

enumeration. Dual color probe signals were assessed keeping CEP17 (green) signals as the internal control. FISH assay was then performed in three basic steps namely, pre-treatment of the slides, co-denaturation and hybridization with FISH probes, and post-hybridization washing. Slides were then examined under the fluorescent microscope for signal enumeration. A dual color probe was used to determine the HER2 and CEP 17 signals in the index case. A total number of 100 cells were scored, of which HER2 signal count was 2500 indicating HER2 gene amplification. Interestingly, total CEP17 signals were also 2500, indicating an amplification of CEP17 signal as well. The case was positive for HER2 according to the average HER2 per cell (25) however, HER2 to CEP17 ratio was 1.0.

FINAL DIAGNOSIS:

According to the ASCO-CAP HER2 guideline recommendation 2013, the case was reported as positive.

The index case highlights the role of CEP17 amplification, and its effect on HER2 results.

DISCUSSION

HER2 belongs to family of epidermal growth factor (EGF) receptor which consists of four members: EGFR (HER1, erbB1), HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). Located on chromosome 17q12, HER2 gene encodes a 185-kDa protein product which is a transmembrane receptor protein with tyrosine kinase activity.¹⁻³ This receptor comprises of an extracellular ligand-binding domain, transmembrane domain, and an intracellular tyrosine kinase catalytic domain. The receptors dimerize on activation by a ligand and undergo transphosphorylation to activate various intracellular signaling pathways which mediate cell proliferation and differentiation.^{4,5} HER2 testing has become mandatory with the introduction of Trastuzumab. It is a humanized monoclonal antibody that targets the extracellular domain of the HER2 protein.

It binds to subdomain IV, which is located near the transmembrane domain and plays a key role in stabilizing and locking the receptor in an open conformation.⁶⁻⁸ On November 16, 2006, the FDA approval of trastuzumab was expanded to all HER2 positive breast cancer patients with node positive status as an adjuvant therapy. There are two main ways to test HER2, immunohistochemistry and FISH. On a technical background, HER2 IHC testing is easier to perform but is subjective and semi-quantitative in nature. Due to lack of an internal control, there