# Molecular cytogenetic aberrations in Thai patients with multiple myeloma

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**ABSTRACT**

Cytogenetic abnormality plays an important role in prognosis of patients with multiple myeloma (MM). Conventional cytogenetic assay reveals limited results. In this study, we performed interphase fluorescence *in situ* hybridization (I-FISH) analysis to explore the incidence of cytogenetic abnormalities in patients with MM. Heparinized bone marrow was evaluated by FISH assay. Four FISH probes specific for deletion 13, deletion 17, t(4;14), and t(11;14) were used. Sixty-six patients from March 2013 to February 2014 were included. The incidence of the abnormality was found in 35 of 66 cases (53.03%). Deletions of the 13q14 region (D13S319) were detected in 19 cases (54.29%). Translocations involving the 14q32 region were observed in 10 cases (28.57%) including t(4;14) and t(11;14) in 6 and 4 cases, respectively. Deletions of the 17p13 region (*TP53*) were detected in 5 cases (14.29%). Furthermore, other abnormalities were observed, such as, 3-4 copies of 11q13 (28.57%), 14q32 deletion (8.57%) and trisomy 17 (2.86%). Results in 8/66 patients (12.1%) were unable to evaluate. Clinical outcome and prognostic values are being followed. The most common cytogenetic abnormality finding in this study is 13q14.3 deletion. The prognostic impact on response to chemotherapy and survival among these patients needs to be determined in long-term follow-up.

**Keywords**: multiple myeloma; fluorescence; FISH; molecular cytogenetic; bone marrow

## INTRODUCTION

Multiple myeloma (MM) is hematopoietic malignancy of plasma cell (PC) in bone marrow and rarely found in other tissues. When the patient has only single plasma cell neoplasm, the disease will be isolated or solitary plasmacytoma. However, in some patients there are more than one plasmacytoma, they become MM (American Cancer Society, 2015). MM represents 1.3% of all cancers and 15% of hematologic malignancies (Jagannath *et al*., 2014).

MM is a clonal plasma cell disorder characterized by the early presence of cytogenetic aberrations (Lom *et al.*, 1993; Put *et al*., 2010). Classic cytogenetic reports were only 30-46 % of MM patients having chromosome abnormalities by karyotype analysis (VanWier and Fonseca, 2005; Put *et al*., 2010). According to low mitotic index and the difficulty of metaphase chromosome preparation of malignant plasma cells, fluoreccent *in situ* hybridization (FISH) technique was applied to detect chromosome abnormalities in these tumor cells (Wiktor *et al.*, 2004; Gmidene *et al*., 2011; Xiao *et al*., 2012). Common genetic changes were 13q14 deletion, 17p13 deletion and 14q32 rearrangement including t(4;14) and t(11;14) (Xiao *et al*., 2012). Therefore, interphase FISH which is more sensitive than conventional cytogenetic method is applied to detect these chromosomal aberrations. Moreover, it was reported that the presence of t(4; 14)(p16;q32), t(14;16)(q32;q23), and -13q14 were associated with shorter survival (Fonseca et al., 2003). The chromosomal abnormalities which allowed prognostic classification of MM patients was 3 distinct categories: (i) poor prognosis group (t(4;14)(p16;q32), t(14;16)(q32;q23), and -17p13), (ii) intermediate prognosis (- 13q14), and (iii) good prognosis group (all others) (Fonseca *et al.*, 2003; Gertz *et al.*, 2005). The purpose of this study was to detect common cytogenetic aberrations, 13q14 deletion, 17q13 deletion, t(4;14) and t(11;14) in Thai MM patients using FISH technique.

## MATERIALS AND METHODS

Heparinized bone marrow aspirates were obtained from 66 patients diagnosed with MM during March 2013 to February 2014 at Human Genetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital. FISH assay was performed on whole bone marrow cells. This work was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 06-57-51). Bone marrow was processed for chromosome studies by standard techniques. The slides were fixed in 100% ethanol for 5 min at room temperature, and subsequently incubated with either goat anti-human κ or human λ light chains conjugated with 7-amino-4-methylcoumarin+acetic acid (AMCA) (Vector Labs, Burlingame, Calif., USA). Slides were hybridized with fluorescent labeled commercial probes obtained from Abbott (Abbott-Vysis, Downers Grove, Ill., USA) according to the manufacturer's instructions. Chromosome 13 deletion signals were identified by Vysis D13S319 SpectrumOrange /LSI 13q34 SpectrumGreen FISH probe Kit. Chromosome 17 deletion was detected by Vysis TP53 SpectrumOrange /CEP 17 SpectrumGreen probes. LSI IGH/CCND1 and IGH/FGFR3 dual-color dual-fusion were further used to detect t(4;14) (p16.3;q32) and t(11;14)(q13;q32), respectively.

Fluorescence images were captured with epifluorescence microscope (Zeiss, Germany) using appropriated filters. One hundred nuclei were scored for each probe. The cutoff levels for positive value of each probe were as follows: 10% for fusion and 20% for numerical abnormalities according of European Myeloma Network FISH workshops recommendations.

## RESULTS

Interphase FISH was performed on a total of 66 MM patients (35 males and 31 females). The median age was approximately 62±10 years. By using the following probes: del13 (13q14), TP53 (17p13), t(4;14) and t(11;14) (Figure 1), 53.03% (35/66) of the patients showed at least one of cytogenetic abnormalities. Deletion of chromosome 13 was the most common structural abnormalities which was found in 19/35 (54.29%) of the patients. Fourteen out of 19 patients (73.68%) had a large deletion spanning both D13S319 and Rb1 (Loss of chromosome 13) whereas 5/19 patients (26.32%) had a small 13q14 deletion with only 1 orange signal detected by FISH with the LSI D13S319 probe. Translocations involving the chromosome 14q32 region were observed in 10/35 cases (28.57%) including 6 cases with t(4;14) and 4 cases with a t(11;14). The other abnormalities were 3 copies of 11q13 and 17p13 deletion. Three copies of 11q13 were detected in 10/35 patients (28.57%). Deletion of 17p13 was detected in 5/35 patients (14.29%). One patient showed an extra signal of the *TP53* gene and centromere 17 (trisomy 17) by FISH (Table 1). Interestingly, 9/19 patients (47.37%) carrying a *RB1* deletion also exhibited a 14q32 rearrangement, and 6 of them also showed the t(4;14) translocation without t(11;14).

## DISCUSSION

Conventional cytogenetic method involves cell culture, metaphase chromosome preparation and chromosome banding. In MM patients, the problems of cytogenetic analysis result from heterogeneities, low mitotic activity and poor growth rate of malignant plasma cells. In contrast, FISH analysis has substantially enhanced the sensitivity of cytogenetic analysis because it is applicable not only to dividing cells but also to interphase nuclei. Here, we performed interphase FISH on fixed bone marrow cells of 66 Thai patients diagnosed with MM, using 4 probes to detect the most common and/or recurrent genetic aberrations, i.e. 13q14 deletion, 17q13 deletion, t(4;14) and t(11;14). In this study, we found that 53.03% of the cases showed at least one of cytogenetic abnormalities (Table 2) as reported by Gmidene *et al*. (2011).

The most frequent cytogenetic abnormalities in the patients were deletion of 13q14 (53.03%) including loss of chromosome 13 (73.68%) and deletion of 13q14 (RB1) (26.32%). This result was concordant with previous reports in patients from the USA and China which the 13q14 deletion was found in 49% and 63.3%, respectively (Wiktor *et al*., 2004; Xiao *et al*., 2012) but discordant from Tunisian patients (Gmidene *et al.*, 2011). It was reported that chromosome 13 aberration was associated with significantly lower response rates, short event-free survival (EFS) and inferior overall survival (OS) in MM (Liebisch *et al.*, 2006; Sawyer, 2011). It was suggested that chromosome 13 has a crucial role in MM as prerequisite for clonal evolution for cancer (Fonseca *et al.*, 2009).

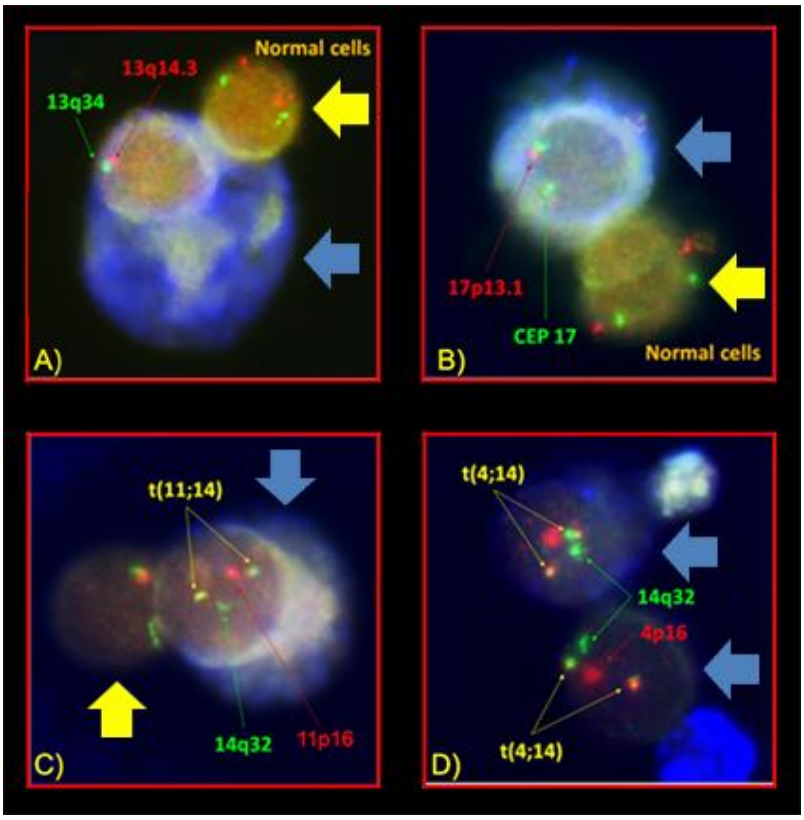


**Figure 1** FISH signals in multiple myeloma patients. (A) A plasma cell with deletion of both 13q14.3 and 13q34. LSI D13S319 probe target located at 13q14 (orange signal) and LSI 13q34 (green signal), (B) A plasma cell showed deletion of 17p13.1. LSI *TP53* probe target located at 17p13.1 (orange signal) and CEP 17 (green signal), (C) Translocation probe set to detect t(11;14), and (D) Translocation probe set to detect t(4;14). Blue and yellow arrow heads indicate plasma cell and normal cell, respectively.

The t(4;14) and t(11;14) are two main IgH translocation. Translocation causing 14q32/IGH rearrangement was seen in 10/35 patients (28.57%) and t(11;14) in 4/35 (11.43%). This finding corresponded to 14q32 rearrangement (33.33%) and t(11;14) (14.17%) in 120 MM patients (Mohamed *et al.*, 2007). The most frequent translocation in MM is the t(11;14) which is found in approximately 15% of patients, and appears to be associated with a favorable outcome in most series. Therefore, t(11;14) is regarded as neutral prognosis. However, t(11;14) is associated with a longer event-free survival (EFS) and overall survival (OS) receiving high dose treatment, whereas the second most frequent translocation, t(4;14), is associated with more aggressive disease and shorter EFS and OS (Xiao *et al.*, 2012).

17p13 deletions occurred in a small proportion of our patients (5/66; 7.58%). Deletion of 17p13 has been identified clinically as an indicator of very poor prognosis. It has been reported that patients with 17p deletion have more aggressive disease, a higher prevalence of extra medullary disease, and overall shorter survival (Sawyer 2011). In addition, Liebisch *et al*. in 2006 reported independently that for the mode of treatment (conventional chemotherapy), deletion of 17p13 identified by FISH is a predictor of shorter survival. Patients with 17p13 deletion receiving conventional or high dose chemotherapy achieved a lower response rate and had significant shorter event-free and overall survival than patients without this deletion (Xiao *et al.* 2012).

In summary, to our knowledge, this is the first report in Thailand to detect chromosomal abnormalities in MM patients with these panel probes. Interphase FISH results provide important information to the hematologists for MM patient management. The prognostic impact on response to chemotherapy and survival among these patients needs to be determined in long-term follow-up.

**Table 1** The incidence of chromosome abnormalities in multiple myeloma patients.

| Patient No. | Age/sex | Specific FISH Probes | | | |
|---|---|---|---|---|---|
| | | **D13S319/13q34** | **TP53/CEP17** | **IGH/FGFR3 DF** | **IGH/CCND1 XT DF** |
| MM01 | 58/F | - | Trisomy 17 | 3 copies of 4p16 | - |
| MM02 | 57/F | - | - | - | 4 copies of 11q13 |
| MM03 | 49/F | ++ | - | - | - |
| MM04 | 37/M | - | - | - | - |
| MM05 | 63/M | ++ | - | - | - |
| MM06 | 55/M | - | - | - | - |
| MM07 | 54/F | - | - | 3 copies of 14q32 | 3 copies of 14q32 |
| MM08 | 52/F | - | - | - | - |
| MM09 | 68/F | - | - | - | - |
| MM10 | 52/M | ++ | - | t(4;14) | - |
| MM11 | 63/F | - | - | - | - |
| MM12 | 70/M | - | - | - | - |
| MM13 | 64/F | - | - | - | - |
| MM14 | 63/F | - | - | - | 3-4 copies of 11q13 |
| MM15 | 57/F | - | - | - | 3 copies of 11q13 |
| MM16 | 66/M | - | - | - | - |
| MM17 | 56/M | | | Low plasma cell | |
| MM18 | 69/M | - | - | - | t(11;14) |
| MM19 | 67/M | ++ | - | t(4;14) | - |
| MM20 | 67/M | - | - | - | - |
| MM21 | 76/M | - | - | Deletion of 4p16 | t(11;14) |
| MM22 | 51/M | - | - | - | - |
| MM23 | 58/M | - | - | - | - |
| MM24 | 60/M | - | - | - | - |
| MM25 | 61/F | - | - | - | - |
| MM26 | 47/M | - | - | - | - |
| MM27 | 69/M | | | Very low plasma cells | |
| MM28 | 77/F | - | - | - | - |
| MM29 | 68/F | ++ | + | - | - |
| MM30 | 62/M | ++ | - | t(4;14) | - |
| MM31 | 83/M | | | Very low plasma cells | |
| MM32 | 52/M | ++ | - | t(4;14) | - |
| MM33 | 59/M | + | - | Deletion of 4p16 | 3 copies of 11q13 |
| MM34 | 75/F | - | + | - | 3 copies of 11q13 |
| MM35 | 60/F | - | - | - | - |
| MM36 | 49/F | - | - | - | - |

**Table 1** (continued).

| Patient No. | Age/sex | Specific FISH Probes | | | |
|---|---|---|---|---|---|
| | | **D13S319/13q34** | **TP53/CEP17** | **IGH/FGFR3 DF** | **IGH/CCND1 XT DF** |
| MM37 | 55/F | ++ | - | 3 copies of 14q32 | 3 copies of 14q32 |
| MM38 | 47/F | Very low plasma cells | | | |
| MM39 | 62/M | ++ | - | - | - |
| MM40 | 72/F | + | - | Deletion of 14q32 | 3 copies of 11q13 |
| MM41 | 61/F | - | - | - | - |
| MM42 | 52/M | - | - | - | - |
| MM43 | 63/F | Trisomy 13 | Tetrasomy 17 | - | Tetrasomy 14 |
| MM44 | 54/F | - | - | - | - |
| MM45 | 62/M | Very low plasma cells | | | |
| MM46 | 63/F | - | - | - | 3 copies of 11q13 |
| MM47 | 62/F | - | - | - | 3 copies of 11q13 |
| MM48 | 56/F | - | - | - | t(11;14) |
| MM49 | 91/M | + | - | - | - |
| MM50 | 53/F | ++ | - | Single fusion t(4;14) | - |
| MM51 | 62/M | ++ | - | t(4;14) | - |
| MM52 | 52/F | ++ | + | Deletion of 4p16 | Deletion of 14q32 |
| MM53 | 62/M | - | - | - | t(11;14) |
| MM54 | 84/M | Very low plasma cells | | | |
| MM55 | 69/M | - | - | - | - |
| MM56 | 54/M | + | - | - | - |
| MM57 | 70/M | Very low plasma cells | | | |
| MM58 | 57/M | - | + | - | 3 copies of 11q13 |
| MM59 | 64/M | Very low plasma cells | | | |
| MM60 | 54/F | Three copies of 13q14.3 | - | - | - |
| MM61 | 74/F | - | - | - | Four copies of 11q13 |
| MM62 | 67/F | - | - | - | - |
| MM63 | 71/M | ++ | - | - | - |
| MM64 | 71/M | ++ | - | - | - |
| MM65 | 71/F | + | ++ | Deletion of 14q32 | Deletion of 14q32 |
| MM66 | 69/F | - | - | - | - |

- = Negative
+ = Deletion of specific region
++ = Deletion of chromosome

**Table 2** Summary of results compared with previous reports.

| Name | Total | Least 1 abnormality | LSI 13 Abnormal | TP53/CEP17 Abnormal | IGH/FGFR3 t(4;14) | IGH/CCND1 t(11;14) |
|---|---|---|---|---|---|---|
| Current result | 66 | 35/66 (53.03%) | 19 (54.29%) | 5(14.29%) | 6(17.14%) | 4(11.43%) |
| Xiao *et al.* (2012) | 60 | 50/60 (83.33%) | 38(76.00%) | 8(16.00%) | 12(24.00%) | 16(32.00%) |
| Gmidene *et al.* (2011) | 70 | 39/70 (55.71%) | 13(33.33%) | 4(10.26%) | 8(20.51%) | 18(46.15%) |
| Put *et al.* (2010) | 321 | 112/321 (34.89%) | 10(8.93%) | - | 1(0.89%) | 7(6.25%) |
| Takimoto *et al.* (2008) | 23 | 13/23 (56.52%) | 12(92.31%) | - | 1(7.69%) | 5(38.46%) |
| Wiktor *et al.* (2004) | 139 | 40/139 (28.78%) | 12(60.00%) | 7(17.50%) | - | 1(2.50%) |

**ACKNOWLEDGEMENT**

The authors would like to thank Ramathibodi Cancer Center for the support of reagents and chemicals.

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