Detection of chromosome 13 (13q14) deletion among Sudanese patients with multiple myeloma using a molecular genetics fluorescent in situ hybridization technique (FISH)

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Abstract

Multiple myeloma (MM) is a neoplastic plasma cell dyscrasia with an incidence of 4-4.5 per 100,000 population per year. It is regarded as the second most prevalent blood cancer (10%) after non-Hodgkin lymphoma. The objective of this study was to investigate the mutational change in chromosome 13 (13q14) among Sudanese MM patients and to identify the association between extent of plasma cell infiltration in the bone marrow, albumin level and deletion of 13q14 by an analytical case control study. Materials and Methods: 15 patients were enrolled in the study. 11 bone marrow samples were collected from MM patients at different stages of the disease and 4 samples were from patients with conditions other than MM as control. Plasma cells were counted from bone marrow smears and fluorescence-in-situ hybridization (FISH) was performed using Fluorophore labeled DLEU1 (13q14) LSI (local specific identifier) probe designed as a dual-colour assay to detect deletion at 13q14. Heparanized sample was taken for estimation of serum albumin in all patients. Results: 13q14 deletion was detected in 6 (54.5%) MM patients while one (9.1%) patient showed monosomy. All relapsed MM (27.3%) had 13q14 deletion. Surprisingly almost all patients studied had normal albumin level. The study could not show whether the deletion is implicated in the pathogenesis of multiple myeloma.

Keywords: multiple myeloma, fluorescence in-situ hybridization, FISH, DLEU1

INTRODUCTION

Multiple myeloma (MM) is a neoplastic plasma cell dyscrasia with an incidence of 4-4.5 per 100,000 population per year.1,2 It is regarded as the second most prevalent blood cancer (10%) after non-Hodgkin lymphoma.3 About 63,000 subjects are reported to die from the disease each year (33,000 males and 30,000 females).4 Death increases slowly over the decades among whites in the western countries.5 It is characterized by malignant proliferation of monoclonal plasma cells with a very complex cytogenetic and molecular genetics aberrations.6

The aetiology of the disease is poorly understood.7,8 Recently investigations of myeloma cells have demonstrated that almost all cases of MM are cytogenetically abnormal.2 IGH translocation occurs as initiating events during the pathogenesis of MM, whereas secondary translocations are involved in progression.2 Most primary IGH translocations result from errors in the B-cell-specific DNA modification process, mostly IGH switch recombination or less often somatic hypermutation.2 The most frequent chromosomal aberrations in multiple myeloma patients are chromosome 13q14 deletion (del 13q14), 1q21 gain (amp1q21), monosomy 13 and 17p13 deletion (del17p13). They play an important role in prognosis of the disease.9 Chromosome 13 spans about 114 million base pairs and represents between 3.5% and 4% of the total DNA in the cells.10 It contains between 300 and 700 genes.10 The deletion is detected in 46% of newly diagnosed and 73% of patients with relapsed MM.11 The objective of this study was to look into the mutational changes in chromosome 13 (13q14) in Sudanese MM patients applying the FISH technique, and to assess the relation...
of chromosome 13q14 deletion and the extent of plasma cell infiltration of the marrow with regard to relapse status and plasma albumin levels.

MATERIALS AND METHODS

This was an analytical case control study conducted in Alzytona Specialized Hospital in Khartoum, Sudan. All patients seen in the hospital from June 2012 to December 2012 and who fulfilled the criteria set by the WHO for multiple myeloma were included in the study.2,6 The disease is characterized by a mature and immature plasma cells count of more than 10% of the bone marrow nucleated cells, monoclonal gammapathy detected by serum protein electrophoresis as well as lytic bony lesions detected radiologically. Clinical, laboratory as well as radiological data was collected through a pretested questionnaire. Cases with inadequate bone marrow biopsies were excluded from the study. The control group was consented patients attending the bone marrow clinic with preliminary clinical, radiological and laboratory features not suggestive of multiple myeloma.

Samples for analysis

Bone marrow aspirate samples were collected from all patients and control group and assessed for percentage of plasma cells by counting cells in the bone marrow smear which was reviewed by two independent hematologists. 0.5 ml of bone marrow was taken in lithium heparin for cytogenetic analysis by fluorescence-in-situ hybridization (FISH) which was performed using Fluorophore labeled DLEU1 (13q14) LSI (local specific identifier) probe designed as a dual-colour assay to detect deletion at 13q14. Serum was taken for measurement of albumin using in vitro quantitative immunoturbidimetric assay.

Testing protocol for FISH

0.5 ml of each bone marrow sample drawn from each patient was subjected to FISH technique by direct harvest in culture and within 24 of collection. The culture employed the addition of 50 µl Colcemid solutions to the culture 1 hour before the harvest. The mixture was centrifuged at 1000 RPM for 10 minutes. The supernatant was removed by a Pasteur pipette leaving 0.5 ml to resuspend the pellet. 8 ml pre-warmed hypotonic solution at 37°C was added to the pellet, mixed gently and kept in the incubator for 50-54 minutes. Centrifugation and supernatant removal were repeated 5 times and then centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and the cells were suspended in 0.5 ml freshly prepared chilled fixative.

A few drops of suspended cells were pipetted to the centre of ethyl alcohol-cleaned slides, allowed to dry, and then examined under the light microscope for the presence of interphase cells. The area of maximum interphase concentration was marked. Pepsin was added to each slide at 37°C for 5 minutes and then immersed in PBS before slides got dry to view under the microscope to assess denaturation. The slides were then placed in a Coplin jar and subjected to pre-warmed 2×SSC in a water bath at 37°C for 20 minutes followed by ethyl alcohol 70%, 90% and 100% concentrations respectively for two minutes each at room temperature. Slides were then allowed to dry. The fluorophore labeled DNA probe, prepared according to the manufacturer’s instruction, was applied on each slide at the marked area and immediately covered with a cover slip to avoid drying. Each slide was wrapped in a piece of parafilm. The slides were then incubated at 80°C for 10 minutes, then at 37°C overnight in a dark pre-warmed humidified chamber (Thermobride) to allow probe hybridization. After 48 hours, the slides were immersed in pre-warmed 0.4×SSC at 75°C in a water bath for 4 minutes followed by immersion in 2×SSC solution with 15 µl NP 40 for 2 minutes at room temperature, then in 2×SSC solution for another 2 minutes followed by PBS for another 2 minutes. Slides were stained using 10-15 µl DAPI II stain (which gives cells a blue color) and cover-slipped.

Interpretation of results

At least 100 interphases in each case were scanned under the fluorescence microscope using oil immersion objective for the detection of deletions. Only metaphase and interphase cells with clear, non-overlapping signals were analyzed. The FISH analysis was performed using DLEU1 (13q14) LSI (local specific identifier). Probe was designed as a dual-color assay to detect deletion involving the 13q14 region showing one red signals, while the control at the Tel 13q region exhibited 2 green signals (Figure 1). Hybridization signals were enumerated in 50 to 100 cells. Results were analyzed independently by two individuals. The cut off for the number of positive cells was set at five i.e. if signals were detected in less than five cells then the
CHROMOSOME 13 DELETION IN MULTIPLE MYELOMA

RESULTS

Interphase FISH analysis using Fluorophore labeled DLEU1 (13q14) LSI probe for 13q14 deletion was successfully performed on all the samples. The number of patients studied were 15, being from 11 multiple myeloma patients and 4 non-MM patients who served as controls. 7 MM patients were males and 4 were females with a male to female ratio of 3.2 to 1. The predominant age distribution was from 39 to 61 years with an average of 50 years. The 4 control samples were used to verify the accuracy of results.

FISH analyses for six (6/11; 54.5%) samples were positive for 13q14 deletion and four (4/11; 36.4%) were negative. One patient (1/11; 9.1%) was found to express monosomy (Figure 2). Both patients (100%) with more than 30% plasma cell marrow infiltration showed 13q14 deletion whereas of those with less than 30% plasma cell infiltration, only 44.4% showed the deletion (Table 1). This difference was statistically significant (p = 0.01). 37.5% of newly-diagnosed and 100% of relapsed MM expressed 13q14 deletion (Table 1). The median duration of relapse was 14 months.

Almost all patients showed normal serum albumin level (Figure 3).

DISCUSSION

Chromosome 13q14 deletion has been shown to be the most frequently encountered chromosomal loss in MM. The percentage of cases with deletion in this study is 54.5% while one case (9.1%) showed monosomy. These findings are comparable with reported literature (30%-80%). Similar findings have been reported by Chang et al in Canada and Fonseca et al from the Mayo clinic, USA, although the techniques used were different.

FISH analysis is necessary for the identification and characterization of most unbalanced de novo structural rearrangements, including marker chromosomes. Numerous acquired aberrations which lead to gains or losses of chromosomal

FIG. 1: Interphase FISH showing normal results: 2 red signals of the LSI 13q14 locus and 2 green signals.

FIG. 2: Interphase FISH analysis showing monosomy of chromosome 13: only one red and one green signal.
material have been described in leukemia, lymphomas and solid tumors. It is important to know whether or not a particular chromosomal region or a particular gene is involved in a chromosomal aberration, so that a correct clinical diagnosis can be made and appropriate treatment initiated. A traditional examination involving metaphase chromosome analysis is often unable to identify features that distinguish one disease from another, due to subtle chromosomal features. FISH can elucidate these differences. It also can be used to detect diseased cells more easily than standard cytogenetic methods which require dividing cells, involve labour and

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<th>p value</th>
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<td>0.04*</td>
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<tr>
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</tr>
<tr>
<td>Relapsed MM</td>
<td>3</td>
<td>3 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>6 (54.5)</td>
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*p value is significant at a value equal or less than 0.05

TABLE 1: Comparison of FISH results with extent of plasma cell infiltration of the marrow and relapse status of MM

FIG. 3: The albumin levels of the 11 patients
There is an increase in the percentage of plasma cell in the bone marrow in the majority of the patients.\textsuperscript{17} The plasma cell labeling index (PCLI) is one of the most important prognostic factors in newly diagnosed MM, and indicates plasma cell proliferative capacity and lower rate of response to treatment. Similar findings of high plasma cells levels have been reported by Hu Y et al in China.\textsuperscript{18,19}

Although low albumin levels are associated significantly with poorer prognosis, none of our patients have abnormal serum albumin levels. This may be due to the shorter duration of disease.\textsuperscript{13,20,21}

The strongest evidence for the aggressiveness of chromosome 13q is the occurrence of relapse among 27.3% of patients. Using a single probe will not enable the differentiation of all chromosomal haploids that are implicated in MM.\textsuperscript{22} This may be a main factor for the finding of 30% of our patients with no deletion. There have been several reports studying 13q14 in MM.\textsuperscript{11,13,21,23} All use interphase FISH. However, none have attempted to delineate the common deleted region. Those studies either employed 1-3 probes for the 13q14 region or, as in one study, used noncontiguous probes spanning the whole of the 13q arm to determine the hot spots of deletion.\textsuperscript{24} Loss of 13q14 sequences is also frequent in chronic lymphocytic leukemia, but is not associated with a poor prognosis.\textsuperscript{14,25}

Conclusion

In this study the detection rate of chromosomal abnormalities was successfully and significantly improved with FISH analysis using DLEU1 (13q14) LSI (local specific identifier) probe designed as a dual-color assay to detect deletion at 13q14. Chromosomal 13q14 deletion was detected frequently (54.5%) among multiple myeloma patients and monosomy was detected in one (9.1%) patients. 13q14 deletion was significantly associated with a hyperproliferative stage of the disease (marrow plasma cell above 30%). All relapsed cases (27.3%) had deletion 13q14. Whether the deletion is implicated in the pathogenesis of multiple myeloma needs to be elucidated.


