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Research Article

KI-67 EXPRESSION IN INVASIVE BREAST CARCINOMA: ITS CO-RELATION WITH PROGNOSTIC PARAMETERS

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ABSTRACT

The number of mitoses and, thus, the proliferative capacity of a tumor is one of the most crucial variables for tumor grading. Molecular markers have been extensively investigated with a view to provide early and accurate information on long-term outcome and prediction of response to treatment of early breast cancer. One of the key features of the tumor progression is now widely estimated by the Immunohistochemical assessment of the nuclear antigen Ki-67. The Ki67 may be considered as an alternative to mitotic counts in grading schemes and as a single parameter. Immunohistochemistry using the anti-Ki-67 antibody [MIB-1] was performed on paraffin blocks of 112 invasive breast cancer. Ki-67 stainings were categorized as low, moderate and high proliferative grade. The results were correlated with Modified Bloom Richardson Histologic [BRG] Grade along with traditional prognostic variables like tumor size, axilliary node metastasis besides predictive markers, Estrogen Receptor, progesterone Receptor and Her2neu status. Ki-67 expression showed statistically significant correlation with tumour grade, nodal status, size of primary tumour, Estrogen Receptor [ER], Progesterone Receptor [PgR] profile and Her2/Neu Expression. Out of 112 invasive breast cancer cases, 53 were node negative , where 11.3% cases showed high and 58.5% moderate Ki67 proliferative grade. Ki-67 detection represents a valuable tool and is a good objective evidence for mitotic counts when used in a grading system. When it is used alone, Ki-67 detection provides significant information and increase the evidence of proliferative fraction for chemotherapy decision in early breast cancer. So besides usual ER, PR, Her2/Neu, Ki-67 can be studied together for appropriate management and better prognosis of patient.

Keywords*: Ki-67, Immunohistochemistry, Proliferative Fraction, Chemotherapy, Prognosis*

INTRODUCTION

Background

Because human breast carcinomas are known to exhibit a broad spectrum of clinical behavior, numerous attempts have been made in the past to establish reliable and reproducible prognostic parameters. .Most commonly, morphologic criteria were used for this purpose, such as the widely accepted tumor grading method according to Bloom–Richardson (Richardson *et al.,* 1957). Histologic grade provides an overview of a number of molecular events that are reflected in morphology. Three major elements are included in histologic grading which includes nuclear morphology, differentiation, and proliferation. Although grading methods were described first over 100 years ago, the efforts to evolve more refined, reproducible and objective criteria which lead to semi quantitative method revised by Nottingham grading method (Trihia *et al.,* 2003; Elston *et al.,* 1991). The overall grade is derived from a summation of individual scores for the three variables and this has been accepted by WHO. Markers of proliferation, and specifically Ki-67-labelling index, were considered important for the determination of prognosis and, to indicate the potential value of the addition of Chemotherapy to breast cancer [BC patients] (Goldhirsh *et al.,* 2007). There is no substantial difference in the underlying tumor biology between node-negative and node-positive disease, and the question that remains in adjuvant chemotherapy [CT] today is in proper patient selection (Herbeck *et al.,* 2011).

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Aims and Objectives

1. To evaluate proliferative fraction of invasive breast carcinoma of females by detecting Ki67 expression pattern
2. To correlate with other prognostic parameters-with special reference to histologic grade[Modified Bloom Richardson Grade], Size of primary tumor, axillary metastasis, both positive and negative tumors, Estrogen Receptor[ER], Progesterone Receptor [PgR] and Her2/neu expression profile
3. To look for possibility of considering Ki67 as an alternative to mitotic counts in grading schemes and as a single parameter.

MATERIALS AND METHODS

The study was approved by Institutional Ethical Committee. All the modified radical mastectomy specimans were examined for general histologic diagnosis, invasiveness of tumor, mitotic counting Modified BRG Grading, confirm axillary lymph node metastasis. Then selected representative tumor area were identified. MIB-1 immunohistochemistry was performed on Formalin fixed paraffin embedded [FFPE] of 112 invasive BC using heat-induced epitope retrieval, and the standard horse radish peroxidase with polymer detection method. Based on Ki-67 nuclear staining, expressions were calculated and categorized as low, moderate and high proliferative grade upto 9/10 HPF is as Low [L] 10-15/10 HPF as Moderate [M] and 16 or more/10 HPF as High[H] similar to mitotic counts of Bloom Richardson Grading criteria which was applied by International Breast Cancer Study Group, Trihia *et al.*, (2003). Then Ki67 expression profile was compared with the mitotic counts component in the Modified BRG grading system and also previously specified parameters.

RESULTS AND DISCUSSION

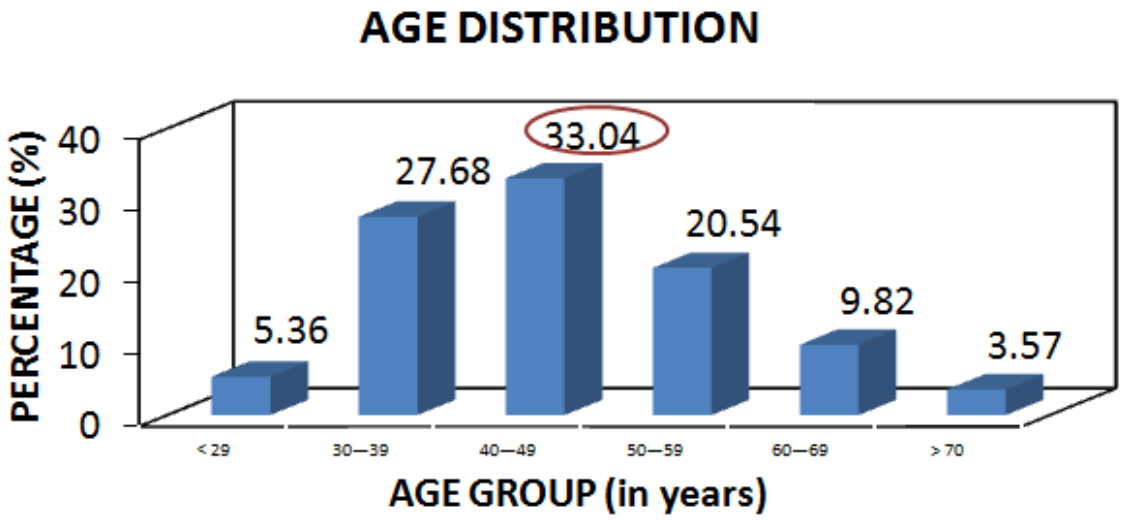
Results

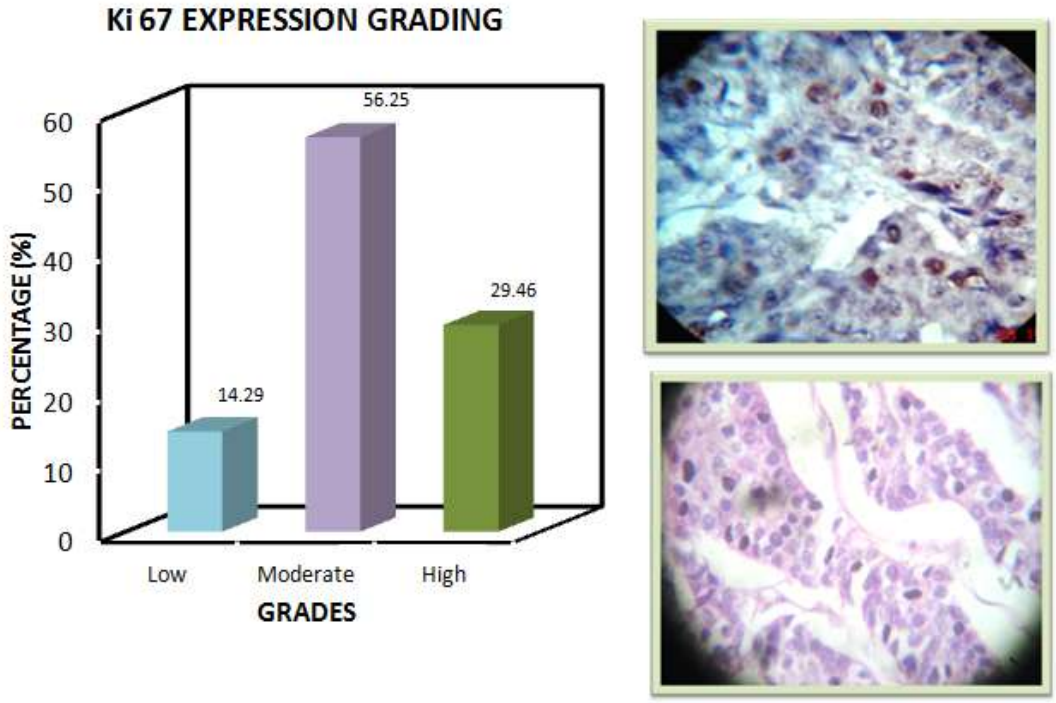
The incidence of invasive breast carcinoma was highest in the age group of 40-49 years [33.04%]. Ki67 expression found to be maximum in moderate grade [56.25%] followed by high grade [29.46%].

DETAILS OF ANTIBODIES USED

ANTIGEN	CATALOG No.	CLONALITY	HOST SPECIES
ER	AM272-2ME	MONOCLONAL (SP1)	MOUSE
PR	AM328-5ME	MONOCLONAL (Y85)	MOUSE
Her2/Neu	AM134-5ME	MONOCLONAL (CB11)	MOUSE
Ki67	275R-18	MONOCLONAL (SP6)	RABBIT
Secondary AB	956D-21/22/23		

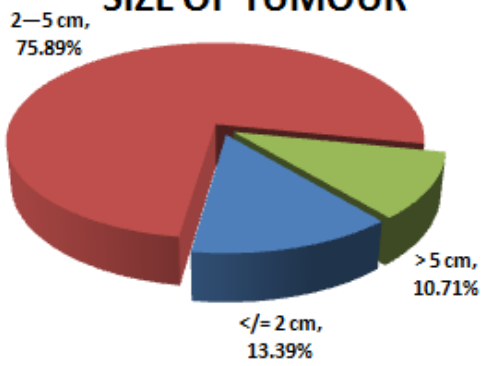
Approximately 80% of invasive tumours size within range of 2-5 centimeter. Ki67 expression didn't show any significant statistical correlation. Axillary node negative breast cancers were found in 47.32% cases and 11% of node negative tumours expressed high Ki67 .Modified Bloom Richardson Histologic Grading of tumours showed Grade II in almost 60 % and Ki67 correlation is statistically significant with P value less than 0.001. When Ki 67 scores were replaced in mitotic counts of BRG system, new Bloom Richardson Ki 67 Grade were derived. 97 of 112 [86.60%] cases showed perfect agreement between adjacent categories.

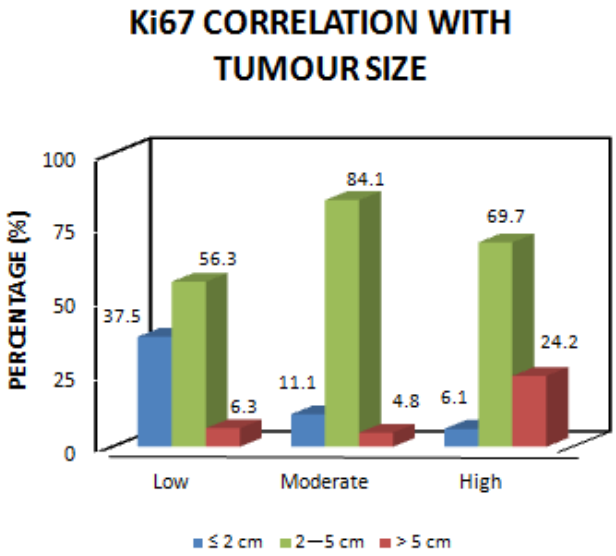




Figures: Ki67 expression above, Mitosis below

Breast cancers were positive for ER and PR in 46% which is lower than Western studies. The hormonal receptors negative cases showed high Ki67 in 44% cases. Again 26.79% tumours were Triple negative breast cancer [TNBC] where 66.67% expressed high Ki67 . Whereas 15 [13.39%] cases showed difference mitotic count in BRG in relation to Ki67 to replace for it. Overall, study showed a enhancement of objective evidence in Grading and proliferative fraction.





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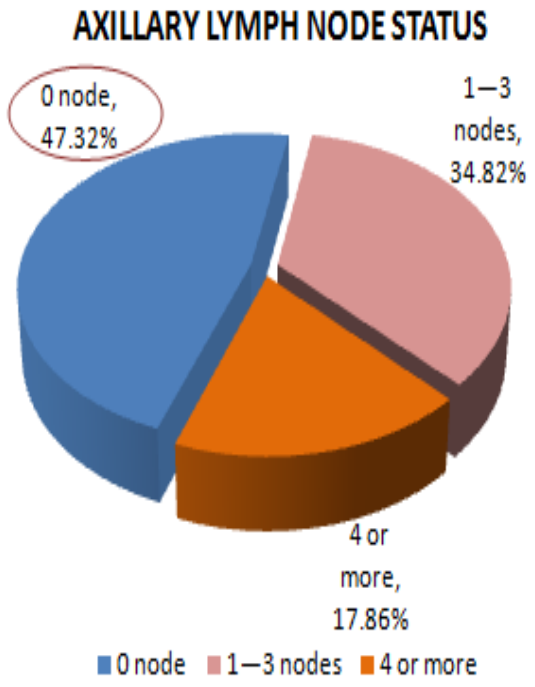

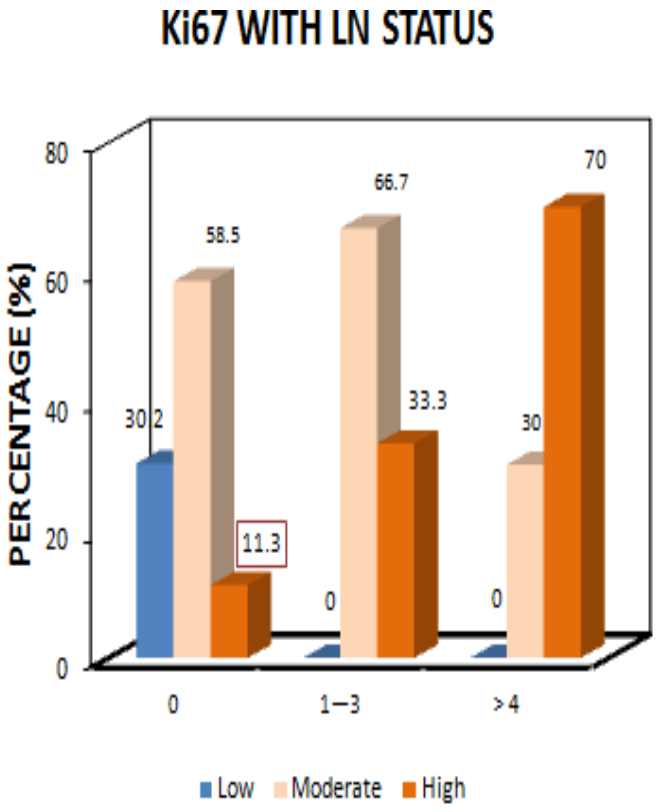

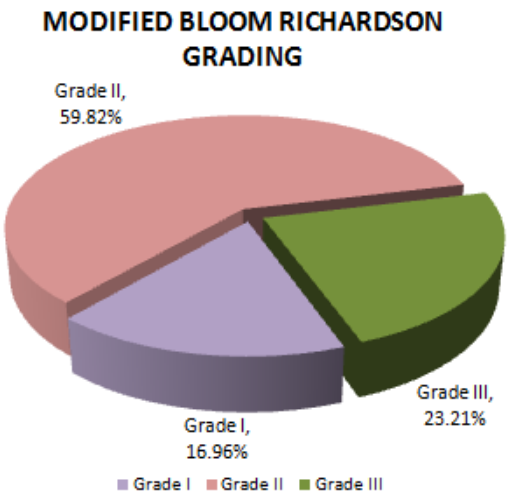

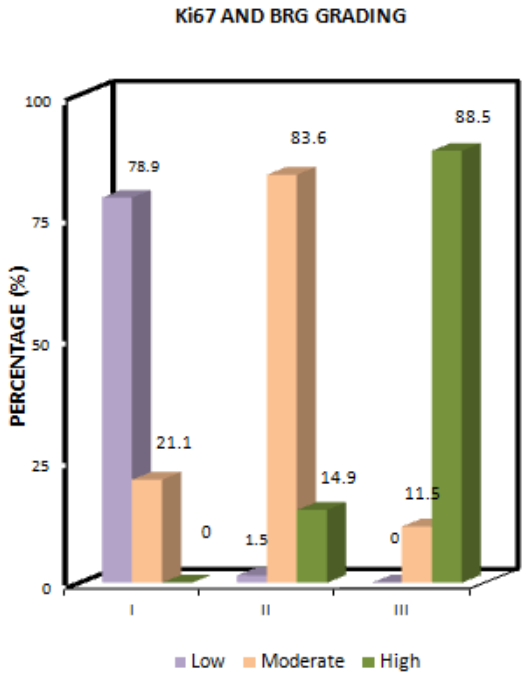

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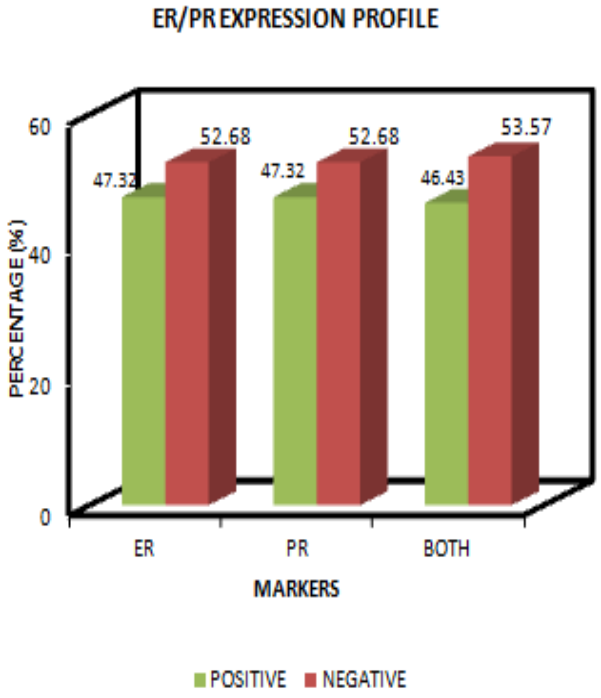
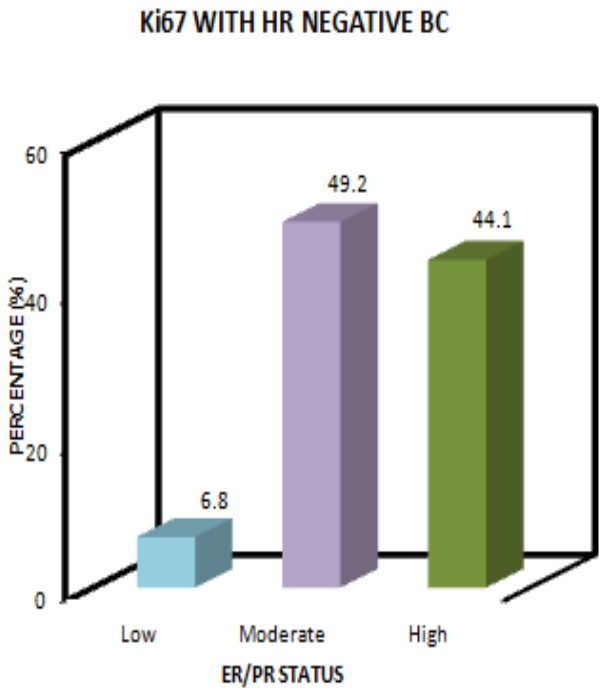
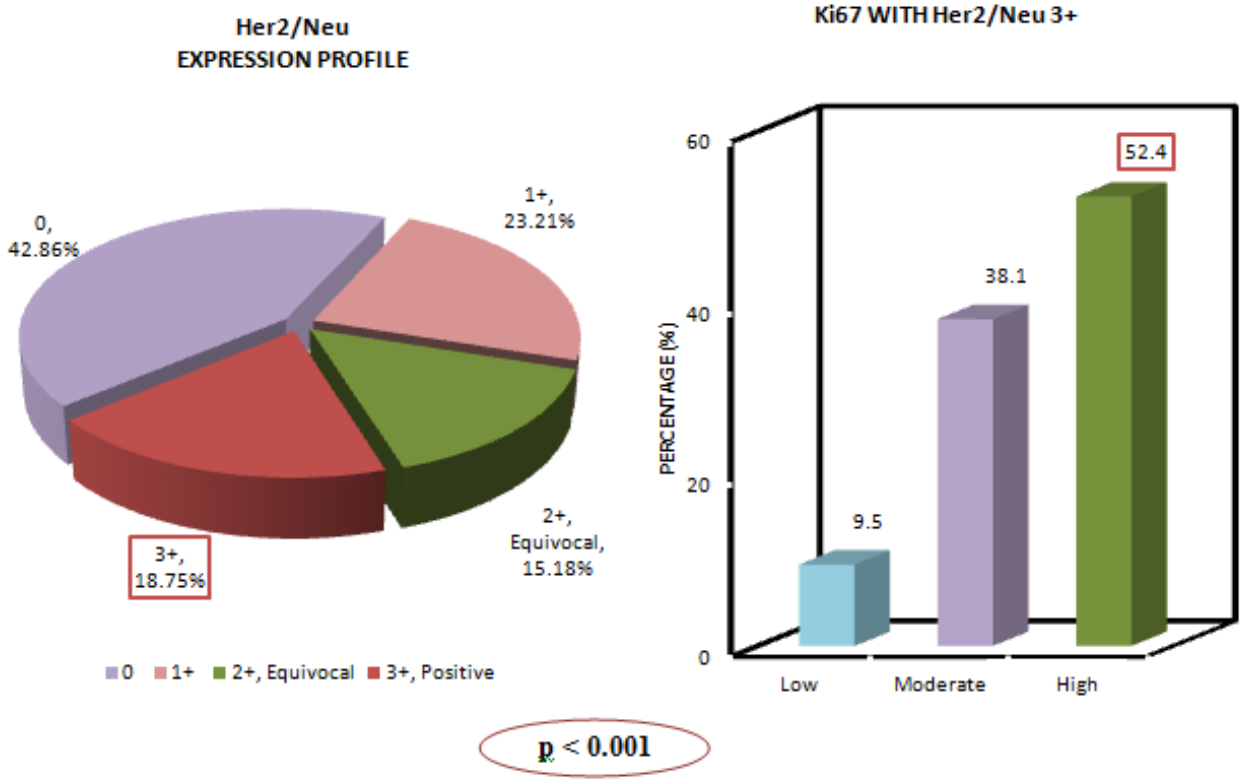
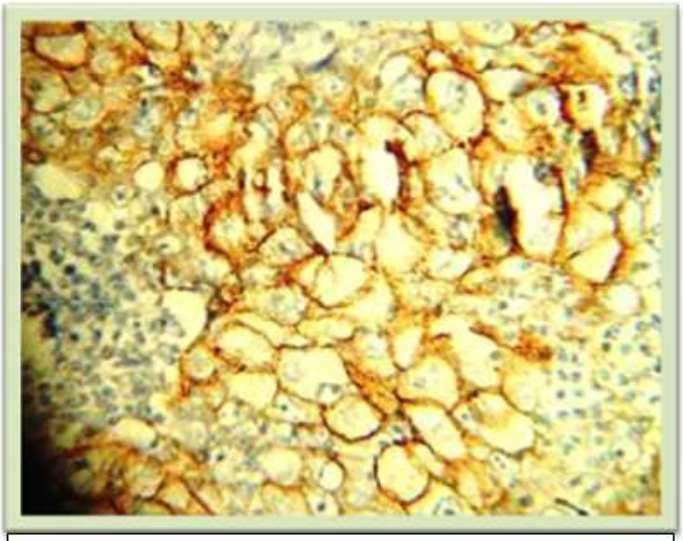
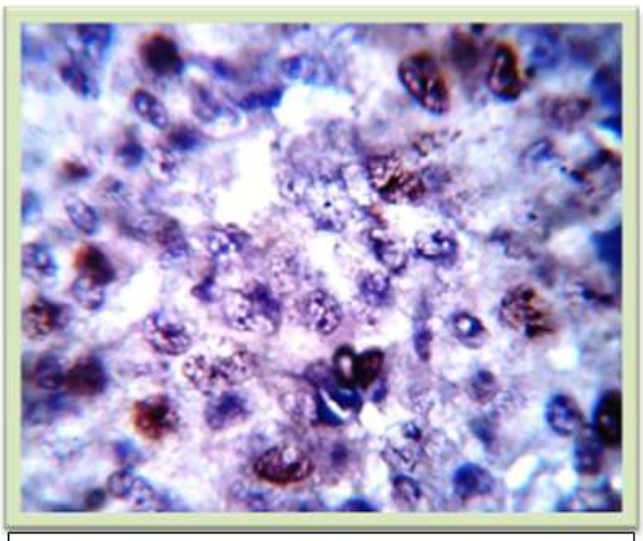




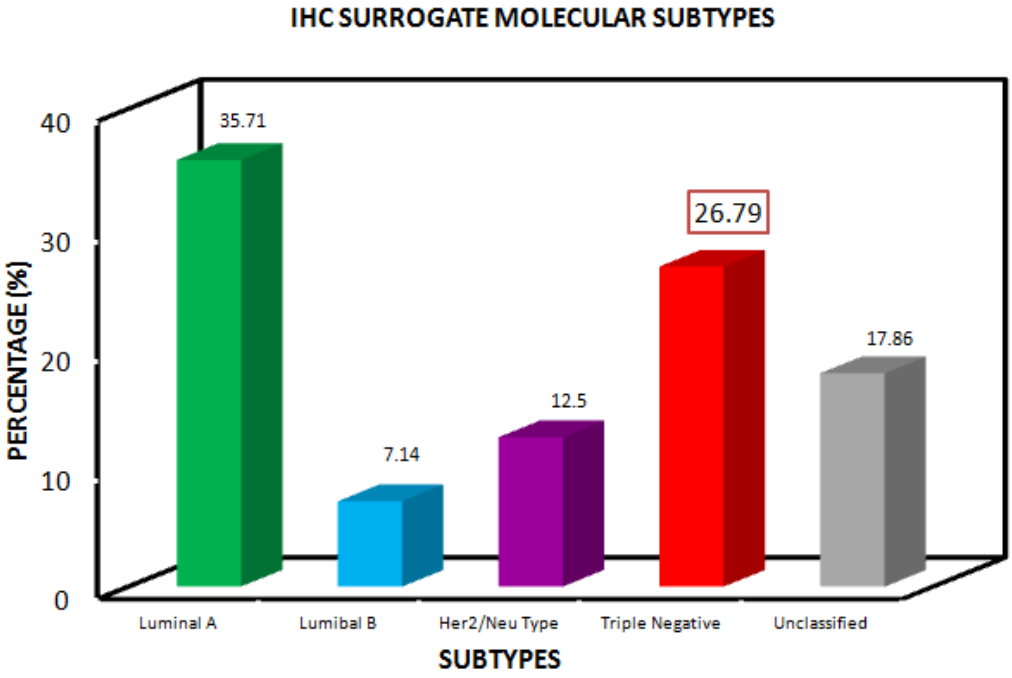


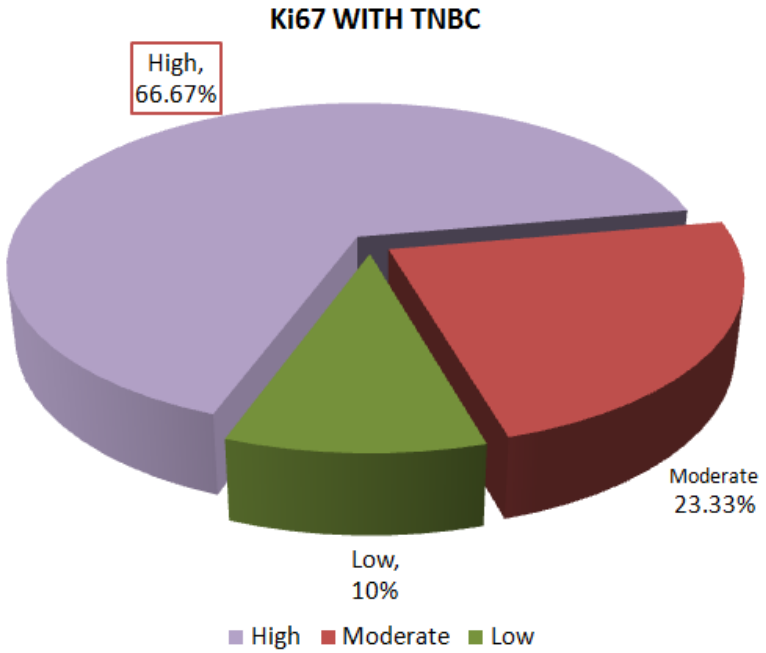


Her2/Neu 3+



High Ki67 Grade





Discussion

The rate of tumor proliferation has long been correlated with its clinical course. Histopathologists, therefore, have sought a means of determining the rate of tumor proliferation as an adjunct to diagnosis.

The simplest and most established of these practices is a count of mitosis (Goldhirsch *et al.,* 2007). The nuclear protein Ki67 (Bloom, 1950) is an indicator of tumor proliferation (Gerdes *et al.,* 1983; Lehr, 1999) and has been found to be a prognostic marker in BC (Sahin *et al.,* 1991; Domagala, 1996; Pietila¨inen *et al.,* 1996; Trihia *et al.,* 2003). MIB-1 antibody also is applicable on routinely fixed, paraffin embedded tissues after microwave pretreatment (Cattoretti *et al.,* 1992). MIB-1 appears to be superior in assessing cycling cells not only because of the simplicity of the technique, but good correlation with frozen (McCormick *et al.,* 1993). In last 20 yrs we have witnessed a marked decline in BC mortality, largely due to earlier diagnosis, a better understanding of the disease, and the advent of ever more effective adjuvant. treatment options (Clarke *et al.,* 2005; Berry *et al.,* 2005). Ki-67 detection represents a valuable tool and in combination with clinical, pathologic, and biologic parameters, it should be analyzed further to produce a grade system (Goldhirsch *et al.,* 2007). An analysis of categorical Ki-67 as an independent parameters revealed that patient with higher Ki-67 % associated with significantly or marginally worse overall Survival (Trihia *et al.,* 2003). The literature on Ki67 though reveals some caveats in regard to inter-observer difference and technicality, but role of application are emphasized by all.

In the future, changes in proliferation rate during or after systemic therapy may be utilized as predictors of response to allow further tailoring of therapy and also necessary for the development of therapeutic reagents (Beresford *et al.,* 2006). Node negative cases are not always suggest a good prognosis. So additional biomarkers are needed to identify cases who would be benefited from CT (Harbeck *et al.,* 2011). Ki67 is a prognostic factor in node negative BC, can be an alternative or complement to Histologic grade and for selection of adjuvant treatment (Klintman *et al.,* 2009). In present study High Ki67 Grade was directly correlated with Node positive , higher size, Her2neu 3+, Triple Negative BC, and negatively correlated with receptor status. Also, Ki67 can be viewed as independent parameters more effectively in stratification of TNBC subgroups where low grade would be spared from aggressive CT.

Conclusion

Besides routine ER, PR, Her2/ neu, Ki67 should be studied together for crucial information of proliferative fraction in a under resourced laboratory where advanced molecular facility still a dream as it is easily available, processable and can be highly applicable in clinical practice

Ki67 is an good reliable objective evidence for mitotic count, so together with BRG become a stronger evidence for treatment decisions. Ki67 could be even more crucial in node negative (early) breast cancer and stratification of TNBC subgroup and a single most parameter or a predictive factor to consider or modify adjuvant chemotherapy.

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