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Research Article

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P53 Gene Mutation Associated With Worse Clinicopathological Characteristic in Breast Cancer

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Abstract

Background: *P53 is a tumour suppresor gene. In breast cancer, p53 gene mutation were noted with frequency of about 30% (range 15 to 71%) and associated with poor prognosis. This study was performed to determine p53 mutation association with clinicopathological characteristic in breast cancer and to assess the suitability of patients' serum to detect p53 autoantibody.*

Methods: *This study conducted in Hospital Seberang Jaya and Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia. Sixty-four breast cancer patients with available fresh breast cancer tissue that been kept under -80°C and with complete clinicopathological, data involve in this study. These fresh breast tissues DNA extracted and 10sampels sent for DNA sequencing. The remaining 54samples proceeded with Polymerase Chains Reaction analysis based on the result from DNA sequencing. The serum of these patients was also taken for p53 autoantibody study using ELISA method.*

Results: *The mean age of the patients in this study was 52.45±9.51 yrs. Most of the patients were Malay with 67.2% followed by Indian and Chinese with 17.2% and 15.6% respectively. 51.6%, of these patients undergone CT scan staging and 14.1% has distant metastases. p53 gene mutation prevalence showed rs1042522 only has 15.7% mutation. There was 54.7% Deletion A and 45.3% Wild Type A detected in rs59758982, 87.5% Deletion A and 12.5% Wild Type A in rs35069695 and 92.2 % recorded for Deletion GAA in rs376546152. There was no significant result between these mutation with breast cancer molecular classification and breast cancer aggressiveness except for rs59758982 shows significant result with p value 0.04 ($p < 0.05$). In regards on for p53 serum autoantibodies, 20.3% of the patients noted to be positive but it has no significant association with p53 gene mutations.*

Conclusion: *In this study, tissue p53 genetic mutation has no significant association with clinicopathological characteristic of breast cancer and the use of serum p53 autoantibody as biomarkers is inconclusive.*

Introduction

Breast cancer is a distressful disease affecting patient overall well-being. It affects both genders and all ethnicity around the world. Each year, this illness affects more than 1 million people worldwide especially women. Worldwide about 1.67million new cases were diagnosed in the year 2012. This represents 12% off all new cases and 25% of all cancer in women [1]. However, the incidence of breast cancers is much lower in men, which account for only 1% of all cancer in men [2].

National Cancer Registry Malaysia (2007) has reported that, breast cancer was the most frequently diagnosed cancer in women in all ethnic groups with a total of 3,242 cases, accounting for 18.1% of all cancer's cases and 32.1% of female cancers [3,4]. Chinese had the highest incidence with an ASR of 46.8 per 100000 population followed by Indian women with an ASR 38.1 per 100000 population and Malay women with an ASR 30.4 per 100000 population. The incidence is steadily increasing with age and peak in the 50-59 age groups [3,4].

According to Penang Cancer Registry report in 2004 until 2008, there were 1699 cases of breast cancer reported from both government and private hospital [5]. Breast cancer was also the commonest cancer notified among women in Hospital Seberang Jaya with 114 cases in 2012 and 103 cases in 2013 [6,7].

Methodology

This is a cross sectional study conducted in Hospital Seberang Jaya and Institut Perubatan dan Pergigian Termaju (IPPT), Universiti Sains Malaysia in Pulau Pinang, Malaysia from January 2015 until September 2017. 64 samples collected from female patients (with consent) diagnosed with breast cancer from Hospital Seberang Jaya that undergone mastectomy with axillary sampling or clearance or wide-local excision with axillary sampling or clearance.

Patient's demographic and clinical data obtained from patient's record from Hospital Seberang Jaya and IPPT. Data was collected by code number using Performa. This data was entered into SPSS and analysed. Patient's fresh breast tissue sent from operating theatre without formalin immersion within 2hour postoperatively to histopathological lab IPPT. Patient's fresh breast cancer tissue was taken by pathologist and kept in -80°C freezer at histopathological lab in IPPT. 3ml of patient's blood were collected in plain tube either before or after surgery. Serum collected kept maximum for 30days under 4°C. Collected patient's blood was sent to regenerative lab IPPT for sample preparation.

Tissue P53- Fresh breast cancer tissues weigh 30mg taken for DNA extraction. First 10 samples of the fresh breast tissue optimised, purified and sent for DNA sequencing by private lab. Upon completion of sequencing, mutation and deletion p53 data obtained. There was 19 mutations and deletions identified from the DNA sequencing. One mutation (rs1042522) and three deletions (rs59758982, rs35069695, and rs376546152) were selected for PCR randomly.

Tissue DNA Extraction - 30mg fresh frozen breast cancer tissue collected in matrix tube. DNA extraction using commercial kit Bioteke Corp.,China protocol performed. 200ml TL Buffer and 200µL preoteinase K was mixed then flipped and incubated at 55°C for 60min. Then 200µL buffer CB mix and incubate for another 10 minutes at 70°C. Sample then cool down before adding Isopropanolol and flip. Supernatant transfer to spin column and centrifuge at 10000rpm for 30minutes, added 500µL Buffer IR re centrifuge at 12000rpm then discard flow through. Similar step proceeded after adding buffer WB for two times. Lastly, collection transfer to clean tube and added 100µL EB buffer (incubate at 65°C) centrifuge at 12000rpm for one minutes for two times. The extracted DNA stored in 4°C freezer before commencing further test.

PCR Protocol- 10samples of extracted DNA were sent for sequencing. The remaining 54samples were preceded with PCR analysis. Before commencing PCR, the master mixed prepared according to protocol by mixing 10XmM PCR Buffer, 25 mM MgCl2, 10mM DNTP, primer forward and reverse, 5U Tag polymerase and double distilled water. Aliquot 19µl also added to each tube. Then, 1µl of DNA template added into 19µl of master mixed. These then mixed using vortex mixer with short spin at 200rpm. Samples then put into PCR machine (SRY gene program); denaturation step, annealing step, extension step and no of cycles, final extending and hold. Each exon mutation and deletion required different temperature and duration of programming. PCR product checked by gel electrophoresis treated with SyberGreen.

Serum p53 autoantibody ELISA kit protocol- The obtained serum proceeded following the product manual and protocol of Human p53 Platinum ELISA ALX-850-057 (Enzolife, Germany). Starting form preparation of wash buffer, assay buffer and human p53 standard preparation, test proceeded using given microwell strips using test protocol. The microwell strips were read by Multiscan Spectrum, Thermo Scientific machine.

Result

Total patient from list were 112 patients. Data of patient from the fresh breast tissue list were collected. Out of 112 patients, only 80 completed data obtained and can be included in the study. Those with complete data were called to get consent and blood for serum p53 autoantibody. Out of these, only 64 patients consented for this study. Six patients have passed away, two patients were uncontactable due to invalid contact number and eight patients refuse to be included in this study. Total patient contacted with complete data was 64 patients.

Profiling Data of Breast Cancer Patient

Table 1 represents the data profiling for this study. Total of 64 patients has been recruited in this research with age mean of 52.45±9.51 yrs. The eldest patient was 72 years old and the youngest was 31years old. Most of the patients were Malay with 67.2% followed by Indian and Chinese with 17.2% and 15.6% respectively. Only one (1) male patient included in this study. Majority of the patients presented with breast lump 90.6%. 12.5% of them were having a family history of breast cancer with 9.4% has first-degree relationship. 51.6% of patients, has right side of tumour and majority has upper outer quadrant lesion; 50%.

Objective: To determine the profiling data (age, ethnic, duration of symptoms, family history, treatment, histological findings and staging) of women with breast cancer

Table 1: Demographic Profiles

Variable (s)		Frequency (%)
Age [mean±SD]	52.45±9.513yrs	
	Max	72 yrs
	Min	31 yrs
Ethnic	Malay	43 (67.2)
	Chinese	10 (15.6)
	Indian	11 (17.2)
Gender	Female	63 (98.4)
	Male	1 (1.6)
Mode of presentation	Breast Lump	58 (90.6)
	Nipple Discharge	2 (3.1)
	Others	4 (6.3)
Family history of breast cancer	Yes	8 (12.5)
	No	56 (87.5)
Relationship	1st Degree	6 (9.4)
	3rd Degree	2 (3.1)
	None	56 (87.5)
Side of tumor	Right	33 (51.6)
	Left	31 (48.4)
Location of tumor	Upper outer quadrant	32 (50.0)
	Lower outer quadrant	12 (18.8)
	Upper inner quadrant	11 (17.2)
	Lower outer quadrant	1 (1.6)
	Center	8 (12.5)
Staging investigation	CT scan	33 (51.6)
	Bone scan	1 (1.6)
	Ultrasound abdomen	30 (46.9)
Metastasis	Yes	9 (14.1)
	No	55 (85.9)
Surgery	Mastectomy and axillary clearance	64 (100.0)
Type	Invansive ductal carcinoma	50 (78.1)
	Invansive lobular carcinoma	8 (12.5)
	Ductal carcinoma in situ	1 (1.6)
	Others	5 (7.8)
Grading	Grade 1	10 (15.6)
	Grade 2	24 (37.5)
	Grade 3	30 (46.9)
Tumor size	T1 (<2cm)	19 (29.7)
	T2 (>2cm - <5cm)	33 (51.6)
	T3 (>5cm)	7 (10.9)
	T4 (tumor with any size with direct extension to skin/ chest wall ; ulcerated or skin nodules)	5 (7.8)

Lymph nodes	0(N0)	29 (45.3)
	1-3 (N1)	22 (34.4)
	4-9 (N2)	8 (12.5)
	> 10 (N3)	5 (7.8)
TNM staging	Stage I	15 (23.4)
	Stage II	27 (42.2)
	Stage III	13 (20.3)
	Stage IV	9 (14.1)
Triple negative	Yes	10 (15.6)
	No	54 (84.4)
HER2 type	Yes	7 (10.9)
	No	57 (89.1)
Luminal A	Yes	40 (62.5)
	No	24 (37.5)
Luminal B	Yes	8 (12.5)
	No	56 (87.5)
Serum p53	Positive	13 (20.3)
	Negative	51 (79.7)

All of the patients in this study undergone mastectomy and axillary clearance. From 64 patients, 78% of them were diagnosed with invasive ductal carcinoma while 12.5% invasive lobular carcinoma. 46.9% of the total patients has histological grading grade 3 and 7.8% has more than 10 positive lymph nodes in axillary tissue. 18.7% has T3, T4, with 10.9% were having tumour size of more than 5 cm, and 7.8% has direct skin or chest wall involvement. All patients had undergone appropriate investigation according to TNM classification staging. 51.6% undergone CT scan staging, 1.6% did bone scan and 46.9% has ultrasound abdomen. From these investigations, 14.1% has distant metastases. In the final TNM staging, 23.4% in Stage I, 42.2% in Stage II, 20.3% in Stage III and 14.1% patients in TNM Stage IV.

Prevalence of p53 mutation in Malaysian`s fresh breast cancer tissue detected PCR technique

The outcomes for determining prevalence p53 mutation in Malaysian`s fresh breast cancer tissue using PCR technique is shown in Table 2. For rs1042522, only 15.7% has mutation with homozygous mutation 6.3% and heterozygous 9.4%. There was 54.7% Deletion A and 45.3% Wild Type A detected in rs59758982. In rs35069695, there was 87.5% Deletion A and 12.5% Wild Type A. Otherwise, there is 92.2 % recorded for Deletion GAA in rs376546152.

Objective: To determine prevalence of tissue p53 mutation in Malaysian`s fresh breast cancer tissue using PCR technique

Table 2: Prevalence of tissue p53 mutation

Variable (s)		Frequency (%)
rs1042522	Homozygous mutation	4 (6.3)
	Heterozygous mutation	6 (9.4)
	No mutation	54 (84.4)
rs59758982	Deletion A	35 (54.7)
	Wild type A	29 (45.3)
rs35069695	Deletion A	56 (87.5)
	Wild type A	8 (12.5)
rs376546152	Deletion GAA	59 (92.2)
	No deletion	5 (7.8)

To Ascertain Fresh Breast Cancer Tissue p53 Mutation Association with Molecular Classification (Luminal A, Luminal B, HER-2 Type and Triple Negative) Status in Breast Cancer Patient

Currently, breast cancer is categorised into Luminal A, Luminal B, HER-2 Type and Triple negative. There was no significant result noted between each exon with Luminal A, Luminal B, Her-2 type and Triple negative as the p-value was more than 0.05 as shown in Table 3 (Table 3a to 3d). For rs1042522 mutation, eight patients are in Luminal A, one patient in Her-2 Type, 1 patient in Triple negative group and none in Luminal B. In rs59758982 Deletion A and Wild Type A, 23patients has Deletion A and 17patients has Wild Type A in Luminal A; three patients have Deletion A and five patients has Wild Type A in Luminal B; four patients have Deletion A and three patients has Wild Type A in HER-2 Type and six patients has Deletion A and four patients has Wild Type A in triple negative. In rs35069695 result showed 35 patients has Deletion A and five patients has Wild Type A in Luminal A; six patients have Deletion A and two patients has Wild Type A in Luminal B; six patients have Deletion A and one patient has Wild Type A in Her-2 Type and ten patients has Deletion A in Triple Negative. For rs376546152, 36 patients have Deletion GAA in Luminal A; eight patients have Deletion GAA in Luminal B; six patients have deletion in HER-2 Type and ten patients have Deletion GAA in Triple Negative.

Objective: To ascertain fresh breast cancer tissue p53 mutation association with Luminal A, Luminal B, HER-2 Type and Triple Negative status in breast cancer patient

Table 3a: Tissue p53 mutation (rs1042522) with Luminal A, Luminal B, HER-2 Type and Triple Negative status in breast cancer patient

Characteristics		rs1042522		P-value
		Homo/Heterozygous mutation	No mutation	
Triple Negative	Yes (%)	1 (10.0)	9 (16.7)	0.491**
	No (%)	9 (90.0)	45 (83.3)	
HER2 Type	Yes (%)	1 (10.0)	6 (11.1)	0.701*
	No (%)	9 (90.0)	48 (88.9)	
Luminal A	Yes (%)	8 (80.0)	32 (59.3)	0.189*
	No (%)	2 (20.0)	22 (40.7)	
Luminal B	Yes (%)	0 (0)	4 (14.8)	0.235*
	No (%)	10 (100.0)	46 (85.2)	

Table 3b: Tissue p53 mutation (rs59758982) with Luminal A, Luminal B, HER-2 Type and Triple Negative status in breast cancer patient

Characteristics		rs59758982		P-value
		Deletion A	Wild Type A	
Triple Negative	Yes (%)	6 (17.1)	4 (13.8)	0.495*
	No (%)	29 (82.9)	25 (86.2)	
HER2 Type	Yes (%)	4 (11.4)	3 (10.3)	0.607*
	No (%)	31 (88.6)	26 (89.7)	
Luminal A	Yes (%)	23 (65.7)	17 (58.6)	0.560**
	No (%)	12 (34.3)	12 (41.4)	
Luminal B	Yes (%)	3 (8.6)	5 (17.2)	0.253*
	No (%)	32 (91.4)	24 (82.8)	

Table 3c: Tissue p53 mutation (rs35069695) with Luminal A, Luminal B, HER-2 Type and Triple Negative status in breast cancer patient

Characteristics		rs35069695		P-value
		Deletion A	Wild Type A	
Triple Negative	Yes (%)	10 (17.9)	0 (0)	0.235*
	No (%)	46 (82.1)	8 (100.0)	
HER2 Type	Yes (%)	6 (10.7)	1 (12.5)	0.627*
	No (%)	50 (89.3)	7 (87.5)	
Luminal A	Yes (%)	35 (62.5)	5 (62.5)	0.659*
	No (%)	21 (37.5)	3 (37.5)	
Luminal B	Yes (%)	6 (10.7)	2 (25.0)	0.260*
	No (%)	50 (89.3)	6 (75.0)	

Table 3d: Tissue p53 mutation (rs376546152) with Luminal A, Luminal B, HER-2 Type and Triple Negative status in breast cancer patient

Characteristics		rs376546152		P-value
		Deletion A	Wild Type A	
Triple Negative	Yes (%)	10 (16.9)	0 (0)	0.235*
	No (%)	49 (83.1)	5 (100.0)	
HER2 Type	Yes (%)	6 (10.2)	1 (20.0)	0.627*
	No (%)	53 (89.8)	4 (80.0)	
Luminal A	Yes (%)	36 (61.0)	4 (80.0)	0.659*
	No (%)	23 (39.0)	1 (20.0)	
Luminal B	Yes (%)	8 (13.6)	0 (0)	0.260*
	No (%)	51 (86.4)	5 (100.0)	

The Association of p53 Mutation with Breast Cancer Aggressiveness i.e. Staging and Grading

In Table 4 (Table 4a to 4d), there is no significant between TNM Staging and characteristics of exon rs1042522, rs59758982, rs35069695 and rs376546152. In metastasis, (Table 5) only rs59758982 shows significant result with p value 0.04 ($p < 0.05$). Other exons were shown to have no statically significant data. In regards of tumour grading, Grade III tumour has the worse prognosis. From the Table 6, all exon has shown non-significant results for each pair. Grade III shows a highest number detection for those rs1042522, rs59758982, rs35069695 and rs376546152.

Objective: To analysed p53 mutation in relations of breast cancer aggressiveness i.e. staging and grading (according to Malaysian Clinical Practice Guidelines)

Table 4a: TNM Staging with tissue p53 Mutation (rs1042522)

Characteristics		rs1042522			P-value
		Homozygous mutation	Heterozygous mutation	No mutation	
TNM Staging	Stage 1 (%)	0 (0)	2 (13.3)	13 (86.7)	0.802
	Stage 2 (%)	3 (11.1)	2 (7.4)	22 (81.5)	
	Stage 3 (%)	1 (7.7)	1 (7.7)	11 (84.6)	
	Stage 4 (%)	0 (0)	1 (11.1)	8 (88.9)	

Table 4b: TNM Staging with tissue p53 Mutation (rs59758982)

Characteristics		rs59758982		P-value
		Deletion A	Wild Type A	
TNM Staging	Stage 1 (%)	9 (60.0)	6 (40.0)	0.083
	Stage 2 (%)	14 (51.9)	13 (48.1)	
	Stage 3 (%)	10 (76.9)	3 (23.1)	
	Stage 4 (%)	2 (22.2)	7 (77.8)	

Table 4c: TNM Staging with tissue p53 Mutation (rs35069695)

Characteristics		rs35069695		P-value
		Deletion A	Wild Type A	
TNM Staging	Stage 1 (%)	14 (93.3)	1 (6.7)	0.360
	Stage 2 (%)	23 (85.2)	4 (14.8)	
	Stage 3 (%)	10 (76.9)	3 (23.1)	
	Stage 4 (%)	9 (100.0)	0 (0)	

Table 4d: TNM Staging with tissue p53 Mutation (rs376546152)

Characteristics		rs376546152		P-value
		Deletion A	Wild Type A	
TNM Staging	Stage 1 (%)	14 (93.3)	1 (6.7)	0.114
	Stage 2 (%)	27 (100)	0 (0)	
	Stage 3 (%)	11 (84.6)	2 (15.4)	
	Stage 4 (%)	7 (77.8)	2 (22.2)	

Table 5: Tissue p53 gene mutation and metastasis

Characteristics		Metastasis		P-value
		Positive (%)	Negative (%)	
rs1042522	Homo/Heterozygous mutation	1 (10.0)	9 (90.0)	0.571*
	No mutation	8 (14.8)	46 (85.2)	
rs59758982	Deletion A	2 (5.7)	33 (94.3)	0.040**
	Wild Type A	7 (24.1)	22 (75.9)	
rs35069695	Deletion A	9 (16.1)	47 (83.9)	0.275**
	Wild Type A	0 (0)	8 (100.0)	
rs376546152	Deletion GAA	7 (11.9)	52 (88.1)	0.141**
	No Deletion	2 (40.0)	3 (60.0)	

*Fisher's Exact Test
** Pearson Chi-Square Test

Table 6: Tissue p53 mutation association with Grading

Characteristics		Grading			P-value
		Grade I (%)	Grade II (%)	Grade III (%)	
rs1042522	Homo/Heterozygous mutation	1(10.0)	5 (50.0)	4 (40.0)	0.653*
	No mutation	9 (16.7)	19 (35.2)	26 (48.1)	
rs59758982	Deletion A	6 (17.1)	13 (37.1)	16 (45.7)	0.933*
	Wild Type A	4 (13.8)	11 (37.9)	14 (48.3)	
rs35069695	Deletion A	7 (12.5)	22 (25.0)	27 (48.2)	0.187*
	Wild Type A	3 (37.5)	2 (25.0)	3 (37.5)	
rs376546152	Deletion GAA	9 (15.3)	21 (35.6)	29 (49.2)	0.442*
	No Deletion	1 (20.0)	3 (60.0)	1 (20.0)	
* Pearson Chi-Square Test					

To Look for Serum p53 Autoantibody Suitability as Biomarkers in Breast Cancer

The test of association in Table 7 has revealed that none of them has significant association between p53 genetic mutation and serum p53 autoantibody where p-value recorded is more than 0.05. The highest positive Serum p53 is recorded for rs1042522 and rs59758982 with 20% maximum. In contradiction, more than 80% of exons are detected to be negative of serum p53.

Objective: To look for serum p53 autoantibodies suitability as biomarkers in breast cancer

Table 7: Tissue p53 gene mutation and serum p53 autoantibodies

Characteristics		Serum p53		P-value
		Positive (%)	Negative (%)	
rs1042522	Homo/Heterozygous mutation	2 (20.0)	8 (80.0)	0.673*
	No mutation	11 (20.4)	43 (79.6)	
rs59758982	Deletion A	7 (20.0)	28 (80.0)	0.946**
	Wild Type A	6 (20.7)	23 (79.3)	
rs35069695	Deletion A	11 (19.6)	45 (80.4)	0.516*
	Wild Type A	2 (25.0)	6 (75.0)	
rs376546152	Deletion GAA	11 (18.6)	48 (81.4)	0.266*
	No Deletion	2 (40.0)	3 (60.0)	
*Fisher's Exact Test ** Pearson Chi-Square Test				

Discussion

Breast cancer is the number one cancer in Malaysia. According to GLOBOCAN 2012, incidence of breast cancer in women is 28%, mortality rates is about 24.7% and 5 year prevalence rate is 39.2% in Malaysia.1 The mean age of patient diagnose with breast cancer in this study was 52.45 +/- 9.513 years. This was the same with national statistic 2006 stating that breast cancer commonly occurs in age group 50 to 60 years old [3]. In regards of race, more Malays were diagnosed with breast cancer in this study compared with Chinese and Indian. Even though National Cancer Statistic (2007) showed that incidence per 100000 (CR) is higher in Chinese, these factors may be influenced by majority of population around respected hospital is Malays compared to Chinese and Indian [3]. In terms of gender, only one male patient diagnosed with breast cancer. The rate for male to develop breast cancer was 1.15 per 100000 compared to female at 42,6 per 100000 [3]. In terms of family history of breast cancer, 12.5% has family history of breast cancer and 9.4% is first degree relatives. First-degree relatives with breast cancer bear more risk than second- and third-degree relatives [3]. Breast cancer commonly located at upper outer quad-

rant similar with this study where 50% of patient has upper outer quadrant lesion.

According to Clinical Practise Guidelines 2010, in asymptomatic early breast cancer screening for metastasis; chest x-ray, liver ultrasound, CT scan and bone scintigraphy should not be performed routinely. Whereas, in advanced breast cancer, further imaging as mention above should be offered to access extend of disease depending on availability of resources [3]. In this study, 51.6% undergone CT scan, 46.9% undergone ultrasound abdomen and 14.1% noted to have distant metastasis. In regards of surgery done, early breast cancer can be treated with wide local excision and axillary dissection, whereas advanced breast cancer is treated with mastectomy and axillary clearance [3]. All patient in this study opted for mastectomy and axillary clearance after consultation.

According to the latest World Health Organisation classification, breast cancer is classified into carcinoma in situ and invasive carcinoma of non-specific type (NST) [8]. Invasive carcinoma of NST include infiltrating ductal carcinoma (70 to 80% of all breast cancer), invasive lobular carcinoma, mucinous carcinoma, medullary carcinoma, ductal lobular carcinoma and tubular carcinoma [9]. From this study, 78.1% has invasive ductal carcinoma and 12.5% invasive lobular carcinoma. Higher grade (grade III), bigger tumour and more lymph nodes involvement implies that the disease has worse prognosis compare to lower grade (grade I), no lymph nodes involvement and smaller tumour size. In this study, 46.9% has higher tumour grade, 18.7% has T3 and T4 tumour size and 7.8% has N3 lymph nodes.

The studies done to determine p53 genetic mutation in breast cancer mainly focus on immunohistochemical staining that is non-specific and less sensitive. In this study, prior to commencing PCR study, DNA sequencing was done onto 10 fresh breast cancer tissue samples. From this, nineteen mutation and deletion were found comparing with normal DNA database. Out of nineteen, one mutation and three deletions were preceded with PCR study randomly. One of the mutations proceeded was rs1042522 (codon72 polymorphism) and has been reported to has correlation with breast cancer. In this study, rs1042522 mutation was found in only 15.7% of the patient with 6.3% is homozygous mutation and 9.4% was heterozygous mutation. In regards rs1042522 association with molecular of breast cancer and clinicopathological characteristic, it has no significant association. Prior study on these genes also shows conflicting result. A meta-analysis done on sixty-one published studies suggest that this mutation is not associated with increased risk of breast cancer [10]. However, most of these studies done using DNA from blood not DNA from fresh breast cancer tissue [11-13]. Other p53 genetic deletion done in this study has no documented study associated with breast cancer but the deletion is documented in National Centre for Biotechnology Information (NCBI) DNA database. Rs59758982 in NCBI ClinVar is documented as hereditary cancer-predisposing syndrome where as rs35069695 and rs376546152 only documented as deletion with no specific condition.

In this study, the prevalence of this deletion is high with rs59758983 found in 54.7%, rs35069695 in 87.5% and rs376546152 in 92.2%. These deletion association with staging and grading was found to be not significant as p value >0.05. The only significant result in this study is rs59758983 deletion with metastasis with p value of 0.04. This cannot be justified because there was no documentation of similar findings before. However, other study on p53 gene mutation documented p53 gene mutation associated with either HER-2 Type or triple negative, grade 3 for histological grade, invasive ductal carcinoma and poor overall survival [14-18].

The prevalence of P53 serum autoantibody in this study was only 20.3%. This is slightly higher than found in another study that was only 9% [19]. However, there was no association found between rs1042522, rs59758982, rs35069695 and rs376546152 with p-value >0.05. This result viability can be inaccurate in view of the serum is extracted from patient after surgery and chemotherapy. Hence, the circulating autoantibodies may be affected as type of chemotherapy and duration from surgery done was not taken into consideration.

Conclusion

In this study, tissue p53 genetic mutation has no significant association with clinicopathological characteristic of breast cancer and the use of serum p53 autoantibody as biomarkers is inconclusive [20-47].

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