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Research Article

Assessment of mRNA Levels of Tumor Antigen (PRAME) and Clinical Outcomes in Newly Diagnosed Cases of Acute Leukemia

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Abstract

Background: Although PRAME's expression in normal tissue is inconsequential, it is an oncogene in many solid tumors and hematological malignancies; nonetheless, its function and mode of action in acute leukemic cells are still up for discussion. We aimed to expose the relationship between PRAME gene expression and acute leukemia patients with clinical outcomes. ***Objectives***: To examine acute leukemia's expression of the primarily expressed antigen of melanoma "PRAME" and its clinical implications. ***Methods***: A real-time PCR using the Cyber Green test was used to determine the amount of PRAME mRNA expression in peripheral blood cells from 40 patients with acute leukemia and 10 samples from healthy individuals. An analysis of the relationship between the clinical outcome and PRAME gene expression was done. ***Results***: Out of the 50 samples that were obtained, 28% were male and 22% were female. The mean age was 34.3 and 34.3 years for the patients and controls, respectively. Of these, 26(52%) had AML, 14(28%) had ALL, and 10(20%) had voluntary health control. In AL patients, PRAME gene expression was significantly higher (0.643) than in healthy people (0.0468). There were no significant differences between the various types of AL. However, the PRAME mRNA levels showed statistically significant correlation with clinical outcomes. ***Conclusions***: In AML, the PRAME gene is highly expressed, and it may be a helpful indicator for monitoring minimal residual illness; on the other hand, it was linked to a poor prognosis for AML patients.

Keywords: Acute Leukemia, Cancer testing antigen, Monitors minimal residual disease, PRAME gene expression.

تقييم مستويات mRNA لمستضد الورم (PRAME) والنتائج السريرية في حالات سرطان الدم الحاد التي تم تشخيصها حديثا

الخلاصة

الخلفية: على الرغم من أن تعبير PRAM في الأنسجة الطبيعية غير منطقي، إلا أنه جين مسرطن في العديد من الأورام الصلبة والأورام الخبيثة الدموية؛ ومع ذلك، فإن وظيفته وطريقة عمله في خلايا سرطان الدم الحادة لا تزال مطروحة للمناقشة. كنا نهدف إلى الكشف عن العلاقة بين التعبير الجيني PRAME ومرضى سرطان الدم الحاد مع النتائج السريرية. **الأهداف**: فحص تعبير ابيضاض الدم الحاد عن مستضد الورم الميلانيني المعبر عنه بشكل أساسي "PRAME" وآثاره السريرية. **الطرائق**: تم استخدام تفاعل البوليميراز المتسلسل في الوقت الفعلي باستخدام اختبار Cyber Green لتحديد كمية تعبير PRAME mRNA في خلايا الدم المحيطية من 40 مريضا مصابا بسرطان الدم الحاد و 10 عينات من أفراد أصحاء. تم إجراء تحليل للعلاقة بين النتيجة السريرية والتعبير الجيني PRAME. **النتائج**: من بين 50 عينة تم الحصول عليها ، كان 28٪ من الذكور و 22٪ من الإناث. كان متوسط العمر 34.3 و 34.3 سنة للمرضى والضوابط ، على التوالي. من بين هؤلاء ، كان 26 (52٪) يعانون من ابيضاض الدم النقوي الحاد، و 14 (28٪) لديهم ابيضاض الدم النقوي الحاد، و 10 (20٪) لديهم رقابة صحية طوعية. في مرضى AL ، كان التعبير الجيني PRAME أعلى بشكل ملحوظ (0.643) منه في الأشخاص الأصحاء (0.0468). لم تكن هناك فروق ذات دلالة إحصائية بين الأنواع المختلفة من AL. ومع ذلك، أظهرت مستويات PRAME mRNA ارتباطا ذا دلالة إحصائية مع النتائج السريرية. **الاستنتاجات**: في ابيضاض الدم النقوي الحاد، يتم التعبير عن جين PRAME بشكل كبير، وقد يكون مؤشرا مفيدا لمراقبة الحد الأدنى من المرض المتبقي؛ من ناحية أخرى، تم ربطه بتشخيص ضعيف لمرضى ابيضاض الدم النقوي الحاد.

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INTRODUCTION

Unusual lymphoid and myeloid cell proliferation is the cause of acute leukemia (AL), a neoplastic illness [1]. The most prevalent heterogeneous acute leukemia in adults is acute myeloid leukemia (AML), which is often linked to a poor prognosis [2,3]. Ikeda et al. used autologous cloning of T-cell epitopes to identify PRAME, a tumor-associated antigen, among patients suffering from metastatic cutaneous melanoma [4,5]. The PRAME gene, which is around 12 kilobases long and has leucine-rich repeat domains, is found on the reverse strand of chromosome 22 (22q11.22) [6]. Based on reports, the PRAME gene is hypomethylated in most cancerous cells but hypermethylated in healthy tissues. The

PRAME gene triggers autologous cytotoxic T-cell-mediated immunological responses and encodes a membrane-bound protein [7]. Many types of cancer have elevated PRAME levels [8]. Leukemic cells have high levels of PRAME gene mRNA expression [9], and there is a correlation between the expression levels and leukemia remission and relapse. [10]. Additionally, the PRAME gene is highly expressed in chronic myeloid leukemia, multiple myeloma, AML, and ALL [11]. According to research by Tanaka *et al*., PRAME boosted leukemia cell proliferation, encouraged cell cycle development, and prevented leukemia cells from undergoing apoptosis. It also changed the expression of genes related to erythroid differentiation [12]. Additionally, Oehler *et al*. demonstrated that PRAME prevented HSPCs and LPCs from differentiating into myeloid cells [13]. Most pathologists believe that evaluating sentinel lymph nodes based only on their appearance is simple. However, it might be difficult to evaluate nodal melanocytic deposits [14-16]. It is currently unclear how PRAME gene expressions are regulated. On the other hand, it has been demonstrated to be altered by epigenetic processes such as DNA methylation, being hypomethylated in cancerous cells and hypermethylated in most healthy tissues [17]. Therefore, by inhibiting RAR (retinoic acid receptor) signaling, PRAME overexpression in various cancers seems to contribute to molecular processes of tumor development and decreased overall survival [18]. Because PRAME is a distinct lymphoma subtype that, even after transplantation, has a poor response and/or a high recurrence rate to standard chemotherapy, it would be a potential target for treatment [19,20]. Leukemic cells' drug resistance and the effects of chemotherapy may be ascertained by ongoing gene expression monitoring throughout treatment [21].

METHODS

Study design and setting

This is a case-control study in which, for the PRAME gene expression analysis, peripheral blood samples were obtained from 40 patients with newly diagnosed "acute leukemia AL and 10 controls as healthy. Of those individuals, 26 had AML (52%), and 14 had ALL (28%), with a control group of 20%. The average proportion of blast cells" in peripheral blood and bone marrow was 77.7% and 67%, respectively (Table 1).

Table 1: Characteristics of Acute leukemia patients and controls Variable Samples n (%) Blast average (%).

Variable		n(%)	Blast-Average (%)
Groups	AML	26(52)	62(15-97)
	ALL	14(28)	59(3-98)
	Control	10(20)	

During follow-up, medical hematologists helped the tumor registry files to capture patient clinical data such as blast percentage in the peripheral blood, "bone marrow, platelet count, hemoglobin, and WBC count non-response (NR), and complete response (CR). Different reactions were seen in every patient treated in accordance with the chemotherapy procedures adopted by the Hematology Unit of the Baghdad Teaching Hospital. Each patient had two rounds of induction and consolidation. Treatment response that was categorized as complete response (CR) persisted for almost six months. In the bone marrow aspirate following chemotherapy induction, the cellularity was over 20%, the blast cell percentage was less than 5%, and leukemia was absent in other areas. After at least two cycles of chemotherapy, leukemia in other areas was suggested, and over 5% of "blast cells" were seen in the bone margin in non-response (NR) patients. "CR and NR" were noted following each induction cycle.

Inclusion criteria

Patients with acute leukemia have recently been diagnosed. The patients' ages ranged from 16-72 years. Assessed the prognostic or clinical outcome related with mRNA level of PRAME gene.

Exclusion criteria

Patients are early diagnosed with pre-leukemia, those with myelodysplastic syndrome (MDS), and those with chronic leukemia, in addition to those who died after the first or second induction, were excluded.

Outcome measurements

We documented complete remission (CR) and non-responding patients (NR) from tumor registry files with the help of consultant hematologists. The patient's clinical data comprised hemoglobin, platelet count, WBC count, and blast in peripheral blood and bone marrow. The reaction of each patient varied based on whether they received treatment in accordance with the chemotherapy regimens recommended in the Hematology Unit of the Baghdad Teaching Hospital. There were two cycles of induction and consolidation for every patient. Under exceptional circumstances, the treatment response lasted for beyond six months and was categorized as complete remission (CR). Bone marrow aspirate required more than 20% cellular and less than 5% blast cells after induction chemotherapy and absent leukemia elsewhere. A patient who has had at least two chemotherapy treatments and more than 5% blast cells in their bone marrow or other signs of leukemia is classified as a non-responder (NR) [22]. We measured NR and CR after each cycle of induction.

Sample preservation

The blood samples were stored in TRIzol at the National Center for Early Detection of Tumors'

genetic laboratory in the "medical metropolis of Baghdad, Iraq. 2 ml of peripheral blood were collected, and all samples were kept at -80°C following TRIzol treatment. The Molecular Oncology Unit at Guy's Hospital, King's College, London, UK, analyzed the samples using real-time PCR and reverse transcription for molecular analysis after first extracting the RNA.

RNA extraction and RT-PCR assay

TRIzol® LS Reagent was used to extract total RNA from each pair of blood samples (Life Technologies-Ambion US) in accordance with the manufacturer's instructions. The reverse transcription of total RNA was performed using the high-capacity cDNA reverse transcription kit (Life Technologies, Ambion, USA) in accordance with the manufacturer's instructions. After then, cDNA was stored until it was needed at -80°C. SYBR Green assessed the gene expression (Table 2) using primers produced using Primer 3 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Table 2: The Sequences of Primers

Primers	Sequences
PRAME-F	5′ - CTTTCCTCGAAGGCCACCT - 3′
PRAME-R	5′- GTTATTGTGAGGACCTTTAACGA - 3′
ABL-F	5′ - TGGAGATAACACTCTAAGCATAACTAAAGGT - 3′
ABL-R	5′ - GATGTAGTTGCTTGGGACCCA - 3′

The cDNA was serially diluted to generate the standard curve. Standard curves for target and endogenous control genes [ABL] were generated. Three duplicates of the quantitative real-time PCR experiments were conducted using the Applied Biosystems 7900 Real-Time PCR equipment.

Real-time quantitative analysis of PCR data

In comparison to an untreated normal control, the quantity of the target PRAME gene was standardized for an endogenous ABL gene also determined using the following formula: 2-ΔΔCt ABI PRISM 7700 Sequence Detection System 1997 (User Bulletin No. 2, 1997). The relative expression level was determined by using the comparative CT technique threshold cycle, and to determine the degree of expression for different blood samples, comparing them with a calibrator. Standard curves for relative quantification are not necessary when using the comparative CT technique if the PCR efficiency of the target and reference genes is similar. The gene expression fold change is calculated via 2-ΔΔCt, where ΔΔCt= ΔCt target- ΔCt untreated for calibration and normalized via ΔCt= Ct target gene – Ct endogenous reference.

The relative effectiveness of the genes ABL and PRAME

The effectiveness of the reference amplification and the target amplification must be roughly comparable for the ΔΔCT calculation to be considered genuine ABI PRISM 7700 Sequence Detection System 1997 (User Bulletin No. 2, 1997) [23]. Using the standard curve for PRAME and ABL required data, the efficiency of the target amplification and the efficiency of the reference amplification were determined using the formula "E= 10^(-1/slop).

Statistical analysis

The mean and standard deviation were used to represent numerical data, whereas frequency and percentage were used to represent qualitative data. The PRAME gene expression in patients and control samples was assessed using a paired sample t-test. Three or more groups were compared using a one-way ANOVA. Chi-square and independent t-tests were used to examine the connection between PRAME gene expression and clinical parameters. A *p*-value <0.05 was deemed statistically significant. The data was analyzed using SPSS version 16.0 (Chicago, USA).

RESULTS

A total number of forty de novo AL patients in this study with ten healthy volunteers were categorized as a healthy group (Table 3); there was no statistically significant difference observed between each group.

Table 3: Summary of the characteristics of the included control and leukaemia patients

Gender	Patients n(%)	Controls n(%)	Total n(%)	*p*-value
Female	17(77.273)	5(22.727)	22(100)	0.669
Male	23(82.143)	5(17.857)	28(100)	0.984
Total%	40(80)	10(20)	50(100)	

The mean ± SD age of patients (ranging from 16 to 72 years) (Table 4). By using qRT-PCR, the PRAME gene expression levels in 50 peripheral blood samples were determined. There is an inverse relationship between the amount of target nucleic acid and the Ct value (cycle threshold) of the PRAME product, e.g., the more target nucleic acid and the lower the Ct level are present.

Table 4: Age groups of leukaemia patients with percentage

		n(%)	Mean±SD
Age group (year)	16-19	10(25)	17.5±0.972
	20-29	9(22.5)	23.667±2.5496
	30-39	6(15)	34.667±2.805
	40-49	7(17.5)	44.857±7.741
	50+	8(20)	57.75±7.7416
Total		40(100)	

Significant positive responses are indicated by Ct values less than 29, which suggests that the target nucleic acid is present in great quantities in the sample. Moderate amounts of the target nucleic acid are indicated by positive responses with Cts between 30 and 37. Weak reactions with tiny amounts of the target nucleic acid are indicated by Cts of 38–40. (Figure 1).

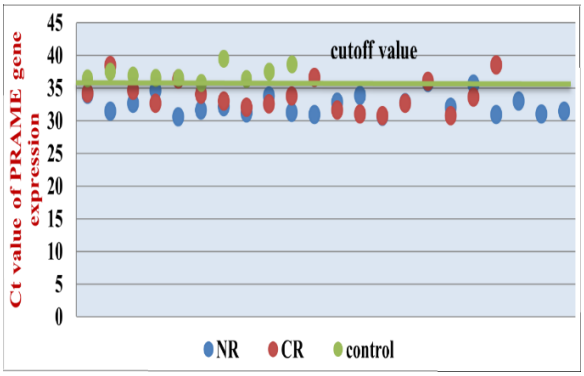

Figure 1: The Ct value of PRAME gene expression.

PRAME mRNA was studied in 40 (80%) cases with acute leukemia and 10(20%) healthy controls. The PRAME mRNA level was between 0.0001 and 6.495 (0.0001-6.495; 0.643±1.465). Fold change in the study group, PRAME showed 0.0003-0.41 (0.0468±1.128) fold change in controls. There was an important difference between PRAME gene expression levels in patients and with control (p=0.0152). On the other hand, when we compare between the PRAME fold change and the clinical outcome, it indicates modest significant associations between the NR AL patients with control (p=0.0802), while no statistically different effect is observed between CR and control (p=0.1009) (Table 5).

Table 5: The comparison between acute patients of PRAME gene expression according to clinical outcome.

Study groups	n(%)	Mean±SD	*p*-value
Patients	40(80)	0.643±1.465	0.0152
Control	10(20)	0.0468±1.128	
Total	50(100		
NR patients	21(42)	0.607±1.38	0.876
CR patients	19(38)	0.682±1.590	
Control	10(20)	0.0468±1.128	0.0802
Total	50(100)		0.1009

According to the types of acute leukemia, the expression levels between AML and ALL patients were observed to be statistically significant in AML compared with ALL (p=0.027) but showed no significant differences with clinical outcome in each type (p=0.968 and 0.182) (Table 6).

Table 6: The comparison between types of acute leukemia of PRAME gene expression with clinical outcome

Patients	n(%)	Mean±SD	*p*-value
AML patients	26(52)	0.931±1.753	0.027
ALL patients	14(28)	0.106±0.233	
Control	10(20)	0.047±013	0.0197
Total	50(100)		0.435
AML-NR Patients	12(30)	0.946±1.77	0.968
AML-CR Patients	14(35)	0.9189±1.809	
ALL-NR Patients	9(22.55)	0.156±0.284	0.182
ALL-CR Patients	5(12.5)	0.017±0.021	
Total	40(100)		

From the total of 26 (52%) cases of AML, the FAB calcification showed the rate of PRAME gene expression was (31% M3, 27% M2, and AML, 8% M5, and 7% M1) (Figure 2).

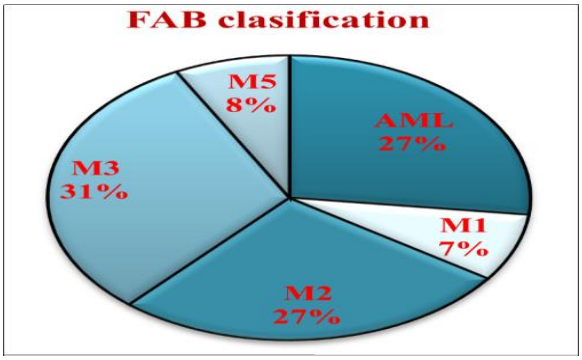

Figure 2: Distribution of AML patients according to FAB classification.

DISCUSSION

PRAME is abundantly expressed in a variety of cancer types and has a role in cell proliferation, apoptosis, differentiation, and metastasis, and cancer patient outcomes. Numerous studies have demonstrated a negative correlation between poor outcomes and elevated PRAME expression [3,8]. According to a study, overexpression of PRAME prevents retinoic acid (RA)-induced cell differentiation, growth arrest, and apoptosis, indicating that PRAME functions as a signaling inhibitor of the retinoic acid receptor (RAR). Therefore, PRAME upregulation promotes carcinogenesis by blocking the RA/RAR signaling pathway. Following PRAME knockdown, DNA microarray analysis of HL cells showed that multiple genes were regulated, including down-regulation of known anti-apoptotic factors. Additionally, increased expression of the retinoic acid metabolizing cytochrome P450 (CYP26B1), a transcriptional target of retinoic acid signaling, indicated increased retinoic acid signaling in these cells [4,5,24,25]. The PRAME gene family is restricted in normal human tissues, but it is transcriptionally active in the germline throughout the whole life cycle and is crucial for gametogenesis. Some of its members are exclusive to either male or female germ cells [6,7,26,27]. In this study, the PRAME gene expression was assessed in peripheral blood samples from ten healthy volunteers and forty leukemic patients. PRAME expressions differ significantly between AML and ALL compared to control (p=0.015). Whereas when we compare clinical outcomes, the results revealed the significant associations between the NR AL patients with control (p= 0.08) and no statistically significant effect between CR and control (p= 0.101). Many studies have shown that the "PRAME gene expression" is limited in normal tissue and highly expressed in hematological malignancy, especially in leukemia, and confirmed that PRAME is a useful target for treating AML [8-10,3,28]. Additionally, PRAME is a useful genetic marker for monitoring MRD and identifying remission in AML patients [4,6]. Epigenetic changes promote high PRAME expression in chemo-resistant HL cell lines, and PRAME hypomethylation may result in elevated expression of PRAME in AML and CML blast

crises, which would raise PRAME's carcinogenic potential [11,12,29,30]. PRAME may be a tumor suppressor in hematological malignancies, according to several controversial publications. According to some research, PRAME expression is linked to a better prognosis for hematological cancers, including AML and ALL [13-15,31-33]. On the other hand, High PRAME expression has been linked to a better prognosis in leukemia, and PRAME overexpression reduces proliferation and enhances leukemia cell death by upregulating p53 and downregulating S100A4 [16,17,34,3]. Based on the FAB classification, the current study revealed that the gene expression rates were 31% M3, 27% M2, 31% AML, 8% M5, and 7% in M1. In 38.2% of the 34 patients with acute myelogenous leukemia, the PRAME gene was expressed. In tune with our study, Ding *et al*. (2012) demonstrated that the rate of gene expression in AML FAB classification was 80% M3, 33.3% M2, and 28.6% in M5 [28]. The specific tumor antigens CA9, WT1, and PRAME are recently interesting indicators as diagnostic and prognostic tools in acute leukemia [35-37].

Study limitations

This study has the following limitations: We were unable to conduct a test set analysis due to the small size of the trial, and the number of patients participating was insufficient for further randomized investigations. Furthermore, differing sample sizes and the limited ability to follow up with AML patients for an extended period may explain these disparities.

Conclusion

Our findings provide persuasive evidence that the PRAME gene tumor antigen could be used as an early indication of the prognosis of acute myeloid leukemia. While a low level of the PRAME gene was associated with a favorable prognosis in AML patients, overexpression of the protein was associated with poor clinical outcomes. Further testing is required, however, because this level did not correspond to their response to induction chemotherapy.

Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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