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Stathmin Expression in Breast Carcinoma as A Severity Marker Related to Staging, Grading and Receptor Evaluation.

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ABSTRACT

Background: Breast cancer is now the most common malignant tumor in women. Pathway specific therapy is the future of cancer management. Stathmin, a microtubule destabilizing cytosolic phosphoprotein which has profound influence on cell proliferation, differentiation and cellular motility is an accurate signature IHC marker of PI3K pathway. From overexpression of Stathmin in breast carcinoma one get information about disease progression, prognosis, drug resistance and change in treatment modality. Objective:1. To study and compare the result of Stathmin with level of Estrogen Receptor, Progesterone Receptor and Her-2-neu expression in breast carcinoma. 2. To study Stathmin expression in relation to staging, grading and type of breast cancer. 3. To study the possibility of role of Stathmin as a therapeutic target in breast carcinoma. **Methods**: A cross- sectional study was done. All cases were grossly and microscopically examined and were subjected to immunohistochemical stains of Estrogen, Progesterone, Her-2-neu, Stathmin and were correlated to staging and grading. **Statistical Analysis**: There were altogether 41 cases. In primary breast carcinoma specimens the Stathmin levels were measured by immunohistochemistry and graded from 0 – 3. Scores more than 3 were high expressors with more than 50% tumor cells showing positivity. **Conclusion:** Stathmin over expression in breast carcinoma seems to co relate with loss of Estrogen receptors and Progesterone receptors.

Keywords: Stathmin, breast carcinoma, hormone receptor.

INTRODUCTION

Pathway specific therapy is the future of cancer management. The oncoprotein phosphatidyl inositol-3 kinase/PI3K is frequently activated in solid tumors including breast cancer. Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in women, with more than 1,000,000 cases occurring worldwide annually. Aberrant PI3K/loss of PTEN which is a negative regulator of this pathway results in robust activation of this pathway. Stathmin, a microtubule modeling cytosolic protein which has profound influence on cell proliferation, differentiation and cellular motility is an accurate IHC marker of this signature pathway. Stathmin performs an important function in regulating rapid microtubule remodeling of the cytoskeleton in response to the cell's needs. In normal cell cycle Stathmin interacts with two molecules of dimeric α,β-tubulin to form a tight ternary complex called the T2S complex. One mole of stathmin binds to two moles of tubulin dimers through the stathmin-like domain (SLD). When stathmin sequesters tubulin into the T2S complex, tubulin becomes non-polymerizable. Without tubulin polymerization, there is no microtubule assembly and thus cell cycle stoppage. Stathmin also promotes microtubule disassembly by acting directly on the microtubule ends. Stathmin shows anti PI3K pathway pharmacodynamics properties both in vitro and in vivo. Regulation of stathmin is cell cycle dependent and controlled by the cell's protein kinases in response to specific cell signals as different hormones, growth factors and neurotransmitters. In neoplastic process Phosphorylation at four serine residues on stathmin named Ser16, Ser25, Ser38 and Ser63,causing weakened stathmin-tubulin binding. Stathmin phosphorylation increases the concentration of tubulin available in the cytoplasm At cytokinesis, the last phase of the cell cycle, rapid dephosphorylation of stathmin occurs to block the cell from entering back into the cell cycle until it is ready for microtubule assembly and thus increased mitosis. Stathmin can also itself be used as a therapeutic agent of cancer treatment .Stathmin expression can be used as an agent for microtubule targeting drugs like taxane and taxol in cancer treatment. From Stathmin expression one can get information about disease progression, prognosis, drug resistance and

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change in treatment modality. However the problem is the pattern and topography of its distribution, especially with regard to the heterogeneity of distribution within the tumor and also that diverse oncoproteins come into play. Another observation is Stathmin over expression in breast carcinoma seems to co relate with loss of Estrogen receptors and Progesterone receptors.

MATERIALS AND METHOD

After approval from the ethical committee Mastectomy specimens were collected from the Operation Theatre of General Surgery Department of R.G. Kar Medical college and hospital to the Pathology Department along with duly filled up consent and case record form will be duly filled up. After receiving mastectomy specimens we examined it grossly with measurement of size, shape, outer surface, cut surface and then fix it in 10% buffered neutral formalin overnight. Then during grossing, sections from the fixed tumor proper were taken. Paraffin blocks were prepared using routine histopathological techniques. Thin sections (5 µ thick) were taken on the slide and staining was done with routine Haematoxylin & Eosin stain. Light microscopic examination were done & results were noted. Different histopathological changes of breast specimens were recorded in histopathology data record form. Histopathological grading was noted. Then again the thin sections on the slides were taken. We used 2% Poly-L-Lysine for section adhesion in case of immunohistochemistry as Poly-L-Lysine does not give background staining in immunohistochemistry. Then sections were dried overnight in a 37°C incubator. Sections were dewaxed in xylene and dipped in absolute alcohol. To ensure complete removal of wax the xylene is warmed to 37°C in an incubator. Endogenous peroxidase activity is blocked by incubating in 0.5% hydrogen peroxide in methanol for 10 minutes. This stage may be performed after the primary antibody has been bound onto the antigenic site. Sections were rehydrated by washing well in running water. Then the tissue was incubated with the Anti-Stathmin antibody for the desired time and temperature as indicated by the manufacturer. Then it is incubated with a secondary conjugated tracer antibody and DAB. Stathmin results were measured. ER, PR and Her-2-neu immunohistochemistry were done and measured in the same way. At the end of the study, Stathmin levels were compared to hormone receptor expressions.

RESULTS

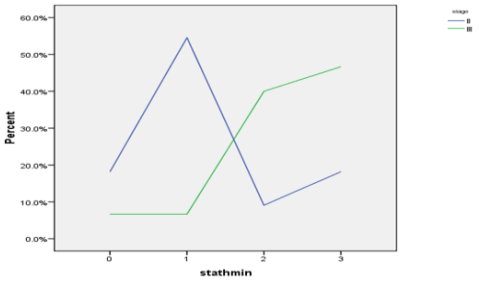
In our study population, 39% of breast carcinoma cases were in the age group of (41-50) yrs, Stage III cases were 73%, and Stage II cases were 27%. In our study population histopathological Grade 3 cases were 42% and Grade 2 cases were 51% and Stathmin grade 3 cases were 39% and Stathmin grade 2 cases were 32%.

Correlation between Stathmin expression and Staging

Table 1: Stathmin and Staging cross tabulation

Count		Stage		Total
		II	III	
Stathmin	0	2	2	4
	1	6	2	8
	2	1	12	13
	3	2	14	16
Total		11	30	41

As the p value is .004 which is less than .05 the study is significant and there is a Spearmans rho positive correlation of .436 calculated by spss software.



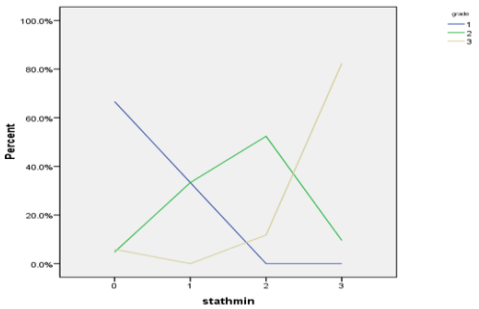
Graph 1: Multilinear graph between Stathmin and Staging

Correlation between Stathmin grade and histopathological grade.

Table 2: Stathmin and Histopathological grade cross tabulation.

Stathmin * Grade Crosstabulation					
Count		Grade			Total
		1	2	3	
Stathmin	0	2	1	1	4
	1	1	7	0	8
	2	0	11	2	13
	3	0	2	14	16
Total		3	21	17	41

As the p value is .000 which is less than .05 the study is significant and the Spearmans rho positive correlation is .719 which is a positive correlation.



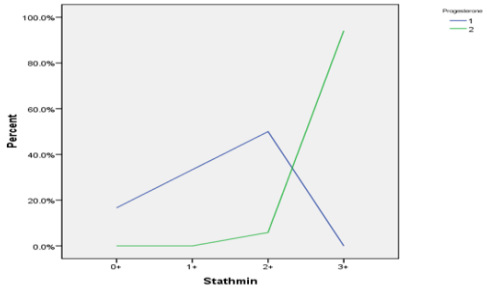
Graph 2: Multilinear graph between Stathmin and Histopathological grading.

Correlation between Stathmin expression and Estrogen/ Progestesterone receptor expression

Table 3: Stathmin and Estrogen/ Progesterone receptor cross tabulation.

Count		ER/PR		Total
		Positive	Negative	
Stathmin	0	4	0	4
	1	8	0	8
	2	12	1	13
	3	0	16	16
Total		24	17	41

As the p value is .000, which is less than .05 the study is significant and the correation coefficient is -.875 there is a spearmans rho negative correlation.



Graph 3: Multilinear graph between Stathmin and Progesterone receptor positive and negative cases.

Correlation between Stathmin expression and Her-2-neu receptor expression

Table 4: Stathmin and Her 2 neu receptor cross tabulation

Stathmin * Her2neu Crosstabulation				
Count		Her2neu		Total
		1	2	
Stathmin	0	0	4	4
	1	4	4	8
	2	6	7	13
	3	10	6	16
Total		20	21	41

As the p value is .083, the study of correlation between Stathmin expression and Her-2-neu is not significant in the study population.

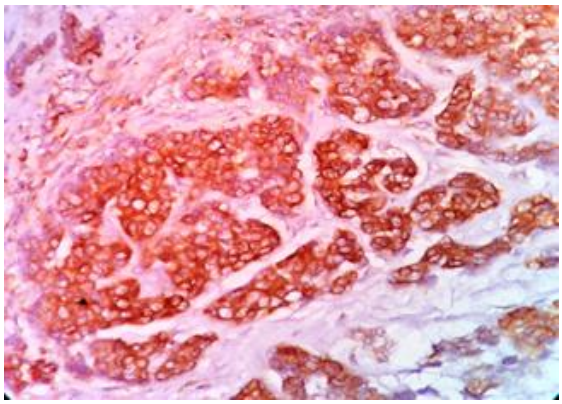


Figure 1: High power view showing cytoplasmic stain of Stathmin.

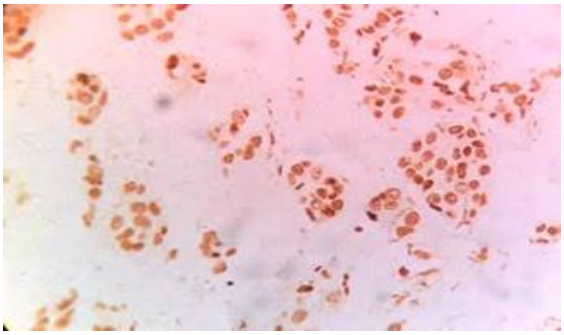


Figure 2: High power view of Progersterone receptor nuclear stain.

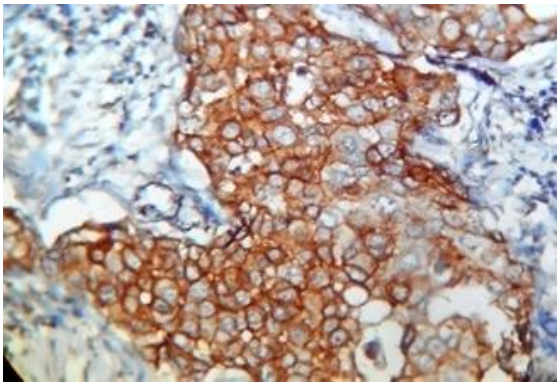


Figure 3: High power view of Her-2-neu showing membrane staining

DISCUSSION

Stathmin is a cytosolic phosphoprotein with a dynamic relation with microtubules necessary for mitosis in cell cycle. It is the signature marker for PI3K pathway activated in neoplasia of solid tumors including breast carcinoma. Increased Stathmin is associated with ER& PR loss signifying metastasis, recurrence and decreased survival. As the sample size is small the study of correlation between Stathmin and Her-2-neu is insignificant. Increased Stathmin is associated with high Stage and grade of breast carcinoma. Thus high Stathmin tells us about disease progression, survival. Taxane, a antimitotic chemotherapeutic drug is given in cancer treatment and high Stathmin gives an idea about high microtubular activity. Stathmin also has a potential to be used as a therapeutic drug in cancer treatment. Thus Stathmin is a new immunohistochemistry agent in the block, with multiple actions and broad scopes of research in cancer prognosis, treatment, disease survival.

CONCLUSION

Breast cancer is now the most common malignant tumor in women. Pathway specific therapy is the future of cancer management. Stathmin, a microtubule destabilizing cytosolic phosphoprotein which has profound influence on cell proliferation,

differentiation and cellular motility is an accurate signature IHC marker of PI3K pathway. From overexpression of Stathmin in breast carcinoma one get information about disease progression, prognosis, drug resistance and change in treatment modality.

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