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Original Article

# Frequency of FLT3 ITD and FLT3 TKD Mutations in Multiple Myeloma Patients (A Case Control Study)

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## A B S T R A C T

**Background and Aims:** Multiple myeloma is a malignant proliferation of plasma cells derived from a single clone. The tumor, its products and the host response lead to organ damages. Some factors that are responsible in its pathogenesis are recognized. As FMS like Tyrosine Kinase 3 receptor (FLT3) mutation has been proved as a determining factor in leukemic patients; the goal of this study was to find association of FLT3 internal tandem duplication (ITD) and FLT3 **t**yrosine kinase domain (TKD) mutations with multiple myeloma**.**

**Materials and Methods:** This case-control study was conducted on 60 paraffin-embedded bone marrow biopsies (30 multiple myeloma and 30 normal bone marrow specimens) in the pathology departments of Ghaem and Imam Reza hospitals in Mashhad. After sections preparation, DNA was extracted and two PCR reactions were set up for detection of FLT3/ ITD and FLT3/TKD mutations.

**Results:** The Mean age of samples was 64±10 years. No FLT3 mutations were detected in multiple myeloma patients.

**Conclusion:** Our findings showed that occurrence of FLT3 mutations seem unusual in multiple myeloma.

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## Introduction

Hematopoiesis is adjusted by cytokine-induced arousal of different signal transductions to instigate differentiation and propagation of various cell lines [1-3]. FMS like Tyrosine Kinase 3 receptor (FLT3) whose gene's position is 13q12 [2, 4], induces normal hematopoiesis in progenitor cells [3-5]. It encodes a protein with 993 amino acids in length [6], which is highly expressed in liver, brain and placenta [3, 6, 7]. FLT3 belongs to class III tyrosine kinase receptor family [4], and is made up of five extracellular immunoglobulin-like domains, a short juxtamembrane intracellular segment and a transmembrane portion [7, 8].

Multiple myeloma (MM) is a kind of hematopoietic neoplasms, which is due to proliferation and differentiation of plasma cells in bone marrow. This disease causes some clinical symptoms such as hypercalcemia, renal failure as a result of high concentration of immunoglobulins in serum, anemia and bone lesions. All these symptoms emerge from proliferation of plasma cells in bone marrow [9].

Different FLT3 activating mutations have been detected, but two types are more common as described in detail previously [3, 10]; 1) internal tandem duplication (ITD) which is seen in 15-35% of adult patients with acute myeloid leukemia (AML) and 2) point mutation in tyrosine kinase domain as known D835 or TKD [1, 4, 6, 8, 10-13]. Both types stimulate FLT3 activation, followed by ligand binding in hematologic malignancy process [1]. ITD mutation was first discovered by Nakao et al. in 1996 [6, 14]. It is very important in prognosis prediction [5, 6]. ITD is associated with high risk of relapse in most AML cases [4, 10, 15, 16] whereas TKD does not have any effect in disease prognosis [7]. Studies state different frequencies for FLT3 ITD mutation. FLT3 ITD has been detected in up to 40% of AML, 10% of chronic myelogenous leukemia (CML), 10% of myelodysplastic syndrome (MDS), 10% of pediatric AML and rarely in acute lymphoblastic leukemia (ALL) cases [8, 12, 13, 17-20].

Limited numbers of studies have evaluated FLT3 mutations in a few MM cases, and little is known about its expression in MM. This study was performed to assess occurrence of FLT3 mutations in MM.

## Material and Methods

### Case selection

The specimens were selected from MM patients' tissue paraffin block in pathology departments of Ghaem and Imam Reza hospitals of Mashhad (Northeast of Iran) during September 2010 to 2014. Preparation, analysis and morphologic assessment of slides, DNA extraction and molecular analysis were performed in Cancer Molecular Pathology Research Center of Mashhad University of Medical Sciences. Plasma cell count of bone marrow was higher than 30% and patients were in symptomatic phase. A full pathological review consisting of bone marrow aspiration and bone marrow biopsy assessment was conducted on all slides to establish malignancy which was previously diagnosed. Repeat sections followed by hematoxylin and eosin staining were done for

specimens not having appropriate slides. Some cases were excluded from this study: specimens with plasma cell count less than 30% in bone marrow biopsies, samples with no sufficient tissue in biopsy paraffin blocks for DNA extraction, and cases with extracted DNA concentration less than 20 ng/ml). Control cases were obtained from normal bone marrow biopsies, matched by age and gender to be compared with MM population. Pathological review was performed for each person.

Finally 30 MM cases and 30 samples belonging to healthy population as control group were included in the study. Ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran approved this research.

**Molecular analysis**

For detection of mutation, DNA was extracted from 60 bone marrow biopsies using QIAamp tissue DNA extraction reagent according to the QIAGEN (USA) protocol. The concentration and quality of the DNA were analyzed with Nano Drop 2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). A ratio value of 1.8 was considered to indicate DNA purity. FLT3 mutations encoded by GATATC nucleotides sequence which exhibit EcoRV restriction were detected by restriction fragment length polymorphism (RFLP), followed by electrophoresis on 2.5% agarose gel and ethidium bromide staining. Primers were designed by Blast software (NCBI.gov website) and synthesized by METABION Company (Germany). The following primers were used for amplified ITD mutation region: 14 Forward 5' GCAATTTAG-GTATGAAAGCCAGC-3' and 15 Reverse 5' CTTTCAGCATTTTGACGGCAACC-3' were used over 35 cycles that included 1 minute 94°C, 30 seconds 60°C and 90 seconds 72°C. The following primers were performed for TKD mutation, too: 17 Forward 5' -CCGCCAGGAACGTGC-TG-3' and 17 Reverse 5'-GCAGCCTCACATTGCCCC-3' over 35 cycles that included 1 minute at 94°C, 1 minute at 66°C and 90 seconds at 72ºC. The master mix was composed of 1µl DNA (1µg/ml), 10 Pmol of each primer, 10mmol dNTP, 2.5 Unit EX-Taq DNA polymerase (Takara, Japan) in the buffer (10 mmol/l Tris–HCl (pH 8.3), 50 mmol/l KCl and 1.5 mmol/l $MgCl_2$). PCR procedure was performed in ABI verity thermocycler machinery. Amplified products were incubated with EcoRV restriction enzyme (thermo scientific, Lithuania) overnight in 37°C, and electrophoresis was performed on agarose gel.

ITD mutations were detected by agarose gel electrophoresis of the PCR products. This protocol usually demonstrates a wild-type band (328bp) and a larger-size band as ITD mutation. TKD mutations were detected by electrophoresis of the amplified products following digestion with EcoRV (Thermo scientific, Lithuania). The amplified products of wild-type alleles were digested into two bands (68 bp and 46 bp) by EcoRV. When amplified products contain TKD mutations, undigested bands (114 bp) are visualized on agarose gel electrophoresis. Inclusion of a negative control is essential to ensure complete digestion by EcoRV thereby eliminating the possibility of false-positive results in patient samples [15]. (Fig.1)

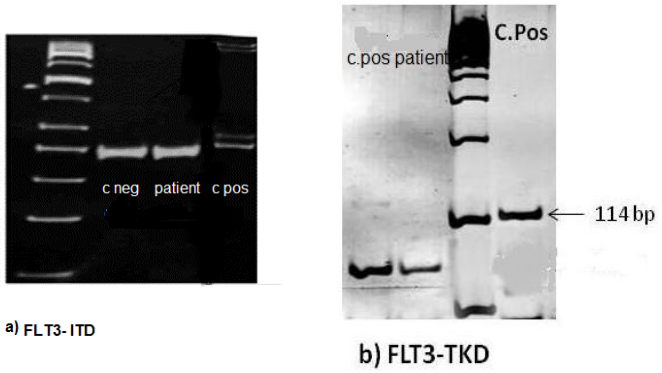


**Fig. 1.** Electrophoresis of PCR products related to FLT3 ITD (a) and TKD (b)
(a): PCR product of the FLT3/ITD mutation in the juxtamembrane domain. Positive control is included. (b): EcoRV restricted positive control related to FLT3-TKD.
C.pos: Positive control; C. neg: Negative control.

### Statistical analysis

Data management and analysis was performed using SPSS (Statistical software for social sciences-version 11.5). Differences between variables were evaluated by the chi-square test and t-test for categorical and continuous variables, respectively. All P-values were two-sided, and values less than 0.05 were considered to be significant.

## Results

No FLT3 mutation, either ITD or TKD was found among 30 multiple myeloma patients and subjects of the control group. Age comparison between the control and test groups is shown in Fig. 2.

Gender population assessment of 30 multiple myeloma cases revealed 14 (46.7%) and 16 (53.3%) as male and female, respectively. This gender ratio was the same in the control group.

Some diagnostic tests of case group are represented in table 1. The mean value of erythrocyte sedimentation rate, creatinine, calcium and lactate dehydrogenase were higher than normal in patients as plasma cell proliferation affects different organs. Due to replacement of erythroid progenitors in bone marrow, hemoglobin was lower than normal range.

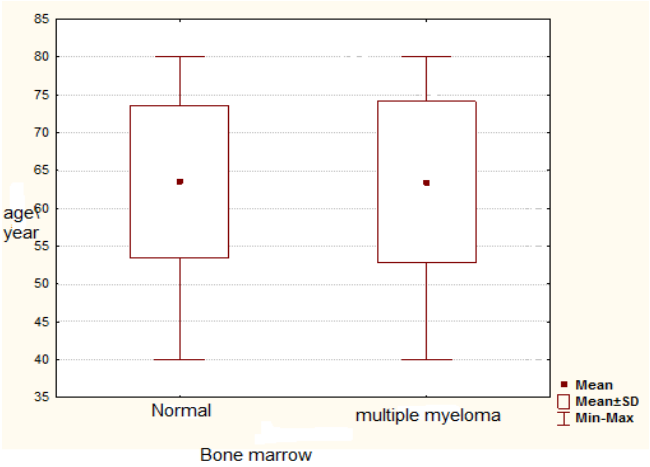


**Fig. 2**. Age distribution in the case and control groups.

**Table 1**. Some diagnostic tests in the patients group

| Variable | Hemoglobin (gr/dl) | Red blood cell ($10^{12}$/liter) | White blood cell ($10^{9}$/liter) | Platelet ($10^{9}$/liter) | Erythrocyte sedimentation rate (mm/h) | Prothrombin time (second) | Partial thromboplastin time (second) | Calcium mg/dl | Creatinine mg/dl | Lactate dehydrogenase U/liter |
|---|---|---|---|---|---|---|---|---|---|---|
| **Mean±SD** | 10.2±2.6 | 3.4±0.8 | 6.5±3.7 | 224.5±109. | 75.6±44.2 | 14.1±1.3 | 31.1±7.1 | 9.4±0.8 | 2.2±2 | 604.9±476.1 |
| **Range** | 6.3-15.5 | 2-5.1 | 0.5-17-3 | 58-534 | 3-132 | 12.5-17.1 | 20-47.5 | 8.1-11.9 | 0.6-9.1 | 240-2012 |

## Discussion

FLT3 mutation has been proved as a determining factor in some hematologic malignancies, and its evaluation may guide proper treatment [10]. In this research no FLT3 mutation either TKD or ITD was found in the study groups. According to table 1, high creatinine levels of serum and low count of white blood cells in MM patients reveals kidney damage and bone marrow involvement. Frequency of this mutation is mentioned in the latest studies as below: AML, 14.4% ITD and 4.1 %TKD [21]; ALL, 1.3% ITD and 1.3% TKD [22]; CML, 3.4% ITD and 1.14%TKD [23]; and MDS, 0.71% ITD and 0.23% TKD [24]. Some slight diversities are seen; these frequencies are similar to some recent studies and are very different from Zeramba's et al. research results about FLT3 mutation and adult T-cell leukemia/lymphoma (ATLL). These data are very different from those of our findings in the field of MM, too. The data suggest that mutations in the listed malignancies may determine prognosis of disease and adopted treatment. The epidemiologic distribution of FLT3 mutations is less known in some proliferative disorders. It's very rare in lymphoma as its frequency is approximately 4% in ATLL [25].

In this study we tried to assess the epidemiologic distribution features of FLT3 ITD mutation in MM patients. So far, it is the first study that has been done to determine frequency of FLT3 mutations in MM patients. Liu et al. (2007) studied the clinical significance of FLT3 mutations in hematologic malignancies and did not found any of them in 3 cases of non-Hodgkin lymphomas and 9 cases of MM [26]. In

Yamamota's study in field of FLT3 mutations prevalence in hematologic malignancies, no mentioned mutations were seen in 40 MM cases. This study is in line with our finding about lack of relation between MM and FLT3 mutations [18]. Yokota's findings show no FLT3 mutations in 38 multiple myeloma patients [27]. That research supports our data, too.

## Conclusions

Although FLT3 mutation occurrence is unusual in Multiple myeloma, it seems that FLT3 gene changes can affect multiple myeloma pathogenesis. This study was a novel project regarding the analysis of FLT3 mutations in the field of multiple myeloma research. More studies of MM patients in combination with other hematologic malignancies may reveal FLT3 mutation presence in the mentioned cases. Evaluations of more cases can achieve more accurate results.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgement

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# References

[1]. Small D. FLT3 mutations: biology and treatment. ASH Education Program Book 2006;2006(1):178-84.
[2]. Xu B, Chen G, Luo X, Tang J. Detection of FLT3/ITD gene mutations in patients with hematologic malignancy and their clinical significance. The Chinese-German Journal of Clinical Oncology 2009;8(2):100-3.
[3]. Meshinchi S, Appelbaum FR. Structural and functional alterations of FLT3 in acute myeloid leukemia. Clinical Cancer Research 2009;15(13):4263-69.
[4]. Chang P, Kang M, Xiao A, Chang J, Feusner J, Buffler P, et al. FLT3 mutation incidence and timing of origin in a population case series of pediatric leukemia. BMC cancer 2010;10(1):513.
[5]. Reilly JT. FLT3 and its role in the pathogenesis of acute myeloid leukaemia. Leukemia & lymphoma 2003;44(1):1-7.
[6]. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood 2002;100(5):1532-42.
[7]. D Kottaridis P, Gale RE, Linch DC. Flt3 mutations and leukaemia. British journal of haematology 2003;122(4):523-38.
[8]. Lin P, Jones D, Medeiros LJ, Chen W, Vega-Vazquez F, Luthra R. Activating FLT3 mutations are detectable in chronic and blast phases of chronic myeloproliferative disorders other than chronic myeloid leukemia. American journal of clinical pathology 2006;126(4):530-533.
[9]. Keneth Kaushansky MAL, Ernest Bentler, Thomas J. Kipps, Uri Seligsohn, Josef T. Prechal. Plasma cell neoplasms, in: Williams hematology. 8 ed: MC Graw Hill Company 2010: 1627-35
[10]. Warren M, Luthra R, Yin CC, Ravandi F, Cortes JE, Kantarjian HM, et al. Clinical impact of change of FLT3 mutation status in acute myeloid leukemia patients. Modern Pathology 2012;25(10):1405-12.
[11]. Bacher U, Haferlach C, Kern W, Haferlach T, Schnittger S. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters—an analysis of 3082 patients. Blood 2008;111(5):2527-37.
[12]. Bains A, Luthra R, Medeiros LJ, Zuo Z. FLT3 and NPM1 Mutations in Myelodysplastic Syndromes Frequency and Potential Value for Predicting Progression to Acute Myeloid Leukemia. American journal of clinical pathology 2011; 135(1):62-9.
[13]. Beitinjaneh A, Jang S, Roukoz H, Majhail NS. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations in acute promyelocytic leukemia: a systematic

review. Leukemia research 2010;34(7):831-36.
[14]. Levis M, Small D. FLT3: ITD does matter in leukemia. Leukemia 2003; 17(9):1738-52.
[15]. Wang L, Lin D, Zhang X, Chen S, Wang M, Wang J. Analysis of FLT3 internal tandem duplication and D835 mutations in Chinese acute leukemia patients. Leukemia research 2005;29(12):1393-98.
[16]. Levis M. Who's dancing with FLT3? Blood 2008;111(5):2503-4.
[17]. Dombret H. Gene mutation and AML pathogenesis. Blood 2011;118(20):5366-7.
[18]. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001;97(8):2434-39.
[19]. Daver N, Strati P, Jabbour E, Kadia T, Luthra R, Wang S, et al. FLT3 mutations in myelodysplastic syndrome and chronic myelomonocytic leukemia. American journal of hematology 2013;88(1):56-9.
[20]. Sino-US SLCG. FLT3 gene mutation and its prognostic implication in patients with acute leukemia. Zhonghua xue ye xue za zhi=Zhonghua xueyexue zazhi 2010;31(1):1.
[21]. Elyamany G, Awad M, Fadalla K, Albalawi M, Al Shahrani M, Al 3Abdulaaly A. Frequency and Prognostic Relevance of FLT3 Mutations in Saudi Acute Myeloid Leukemia Patients. Advances in hematology 2014;6:337-49.
[22]. Elyamany G, Awad M, Alsuhaibani O, Fadalla K, Al Sharif O, Al Shahrani M, et al. FLT3 Internal Tandem Duplication and D835 Mutations in Patients with Acute Lymphoblastic Leukemia and its Clinical Significance. Mediterranean journal of hematology and infectious diseases 2014;6(1): e2014038..
[23]. Annamaneni S, Kagita S, Gorre M, Digumarti RR, Satti V, Battini MR Incidence of internal tandem duplications and D835 mutations of FLT3 gene in chronic myeloid leukemia patients from Southern India. Hematology 2014;19(3):129-35.
[24]. Daver N, Strati P, Jabbour E, Kadia T, Luthra R, Wang S, et al. FLT3 mutations in myelodysplastic syndrome and chronic myelomonocytic leukemia. American journal of hematology 2013;88(1):56-9.
[25]. Zaremba CM, Oliver D, Cavalier M, Fuda F, Karandikar NJ, Chen W. Distinct immunophenotype of early T-cell progenitors in T lymphoblastic leukemia/lymphoma may predict FMS-like tyrosine kinase 3 mutations. Annals of diagnostic pathology 2012;16(1):16-20.
[26]. Liu H, Yu H, Jia HY, Zhang W, Guo CJ. Detection of FLT3 gene mutation in hematologic malignancies and its clinical significance. Hematology/Chinese Association of Pathophysiology 2007; 15(4):709-13.
[27]. Yokota S, Kiyoi H, Nakao M, et al. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic Syndrome among various hematological malignancies: a study on a large series of patients and cell lines. Leukemia 1997; 11:1605-1609.