Original Article

Toll-Like Receptor 4, 2, and Interleukin 1 Receptor Associated Kinase4: Possible Diagnostic Biomarkers in Myelodysplastic Syndrome Patients

Parvin Khalilian[1], Nahid Eskandari[1,2], Mohammad Jafar Sharifi[3], Mohammad Soltani[1], Pardis Nematollahi[4]

[1]Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Applied Physiology Research Center, Isfahan Cardiovascular Research Institute, Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Laboratory Sciences, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran, [4]Department of Pathology, School of Medicine, Cancer Prevention Research Center, Seyyed Al-Shohada Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Myelodysplastic syndrome (MDS) is a clonal hematologic disorder that requires the integration of morphologic, cytogenetic, hematologic, and clinical findings for a successful diagnosis. Trying to find ancillary tests such as biomarkers improve the diagnosis process. Several studies showed that a disordered immune system is associated with MDS. The chronic activated innate immune system, particularly the Toll-like receptors (TLRs) pathway could be involved in the induction of the inflammation.

Materials and Methods: In the present study, we investigated the expression of *TLR2, TLR4*, and *IRAK4* in bone marrow (BM) of MDS patients, the leukemia group, and the healthy group. For this purpose, we assessed the expression of *TLR2, TLR4*, and *IRAK4* by real time-PCR.

Results: In line with new findings, we demonstrated that the expression of *TLR2, TLR4*, and *IRAK4* significantly increased in MDS BM compared with the healthy group. Moreover, *IRAK4* expression raised significantly in MDS patients compared with other studied hematologic neoplasms. Also, the expression levels of *TLR2* and *TLR4* significantly increased in MDS in comparison to some studied non-MDS malignancies ($P < 0.05$). Receiver operating characteristics (ROC) analysis and area under the curve (AUC) suggested that the expression of *TLR2, TLR4*, and *IRAK4* (AUC = 0.702, AUC = 0.75, and AUC = 0.682, respectively) had acceptable diagnostic values to identify MDS from the other understudied leukemias.

Conclusion: Overall, the expression of *TLR2*, *TLR4,* and *IRAK4* could be potential biomarkers for discriminating MDS from some hematologic disorders.

Keywords: IRAK4, myelodysplastic syndrome, TLR2, TLR4

Address for correspondences: Dr. Nahid Eskandari, Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: neskandari@med.mui.ac.ir
Submitted: 21-Feb-2023; **Revised:** 02-May-2023; **Accepted:** 10-May-2023; **Published:** 26-02-2024

Introduction

MDS is a preleukemia disorder characterized by peripheral blood cytopenia, infective hematopoiesis, and dysplastic morphology in one or more lineages. Impaired proliferation and differentiation by hematopoietic stem cells induce apoptosis in bone marrow (BM). A serious concern about MDS patients is the possibility of disease progression and transformation to AML.[1]

The spectrum of symptoms is highly variable and patients can be asymptomatic at the time of diagnosis.[2] MDS can occur at any age; however, the risk of MDS increases with age. The onset of the disease is about 65–70 years old in most populations but is earlier in some Asian populations.[3,4]

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_67_23

How to cite this article: Khalilian P, Eskandari N, Sharifi MJ, Soltani M, Nematollahi P. Toll-like receptor 4, 2, and interleukin 1 receptor associated kinase4: Possible diagnostic biomarkers in myelodysplastic syndrome patients. Adv Biomed Res 2024;13:17.

Diagnosis of MDS and discrimination from the other types of cytopenia and mimic malignancies could be challenging.[2] Diagnosis of MDS is based on the morphological assessment of peripheral blood, BM aspirate, and biopsy after observation of abnormal complete blood count (CBC) evidence. Karyotype, next-generation sequencing (NGS), and flow cytometry can also help to improve diagnosis.[5,6]

The pathogenesis of MDS is complex and heterogeneous. MDS pathology is associated with genetic mutations, chromosomal abnormality, epigenetics alteration, and changes in the microenvironment ofBM. Moreover, the chronic inflammation induced by dysregulation of the immune system could be considered a critical factor in the pathogenesis of this disorder because inflammation could influence on microenvironment and damage precursors of the hematopoietic cells.[7-9]

Recent studies reported dysregulation of the innate immune system factors such as TLRs pathways in MDS. TLRs signaling could regulate hematopoietic homeostasis.[8,10,11] Long-term activation of TLR pathways signal impaired normal hematopoiesis.[12]

Several studies showed that TLRs and their effector molecules are overexpressed in MDS patients.[13-15]

TLR2 and 4 produce inflammatory cytokines that maintain TLR pathways as a positive feedback loop.[16]

Administration of TLR2 agonist in isolated cells from normal BM diminishes the population of erythroid progenitor cells. Also erythroid colony differentiation increases following TLR2 inhibition in low-risk MDS patients.[17]

Constitutive and chronic TLR4 signaling leads to production of reactive oxygen species (ROS) and DNA damage. Accumulation of damaged DNA might be the basis of malignancy development.[18]

IRAK4 is an important downstream molecule in TLRs signaling and interacts with MYD88. The IRAK4 participates in myddosome formation and activation of NF-κB, JNK, p38, and MAPK in TLRs pathway.[19] Cheng *et al*.[20] reported IRAK4 can be related to malignancy and patient overall survival. Moreover, long isoform of IRAK4 (IRAK4-L), which is a mutated form in some malignancies such as AML and MDS, can boost NF-κB inflammatory responses.[21,22]

In the present study, we investigate the expression levels of *TLR2, TLR4*, and *IRAK4* in BM cells of MDS patients, healthy donors, and some leukemia patients. We assessed the potential capacity of TLR2, TLR4, and IRAK4-L expression as biomarkers to discriminate MDS from other hematologic disorders. Biomarkers could improve the diagnosis and differentiation of MDS from other hematologic disorders.

Materials and Methods

Patient characteristics

Patients with myelodysplastic syndromes (MDS), non-myelodysplastic syndrome hematological cancers, and healthy controls who attended Seyed-Al Shohada Hospital, Isfahan, Iran were enrolled in the study. The demographic data of patients are summarized in Table 1.

BM samples of 53 MDS suspected patients were collected. After the final approval, 27 newly diagnosed, MDS patients entered the study. The diagnosis of MDS patients was based on morphological criteria presented by the 2016 revision of the WHO Classification of Myelodysplastic Syndromes.[23] None of the MDS patients have autoimmune diseases, childhood MDS, or malignancy history. The most frequent symptoms among patients with MDS were weakness and lethargy.

Patients with non-MDS hematological cancers including AML (n = 14), ALL (n = 10), CLL (n = 7), and MM (n = 6). Non-MDS hematological cancers were selected based on the laboratory findings, physician diagnosis, and no prior treatment. Patients with cytopenia due to megaloblastic anemia, Idiopathic Thrombocytopenic purpura (ITP), autoimmune disorder, drug use, dysplasia associated with megaloblastic anemia, and malignancies history were excluded.

Also, the healthy group included five persons with normal BM and CBC results, and no history of cancer.

Of note, the age difference between the groups was not statistically significant.

Sample collection

150–400 μL EDTA samples of BM aspirated from volunteers were collected before treatment, and then samples were stored at −70°C. Sample collection was performed from September 2020 to November 2021.

RNA isolation and quantitative real-time PCR

Total RNA from bone marrow aspirated samples were extracted using the total RNA extraction kit (Roje technologies, RNjia phenol free pb kit, Yazd, Iran) according to the manufacturer's protocol. We assessed RNA integrity by gel electrophoresis and evaluated its concentration at 260 nm. Purity was assessed by calculating the ratio for absorbance at 260 nm versus 280 nm (A260 nm/A280 nm) by using a Nanodrop.

Complementary deoxyribonucleic acid (cDNA) synthesis was performed by reverse transcription kit (Pars Tous, Mashhad, Iran) following to manufacturer's protocol. Quantitative real-time polymerase chain reaction (qPCR) was performed using an ABI7700 machine (Applied Biosystems, Foster City, CA, USA) and by the SYBR Green Master Mix (Biofact Co. South Korea) according to the manufacturer's instructions. The reaction mixture contained 5 μL SYBER Green Master Mix, 0.5 μL of each primer, 3 μL deoxyribonuclease (DNase)-free and ribonuclease (RNase)-free water and, 1 μL cDNA in total a volume of 10 μL.

The *TLR-2, TLR-4, IRAK4*, and housekeeping gene (GAPDH) specific primers sequences are listed in Table 2. All genes were

Table 1: Study participants demographic data

	Age; Mean (SD), years	Gender Male/Female	Numbers	Sub-group
MDS	69±11	18/9	27	MDS-SLD (5) MDS-MLD (8) MDS-RS (2) MDS-EB-1 (2) MDS-EB-2 (3) MDS-U (7)
Non-MDS hematologic disorder	60±20	25/12	37	AML (14) ALL (10) CLL (7) MM (6)
Healthy group	56±4	2/3	5	

AML: acute myeloid leukemia, ALL: acute lymphocytic leukemia, CLL: chronic lymphocytic leukemia, MM: multiple myeloma, MDS-SLD: Myelodysplastic syndrome with single lineage dysplasia, MDS-MLD: Myelodysplastic syndrome with multilineage dysplasia, MDS-RS: Myelodysplastic syndrome with ring sideroblasts, MDS-EB-1: Myelodysplastic syndrome with excess blast, subtype 1, MDS-EB-2: Myelodysplastic syndrome with excess blast, subtype 2, MDS-U: myelodysplastic syndrome-unclassifiable

Table 2: Specific primers sequencies for real-time PCR

Target gene	Forward primer	Reverse primer
TLR-2	CAAATGACGGTACATCCACG	GGGTAAATCTGAGAGCTGCG
TLR-4	GTCGTGCTGGTATCATCTTC	TGTACCCACTGTTCCTTCTG
IRAK4[21]	GCTGCCTCAATGTTGGACTA	TCTGGACTTGAGGAGTCAGG
GAPDH	ACAGCCTCAAGATCATCAGC	TAGAGGCAGGGATGATGTTC

normalized with *GAPDH* as endogenous control. Relative quantification in comparison control groups was measured by pfaffl method ($R = \frac{E\ target^{\Delta CT\ target(control-sample)}}{E\ ref^{\Delta CT\ ref(control-sample)}}$ due to different efficiency of primers.[24]

Statistical analysis

All statistical analysis was performed with GraphPad Prism 9.3.0 (GraphPad Software, San Diego, CA). Numerical data were expressed as the mean ± standard error bar (SEM). The Kolmogorov–Smirnov test was applied to evaluate the normality of data. Independent sample *t*-test was performed for data with a normal distribution, while the comparisons of the groups with non-normal distribution were done using Kruskal-Wallis and Mann-Whitney test.

Biomarker efficiency and assessment of sensitivity and specificity was calculated by ROC) and AUC. The Youden index is used to evaluate optimal cutoff points. *P* value < 0.05 was considered statistically significant.

Results

Expression of TLR2, TLR4, and IRAK4 mRNA in MDS patients and healthy controls

The levels of *TLR2, TLR4,* and *IRAK4* expression were evaluated by real-time PCR in 27 MDS patients and 5 healthy controls. *TLR2* gene expression was significantly increased in BM cells of MDS patients in comparison with normal BM ($P = 0.004$). Additionally, the expression of *TLR4* and *IRAK4* genes was higher in the patients than in the control groups ($P = 0.048$, $P = 0.026$), respectively [Figure 1].

Expression of TLR2, TLR4, and IRAK4 mRNA in MDS patients and other hematologic malignancies

The levels of TLR2 mRNA expression among disease groups were highest in MDS (n = 27) compared with all other non-MDS hematologic malignancies, including CLL (n = 7; $P = 0.005$), MM (n = 6; $P = 0.003$) [Figure 2a], and no statistical difference in expression of *TLR2* was found between MDS and ALL (n = 10; $P = 0.160$) and AML (n = 14; $P = 0.564$) Then, we compared the expression of *TLR4* in BM cells of MDS patients versus other non-MDS malignancies. The level of *TLR4* in MDS enhanced as compared with AML ($P = 0.0493$), CLL ($P = 0.0476$), and MM ($P = 0.0477$) [Figure 2b]. So, we found that mRNA expression of *TLR4* increased in MDS patients compared with non-MDS hematologic malignancies except ALL patients ($P = 0.342$). In the present study, the expression of *IRAK4* in MDS patients was up-regulated compared with AML ($P = 0.0108$), ALL ($P = 0.0479$), CLL ($P = 0.0350$), and MM ($P = 0.0192$) [Figure 2c].

Discrimination of MDS from other hematologic disorders

To determine the diagnostic value of *TLR2, TLR4*, and *IRAK4* expression, groups with no significant differences were excluded and then a ROC/AUC analysis was performed to clarify the specificity and sensitivity. ROC curve analysis showed the potential of *TLR2* gene expression as a biomarker in distinguishing MDS from non-MDS disorders except AML and ALL ($P = 0.046$, AUC = 0.702). Furthermore, ROC curve

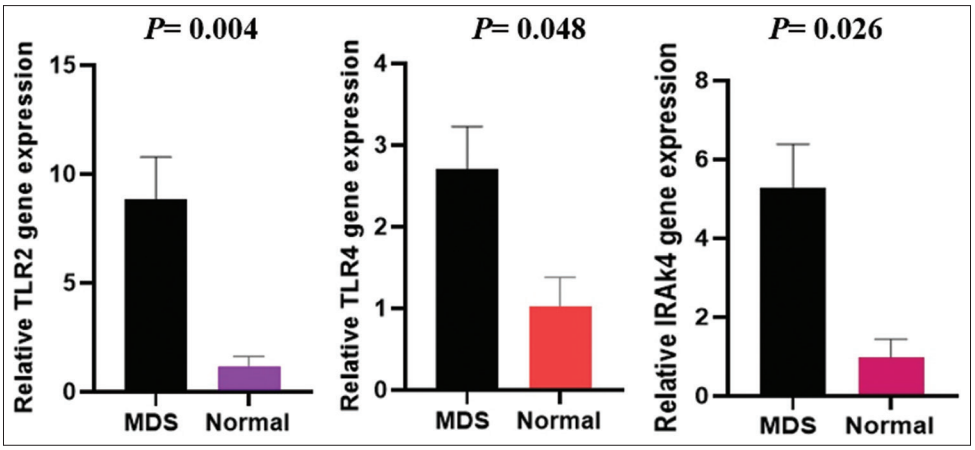

Figure 1: Comparison of *TLR2, TLR42, and IRAK4* gene expressions in BM cells of MDS patients and health controls by the quantitative reverse transcriptase-polymerase chain reaction. The healthy controls included five persons with normal bone marrow and no history of cancer. Error bars correspond to mean ± SEM; $P < 0.05$ is significant. MDS: myelodysplastic syndrome

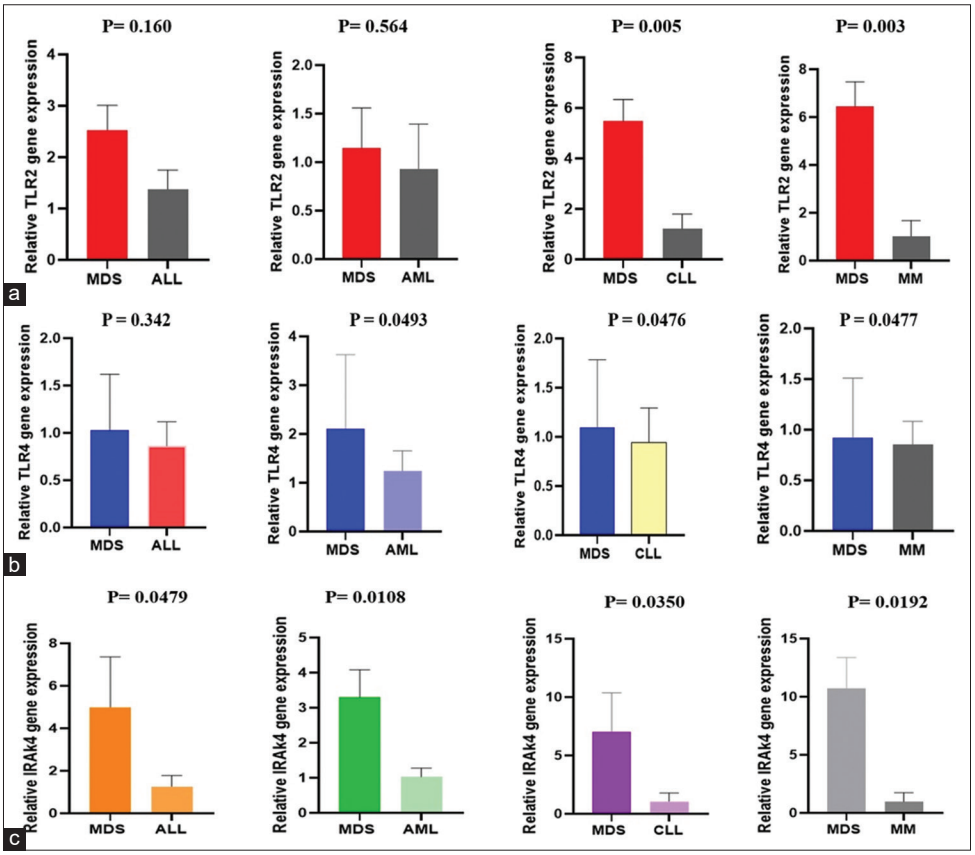

Figure 2: Quantification of *TLR2* (a), *TLR4* (b), and *IRAK4* (c) in BM cells of MDS and non-MDS disorders by the quantitative reverse transcriptase-polymerase chain reaction. Error bars correspond to mean ± SEM; $P < 0.05$ is significant. MDS: myelodysplastic syndrome, AML: Acute myeloid leukemia, ALL: Acute Lymphoblastic leukemia, CLL: Chronic Lymphoblastic leukemia, MM: Multiple myeloma

analysis suggested the *TLR4* gene expression as a potential biomarker to discriminate MDS from non-MDS malignancies except AML ($P = 0.006$, AUC = 0.75). Also, we got an acceptable diagnostic value of *IRAK4* gene expression in MDS differentiation from other hematologic disorders ($P = 0.031$, AUC = 0.682) [Figure 3]. Sensitivity, specificity, and other data of ROC curve analysis are summarized in Table 3.

Discussion

MDS diagnosis is complex and challenging due to the clinical symptoms' heterogeneity and nonspecific findings. Some drugs such as methotrexate or azathioprine, nutritional deficiencies (e.g. including Vitamin B12, iron, and copper deficiency), or infections-induced cytopenia, must be excluded from MDS

Table 3: Receiver operating characteristic (ROC) analysis of TLR2, TLR4, and IRAK

	AUC	95% CI	Sensitivity (%)	Specificity (%)	cutoff	PPV	NPV
TLR2	0.702	0.5137 to 0.8910	100	38.46	>0.071	0.74	1
TLR4	0.750	0.5834 to 0.9166	77.78	75	<0.372	0.70	0.81
IRAK4	0.682	0.5368 to 0.8408	88.24	45.16	>0.563	0.46	0.87

AUC: area under curve; CI: confidence interval; PPV: positive predict value; NPV: negative predict value

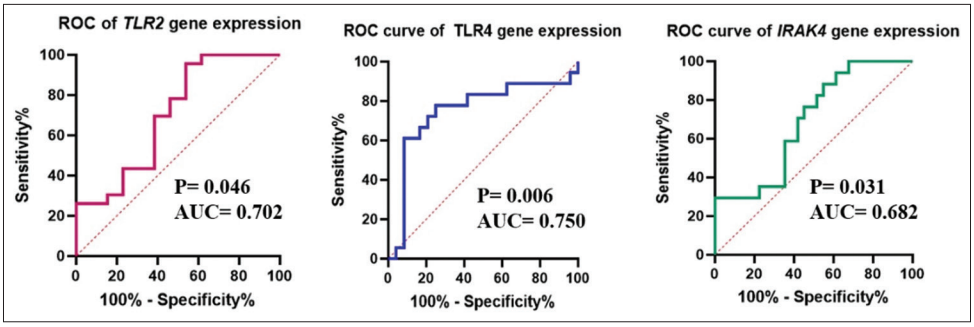

Figure 3: Receiver operating curve (ROC) analysis of *TLR2, TLR4*, and *IRAK4* gene expression to determine diagnostic accuracy in differentiation of MDS patients with other hematologic disorders. AUC: area under the curve

diagnosis.[25] Several methods are used to achieve a definitive diagnosis including morphology examination, cytogenetics, flow cytometry, and molecular tests. All of them provide different information to confirm the MDS diagnosis; however, the final diagnosis will be made by morphologic examination.[2]

In the current study, according to the role of the innate immune system in MDS, we investigated the expression of *TLR2*, *TLR4*, and *IRAK4* in a group of MDS patients and healthy subjects.

We found a significant increase in the expression of *TLR2* and *TLR4* genes in BM of MDS patients compared with normal groups (*P*value = 0.004, *P* value = 0.048, respectively). Similarly, Maratheftis *et al*.[13] reported elevated levels of *TLR4* expression in $CD34^+$ cells of BM and mononuclear cells of 21 MDS patients by reverse transcription-PCR. However, *TLR2* expression only increased in mononuclear cells of BM. Other similar studies reported that RNA expression of *TLR2* was significantly enhanced in $CD34^+$ cells of BM in MDS patients by Quantitative RT-PCR.[17,26] Velegraki *et al*.[27] showed that *TLR4* gene expression was significantly increased in BM $CD14^+$ cells population of 27 MDS patients compared with healthy controls by flow cytometry. They also reported the overexpression of other TLRs including TLR1, TLR2, TLR3, and TLR9 in these patients. However, this increase was not statistically significant. Recently, Paracatu *et al*. showed that *TLR2* upregulated in diverse BM cell populations such as $CD14^+$ cells, T and B lymphocytes, and $CD34^+$ cells of low/intermediate-risk MDS compared with high-risk and normal groups by mass cytometry.[28]

To determine the expression of the long isoform of IRAK4 in MDS BM, we used a pair of IRAK4 primers with flanking exon 4 in the Smith study. In the case of MDS and AML, Smith *et al*. found that IRAK4-L is generated by RNA splicing factor U2 small nuclear RNA auxiliary factor 1 (U2AF1) mutation that preserved exon 4.

IRAK4-L gained oncogenic activity because of N-terminal domain maintenance that interacts with MYD88 directly and induced maximum activation of NF-κB.[21] However, we did not succeed to measure the long isoform of *IRAK4* in MDS BM. This discrepancy with the Smith study may be due to CD34+ cell isolation in their work. In the present study, *IRAK4* expression was upregulated in MDS patients in comparison to normal BM ($P = 0.026$). *IRAK4* is a member of IRAKs family and a downstream molecule in signaling pathways of TLRs, IL-1R, and IL-18R. Deletion of *IRAK4* in mice models leads to disruption in TLRs pathways signaling.[29] Particularly, the administration of IRAK4 inhibitor reduced proliferation, viability, and cytokine production in cells isolated from CLL patients.[30] Moreover, treatment with IRAK4 inhibitor was assessed in immune-related diseases such as rheumatoid arthritis and psoriasis. So, it seems that IRAK4 inhibitor could be effective in MDS and AML treatment.[19,21]

High expression of *TLR2, TLR4*, and *IRAk4* in MDS BM cells is associated with inflammation promotion through hyperactivation of mitogen-activated protein kinase (MAPK) and NF-κB pathway. Several studies reported that NF-κB activation is increased in MDS cell lines and MDS patients.[14,31,32] Continuous activity of NF-κB and the subsequent inflammatory response may disrupt normal hematopoietic cells and causes cytopenias in MDS.[16,22,33]

Previous studies also showed that the mediator molecules of TLRs pathway such as E3 ubiquitin ligase TRAF6 and the TLR IL-1 receptor domain-containing adaptor protein (TIRAP) are enhanced in $CD34^+$ cells of MDS patients.[34-36]

To determine the diagnostic value of *TLR2, TLR4*, and *IRAK4* expression in MDS patients compared with understudied

leukemias, we compared the expression of *TLR2*, *TLR4*, and *IRAK4* between MDS and other leukemia patients including AML, ALL, CLL, and MM. Results showed that the expression of *TLR4*, *TLR2*, and *IRAK4* was significantly higher in MDS patients than in many other hematologic malignancies [Figure 2]. Ward *et al*.[37] also showed that the oxidized mitochondrial DNA level was significantly enhanced in the peripheral blood of MDS patients compared with other hematologic malignancy patients except CLL. Despite the molecular pathology similarities in MDS and AML, we found significant differences in *IRAK4* expression between AML and MDS patients.[21] being in the early stage of MDS may be the reason.

Also, we did not observe significant differences in *TLR2* expression in MDS patients compared with AML and ALL patients and *TLR4* gene expression in MDS and ALL groups. Other studies on *TLR2* and *TLR4* expression were not found in MDS and other leukemia to compare the result.

Biomarker identification would improve diagnosis and can be shortened this process, especially in low-risk MDS. The central role of the immune system in MDS was demonstrated by previous studies.[38]

Moreover, we assessed the diagnostic value of *TLR2, TLR4*, and *IRAK4* by ROC curve analysis. ROC analysis of *TLR2* gene expression showed an acceptable value to discriminate MDS from CLL and MM (AUC = 0.702, Se = 100%, Sp = 38.46%). The ROC/AUC analyses showed that *TLR4* gene expression (AUC = 0.75, Sensitivity = 77.78%, Specificity = 75%) can be effective in MDS discrimination from understudied leukemia except ALL. Furthermore, *IRAK4* gene expression (AUC = 0.682, Se = 88.24%, Sp = 45.16%) has an acceptable diagnostic value to identify MDS from other reviewed leukemia. The AUC results of *TLR2*, *TLR4*, and *IRAK4* were not perfect which may be due to the small sample size of leukemia groups.

However, recently more investigations conducted to find potential diagnostic biomarkers in MDS. Another study represented that plasma Oxidized mitochondrial DNA could be considered as a biomarker for MDS patients and hematologic malignancies except CLL.[37] The diagnostic utility of pyroptosis biomarkers in a cohort of MDS patients was confirmed by another study. Basiorka *et al*.[39] showed that plasma ASC could be a potential biomarker of pyroptosis in MDS.

In other studies, *TLRs* and *IRAk4* expression were assessed in most diseases. Evaluation of TLRs expression in breast cancer patients represented the higher expression of *TLR4* which is related to poor prognosis. as well as suggested that *TLR4* expression level could be a prognostic and survival biomarker in breast cancer.[40] Wang *et al*.[41] showed that *IRAK4* expression was higher in glioma tissue samples than in normal brain cells, and IRAK4 level was associated with poor survival of patients and would be the potential prognostic marker. In another study, *TLR4* expression has been indicated as a diagnostic biomarker in diabetic peripheral neuropathy.[42]

It seems that the potential capacity of *TLR2, TLR4*, and *IRAK4* expression as biomarkers could be considered in MDS diagnosis because they are candidates as diagnostic or prognostic biomarkers in different diseases and malignancies.

In previous studies, expression of TLRs effectors was performed in MDS patients.[10,43] We investigated the expression level of TLRs pathway throughout BM in MDS patients compared with other hematologic malignancies allowing exploitation for novel BM biomarkers that can be used as diagnostic tools and also, lead to manipulation as therapeutic strategies.

The limitation of our work is the small sample size due to the low incidence of disease and the assessment of whole BM cells. The different populations of BM cells must be isolated. TLRs expression may vary in different cell populations in any kind of leukemia that affects the results. Also, BM population frequencies must be considered between MDS and other leukemia. We suggested further studies with a larger sample size to assess the validity of these molecules as a biomarker. Alternative methods such as flow cytometry can be used to evaluate the accurate result. Also, next studies can evaluate the potential role of other TLRs in peripheral blood cells and BM as biomarkers in the diagnosis of MDS patients.

Conclusion

In the current study, we observed that the gene expression of *TLR2, TLR4*, and *IRAK4* are upregulated in MDS BM compared with healthy BM. Also, the expression of *TLR2, TLR4,* and *IRAK4* could be potential biomarkers for discrimination of MDS from some types of leukemias.

The study was approved by the Ethics Committee of the Isfahan University of Medical Sciences (IR.MUI.REC.1399.132), Isfahan, Iran.

Financial support and sponsorship

This study was supported by Isfahan university of medical sciences, Isfahan, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Gangat N, Patnaik MM, Tefferi A. Myelodysplastic syndromes: Contemporary review and how we treat. Am J Hematol 2016;91:76-89.
2. Weinberg OK, Hasserjian RP. The current approach to the diagnosis of myelodysplastic syndromes. Semin Hematol 2019;56:15-21.
3. Matsuda A, Germing U, Jinnai I, Misumi M, Kuendgen A, Knipp S, *et al*. Difference in clinical features between Japanese and German patients with refractory anemia in myelodysplastic syndromes. Blood 2005;106:2633-40.
4. Tinsley SM, Sutton SK, Thapa R, Lancet J, McMillan SC. Treatment choices: A quality of life comparison in acute myeloid leukemia and high-risk myelodysplastic syndrome. Clin Lymphoma Myeloma Leuk 2017;17S: S75-9.
5. Montalban-Bravo G, Garcia-Manero G. Myelodysplastic syndromes:

2018 update on diagnosis, risk-stratification and management. Am J Hematol 2018;93:129-47.
6. Hasserjian RP. Myelodysplastic syndrome updated. Pathobiology 2019;86:7-13.
7. Yang L, Qian Y, Eksioglu E, Epling-Burnette PK, Wei S. The inflammatory microenvironment in MDS. Cell Mol Life Sci 2015;72:1959-66.
8. Trowbridge JJ, Starczynowski DT. Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J Exp Med 2021;218:e20201544.
9. Matos A, Magalhães SMM, Rauh MJ. Immune dysregulation and recurring mutations in myelodysplastic syndromes pathogenesis. Adv Exp Med Biol 2021;1326:1-10.
10. Paracatu LC, Schuettpelz LG. Contribution of aberrant toll like receptor signaling to the pathogenesis of myelodysplastic syndromes. Front Immunol 2020;11:1236.
11. Cannova J, Breslin SJP, Zhang J. Toll-like receptor signaling in hematopoietic homeostasis and the pathogenesis of hematologic diseases. Front Med 2015;9:288-303.
12. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. Blood 2018;132:1553-60.
13. Maratheftis CI, Andreakos E, Moutsopoulos HM, Voulgarelis M. Toll-like receptor-4 is up-regulated in hematopoietic progenitor cells and contributes to increased apoptosis in myelodysplastic syndromes. Clin Cancer Res 2007;13:1154-60.
14. Wang C, Yang Y, Gao S, Chen J, Yu J, Zhang H, *et al*. Immune dysregulation in myelodysplastic syndrome: Clinical features, pathogenesis and therapeutic strategies. Crit Rev Oncol Hematol 2018;122:123-32.
15. Dimicoli S, Wei Y, Bueso-Ramos C, Yang H, Dinardo C, Jia Y, *et al*. Overexpression of the toll-like receptor (TLR) signaling adaptor MYD88, but lack of genetic mutation, in myelodysplastic syndromes. PLoS One 2013;8:e71120.
16. Banerjee T, Calvi LM, Becker MW, Liesveld JLJBr. Flaming and fanning: The spectrum of inflammatory influences in myelodysplastic syndromes. Blood Rev 2019;36:57-69.
17. Wei Y, Dimicoli S, Bueso-Ramos C, Chen R, Yang H, Neuberg D, *et al*. Toll-like receptor alterations in myelodysplastic syndrome. Leukemia 2013;27:1832-40.
18. Walter D, Lier A, Geiselhart A, Thalheimer FB, Huntscha S, Sobotta MC, *et al*. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature 2015;520:549-52.
19. Bahia MS, Kaur M, Silakari P, Silakari O. Interleukin-1 receptor associated kinase inhibitors: Potential therapeutic agents for inflammatory- and immune-related disorders. Cell Signal 2015;27:1039-55.
20. Cheng BY, Lau EY, Leung HW, Leung CO, Ho NP, Gurung S, *et al*. IRAK1 augments cancer stemness and drug resistance via the AP-1/AKR1B10 signaling cascade in hepatocellular carcinoma. Cancer Res 2018;78:2332-42.
21. Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, *et al*. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol 2019;21:640-50.
22. Gonzalez-Lugo JD, Verma A. Targeting inflammation in lower-risk MDS. Hematology Am Soc Hematol Educ Program 2022;2022:382-7.
23. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405.
24. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45.
25. Bejar R. Myelodysplastic syndromes diagnosis: What is the role of molecular testing? Curr Hematol Malig Rep 2015;10:282-91.
26. Zeng Q, Shu J, Hu Q, Zhou SH, Qian YM, Hu MH, *et al*. Apoptosis in human myelodysplastic syndrome CD34+cells is modulated by the upregulation of TLRs and histone H4 acetylation via a β-arrestin 1 dependent mechanism. Exp Cell Res 2016;340:22-31.
27. Velegraki M, Papakonstanti E, Mavroudi I, Psyllaki M, Tsatsanis C, Oulas A, *et al*. Impaired clearance of apoptotic cells leads to HMGB1 release in the bone marrow of patients with myelodysplastic syndromes and induces TLR4-mediated cytokine production. Haematologica 2013;98:1206-15.
28. Bennett J, Starczynowski DT. IRAK1 and IRAK4 as emerging therapeutic targets in hematologic malignancies. Curr Opin Hematol 2022;29:8-19.
29. Wang Z, Wesche H, Stevens T, Walker N, Yeh WC. IRAK-4 inhibitors for inflammation. Curr Top Med Chem 2009;9:724-37.
30. Giménez N, Schulz R, Higashi M, Aymerich M, Villamor N, Delgado J, *et al*. Targeting IRAK4 disrupts inflammatory pathways and delays tumor development in chronic lymphocytic leukemia. Leukemia 2020;34:100-14.
31. Fabre C, Carvalho G, Tasdemir E, Braun T, Adès L, Grosjean J, *et al*. NF-kappaB inhibition sensitizes to starvation-induced cell death in high-risk myelodysplastic syndrome and acute myeloid leukemia. Oncogene 2007;26:4071-83.
32. Ping Z, Chen S, Hermans SJF, Kenswil KJG, Feyen J, van Dijk C, *et al*. Activation of NF-κB driven inflammatory programs in mesenchymal elements attenuates hematopoiesis in low-risk myelodysplastic syndromes. Leukemia 2019;33:536-41.
33. Rupec RA, Jundt F, Rebholz B, Eckelt B, Weindl G, Herzinger T, *et al*. Stroma-mediated dysregulation of myelopoiesis in mice lacking I kappa B alpha. Immunity 2005;22:479-91.
34. Gopal A, Ibrahim R, Fuller M, Umlandt P, Parker J, Tran J, *et al*. TIRAP drives myelosuppression through an Ifnγ-Hmgb1 axis that disrupts the endothelial niche in mice. J Exp Med 2022;219:e20200731.
35. Choudhary GS, Pellagatti A, Agianian B, Smith MA, Bhagat TD, Gordon-Mitchell S, *et al*. Activation of targetable inflammatory immune signaling is seen in myelodysplastic syndromes with SF3B1 mutations. eLife 2022;11:e78136.
36. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood 2019;133:1039-48.
37. Ward GA, McGraw KL, Abbas-Aghababazadeh F, Meyer BS, McLemore AF, Vincelette ND, *et al*. Oxidized mitochondrial DNA released after inflammasome activation is a disease biomarker for myelodysplastic syndromes. Blood Adv 2021;5:2216-28.
38. Gañán-Gómez I, Wei Y, Starczynowski DT, Colla S, Yang H, Cabrero-Calvo M, *et al*. Deregulation of innate immune and inflammatory signaling in myelodysplastic syndromes. Leukemia 2015;29:1458-69.
39. Basiorka AA, McGraw KL, Abbas-Aghababazadeh F, McLemore AF, Vincelette ND, Ward GA, *et al*. Assessment of ASC specks as a putative biomarker of pyroptosis in myelodysplastic syndromes: An observational cohort study. Lancet Haematol 2018;5:e393-402.
40. Shi S, Xu C, Fang X, Zhang Y, Li H, Wen W, *et al*. Expression profile of Toll-like receptors in human breast cancer. Mol Med Rep 2020;21:786-94.
41. Wang J, Liu B, Yao J, Liu Z, Wang H, Zhang B, *et al*. Interleukin-1 receptor-associated kinase 4 as a potential biomarker: Overexpression predicts poor prognosis in patients with glioma. Oncol Lett 2021;21:254.
42. Zhu T, Meng Q, Ji J, Lou X, Zhang L. Toll-like receptor 4 and tumor necrosis factor-alpha as diagnostic biomarkers for diabetic peripheral neuropathy. Neurosci Lett 2015;585:28-32.
43. Paracatu LC, Monlish DA, Greenberg ZJ, Fisher DAC, Walter MJ, Oh ST, *et al*. Toll-like receptor and cytokine expression throughout the bone marrow differs between patients with low- and high-risk myelodysplastic syndromes. Exp Hematol 2022;110:47-59.