Acute promyelocytic leukemia (APL) is a distinct and rare subtype of acute myeloid leukemia accounting for 10–15% of all acute myeloid leukemias (AMLs) in a year \(^1\). In 90% of cases, APL is characterized by the reciprocal translocation t (15;17) (q22; q21) (retinoic acid receptor-alpha (RARA) gene on chromosome 17 is involved in a translocation with the promyelocytic leukemia gene (PML) on chromosome 15). t(15;17) giving rise to a PML/RARA gene fusion product which prevent the expression of genes involved in granulocytes proliferation and differentiation, cease the differentiation of myelocytes, and cause endless proliferation \(^2, 3\). The most effective drugs for APL treatment are All-trans retinoic acid (ATRA) and arsenic trioxide (As2O3). They induce PML/RARα chimeric oncoprotein

**Introduction**

Acute promyelocytic leukemia (APL) is a distinct and rare subtype of acute myeloid leukemia accounting for 10–15% of all acute myeloid leukemias (AMLs) in a year \(^1\). In 90% of cases, APL is characterized by the reciprocal translocation t (15;17) (q22; q21) (retinoic acid receptor-alpha (RARA) gene on chromosome 17 is involved in a translocation with the promyelocytic leukemia gene (PML) on chromosome 15). t(15;17) giving rise to a PML/RARA gene fusion product which prevent the expression of genes involved in granulocytes proliferation and differentiation, cease the differentiation of myelocytes, and cause endless proliferation \(^2, 3\). The most effective drugs for APL treatment are All-trans retinoic acid (ATRA) and arsenic trioxide (As2O3). They induce PML/RARα chimeric oncoprotein

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**Abstract**

**Background** Differentiation syndrome (DS) is an inflammatory complication seen in some patients with acute promyelocytic leukemia (APL) undergoing differentiation therapy with all-trans retinoic acid (ATRA) and/or arsenic trioxide (ATO). It is unknown how DS occurs, but it is believed that it is caused by inflammatory cytokines release from differentiating leukemic cells. High mobility group box-1 (HMGB1) is a DNA-binding protein that acts as a cytokine outside of cells and may play a role in inflammation. This study was conducted to determine whether HMGB1 polymorphisms (rs1360485, rs2249825 and rs1060348) are associated with the incidence of differentiation syndrome in acute promyelocytic leukemia patients treated with all-trans retinoic acid and arsenic trioxide.

**Methods** One hundred and thirty APL patients and 100 healthy controls were included. Seventeen patients with differentiation syndrome were selected according to the PETHEMA criteria. Tetra-primer ARMS polymerase chain reaction (tetra-ARMS PCR) was used to determine the genotype distribution of polymorphisms. DNA sequencing was done to validate the results.

**Results** In both healthy and APL patients, AA was the most frequent genotype in rs1360485 followed by AG and GG. CC, CG, and GG were the most frequent genotypes in rs2249825 polymorphism in the order mentioned. CC was more frequent than CT, and CT was more frequent than TT in rs1060348. There was no correlation between HMGB1 polymorphisms and the incidence of differentiation syndrome based on genetic models (p-value > 0.05).

**Conclusions** HMGB1 polymorphisms are not probably associated with DS development in APL patients treated with ATRA and ATO.

**Keywords** Acute Promyelocytic Leukemia · Differentiation syndrome · HMGB1 · SNPs
APL was formerly considered one of the most fetal subtypes of AML, but treatment with arsenic trioxide and all-trans retinoic acid has improved the outcomes in recent years [6]. In some APL patients, ATRA and/or arsenic trioxide administration may results in a life-threatening complication that is called differentiation syndrome (DS) [7, 8]. This complication occurs in massive leukemic blast presence not during consolidation or maintenance therapy. The main characteristic features of DS are: fever, acute respiratory distress with interstitial pulmonary infiltrates, and/or a vascular capillary leak syndrome leading to acute renal failure. These main symptoms may primarily cause by cellular migration, endothelial activation, release of interleukins, and vascular factors responsible for tissue damage [9, 10]. Montesinos et al., reported 24.8% treated patients experienced DS, 12.6% were severe and 12.2% moderate cases. [11]. The pathogenesis of DS is not fully understood, but it is believed cytokines released from differentiating leukemic cells lead to systemic inflammatory response syndrome (SIRS) in DS [12, 13].

High mobility group box1 (HMGB1), also called amphoterin and p30, is a highly conserved protein that belongs to the HMGB family. It is extremely versatile and unique with extracellular and intracellular functions [14]. Inside cells, in the nucleus, it binds to the DNA with no sequence specificity and acts as a chaperon and regulates the transcription of various genes. However, it acts as a cytokine and may cause inflammation out of cells. Several studies have shown that HMGB1 polymorphisms play an important role in inflammatory diseases and cancer development [15–21]. It can also play role in hematopoietic malignancies like leukemia, lymphoma, myelodysplastic syndrome and multiple myeloma [22]. Several studies have shown that HMGB1 and its polymorphisms play important role in inflammatory diseases [14, 19, 21, 23, 24].

Due to the importance of HMGB1 gene variations and their role in inflammatory diseases, this study was conducted to assess the association between rs1360485, rs2249825, and rs1060348 polymorphisms of the HMGB1 gene and DS incidence in APL patients treated with ATRA and ATO.

### Materials and methods

**Participants**

The Ethical Committee of Tehran University of Medical Sciences approved the study (IR.TUMS.SPH.REC.1397.271). This study was conducted on APL patients who presented to the Hematology, Oncology, and Stem Cell Transplantation Research Center of Shariati Hospital, Tehran, Iran from 2012 to 2017. Real-time PCR was done for the presence of...
Results

Baseline characteristics

One hundred and thirty APL patients (70 males and 60 females) and 100 healthy controls (47 males and 33 females) were studied. The mean age of the APL patients and control subjects was 36.5 and 36.9 years respectively, indicating no significant difference (P value > 0.05). The hematologic and laboratory findings of the APL patients with and without DS are presented in (Table 2). The allele and genotype distribution of rs1360485, rs2249825 and rs1060348 did not deviate from the Hardy–Weinberg equilibrium in APL patients and normal subjects (P value > 0.05). Chi-square and Mann-Whitney U tests showed no significant differences in sex and age between DS and non-DS patients, respectively (P value > 0.05).

Electrophoresis results

Electrophoresis results are presented in (Fig. 1). The product of tetra ARMS-PCR for rs1360485 contained three fragments in heterozygote samples (433 bp, 284 bp (G allele) and 205 bp (A allele)). Two fragments of 433 bp and 284 bp were seen in the GG mutant homozygous samples, and the AA homozygous samples contained 433 bp and 25 bp fragments (Fig. 1 A). In rs2249825, heterozygous samples contained 481 bp, 293 bp (G) and 245 bp (C) fragments. The CC homozygous wild type contained two fragments of 481 bp and 245 bp and the GG homozygous mutant type contained two fragments of 481 bp and 293 bp (Fig. 1 B). In rs1060348, heterozygous samples contained three fragments of 267 bp, 189 bp (C) and 132 bp (T). CC homozygous wild samples contained two fragments of 267 bp and 189 bp and TT homozygous mutant samples contained tow fragments of 267 bp and 132 bp (Fig. 1 C).

| Table 2 Demographic characteristics and peripheral blood counts in APL patients with and without DS |
|-------------------|---------|---------|-----------|
|                  | Non-DS  | Ds      | Total     | P Value  |
| Men               | 63      | 7       | 70        | 0.7      |
| Women             | 50      | 10      | 60        |          |
| Age               | 35 ± 13 | 38.5 ± 15 | 36.13 ± 5.35 | 0.3       |
| Hb                | 9.2 ± 2.6 | 8 ± 1.7  | 9.2 ± 4.6  | 0.4       |
| WBC ×10^3         | 65.51 ± 12.23 | 61.33 ± 9.87 | 64.19 ± 9.96 | 0.6       |
| PLT ×10^3         | 76.2 ± 51.6 | 44.9 ± 33.7 | 73.15 ± 42.6 | 1.3       |

Values presented as mean (±SD), Hb: Hemoglobin, WBC: White Blood Cells, PLT: Platelet
Correlation of HMGB1 gene polymorphisms with differentiation syndrome in APL patients

We further analyzed the association between the rs1360485, rs2249825 and rs1060348 polymorphisms and the risk of DS under five genetic models (codominant, dominant, recessive, over dominant and log-additive) in APL patients treated with ATRA and ATO using multi variant logistic regression analysis to estimate the OR and 95% CI of this association. Based on five genetic models used, there was no significant association between rs1360485, rs2249825 and rs1060348 HMGB1 polymorphisms and DS (P value > 0.5).

Five genetic models that were used to study rs1360485 and rs2249825 are shown in (Table 4). The results showed no significant association between rs1060348 and DS development based on genetic models (data not shown).

Discussion

The results of the genetic models in the present study revealed that there were no significant associations between HMGB1 SNPs (rs1360485, rs2249825 and rs1060348) and DS susceptibility in APL patients. However, several studies have indicated the role of HMGB1 in progression of inflammatory diseases [16, 18, 19, 21, 26–32].

Tang et al. found HMGB1 stimulated inflammatory cytokines such as TNF-α and IL1-β secretion by MEK/ERK signaling and led inflammation in DS [27]. It has been demonstrated that some HMGB1 polymorphisms play an important role in inflammatory diseases and cancers. Wang et al. found that the presence of the G allele in rs2249825 reduced HMGB1 gene expression and was associated with a lower
risk of rheumatoid arthritis [16]. In 2015, Jin reported that rs2249825 C/G SNP increased the expression of HMGB1 and led to recurrent pregnancy loss HMGB1 as an inflammatory protein is present in chorionic villi and inflammation in chorionic villi can cause recurrent pregnancy loss [19]. The other study indicated that the presence of one G allele in rs1360485 and one G allele in rs2249825 increased the risk of breast cancer development [18].

Severe systemic inflammations may result in systemic inflammatory response syndrome (SIRS). The underlying mechanism of the disease is not well understood but Kornblit et al. showed some HMGB1 gene polymorphism affect the mortality. They found that the presence of rs1060348 (982 C>T) in exone 4 of the HMGB1 gene decreased the serum concentration of HMGB1 and was associated with SIRS development and patient survival [28]. In addition, Coa revealed that rs1360485 polymorphism plays role in neonatal necrotizing enterocolitis in Chinese neonates [29].

Trauma is life threatening and improper immune inflammatory response causes sepsis and multiple organ dysfunction syndrome (MODS). It seems that genetic variations in cytokines play a great role in immune inflammatory response as Zeng showed HMGB1 polymorphism (rs2249825) was associated with sepsis and MODS in trauma patients and patients with G allele were more susceptible to sepsis mortality [30].

In another study, the association between hepatitis B virus infection and HMGB1 polymorphisms was evaluated. The results showed that homozygosity of rs2249825 C/G minor allele might increase overall survival and progression-free survival of patient with hepatitis B virus infection [31].

It has been shown that genetic variation in cytokine genes regulates the immune reactions after allogeneic hematopoietic cell transplantation (HCT). The interaction between antigen-presenting cells (APCs) and donor T lymphocytes is a crucial property of allogenic HCT. In addition, HMGB1 is important for the activation of APC. Kornblit showed that patient homozygosity for the rs2249825 C/G minor allele was associated with increased overall survival, progression free survival [32]. The results of the present study showed that the A allele was more frequent in the rs1360485 SNP and the C allele was more frequent in rs2240825 and rs1060348 SNPs in APL patients.

In consistence with our study, Choi studied the effect of rs2249825 (HMGB1 polymorphism) on severity of Febrile seizer (FS). This disease is caused by fever in children younger than 6 years and the fever is closely related with inflammatory cytokines release. It has been reported that HMGB1 level was high in these patients. The results showed that this polymorphism was not associated with FS [33, 34].
DS symptoms are not specific and different criteria are used to diagnose this complication. In the present study, strict diagnostic criteria were used to select DS patient, which limited the DS population and could have influenced the results.

**Conclusions**

Based on the results, the HMGB1 polymorphisms probably are not associated with DS development in APL patients treated with ATRA and ATO, however, larger studies are required to confirm our results.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07386-1.

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**Authors’ Contributions** GH: Acquired data, Drafted the manuscript.

AO: Originated the study and substantively revised manuscript.

BC: Acquired and interpreted data.

ZM: Performed statistical analysis, interpreted data.

KAM: Design some part of the work.

SAM: Design some part of the work.

SHR: Made substantial contributions to the conception, design of the work.

All authors have approved the submitted version and have agreed both to be personally accountable for the author’s own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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**Table 4 Analysis of association of HMGB1 polymorphism (rs1360485 and rs2249825) with the development of DS in APL patients**

| Models       | Genotype | With DS (%) | Without DS (%) | OR*(95%CI*) | P-value | AIC*   | BIC*   |
|--------------|----------|-------------|----------------|-------------|---------|--------|--------|
| rs1360485    |          |             |                |             |         |        |        |
| Codominant   | AA       | 12 (70.6)   | 78 (69)        | 1           | 0.47    | 107.2  | 118.7  |
|              | AG       | 5 (29.4)    | 30 (2.6)       | 0.95 (0.31–2.96) | 0.85    | 106.7  | 115.3  |
|              | GG       | 0           | 5 (4.4)        | 0           |         |        |        |
| Dominant     | AA       | 12 (70.6)   | 78 (69)        | 1           | 0.85    | 106.7  | 115.3  |
|              | AG-GG    | 5 (29.4)    | 35 (31)        | 1.11 (0.36–3.43) | 0.83    | 106.7  | 115.3  |
| Recessive    | AA-AG    | 17 (100)    | 108 (95.6)     | 1           | 0.22    | 105.2  | 113.8  |
|              | GG       | 0           | 5 (4.4)        | 0           |         |        |        |
| Overdominant | AA-GG    | 12 (70.6)   | 83 (73.5)      | 1           | 0.83    | 106.7  | 115.3  |
|              | AG       | 5 (29.4)    | 30 (26.6)      | 0.89 (0.29–2.74) | 0.63    | 106.5  | 115.1  |
| Log-additive | ---      | ---         | ---            | 1.27 (0.47–3.44) | 0.63    | 106.5  | 115.1  |

| Models       | Genotype | With DS (%) | Without DS (%) | OR*(95%CI*) | P-value | AIC*   | BIC*   |
|--------------|----------|-------------|----------------|-------------|---------|--------|--------|
| rs2249825    |          |             |                |             |         |        |        |
| Codominant   | CC       | 12 (70.6)   | 82 (72.6)      | 1           | 0.35    | 106.6  | 118.1  |
|              | CG       | 5 (29.4)    | 25 (22.1)      | 0.73 (0.23–2.82) | 0.88    | 106.7  | 115.3  |
|              | GG       | 0           | 6 (5.3)        | 0           |         |        |        |
| Dominant     | CC       | 12 (70.6)   | 82 (72.6)      | 1           | 0.88    | 106.7  | 115.3  |
|              | CG-GG    | 5 (29.4)    | 31 (27.4)      | 0.92 (0.30–2.82) | 0.51    | 106.3  | 114.9  |
| Recessive    | CC-CG    | 17 (100)    | 107 (94.7)     | 1           | 0.18    | 104.9  | 113.5  |
|              | GG       | 0           | 6 (5.3)        | 0           |         |        |        |
| overdominant | CC-GG    | 12 (70.6)   | 88 (77.8)      | 1           | 0.51    | 106.3  | 114.9  |
|              | CG       | 5 (29.4)    | 25 (22.1)      | 0.68 (0.22–2.11) | 0.79    | 106.6  | 115.2  |
| Log-additive | ---      | ---         | ---            | 1.13 (0.44–2.93) | 0.79    | 106.6  | 115.2  |

OR, odds ratio; CI, confidence interval; AIC: Akaike Information criterion; BIC: Bayesian Information Criterion.
Declarations

Conflict of interest All authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants (Healthy Controls and patients) included in the study.

References

1. de Thé H (2018) Differentiation therapy revisited. Nat Rev Cancer 18:117–127
2. Deschler B, Lübbert M (2006) Acute myeloid leukemia: epidemiology and etiology. Cancer 107:2099–2107
3. Wang Z-Y, Chen Z (2008) Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 111:2505–2515
4. Dore AI, Santana-Lemos BA, Coser VM, Santos FL, Dalmazzo LF, Lima AS et al (2007) The association of ICAM-1 Exon 6 (E469K) but not of ICAM-1 Exon 4 (G241R) and PECAM-1 Exon 3 (L125V) polymorphisms with the development of differentiation syndrome in acute promyelocytic leukemia. J Leukoc Biol 82:1340–1343
5. Luersink M, Pennings JL, Wissink WM, Linssen PC, Muus P, Pfu¨ndt R et al (2009) Chemokine induction by all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia: triggering the differentiation syndrome. Blood 114:5512–5521
6. Ryan MM (2018) Acute Promyelocytic Leukemia: A Summary. J Adv Pract Oncol 9:178–187
7. Tsai W, Shih C, Lin C, Hsu F, Hsu H (2008) MCP-1 in the migration of differentiated leukemic cells toward alveolar epithelial cells. European Respiratory Journal 2008
8. Rogers JE, Yang D (2012) Differentiation syndrome in patients with acute promyelocytic leukemia. J Oncol Pharm Pract 18:109–114
9. Montesinos P, Sanz MA (2011) The differentiation syndrome in patients with acute promyelocytic leukemia: experience of the pethema group and review of the literature. Mediterr J Hematol Infect Dis 3:e2011059
10. Jimenez JJ, Chale RS, Abad AC, Schally AV (2020) Acute promyelocytic leukemia (APL): a review of the literature. Oncotarget 11:992–1003
11. Montesinos P, Bergua JM, Vellenga E, Rayon C, Parody R, de la Serna J et al (2009) Differentiation syndrome in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline chemotherapy: characteristics, outcome, and prognostic factors. 113: 775 – 83
12. Rego EM, De Santis GC (2011) Differentiation syndrome in promyelocytic leukemia: clinical presentation, pathogenesis and treatment. Mediterr J Hematol Infect Dis 3:e2011048
13. Stahl M, Tallman MS (2019) Differentiation syndrome in acute promyelocytic leukaemia. British journal of haematology 187: 157 – 62
14. Kianian F, Kadkhodaei M, Sadeghipour HR, Karimian SM, Seifi B (2020) An overview of high-mobility group box 1, a potent pro-inflammatory cytokine in asthma. J Basic Clin Physiol Pharmacol 31
15. Jiang M, Li X, Quan X, Li X, Zhou B (2018) Single Nucleotide Polymorphisms in HMGB1 Correlate with Lung Cancer Risk in the Northeast Chinese Han Population. Molecules (Basel, Switzerland) 23.
16. Wang LH, Wu MH, Chen PC, Su CM, Xu G, Huang CC et al (2017) Prognostic significance of high-mobility group box protein 1 genetic polymorphisms in rheumatoid arthritis disease outcome. Int J Med Sci 14:1382–1388
17. Qiu P, Wang L, Ni J, Zhang Y (2019) Associations between HMGB1 gene polymorphisms and susceptibility and clinical outcomes in Chinese Han sepsis patients. Gene 687:23–29
18. Huang BF, Tzeng HE, Chen PC, Wang CQ, Su CM, Wang Y et al (2018) HMGB1 genetic polymorphisms are biomarkers for the development and progression of breast cancer. Int J Med Sci 15:580–586
19. Jin H, Wu J, Yang Q, Cai Y, He W, Liu C (2015) High mobility group box 1 polymorphism affects susceptibility to recurrent pregnancy loss by up-regulating gene expression in chorionic villi. J Assist Reprod Genet 32:1123–1128
20. Wang Y, Li XP, Yin JY, Zhang Y, He H, Qian CY et al (2014) Association of HMGB1 and HMGB2 genetic polymorphisms with lung cancer chemotherapy response. Clin Exp Pharmacol Physiol 41:408–415
21. Kornblit B, Mumthe-Fog L, Madsen HO, Strom J, Vindelov L, Garsed P (2008) Association of HMGB1 polymorphisms with outcome in patients with systemic inflammatory response syndrome. Crit Care 12:R83
22. Yuan S, Liu Z, Xu Z, Liu J, Zhang J (2020) High mobility group box 1 (HMGB1): a pivotal regulator of hematopoietic malignancies. J Hematol Oncol 13:91
23. Ulloa L, Messmer D (2006) High-mobility group box 1 (HMGB1): protein: friend and foe. Cytokine Growth Factor Rev 17:189–201
24. Magna M, Pisetsky DS (2014) The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. Mol Med 20:138–146
25. Mohammadzadeh Z, Omidkhoda A, Chahardouli B, Hoseinzadeh G, Moghaddam KA, Mousavi SA et al (2021) The impact of ICAM-1, CCL2 and TGM2 gene polymorphisms on differentiation syndrome in acute promyelocytic leukemia. BMC Cancer 21:46
26. Cardinale L, Asteggianno F, Moretti F, Torre F, Ulisciani S, Fava C et al (2014) Pathophysiology, clinical features and radiological findings of differentiation syndrome/all-trans-retinoic acid syndrome. World J Radiol 6:583–588
27. Tang L, Chai W, Ye F, Yu Y, Cao L, Yang M et al (2017) HMGB1 promotes differentiation syndrome by inducing hyperinflammation via MEK/ERK signaling in acute promyelocytic leukemia cells. Oncotarget 8:27314–27327
28. Goodwin GH, Sanders C, Johns EW (1973) A new group of chro-matin-associated proteins with a high content of acidic and basic amino acids. Eur J Biochem 38:14–19
29. Cao H, Guo D (2021) Association of High-Mobility Group Box 1 (HMGB1) Gene Polymorphisms with Susceptibility and Better Survival Prognosis in Chinese Han Neonatal Necrotizing Enterocolitis. Med Sci Monit 27:e930015
30. Zeng L, Zhang AQ, Gu W, Chen KH, Jiang DP, Zhang LY et al (2012) Clinical relevance of single nucleotide polymorphisms of high mobility group box 1 protein gene in patients with major trauma in southwest China. Surgery 151:427–436
31. Deng CQ, Deng GH, Wang YM (2013) HMGB1 gene polymorphisms in patients with chronic hepatitis B virus infection. World J Gastroenterol 19:5144–5149
32. Kornblit B, Masmas T, Petersen SL, Madsen HO, Heilmann C, Schjøbel L et al (2010) Association of HMGB1 polymorphisms with outcome after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 16:239–252
33. Choi J, Choi SA, Kim SY, Kim H, Lim BC, Hwang H et al (2019) Association Analysis of Interleukin-1β, Interleukin-6, and HMGB1 Variants with Postictal Serum Cytokine Levels in Children with Febrile Seizure and Generalized Epilepsy with Febrile Seizure Plus. J Clin Neurol 15:555–563

34. Zhu M, Chen J, Guo H, Ding L, Zhang Y, Xu Y (2018) High Mobility Group Protein B1 (HMGB1) and Interleukin-1β as Prognostic Biomarkers of Epilepsy in Children. J Child Neurol 33:909–917

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