INTRODUCTION

Serum appearance of p53 antibodies can be appreciated as a similarly identified aspect of p53 gene mutations (1, 2), and its significance as an indicator of progressive potential of the tumors has been reported in some human malignant tumors including breast cancer (2, 3).

We also recently reported the close correlation of serum appearance of p53 antibodies with an incidence of triple negative breast cancer that is well known to be correlated with more malignant potential of the tumor causing worse outcome of the patients (2). Moreover we have proposed a newly devised criteria based on the incidence of triple negative breast cancer and nuclear grade to predict tumor recurrence of invasive ductal carcinoma of the breast (4).

In this study, we investigated to find out a further evidence to demonstrate the significance of serum appearance of p53 antibodies as an indicator of progressive potential in invasive ductal carcinoma of the breast.

PATIENTS AND METHODS

Patients

One hundred and twenty nine women with breast IDC, who had been treated with surgical resection between April 2008 and October 2013 in our institute, were studied in the current study. This study was approved by the institutional ethic committee of Fukuoka Higashi Medical Center.

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Pathologic investigation

Pathological features were presented according to the general rules for clinical and pathological recording of breast cancer established by the Japanese Breast Cancer Society (5) and TNM classification of malignant tumors prescribed by the International Union Against Cancer (6).

Triple negative breast cancer (TNBC)

Expression of estrogen receptor (ER), Progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) were examined using an usual immunohistochemical methods and an additional FISH examination was performed to find out HER2 expression for cases with an immunohistochemical expression of HER2 2+. Then, tumors which are found to be absolutely negative for expression of hormone receptors (ER and PgR) and HER2 could be diagnosed as TNBC.

Definition of nuclear grade

Nuclear grade (NG) was determined as an aggregate of nuclear atypia score and mitotic count score according to the criteria was shown in Table 1 (7).

Determination of TGS (Triple negative cancer and nuclear grade 3 score)

Definition of TGS was shown in the previous report (4). In brief, patients who had both pathological characteristics of TNBC and NG 3 were allocated a TGS 2. Patients who had one of these characteristics were allocated a TGS 1, and patients who had none were allocated a TGS 0.
Table 1. Decision of nuclear grade

Nuclear grade is decided by the sum of the nuclear atypia score and the mitotic counts score that are defined as follows.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Score 1</td>
<td>Nuclei are relatively uniform in size and shape, and chromatin in inconspicuous.</td>
</tr>
<tr>
<td>2</td>
<td>Score 2</td>
<td>Intermediate between 1 and 3</td>
</tr>
<tr>
<td>3</td>
<td>Score 3</td>
<td>There is considerable variation in size and shape of nuclei and the increase and unevenness of chromatin, sometimes having giant nucleoli.</td>
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Mitotic counts score

After selecting the area in which mitoses are most abundant by small or intermediate magnification, the mitoses are counted in greater magnification. When using a 20 mm field of view, count 10 high-power fields (40x objective).

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>0-4 mitoses</td>
</tr>
<tr>
<td>2</td>
<td>5-10 mitoses</td>
</tr>
<tr>
<td>3</td>
<td>11 or more than 11 mitoses</td>
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Assay of serum anti-p53 antibodies

Serum were drawn from the patients preoperatively and serum anti-p53 antibodies (p53Abs) were assessed by an enzyme-linked immunosorbent assay (ELISA) using anti-p53 EIA kit II (MESACUP anti-p53 test (Medical and Biological Laboratories Co., Ltd., Nagoya, Japan) (8). The samples were added to the wells of a microtiter plate coated with either wild-type human p53 or the control protein and then they were incubated for 60 minutes at 37°C. After washing with the washing solution, goat anti-human immunoglobulin (IgG) antibody conjugated with peroxidase was added and incubated for 60 minutes at 37°C. Next, the substrate solution was added and incubated for 10 minutes. After adding the stop solution, light absorption was measured at the length of 450 nm using the photospectrometer. When the optic density was more than the lowest positive control samples, the samples were considered positive for p53Abs.

Statistical analysis

Then, correlation of the serum appearance of p53Abs with clinicopathologic features including TGS values was examined. Values were expressed mean ± SEM. The Fisher’s exact test and non-parametric test (Mann-Whitney U test) were used to compare the clinicopathologic data. A P value of less than 0.05 was considered significant.

RESULTS

p53Abs was detected in 11.6% (15 out of 129) of the sera of patients with breast IDC. Relationship between serum appearance of p53Abs and clinicopathologic characteristics were demonstrated in Table 2. No significant difference was observed regarding the age, menstruation, operative procedures, histologic type, size of the tumors, lymph node metastasis and the tumor stage. There was a significant correlation between appearance of p53Abs and recurrence of the tumors (P = 0.035).

DISCUSSION

The p53 protein accumulation in tumor cells can be recognized in many human tumors (9), and this accumulation of the mutational p53 can subsequently induce serum circulating anti-p53 antibodies (p53Abs) as the results of the immunological response to p53 (10). On the other hand, in a previous investigation (11), it was mentioned that while a development of p53 antibodies in sera of patients with malignant tumors is derived from the type of p53 gene mutation, p53 accumulation and/or p53 gene mutation does not correlate with the expression of hormonal receptor, ER and PgR, and HER2, incidence of TNBC, NG, and TGS was shown in Table 3. Significant correlation of serum appearance of p53Abs with negative expression of ER (P = 0.011). The proportion of TNBC was significantly higher among patients with serum appearance of p53Abs (P = 0.013). There was also a significant correlation between serum appearance of p53Abs and NG (P = 0.017). Moreover a significant correlation was observed between serum appearance of p53Abs and TGS (P = 0.0065).
In the current study, although no significant correlation was found between serum appearance of p53Abs and such pathologic factors as incidence of lymph node metastasis and tumor size (T factor), serum appearance of p53Abs was significantly correlated with NG 3, incidence of TNBC and also with TGS score. Moreover, tumor recurrence was found to be significantly higher among patients with serum appearance of p53Abs. On the other hand, no significant correlation of such pathologic factors regarding malignant potential of breast IDC as NG, TNBC, and TGS with serum elevation of such tumor markers as CEA and CA15-3 (data not shown).

TGS was constructed by two well-known prominent indicators of malignant potential of breast cancer of an incidence of triple negative cancer and nuclear grade of the tumor. And moreover the fact that TGS could be significantly correlated with serum appearance of p53Abs can compensate for the further clinical significance of serum appearance of p53Abs as an indicator of malignant potential of breast cancer.

The data regarding malignant potential of the tumor by measuring serum p53Abs can be brought about anytime while such pathologic factors as TNBC and NG would be definitively obtained at the timing of surgical treatment. Moreover one another advantage in measuring serum p53Abs is possibly to acquire the more objective and reproducible data. These might be helpful to both patients with breast IDC and physicians.

In conclusion, serum appearance of anti-p53 antibody, that can be easily measured, would be utilized to predict malignant potential of breast IDC.

**DISCLOSURE**

The authors declare that we have no financial interest or conflict of interest.

**REFERENCES**


