who failed to treatment had presented with variant CT genotype with no statistical significance as P value 0.3 see table 6

**Table 6: showed correlation between genotypes distribution of VEGFR-2 genotype**

| Variables | (rs1531289 T>C) | P value | (rs1870377 | P value | (rs2305948 | P value |
|---|---|---|---|---|---|---|

| | n | | | | T>A) | | | C>T) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TT | TC | CC | **0.04* S** | TT | TA | 1.0* NS | CC | CT | 0.3* NS |
| Molecular Response(n=28) | 4 | 14 | 10 | | 22 | 6 | | 18 | 0 | |
| Failure (n=12) | 3 | 1 | 8 | | 10 | 2 | | 11 | 1 | |
| SUM (40 patients) | 7 | 15 | 18 | | 32 | 8 | | | | |

## Discussion

The mean age of the CML patients included in our study was: 43.4, which is close to other two Iraqi studies which had been done by Kawa Muhamed amin Hasan , in which the means age were 43.3 years respectively (10) and close to that in the neighboring countries like Jordan (11) also close to that in the other countries like Brazil(12). Moreover, the highest distribution of patient in our study was found in the age group of 40 – 60 years, which revealed a younger age distribution among CML patients when compared to those in most western countries(8) .This lack of correspondence in age distribution can be explained by ethnic variances in addition to overall younger population demographics in our country.

Although its well documented that CML is slightly more common in male, the gender distribution of patients in our study also showed male predominance with male to female ratio of 1.2:1; However, disagreed with another Iraqi study done by Yaseen M. Taher, Ali M. Almothaffar1, Bassam Francis Matti, Alaa Fadhil Alwan that showed slightly higher CML incidence in female(10) This can be attributed to different gender distribution in Iraqi population, different sample size (Annual Statistical Report, 2017), and different inclusion or exclusion criteria in other studies.

As regards to clinical presentation, 95% of enrolled patients were symptomatic at time of presentation. Most of them showed symptoms related to hyper metabolic state and splenomegaly as weight loss, fever and abdominal pain. Only 5% were asymptomatic and incidentally had been discovered during routine check-up. However, data obtained from western countries reported that approximately 40% of CML cases were asymptomatic at diagnosis and detected during routine blood tests they discovered. This discrepancy in findings is probably related to a higher degree of public health awareness in developed countries, in addition to better medical care practices that favors early detection and diagnosis.

Our results revealed that :SNP1 of VEGFR-2 the frequency of the combined variant genotypes (TC+CC) was higher than wild TT genotype in both CML patients and in controls( 82.5% & 80% respectively)with no significance which agreed with another study (9) while SNP2 the majority of patients had presence of wild TT genotype 80% while in controls was 62.5% ,it was found that CML patients never showed the homozygous AA genotype while it was present in controls in about 12.5%which showed statistical significance ,disagreed with another study which showed that presence of variants genotypes of SNP2 was higher in control than patients Samyuktha Lakkireddy & D. H. Kim (2) &(9).SNP3 the frequency of wild CC genotype presented in most of patients (97.5%)and only one patient had presence of the variant heterozygous CT genotype which was slightly higher in controls (12.5%)was close to be statistically significant ,whole studied group didn't show the presence of variant homozygous TT which disagree with a study done by Dongxing Liu in China(13) these differences may be due to ethnical or geographical variations between our study and other compared ones.

Our results also revealed a higher prevalence of the genotypes combination of the 3 SNPs was (TC/CC + TT+CC) in CML patients with a percentage of (62.5%) while the lowest presence combination was (TT+TT+CT/TT)with a percentage of (2.5%).

The five heamatological parameters of 40 IM treated CML patients along with those of 40 healthy individuals were measured including [total white blood cell count, haemoglobin, platelet count, basophil and eosinophil percentage]. There was no statistically significant difference between the two study groups which can explained by the fact that our patient group included an already treated CML patient who had at least 12 months of IM treatment prior to collection of blood. The total WBC count of the CML patient group ranged from 3.2×103µl to 20.7×103µl with mean of 7.9±3.17 x103, while mean Hb value 12.9±2.4 g/dl ranging from 4.6 gm/dl to 18.1 gm/dl. Whereas platelet count of the patients was ranging from 31 to 589 ×103 µl with mean of (230.6±86.7 x109) µl. Mean basophil and eosinophil percentage were 0.63%± 0.008 and 3.53% ± 0.032 respectively. These findings were parallel to Suadian study by Farjah H. Algahtania, in which the mean of WBC count, Hb, platelet count, were: 7.7 ± 5.4, 13.3 ± 9.6, 298 ± 56.9,respectively(14) In addition another Iraqi study by Alauldeen showed quite similar results with mean WBC count of 6.7 ± 1.8 , and mean platelet count of 217.9 ± 47.4(15) This disproportional finding is probably related to differences in duration of treatment with IM, which ranged from one year to 19 year in our study. It also might be attributed to use of hydroxyurea in addition to IM during treatment course in some patients. Five heamatological parameters [total white blood cell count, haemoglobin, platelet count, basophil and eosinophil percentage]. presented in correlation to VEGFR-2 genotypes ; SNP1 rs1531289 showed highest percentage on the variants genotypes TC+CC; whereas in SNP2 rs1870377 showed highest presence in wild TT genotype ;same for SNP3 rs2305948 majority of symptoms presented in whom with wild CC genotype;those findings where disagreed for SNP2 in another study (2) That's probably due to ethnical or geographical differences of genotypes variations.

As regards to heamatological response of patients to treatment with correlation to VEGFR-2 polymorphisms, generally (85%)of patients showed CHR that is lesser than showed in 2 other studies had been done by D. H. Kim and Namrata Bhutani1, where CHR was found to be 96%(16)(17)

A study by Namrata Bhutani1, have showed a slightly higher percentage of patients (56.66%) achieving MMR with IM as frontline treatment after 12 months of treatment(17) Since CML patients who achieved MMR had a significantly lower risk of disease progression, Current ELN recommendations for the management of CML are essentially targeted toward a main goal of achieving MMR within 12 months(18)

we noticed that the prevalence of treatment failure was significantly higher in patients carrying the variant genotype CC for 1st SNP(rs1531289) at (44.4%) when compared to wild TT genotype which not much different (42.9%) not indicating a risk of treatment failure among patients exhibiting the variant genotype. Regarding 2nd SNP(rs1870377) difference was not significance between wild and variant genotypes TT& TA as both presence was about (31.3% & 25%) respectively.3rd SNP (rs2305948)also no significant variation as majority of patient with failure presented with wild CC genotype (30% )and only one patient with variant CT genotype . So as 70% of patients showed molecular response (optimal and non-optimal response) when they had compared with those who had failure in association with VEGFR-2 genotypes ;1st SNP(rs1531289) showed significant association of response associated with variant genotypes TC+CC at(93.3%+55.6%) with significant p value =0.04,and this is agreed by D. H. Kim which had been done for 228patients (16) whereas the 2nd SNP(rs1870377) majority of responding patients showed the presence of wild TT

genotype (68.7%) and all of whom presented with variant genotype TA without significance if compared to whom had failure which disagreed with both D. H. Kim(which had been done for 228patients ) & Samyuktha Lakkireddy(which had been done for 208patients ) (16)(2) which is mostly due to different presence of genotypes in our patients who never showed presence of variant AA and majority had wild TT while in the mentioned studies majority showed presence of AA variant and majority associated response to treatment with this variant .3rd SNP(rs2305948 C>T) had showed only 1 patient with variant CT genotypes who had failed to respond to treatment ,all remaining had wild CC genotype with no statistical significance which agreed with D. H. Kim(16) disagreed with Malaysian study by Siti Mariam Ismail(19)

In taking combination of 1st SNP and 2nd SNP (3rd SNP not taken as only patient presented with variant CT and had failure) we found that majority of patients who had optimal response (31.8%) presented with both of variant TC of 1st SNP +wild TT of 2nd SNP, whereas the majority who had non-optimal response (warning &failure) at 50% presented with variant CC of 1st SNP +wild TT of 2nd SNP .

We could not compare our data with other reports as up to our knowledge there are no other reports available on aforementioned notion.

## Conclusion

Our data showed that VEGFR-2 polymorphisms(specifically SNP2 rs1870377 absence of variant AA) might be a potential risk factor for development of CML. The present study revealed that polymorphic variation in VEGFR-2 gene might contribute to heterogeneous responses to Imatinib treatment among CML patients favoring poor response and inferior treatment outcome. The combined variant TC+CC genotypes of SNP1 rs1531289 was found in more three-quarters of the patients, while the wild genotype TT was exhibited in less than one-quarter of patients indicating a higher frequency of the mutant genotype among studied Iraqi CML patients; the wild TT genotype of SNP2 rs1870377 presented in eighty percent of patients while remaining only variant TA with no presence of variant AA , indicating a higher frequency of the wild genotype among studied Iraqi CML patients; only one patient had variant CT genotype of SNP3 rs2305948 whereas remaining others had wild CC genotype.

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