TGFB 2 RS79375991 GENE POLYMORPHISM IN IRAQI CHRONIC MYELOID LEUKEMIA PATIENTS INFECTIOUS WITH HUMAN HERPES VIRUS-8

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Abstract

Background: HHV8 + primary effusion lymphoma (PEL), EB-LBCL has no known association with HIV or HHV8 infection. Plural or pericardial effusions complicate BCR::ABL1-positive (CML) in around one-third of patients. According to recent findings, the maintenance of cancer stem cells is influenced by transforming growth factor-β (TGF-β) in both positive and negative ways, depending on the kind of cell and the setting. Currently, little is understood about the relationship between Bcr-Abl expression and TGFbeta and downstream Smad transcription factors and CML cell growth.

Objective: cross-sectional case-control study was aimed to determine the association between TGFβ 2 rs79375991 polymorphism; BCR protein level; biochemical parameters and the percentage of HHV-8 in patients with CML.

Patients and methods: A cross-sectional case-control study included one tow hundred (200) blood specimens enrolled in the current research, including 120 CML (20 patients diagnosed as newly diagnosed; 100 patients treated CML 65 out of 100 patients on imatinib while 35 were treated with other tyrosine kinase inhibitors (Nilotinib and Bosutinib); as well as eighty (80) blood specimens collected from persons as apparently healthy control group were aged 16 to 65 years. Conventional PCR was chosen for the DNA- HHV-8 detection as well as TGFβ 2 rs79375991 gene polymorphism by sequencing. Lastly, ELISA used serum to detect the BCR protein level among studied groups.

Results: The current study included 20 patients diagnosed as newly diagnosed CML with mean age 35±12 years and 100 patients treated CML with mean age 51±11.89 years. While, mean age of apparently healthy-looking persons 44±13.7 years. There are statistically differences between new diagnosis CML group and controls group in serum level of (Urea, Creatinine, ALT, AST and LDH), p-value (0.004, 0.014, <0.001, <0.001 <0.001), respectively. The positive result of HHV-8 according to PCR shows 49.3 % (33 out of 67 cases) as positive, while 50.7% (34 out of 67 cases) as negative,. While, not found positive of HHV-8 infection in all examined apparently healthy specimens. HHV-8-PCR detection results from patients with various forms of CML were 3.3%, 16.7%, and 25.8% of new diagnosis CML, patients respond to treatments and relapse groups, respectively, showed positive PCR results for HHV-8 detection. The results of TGFβ 2 rs79375991 gene polymorphism showed difference in frequency of genotype distribution of the polymorphism between patients and controls groups was statistically significant. According to BCR protein level, there are highly statistically significant differences between new diagnosis group and response to treatment group, while non-significant differences between respond to treatment and relapse group (p-value: 0.64).

Conclusion: The significant correlation between the gene polymorphism of TGFβ 2 rs79375991; BCR protein with HHV-8 infection could indicate highly important role of these molecular factors in patients suffering from CML.

Keyword: HHV-8; TGFβ 2 rs79375991; sequencing; BCR protein level; ELISA; Chronic myeloid Leukemia.

Introduction

Clinically, CML presents with a triphasic course. The chronic phase (CP), which is the initial stage of CML diagnosis, accounts for more than 90% of cases. At this point, regular blood tests are used to diagnose the condition in up to 50% of people who are asymptomatic. Common indicators during diagnosis include fatigue, anemia, thrombocytosis, bleeding, splenomegaly, stomach fullness, leucocytosis, and purpura (1).

ABL1 TKIs are a successful treatment option for people with CP CML. Imatinib mesylate was the first to be launched; in the critical IRIS research, it demonstrated a high rate of response and an acceptable side effect profile when evaluated as initial therapy for newly diagnosed CP CML. Nevertheless, some patients may experience a relapse following an initial response to imatinib, while others may not respond to the medication at all (primary resistance). Only a small percentage of instances have resistance development occurring simultaneously with, or soon after, the progression to BP. The primary cause behind the creation of second-generation TKIs (2G-TKIs), which were initially approved as frontline therapy for patients who were resistant to imatinib, was the issue of resistance. There are currently three 2G-TKIs on the market: bosutinib, dasatinib, and nilotinib. Randomized clinical trials have shown that these drugs have a considerable clinical advantage over imatinib (2).

A third-generation tyrosine kinase inhibitor (TKI), known as ponatinib, has been developed to address the issue of TKI resistance in patients with a specific mutation (T315I) in the BCR\ABL1 gene. This mutation renders first-generation TKIs

like imatinib and second-generation TKIs ineffective. In cases where no other TKI treatment is suitable, ponatinib may be considered as an alternative option (3).

HHV-8 may also be linked to multicentric Castleman's disease and primary effusion lymphoma, two other B-cell lymphomas. Numerous genes that can lead to immunological problems and neoplastic transformation are present in the HHV-8 genome (4). The molecular effects of HHV8 gene expression on B cells are being studied using cell lines that were generated from HHV8-infected Primary Effusion Lymphoma (PEL) tissues (5).

Higher seropositive rates for HHV-8 immunoglobulin G antibody were seen in patients with lymphoma, leukemia, autoimmune cytopenias, and myeloproliferative diseases in Taiwan (6). The transforming growth factor β-Smad pathway is a multifunctional molecular system that controls various cellular processes, including angiogenesis, differentiation, proliferation, apoptosis, metamorphosis, and extracellular matrix remodeling. Pleiotropic cytokine TGFβ1 attaches itself to the receptor TGFβR2, which then draws TGFβR1 to itself. The receptor-Smads (R-Smads), Smad2 and Smad3, are phosphorylated by the activated TGFβR2/TGFβR1 complex. Target gene expression occurs when R-Smads phosphorylate, form a higher order complex with SMAD4 and common-Smad (Co-Smad), and translocate to the nucleus. Smad6 and Smad7, which are inhibitory Smads (I-Smads), stop TGFβR1 from phosphorylating R-Smads. It is shown that Smad6 functions in the BMP-Smad pathway, while SMAD7 contributes to the TGFβ-Smad pathway (7).

This study was aimed to determine the association between *TGFβ* 2 rs79375991 polymorphism; BCR protein level; biochemical parameters and the percentage of HHV-8 in patients with CML.

Material and Methods

This study is designed as a cross-sectional case-control study.

A. Study groups

The studied CML blood was obtained from those patients aged from 18 to 68 years. Blood from each study group of Patients with CML should be enrolled, that classified into: -

1. Group of 120 blood samples from Patients with CML including 20 patients diagnosed as newly diagnosed CML and 100 patients treated with CML 65 out of 100 patients on imatinib while 35 were treated with other tyrosine kinase inhibitors (Nilotinib and Bosutinib); 69 out of 100 treated patients responded to treatment while 31 did not respond.
2. Blood from 80 apparently healthy persons as a control group were aged 16 to 65 years.

B. Sample Collection

Aseptic venous blood collection of five milliliters was performed on each patient utilizing gel tubes for gated blood serum and EDTA tubes for buffy coats, respectively. In patients with CML and AHC groups, viral genetic identification of HHV-8 was observed.

-Human total DNA in order to detect *TGFβ 2* gene Polymorphism in patients with CML and AHC.

- Serology analysis of *biochemical parameters as well as BCR* protein level in patients with CML and AHC groups.

C.PCR analysis for HHV-8:

In compliance with the guidelines supplied by the manufacturer (Intron / Korea), the viral genome was isolated from whole blood samples utilizing a blood and tissue kit. Prior to being used, the extracted DNA/RNA was stored at a temperature of -20°C. For the aim of performing PCR analysis on HHV-8 DNA, a total of 500 nanograms of DNA were taken from freshly frozen blood specimens.

K1-HHV-8 (IF): CAGTCTGGCGGTTTGCTTTC;
K1-HHV-8 (IR): GTAGGTGCGGTTGCAAATGT

D. Genotyping of *TGFβ* 2 rs79375991:

DNA was isolated from blood samples using the DNeasy blood and tissue kit, following the guidelines provided by the manufacturer (Intron / Korea). The DNA that was obtained was subsequently preserved at a temperature of -20°C until it was ready for utilization. The detection of *TGFβ* 2 rs79375991gene polymorphism was accomplished through the utilization of polymerase chain reaction, and PCR products were subsequently employed to investigate the presence of genetic polymorphisms within this gene.

TGFβ 2 rs79375991 F: AAGTATTCCAGATTGCCTTTCTGTC
TGFβ 2 rs79375991 R:CACCAGCTGAATGAGCTCCTAA

E. Measurement of concentration and purity of extracted DNA

By (Nanodrop) at the absorbance at 260 nm and 280 nm, respectively, can determine the DNA quantity and purity.

F. The amplification of PCR

A standard heat cycler (Biometra, Germany) was used for PCR amplification. Two microliters of template DNA were added to PCR master mix tubes, and then one microliter of forward and reverse primers were added to the same tubes. The PCR-premixed tubes were filled with a volume of 25 µl of distilled water, as indicated in Table (1).

Table (1): Recommended volumes and concentrations for applying PCR into AccuPower® PCR tubes.

No.	Contents of PCR Reaction Mixture	Volume/ µl
1	Master mix	10 µl
2	Forward primers (each one of snps)	4 µl
3	Reverse primers (each one of snps)	4 µl
4	Template DNA	2 µl
5	Nuclease free water	5 µl
	Total	25 µl

G. The Conditions of Thermal Cycles:

The required cycling conditions were manually configured before the master mix solutions were put into a Biometra-Germany thermal cycler that had been preheated to 94°C. The amplification of the target regions of HHV-8 and *TGFβ* 2 rs79375991 polymorphism was performed using particular primers, according to mentioned conditions in Table (2).

Table (2): The study conditions both for amplification of HHV-8 and *TGFβ 2*genes

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
HHV-8	95C^0 /4 min	95C^0 / 1 min	59 C^0 /45 Sec	72 C^0 / 2 min	72 C^0 /5min	40

TGFβ 2	95C^0 /5 min	95C^0 / 1 min	64 C^0 /45 sec	72 C^0 / 2min	72 C^0 /5min	40

H. Sequencing of PCR Products

The phrase "DNA sequencing" refers to techniques utilized for ascertaining the sequence of nucleotide bases, adenine, guanine, cytosine, and thymine, within a DNA molecule. In the early 1970s, Academic researchers employed laboratory techniques utilizing 2-dimensional chromatography to successfully get the initial DNA sequence. The advancement of dye-based sequencing techniques with automated analysis has facilitated the simplification and acceleration of DNA sequencing processes. The understanding of DNA sequences pertaining to genes and other components of an organism's genome has become essential in fundamental scientific investigations exploring biological mechanisms, as well as in practical domains like diagnostic or forensic research.

J. Statistical Analysis:

This study used the Chi-square test to determine the statistical analytic significance between the studied variables. All statistical analyses were performed using the Version-26 SPSS program, and a significance level of p 0.05 was deemed present.

Results

I. Distribution of study CML group according to their age stratum and sex:

The highest male frequency (18) was found in the 51–60 year age group; Also the highest female frequency (16) was found in the 51–60 year age group Table (1).

Table 1: Patients with CML according to their age and sex.

Age	Sex		Total	
	Male	Female		
	No.	No.	No.	%
18-30	8	6	14	11.7
31-40	12	11	23	19.2
41-50	16	14	30	25
51-60	18	16	34	28.3
61-68	10	9	19	15.8
Total CML Patients	64	56	120	100

II. Comparison among study groups according to biochemical parameters

Based on the assessment of biochemical parameters including Urea, creatinine, ALT, AST, and LDH serum levels in patients with new diagnosis CML, those responding to treatments, and the relapse group, it was determined that there are no statistically significant differences except for the serum levels of creatinine between the new diagnosis CML group and the relapse group, where a p-value of 0.01 was observed in Table 2.

Table 2: Comparison between new diagnosis CML, treated patients respond to treatment and relapse according to biochemical parameters.

Parameters	New diagnosis (n=20)	Treated (n=100)		p-value
		Response to treatment (n=69)	Relapse (n=31)	
Urea±SD mg/dl	31.5±7.8	33.3±18.2	30.3±12.1	*0.615 **0.69 ***0.399
Creatinine±SD mg/dl	0.71±0.51	1.0±0.73	0.9±0.33	*0.059 **0.01 ***0.329
ALT ±SD IU/L	29.5±10.9	30.5±10.79	28.3±12.3	*0.73 **742 ***418
AST ±SD IU/L	33.6±12.6	32.2±12.7	33.2±15.6	*0.671 **931 ***733
LDH ±SD IU/L	478±201	485±278	438±20.4	*0.909 **0.49 ***0.39
*** Comparison between New diagnosis CML and response to treatment patients.** **** Comparison between New diagnosis CML patients and relapse patients.** ***** response to treatment patients and relapse patients.**				

III. Detection of (HHV-8) DNA by PCR

The positive result of HHV-8 according to PCR shows 49.3 % (33 out of 67 cases) as positive, while 50.7% (34 out of 67 cases) as negative, as shown in Table (3) as well as Figure (1). While, not found positive of HHV-8 infection in all examined apparently healthy specimens. Statistically significant differences (p = 0.04) among patients group.

Table 3. Percentage of HHV-8 positive signals in patients with CML by using PCR technique.

Total Viral genome	Patients With CML No. (%)	AHC No. (%)	Chi-Square (P-value)
HHV-8 Positive	33 (48.4%)	0 (0.00%)	***P=0.001 H.Sig (P>0.05)***
HHV-8 Negative	34(51.6%)	3(100%)	

Total	67(100%)	3 (100%)	

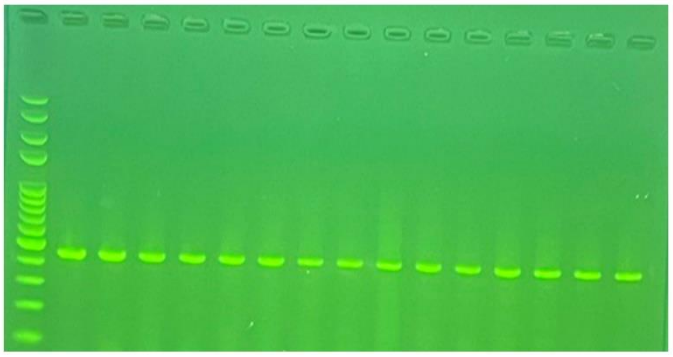

Figure 1: The electrophoresis pattern of HHV-8 DNA (592bp) detection in blood sample of CML patients. Lanes (47,4,31 and others) refers to HHV-8 DNA specimens; Electrophoresis conditions, 1.5% agarose,85 V, for 1h.

IV. Distribution of HHV-8 Infection according to the new diagnosis CML, patients respond to treatments and relapse groups:

Table (4) shows positive HHV-8-PCR detection results from patients with various forms of CML, were 3.3%, 16.7%, and 25.8% of **new diagnosis CML, patients respond to treatments and relapse groups**, respectively, showed positive PCR results for HHV-8 detection. The statistical analysis of different types of CML with HHV-8 positive showed significant differences ($p<0.05$) (Table 4).

Table 4. Distribution of HHV-8 Infection according to the new diagnosis CML, patients respond to treatments and relapse groups

CML Patients	No. of cases	HHV-8		p-value
		Positive	%	
New diagnosis	20	4	3.3%	
Response to treatment	69	20	16.7%	
Relapse	31	9	25.8%	
Total	120	33	27.5%	

V. Genotyping of *TGFβ* 2 rs79375991 Polymorphism

The results of *TGFβ* 2 rs79375991 gene polymorphism showed that DNA polymorphism distribution were DNA polymorphism distributions according to A\A; A\T; and A\G were 42%; 56% and 2%; respectively in patients with CML and 66.7%; 26.7%; and 6.6%; respectively in AHC group. The difference in frequency of genotype distribution of the polymorphism between patients and controls groups was statistically significant Table (5).

Table 5: Comparison between patient with and without CML based on percentages of *TGFβ* 2 rs79375991 expressed gene polymorphism.

Conformational Polymorphism of *TGFβ* 2 rs79375991 gene	Type of Mutation	Study group		OR Patients		*P value*	95% C.I for OR Patients	
		CML NO. (%)	AHC NO. (%)	0.65 (0.50-1.99)		0.04	*Lower*	Upper
A\A	Transition	21 (42%)	20 (66.7%)	1.08 (0.85-2.90)		0.03	1.0	**2**
A\T	Transversion	28 (56%)	8 (26.7%)	0.75 (0.60-1.70)		0.04	***1.2***	**1.8**
A\G	Transition	1 (2%)	2 (6.6%)				***1.4***	**1.7**
Allele Frequency								
A		**95**	**67**			0.03		
T		**5**	**33**					

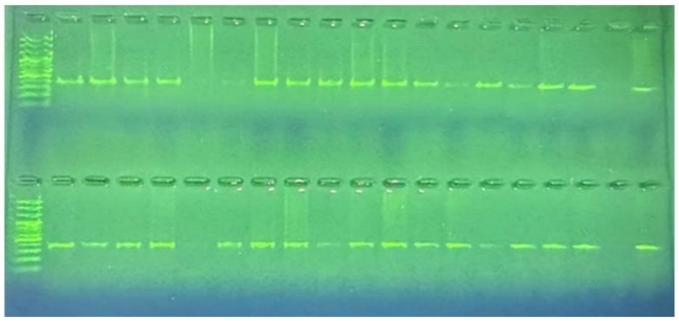

Figure 2: The SNP's novelty checking of *TGFβ* 2 rs79375991 genetic single nucleotides polymorphisms using the dbSNP server. The position of the targeted sequences was found in the negative strand. 1.5% agarose gel electrophoresis, TBE 1X , at voltage 75 volt for 45 min.

Samples were submitted in NCBI, and the accession number of nucleotide sequences of TGFβ 2 rs79375991 as new recording:

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VI. Comparison between new diagnosis CML, treated patients respond to treatment and relapse in BCR proteins level.

According to BCR protein level, there are highly statistically significant differences between new diagnosis group and response to treatment group (p-value: <0.001) also between new diagnosis group and relapse patients group (p-value: <0.001) while non-significant differences between respond to treatment and relapse group (p-value: 0.64) Table (6).

Table 6: Comparison between new diagnosis CML, treated patients respond to treatment and relapse in BCR proteins level.

Parameters	New diagnosis (n=20)	Treated (n=100)		p-value
		Response to treatment (n=69)	Relapse (n=31)	
BCR ±SD protein level	5.3±2.5	8.9±2.9	8.63±2.2	*<0.001 **<0.001 ***0.64

*** Comparison between New diagnosis CML and response to treatment patients.**
**** Comparison between New diagnosis CML patients and relapse patients.**
***** response to treatment patients and relapse patients.**

VII. Correlation among study parameters

The correlation coefficient among study parameters shows: Urea and Creatinine have a positive correlation (r = 0.222, p = 0.015), indicating a weak association between them. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) show a moderate positive correlation (r = 0.680, p < 0.001). This suggests that higher levels of ALT are associated with higher levels of AST. Lactate dehydrogenase (LDH) and BCR protein have a negative correlation (r = -0.178, p = 0.052). However, the association is weak and not statistically significant.

BCR protein after treatment exhibit a strong positive correlation (r = 0.469, p < 0.001), suggesting that the ratio at diagnosis is related to the ratio after treatment. Other non-significant relationships are shown in table (7) below.

Table 7: The correlation coefficient among study parameters

Parameters		Urea	Creatinine	ALT	AST	LDH	BCR protein
Urea	r	1	0.222*	0.036	-0.097	0.020	-0.178
	p		0.015	0.697	0.290	0.832	0.052
Creatinine	r	0.222*	1	0.069	0.044	0.279**	0.158
	p	0.015		0.454	0.631	0.002	0.085
ALT	r	0.036	0.069	1	0.680**	0.130	-0.085
	p	0.697	0.454		<0.001	0.156	0.357
AST	r	-0.097	0.044	0.680**	1	0.163	-0.036
	p	0.290	0.631	<0.001		0.075	0.693
LDH	r	0.020	0.279**	0.130	0.163	1	0.007
	p	0.832	0.002	0.156	0.075		0.942
BCR protein	r	-0.178	0.158	-0.085	-0.036	0.007	1
	p	0.052	0.085	0.357	0.693	0.942	

Discussion

According to the Surveillance, Epidemiology, and End Results (SEER) research, there was a comparable improvement in survival rates across all age cohorts. Comprehensive research conducted in Sweden, which included all patients treated in the nation from 1958 to 2008, revealed that the overall survival rates had shown improvement, except for those aged 79 years and older. The registry data did not provide sufficient information to analyse the factors contributing to the age-related disparity. It is conceivable to posit that the interplay between competing causes of mortality and suboptimal healthcare provision attributable to advanced age may have had a significant influence. The findings of the Italian research, which included 31 medical centres, indicate that individuals aged 75 and above seemed to get comparable benefits from TKI therapy as their younger counterparts (8).

The current results was found the highest male frequency (18) was found in the 51–60 year age group; Also the highest female frequency (16) was found in the 51–60 year age group. Lokesh and colleagues ,(9) demonstrated comparable patterns that have been documented in existing research pertaining to first generation TKI. The available Indian literature reports that a significant proportion of the patient population consisted of individuals under the age of 60.

Data from the SIMPLICITY study (10) indicate that women are more likely than men to switch tyrosine kinase inhibitors (TKIs) throughout this era of treatment.

The primary focus of this research was on the therapy and its impact on the levels of urea, creatinine, ALT, AST and LDH. There were no significant differences seen in the levels of treated CML patients.

The findings of this study are consistent with those of Jacob and Sheba (11) who postulated that mild to moderate hepatomegaly represents the initial presentation for about 50% of individuals diagnosed with chronic myeloid leukemia (CML), with no changes in liver function.

Moreover, the higher enzyme levels seen in CML patients align with earlier findings by Murakami et al. (12), which proposed that liver enlargement and elevated serum alkaline phosphatase (ALP) levels could be caused by immature cells infiltrating the liver sinusoids during the blastic crisis phase of CML. Drug-induced damage to these organs may be shown by elevated liver function tests (e.g., alanine transaminase, ALT, and aspartate transaminase, AST) and renal function markers (13).

Pleural effusion (PE) (28-33%) is a common side effect of dasatinib, a tyrosine kinase inhibitor (TKI) used to treat chronic myeloid leukemia (CML). PE is caused by exudates that are predominantly lymphocytes (14). Six HHV8-negative EBL cases that occurred while receiving dasatinib have been documented to date (15; 16).

Nevertheless, to the best of our knowledge, our investigation into HHV-8 in CML patients is the first study conducted in the Mid-Euphrates Governorates of Iraq intended to examine the relationship between HHV-8 and CML through the use of the PCR method.

The current study was found 49.3 % (33 out of 67 cases) as positive, while 50.7% (34 out of 67 cases) as negative, as shown in Table (4-6) as well as Figure (1). These findings conflict with those of Chao-Hsien et al. (18), who found HHV-8 DNA in PBMCs in 8.94% (11/123) of the relatives' cases and 10.29% (14/136) of the leukemia cases.

There was no discernible difference in the frequency of HHV-8 DNA in PBMCs between these two groups (P = 7.31). HHV-8

DNA was found in PBMCs of 14 (10.29%) of the Taiwanese leukemia patients overall; this is greater than the percentage of plasma PCR-positive HHV-8 cases (5.8%, 29/501) among patients with malignant lymphoma in Spain (17).

In contrast, Christe et al.,(15), discovered that HHV8 (LANA-1) was negative. Patients with CML were found to have rearranged IG genes in a monoclonal manner. After the disease was brought under control with drainage, HHV8-negative EBL recurred rather quickly. This could have been caused by the patient's weakened immune system as a result of the CML.

The percentage of HHV-8 DNA in PBMCs of leukemia patients (10.29%, 14/136) and relatives' cases (8.94%, 11/123) discovered by Lin et al. (20) was comparable to our earlier findings that 8.9% of Taiwanese patients with HIV were seropositive for HHV-8 DNA in plasma.

Both Vey et al. (18) in France and Tattevin et al. (22) report a patient with multiple myeloma and chronic myelogenous leukemia who is infected with HHV-8.

Since it encodes a large number of oncoproteins, or cell signaling proteins, human herpesvirus type 8 is unique among herpesviruses. A substantial amount of research has connected HHV-8 to at least three cancers: primary effusion lymphomas, multicentric Castleman's disease, and Kaposi's sarcoma. The first infection of susceptible hosts is most likely the starting point for HHV-8 infections. After this, latency is established (mostly in B cells), from which periodic reactivation of replication is conceivable. Comprehending the frequency of HHV-8 in various populations and patient groups is essential as it can aid in devising preventive methods to lower the rates of viral transmission from infected individuals (19).

Viruses are seldom complete carcinogenesis and are essential but not sufficient factors even in those with viral carcinogenesis such as HPV, EBV, HTLV-1and HHV-8 related carcinogenesis (20).

The most significant of these are the wide range of illnesses that fall under the umbrella of CML, each of which shows a unique rate of association with EBV and HHV-8. Additionally, the prevalence of these illnesses varies across different geographic areas, a phenomenon that can be attributed to both genetic and environmental etiologic factors. The degree to which various forms of CML compromise immunity, particularly those that result in faulty B-cell control, was also a significant determinant. Furthermore, only a small number of diseases have been examined in some of these research, and the relevance of these findings and post-transplant lymphoproliferative diseases is undoubtedly influenced by the number of cases in the other cohorts studies (21).

The BCR-ABL gene is used to diagnose CML, and imatinibmesylate (TKI) is the first-line treatment for the condition. The etiology of IM resistance in CML is changes in BCR-ABL dependent and independent pathways (22). One of the main BCR-ABL independent routes, TGFβ-Smad, has been thoroughly investigated in both normal and aberrant hematopoiesis. Although changes in this route have been linked to myeloid and lymphocytic leukemias, its significance in chronic myeloid leukemia is still unclear (23). We are the first to report the *TGFβ* 2 rs79375991 mutation in a group of Iraqi patients with CML to the best of our knowledge.

the results of *TGFβ* 2 rs79375991 gene polymorphism showed that DNA polymorphism distribution were DNA polymorphism distributions according to A\A; A\T; and A\G were 42%; 56% and 2%; respectively in patients with CML and 66.7%; 26.7%; and 6.6%; respectively in AHC group. These findings are in agreement with those of Shokeen et al. (7), who discovered that the most significant genetic variants were g.46474746C>T in SMAD7, c.69A>G in TGFβ1, and c.1024+24G>A in TGFβR1. These variants were present in 10/20, 8/20, and 7/20 individuals, respectively.

It is well established that TGFβ-Smad signaling makes CML cells more hyperresponsive, which improves response via BCR-ABL inhibition. While the route prevents the activation of AKT, a downstream element of the BCR-ABL pathway, it also releases FOXO's inhibitory sequestration, which encourages CML stem cells to remain quiescent and eventually leads to TKI resistance (23). The goal of the current study was to investigate possible direct correlations between changes in TGFβ 2 rs79375991SNPs and CML patients. The homozygous genotype CC at codon 10 or at codon 25 is highly related with poor production, whereas the homozygous genotype GG at codon 25 is associated with high production, according to Amirzargar et al. (24).

The substantial excess of GG homozygotes seen in our case may be explained if the "high producer" GG genotype had a genuine selective benefit. An intermediate production is shown when the GG and CC genotypes coexist, suggesting that the two alleles interact or have a dosage impact. According to BCR protein level, there are highly statistically significant differences between new diagnosis group and response to the treatment group (p-value: <0.001) also between new diagnosis group and the relapse patients group (p-value: <0.001) while non-significant differences between the respond to the treatment and relapse group (p-value: 0.64).

Jianchao *et al*.,(22) find that the reduction level of BCR–ABL can be easily adjusted by substituting different amino acids. Furthermore, a single PEG linker is found to achieve the best proteolytic effect.

The levels of the BCR protein in newly diagnosed patients exhibit notable variations compared to both treated patients and a control group. This finding aligns with previous research indicating that the breakpoint cluster region (Bcr) protein is abundant in neurons and plays a role in neural activities in healthy individuals. Furthermore, this protein is involved in various cellular processes, including the regulation of cell cycle, differentiation, and morphogenesis (23).

The observed differences in BCR protein levels may be attributed to the translocation event between chromosome 9 and chromosome 22, which results in the formation of the fusion gene BCR\ABL. The Bcr\Abl fusion protein exhibits a persistent activation of Abl tyrosine kinase activity, leading to the unregulated proliferation seen in chronic myeloid leukaemia (24).

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