

Absence of BRCA1 185delAG, BRCA1 5382InsC and BRCA2 6174delT among Hereditary Breast Cancer Patients in North Sumatera, Indonesia

Yolanda Sitompul^a, Sumaryati Syukur^{b*}, Syafrizayanti^c, Endang Purwati^{d*}

^a*Department of Radiology, Faculty of Medicine, University Prima Indonesia, Medan, Indonesia*

^{b,c}*Laboratory of Biochemistry and Biotechnology, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Andalas, Padang, Indonesia*

^d*Laboratory of Biotechnology, Department of Nutrition, Faculty of Animal Husbandary, University of Andalas, Padang, Indonesia*

^b*Email: Sumaryatisyukur_unand@yahoo.co.id; sumaryatisyukur@fmipa.unand.ac.id*

^d*Email: purwati17@gmail.com ; purwati17@yahoo.co.id*

Abstract

One causes of breast cancer is mutations in tumor suppressor genes, namely BRCA1 and BRCA2. One of the most common types of mutations is 185delAG, 5382InsC in the BRCA1 and 6174delT in the BRCA2 gene. This mutation is called the foundation mutation which the frequency were high in the Jewish Ashkenazi population. The aim of this study was to detect the frequency of founder mutation in hereditary breast cancer patients in the North Sumatran population using the PCR-RFLP method and confirmation by sequencing. The results showed that there was no mutation in the population of north sumatera, this may be due to a small number of samples or non-specific mutations occur.

Keywords: BRCA1; BRCA2; mutation; breast cancer; PCR-RFL.

1. Introduction

Breast cancer is one of the most common type of cancer and contributes to causing high mortality among women. Breast cancer is the most common cancer in women after lung cancer [1].

* Corresponding author.

About 10% of breast cancer are hereditary breast cancer and approximately 25% of breast cancer of this type are caused by mutations in BRCA1 and BRCA2 genes [2]. These genes has an important role in DNA repair who carry mutations in these genes will increase the risk of cancer at early age [3].

Women carrying genetic mutation in BRCA1 and BRCA2 genes will increase the risk of developing breast cancer in their lives particularly at the age of 70 years old (45-85%) compared with general population (12,5%) [4].

Although the understanding of how mutation in BRCA1 and BRCA2 genes increasing with the number of studies, the variation of mutation frequency in some ethnic groups still need to be investigated. 185delAG and 5382InsC mutation in BRCA1 and 6174delT mutation in BRCA2 are the three most frequent mutations , and these mutations are higher in Ashkenazi Jewish [5]. These founder mutation is also found in several countries or ethnic including Turkey [6], Canada, Franch and several population in Europe [7].

A number of studies on the BRCA1 and BRCA2 gene mutations in Indonesia have distinctive factors related to dietary factors and heredity. Study related about mutation detection on hereditary breast cancer patients in North Sumatera, Indonesia have not been investigated. This makes the study about mutation of BRCA1 and BRCA2 genes in hereditary breast cancer are necessary to investigated the effect of these gene mutations on the prevalence of breast cancer in population in North Sumatera.

2. Material and Methods

Materials used in this study are blood samples from breast cancer patients, Blood DNA extraction kit (geneaid), Gel Red, Primer, DNA Lader, restriction enzyme, agarose, dH₂O. Instrument used in this study are autoclave, waterbath, vortex, Biodoc analyze (Biorad), electrophoresis (Biorad), thermocycler, micro pippet, and glass ware.

The method used in this study is retrospective where medical records of breast cancer patients are obtained from 2 tertiary hospitals in North Sumatera. With the approval of the Director of the hospital, we obtained a medical record of breast cancer patients from 2016 to 2017 as 29 patients.

Each patients have to fill informed consent and we explain the objectives of the research. After we obtained the approval of the patients we started the experimental section by extracting DNA from peripheral blood.

DNA Extraction and Gene amplification

DNA was extracted from peripheral blood using manufacture's protocols. The DNA were stored in freezer at -20°C until analysis.

The isolated DNA was checked by gel electrophoresis and then DNA was processes for the PCR amplification. The coding regions of BRCA1 and BRCA2 gene were amplified using specific primers (Table 1).

Table 1: Primers used for each mutation

| Primers name | Primers sequence |
|------------------|--------------------------------------|
| BRCA1 185delAG-F | 5'TGA CTT ACC AGA TGG GAG AC 3' |
| BRCA1 185deAG-R | 5'GAA GTT GTC ATT TTA TAA ACC TTT 3' |
| BRCA1 5382InsC-F | 5' GGG AAT CCA AAT TAC ACA GC 3' |
| BRCA1 5382InsC-R | 5' CCA AAG CGA GCA AGA GAA TCT 3' |
| BRCA2 6174delT-F | 5' TGG GAT TTT TAG CAC AGC ACG 3' |
| BRCA2 6174delT-R | 5' CTG GTC TGA ATG TTC GTT AC 3' |

The program of PCR for BRCA1 185delAG were consisted of denaturation, annealing, extension and final extension. The program were given in table 2.

Table 2: PCR program for BRCA1 185delAG

| PCR steps | Temperature (°C) | Duration | Cycle |
|----------------------|------------------|----------|-----------|
| Initial Denaturation | 95 | 7 min | |
| Denaturation | 95 | 30 s | |
| Annealing | 59,4 | 30 s | |
| Extension | 72 | 30 s | |
| Final extension | 72 | 7 min | 35 cycles |

The program of PCR for BRCA1 185delAG were given in table 3.

Table 3: PCR program for BRCA1 185delAG

| PCR steps | Temperature (°C) | Duration | Cycle |
|----------------------|------------------|----------|-----------|
| Initial Denaturation | 95 | 3 min | |
| Denaturation | 95 | 30 s | |
| Annealing | 52 | 30 s | |
| Extension | 72 | 30 s | |
| Final extension | 72 | 10 min | 35 cycles |

And the PCR program for BRCA2 6174delT were given in table 4.

Table 4: PCR program for BRCA2 6174delT

| PCR steps | Temperature (°C) | Duration | Cycle |
|----------------------|------------------|----------|-----------|
| Initial Denaturation | 95 | 7 min | |
| Denaturation | 95 | 30 s | |
| Annealing | 59,4 | 30 s | |
| Extension | 72 | 30 s | |
| Final extension | 72 | 7 min | 35 cycles |

The PCR product then proceed with electrophoresis agarose gel 1,5% which already mixed with 3µL Gel Red. After the electrophoresis completed, proceed with visualization using gel documentation.

Mutation Detection of BRCA1 and BRCA2 gene using PCR-RFLP

To detect mutation in BRCA1 and BRCA2 genes, the PCR-RFLP method was used using specific restriction enzymes. The restriction enzymes used for BRCA1 185delAG, BRCA1 5382InsC and BRCA2 6174delT were HinfI, DdeI and PmII respectively. The components for the reactions consisted of 13µL ddH₂O, 2µL Buffer Tango, restriction enzyme 1µL and PCR product were mixed into PCR tubes. Spin down for a few seconds so that the components are at the bottom of the tube. Incubation conducted at 37°C for 16hours the inactivation of the enzyme was conducted at 65°C for 20 minutes. The result of RFLP were observed with electrophoresis using 2% agarose gel which has been added with Gel Red. The interpretation of electrophoregram resulted for BRCA1 185delAG restriction product is if there are 2 DNA bands (150bp and 20bp) then the sample has wild type allele. If 3 DNA bands are obtained (170bp, 150bp and 20bp) then the sample has a heterozygous mutant allele.

The interpretation of electrophoregram resulted for BRCA1 5382InsC restriction product is if there are 3 DNA bands (214 bp, 36bp and 20bp) then the sample has wild type allele. If there are 2 DNA bands (36bp and 234bp) the the samples has mutant homozygous allele and if there are 4 DNA bands (26bp, 234bp, and 214bp and 20bp) then the samples has heterozygous mutant allele.

The interpretation of electrophoregram resulted from BRCA2 6174delT restriction product is if there are 2 DNA bands resulted (128bp and 20bp) the the samples have wild type allele, if there is 1 DNA band (148bp) the the samples has mutant homozygous allele and of there are 4 DNA bands (148bp, 128bp and 214bp and 20bp) then the samples has mutant heterozygous allele. After the PCR-RFLP were conducted, PCR product the purified and send to Macrogen (Korea) for sequencing.

3. Results

Restriction reaction BRCA1 185delAG using HinfI Restriction Enzyme

The electrophoregram of restriction result of BRCA1 185delAG using *HinfI* was showed in Figure 1, below.

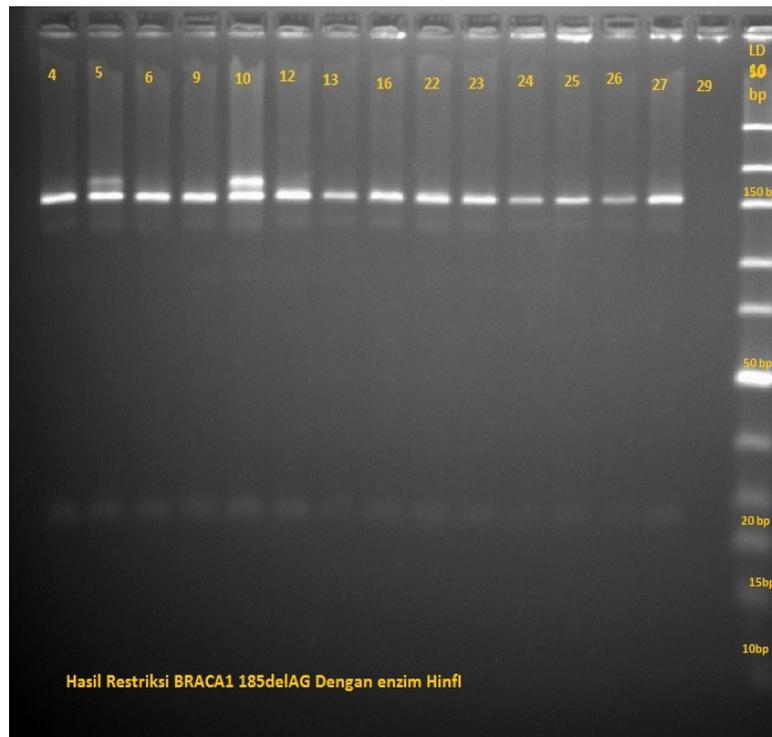


Figure 1: Visualization of PCR-RFLP BRCA1 185delAG after digested by *HinfI*

From figure above it is showed that wells number 4,5,6,9,10,12,13,16,22,23,24,25,26 and 27 are samples that digested by *HinfI*, LD is DNA ladder. From the result of PCR-RFLP for BRCA1 185delAG, there are 5 samples from 29 (17,24%) patients which have heterozygous mutant allele (170bp, 150bp and 20bp).The sequence of PCR-RFLP produk of BRCA1 185delAG was showed in Figure 2.

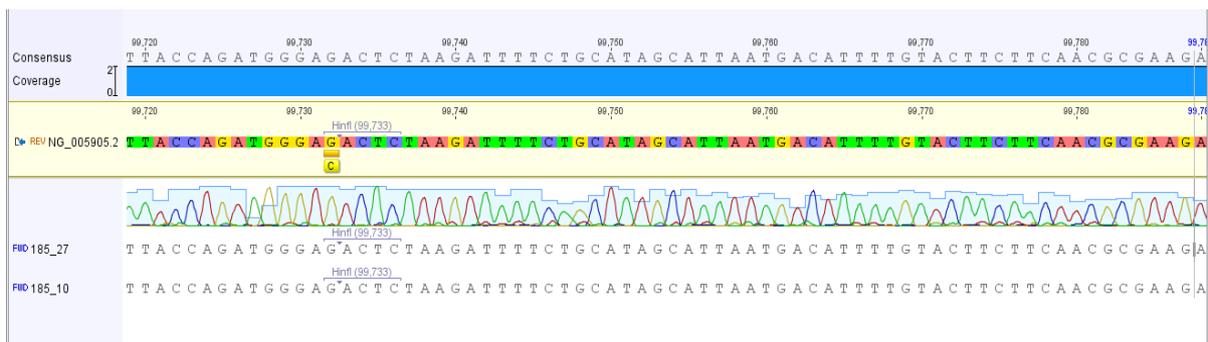


Figure 2: sequence of BRCA1 185delAG after digested with restriction enzyme *HinfI*

From figure 2 it is indicate what there is a restriction site of G*ACTC by restriction enzyme, resulting 2 DNA bands of wild type allele. Murray and his colleagues [8] reported that the *HinfI* restriction enzyme would cut on the G*ANTC, since N= A, C, G or T. Based on PCR-RFLP result, from 29 hereditary breast cancer patients, there were 5 samples with heterozygous mutant alleles, thus the percentage of heterozygous mutant alleles was

17,24%. Based on the result it could be explained that the gene sequencing could not be represented to determine mutation in the form of deletion. BRCA1 185delAG mutations occur in the case of deletion of AG starting at the 185th nucleotide position occurring in the BRCA1 exon 2 [9].

Restriction reaction BRCA1 5382InsC using DdeI Restriction Enzyme

The electrophoregram of restriction result of BRCA1 5382InsC using DdeI was showed in

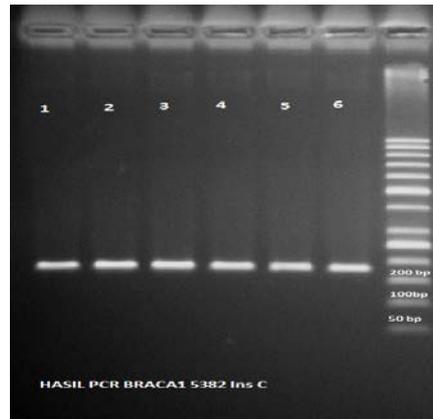


Figure 3: visualization of PCR-RFLP BRCA1 5382InsC digested by restriction enzyme DdeI

From figure 3 it showed that all of the PCR-RFLP product of BRCA1 5382InsC was 1 single band of DNA (270bp). The similar result were also reported by Jara and his colleagues [10], which reported the 5382InsC mutations in healthy Chilean women with a family history of breast cancer. All samples from healthy women with a history of breast cancer then isolated, amplified and digested with restriction enzymes. The RFLP resulted a 270 bp DNA band. The sequences of PCR-RFLP BRCA1 5382InsC was showed in figure 4.

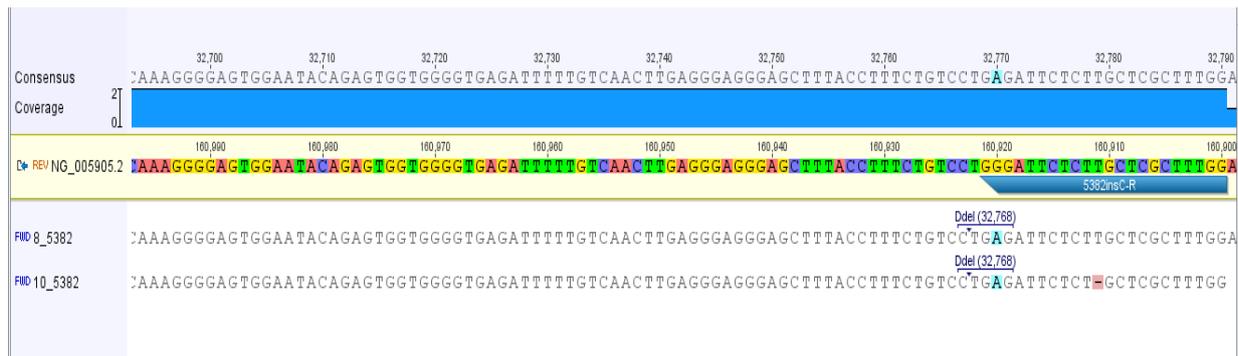


Figure 4: sequence of BRCA1 5382InsC after digested with restriction enzyme DdeI

The restriction enzyme DdeI wil cut on the CT*GAG sequence. Based on PCR-RFLP and sequencing result, there is no mutation found in all samples of hereditary breast cancer. The absence of these gene mutations is likely due to the small number of samples or caused by non specific mutation for this population.

Restriction reaction BRCA2 6174delT using PmII Restriction Enzyme

The electrophoregram of restriction result of BRCA2 6174delT using PmII was showed in Figure 4.

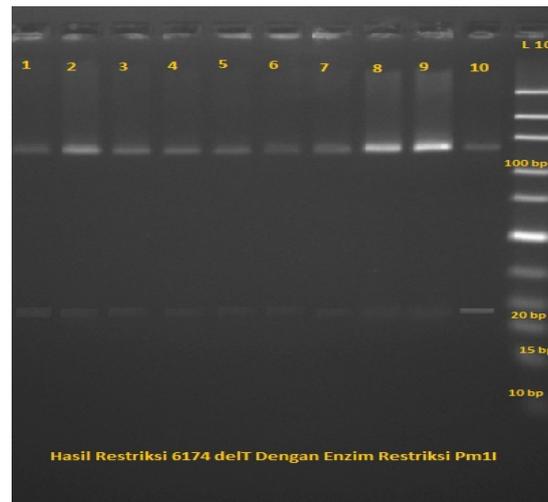


Figure 5: visualization of PCR-RFLP BRCA2 6174delT after digested by restriction enzyme PmII

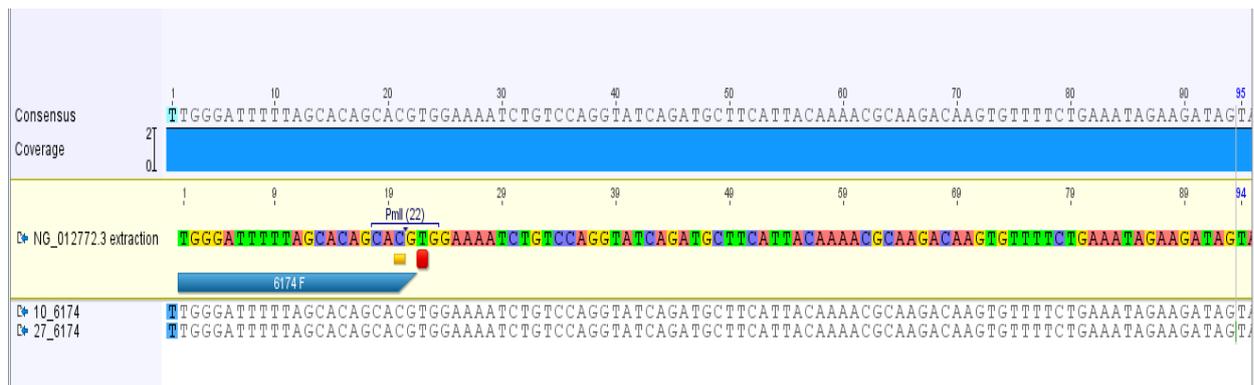


Figure 6: sequencing result of PCR RFLP product of BRCA2 6174delT after digested with PmII

From the figure it is known that the visualization of digested PCR product (well 1-10) resulting in 2 DNA bands of 20bp and 128bp (wild type allele). Result of digested PCR product from patients with hereditary breast cancer showed no mutation in the population. The sequences of PCR-RFLP BRCA2 6174delT was showed in figure 6.

Based on figure 6, the restriction enzyme will cut on the CAC*GTG sequence, so if the allele is wild type allele, it will cut the into 2 parts with the size 20bp and 128bp. From the sequencing result there was no mutation 6174delT among 29 patients with hereditary breast cancer.

The first step in PCR-RFLP analysis is amplification of a fragment containing the variation. This is folloer by treatment of the amplified fragment with an appropriate restriction enzyme. Important advantages of PCR-RFLP technique include inexpensiveness and lack of requirement for advanced instruments. In addition, the design of PCR-RFLP analysis generally is easy and can be accomplished using public available programs. Disadvantages including the requirement for specific endonucleases and difficultis in identifying the exact variation in the

event that several SNP's affect the same restriction enzyme recognition site. Another disadvantages is this method requires that a variation generates or abolishes a restriction enzyme recognition site [11].

The same result were also reported by Ghanim and his colleagues [12]. In their research, it was reported that the frequency of 185delAG and 5382InsC mutations was not present in patients with hereditary breast cancer in Iraqi population. As many as 80 breast cancer patients are withdrawn their blood samples for the analysis of mutation frequency using multiplex PCR. It can be explained that the possibility of other types of mutations has a higher frequency than the mutations observed in the study.

3 specific mutations responsible for the BRCA1 gene mutations (185delAG and 5382InsC) and BRCA2 (6174delT) accounted for 85% of the Jewish Ashkenazi ethnicity. The risk for breast and ovarian cancer is significantly increased in women who carry one or more of these 3 mutations [13].

4, Conclusion

Based on PCR-RFLP method and sequencing of BRCA1 185delAG mutation, BRCA1 5382 insC and BRCA2 6174delT on hereditary breast cancer patient in North Sumatera. The absence of these three mutations in breast cancer patients in this study may be influenced by ethnic, mixed ethnicity through marriage and geographic location.

References

- [1] Ginting S F, Syukur S, Ibrahim S, and Tjong D H, The Influence of Estradiol and HDL-Cholesterol levels on Breast Cancer in Pre-menopause and Menopause Women and Its Correlation with BRCA1 Gene C61G Mutation, *American Scientific Journal for Engineering Tehnology and Sciences (ASRJETS)*, 38(1), (2017), pp 250-257
- [2] Sihombing B, Syukur S, Ibrahim S, Cong HD and Setyoheryanto D, Gene Expression, P53 and Bcl-2 as Markers of Prostate Cancer Progression, *American Scientific Journal for Engineering Tehnology and Sciences, (ASRJETS)*, 35, (1), (2017), pp 8-14
- [3] D.Purnomosari, G.Pals, A.Wahyono, T.Aryandono, TW.Manuaba, SJ.Haryono, PJ.van Diest."BRCA1 and BRCA2 germline mutation analysis in the Indonesian population." *Breast Cancer Res Treat*, vol (106), pp297-304.2006
- [4] A. Kwong."Genetic testing for hereditary breast cancer in Asia-moving forward." *Chinese Clinical Oncology*, vol 5(3), pp 1-5. 2016
- [5] A. Chakraborty, D.Banerjee, J.Basak, A.Mukhopadhyay."Absence of 185delAG and 6174delT Mutations among Breast Cancer Patients of Eastern India." *Asian Pac J Cancer Prev*, vol 16(17), pp 7929-7933, 2015

- [6] H.Yazici, O.Bitisik, E.Akisik, N.Cabioglu, P.Saip, et al." BRCA1 and BRCA2 mutations in Turkish breast/ovarian families and young breast cancer patients."British Journal of Cancer, vol 83(6), pp 737-742, 2000
- [7] HA.Anis. "Mutations and Cancer Genesis Highlights on BRCA1 and BRCA2 Genes.". Journal of Cancer Prevention & Current Research, vol 2(6), pp 1-2, 2015
- [8] IA.Murray, SK. Stickel, RJ. Roberts."Sequence-specific cleavage of RNA by type II restriction enzymes." Nucleic Acids Res, vol 38(22), pp 8257-8268, 2010.
- [9] J. Hansa, R.Kannan, SK. Ghosh." Screening of 185delAG, 1014deGT and 3889DelAG BRCA1 Mutations in Breast Cancer Patients from North-East India." Asian Journal of Cancer Prevention, vol 13, pp 5871-5873, 2012.
- [10] L. Jara, S.Ampuero, L.Seccia, M.Bustamante, R.Blanco, JM.Ojeda." Analysis of 5382InsC (BRCA1) and 6174delT (BRCA2) mutations in 382 healthy Chilean women with a family history of Breast Cancer." Breast Cancer.Biol.Res, vol 35(1), pp 1-3, 2002
- [11] HB.Rasmussen." Gel-Electrophoresis-Principles and Basics. InTech. Pp 315-328, 2012
- [12] M. Ghanim, N. Alwan, F.MacDonald."Low prevalence of BRCA1 and BRCA2 Common Founder Mutations among Iraqi Breast Cancer Cases and At Risk Families. JJ Cancer Sci Res. Vol 2(8), pp 047-048
- [13] MR.MacClain, KL. Nathanson, GE.Palomaki, JE.Haddow."An evaluation of BRCA1 and BRCA2 founder mutations penetrance estimates for breast cancer among Ashkenazi Jewish women." Genetics in Medicine, vol 7(1), pp 123-127, 2005