INTRODUCTION

Prostate cancer in 2020 is estimated to have 1,414,000 new cancer cases and people deaths due to prostate cancer at around 375,304 deaths. This state makes prostate cancer the second most commonly diagnosed cancer and the fifth leading cause of death among men worldwide. Prostate cancer is the most commonly diagnosed in 112 countries and is the leading cause of cancer death in 48 countries. The risk factors of getting prostate cancer are Advancing age, black race, and family history.

Previous studies have reported large fluctuations in prostate cancer incidence and mortality. These variations reflect the regional distribution of populations with different genetic susceptibilities, such as Africans having a higher risk than Asians and differences in medical availability and access.

Hormonal factors have an important role in prostate cancer progression through estrogen synthesis, metabolism, and signaling pathways. Studies suggest that genetic factors such as mutations in hormonal genes have been a major player in the development of prostate cancer. Various genes associated with prostate cancer and risk have been identified, including AR, CYP17 / 19, NOS, PSA, and ESR1 / 2.

Estrogens normally created from androgens through steroidogenesis have been connected to prostate cancer development. It is found that increased levels of estrogens and their synthesis are associated with the aggressiveness of prostate cancer. This modulation of prostate cancer development is suggested as the work of the estrogen receptors ERα and ERβ. Some studies thought that ERα has oncogenic activities because, when activated, it will increase the proliferation of cancer cells. Otherwise, ERβ is suggested to have tumor suppression function due to its absence will pro, promoting hyperplasia and the development of the disease. Other shreds of evidence to support this are the expression of ERα is increased, and ERβ is decreased during prostate cancer progression.

Estrogen receptors (ERs) comprise nuclear ERs, extra-nuclear ERs, and G protein-coupled ERs (GPERs). Nuclear ERs, counting estrogen receptor α (ERα) and estrogen receptor β (ERβ), are found within the core and are encoded by ESR1 and ESR2, respectively. Estrogen receptor 1 (ESR1) is found on chromosome 6, locus 6p25.1, and ranges roughly 300 kb in length, counting eight exons and seven introns. ESR1 capacities as a ligand-activated translation composed of a few spaces critical for hormone binding, DNA binding, and
The samples taken from 40 FFPE include fifteen samples diagnosed with Benign Prostate Hyperplasia (BPH), six samples diagnosed with non-metastatic prostate cancer (Non-MPCa), and nineteen samples of metastatic prostate cancer (MPCa). The mean age of the patients was 67.1 years. The mean Expression was 10.8. Shapiro Wilk tested the distribution data of all samples, and the distribution data of ESR1 was normally distributed. Mean Expression of ESR1 was high in Benign Prostate Hyperplasia (BPH) and MPCa groups but not in non-MPCa. The highest expression of ESR1 to the lowest was the MPCa group, BPH group and Non-MPCa group, respectively.

Based on the One Way ANOVA table above, the expression of ESR1 in BPH compared to MPCa and Non-MPCa shows a significant difference. Besides that, expression of ESR1 in Non-MPCa compared to MPCa also shows a significant difference in which expression MPCa shows an increased level of ESR1 expression.

DISCUSSION
This research shows a variety of ESR1 expressions between BPH, non-MPCa, and MPCa with a significant difference. The level of ESR1 expression MPCa is activation of translations. It can also be associated with estrogens receptors to invigorate multiplication of mammary epithelial tissue and change the expression of downstream qualities.23

As stated above, Estrogen Receptor 1 (ESR1) expression has appeared to have a significant impact on the advancement of prostate cancer, and this study aims to compare the expression of ESR1 in BPH, metastatic prostate cancer, and non-metastatic prostate cancer.

METHOD
Cluster Random Sampling was used as the sampling method. The FFPE sample is taken from the patient’s tissue diagnosed with prostate cancer histopathologically. Exclusion criteria include no baseline Total PSA data, no baseline Total Testosterone data, FFPE age greater than three years old, and invalid DNA integrity.

RNA Extraction
The RNA genome was extracted from formalin-fixed paraffin-embedded (FFPE) prostate tissue. In FFPE tissue specimens, a deparaffinization procedure was carried out using deparfin liquid, and RNA was extracted using the Hybrid-R miRNA kit.

RT-qPCR
The RNA extraction product was examined by RT-qPCR using the Bioner Accupower Greenstar RT-qPCR Master Mix. The PCR was performed using Veriti Thermal Cycler under the following conditions: Reverse Transcription at 50-70°C for 15 minutes followed by one cycle of pre-Denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, 40 cycles Annealing/Extension/Detection at 55-60°C for 30 seconds and one cycle of melting. Primer sequences for the ESR1 gene for methylated sequences (M) were the following: forward primer 5'-TGCACTTGCTCCCGGCTG-3' and reverse primer 5'-AACCAGCGGGGCAACCTGGAA-3'. The primer sequences for the ESR1 unmethylated sequences (U) were the following: forward primer 5'-GATTGTATTTGTTTTGTTGGT-3' and reverse primer 5'-AACCAACAAACCCACCTAAAAA-3'.

Statistical Analysis
Data analysis methods include both descriptive and analytic methodologies were employed. To check the data normality, an analytical test was run. If the number of samples used is greater than 50, the Kolmogorov Smirnov test is used and is compared to the Shapiro Wilk Test. The comparison was analyzed using the One Way ANOVA.

Table 1. Sample characteristic and data distribution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>BPH</th>
<th>Prostate Cancer</th>
<th>Non-MPCa</th>
<th>MPCa</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n (%)</td>
<td>40</td>
<td>15 (37.5)</td>
<td>6 (15)</td>
<td>19 (47.5)</td>
<td></td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Mean Age, years ± SD</td>
<td>67.1 ± 7.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Expression ESR1 ± SD</td>
<td>10.8 ± 9.5</td>
<td>6.9 ± 3.8</td>
<td>2.6 ± 1.0</td>
<td>12.7 ± 9.5</td>
<td></td>
<td>&gt;0.05*</td>
</tr>
</tbody>
</table>

Table 2. Comparison of ESR1 expression in BPH, non-MPCa, MPCa.

<table>
<thead>
<tr>
<th>(I) M</th>
<th>(J) M</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH</td>
<td>Non-MPCa</td>
<td>4.31346</td>
<td>1.06659</td>
<td>.002</td>
<td>1.5897 - 7.0373</td>
</tr>
<tr>
<td>BPH</td>
<td>MPCa</td>
<td>-5.77873</td>
<td>2.39870</td>
<td>.060</td>
<td>-11.7582 - .2007</td>
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<td>Non-MPCa</td>
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<td>2.22785</td>
<td>.001</td>
<td>-15.7462 - 4.4382</td>
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</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
* One Way ANOVA test.
also significantly increased compared to non-MPCa. The previous study shows an increased level of ESR-1 expression in BPH and prostate cancer, suggesting the association of estrogen receptors in hyperplasia and cancer.24,25

The estrogen receptor (ESR1) is involved in the metabolism of sex steroids and is responsible for carrying out appropriate cellular responses.26 Increasing evidence also suggests that estrogen and estrogen receptors play an important role in the development and progression of prostate cancer.27 ESR1 is expressed in prostate stromal cells and is thought to stimulate the release of growth factors and cause epithelial cell proliferation.2 A study suggested that their expression imbalances are likely to be important in determining the effect of estrogen on prostate cancer cells.28 Another study shows that overexpression of ERα in prostate cancer is related to worse survival outcomes.25 These findings indicate that ESR1 in the human prostate has an oncogenic function.29

In this study, there were significant differences in ESR1 expression in the BPH group, non-MPCa group, and MPCa group, in which the highest expression of ESR1 was found in the MPCa group. This finding was correlated with other studies in which the highest ESR1 expression was found in the MPCa group. Interestingly, the BPH group has higher ESR1 expression than the non-MPCa group. Further research is needed to find the role of ESR1 expression in targeted therapy. This study also has a small sample size limitation, so we suggest conducting a further study with larger sample size.

CONCLUSION

This study shows that ESR1 has significant expression differences in Benign Prostatic Hyperplasia, non-Metastatic Prostate Cancer, and Metastatic Prostate Cancer. The highest ESR1 expression is found in Metastatic Prostate Cancer. We can conclude higher ESR1 expression is related to worse clinical outcomes in prostate cancer.

ACKNOWLEDGMENTS

We are thankful to all the staff for helping with the data retrieval.

ETHICAL CLEARANCE

This study has obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada – Dr. Sardjito General Hospital Ref. No: KE/FK/0017/EC/2021 before the study conducted.

CONFLICT OF INTEREST

We declare that there were no conflicts of interest in this study.

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AUTHOR CONTRIBUTION

All of the authors equally contributed to the study.

REFERENCES


