RESEARCH ARTICLE

BRCA1 Gene Mutation Screening for the Hereditary Breast and/or Ovarian Cancer Syndrome in Breast Cancer Cases: a First High Resolution DNA Melting Analysis in Indonesia

Farmaditya EP Mundhofir1*, Catharina Endah Wulandari1, Yan Wisnu Prajoko2, Tri Indah Winarni1

Abstract

Specific patterns of the hereditary breast and ovarian cancer (HBOC) syndrome are related to mutations in the BRCA1 gene. One hundred unrelated breast cancer patients were interviewed to obtain clinical symptoms and signs, pedigree and familial history of HBOC syndrome related cancer. Subsequently, data were calculated using the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) risk prediction model. Patients with high score of BOADICEA were offered genetic testing. Eleven patients with high score of BOADICEA, 2 patients with low score of BOADICEA, 2 patient’s family members and 15 controls underwent BRCA1 genetic testing. Mutation screening using PCR-HRM was carried out in 22 exons (41 amplicons) of BRCA1 gene. Sanger sequencing was subjected in all samples with aberrant graph. This study identified 10 variants in the BRCA1 gene, consisting of 6 missense mutations (c.1480C>A, c.2612C>T, c.2566T>C, c.3113A>G, c.3548 A>G, c.4837 A>G), 3 synonymous mutations (c.2082 C> T, c.2311 T> C and c.4308T>C) and one intronic mutation (c.134+35 G>T). All variants tend to be polymorphisms and unclassified variants. However, no known pathogenic mutations were found.

Keywords: HBOC syndrome - BRCA1 gene - Indonesian population - PCR-HRM - BOADICEA

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Introduction

Breast cancer is the most common cancer in Indonesian females with incidence rate approximately 36.2 in 100,000 and in the top ten of mortality cause with mortality incidence is 18.6 in 100,000 among other diseases (Azis et al., 2009; Wahidin et al., 2012). Roughly 5-10% of breast cancer cases related to the Hereditary Breast and/or Ovarian (HBOC) syndrome. Individuals with HBOC syndrome have significantly higher lifetime risk of breast cancer development compare to general population (lifetime risk to age 70 for breast cancer is 60-80%). Specific pattern of HBOC syndrome is related to specific mutation in the BRCA1 or BRCA2 gene (Mary et al., 2012; Petrucelli et al., 2013).

Compared to BRCA2, BRCA1 has higher prevalence related to HBOC; it is 1 in 300 per 100,000 population, while BRCA2 gene only 1 in 800 (Mary et al., 2012). BRCA1 gene is located in chromosome 17q21. The main function is maintaining chromosome stability through DNA damage repair process and regulation process of cell cycle checkpoint as the response to DNA damage.

Mutation in genomic region of BRCA1 contributes to genetic instability. Chromosomal instability caused by deficiency of BRCA1 thought to be pathogenic basic in breast cancer development (NCBI, 2013; Petrucelli et al., 2013).

Identification of hereditary breast cancer through family history and pedigree construction are cost-effective method. Several models and scoring systems have been designed to assess the probability of BRCA1 mutation in an individual based on family history. However, among of these models, the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) appeared to be the most accurate for assessing the risk of breast cancer (Evans et al., 2007; Stahlbom et al., 2012). Data from BOADICEA provides risk estimation of BRCA1 gene mutation for each individual which is included in pedigree construction. These data could direct clinicians to consider the highest risk-individual to undergo genetic testing for further assessment.

This study was designed to identify patient who were predisposed to BRCA1-related HBOC syndrome in Indonesia using BOADICEA. Genetic testing was

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performed in individual with high score of BOADICEA result by using Polymerase Chain Reaction-High Resolution Melting Analysis (PCR-HRM). This is the first study using PCR-HRM in Indonesia.

Materials and Methods

Study population

One hundred unrelated breast cancer patients from Oncology surgery and Chemotherapy Department of Dr. Kariadi Hospital were included. Data obtained from interview including history of the disease, pedigree and family history of cancer related HBOC syndrome were analyzed by BOADICEA risk prediction software. The study was conducted in compliance with the Helsinki Declaration. Ethical clearance had been obtained from Ethical Committee for Medical Research Faculty of Medicine, Diponegoro University - Dr. Kariadi Hospital and written informed consent was obtained from all participants involved in this study.

DNA extraction and high resolution melting (HRM)

Genomic DNA was extracted using salting out method from venous blood samples. DNA quantification and qualification were estimated using Spectrophotometer (GE-NanoVue). To cover all exons of BRCA1 gene, 41 primers were used according to previous study (Ava Kwong et al., 2012) for HRM mutation screening. DNA amplification was performed by 36 plates RotorGeneQ 5Plex HRM (Qiagen, California, USA). RotorGene Q 5Plex HRM (Qiagen, California, USA) was used for PCR-HRM amplification. Each reaction was performed in a final volume of 20 μl containing 10 μl Type-It HRM Master Mix (Qiagen, California, USA), 4 μl primer (5 pm forward and reverse primer), 2 μl DNA 10 ng and H2O up to 20 μl. The PCR profile was performed as follows: pre-activation at 950C for 5 minute, followed by 40 cycles of denaturation at 950C for 10 seconds, 30 seconds annealing at 54-620C and 10 seconds extension at 720C. HRM amplification were done at 60-980C with 0.10C temperature increment. Melting curve was analyzed by Rotor Gene - Pure Detection version 2.1.0 (build 9) (Qiagen, California, USA) software. Afterwards, aberrant patterns were sequenced using Sanger sequencing method.

Mutation analysis

The sequence analysis was compared to the reference from NCBI BRCA1 referred sequence: NC_000017.10. The previous known mutations were accessed in The UMD-BRCA1 mutations database website (http://www.umd-be/BRCA1/4ACTION/W_DMDT1/11) last updated June 2015, NCBI (http://ncbi.gov.nih) and Ensembl (http://ensembl.org). The Alamut version 2.4-7 software has been used for the interpretation of the new sequence variants and for the detection of splicing aberrations caused by the new unclassified variants detected in our present study. Alamut can predict the severity of amino acid substitutions by integrating nucleotide and amino acid conservation, by prediction methods including the Sorting Intolerant From Tolerant (SIFT), Align-Grantham Variation with Grantham Deviation (A-GVGD), and Mutation Taster.

Results

A hundred samples had been collected from August to December 2014. Data collection was done through interview about clinical history, risk factors, pedigree construction and family history of HBOC syndrome related cancer. Collected data then were calculated using BOADICEA software risk prediction model to determine the suspect of hereditary breast cancer. Fourteen of 100 patients with BOADICEA score ≥1.5 were offered for genetic testing. However, 4 of them refused to do genetic testing. There was one patient with diagnosis of Fibro...
## Table 1. Variants in Samples and Control

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence variant</th>
<th>Number of Samples</th>
<th>Sample ID</th>
<th>Type of mutation</th>
<th>Protein change</th>
<th>Pathogenicity</th>
<th>Mutation Taster</th>
<th>AGVCD</th>
<th>SIFT</th>
<th>UMD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.4</td>
<td>c.1480C&gt;A</td>
<td>3</td>
<td>S2, S5, S7</td>
<td>Missense mutation</td>
<td>p.Gln494Lys</td>
<td>Deleterious</td>
<td>(GV: 223.30 - GD: 53.3%)</td>
<td>53.3%</td>
<td>?</td>
<td>8</td>
</tr>
<tr>
<td>11.10</td>
<td>c.2567C&gt;T</td>
<td>11</td>
<td>S1, S2, S5, S6, S8, FS3.2</td>
<td>Missense mutation</td>
<td>p.Tyr856His</td>
<td>Benign</td>
<td>(GV: 97.83 - GD: 0.00)</td>
<td>0.00</td>
<td>?</td>
<td>11</td>
</tr>
<tr>
<td>11.13</td>
<td>c.3113A&gt;G</td>
<td>12</td>
<td>S1, S2, S5, S6, S8, S9, S10, LS2, FS3.1, FS3.2</td>
<td>Missense mutation</td>
<td>p.Glu1038Gly</td>
<td>Benign</td>
<td>(GV: 97.83 - GD: 0.00)</td>
<td>0.00</td>
<td>?</td>
<td>11</td>
</tr>
<tr>
<td>11.15</td>
<td>c.3548A&gt;G</td>
<td>2</td>
<td>S1, S3, S9, S10, S11, LS1, LS2, FS3.1, FS3.2</td>
<td>Missense mutation</td>
<td>p.Lys1183Arg</td>
<td>Benign</td>
<td>(GV: 124.29 - GD: 0.00)</td>
<td>0.00</td>
<td>?</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>c.134+35G&gt;T</td>
<td>2</td>
<td>S3, FS1.2</td>
<td>Intronic mutation</td>
<td>?</td>
<td>?</td>
<td>(GV: 74.82 - GD: 22.66)</td>
<td>22.66</td>
<td>?</td>
<td>3</td>
</tr>
</tbody>
</table>

**Note:** K: Control (Normal individual); S: Sample; LS: Low score; FS: Family member; UMD*: number of times reported in UMD database (http://www.umd.be/BRCA1/); **: mutations not found in previous studies based on Alamut, BIC database, the Universal Mutation Database BRCA1 (updated 2015, April) and Ensemble.
Adenoma Mammae (FAM) included in this study due to family history of cancer-related HBOC syndrome (grandmother of the father and two father’s cousins suffering from breast cancer as well as having two older sisters with FAM). There were two family members of patients who did not have breast cancer but had high score of BOADICEA (sample ID: FS3.1 and FS3.2) and 2 patients with low score of BOADICEA (Sample ID: 

<table>
<thead>
<tr>
<th>Initial</th>
<th>Sample ID</th>
<th>Age</th>
<th>Onset of breast cancer</th>
<th>Diagnosis</th>
<th>Stadium</th>
<th>Family history of cancer</th>
<th>Hormonal testing result</th>
<th>BOADICEA score*</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT</td>
<td>S1</td>
<td>40</td>
<td>37 (MD) 39 (MS)</td>
<td>IDC grade II MD post op+ IDC grade II MS</td>
<td>IIIC</td>
<td>Uncle: soft tissue carcinoma of neck</td>
<td>-</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td>S2</td>
<td>56</td>
<td>54</td>
<td>IDC grade II MS</td>
<td>IV</td>
<td>Sister, mother: BC</td>
<td>-</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>S3</td>
<td>51</td>
<td>46 (MS) 49 (MD)</td>
<td>IDC grade II MS post op+ IDC grade II MD</td>
<td>IIIC</td>
<td>Uncle, cousin: soft tissue carcinoma</td>
<td>ER: neg PR: neg HER2: neg</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>S4</td>
<td>33</td>
<td>28</td>
<td>Adeno Ca mammae mucoides MS</td>
<td>IIIB</td>
<td>Sister: FAM</td>
<td>-</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>S5</td>
<td>43</td>
<td>43</td>
<td>IDC grade III MS</td>
<td>IIIB</td>
<td>Cousin: BC</td>
<td>-</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>-</td>
<td>34</td>
<td>32</td>
<td>IDC grade III MD</td>
<td>IIIB</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>JM</td>
<td>S6</td>
<td>60</td>
<td>40 (MS) 52 (MD)</td>
<td>IDC grade II III MS, IDC grade II MD</td>
<td>IV</td>
<td>Grandmother: BC</td>
<td>-</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>KT</td>
<td>S7</td>
<td>40</td>
<td>40</td>
<td>IDC grade II MD</td>
<td>IIIB</td>
<td>Mother’s sister: BC</td>
<td>-</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td>-</td>
<td>32</td>
<td>30</td>
<td>IDC grade II</td>
<td>IIIB</td>
<td>Mother’s sister: BC</td>
<td>-</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>UY</td>
<td>-</td>
<td>35</td>
<td>34</td>
<td>IDC grade III MD</td>
<td>IIIB</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>S9</td>
<td>44</td>
<td>44</td>
<td>IDC grade II MS</td>
<td>IIIB</td>
<td>Mother’s sister: BC</td>
<td>-</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>BN</td>
<td>S10</td>
<td>40</td>
<td>38 (MS) 40 (MD)</td>
<td>ILC MS</td>
<td>IV</td>
<td>Mother: Bilateral FAM</td>
<td>-</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>S11</td>
<td>39</td>
<td>39</td>
<td>IDC grade II MS</td>
<td>IIIB</td>
<td>Mother’s sister: BC</td>
<td>ER: neg PR: neg HER2: +3</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>-</td>
<td>43</td>
<td>40</td>
<td>IDC grade II MS</td>
<td>IIIB</td>
<td>Father’s sister: BC</td>
<td>ER: neg PR: neg HER2: neg</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

*Score of ≥1.5 was defined as high risk of harboring BRCA1 gene mutation*
LS1 and LS2) were randomly selected for genetic testing. Controls in this study were 15 women without breast cancer sign and familial history of cancer related to HBOC syndrome. In total there were 15 patients and 15 controls were carried out for genetic testing. The characteristics of patients and risk factors for breast cancer was shown in supplementary data, Table 1 and the characteristics of patients who have a high score for BOADICEA risk prediction model result was summarized in Table 2.

According to classification in Universal Mutation Database for \textit{BRCA1} gene mutation and using Alamut 2.4.7 software for the interpretation of the new sequence variants, we identified 10 variants consist of 6 missense mutations, 3 silent/synonymous mutations and 1 intronic mutation. Five out of six variants (c.2612 C>T, c.2566 T>C, c.3113 A>G, c.3548 A>G and c.4837 A>G) and three variants of synonymous mutation (c.2082 C>T, c.2311 T>C and c.4308T>C) have been reported in previous studies as polymorphism with uncertain clinical significance; however it was suspected to be benign. While variant of c.1480C>A/p.Gln494Lys found in this study was novel. In present study, these variants were found in the most of samples (both normal controls and patients with breast cancer). Therefore, these variants tend to be polymorphism in Indonesian population.

One missense mutation has not been recorded in the database and has not been reported by previous studies. Variants of c.1480 C>A was predicted by SIFT as deleterious (0). However according to AGVGD it was in Class C0 (GV: 223.30 - GD: 36.80) and polymorphism by Mutation tasters. This mutation was found in most of the controls (8 of 15 control) and 3 patients.

Intronic mutation (c.134+35 G>T) was found in three controls and two patients. This mutation was not on the splicing site according to Human Splice Site Finder Software version 3.0 (available online in http://www.umd.be/HSF3/). Therefore, it did not alter the protein function of the protein and has no clinical impact.

To confirm the result, we performed sequencing of all \textit{BRCA1} gene exons in one patient with highest BOADICEA score (sample

**Discussion**

To date, only few reports have been published about the spectrum of \textit{BRCA1} sequence variants in Indonesian population (Purnomosari et al., 2007; Kwong et al., 2015). It is interesting that the younger onset of breast cancer was identified in 68% (68 of 100 patients). Furthermore, 85.3% of them (58 of 68 patients) did not have a family history of HBOC syndrome-related cancer. Several studies have been reported that breast cancer in Asia presents at a younger mean age compared to Western population. For the European population, the age specific rates peaked between 55 to 75 years and then slightly decreased (Matsuno et al., 2007; Leong et al., 2010). This younger mean age is likely due to the population pyramid structure in developing countries, which have a broad base indicating a higher fertility rate. Therefore, the proportion of women in the older age groups are lower compared to Western countries (Ng et al., 2011).

Although breast cancer in Asia tend to have younger onset, breast cancer on age fewer than 50 years old at the time of diagnosis during 2008 were less than 50% (incidence rate 42% in Asia-Pacific region and 47% within the sub region of South-Eastern Asia) (Youlden et al., 2014). Malaysia as neighbor country of Indonesia only has incidence of breast cancer 48.1% below age 50 (Ng et al., 2011). It may suggest that other factor than genetic play a role in the tendency of younger onset in Indonesian breast cancer population.

There are several studies suggested that oral contraceptive was associated with an increased breast cancer risk (Gierisch et al., 2011; Beaber et al., 2014). In Indonesia, hormonal contraceptive use increases risk of cancer (approximately 2 times) (Harianto et al., 2005; Sirait et al., 2009). Mammography screening implementation can affect the onset of breast cancer (Glass et al., 2007; Brown et al., 2009). Currently, Indonesia does not have population-based breast cancer screening program. This is due to socio-economic development problems, hence breast cancer probably considered as ‘low priority’ compared to infectious diseases by the health care system in Indonesia. Thus, it leads them to be less responsive to breast cancer care in terms of early detection, breast health education and creating awareness (Ng et al., 2011). In addition, other recognized barriers for early detection of breast cancer in the Asian region include belief in traditional medicine and lack of autonomy in decision making (Norsa’adah et al., 2011; Taib et al., 2011). These barriers may be more prevalent in poorer countries (Ng et al., 2011). Other factors that can increase risk of younger onset in breast cancer were BMI index and environmental factors (Nichols et al., 2009; Cecchini et al., 2012; Assi et al., 2013). No previous study in Indonesia about these factors and their impact in breast cancer risk. Therefore, future studies are warranted.

A number of variants have been identified in this study. There are five missense mutations that have been reported in previous studies as polymorphism (p.Pro871Leuc.2612 C>T (Cherbal et al., 2012; Akilzhanova et al., 2013), p.Tyr856His c.2566 T>C (Herrick et al., 2006), p.Glu1038Gly c.3113 A>G (Akilzhanova et al., 2013), p.Lys1183Arg c.3548 A>G (UMD-\textit{BRCA1}), 2015) and p.Asn1613Asp c.4837 A>G (Cherbal et al., 2012). Silent mutation at c.2082C>T was found in most of the samples, either homozygous or heterozygous. This variant has been reported by previous studies in a population of Argentinian and Kazakhstan populations as single nucleotide polymorphism (Cherbal et al., 2012; Akilzhanova et al., 2013). This variant has also been recorded in the ensemble data base as a normal variant (rs1799949). Variant in c.2311T>C have been reported previously in several studies as a polymorphism (Keshavarzi et al., 2012; Berzina et al., 2013). Therefore, the existence of both mutations in this study tends to be normal variant that will not cause disease.

To date, several studies have evaluated risk associated
of breast and/or ovarian cancer with SNPs in BRCA1. However, results from these studies showed conflicting evidence (Dombernowsky et al., 2009; Pilato et al., 2010; Medimegh et al., 2014). Variants of c.2082C>T, c.3113A>G and c.3548A>G SNPs were not associated with breast cancer disease with P value <0.05. Whereas variants of c.2311T>C, c.2612 C>T and c.4308T>C are clearly associated with familial breast cancer with an odds ratio ranging from 2.49 to 4.66. Among these SNPs, the c.2612 C>T variant could have an effect on amino acid change (Proline to Leucine) at position 871, suggesting an alteration on the protein function that can play a role in familial breast cancer susceptibility (Medimegh et al., 2014). However, in other study evaluated risk associated of breast and/or ovarian cancer by missense polymorphisms in BRCA1 c.2612C>T, c.3113A>G and c.4837A>G, found no association between heterozygosity or homozygosity for those polymorphisms and risk of breast and/or ovarian cancer (Dombernowsky et al., 2010). The other study found that SNPs BRCA1 c.3548A>G/ p.Lys1183Arg was more frequently present in breast cancer relatives who were tested negative (Pilato et al., 2010).

In this study, those known missense polymorphism were found in many patients; heterozygous mutation of c.2612C>T (10 patients, 66.7%), heterozygous mutation of c.2566T>C (10 patients, 66.7%), heterozygous mutation of c.3113A>G (13 patients, 86.7%), heterozygous mutation c.3548 A>G (15 patients, 100%), heterozygous mutation c.4837 A>G (7 patients, 46.7%), c.2082C>T (homozygous: 2 patients, 13.3%; heterozygous: 8 patients, 53.3%), c.2311T>C (2 patients, 13.3%), and heterozygous mutation of c.4308T>C (8 patients, 53.3%), respectively. Interestingly, all patients have multiple variants and no known pathogenic mutation found. Several missense polymorphisms detected in our patients who were tested negative for BRCA1 gene mutations, may have a role in breast cancer susceptibility marker. However, evaluation study with larger samples and healthy controls are required.

Variant of c.1480C>A p.Gln494Lys has not been found in previous study. This variant was found in 3 patients (20%) and 8 normal controls (53.3%). It is suggested a polymorphism although on In Silico analysis, c.1480C>A is Deleterious (0) by SIFT, but Class C0 (GV: 223.30 - GD: 36.80) by AGVGD and prediction polymorphism by Mutation Taster. There are 2 patients with this variant having young onset of breast cancer (40 and 43 years). Both are having invasive ductal carcinoma in histopathological finding and have family history of cancer related HBOC syndrome. Further study is required to investigate whether this mutation is only polymorphism or potential to be pathogenic.

Intronic mutation (c.134+35 G>T) was found in three controls and two patients. There is no previous studies that mention this mutation. This mutation was not found on the splicing site according to Human Splice Site Finder Software version 3.0 (available online in http://www.umd.be/HSF3/). Therefore, it not alters the protein function of the protein and has no impact clinically.

Specific pattern of HBOC syndrome are linked to mutation in BRCA1 or BRCA2 genes. However, there are many other genes such as TP53, PTEN, also related to hereditary breast cancer in a rare case (Pilarski et al., 2009; Schneider et al., 2010; Petrucci et al., 2013). Several studies have been conducted and they indicate that mutations of BRCA genes in Asian breast cancer patients are occur at similar rates compared to other racial groups, with prevalence rate 12.7%. Except for Pakistani and Indian patients, BRCA2 mutations in the Asian population were detected equally, or more frequently than BRCA1 mutations when compared to other ethnicities (Haffty et al., 2009; Hall et al., 2009). BRCA1 gene mutation in breast cancer patients have been studied in Indonesia using multiplex ligation-dependent probe amplification (MLPA) method. Samples were 136 breast cancer patients from Jakarta, Yogyakarta and Denpasar. That study found three pathogenic mutation (c.2784_2875insT, p.Leu1415x and del exon 13-15) and 20 “unclassified variants” with uncertain clinical consequences. However, only 7.8% of patients at high risk for hereditary breast cancer had BRCA1/2 mutation (BRCA1: 2.6%, BRCA2: 5.2%) (Purnomosari et al., 2007). A Pilot Genome-wide Association Study of Breast Cancer Susceptibility Loci in Indonesia was performed in 89 breast cancer patients. It was reported 11 chromosome loci that possessed suggestive associations with breast cancer risk. However all subjects with breast cancer were negative for mutations in BRCA1 and BRCA2 genes (Haryono et al., 2015). This result suggesting that probably BRCA1/2 genes are not common related to HBOC syndrome in Indonesia. However further study with larger sample are required.

In conclusion, we identified 10 variants in BRCA1 gene that tend to be polymorphism and unclassified variants. No known pathogenic mutation found. This is the first study in Indonesia for risk and mutational analysis using PCR-HRM in breast cancer patients. Limitation of this study was small size of samples due to financial constraints. In addition, hormonal data (ER, PR and HER2) could not be obtained since they are not common diagnostic test in Indonesia for breast cancer individuals. These hormonal data affect to the BOADICEA calculation though. Our study shows that PCR-HRM can be considered as cost-effective screening method especially for low-income country like Indonesia.

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