Quantitative Histological Evaluation of Invasive Breast Carcinoma of Filipinos with TP53 Mutation

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Abstract

This study utilized stereological technique and standard morphometry in the characterization of TP53 population. The study analyzed the relationship of age and histological grade on the morphometric and stereologic characters.

The morphometric characters studied were cell size, nuclear size, and lymph node and microvessel density. Cell sizes showed correlation with histological grade and marginal correlation was observed between histological grade 3 age and cell size. Nuclear sizes increased as histological grade increased too. Lymph node status showed no correlation with histological grade. Histological grade 3 shows age-lymph node marginal correlation. Microvessel density showed correlation with histological grade but weak correlation was observed between histological grade 3 age and microvessel density.

The stereologic parameters were mean nuclear volume, mean nuclear volume fraction,

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mean nuclear index, mitotic index and mean nuclear profile area. Measurements were performed in microscopic fields that were sampled systematically from histological sections. Using a test system with point and counting frames, estimates of the nuclear values and mitotic index were made.

Mean nuclear volume increased as histological grade increased too. Histological grade 3 age and mean nuclear volume showed low correlation. Nuclear volume fraction showed relationship with histological grade. Age factor showed no correlation with nuclear volume fraction. Nuclear index in both wild type and mutant population did not show relationship with histological grade and age. Mean nuclear profile area showed correlation with histological grade and no correlation with age and nuclear profile area. Mitotic index showed correlation with histological grade and low correlation between histological grade 3 age and mitotic index. This study suggests to confirm the results with more samples.

Keywords: Histological Evaluation, Invasive Breast Carcinoma, TP53 Mutation, stereology, morphometry

Introduction

Cancer is a dreaded disease that has claimed millions of lives for the last fifty years. In spite of the billions of dollars that were poured into research for the discovery of the cure, the mortality rate has not decreased.

Cancer results from interaction between the genes and environment. It is recognized that environmental agents that modify the action of genes cause the vast majority of cancers. It has been estimated that 80-90% of human cancers are caused by environmental agents and diet accounts for more than 30% of this risk. Evidences from epidemiological studies have shown, too, that those people who consume large amount of fruits or vegetables daily have a much lesser cancer risk. The dietary antimutagens in food act as chemical in activators, free radical scavengers, and antioxidants. Only 10-20% of cancer cases are genetically inherited. Among these are the BRCA1 gene in chromosome 17 in breast cancer, BRCA2 in chromosome 13q12 in breast cancer, WT in chromosome 11 in Wilm's tumor, NF_2 in chromosome 22 in neuromas and

meningomas, RBI gene in chromosome 13q14.3 in retinoblastoma, APC in chromosome 5q21 in colorectal cancer, MET in chromosome 7q31 in renal cancer and RET in chromosome 10q11.2 in thyroid cancer.

Mutations on TP53, is implicated in 51 cases of human cancer, it is prevalent in cervical cancer (90%), colon (70%), liver (65%) ovarian cancer (60%), brain cancer, leukemia (60%), stomach (60%), lung cancer (50%), breast cancer (40%), lymphoma (35%), melanoma (35%).

Breast cancer is the most common malignancy in women worldwide. It is estimated that one in eight women will develop breast cancer in a lifetime; around 30% of them will ultimately die. The most recent IARC (International Agency for Research on Cancer) survey found that breast cancer incidence had increased by 26 percent from 1980 to 1995. For the year 2000 IARC estimates 1.2 million new cases and 500,000 deaths from breast cancer worldwide. In Asia, report shows that the Philippines have the highest incidence of breast cancer. Between 1992-1998, there were 5674-recorded cases, a threefold increase over the preceding 5-year period. This alarming increase is attributed to lifestyle and diets.

Review of Related Literature

1. Breast Morphology

Breast refers to the mammary gland and the additional connective tissues and fat that surrounds the gland. The mammary gland is a compound alveolar gland that consists of fifteen to twenty lobes separated by broad bands of dense connective tissue. The fifteen to twenty main lactiferous ducts pass through the nipple and open on its surface. At the base of the nipple each main duct presents a sac-like dilatation, the ampulla, which is a reservoir for the storage of milk (Figures 1A, 1B).

The areola, nipple and mouths of the main lactiferous ducts are covered by stratified squamous epithelium. A circular area, the areola, which is characterized by greater skin pigmentation and thinness of epidermis, surrounds the nipple. In both the areola and nipple the dermis possesses high papillae, and is rich in smooth muscle fibers.

2. Breast carcinoma

Human mammary carcinoma affects breast epithelia. The earliest histologically recognizable aberration is epithelial hyperplasia which is due to an increase in the number of cells and is accompanied by varying degrees of disarrangement and anaplasia. Mammary carcinoma may spread within the breast either by formation of new invasive nodules from intraductal carcinoma or by local lymphatic spread, or both.

Breast cancer originates either from the epithelial lining of the large or intermediate-sized ducts (ductals) or from epithelium of the terminal ducts of the lobules (lobular). Ductal carcinoma that has not invaded the extraductal tissue is commonly called intraductal or in *situ ductal*, otherwise it is an invasive type. Lobular carcinoma is either invasive or in situ (Giuliano, 1991).

3. Types of Carcinoma

A. *Noninvasive (in situ) carcinoma*

A.1 Intraductal carcinoma or ductal carcinoma in situ. This accounts for 15 to 20 percent of all breast carcinomas (Schnitt et al., 1988). The ducts are filled with profuse, sometimes papillary growth of large atypical cells. Focal disruption, thinning or absence of duct basement membrane occurs. Some show obvious periductal infiltration (Miller, 1978).

A.2. Papillaryadenocarcinoma or intraductal papillary carcinoma. This type constitutes less than five percent of breast carcinoma, which usually arises *de novo* in a large lactiferous duct, sometimes from a preexisting duct papilloma. The papillary pattern consists of epithelium, which varies from a single layer to large sheets of columnar cells showing moderate hyperchromatism, lack of polarity, and frequent mitosis. Frequently, intraductal carcinoma showing a cribriform (sievelike) pattern is found in ducts adjacent to a papillary carcinoma (Miller, 1978; Cotran et al., 1994).

A.3. Comedocarcinoma. Tumor is frequently multicentric in origin, which has comedos or small casts of desquamated tumor cells that extrude from the cut surface of the ducts (Wilson, 1986). It constitutes less than five

percent of breast carcinoma. Under the microscope, the ducts are filled with darkly staining, a typical epithelial cells and contain a central granular, amorphous and eosinophilic necrotic area (Miller, 1978; Cotran et al., 1994).

A.4. Lobular carcinoma in situ. This variant comprises about five percent of breast cancers and is the only type of carcinoma that does not occur in male breast (Miller, 1978). This is manifested by proliferation in one or more terminal ducts and/or ductules (acini), of cells that are loosely cohesive, larger than normal, and have rare mitoses and oval round nuclei with small nucleoli (Cotran et al., 1994). A black area of calcification in one lobule may occur (Hutter & Foote, 1969).

B. *Invasive (infiltrating) carcinoma*

B.1. Invasive ductal carcinoma-not otherwise specified. This is the most common type of carcinoma of the breast, accounting for 65 to 80 percent of all mammary cancers. These tumors are devoid of special characteristic of the other types thus the designation of "not otherwise specified" or NOS is utilized (Fisher et al., 1975). Microscopically, the tumor consists of malignant duct lining cells disposed in cords, solid cell nests, tubules, glands anastomosing masses, and mixtures of all these. There is marked fibrosis with minute, angular, cleff-like space containing closely packed, atypical epithelial cells in thin columns. Frequently, invasion of perivascular and perineural spaces as well as blood and lymphatic vessels is readily evident (Miller, 1978; Cotran et al., 1994).

B.2 Medullary carcinoma . This form accounts for 1 to 5 percent of all mammary carcinoma. The average size is 2 to 4 cm in diameter. The malignancy is characterized by solid, syncytium-like sheets of large cells with vesicular, often pleomorphic nuclei, containing prominent nucleoli and frequent mitoses, in which the syncytial cells occupy more that 75 percent of the tumor. Another distinct characteristic is the presence of a moderate-to marked lymphocytic infiltrate between the sheets with a scant fibrous component (Fisher et al., 1975; Cotran et al., 1994).

B.3 **Mucinous or colloid carcinoma.** This kind of carcinoma, also designated as mucoid, gelatinous, or colloid carcinoma, represents a distinct histopathologic type of mammary cancer (Fisher et al., 1975). This includes those breast carcinomas in which there is marked mucin secretion (Miller, 1978). It may occur in pure from, in, which at least 75 percent of the tumor are mucinous, or mixed, which is in association with other types of infiltrating duct carcinoma. In mucinous carcinoma, there are large lakes of lightly staining, amorphous mucin that dissect and extend into contiguous tissue spaces and plane of cleavage. Floating within this mucin are small islands and isolated neoplastic cells, sometimes forming glands and vacuoles. In "mixed" mucinous tumors, on the other hand, the tumor exhibits large areas with mucin as well as areas of typical nonmucinous invasive duct carcinoma (Cotran et al., 1994).

B.4. **Paget's disease.** Paget's disease of the breast is associated with an intraductal carcinoma. It arises in the main excretory ducts of the breast and extends intraepithelially to involve the skin of the nipple and the areola (Cotran et al., 1994). Many vacuolated, large pale cells with hyperchromatic nuclei and frequent mitotic figures (Paget cells) occur in the epidermis occur. These cells frequently contain mucin and melanin granules in the cytoplasm. The dermis is edematous, and infiltrated by plasma cells and lymphocytes (Fisher et al., 1975; Miller, 1978; Wilson, 1986).

B.5. **Infiltrating lobular carcinoma.** This variant of constitutes of 5 to 10 percent of all breast carcinomas and does not occur in the male breast. It arises from alveolar epithelium (Miller, 1978 and probably from the terminal ductules of the breast lobule (Cotran et al., 1994). It consists of the dispersion of small, sometimes innocuous appearing neoplastic cells singly or at most in small clusters in an "Indian file" or targeted pattern, often about non-neoplastic ducts (Fisher et al., 1975). The cells are small and exhibit uniform staining with relatively little cytologic pleomorphism. Irregularly shaped, solid nests and sheets may also occur in continuity with the targeted pattern. The tumor cells are frequently arranged in concentric rings about normal ducts (Yeatman et al., 1995).

B.6. Tubular carcinoma. Tubular carcinoma of the breast, also known as well differentiated or orderly carcinoma is an uncommon from of breast cancer, occurring mostly in women in their fifties. It ranges from 0.5 to 12 cm in diameter (Miller, 1978). A single layer of well-differentiated epithelium typically lines tubular structures. Electron microscopy confirms that tubular carcinoma arises from duct or ductular epithelium and lack of a basement membrane about the tubules. The luminal border of the tumor cells frequently contain bulbous projections regarded by some as representing so-called "apocrine snouts" (Fisher et al., 1975).

B.7. Invasive papillary carcinoma. The truly papillary invasive carcinoma is a rare neoplasm. It is a progression of the noninfiltrating type and does not originate from a preexisting intraductal papilloma (Fisher et al., 1975; Wilson 1986). Frond-like projections characteristic of this pattern may contain fibrous supporting stalks. Indeed, this delicate or nonexistenct fibrovascular core, nuclear hyperchromatism, and absence of double layer of cells and "apocrine" changes features distinguishing papillary carcinoma from intraductal papillomas (Fisher et al., 1975).

Materials and Methods

Materials

Specimens included only the breast cancer tissues that were collected from Philippine General Hospital (PGH), Veteran's Memorial Clinic, Armed Forces of the Philippines Medical Center, East Avenue Medical Center, Ospital ng Maynila and Veterans Memorial Hospital. These specimens that are positive for p53 mutations are either formalin-fixed or frozen samples (Appendix 1). These specimens were screened for p53 mutations by the Biomedical Research team at the Philippine Nuclear Research Institute using PCR-TTGE (Polymerize Chain Reaction-Temporal Temperature Graded Electrophoresis).

Histological Analysis

A. Morphometry

Morphometric characters that were studied were nuclear size, cell size, and microvessel density, following the methodology of Baak and Diest (1991).

One hundred cells per slide were randomly selected and used for the morphometric analysis. The nuclear and cell sizes were measured using a calibrated micrometer eyepiece. The longest diameter of the cell and that of the nucleus were measured for each cell. Microvessel density (number of blood and lymph vessels per sq. cm) was estimated through absolute counting of blood vessels per slide divided by the area of the tissue mounted on the slides (in square centimeter).

B. Stereology

The stereologic parameters that were studied were mean nuclear volume, nuclear volume fraction, nuclear index, mitotic index and mean nuclear profile area.

Slides of tumor were viewed under a Nikon Research microscope equipped with projection attachment and 25" JVC Monitor TM-290ZE for estimation of quantitative histopathologic parameters. Fifteen fields of vision were selected based on the principles stipulated by Sorensen, F.B. (1990, 1992, and 1995) and Ladekarl, M. and F.B. Sorensen (1993). The first field of vision was selected at random and the subsequent fields were sampled systematically by adjusting the distance between individual fields of vision roughly proportional to the overall tumor sectional area. Areas showing inflammation, necrosis, or nuclear pyknosis were excluded from the measurements. Only cancer nuclei that were in focus in one selected focal plane were considered for sampling and were projected and measured on a Test system with 2 Counting frames (appendix 3A). The test system was constructed in an acetate film. This system was made up of one big counting frame (420*mm*x300*mm*) and one inner small counting frame (140*mm* x130*mm*) where nuclei hit by points inside the

frame were scored using an ordinary ruler on a data sheet. Only those cells whose nuclei were hit by points were sampled (Ladekarl and Sorensen, 1993). The number of nuclei evaluated depended on the number of those nuclei that were hit by points.

B.1 Mean Nuclear Volume

The mean nuclear volume, v_v (nuc), was determined using point-sampled intercepts. A test probe with points was randomly thrown on the projected image of tumor tissue. If a test point fell within a nuclear profile of a cancer cell, the intercept through the nucleus and through the test point was measured with an ordinary ruler in one arbitrary direction. The mean of individually cubed nuclear intercept lengths was multiplied by $\pi/3$ to obtain an unbiased estimate of nuclear volume.

$$V_v(\text{nuc}) = \frac{\pi}{3} \cdot l^3$$

B.2 Nuclear Volume Fraction

Using the area associated with each point in the test probe, a (p), the nuclear volume fraction Vv (nuc/tis), that is, the fraction of the total tumor volume occupied by neoplastic nuclei, was estimated by

$$Vv\ (\text{nuc/tis}) = Pp\ (\text{nuc/tis}) = \frac{N \cdot a(p)}{n \cdot A}$$

Were N was the total number of nuclei hit by points, n was the number of fields of vision with area A; and, Pp (nuc/tis), the number of points hitting nuclei as a fraction of points hitting tumor tissue. Two counting frames were integrated in the test probe.

B.3 Nuclear Index

The small frame (of area A_1) was used to obtain the nuclear index, N1 that represents an estimate of the number of nuclear profiles per tissue area. The formula was:

$$NI = \frac{Q_1(nuc)}{n_f . A1}$$

Were Q_1 (nuc) was the total number of nuclear profiles counted in n_f fields of vision within the small frame of area A_1. The number of mitotic profiles was estimated using the large counting frame (with area A) in the same way as described for NI. The computation is shown in appendix 7.

B.4 Mitotic Index

The mitotic index (MI), which expresses the number of mitotic profiles per thousand neoplastic nuclear profiles, was solved by the following formula:

$$MI = \frac{Q(mit).A}{Q_1(nuc).A_1}$$

Q where (mit) was the total number of mitoses counted;

A the large counting frame;

A_1 the small counting frame; and

Q_1 (nuc) – total number of nuclear profile.

The computation of the mitotic index is shown in appendix 8.

B.5 Mean Nuclear Profile

The mean nuclear profile area, $a_H(nuc)$, was estimated by this formula:

$$a_H(nuc) = \frac{a(p) \cdot N \cdot A_1}{Q1\ (nuc) \cdot A}$$

H indicates that the nuclei are sampled with a chance proportional to their height perpendicular to the section.

Where a(p) was the fraction of the total tumor volume occupied by neoplastic nuclei;

N, the total number of nuclei hit by points; and

Q_1 (nuc) the total number of nuclear profiles.

Histological grading

Tumors were graded according to Elston modification of the Scarff, Bloom and Richardson method (1987). The tubule formation was coded 1 when these structures were seen throughout the tumor (> 75%), 3 if no such formation were found (<10%) and 2 if they were intermediate (10-75%). The nuclear pleomorphism (anisonucleosis) was coded 1 if the nuclei were regular (mild), 3 if they were distorted in size or irregular (severe) and 2 if they were intermediate (moderate). The mitotic frequency was observed in the tumor periphery to identify the areas most abundant in mitosis. Under x400, less than one or one mitosis/field was scored 1 (>9 per 10 HPF), two or three mitoses was scored 2 (10-19 per 10 HPF) and more than 3 mitoses was scored 3 (> 20 per 10 HPF) The grade was determined on the following basis: 3 to 5 points was regarded as well differentiated (grade 1), 6 to 7 points as moderately differentiated (grade 2) and 8-9 point as poorly differentiated (grade 3).

Histological grade 1 is well-differentiated stage. Glands are well differentiated, have intact basal membrane and the cells have not yet invaded the stroma. Histological grade 2 is the moderately differentiated stage. Glands consist of irregular sheets and cords of ductal cells actively invade the mammary stroma Histological grade 3 is the poorly

differentiated stage. The tumor cells appear as small basophilic cells permeating the stroma near adipose tissues. There is no definite tubule formation or the atypical appearances of the tumor cells exhibiting large, dark-staining, sometimes pale nuclei cells actively invade the mammary stroma. Histological grade 3 is the poorly differentiated stage. The tumor cells appear as small basophilic cells permeating the stroma near adipose tissues. There is no definite tubule formation or the atypical appearance of the tumor cells exhibiting large, dark-staining, sometimes pale nuclei surrounded by eosinophilic cytoplasm. The edge of the mass of tumor cells advances toward the lymphoid stroma indicating active invasion. Some tumor metastasis with more extensive involvement of the lymph node. The remaining normal lymphoid stroma is seen as a thin rim of bluish area in sharp contrast to the pinkish mass of tumor cells

Results

Cancer Types

A total of 52 cases that were studied included 51 women and 1 male. All of these expressed invasive ductal carcinoma (IDC). Twenty-eight showed wild type TP53 and 24 had TP53 mutations. Ages of the sample cases ranged from 23-70 years.

Histological Grades

Out of 52 samples, 24 were p53 mutants and 28 were p53 wild type. Out of 24 mutant samples, one showed a histological grade 1, nine showed histological grade 2 and ten belonged to histological grade 3. In wild type population, there were five samples with histological grade 1, eight with histological grade 2 and 12 with histological grade 3.

Histological grade 1 has fairly uniform ductal cells disposed in loose aggregates within spaces and display necrotic center. Histological grade 2 has irregular sheets and cords of ductal cells actively invade the mammary stroma. Histological grade 3 has small cells with large dark staining nuclei. Tumor cells were observed in the lymphoid stroma.

Morphometry

A.1 Cell size

Cell sizes 5-10 um were seen in 50% of the wild samples. Sizes 10 15 *um were* observed in 67% of the mutant population. Histological grade 3 registered larger sizes, 11-20 *um*, compared to histological grades 1, of which 2. 37% were seen in samples aged 40-55 years. Cell sizes showed correlation with histological grade but no correlation with age. Marginal correlation was observed between histological grade 3 age and cell size with Multiple R=0.5047804 and Significance F=0 .01367438.

A.2 Nuclear size

Nuclear sizes from 5 –10 *um* were observed in *54%* of the wild type population, 29% of which were observed on samples aged 40-55 years. Nuclear sizes 5-10 *um* were observed in 46% of the mutant group, 42% of which were seen in samples aged 40-55 years. Histological grade 3 showed sizes 10-19 *um, which are larger* compared to those in histological grades 1 and 2 in mutant population. Wild type histological grade 3 showed sizes that range from 9-17 *um which are larger* compared to those in histological grades 1 and 2. Based on the samples collected, as histological grade increased, nuclear sizes increased too. Nuclear sizes showed no trend with age. Through regression analysis of histological grade 3 cases low correlation is shown between age and nuclear size, (multiple R=0.3446357 and Significance F=0.272622409).

A.3 Lymph node

Zero lymph node was observed in 35.7% of the wild type population. Majority of these, 67%, was observed on ages 40-55 years. In the mutant samples, 67% showed zero lymph node and 56% of these samples were observed in ages 40-55 years. Lymph node status showed no correlation with histological grade and age. Histological grade 3

samples, through linear correlation analysis, showed marginal correlation (R=0.61743218,SF=0.002202) between age and lymph node.

A.4 Microvessel Density

Thirty-six percent of wild type samples showed 10-15 per square cm. and 29% showed 6-11 per square cm. Forty-six percent of the mutant samples showed 5-10 per *square cm. microvessel* density while *40%* of wild type and mutant populations showed a microvessel density of 5—10 per square cm. 68% of these were observed on ages 40-55 years. Microvessel density in mutant samples showed correlation with histological grade and no correlation with age. Linear regression analysis showed weak correlation (R=0.2429562, F=0.25405607) among histological grade 3 microvessel density and age.

B. Stereology

B.1 Mean nuclear volume

The dominant range, 400-600 um, in wild type population were observed in 67% of the samples clustered on ages 50-55 years and showed diverse ranges. Size 600-800 um in mutant populations were observed in 46% of the samples clustered on ages 45-55 years. Nuclear volume increased with increase in histological grade. Age did not show relationship with nuclear volume. Histological grade 3 age and mean nuclear volume showed low correlation (R= 0.261842277,F=0.41101069).

B.2 Nuclear volume fraction

Figures 53, 54 and 55 show the nuclear volume fraction for both mutant and wild type populations. The wild type population showed range 10-21 *um*. The ranges on mutant were 11-21 *um* and 13-15 *um* the dominant range (50%) clustered population on age group 40-50 years. Thirty two percent of same age group on wild type population exhibited 13-15 um ranges. Nuclear volume fraction increased with the increase in histological grade. Age factor showed no relationship with nuclear

volume fraction. Histological grade 3 age and nuclear volume fraction showed no correlation (R=0.102354912. F= 0.6503636230).

B.3 Nuclear index

Mean nuclear index in both wild type and mutant populations showed a dominant range, 0.0003-. 0004 um. In the wild type group, 67% exhibited these values and 66% in mutant group exhibited the same range. Nuclear index did not show relationship with histological grade and age. Histological grade 3 age and mean nuclear index showed no correlation through linear regression (R=0.0262040783, F=0.4655057).

B.4 Mean nuclear profile area

The mean nuclear profile area is shown in Figures 71, 72 and 73. The dominant mean nuclear profile, 6000-7000 um, was observed in 42.8% of the samples in wild type and 50% in mutant populations. This range was dominant, too, in ages 40-55 years in both wild type and mutant groups. Nuclear profile area showed correlation with histological grade but no correlation with age and nuclear profile area. Histological grade 3 registered the highest mean nuclear profile area in both populations. Histological grade 3, age and mean nuclear profile area showed no correlation (R= 0.0139389379, F= 0.536138388).

B.5 Mitotic index

In wild type population, mitotic index was observed in 42.9% of the samples. The mutant group did not show any dominant mitotic index value. Mitotic index showed correlation with histological grade. Histological grade 3 showed the highest mitotic index in both wild and mutant populations. Age showed no correlation trend with mitotic index. Linear regression analysis shows low correlations between histological grade 3 age and mitotic index in both wild (R=0.2205260292) and mutant R=0.222808708) populations.

Discussions

Cancer Incidence

Breast cancer is the most common cancer in women. It also occurs in one male for every 100 females affected. One difference between female and male breast cancer is that the latter is more likely to be grade 3 tumors and hormone receptor positive (Willsher, et al., 1997) Germ line mutations in BRCA 2 and androgen receptor (AR) genes are thought to be responsible for a greater proportion of male breast cancer cases (Kwiathwaska et al., 2001).

In this study of 52 cases of invasive ductal carcinoma, there is only one incidence of male cancer. This concurs with previous studies on the rare occurrence of breast cancer in men. This male patient, unlike all females, expresses two types of cancer-invasive ductal carcinoma, grade 3 and Paget's disease, which is characterized by lesions in the skin, nipple and subareolar area. The male in this study exhibited bleeding nipple.

A number of studies have shown that TP53 mutations are associated with poor prognosis. The presence of mutations in one-fifth of breast cancers cases (Sjoegren et al., 1996) and in 30% of the invasive types (Chang et al., 1995) have been associated with aggressive biological behavior and poor prognosis. In this study, morphometric and stereologic characters which are associated with poor prognosis are exhibited by mutants. In this population, 50% belong to histological grade 3, a poorly differentiated stage. AJCC describes this as a metastatic stage - the small cells have large, dark staining and pleomorphic nuclei and cells were observed in the lymphoid stroma.

Epidemiological studies show that breast cancers have age-specific incidence-45-55 years. My results show similar high incidence in the same age groups. A small proportion of breast carcinomas occur in young women. In a study by Thike et al., (2001), the youngest patient is under 30 years, in Australia; cancer is uncommon in women less that 35 years (Adami et al., 1986). In this study the youngest patient is 23 years old, which concurs with the epidemiological data.

Breast cancers in younger women express aggressive features such as high histological grade and high mitotic rate, and lymph node (Querzoli et al., 1998, Agnarsson et al., 1998). The lone 23–year old patient in the study expresses histological grade 2, which is

characterized by loss of tubular formation. Stereologic values-nuclear volume, nuclear volume fraction and nuclear index closely approximate those of ages 40-55 years old. A proportion of carcinomas in young patients are attributed to hereditary predisposition to disease caused by defects in BRCA 1 and TP53 (Querzoli et al., 2001). The young patient in my study has wild type TP53. Other factors such as loss of function mutations in other tumor suppressor genes or gain-of-function in protooncogenes may have occurred in this young woman.

Morphometric characters:

Lymph node status does not show statistical correlation with histological grade and age. This absence of correlation was observed, too, in a number of studies. Azura et al, (2001) in their studies on environment and breast cancer in Malaysian population observed no statistically significant correlation between lymph node status and pS2 expression. Axillary lymph node metastases show no significant correlation with cathepsin in tumor specimens of invasive ductal carcinoma (Perrez et al., 2001).

In spite of the now-existence of statistical correlation between lymph node and some morphometric characters and biomarkers, lymph node status has been accepted as a significant prognostic factor in breast cancer. Among women who have negative lymph node, 70% to 80% have a good likelihood of long-term survival while 80% of women with lymph node involvement have some metastasis in later life (Noguchi and Miyazaki, 2001). Also, the zero node status may represent an occult spread, which cannot be identified by ordinary clinical protocol. Immunostaining with E-cadherin (Hoffman and cytokeratin (Kamath et al., 2001) detects micrometastases and predicts nodal metastasis. The negative node status particularly in high histological grade is a marker for the potential spread of cancer (Looi et al, 2001). It is also a predictor of aggressive disease that may be due to the presence of host-derived or tumor-derived lymphangiogenic cytokines (Camp et al, 2000).

This study shows that high microvessel density (MVD) is associated with high histological grade and is also observed in invasive ductal carcinoma (Sales et al., 1999), prostate cancer (de la Tarlle et al.,

2001) and endometrial carcinoma (Salvesen and Alslen, 1999). The importance of MVD in cancer pathology is its role in angiogenesis – the formation of new blood vessels. Angiogenesis is the route for the progression of cancer into a metastatic stage, for new blood vessels lack complete basement membranes that facilitate entry of cancer cells.

Cancer cells stimulate angiogenesis by directly secreting angiogenic factors in the extracellular matrix (de la Taille, 2000). Neovascularity is also caused by angiogenic facts such as vascular endothelial cell growth factor (VEGF), epidermal growth factor (EGF), Tumor necrosis factor-alpha (TNF-α) and extracellular matrix (ECM) degrading enzymes such as urikinase type plasminogenactivator (http://www/ndch.o.x)

Based on the fifty-two samples, mean nuclear volume, mean nuclear volume fraction and mean nuclear profile area and mitotic index show close association with histological grade, the biological marker in my study. Histological grade 3, a poor differentiated stage, is characterized by high proliferative activity (Talkashi et al, 1996). The high proliferating cell nuclear antigen (PCNA) indicates aggressive phenotype of histological grade 3 tumors (Haerslev & Jacobsen 1995). These result to high values of mean nuclear volume, mean nuclear volume fraction and mean nuclear profile area.

In a number of studies, significant correlations have been reported between stereological parameters and other morphometric characters. In aspiration smears of breast carcinoma, mean nuclear volume shows significant correlations with tumor grade. Stereologically measured nuclear characters have been utilized as index of prognosis for appropriate clinical management. Mean nuclear volume in conjunction with histological grade may be a prognostic factor in lung squamous cell carcinoma (Kargi & Ozkal, 2000).

Mean nuclear volume is an objective method for the cytologic grading of ductal carcinoma of the breast and has independent prognostic value in relation to nodal status higher than those of tumor diameter and cytologic grade (Martin et al., 1999). The estimation of mean nuclear volume is an important prognosicator, in conjunction with post treatment nadir PSA level, in prostate cancer (Fujikawa et al., 1999).

Stereological estimates of the mean nuclear volume and morphometric estimates of the mitotic profile frequency are of

independent prognostic value for patients with ductal breast cancer with positive axillary lymph nodes (Ladekarl, M., 1995). Quantitative histopathologic variables are of value for objective grading of malignanacy in lobular carcinomas. The new parameter estimates of the mean nuclear volume are highly reproducible and suitable for routine use. However, larger and prospective studies are needed to establish the true value of quantitative histopathologic variables in the clinical management of patients with breast cancer (Ladekarl and Sorensen, 1993). The nuclear volume possesses excellent prognostic information and may be suitable for objective malignancy grading (Sorensen et al., 1990). Mean nuclear volume was significantly correlated with Clark's level in primary malignant melanomas (Zer and et al., 1997). Mean nuclear volume is also a superior, efficient, and sensitive estimator for distinguishing melanocytic cutaneous tumors.(Sorensen et al., 1989).

The results from this and earlier studies emphasize the strong prognostic value of easy to assess and highly reproducible morphometric nuclear features in ovarian tumors and offer a useful instrument for the definition of patient groups for future clinical trials (Brugghe et al., 1998). Most investigators agree that the most common antecedent of cancer in a breast is cancer of the opposite site.

However, Moriki et al., (1996) found out there was no significant correlation between the expression of p53 and tumor size, nodal involvement, age or histological type. This contradicts the result of Molina et al., (1998) which said that p53 expression was clearly related to histological grade and steroid receptors The histological grade does represent the proliferative activity of tumor cells and that adding the histological grade to the pathological diagnosis in invasive ductal breast carcinoma may he useful from the clinicopathological aspect concerning tumor behavior (Morili et al. 1996). In univariate analysis p53 immunoreactivity was positively correlated with that absence of tubule formation, high histological grade (poor differentiation), absence of estrogen receptor, and a high proliferating cell nuclear antigen (PCNA) score (Haerslev &Jacobsen et al., 1195).

Mean nuclear volume is the strongest single prognostic factor for overall survival on ovarian cancer patients without post operative treatment (Brugghe et al., 1998). Mean nuclear volume of lung tumor cells shows statistically significant differences between operable and non-operable cases (Kargi and Ozkal, 2000). Mitotic activity and mean

nuclear volume were measured to evaluate on the effects of tamarifen on breast cancer (Nazario et al., 1998). Mean nuclear volume is significantly larger in patients with lymph node metastasis than those without in primary transitional cell carcinoma of the upper urinary tract (Fukyaron et al, 2000).

Since my study does not include prognostic value of stereologic values of nuclear measurements, future researchers can utilize the data for prognostic evaluation.

Summary and Conclusions

This study utilizes stereological technique as well as standard morphometry in the characterization of TP53 wild type and mutant populations. The study analyzes the relationship of age and histological grade on the morphometric and stereological characters. The following are the findings:

1. Among the 52 cases of invasive ductal carcinoma there is only one incidence of male cancer. Out of 52 cases, 24 were TP53 mutants type and 28 were wild type.
2. Histological grade 1 has fairly uniform ductal cells disposed in loose aggregates within spaces and display necrotic center. Histological grade 2 shows irregular sheets and cords of ductal cells actively invading the mammary stroma. Histological grade 3 shows small cells with large, dark staining nuclei. Tumor cells were observed in the lymphoid stroma.
3. Ages of the samples ranged from 23-70 years old. The youngest case is 23 years old with histological 2. Most of the breast cancer incidence occurs at the age of 40-55 years old.
4. The morphometry studied were cell size, nuclear size, and lymph node and microvessel density. Cell sizes showed correlation with histological grade while marginal correlation was observed between histological grade 3 age and cell size. Nuclear sizes increased as histological grade increased too. Marginal correlation was observed between histological grade 3 age and cell size. Lymph node status showed no correlation with histological grade

but with a marginal correlation with age. Microvessel density showed correlation with histological grade.

5. The stereologic parameters were mean nuclear volume, mean nuclear volume fraction, nuclear index, mitotic index, mean nuclear profile area. Mean nuclear volume increased as histological grade increased. Mean nuclear volume fraction showed relationship with histological grade. Age factor showed no correlation with nuclear volume fraction. Nuclear index in both wild type and mutant population did not show relationship with histological grade and age. Mean nuclear profile area showed correlation with histological grade but no correlation with age. Mitotic index showed correlation with histological grade.

Recommendations

More samples on histological grade 1, 2 and 3 are needed to establish the reliability of the statistical analysis. A possible statistical procedure is to combine histological grade 1 and grade 2 and perform binary study. A study can also be done aimed at developing user-friendly computer programs and software for quick estimation of stereological characters. To assess the prognostic value of stereological estimated parameters, retrospective studies on the 5 year survival rate of the patients is also recommended.

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Literature Cited

Adami HO, and Malker B. The relation between survival and age at diagnosis in breast cancer. New England Journal of Medicine. 315:559-563 (1986).

Agnarsson BA, Jonassan JG, Bgomdottir IB, Barkardotter RB, Egilsson V, and Sigurdson H. Inherited BRCA2 mutations associations with high grade breast cancer. Breast Cancer Re. Treat. 477-121-127 (1998).

Aziz, S, Pervez Z, Kayani N, and Rahbar M. Immunohistochemical cathepsin depression in breast cancer- correlation with established pathological parameters and survival. Pathology, Research & Practice. 197(8): 551- 557 (2001).

Baak, J.P.A. and P.J. Van Diest. Morphometry. Manuala\ of Quantitative Pathology in Cancer Diagnosis and Prognosis. Berlin, Springer-Verlag, (1991).

Bentzen, JK, Hanssen HS, and Nielsen HW. The prognostic importance of volume - weighted mean nuclear volume, mitotic index, and other stereologically measured quantitative parameters in supraglottic laryngeal carcinoma.Cancer. 86(11):2222-8 (1999).

Begley, S. The Cancer Killer. Newsweek (January 13): 42-43 (1997).

Brugghe J, Baak JPA, Wiltshaw E, Brinkhuis M, Meijer GA, and Fisher C. Quantitative prognosic features in figo 1 ovarian cancer patients without postoperative treatment. Gynecologic Oncology 68 (1): 477-53 (1998).

Camp RL, Rimm EB, and Rimm DL. A high number of tumor free axillary lymph nodes from patients with lymph node negative breast carcinoma is associated with poor outcome. Cancer 88 (1): 218-9 (2000).

Cattoretti G. and Bartek J. p53 expression on breast cancer. Int J Cancer. 41:178-183 (1988).

Chang, F. Implication of the p53 tumor suppressor gene in clinical oncology. Journal of Clinical Oncology. 13:1010 (1995).

Cotran RS, and Kumar V, Robbins S. Robbins' pathologic basis of disease. 5th Ed.WB. Saunders Company. Philadelphia (1994).

Davidoff AM, Humphrey PA, Ighlehart JD, and Marks JR. Genetic basis for p53 overexpression in human breast cancer. Proc. Nat'l Acad. Sci. USA. 88:5006-5010 (1991).

De la Tallie A, Katz A E, Bagiella E, Buttyan R, Sharir S, olsson C, Burchardt T, Ennis R, and Rubin MA. Microvessel density as a predictor of PSA recurrence after radical prostatectomy: a comparison of CD34 and CD31. Anatomic Pathology. 1-5 (2001).

De Jong JS, Van Diest PJ, and Baak JP. Hot spot microvessel density and the mitotic index are strong additional prognostic indicators in invasive breast cancer. Histopathology. 36 (4): -12 (2000).

Elston, CW. Grading of invasive carcinoma of the breast. In: Page DL, Anderson TJ, editors. Diagnostic histopathology of the breast. New York:Church Chill-Living Stone. 300-11 (1987).

Elston CW and Ellis IO. Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer. Histopathology journal. 19: 403-410 (1991).

File://H:sigmetric morphometryandstereology.htm

Fisher ER, Gregorio B, Fisher C, Redmond F, Vellios F, and S.C. Sommers.The Pathology of Invasive Breast Cancer: a syllabus derived from findings of National Surgical Adjuvant Breast Project (Protocol No. 4). Cancer. 36: 1- 85 (1975).

Forrester K, Lupold S.E. Ott VI, Chay Ch, Band V. and Wang XW. Effects of p53 mutants on wild-type p53 mediated transactivation are cell-type independent. Oncogene. 10:2103-11 (1995).

Fujikawa K, Aoyama T, Nishio Y, Miyakawa M, and Sasaki M. The role of volume-weighted mean nuclear volume in predicting disease outcome in patients with stage MI prostate cancer. APMIS. 105 (4): 395-400 (1999).

Giuliano, A. E. Breast in Current: Medical Diagnosis and treatment. 30th ed. Edited by S.A. Schroeder et. al., Appleton and Large, Prentice Hall Pub., Connecticut (1991).

Groenendijk RP, Bult P, Tewarie L, Peer PG, Van der Sluis RF, Ruers and TJ.Wobbes T. Screen-detected breast cancers have a lower mitotic activity index. Br J Cancer. 82 (2): 381-4 (2000).

Haerssley T, and Jacobson GK. A immunohistochemical study of p53 withcorrelations to histopathological parameters, C-ERBB-Human Pathology. 26 (3): 295-301 (1995).

Hartmann A, Blaszy H, Kovach JS, and Sommer SS. The molecular epidemiology of p53 gene mutations in human breast cancer. TIG. 13:27-33 (1997).

Holstein M, Sidransky D, Vogelstein B, and Harris CC. P53 mutations in human cancers. Science. 253:4953 (1991).

http://breastdoctor.com.breast/anatomy.htm

Kamath VJ, Rammath EM, and Dupont EL. The role of lymphoscintigraphy in the management of the patient with breast cancer. Ann Surgical Onco. 8:354-60 (2001).

Kargi A, Ozcal S, and Sagol. Stereologically estimated mean nuclear volume and histopathologic malignancy grading as predictors of disease extent in non-small cell lung carcinoma. Pathology Research & Practice. 196 (10): 683-689 (2000).

Kwiatkowska E, Terreiak M, and Lamperska KM. BRCA 2 germline mutation in male breast cancer patients in the polish population. Contemporary Surgery. 57 (9): 427-428 (2001).

Ladekarl M. Quantitative histopathology in ductal carcinoma of the breast prognostic value of mean nuclear size and mitotic counts. Cancer. 72 (8): 2114-2122 (1995).

Ladekarl M, and Sorensen FB. Prognostic quantitative histopathology variables in lobular carcinoma of the breast. Cancer. 72:2602-11 (1993).

Lasky T. and Silbergeld E. p53 mutations associated with breast, colorectal, liver, lung, and ovarian cancers. Environ Health Perspect. 104 (12): 1324-1331 (1996).

Li P, Bui T, Gray D, and Klamut HJ. Therapeutic potential of recombinant p53 over-expression in breast cancer cells expressing endogenous wild type p53. Breast Cancer Res Treat. 48 (3): 273-86 (1998).

Looi LM, Azura WW, Cheah PL, and Ng NH. PS2 expression in infiltrating ductal carcinoma of the breast correlated with estrogen receptor passivity but not with histological grade and lymph node status. Pathology. 33 (3): 283-286 (2001).

Martin MP, Fishback K, Osborne CK, Allred DC and O'Connell P. The inverse relationship between microvessel counts and tumor volume in breast cancer. Cancer. 61 (9): 3578-80 (1999).

Miller FF. Jr. Miller's Pathology. Dynamic introduction to Medicine and Surgery. 3rd ed. Little Brown Company, Boston (1978).

Mukhopadhyay, T. S. A. Maxwell, and J. A. Roth 1995. p53 Suppressor gene. R.G. Landes Company, Texas (1995).

Nazario, ACP, Uehara, Delima GR, Simeos MJ, Juliano Delima GR, Simeos MJ, Juliano and Gebrim LH. Effects of tamoxifen on the breast in the luteal phase of the menstrual cycle. International Journl of Gynecology & Obstetrics. 62 (1): 77-82 (1998).

Nielsen K. Stereological estimates of nuclear volume in normal mucosa and carcinoma in situ of the human urinary bladder. Virchow Arch.52:41-54 (1986).

Nigro J.M., S.J. Baker, A.C. Preisinger, J.M. Jessup, R. Hostetter, K. Cleary, S.H. Beigner, N. Davidson, S. Baylin, P. Devilee, T. Glover,F.S.Collins, A. Wetson, R. Modali, C.C. Harris, and B. Vogelstein. Mutations in the p53 gene occur in diverse human tumor types. Nature. 342:705-708 (1989).

Noguchi M and Miyazaki. Prognostic factors in breast cancer and their controversies (Review). Oncology Reports. 1 (60: 1157-1163 (1994).

Ouchi K T, Sugawara H, Ono T, Fujiya Y, Kamiyana Y, Kakugawa J, Mikuni H, Yamanami S, Komatsu A, and Horikoshi A. Mitotic index is the best predictive factors for survival of patients with research hepaocellular carcinoma. Digestive Surgery. 17 :42-48 (2000).

Querzoli P G, Albonico MR, Lasio S, Ferretti R, Rinalde R, Cariello M, Pedriale M, Matteuzi I, Maestri I, and Nenci L. Biophenotypes and survival of BRCA1 and Tp53 deleted breast cancer in young women. Breast Cancer and Treatment. 83:89-97 (1998).

Querzoli P, Albonico G, Ferretti S, Rinaldi R, Baccati D, Corcione S, Indelli M, and Nenci L. Modulation of biomakers in minimal breast carcinoma: a model for human breast progression. Breast Cancer Treatment. 83:89-97 (1998).

Reed, MG and Howard V. Unbiased stereology: Three-Dimensional Measurement in microscopy.

http://www.amazon.comexec/obodos/tg/stores/detail/-books.0387915168.reader/8/ref=lih_dpTT01/102-47238312- 0900129 (2002).

Sales A. and Bosch-Ilonbart. Comparative morphometric evaluation of microvessel density and nuclear area in ductal carcinoma in breast. webmaster@harcourt-international com. (1999).

Salvassen HB and Alslen LA. Significance of tumor-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumor angiogenesis and prognosis in endometrial carcinomas. International Journal of Cancer. 84 (5): 539-543 (1999).

Seshadri R, Leong, ASY, McCaul K, Firgaira FA, Setlur V, Horsfall DJ. Relationship between p53 gene abnormalities and other tumor characteristic in breast cancer prognosis. Nature. 69 (2): 135-141 (1996).

Sorensen, F.B. Unbiased stereologic techniques for practical use in diagnostic. Histopathology Pathological. 87:263-278 (1995).

Sorensen, F.B. Biology of Disease Quantitative Analysis of nuclear size for objective malignancy grading: a review with emphasis on new, unbiased stereologic methods. Laboratory Investigation. 66(1):4-23 (1992).

Sorensen, F.B. Stereological estimation of the mean and variance of nuclear volume from vertical sections. Journal of Mircroscopy 162 nuclear volume from vertical sections. Journal of Mircroscopy. 162:203-229 (1991).

Takahasshi T, Moriki T, Hiroi M, Yamane T, and Hara H.Histological grade in invasive ductal carcinoma of breast correlated with the

proliferative activity including correlation with p53, C-ERBB-2 and estrogen receptor status. Pathology International. 46 (6): 417-425 (1996).

Thike A. A, Chang M, Chong SF and Tan PH. Immunohistochemical expression of hormone receptors in invasive breast carcinoma: correlation of results of h-score with pathological parameters. Pathology. 33:21-25 (2001).

Voglino G, Castillo D, Silengo L. Stefanuto G, Friad O, Ferrara G, and Fessia L. An intronic deletion in Tp53 gene causes exon 6 skipping in breast cancer. European Journal of Cancer. 33(9):1479-1483 (1997).

Willsher PC, Leach LH, Ellish IO, Bell JA, Elston CW, Bourke JB, BlamenyRW and Robertson JPR. Male breast cancer – pathological and immunohistochemical features. Anti cancer Research. 17 (3C):2335-2338 (1997).

Wilson RE. The Breast in Textbook of Surgery: The Biological Basis of Modern Surgical Practice. Vol. 1. 3rd ed. Edited by D. C. Sabiston. W. B. Company, Pheladelphia (1996).

World Health Organization. International classification of tumors. Histologic types of breast tumors. Geneva: World Health Organization (1981).

Yeatman TJ, Cantor AB, Smith SK, Reintgen DS, Miller MS, Ku NK, and Baekley CE. Tumor biology of infiltrating lobular carcinoma: implications for management. Ann.Surg. 222:549 (1994).

Zer E, Sagol Z and Kuyucuoglo F. ki-67 immunostaining and stereological estimation of mean nuclear volume in melanocytic cutaneous tumors. Turkish Journal of Dermatopathology. Vol 1:3-4 (1997).