INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal disease that results from an acquired genetic change in a pluripotent haemopoietic stem cell. This altered stem cell causes increased proliferation of myeloid cells predominantly granulocytic series both in peripheral blood and bone marrow. It is the most common myeloproliferative neoplasm, which accounts for about 15 - 20% of adult leukemias, but less than 5% of all childhood leukemias. Its incidence is about 1.5 cases per 100,000 persons per annum, being almost the same throughout the world. CML passes through three phases during the course of the disease progression: from chronic phase (CP) to an accelerated phase and then finally blast transformation. Patients of CML usually present in chronic phase that is a stable and indolent state. About 10-30% of patients are asymptomatic at the time of diagnosis and are picked up incidentally on examination of the peripheral blood film. Clinical features include weight loss, increased sweating, symptoms of anaemia, abdominal discomfort secondary to splenic enlargement, bruising, epistaxis or other haemorrhagic sequelae. On examination, the most common feature is splenomegaly comprising 50 - 70% of patients at diagnosis. A few patients may have palpable liver or ecchymosis on their body. CML is unique in a sense that it was the first human cancer consistently associated with a chromosomal abnormality, the Philadelphia (Ph) chromosome. It was also the first malignancy that was faithfully reproduced in an animal model based on precise knowledge of the causal molecular lesion. Lastly, CML was the first malignant condition where the identification of the causal abnormality led to a specifically targeted therapy. The history of CML begins in 1845, when Bennet and Virchow reported cases with enlarged spleen, severe anaemia and markedly raised leucocyte count in their
counts were performed using Sysmex KX 21 automated acetic acid (EDTA) for CBC and PCR. Complete blood were taken the antecubital vein by aseptic technique. Half was taken the study. After an informed consent and recording the Declaration of 1975, as revised in 2000. All newly diagnosed patients of CML were recruited in human experimentation and with the Helsinki ethical standards of the responsible committee on all the procedures followed were in accordance with the Institute's Ethical Committee for Medical Research, 2012 to February 2014. After the approval of the study Forces Institute of Pathology, Rawalpindi, from January. It was a cross-sectional study carried out at Armed METHODOLOGY

It was a cross-sectional study carried out at Armed Forces Institute of Pathology, Rawalpindi, from January 2012 to February 2014. After the approval of the study by Institute's Ethical Committee for Medical Research, all the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

All newly diagnosed patients of CML were recruited in the study. After an informed consent and recording demographic data, 5 ml of venous blood was drawn from the antecubital vein by aseptic technique. Half was taken in heparin for FISH and half in ethylenediamine tetra-acetic acid (EDTA) for CBC and PCR. Complete blood counts were performed using Sysmex KX 21 automated haematology analyser. Routine diagnostic procedures for these patients consisted of morphology and differential white blood cell counts on bone marrow as well as on peripheral blood film. Diagnosis of CML was made on the basis of clinical history, bone marrow aspirate examination, and neutrophil alkaline phosphatase score. Patients with other forms of myeloproliferative neoplasms like polycythemia vera, essential thrombocytopenia, and those patients who were already on treatment for CML were not included.

For dual colour dual fusion FISH analysis, 0.5 ml of heparinized blood was mixed with 7 ml of RPMI-1640 culture medium in culture vessel and then incubated at 37°C for 24 hours. Analysis was performed on the cultured samples of peripheral blood/bone marrow following manufacturer's protocol (Vysis Inc. Abbot, Germany). Slides were evaluated using BX51 fluorescence microscope for dual fusion signal of yellow colour. Samples having 20 or more interphases positive for dual fusion signals were taken as positive (Figure 1).

PCR was considered the gold standard in the diagnosis of CML. Strict precautions were taken to avoid contamination. RNA extraction was performed using Trizole LS reagent (USA). Complimentary DNA (cDNA) was synthesised by mixing 8 µl of extracted RNA with deoxynucleotide triphosphates (dTTPs), RT buffer, M-MLV enzyme and RNAase inhibitor. A gene specific primer was added to the tube. The reaction tube was incubated at 37°C for 2 hours. BCR-ABL1 fusion gene analysis was done as follows. The synthesised cDNA was mixed with PCR mixture, that contains dTTPs, magnesium chloride etc. along with DNA Taq polymerase and following BCR-ABL primers were used.

BCR3-b3F  5’-CGTCCACTCAGCCACTGGAT
BCR2-b2F  5’-TGCAGATGTCAGCAACTCTG
ABL-a2-R  5’-TCCAACGACGGCTTAC
ABL-F  5’-GTCTGATGTCAGCCGCTCGT
b3a2b2a2P  6 FAM-CAGTAGCATCTGACTTTGAGCC
ABL-P  IC-TGGACCAGTGAAAAATGACC
CAACC-TAMRA

Samples were analysed by running on ABI 7500 sequence detection system (Applied Biosystem). Thermal cycling parameters for RNA quantification assay hold cDNA for 10 minutes at 95°C and then followed by biphasic cycles of 15 seconds at 95°C for denaturation and 1 minute at 60°C for annealing and extension. Forty PCR cycles were repeated. Positive samples were those in which fluorescence exceeds threshold before 35 cycles. Those samples that exceeded threshold after 35 cycles were repeated. Positive and negative controls were run along with test samples.

All the collected data was analysed in Statistical Package for Social Sciences (SPSS) version 21. The
analysed variables included numerical data like age and qualitative data like gender. Results for BCR-ABL1 in FISH and PCR were compared by chi-square test and p-value < 0.05 was taken as significant. Sensitivity and specificity were calculated using PCR as gold standard.

RESULTS

A total of 87 patients of CML were included during the study period of 24 months, from January 2012 to February 2014. Out of these 87 CML patients, 56 (64%) were males and 31 (36%) were females with male to female ratio of 1.8:1. Mean age of patients was 37 ± 10 years and majority of them ranged between 30 to 50 years. On clinical examination of these patients, mean spleen size was 10.4 cm ± 4 ranging from non-palpable to a maximum size of 18 cm below left costal margin. The mean TLC in these patients was 157x10^9/L. Minimum TLC was 19 x 10^9/L and maximum was 510 x 10^9/L. LAP score ranged from 0 to 22 ±5. In PCR out of 87 subjects, 85 (97.7%) were BCR-ABL1 positive and 2 (2.3%) were negative. In FISH out of 87 patients, 83 (95.4%) were positive for BCR-ABL1 gene mutation. Sensitivity and specificity of FISH were 97.6% and 100%, respectively. Results of FISH and PCR were compared and shown in Table II.

DISCUSSION

Chronic myeloid leukemia is the most common myeloproliferative neoplasm that results from an acquired genetic defect known as the Philadelphia (Ph) chromosome. This translocation results in transcription and translation of the constitutively active BCR-ABL1 tyrosine kinase, which is central to CML pathogenesis. The discovery of tyrosine kinase activity as a part of pathogenesis in CML led to introduction of imatinib, a tyrosine kinase inhibitor that has changed the course of the disease. But at the same time importance of detection of BCR-ABL1 fusion gene has also increased as the product of this gene is the target of the agent. The available methods for the detection of this chromosomal abnormality are conventional karyotyping and molecular analysis. Although PCR is the most sensitive technique, it has two concerns. One is false positive result due to contamination of a negative specimen by amplicons produced from prior positive amplification reactions; and second is physiological positive results that mimic tumour-associated positive results. Normal individuals have only infinitesimal amounts of BCR-ABL1 transcripts and PCR very rarely yield positive results in healthy individuals. In comparison, FISH is another molecular technique, which can be applied to avoid such concerns. It allows analysis of both proliferating (metaphase cells) and non-proliferating (interphase nuclei) cells to detect specific nucleic acid sequences by using fluorescent specific DNA probes. Though its cost is little higher, it plays a pivotal role in those cases where results by other techniques are equivocal.

In this study, the gender distribution shows slight male predominance that is comparable with local and international data. The median age at presentation was younger than what mentioned in international data but comparable with the local data available. A local study done by Jamil et al. showed median age of 36 years while study of Usmani et al. showed it to be 31 years. This variation in age may be due to some biological variation as compared to western population.

The prime purpose of the study was to evaluate the role of FISH in diagnosis of CML. This study showed
sensitivity of FISH as 97.6% with an overall positivity of 95.4% in CML cases. These results closely matched with results of international studies. There is paucity of local data showing role of FISH in diagnosis of CML, due to unavailability of advance facilities and financial constraints. A study done by Guo et al. in China showed sensitivity of FISH as 94.4%, while studies done by Bao et al. in USA and by Reena et al. in Malaysia showed it to be 100% each.19,20

CONCLUSION

FISH is a reliable, accurate, and quick method for detecting BCR-ABL1 gene mutation in CML; and it can be used as a supplementary test to PCR for the diagnosis of CML.

REFERENCES