ORIGINAL ARTICLE

Genetic polymorphism and immunological evaluation of PD-1 in Iraqi patients with acute myeloid leukemia

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ABSTRACT

PD-1 has a noteworthy function in developing acute myeloid leukemia (AML). The expression of PD-1 on effector T cells is regulated at the protein level depending on the interactions between cells. The objective of the study was to evaluate the PD-1 concentration levels and the polymorphism genetic variants (rs36084323 G/A) in Iraqi Arab patients with AML. Sanger's DNA sequencing was used, and the assessments were done by enzyme-linked immunosorbent assay and PD-1 gene polymorphism SNP rs36084323 G/A. The frequency of rs36084323 was significantly different between AML and control, with a lower risk for AML seen in patients with GA genotype (odds ratio; 95% confidence interval: 0.53; 0.32–0.87). PD-1 elevated AML compared to control (213.1 pg/mL vs. 178.8 pg/mL). in AML patients, there is upregulation in PD-1, which indicates that PD-1 is a possible biomarker for AML. PD-1 rs36084323 G/A may have a role in AML risk.

Key words: Acute myeloid leukemia, gene polymorphism, programmed cell death protein 1 gene, SNP

INTRODUCTION

Acute myeloid leukemia (AML) accounts for more than 80% of documented cases in adults.[1] AML develops when a growing marrow cell's DNA (genetic material) undergoes acquired alterations, leading to immature cells becoming the dominant type.[2] Blood cell counts have a tendency to be lower than usual when AML is diagnosed.[1]

To control how the immune system reacts to human cells, programmed cell death protein 1 (programmed cell death protein 1) location on B and T lymphocyte surfaces. It promotes self-tolerance by reducing inflammatory activity in T lymphocytes and downregulating the immune system; this stops autoimmune illnesses in their tracks.[3] PD-1 protects against autoimmunity in two ways: as an immunological checkpoint, it starts by encouraging lymph node antigen-specific T-cells to undergo apoptosis, which involves programmed cell death.[4-6] Second, it decreases the death of regulatory T cells, which are anti-inflammatory and suppressive.[5] Thus, targeting the axis of PD-1 shows promise as an auxiliary treatment for hematologic malignancies.[7]

The PD-1/PD-L1 pathway in regulating the initiation and preservation of immunotolerance for tumors. PD-1 and its ligand functions involve cellular activation, cell proliferation, cellular cytotoxic mediators production, and release in tumors.[8] B lymphocytes activated B and T cells, natural killer, monocytes, dendritic cells, and macrophages express the innate immune response inhibitor PD-1.[8-11]

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Recently, several Iraqi researchers examined the genetic polymorphisms in AML, such as APE1 gene's (rs1130409; Aspl48Glu, T/G in Exon 5) polymorphism,[12] and tumor suppressor gene (TP53) (rs1042522 and rs1642785) in AML.[13] This research aims to ascertain the immunological concentration of PDCD protein 1 in patients with AML who have the genetic polymorphism rs36084323.

METHODS

Study design

A retrospective case–control study that included 200 participants was divided into two groups: 100 patients diagnosed with AML and 100 healthy participants; consultant hematologists diagnosed the patients, and all patients were recruited from Baghdad Teaching Hospital.

The patients were subgrouped according to (1) sex (male and female), (2) classification: FAB classification (M0–M7), and UC, which is defined as AML not otherwise specified,[14] and (3) cause of treatment: newly diagnosed, under treatment for AML (MD), and relapse (RL).

Study settings

The study was conducted in the biotechnology department of the College of Science, Baghdad University. Patients were recruited from the period November 15, 2023, to January 15, 2024.

Inclusion criteria

Adult patients with a diagnosis of AML.

Exclusion criteria

Patients with chronic disorders (liver, renal, thyroids, endocrine, heart, etc.), active infection, other types of malignancies, and missing data.

Analytical procedure

PD-1 serum levels

A human PD-1 enzyme-linked immunosorbent assay kit (sunLong Biotech Co. Ltd, China) was used to measure soluble PD-1 protein according to the manufacturer's instructions. Every subject had drawn 5 ml of venous blood into a clear tube. The tube was centrifuged (3000 rpm for 15 min at 4°C) after the blood had clotted, and the serum was then removed and stored at − 20°C until the analysis.

PD-1 gene polymorphisms

ReliaPrep™ DNA extraction kit Blood Promega Corporation, USA, was utilized to extract Genomic DNA isolated from venous blood using the solid-phase DNA extraction method of EDTA. After evaluating concentration and purity, it was deported in agarose gel electrophoresis (1% agarose) Bio-Rad Experion Automated Electrophoresis System (RRID: SCR_019691), it was exposed to amplifications of polymerase chain reaction (PCR) (TProfessional TRIO combi, Biometra GmbH, Germany). Two primers were developed for use (Reverse: 5′-CTCAAC CCCACTCCCATTCT-3′ and Forward: 5′-TTCTAGCCTCGCTTCGGTTA-3′) for genotyping of rs36084323 SNP of PD-1. A 20 µl of final volume was used for the reaction, which included 10 µl of Luna Script PCR master mix, 0.5 µl; 10 µM of forward primer, 0.5 µl; 10 µM of reverse primer, 5 µl of 50 ng DNA sample, and nuclease-free distilled water (4 µl). The PCR conditions included a 95°C-enzyme activation step of 10 min, a 95°C-denaturation step of 15 s, and a 60°C-annealing/extension step of 1 min. Sanger sequencing was used for the amplified PCR fragments, sequenced according to the instruction manuals of the sequencing company (Macrogen Inc. Geumcheon, Seoul, South Korea). The PCR product was sent for Sanger sequencing using ABI3730XL, an automated DNA sequence, by Macrogen Corporation (Biomatters Ltd., Auckland, New Zealand; www.geneious.com). Sequence alignment with a reference in the Gene Bank allowed the program to uncover the genotypes.

Ethical considerations

Written consent was taken from all subjects, and the Research Ethical Committee of the College of Science, Baghdad University (Approval number: CSEC/1123/0110, date: November 12, 2023) give approval for the study.

Statistical analysis

The frequencies of each allele and genotype were reported as percentages. The first step was to check for Hardy—Weinberg equilibrium (HWE) agreement with genotype frequencies. The association between PD-1 SNP rs36084323 and AML was presented. Median and interquartile range (IQR), odds ratio (OR). Receiver operating characteristic analysis was used to analyze the diagnostic utility of PD-1 levels. All calculations were performed using SPSS 24.1 software (IBM Corp., Armonk, NY. USA).[15]

RESULTS

In both AML patients and controls, the PD-1 rs36084323 G/A SNP was found with two genotypes, GG and GA, corresponding to G and A alleles. There was no statistically significant variation in the genotype frequencies of the PD-1 rs36084323 G/A SNP in patients with AML, according to the HWE analysis ($P = 0.078$). When AML patients were compared to controls, the homozygous genotype (GG) showed an increased frequency in patients (GG 70% vs. 50%, respectively), whereas (GA 30% vs. 50%, respectively, OR; 95% confidence interval (95% CI): 0.43, 0.24–0.76. Similarly, the frequency of mutant allele (A) was 30 (15.0%), which was significantly lower in AML compared to the control group; OR; 95% CI: 0.53; 0.32–0.87, as illustrated by Table 1. The sequence chromatogram of PD-1 rs36084323 SNP showing two genotypes, GG and GA, is shown in Figure 1.

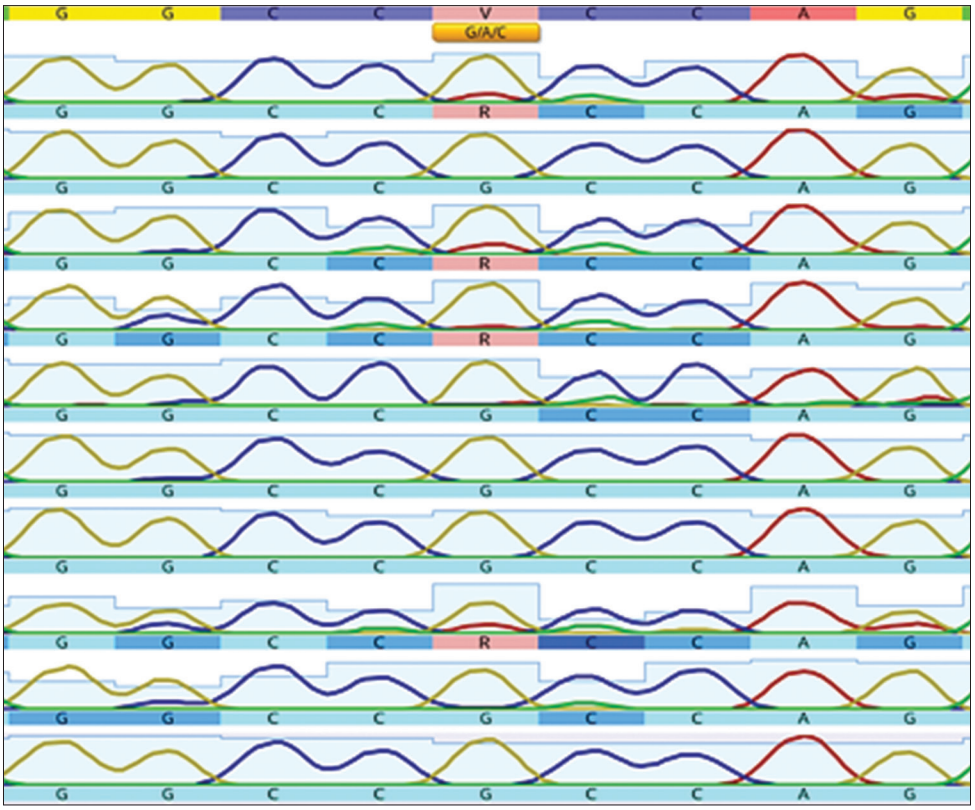

Figure 1: PD-1 rs36084323 SNP DNA sequence chromatogram displaying GA and GG genotypes. PD-1: Programmed cell death protein 1

The median levels of PD-1 were significantly increased in AML patients in comparison with healthy control (HC) (213.1 [IQR: 76.9–242.1] pg/mL vs. 178.8 [IQR: 132.6–202.1] pg/mL; $P < 0.001$), as shown in Figure 2.

PD-1 showed a good ability to differentiate between AML and healthy control, with optimal cutoff >202.9 pg/ml to predict AML; in addition, PD-1 also showed good sensitivity and specificity as a predictor, as illustrated in Figure 3.

Serum PD-1 levels showed no significant difference between male and female AML patients (263.6 [IQR: 200.7–708.3] pg/mL vs. 237.9 [IQR: 217.9–444.5] pg/mL; $P = 0.42$), as illustrated in Figure 4a. Serum PD-1 levels showed no significant difference between AML patients distributed according to AML classification ($P = 0.72$), as illustrated in Figure 4b. Serum PD-1 levels showed no significant difference according to the course of the disease treatment, as illustrated in Figure 4c. Serum PD-1 levels showed no significant difference between AML patients classified by rs36084323 GG and GA genotypes (251.9 [IQR: 226.7–494.1.3] pg/mL vs. 242.1 [IQR: 130.2–1131.0] pg/mL; $P = 0.32$), as illustrated in Figure 4d.

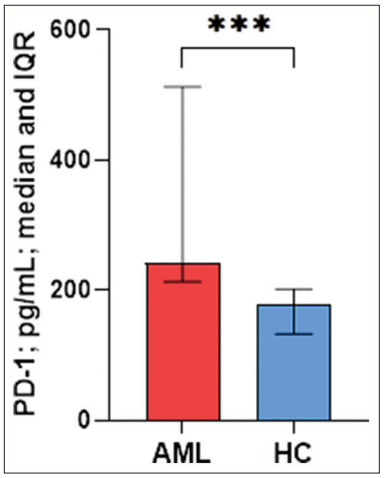

Figure 2: PD-1 levels in patients with acute myeloid leukemia and HC. AML: Acute myeloid leukemia, IQR: Interquartile range, PD-1: Programmed cell death protein 1, HC: Healthy control. ***Indicate *P*-value <0.001

DISCUSSION

PD-1 gene rs36084323 polymorphism in the Iraqi sample was investigated by DNA sequencing in 100 AML patients; this polymorphism has location in the promoter region which showed 70% GG and 20% GA polymorphism, with the presence of GA associated with the lower risk of AML compared to healthy control (OR, 95% CI: 0.43, 0.24–0.76). In addition, to study the polymorphism of PD-1 rs36084323 polymorphism, the level of PD-1 was also investigated, and it was statistically higher in AML compared to HC (213.1 pg/mL vs. 178.8 pg/mL); however, the serum PD-1 levels showed no significant difference between

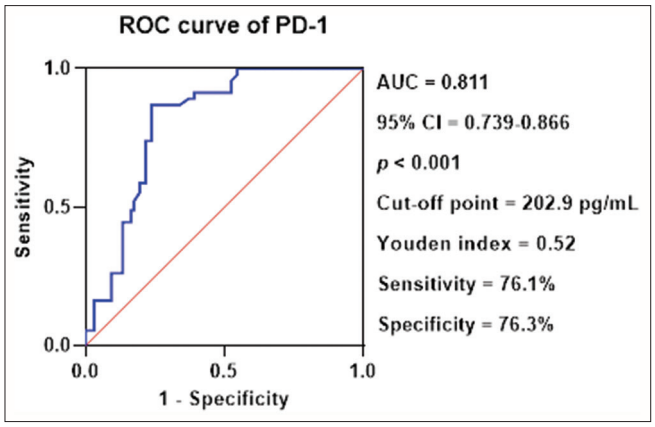

Figure 3: Receiver operating characteristic curve analysis of PD-1 levels in acute myeloid leukemia patients versus healthy controls. ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval, PD-1: Programmed cell death protein 1

AML patients when classified by rs36084323 GG and GA genotypes (251.9 pg/mL vs. 242.1 pg/mL).

Globally, the wild-type G allele frequency was 96.5% (data came from 18,890 subjects), which varies according to the population from 48% (data from 86 subjects) in East Asia[16] to 52.7% (data came from 112 subjects) in the Asian general population,[16] 96% in South Asian population (data came from 98 subjects),[16] 95% in the African population (data came from 2946 subjects),[16] and 98.4% from the European population (data came from 14,286 subjects),[16] with no reported study in the ALFA project in the dbSNP database that examined the Southeast Asian population involved in the study,[17] in the current study, the G allele frequency in AML was 85%, and in healthy control, 75%, which indicates that it is slightly lower than the global frequency and higher than the general Asian frequency.

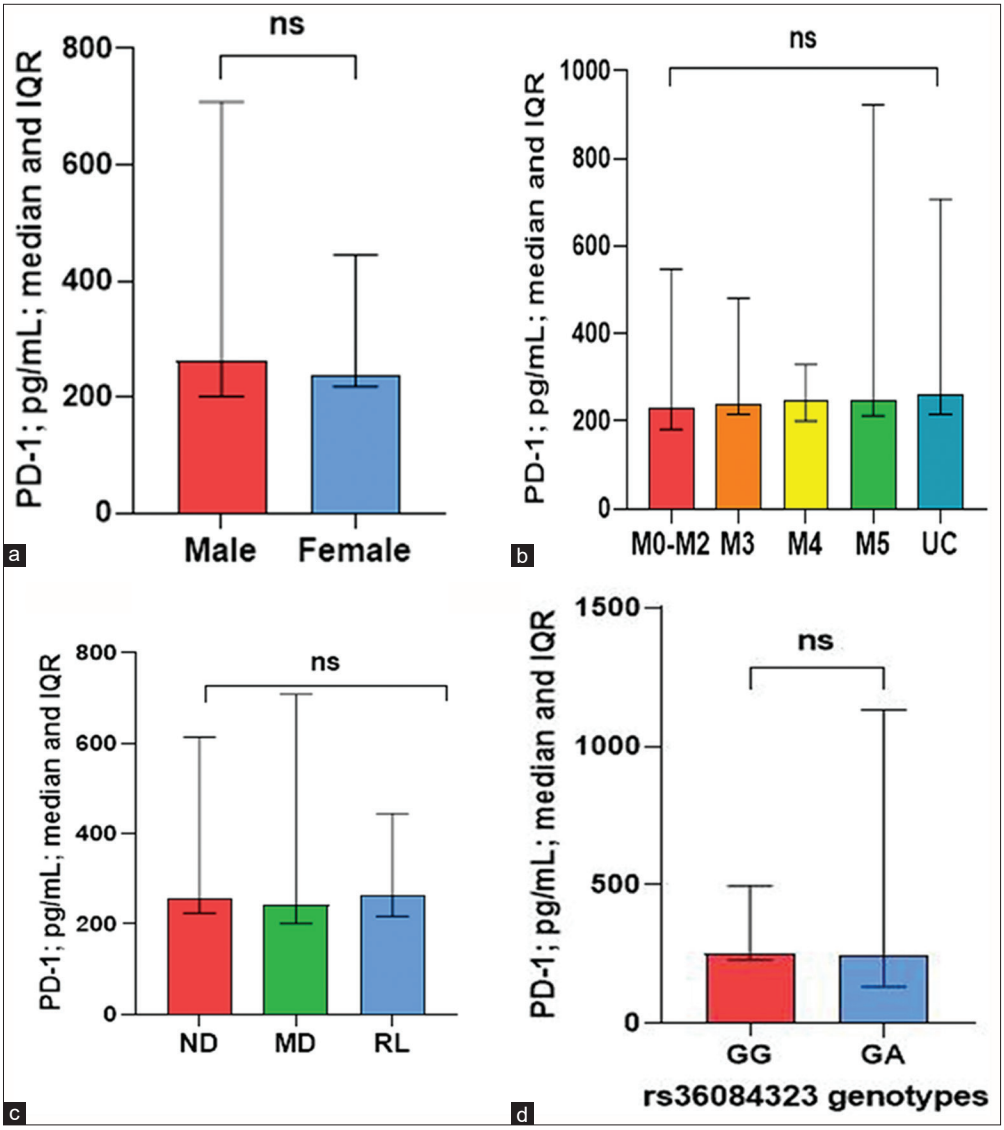

Figure 4: Assessment of PD-1 level in acute myeloid leukemia (AML) patients. (a) Classified by sex, (b) According to FAB classification, (c) According to AML course of treatment, (d) Classified by rs36084323 genotypes. IQR: Interquartile range, PD-1: Programmed cell death protein 1

Table 1: Allele and genotype frequencies of rs36084323 single-nucleotide polymorphism in acute myeloid leukemia patients and healthy controls

Allele/ genotype	AML patients, *n* (%)	Control, *n* (%)	OR	95% CI	*P*
G	170 (85.0)	150 (75.0)	Reference		
A	30 (15.0)	50 (25.0)	0.53	0.32–0.87	0.017
GG	70 (70.0)	50 (50.0)	Reference		
GA	30 (30.0)	50 (50.0)	0.43	0.24–0.76	0.006

AML: Acute myeloid leukemia, OR: Odds ratio, CI: Confidence interval, *n*: Number

Human malignancies exhibit various genetic and epigenetic alterations, resulting in the formation of neoantigens that have the potential to be detected by the immune system.[18] Tumors develop multiple resistance mechanisms, including local immunosuppression, tolerance generation, and systemic T-cell signaling dysfunction.[19-21]

Moreover, cancers employ many mechanisms to evade immune destruction. Several checkpoints are implemented to regulate this early immune response and prevent the development of anti-tumor immune reactions. Immune checkpoint pathways that have the potential to be targeted for anticancer therapy include PD-1 which is responsible for regulating T-cell activation and peripheral tolerance and preventing tissue damage during immune responses. Significant endeavors have been made to advance immunotherapeutic strategies for the treatment of cancer, which have stemmed from these discoveries. These include the development of immune checkpoint pathway inhibitors, such as anti-PD-L1 therapy.[22-24]

In the current study, levels of PD-1 did not show significant differences according to sex, type of AML, and clinical course of the disease. In a study by Chen *et al*., G allele of rs36084323 was found to be associated with the risk of relapse in ALL and AML patients;[25] since this SNP is located in the promotor region of the PD-1, the author suggest that it may impact transcriptional expression and modify the coding of amino acids,[25] this was observed in the current study with the levels of PD-1 was significantly higher in the AML patients.

A recent study that examined PD-1 gene polymorphism in AML found that it was several SNPs (rs10204525[26] and rs2227982) associated with increased susceptibility for AML, the prognosis of AML, and rs10204525 associated with anthracycline induction response[27] (rs10204525 located in the 3′ UTR of the PD-1 gene, and rs2227982 is located in the 5th exon[28]), this study suggests the importance of polymorphisms in PD-1 gene in AML.

The activation of the MAPK/ERK signaling pathways by a subset of murine and human AML blasts was demonstrated in a previous study.[29] Programmed death-1 (PD-1) is a cell surface receptor that regulates T-cell exhaustion and functions as a T-cell checkpoint. Evidence also suggests that PD-1 (expressed on melanoma cells) is critically important for cancer progression. While PD-1 involvement in host T-cells has received much attention in AML, its function in AML cells has received little attention.[30] The current study showed that serum PD-1 levels have no significant difference in AML patients classified by rs36084323 polymorphisms GG and GA genotypes (251.9 vs. 242.1).

CONCLUSION

The rs36084323 on PD-1 is an important diagnostic marker in AML, with G allele and GA SNP associated with increased risk of AML; this finding is important for further studies about its prognostic and therapeutic benefits for AML.

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Conflicts of interest
There are no conflicts of interest.

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