ORIGINAL ARTICLE

# Immunogenetic Analysis of CTLA4 Polymorphisms, Serum Levels, and Hepatitis B Virus Coinfection in Iraqi Patients with Chronic Myeloid Leukemia

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***Key words:***
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## ABSTRACT

***Background:*** *Chronic myeloid leukemia (CML) is driven by the BCR-ABL1 oncogene and characterized by dysregulated myeloid cell proliferation. Cytotoxic T-lymphocyte antigen-4 (CTLA4), an immune checkpoint protein, modulates T cell activity and may influence cancer progression. Tyrosine kinase inhibitors (TKIs) are effective in treating CML.* ***Objectives****: This study aimed to assess CTLA4 gene polymorphisms, serum CTLA4 levels, and HBV infection as potential risk factors in patients with chronic myeloid leukemia (CML).* ***Methodology:*** *A case-control study included 220 participants: 120 CML patients (20 newly diagnosed and 100 receiving treatment) and 100 age/sex-matched healthy controls. DNA was extracted from the blood samples, and CTLA4 polymorphisms (rs231775) were analyzed by PCR and Sanger sequencing. Serum CTLA4 levels were measured using ELISA. HBV DNA was detected by PCR. Statistical analyses included the chi-square test, Hardy-Weinberg equilibrium, and logistic regression.* ***Results:*** *CTLA4 genotype distributions differed significantly between the groups: CML patients exhibited 20% AA, 30% AT, and 50% TT, whereas controls showed 60% AA, 0% AT, and 40% TT. The AT genotype was exclusive to all patients (p=0.06, OR=0.05). Serum CTLA4 levels were higher in patients (32.47 ± 14.2 pg/mL) than controls (23.83 ± 9.03 pg/mL), though statistically significant (p=0.0387). HBV DNA was not detected in any sample. Biochemical parameters (urea, ALT, AST, and LDH) showed no significant differences, except for creatinine (p=0.01).* ***Conclusion:*** *CTLA4 polymorphisms, particularly the AT genotype, may confer a protective effect against CML development in Iraqi patients Elevated serum CTLA4 levels suggest immune dysregulation in CML; however, no association with HBV infection was observed. These findings do not support routine HBV screening in this cohort.*

## INTRODUCTION

Hematopoietic stem cells (HSCs) are associated with chronic myeloid leukemia (CML). Cancer starts in bone marrow blood-producing cells and spreads via the bloodstream. It is also known as chronic myelogenous leukemia. It is a myeloproliferative neoplasm, with 1–2 occurrences per 100,000 individuals. It is rare and accounts for 15% of newly diagnosed adult leukemia cases[1]. Philadelphia chromosome (Ph) is a defining feature of myeloproliferative neoplasms (MPN).

Bone marrow cells translocate sections of chromosomes 9 and 22. This translocation occurs during the cell division. In contrast, chromosomes 9 and 22 were connected to each other. Thus, Chromosome 9 lengthens, whereas chromosome 22 shortens. The Philadelphia chromosome is chromosome 22, which produces a BCR-ABL1 fusion gene due to reciprocal translocation [t(9;22)]. Most of the patients were [2].

The Philadelphia chromosome is the main risk factor for CML, along with sex, age, radiation exposure, and chemical exposure [3]. Activated T cells include CTLA-4, an inhibitory checkpoint [4]. It is usually observed on activated T cells [5], also known as CD152 [6]. An extracellular immunoglobulin V-like domain that binds B7 ligands (CD80/CD86) with a higher affinity than CD28, a transmembrane region, and a cytoplasmic tail with a conserved YVKM motif that recruits inhibitory signaling phosphatases such as SHP-2 and PP2A make up CTLA-4 [7,8]. acts as a negative regulator of T-cell-mediated immune responses by directly sending a negative signal to effector T cells and interacting with regulatory T cells (Tregs) [9]. CTLA-4 is polymorphic, with over 100 SNP. Many studies have found that these SNPs increase the incidence of several malignancies, including colon cancer[10], oral squamous cell carcinoma, and cervical, lung, and breast cancer [11]. Hepatitis B Virus (HBV) infection is the leading cause of death in the Asia-Pacific region and is connected to the liver. Therefore, it is an important public health concern.

HBV is a small, enclosed DNA virus within the family Hepadnaviridae [12].

According to a number of studies, cytotoxic chemotherapy and immunosuppressive drugs have the potential to hasten the reactivation of chronic HBV infection, putting patients undergoing these treatments at an increased risk for adverse effects [13,14].

According to a comprehensive analysis, individuals who are undergoing cytotoxic therapy for solid organ malignancies have a likelihood of HBV reactivation that is greater than ten percent, according to a comprehensive analysis. A great amount of attention has been drawn to rituximab because of the significant risk of hepatitis B reactivation that it poses in patients who are only positive for hepatitis B core antibody (HBcAb) at the beginning of their treatment [15–17]. The current study aimed to determine the association of CTLA4 with CML in patients infected with HBV, as well as to detect the role of HBV in CML.

## METHODOLOGY

**Study Population:**

Two hundred and twinty (220) blood samples were obtained from CML patients (120) (20 newly diagnosed and 100 receiving treatment) collected from the Iraqi Hematology Center as well as general hospitals in Middle Euphrates– Iraq, Also 100 from AHC who had no prior history of CML collected from different Babylon populations. (as controls). The ages for both the AHC and patient groups ranged from 20 to 60 years.

**Estimation the levels of HBV and CTLA-4**

Around (3 ml) of venous blood was placed into a gel tube and centrifuged to obtain serum, which was instantly stored at freezing (-20) until use. HBV and CTLA-4 levels in the serum of both patients and AHC groups were evaluated using an ELISA Kit (BT LAB/China).

**DNA extraction**

*Total DNA Extraction:*

To investigate the polymorphism of CTLA-4 gene we used the total DNA extraction G-SpinTM Total DNA Extraction Mini Kit (iNtRON Biotechnology Co., Korea), which optimized to extract (20-30kb) DNA fragments also it help extract up to 50kb fragments. Approximately (2 ml) of venous blood was placed into an EDTA tube that was collected from CML patients, then stored immediately at -20 °C until total DNA was extracted.

*Viral Genome Extraction*:

The viral genome was extracted using 3 ml of venous blood from patients infected with HBV that had already have CML put into an EDTA tube, which was then stored until use at (-20˚C). The viral genome was extracted using the Patho Gene-spinTM DNA/RNA Extraction Kit (iNtRON Biotechnology Co., Korea), which was designed to isolate high-quality nucleic acids from a variety of pathogens and specimens using low elution volumes that allow sensitive downstream analysis.

**Primer selection**

The primers sequence used in these research was designed for *CTLA-4* gene

F- “AGTTAGGGAATGGCACAGCC”

R- “GCCCCAAAGCACATGTCAAC”

HBVprimer sequence

F- “ACATGGAGAACATCGCATCA”

R- “AGGACAAACGGGCAACATAC”.

**PCR Technique**

The current process known as Polymerase chain reaction (PCR) was conducted using a conventional thermal cycler (Biometra-Germany) with a total volume of approximately 25 µL, which consisted of a master mix (12.5µl), forward and reverse primers (1µl µL each), nuclease-free water (5.5µl), and extracted DNA (5µl)

**DNA genotyping and Sequencing**

The PCR reactions for amplifying HBV and CTLA-4 polymorphisms were conducted using a preheated thermal cycler (Biometra, Germany) with distinct cycling parameters. HBV amplification targeted 35 cycles, beginning with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 1 min), annealing (59°C, 45 s), extension (72°C, 2 min), and a final extension at 72°C for 5 minutes. CTLA-4 amplification used 40 cycles with similar steps but a slightly lower annealing temperature (58°C, 45 s). Post-amplification, products were electrophoresed on a 1.5% agarose gel and visualized via gel documentation. For CTLA-4 sequencing, the forward PCR primer was utilized, and automated sequencing was performed by Macrogen Company (Seoul, South Korea). Sequences were analyzed using Geneious Bioinformatics software (v2) and aligned against NCBI reference databases for verification and processing.

**Statistical analysis**

To assess the significance of the variables examined in this study, the chi-square test was used. All statistical analyses were conducted using SPSS program version 23. Statistical significance was set at $P < 0.05$. The Hardy-Weinberg equilibrium was used to analyze the CTLA-4 gene polymorphism.

**Ethical certification**

This study adhered to the principles of the Declaration of Helsinki. Before sample collection, verbal and written consent was obtained from all patients. A local ethics commission reviewed and approved the study protocol, consent form, and subject information on septemper 10,2024, under project number M240902.

## RESULTS

The features of the 120 patients in the CML and 100 in the AHC groups, are illustrated in (Table1), the mean ages of the newly diagnosed and treated CML patients were 44±13.3 and 48±11.43, respectively. While, AHC group was 46±12.9, Also, this table show the sex distribution in study groups. Non- Significant differences were found between CML patients and AHC groups according to their age. Also, was found nen CML patients and AHC groups according to their sex as shown in (Table 1).

According to biochemical parameters (Urea creatinine, AST, ALT and LDH) serum levels, there are no statistically significant differences among new diagnosis CML, patients respond to treatments and relapse group accept in serum level of creatinine between new diagnosis CML group and relapse group there is statistically significant differences p value (0.01) ( Table 2).

In the current study, CTLA4 genetic sequences located on chromosome 2 were targeted. The 537 bp amplicon analyzed in this study represents part of the exon region of the CTLA4 molecule (Figure 1).

**Table 1. Age and Sex distribution between patients with CML groups and controls groups.**

| Parameters | | Patients (n=120) | | Controls (n=100) | p-value |
|---|---|---|---|---|---|
| | | New diagnosis (n=20) | Treated (n=100) | | |
| Age ±SD | | 44±13.3 | 48±11.43 | 46±12.9 | 0.57 |
| Sex | Male | 12(60%) | 52(52%) | 56(56%) | 0.59 |
| | Female | 8(40%) | 48(48%) | 44 (44%) | |
| sex | Ratio | 1.5:1 | 1.08 | 1.5 | |

**Table 2: Comparison between newly diagnosed 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<br>**0.69<br>***0.399 |
| Creatinine±SD mg/dl | 0.71±0.51 | 1.0±0.73 | 0.9±0.33 | *0.059<br>**0.01<br>***0.329 |
| ALT ±SD IU/L | 29.5±10.9 | 30.5±10.79 | 28.3±12.3 | *0.73<br>**742<br>***418 |
| AST ±SD IU/L | 33.6±12.6 | 32.2±12.7 | 33.2±15.6 | *0.671<br>**931<br>***733 |
| LDH ±SD IU/L | 478±201 | 485±278 | 438±20.4 | *0.909<br>**0.49<br>***0.39 |

* Comparison between newly diagnosed CML and response to treatment patients. ** Comparison between newly diagnosed CML patients and relapse patients.; *** Response to Treatment and relapse.

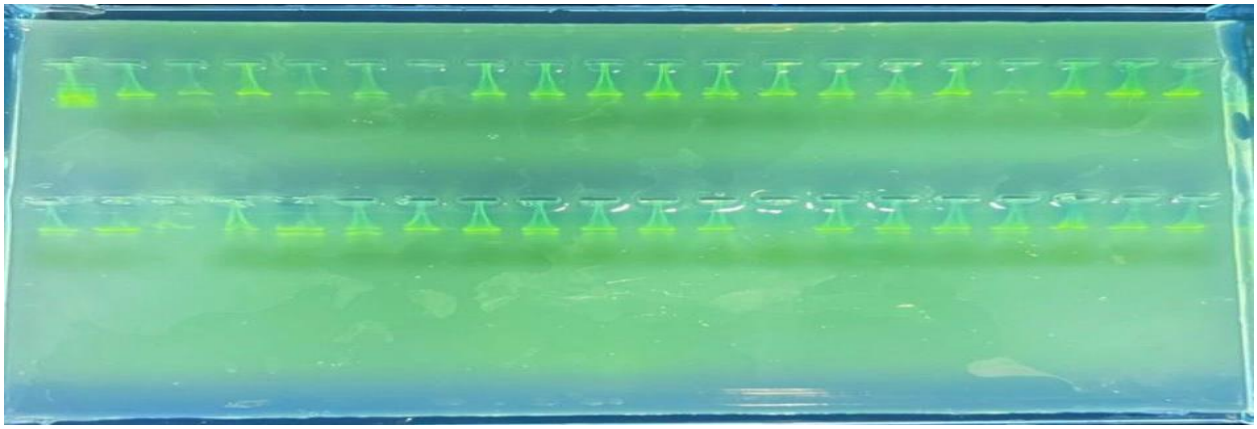

**Fig. 1:** PCR detection of the CTLA4 gene; in CML patients. M: A 100–1100 bp DNA ladder. After migrating into 2% agarose at 75V and 20 mA for 120 minutes, the PCR-amplified products were stained with ethidium bromide and placed in 15 µl per well.

The present results showed that the DNA polymorphism distribution was according to AA; AT and TT were 20%, 30%, and 50% in patient with CML, respectively, in patients with CML and 60%, 0.0%, and 40%, respectively, in the control group. There were no statistically significant differences ($p< 0.05$) between the different groups according to CTLA-4 genotyping. Statistical analysis of AA indicated a borderline non-significant association ($P = 0.06$) with an odds ratio (OR) of 0.05 (95% confidence interval [CI]: 0.002–1.20), suggesting a potential but not statistically confirmed protective effect, as shown in table (3). Statistical analysis of the AT genotype indicated a borderline non-significant association ($P = 0.06$), with an odds ratio (OR) of 0.05 (95% confidence interval (CI): 0.002–1.20], suggesting a potential but not statistically confirmed protective effect.

Analysis of the TT genotype demonstrated no significant association with disease susceptibility ($P = 0.13$), with an OR of 0.26 (95% CI: 0.04–1.48), indicating a possible but statistically non-significant trend toward reduced disease risk in individuals carrying the TT genotype compared to those carrying the AA genotype. The A allele was present in 14 patients (35%) and 12 control alleles (60%) and served as the reference allele. The T allele was observed in 26 patients (65%) and 8 control alleles (40%). Statistical analysis showed a borderline non-significant association between the T allele and disease risk ($P = 0.06$), with an OR of 0.35 (95% CI: 0.11–1.08), as shown in table (3).

A novel partial sequence of the CTLA4 gene was identified, covering 537 bp and located on chromosome 2. The samples were collected from blood-derived leukocytes and submitted to the DDBJ/EMBL/GenBank databases under accession numbers LC86765, LC867666, and LC867667. In the current study, the genetic sequence of CTLA4 was analyzed. For the currently investigated 537 bp gene amplicon, BLASTn analysis revealed a high similarity with the reference human CTLA4 gene sequences available in NCBI, including coding and adjacent regulatory regions, according to human genome annotation (GenBank version: LC867665.1, LC867666.1, and LC867667.1). HBV DNA was not found in any of the blood samples of patients in the CML or AHC groups shown in (Table 4) and (Figure 2).

The (table5) compares serum CTLA-4 levels in the same cohort of 120 CML patients and 100 AHC. CML patients showed higher mean CTLA-4 levels (32.47 ± 14.2) than the AHC group (23.83 ± 9.03). The difference was statistically significant ($p=0.0387$), suggesting dysregulated CTLA-4 expression in CML pathogenesis.

**Table 3: Genotyping of (CTLA-4) gene in CML patients and AHC groups.**

| Genotype CTLA4 | Patients No. (%) | Control No. (%) | z- statics | Sig. | OR (95%) |
|---|---|---|---|---|---|
| AA [a] | 4 (20%) | 6 (60%) | References | | |
| AT | 6 (30%) | 0 (0.0%) | 1.48 | 0.06 | 0.05(0.002-1.20) |
| TT | 10 (50%) | 4 (40%) | 1.51 | 0.13 | 0.26(0.04-1.48) |
| Total number | 20 | 10 | | | |
| A allele | 14 (35%) | 12 (60%) | References | | |
| T allele | 26 (65%) | 8 (40%) | 1.81 | 0.06 | 0.35(0.11-1.08) |

**Table 4: The Results of PCR for HBV-DNA infection in patients with CML**

| HBV | No. | % | P value |
|---|---|---|---|
| **Positive** | **0** | **O.OO%** | **P=0.03** |
| **Negative** | **120** | **73%** | **Sign** |
| **Total** | **100** | **100%** | **>0.05** |

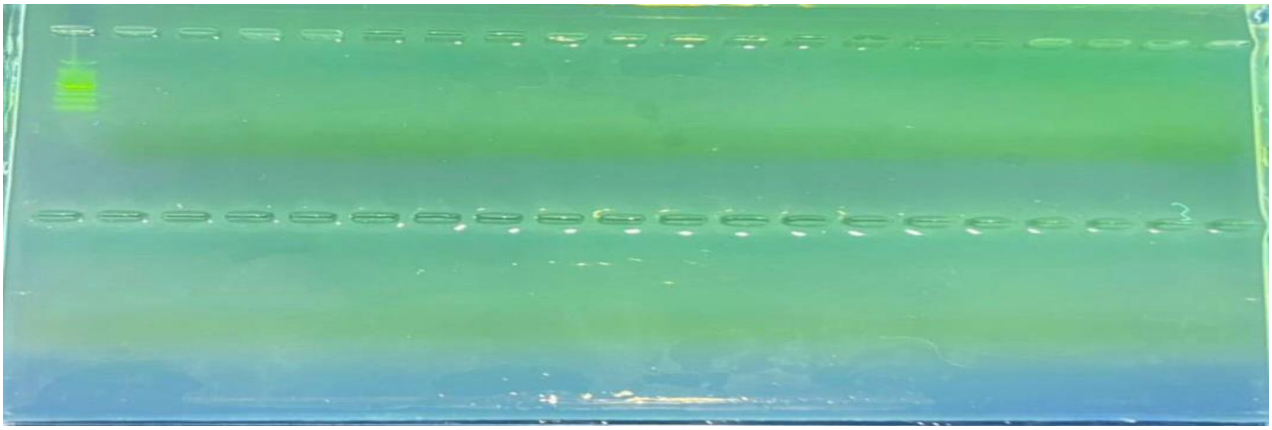

**Fig. 2:** NO-PCR detection of the HBV gene; in CML patients. M: A 100–1100 bp DNA ladder. After migrating into 2% agarose at 75V and 20 mA for 120 minutes, the PCR-amplified products were stained with ethidium bromide and placed in 15 µl per well.

**Table 5: Comparison of Serum CTLA-4 Levels Between CML Patients and AHC**

| Groups study | No. of cases | Levels of CTLA-4 (Mean ±SD) | Sig. |
|---|---|---|---|
| Patients | 120 | 32.47 ± 14.2 | |
| AHC | 100 | 23.83 ± 9.03 | 0.0387 |

## DISCUSSION

Clonal proliferation of progenitor cells in hematopoietic tissue is a characteristic feature of chronic myeloid leukemia (CML). This phenomenon leads to an increase in the generation of myeloid blasts and a decrease in the rate of apoptosis[18].

According to the research, newly diagnosed patients are 35 years old, whereas the group averages 51 years old. However, the age difference between the groups was not statistically significant (p = 0.62). The sick group had slightly more males than the control group. However, the difference was not statistically significant. The data suggest that the patient groups were male. This applies to gender. Epidemiological studies suggest that the age of patients with CML changes during the study period and that the median age ranges from 52 to 64 years. These findings are in agreement with ALgahtany et al [19].

Another study reported that Asians commonly develop CML at 35–45 years of age. CML affects 15 per 1,000,000 people worldwide, with a 1.34 male-to-female ratio. Indian studies showed that most patients were under 60 years of age. The current study found no statistically significant change in clinical parameters of older CML patients[20,21].

Berger et al [22]. observed that women had higher platelet counts and smaller spleens than men. Men with CML have a lower survival rate and more genetic abnormalities than women. This did not correlate with the Sokal score-based risk assessment, and the gap was greater in low- and moderate-risk patients.

Despite women switching TKIs more frequently during TKI therapy, the SIMPLICITY trial found this to be true [23]. No data suggest a sex difference in TKI ability to induce dramatic cytogenetic and molecular remissions. Similarly, there is no evidence that sex affects the overall survival of patients with CML during TKI therapy.

TKIs including imatinib, dasatinib, and nilotinib are used to treat chronic myelogenous leukemia. However, these medicines may have negative effects. These drugs cause hepatotoxicity and nephrotoxicity in CML patients. These adverse effects have been reported in several studies. Drug-induced liver and kidney damage may elevate ALT, AST, and renal function indicators[24].

A decrease in the SOD potential from 34.63 to 26.12 U/mg proteins was also observed in CML-PBM cells as a result of the addiction to Li. (CML-PBM) cells showed a significant decrease in SOD activity as a result of in vivo treatment with IM, DAS, and NIL[25].

Activated T cells express CTLA-4, which inhibits T cell activation and proliferation. This has been proven by a significant amount of research. CTLA-4 controls T cell-mediated immunological responses by

competitively binding to costimulatory B7 protein and activating FAS-dependent cell death. Previous studies [26] have demonstrated this. Recent studies have shown that many tumors exhibit aberrant CTLA-4 gene expression. This supports the idea that this gene may contribute to cancer formation and progression [27–29].

In the present study, a unique CTLA4 gene mutation at LC86765, LC867666, and LC867667 (537 bp) exhibited polymorphisms that differed between patients with CML and controls. AA was more common in the control group (60%) than in the AT group and was only found in patients with CML (30%). These data were non-significant (p = 0.06), but showed that some CTLA4 alleles may be protected. This supports earlier findings that CTLA-4 gene polymorphisms may affect colorectal, lung, and breast cancer risks [10]. The immune-suppressive role of CTLA-4 in cancer is supported by the increased mean blood levels of CTLA-4 in CML patients compared to controls, albeit being statistically insignificant. CTLA-4 reduces T-cell activation by competing with CD28 for B7 ligands on antigen-presenting cells [30]. CTLA-4 upregulation is a well-known tumor immune escape mechanism, especially in hematologic malignancies [31].

Unlike other cancers, lymphoid tumors are immune system-derived. This distinguishes them from lymphoid malignancies. Most lymphoid lung malignancies originate from mature B and T cells[23]. Thus, the immune system plays a complex role in lymphadenopathy. CTLA-4, a typical immunological regulatory checkpoint, is essential for T cell anergy, T and B cell inhibition, and other immune responses [26]. Thus, aberrant CTLA-4 expression may contribute to lymphoid malignancies. Additionally, single nucleotide polymorphisms (SNPs) in CTLA-4 are thought to influence promoter activity and CTLA-4 production. CTLA4 on chromosome 2q33 negatively regulates T-cell growth and activation. It does this by competing with CD28 for binding to B7-1/B7-2, thereby reducing T-cell responses and peripheral tolerance[32]. Overexpression of CTLA4 produces inhibitory signals that are larger than tumor cell surface antigen immunogenicity. T-cell activation is downregulated or stopped, allowing the immune system to escape the tumor [33]. Several types of cancers have aberrant CTLA-4 gene expression, which may contribute to cancer formation and progression [34]. Many studies have linked CTLA-4 polymorphisms to a wide spectrum of cancer-related disorders. Genetic variation can alter gene expression and protein function. HBV can cause persistent infection, immunological modulation, liver cirrhosis, and hepatocellular cancer. Covalently closed circular DNA (cccDNA) allows viral reproduction despite immune responses and antiviral medicines, helping the virus to survive. [(38)].

In the current study, HBV DNA was not found in any of the blood samples of patients in the CML or AHC groups. These results agree with Ikeda et al [35] It has been well established that individuals who were undergoing chemotherapy experienced reactivation of chronic HBV infection. However, reactivation that occurs during treatment with imatinib mesylate has not been described.

In addition, this contradicts an epidemiological research showing that prolonged inflammation or immunological modulation from viral infections may cause leukemia Dalia et al [37]. The lack of a substantial relationship may be related to CML phase or HBV replication in these patients. According to recent studies, hematological cancers may be affected by it. Patients undergoing therapy for hematological malignancies are treated with TKI. A comprehensive investigation by Barone et al [36]. showed the risk of HBV reactivation in these patients. The study shows that HBV reactivation in patients with resolved infection is rare, but it can be affected by factors such as hematologic malignancy, medication, ethnicity, and HBV genotype. This emphasizes the need for HBV monitoring in patients with CML receiving TKI therapy.

CML patients demonstrated significantly elevated serum CTLA-4 levels compared to the AHC with a p-value of 0.0387. This statistically significant difference suggests that CTLA-4 expression is upregulated in CML, potentially reflecting an underlying immune dysregulation associated with disease pathogenesis. The increased CTLA-4 levels may be related to T-cell exhaustion or impaired immune surveillance mechanisms in these patients. The absence of HBV DNA in all samples suggests a low viral reactivation in this cohort. Despite the lack of a direct connection, CTLA-4 levels may indicate immune dysregulation in CML patients. Conclusion: This study highlights the potential protective role of CTLA4 polymorphisms, particularly the AT genotype, in Iraqi CML patients, along with elevated, yet significant, serum CTLA4 levels. The absence of HBV DNA in both patients and controls contradicts prior hypotheses linking HBV infection to CML progression in this population. These results emphasize the need for population-specific genetic studies on CML and suggest that HBV reactivation may not be a critical concern in Iraqi patients undergoing TKI therapy.

## Declarations

### Ethical approval

This study adhered to the principles of the Declaration of Helsinki. Before sample collection, verbal and written consent was obtained from all patients. A local ethics commission reviewed and approved the study protocol, consent form, and subject information on septemper 10,2024, under project number M240902.

**Availability of data and material** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests** The author declare that they have no competing interests
**Funding** No funds were received to fulfil this work.
**Author Contribution** The authors were contributed equally in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.

## REFERENCES

1. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2022 update on diagnosis, therapy, and monitoring. Am J Hematol. 2022;97(9):1236-1256.
2. Deininger MW, Shah NP, Altman JK, et al. Chronic myeloid leukemia, version 2.2021, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2020;18(10):1385-1415.
3. Szuber N, Orazi A, Tefferi A. Chronic neutrophilic leukemia and atypical chronic myeloid leukemia: 2024 update on diagnosis, genetics, risk stratification, and management. Am J Hematol. 2024;99(7):1360-1387.
4. Sakamoto Y, Ishida T, Masaki A, et al. Clinicopathological significance of CD28 overexpression in adult T-cell leukemia/lymphoma. Cancer Sci. 2022;113(1):349-361.
5. Van Nguyen S, Shamoun L, Landerholm K, Andersson RE, Wagsater D, Dimberg J. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene polymorphism (rs3087243) is related to risk and survival in patients with colorectal cancer. In Vivo (Brooklyn). 2021;35(2):969-975.
6. Hossen MM, Ma Y, Yin Z, et al. Current understanding of CTLA-4: from mechanism to autoimmune diseases. Front Immunol. 2023;14:1198365. doi:10.3389/fimmu.2023.1198365
7. Sobhani N, Tardiel-Cyril DR, Davtyan A, Generali D, Roudi R, Li Y. CTLA-4 in regulatory T cells for cancer immunotherapy. Cancers (Basel). 2021;13(6):1440.
8. Van Coillie S, Wiernicki B, Xu J. Molecular and cellular functions of CTLA-4. Regul Cancer Immune Checkpoints Mol Cell Mech Ther. 2020:7-32.
9. Lisi L, Lacal PM, Martire M, Navarra P, Graziani G. Clinical experience with CTLA-4 blockade for cancer immunotherapy: From the monospecific monoclonal antibody ipilimumab to probodies and bispecific molecules targeting the tumor microenvironment. Pharmacol Res. 2022;175:105997.
10. Zou C, Qiu H, Tang W, Wang Y, Lan B, Chen Y. CTLA4 tagging polymorphisms and risk of colorectal cancer: a case–control study involving 2,306 subjects. Onco Targets Ther. 2018:4609-4619.
11. Chen Y, Fu M, Li H, Wang L, Liu R, Liu Z. Molecular characterization of the Acyl-CoA-Binding protein genes reveals their significant roles in Oil Accumulation and Abiotic stress response in cotton. Genes (Basel). 2023;14(4):859.
12. Wang F, Song H, Xu F, et al. Role of hepatitis B virus non-structural protein HBx on HBV replication, interferon signaling, and hepatocarcinogenesis. Front Microbiol. 2023;14:1322892.
13. Shi Y, Zheng M. Hepatitis B virus persistence and reactivation. Bmj. 2020;370.
14. Hsu C, Tsou H, Lin S, et al. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. Hepatology. 2014;59(6):2092-2100.
15. Deepan N, Maung ST, Decharatanachart P, Chaiteerakij R. Hepatitis B Virus Reactivation in Cancer Patients Receiving Chemotherapy—A Systematic Review and Meta-Analysis. In: Seminars in Oncology. Elsevier; 2024.
16. Tsutsumi Y, Yamamoto Y, Ito S, et al. Hepatitis B virus reactivation with a rituximab-containing regimen. World J Hepatol. 2015;7(21):2344-2351. doi:10.4254/wjh.v7.i21.2344
17. Hu J, Protzer U, Siddiqui A. Revisiting hepatitis B virus: challenges of curative therapies. J Virol. 2019;93(20):10-1128.
18. Al-Bayati A, Al-Bayti A, Husain V. A short review about chronic myeloid leukemia. J Life Bio Sci Res. 2023;4(01):15-19.
19. Algahtani FH, Alqahtany FS. Evaluation and characterisation of Chronic myeloid leukemia and various treatments in Saudi Arabia: A retrospective study. J Infect Public Health. 2020;13(2):295-298.
20. Smith AG, Painter D, Howell DA, et al. Determinants of survival in patients with chronic myeloid leukaemia treated in the new era of oral therapy: findings from a UK population-based patient cohort. BMJ Open. 2014;4(1):e004266.
21. Berger U, Maywald O, Pfirrmann M, et al. Gender aspects in chronic myeloid leukemia: long-term results from randomized studies. Leukemia. 2005;19(6):984-989.
22. Suttorp M, Millot F, Sembill S, Deutsch H, Metzler M. Definition, epidemiology, pathophysiology, and essential criteria for diagnosis of pediatric chronic myeloid leukemia. Cancers (Basel). 2021;13(4):798.
23. Hashimoto R, Okada T, Kato T, et al. The breakpoint cluster region gene on chromosome

22q11 is associated with bipolar disorder. Biol Psychiatry. 2005;57(10):1097-1102.

24. Sasaki K, Lahoti A, Jabbour E, et al. Clinical Safety and Efficacy of Nilotinib or Dasatinib in Patients With Newly Diagnosed Chronic-Phase Chronic Myelogenous Leukemia and Pre-Existing Liver and/or Renal Dysfunction. Clin Lymphoma Myeloma Leuk. 2016;16(3):152-162. doi:10.1016/j.clml.2015.12.003
25. Ciarcia R, Damiano S, Puzio MV, et al. Comparison of dasatinib, nilotinib, and imatinib in the treatment of chronic myeloid leukemia. J Cell Physiol. 2016;231(3):680-687.
26. Walker LSK. CTLA-4 and autoimmunity: new twists in the tale. Trends Immunol. 2015;36(12):760-762.
27. Charbonneau B, Moysich KB, Kalli KR, et al. Large-scale evaluation of common variation in regulatory T cell–related genes and ovarian cancer outcome. Cancer Immunol Res. 2014;2(4):332-340.
28. Liu J, Liu J, Song B, et al. Genetic variations in CTLA-4, TNF-α, and LTA and susceptibility to T-cell lymphoma in a Chinese population. Cancer Epidemiol. 2013;37(6):930-934.
29. Ramzi M, Arandi N, Saadi MI, Yaghobi R, Geramizadeh B. Genetic Variation of Costimulatory Molecules, Including Cytotoxic T-Lymphocyte Antigen 4, Inducible T-Cell Costimulator, Cluster Differentiation 28, and Programmed Cell Death 1 Genes, in Iranian Patients With Leukemia. Exp Clin Transplant Off J Middle East Soc Organ Transplant. 2018;18(6):719-724.
30. Kennedy A, Robinson MA, Hinze C, et al. The CTLA-4 immune checkpoint protein regulates PD-L1: PD-1 interaction via transendocytosis of its ligand CD80. EMBO J. 2023;42(5):e111556.
31. Jiménez-Morales S, Aranda-Uribe IS, Pérez-Amado CJ, Ramírez-Bello J, Hidalgo-Miranda A. Mechanisms of immunosuppressive tumor evasion: focus on acute lymphoblastic leukemia. Front Immunol. 2021;12:737340.
32. Zhang H, Dai Z, Wu W, et al. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. J Exp Clin Cancer Res. 2021;40(1):184.
33. Lee J, Kim EH. Mechanisms underlying response and resistance to immune checkpoint blockade in cancer immunotherapy. Front Oncol. 2023;13:1233376.
34. Zhang C, Chen J, Song Q, et al. Comprehensive analysis of CTLA-4 in the tumor immune microenvironment of 33 cancer types. Int Immunopharmacol. 2020;85:106633.
35. Ikeda K, Shiga Y, Takahashi A, et al. Fatal hepatitis B virus reactivation in a chronic myeloid leukemia patient during imatinib mesylate treatment. Leuk Lymphoma. 2006;47(1):155-157.
36. Barone M. Risk of hepatic decompensation from hepatitis B virus reactivation in hematological malignancy treatments. World J Gastroenterol. 2024;30(25):3147.
37. Dalia S, Chavez J, Castillo JJ, Sokol L.Hepatitis B Infection Is Associated with an Increased Risk of Non-Hodgkin Lymphoma: A Meta-Analysis. Blood, 2012; 120(21), 2658.