

cytogenetic hallmark of CML, the Philadelphia chromosome (Ph), is formed as a result of reciprocal translocation between chromosomes 9 and 22, leading to the uncontrolled proliferation of the bone marrow cells ⁽³⁾.

Angiogenesis is the formation of new blood vessels from pre-existing vessels during adult life ⁽⁴⁾. Many studies suggest a role for angiogenesis not only in the pathogenesis of solid tumors but also in hematological malignancies like acute and chronic leukemia, lymphoma, myelodysplastic syndromes, myeloproliferative neoplasms, and multiple myeloma ⁽⁵⁾; furthermore, WT1 is also expressed in a large variety of tumour blood vessels ⁽⁶⁾, as it is involved in endothelial cell proliferation, vascular formation and migration, indicating that it might be a general marker for angiogenesis ⁽²⁾. Since WT1 is a marker of angiogenesis and it is believed to be relevant in the maintenance of the malignant phenotype of the tumour cells ⁽²⁾, this study will assess the expression of WT1 in chronic myeloid leukemia, and investigate if there is a correlation between the WT1 expression and angiogenesis (as marked by CD31 expression) in CML which might help for future therapeutic trials.

Methods

Patients and sampling

This cross sectional case control study was conducted from March 2011 to July 2012 on the trephine biopsy of 16 newly diagnosed CML patients including 10 in chronic, 3 in blastic, and 3 in accelerated phases. In addition to 20 age matched control cases with benign reactive marrow with no evidence of hematological malignancy, the cases were collected from The Hematology Ward of Baghdad Teaching Hospital. This study was ethically approved by the Ministry of Health. Clinical and laboratory information regarding age, sex, packed cell volume (PCV), white blood cell (WBC) count, platelets count, blasts percent, were obtained directly from the patient through taking history and examination at time of diagnosis during the clinical course and before taking chemotherapy.

From each formalin fixed paraffin embedded bone marrow biopsy used in this study, 3 sections of 4 µm thick were taken; one representative section was stained with Hematoxylin and Eosin (H&E) stain and was reviewed, while the other sections were stained immunohistochemically with WT1 and CD31 monoclonal antibodies respectively.

Immunohistochemistry

The primary antibodies used in this study were: monoclonal mouse anti-human Wilms' Tumor 1 (WT1) protein, clone 6F-H2 (Dako Cytomation), prediluted monoclonal mouse antiendothelial cell marker (CD31) antibody, clone JC70A (Dako Cytomation); while the immunohistochemistry (IHC) secondary detection kit used was immunoperoxidase secondary detection kit (DakoCytomation IHC kit LSAB2 System-HRP, code K0679) which was purchased from DAKO, Denmark. The immunohistochemical staining procedure was done according to the manufacturer's instructions. Positive staining is expressed as a brown color, in which brown cytoplasmic staining of endothelial cells is considered positive reaction for CD31 ⁽⁷⁾; and staining of either the nucleus and/or the cytoplasm indicated a positive result for WT1 ⁽⁸⁾. For IHC technical quality control: tonsils tissue which was taken from a healthy young patient who had no other known disease other than inflamed tonsils that required tonsillectomy were used as a positive control tissue for CD31, while Wilms Tumor tissues were used as a positive control tissue for WT1 staining. Technical negative control was performed by omission of the primary antibody.

Scoring of immunohistochemical staining

Scoring of immunohistochemical staining was performed using specialized automated cellular image analysis system, Digimizer software, version 3.7.0, that allows precise manual measurements as well as automatic object detection with measurements of object characteristics (Fig. 1) ⁽⁹⁾.

For purpose of statistical analysis, the following variables were used: