

## Expression of Immunoglobulin G in Breast Cancer on Immunohistochemistry

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### ABSTRACT

**Objective:** To determine the frequency of expression of Immunoglobulin G immunohistochemical stain in breast cancer.

**Methodology:** It was a descriptive cross-sectional survey. The study was conducted in the Department of Histopathology, Fatima Memorial Hospital, Lahore from 10th July 2015 to 9th January 2016. The study was approved by the ethical committee of the hospital. A total of 70 cases of breast cancer presented and diagnosed at Fatima Memorial Hospital were included in this study. The demographic details of the patients such as name and age were noted. The received specimens were fixed in 10% buffered formalin, grossed and slides were prepared from tissue blocks which were stained with hematoxylin and eosin. The morphology was reviewed and immunohistochemical staining for IgG was performed. Immunohistochemical IgG staining was assessed independently and it was recorded in terms of IgG positive and IgG negative cases. Results were verified by consultant histopathologist. The data was analyzed using SPSS version 24.

**Results:** In our study, out of 70 patients of breast cancer, 57.1% (n=40) were between 25-50 years of age while 42.9% (n=30) were between 51-85 years of age. Sixty nine (98.6%) patients were females and 1 (1.43%) patient was male. Invasive mammary carcinoma of no special type was the commonest tumor type and seen in 66 (94.3%) cases. Grade 2 tumors were recorded as 65.71% (n=46) and grade 3 tumors were recorded as 34.29% (n=24). Out of 70 cases, 92.75% (n=64) were not associated with ductal carcinoma in situ whereas 7.25% (n=5) had an associated ductal carcinoma in situ. Receptor studies showed that 52.27% (n=23) cancers had positive estrogen receptor expression, 52.27% (n=23) had positive progesterone receptor expression and 56.81% (n=25) had Her2/neu positive expression. The frequency of expression of immunoglobulin G immunohistochemical stain was recorded as positive in 55.71% (n=39) breast cancers.

**Conclusion:** The frequency of expression of Immunoglobulin G immunohistochemical stain is less (55.7%) in breast cancer in our population but has significant value (p-value < 0.05) with regards to higher grade and histological subtype of breast cancers.

**Keywords:** Breast cancer. Immunoglobulin G. Immunohistochemical stain.

### INTRODUCTION

Breast cancer is the most prevalent malignancy among women. Accounting for nearly 1 in 3 cancers diagnosed among women, it is the second leading cause of cancer death among women.<sup>1</sup> The incidence of breast cancer in Pakistan is highest in Asians. It is 2.5 times higher than that in neighboring countries like Iran and India accounting for 34.6% of female cancers.<sup>2</sup> A lot of research has been focused on breast cancer pathogenesis which not only helps in understanding oncogenesis but also assists in modifying better prognostic and therapeutic targets.

In recent times, it has been established that epithelial cancer cells produce immunoglobulin which was conventionally perceived to be only produced by mature B lymphocytes and plasma cells. The immunoglobulins expressed by cancer cells consisted of IgG, IgM and IgA.<sup>3-5</sup> Various neoplasms have been identified which produce IgG including colon carcinoma, cervical carcinoma, liver carcinoma, prostatic carcinoma and lung carcinoma along with several soft tissue tumors such as leiomyosarcoma,

rhabdomyosarcoma, fibrosarcoma and malignant fibrous histiocytoma.<sup>9</sup>

The immunoglobulin IgG is detected by immunohistochemical staining (IHC) of IgG, in situ hybridization (ISH) and PCR in plasma cells, tumor infiltrating lymphocytes mainly in cancer tissue and not in normal tissue. Tumor-derived IgG is found to be involved in the survival and growth of epithelial tumor cells.<sup>9</sup> Immunoglobulin G production has also been detected in breast cancer cells mainly in poorly differentiated breast cancer cells.<sup>10</sup>

Immunoglobulin G may be involved in tumor progression. There is a correlation between the level of IgG expression in tumor grade and stage which shows that IgG has a tumor growth promoting function.<sup>6</sup> In the breast, IgG expressing cancer cells are found to be mainly present in the periphery and margin area of primary cancer nests and these cells show more cellular atypia and nuclear pleomorphism. Also there are plenty of IgG expressing cancer cells in metastatic breast lesions compared with the primary lesion.<sup>8</sup>

Tumor cells that express IgG have a more violent disposition and could have significance as an indicator of evolution and metastasis of disease. Since IgG that is derived from breast cancer may be related to pathogenesis, development and prognosis of cancer, it may serve as a probable therapeutic target.<sup>6,7</sup>

The rationale of this study is to determine the frequency of IgG expression in breast cancer patients in Pakistan. This will increase our understanding of breast cancer

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pathogenesis in Pakistani population, the effect of IgG on tumor behaviour and can potentially help in directing towards future clinical therapies against breast cancer.

### METHODOLOGY

It was a descriptive cross-sectional survey. The study was conducted in the Department of Histopathology, Fatima Memorial Hospital, Lahore from 10th July 2015 to 9th January 2016. The study was approved by the ethical committee of the hospital. A total of 70 cases of breast cancer presented and diagnosed at Fatima Memorial Hospital fulfilling inclusion criteria were included in this study by non-probability consecutive sampling technique. All breast cancers detected on histopathological examination (by taking representative sections from specimens) of patients in the age range 25-85 years were included in the study.

The demographic details of patients such as name and age were noted. The received specimens were fixed in 10% buffered formalin, grossed and stained with hematoxylin and eosin. The morphology was reviewed by a histopathologist to establish the diagnosis. Immunohistochemical staining for IgG was performed according to the specifications given by the manufacturer including appropriate positive and negative controls for staining. Immunohistochemical staining was assessed independently and IgG staining was recorded in terms of IgG positive and IgG negative.

### STATISTICAL ANALYSIS

The collected data was entered and analyzed by using the Statistical Package for Social Sciences (SPSS) version 24. The quantitative variables like age were presented as the mean & standard deviation. The qualitative variables like a positive/negative expression of IgG were presented as percentages and frequencies. The data was stratified for age, grade of tumor & histological subtypes to address the effect modifiers. Post-stratification chi-square test was applied to check the significance with a p-value  $\leq 0.05$  considered as statistically significant.

### RESULTS

A total of 70 cases fulfilling the inclusion/exclusion criteria were enrolled to determine the frequency of expression of IgG immunohistochemical stain in breast cancer. The mean age of the patients was  $48.06 \pm 13.49$  years. The age distribution showed that 57.1% (n=40) patients were between 25-50 years of age while 42.9% (n=30) were between 51-85 years of age. Sixty nine (98.6%) patients were females and only 1 (1.4) was male.

Invasive mammary carcinoma of no special type was the commonest tumor type and was diagnosed in

66 (94.3%) cases. The most common histologic subtype of invasive mammary carcinoma was invasive ductal carcinoma diagnosed in 75.7% (n=53) cases. Invasive lobular carcinoma was seen in 24.3% (n=17) cases.

The frequency of tumor grade was recorded as 65.71% (n=46) cases with grade 2 and 34.29% (n=24) had grade 3 tumor. Majority of the cancers i.e 92.75% were not associated with ductal carcinoma in situ whereas 7.25% (n=5) had an associated ductal carcinoma in situ. Component receptor studies were performed in only 44 patients of which 52.27% had positive estrogen receptor expression, 52.27% had positive progesterone receptor expression and 56.81% had Her2/neu expression positive.

The frequency of expression of immunoglobulin G immunohistochemical stain in breast cancers was recorded as positive in 55.71% (n=39) while 44.29% (n=31) had negative expression. The data was stratified for age, grade of tumor, histological subtypes and receptor studies to address the effect modifiers.

### DISCUSSION

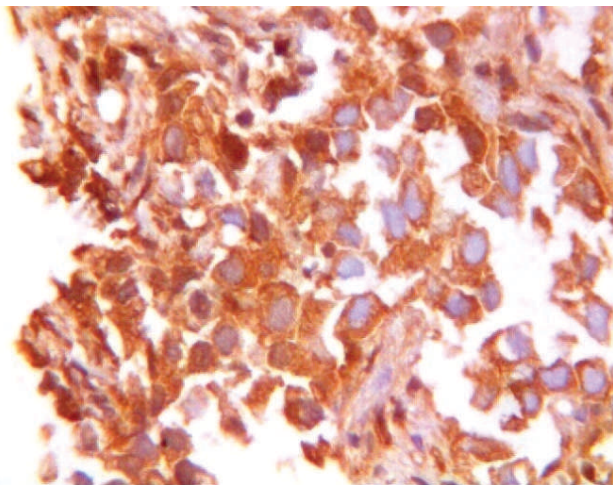
Increased expression of immunoglobulin G (IgG) in various non-B lymphocyte cell malignancies and their unpredictable roles in promoting survival and spread of cancer cells have been established. Cells with high IgG expression demonstrated higher tendency to migrate, increased invasiveness & metastasis, boosted self-regenerative and tumor genetic capability both in vitro and in vivo. Depletion of IgG in breast cancer significantly leads to reduced adhesion, invasion and self-renewal and increased apoptosis of cancer cells.

In our study, out of 70 cases of breast cancer, 57.1% (n=40) were between 25-50 years of age while 42.9% (n=30) were between 51-85 years of age, the mean age being  $48.06 \pm 13.49$  years. Most of the patients were females (98.57%). Frequency of grade of tumor was recorded as 65.71% (n=46) with grade 2 and 34.29% (n=24) had grade 3 tumor. Majority of the breast cancers (92.75%) were not associated with ductal carcinoma in situ.

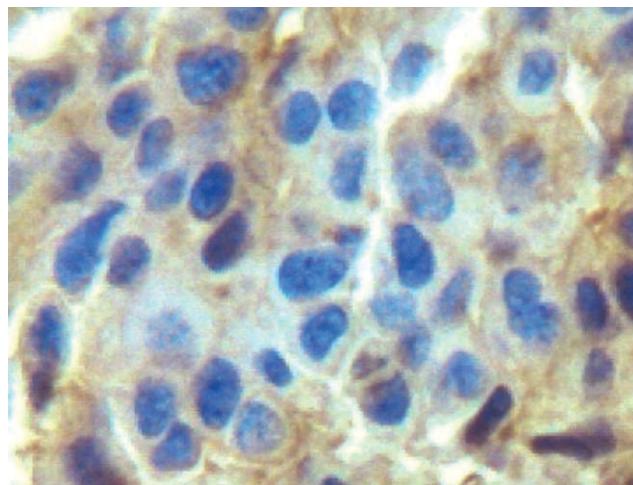
Receptor studies were recorded as 52.27% (n=23) with positive estrogen receptor expression, 52.27% (n=23) with positive progesterone receptor expression and 56.81% (n=25) with Her2/neu expression only. The frequency of expression of immunoglobulin G immunohistochemical stain in breast cancers was recorded as positive in 55.71% (n=39).

A p-value was calculated for expression of IgG immunohistochemical stain with regards to age, grade, histological subtype and receptor status and was found to be significant ( $< 0.05$ ) in relation to higher grade of breast cancer and histological subtype (more in ductal origin).

In a previous study by Chang et al., out of 186 breast



**Figure 1: Positive IgG Expression in Invasive Ductal Carcinoma Grade III Showing Diffuse Cytoplasmic Staining of Immunoglobulin G Immunohistochemical Stain**



**Figure 2: Negative IgG Expression in Invasive Ductal Carcinoma Grade III Showing Weak Membranous Staining by Immunohistochemical Stain IgG**

**Table 1: Frequency of Expression of Immunoglobulin G Immunohistochemical Stain in Breast Cancer (n=70)**

Expression of Immunoglobulin G	No. of patients	Percentage
Positive	39	55.71
Negative	31	44.29
Total	70	100

**Table 2: Expression of Immunoglobulin G Immunohistochemical Stain in Breast Cancer According to Study Variables**

Study Variables	Expression of Immunoglobulin G		
	Positive	Negative	p-value
<b>Tumor Grade</b>			
2	19	27	0.000
3	20	4	
<b>Histological Subtype</b>			
Invasive Lobular Carcinoma	4	13	0.002
Invasive Ductal Carcinoma	35	18	
<b>Receptor Studies</b>			
Estrogen Receptor	6	17	0.0290
Progesterone Receptor	6	17	
Her2/neu Receptor	15	11	

cancer cases of various subtypes, 172 expressed IgG on immunohistochemistry. The expression of IgG was associated with aggressiveness of the breast cancer and histological subtypes of carcinoma.<sup>8</sup> Their study showed higher positive expression than recorded in our study. Another study investigated the pathophysiological importance of breast cancer expressing IgG and examined the appearance of IgG in 68 breast cancers which included 40 cancers without metastasis and 28 cancers with lymph node metastases. Genetic expression of IgG was identified in all these cases.<sup>7</sup>

In 2003, a study carried out by Qiu et al. showed IgG

expression in breast cancer specimens, breast cancer cells and a specific breast cancer cell line.<sup>9</sup> They identified both the protein and mRNA of IgG using tests such as Western blotting, immunohistochemistry (IHC) and in situ hybridization (ISH).<sup>13-17</sup> Babbage et al. made an observation that the genetic transcription of IgG heavy chain variable region detected by nested reverse transcription PCR was seen in 4 of 6 breast cancer cell lines.<sup>10</sup>

Chen et al. demonstrated the production of IgG in breast cancers with immunohistochemistry and laser microdissection (LM)-RT-PCR.<sup>11</sup> A study by Zheng et al. evaluated heavy chain gene repertoire VH(D)JH of

IgG in breast carcinoma and other cancers and concluded that IgG derived by cancer cells might play a significant role in pathogenesis and carcinogenesis.<sup>12</sup>

A study by Manzo et al. revealed different IgG subclasses in 37(35.58%) cases out of 104 preoperated breast cancer patients.<sup>18</sup> The sera of patients containing IgG1 and IgG3 immune complexes were identified by monoclonal antibodies by an indirect immunoenzymatic assay procedure.<sup>19,20</sup>

The particular differences between IgG generated by cancer cells and that produced by B lymphocyte/plasma cell are entirely not clear. However, a study by Lee and associates revealed that IgG (named CA215) produced by cancer cells was indistinguishable from IgG produced by B lymphocyte except that cancer generated IgG had a heavy chain of the molecule with an attached carbohydrate side chain. Against the carbohydrate epitope of the molecule, a monoclonal antibody (named RP215) was developed. The main functional importance of this contrast has not yet been interpreted. Though, CA215 is a collection of IgG superfamily molecules and is most likely only demonstrating a certain proportion of IgG formed by cancer.<sup>20,21</sup>

### CONCLUSION

We concluded that the frequency of expression of IgG immunohistochemical stain is less (55.7%) in breast cancer in our population compared to international data (92.4%) but has significant value (p-value <0.05) with regards to higher grade and histological subtype of breast cancer.

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