Selected Peer-Reviewed Articles from the
1st Annual International Conference and Exhibition
Indonesian Medical Education and Research
Institute (ICE on IMERI), Central Jakarta,
Indonesia, 14–16 November, 2016

The 1st Annual International Conference and Exhibition
Indonesian Medical Education and Research Institute (ICE on
IMERI) is an event that provides exposure of the research and
a research topic that is a trend in the world by integrating all
the disciplines that support health. ICE on IMERI integrates
multidisciplinary medical subjects and proposes the following
value prepositions: cutting edge medical science and technol-
yogy, academic ambience, excellence and society impact. This
conference will cover biomedical and clinical sciences on sev-
eral topics as follow: Infectious disease, Stem cell, Cancer,
Reproduction, Neuroscience, Medical technology, Drug develop-
ment, Occupational health, Sport studies, Hydration science,
Clinical epidemiology, Human nutrition, Human genetics, and
Cardiovascular-metabolic-aging. In addition, the project was to
provide ideas to increase research conducive atmosphere among
academicians in Indonesia. ICE on IMERI aims to improve
health and provide a forum to share the findings of the study in
terms of health and medicine. In addition, the conference also
aims to provide an opportunity of meeting colleagues who works
in the same area so as to strengthen relations and professional
collaboration.

ICE on IMERI is arranged annually to become our stage show-
ing and exchanging medical education and research achievement
among national and international partners. We really hope this
conference will bring us to a very fruitful scientific discussion
and sharing experiences among the students, scientists, industry
and faculty members. Our collaboration will be strengthening
and facilitating the achievement of Indonesian Medical Education
and Research Institute, Faculty of Medicine, Universitas
Indonesia.

I would be gladened if the current special section is useful
in updating the readers’s biomedical and clinical sciences. It is
hoped that this special sections will make a good reference mate-
rial and be of great use for biomedical and clinical scientist.

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Analysis of Protein Oxidative Damage and Pregnancy Associated Plasma Protein-A (PAPP-A) Expression in Preeclamptic Placenta

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Preeclampsia is the biggest cause of maternal and fetal mortality. Ischemia Uteroplacental that cause oxidative stress in preeclampsia lead to dysfunction of placental cells. PAPP-A is an enzyme to cleaved the bonding between the IGF binding protein and IGF, free IGF causes trophoblast cells in order to improve placental tissue. The PAPP-A expression and its relationship to oxidative stress is still in question. This study was conducted using 34 samples each of preeclampsia and normal placental tissue. mRNA relative expression of PAPP-A was measured by qRT-PCR, protein PAPP-A was measured by ELISA. Carbonyl measured by dinitrofenil hydrazine method. The mother age was 30.76±6.0 years; gestational age 39 (26–42) weeks. The mRNA relative expression of PAPP-A was lower in the pre-eclampsia. PAPP-A protein levels preeclampsia groups 0.101 (0.048 to 0.425) ng/mL; normal group 0.066 (0.044 to 0.178) ng/mL. Elevated levels of PAPP-A protein is not statistically significant. Placenta Carbonyl has no significant difference between normal and preeclampsia. There were no significant differences between PAPP-A protein and carbonyl in preeclampsia and normal placental, but there is a significant negative correlation between the levels of PAPP-A and carbonyl in preeclampsia placenta (p = 0.003, r = −0.354).

Keywords: Preeclampsia, PAPP-A, Carbonyl.

1. INTRODUCTION

Preeclampsia become the highest underlying of maternal mortality up to 3–5% of pregnancies in the development country.1 Preeclampsia is characterized by placental abnormalities, cerebral edema, renal failure, and HELLP (hemolysis, Elevated Liver enzymes and Low Platelets).2 The disorder is often associated with uteroplacental ischemia and release of toxic substances (E-chaderin and integrin) from the placenta into the maternal circulation.3

Pregnancy-associated plasma protein A (PAPP-A) is a protein synthesized by sincytiotrofoblast placenta and often used as a marker on a routine examination for early detection of Down syndrome in the first trimester of pregnancy. In maternal blood flow, protein increases as pregnancy developed and decreasing after childbirth. PAPP-A can be detected on day 28 after implantation in the maternal blood.4

Protein PAPP-A serves as the enzyme protease for cleavage the binding of IGFBP-4 (insulin-like growth factor binding protein) and IGF, therefore IGF released and can interact with its receptors, to help trophoblastic invasion and development of placental vascularization, and modulate the process of placenta development in the first trimester of pregnancy.4

In preeclampsia, uteroplacental ischemia is a condition due to a decrease of blood supply. In ischemia or hypoxia decreased blood supply cause decrease of oxygen pressure, and ratio of ATP/ADP will increased. Degradation of ATP lead to elevate the amount of hypoxanthine, then will follow increase activity of xanthine oxidase. Xanthine oxidase activity causes uric acid and hydroperoxyde production. Metal ion such as iron and hydroperoxides interaction will produce hydroxyl radical that is the very potent free radical that could damage the lipid membrane, protein and DNA. Carbonyl compounds as a product of protein damaged could be used to indicate oxidative stress in placental tissue. Therefore decrease of blood supply involved in tissue hypoxia, and cause tissues damages, and lead to premature birth.5,6

Examination of PAPP-A maternal serum in early detection of preeclampsia syndrome have been studied, but there were
differences of view with the expression levels of PAPP-A, and there were few research publications of preeclampsia placental tissue PAPP-A. Therefore, we conducted research to observe the PAPP-A marker protein of placental insufficiency through analysis of its mRNA expression, and placenta carbonyl as indicator of oxidative stress damages in preeclampsia pregnancies at 20–40 weeks of gestation and normal gestation >37 weeks.

2. METHOD
This research samples were placental tissue of women with normal pregnancy and preeclampsia syndrome. Samples were obtained in the delivery room and Budi Kemuliaan Hospital and Cipto Mangunkusumo Central Hospital Jakarta along August–September 2015. This study was approved by the ethics committee of the Faculty of Medicine, University of Indonesia (number 597/UN2.F1/ETHICS/2015). Subjects who were participated in the study signed an informed consent. Exclusion criteria were preeclampsia syndrome with infections such as chorioamnionitis and gestational hypertension.

This preliminary study design was a cross-sectional and set minimum sample size of each group is 12 samples. This research is preliminary study because there was no standard deviations obtained from the literature. 7

This study was conducted in Oxidative Stress Laboratory of Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. The tissue samples obtained were then stored in a deepfreezer –80 ° C. The mRNA relative expressions of PAPP-A were measured by qRT-PCR used Livak method, and protein PAPP-As were measured by ELISA. Carbonyl compounds were measured by spectrophotometric method used dinitrofenil hydrazine.

Data assessed by Kolmogorov-Smirnov test to determine normality. Normal data were presented as mean and standard deviation, not normal data distribution showed in median and minimum–maximum range. Un-pair t-test used to determine the difference between two variables normal distribution data. Not normal distribution data determined using the Mann-Whitney test. To assess the correlation between the two variables we used Spearman rank. Limit of significance used was p < 0.05. All data is processed using statistical software package for social science (SPSS) for Windows version 20.

3. RESULT
Sixty eight samples of placental tissues 34 normal and 34 preeclampsia pregnancies were measured and analyzed. Table I showed the distribution of maternal characteristics (maternal ages and age of gestation).

Analysis of PAPP-A mRNA relative expression: Total RNA isolation of placental tissues were measured using varioscan at 260 nm and 280 nm wavelength. The absorbances were used to determine the concentration and purity index of RNA. Result show total RNA samples was 106 (19.975–538.4), and the purity index was 1.981 (1.91–2.099).

Primer Design of Human PAPP A mRNA: The result of PAPP primers was 150 bp of each. Selection of primers based on the requirement that large qRT-PCR amplicons were about 100–200 bp. Primer specificity was analyzed by the melting curve during the qRT-PCR process. We determined PAPP and 18S-rRNA primers (Table II).

PAPP mRNA primer annealing temperature: Optimization was done to get optimum temperature in the annealing process, will be used in amplification of cDNA. Annealing temperature of primer melting of 18S rRNA has been measured in previous study was 59 °C for 18S RNA, besides PAPP 55–62 °C. Optimum temperature was 56.56 °C base on the highest peak, and the value of cycle threshold (Ct) was 26.36. The PAPP melting curve formed one peak (Tm) at 87 °C.

Amplification of cDNA using Two Step qRT-PCR: In this study, normal pregnancy placentas were used as calibrator to preeclampsia. Expression of PAPP and 18 S rRNA were compared, and 18 S rRNA was used as gene referent in this study.

PAPP mRNA relative expression: In this study the PAPP in preeclampsia placentas age of 20–40 weeks were compared to normal placentas (Fig. 1).

The mRNA relative expressions were measured using Livak formula. Figure 1 show that the PAPP mRNA relative expression of preeclampsia were decreased compared to normal placentas but not significant (p > 0.05).

### Table I. Characteristic of samples normal and preeclampsia.

<table>
<thead>
<tr>
<th>No</th>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mother age</td>
<td>30.76 ± 6.0 year</td>
</tr>
<tr>
<td>2</td>
<td>Gestational age</td>
<td>39 (26–42) week</td>
</tr>
</tbody>
</table>

### Table II. Characteristic of human PAPPA and 18S-rRNA primers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Length base</th>
<th>Product</th>
<th>%GC (%)</th>
<th>Tm °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPPA human</td>
<td>F: 5'-TGA TCA CAG GCC TGT ATG ACA AA-3'</td>
<td>23</td>
<td>150</td>
<td>43.5</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>R: 5'-GTA TAC CCA CTG GCC TGG GA-3'</td>
<td>20</td>
<td>60</td>
<td>58.6</td>
<td></td>
</tr>
<tr>
<td>18S rRNA</td>
<td>F: 5'-AAA CCG GTA CCA CAT CCA AG-3'</td>
<td>20</td>
<td>155</td>
<td>50</td>
<td>59.99</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CTT CCA ATG GAT CTT GTA TA-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. PAPP mRNA relative expression in preeclampsia and normal placenta.
Fig. 2. Distribution of PAPPA mRNA relative expression in preeclampsia placentas.

Distribution of PAPPA mRNA relative expression in preeclampsia placentas showed in Figure 2. Figure 2 show the number of placentas with low (<1) PAPPA mRNA relative expression was the highest (=26). It means that PAPPA mRNA relative expressions were low in preeclampsia placental tissues.

Placental PAPPA protein: Samples were homogenized and measured for protein concentration. PAPPA protein standard curve was created every each measurement to determine PAPPA protein concentration. Standard curve of PAPPA show $R^2 = 0.9984$ and the concentration were expressed in ng/ml. PAPPA placental protein concentration than divided with total protein content of placenta.

Figure 3 show the median value of preeclampsia PAPPA protein was tend higher than normal placentas but not significant in correlation (Spearman, $p = 0.79$; $r = -0.046$). Data distribution of PAPPA protein was not normal, preeclampsia PAPPA protein was 0.101(0.048–0.425) ng/ml and 0.066(0.044–0.178) ng/ml for normal control placenta.

Carbonyl compound concentration: Measurement of carbonyl compound used Levine et al. method. Concentration of carbonyl compound was related to oxidative stress in protein or protein damages. The peptide chain of protein transformed to carbonyl compounds. The result of carbonyl compound amount could be seen in Figure 4.

In Figure 4, it show that carbonyl concentration in preeclampsia placental tissue tend to decrease, but statistically not significant ($p = 0.333$) compared to normal placental tissues. Carbonyl concentration were presented in median and range, preeclampsia was 4.545(0.591–40.909), normal was 5.681(0.636–24.909).

Comparison between mRNA and protein concentration of placental PAPPA and carbonyl compound.

Figure 5 show there was a decrease in oxidative stress marker (carbonyl compounds) in preeclampsia placentas compared to normal placenta.

Carbonyl compound used as oxidative stress marker of protein damages. There was a significant negative correlation between carbonyl compound and PAPPA protein ($p = 0.003$; $r = -0.354$).

In Table III the correlation statistical analysis show a not significant correlation between PAPPA mRNA relative expression and its protein ($p = 0.127$; $r = 0.187$). Correlation between PAPPA mRNA relative expression and carbonyl compound was not significant ($p = 0.402$; $r = -0.103$).

4. DISCUSSION

Several studies of the pre-eclampsia known that abnormal vascularization due to endothelial dysfunction in the maternal and placental trophoblast caused placental insufficiency and ischemia. Estrogen can induce implantation by producing growth factors in the uterus. Insulin-like growth factors (IGFs) is a growth factor for the proliferation of the uterus induced by the hormone estrogen. IGF regulated by PAPP-A a proteolytic enzyme for Insulin-like growth factors binding protein-4 (IGFBP-4) therefore IGF released and could interact with its receptor to help trophoblast invasion and development of vascularization of the placenta. Furthermore it can modulate the process of forming the bearing of
placenta in the first trimester of pregnancy and affects metabolic function by stimulating the uptake of glucose and amino acids and then transferred to placenta.\textsuperscript{3,9} This proteolytic (PAPP-A) enzyme of IGFBP-4 can provide an additional mechanism to increase IGF availability, therefore changes in the level of this protease lead to placental and fetal growth disturbances.\textsuperscript{9}

This study conducted on placental tissue after pregnancy termination and obtained the following results: the PAPP mRNA relative expression decreased in preeclampsia compared to normal controls (Fig. 1) were found most in pre-eclampsia gestational age of 20–40.

Spencer et al.\textsuperscript{10} concluded that decreased levels of PAPP-A maternal serum early second trimester in the group of preeclampsia, compared to normal pregnancies, and Odibo et al.\textsuperscript{11} known PAPP-A levels were lower in pre-eclampsia than the control group, used to predict at the beginning or all of pre-eclampsia, but it was only using to probe PAPP-A in maternal serum. The results of our study were to detect PAPP-A in placental tissue aligned with the results of Lin et al.\textsuperscript{12} study which concluded that the PAPP-A protein levels increased in maternal serum pregnancy preeclampsia with hypertension and albuminuria, this occurs in the final trimester of pregnancy with preeclampsia than normal pregnancies. In our study examinations were done after obtain the placenta due to termination of pregnancy. There was a decrease mRNA relative expression of PAPP-A, and PAPP-A protein increase in placental tissues of 20–40 weeks gestational age with pre-eclampsia syndrome. It indicates that PAPP-A protein are needed to improve placental perfusion and growth. Few studies of PAPP-A is used as a marker protein of preeclampsia in the maternal circulation show a concentration decreased, likely due to protein PAPP-A increased only in placental tissue in an attempt to release the IGF from the binding protein (BP).

In this study the comparison between carbonyl compound content of placental tissue ages ≤37 weeks of normal pregnancy and pre-eclampsia in gestational age of 20–40 weeks showed no significant different \( p = 0.333. \) (Fig. 5) Concentration of carbonyl compounds which not different in the group of normal pregnancy and pre-eclampsia syndrome indicates hypoxia does not only occur in pathological conditions, but also on the physiological state, which is useful for embryogenesis. Along increasing of gestational age and growing utero placental circulation, will increase the oxidative stress in the placenta. In the preeclampsia syndrome in accordance with the conclusions of Burton and Jauniaux;\textsuperscript{13,14} Raijmakers et al.,\textsuperscript{15} when the imbalance between the oxidant and antioxidant capacity caused oxidative stress, will stimulates trophoblast invasion disorders, disorders of spiral artery remodeling and ischemia reperfusion phenomenon that leads to chronic oxidative stress in the placenta.

In our study the comparison between the concentration of PAPP-A protein with carbonyl compounds of pre-eclampsia placental tissue at age of 20–40 weeks of pregnancy had significant and negative correlation \( (p = 0.003) \ (r = -0.354) \) (Fig. 6).

Markers of oxidative stress perform by carbonyl compounds were increased as a result of tissue protein damage, but trophoblast cells were still could synthesize PAPP-A protein to maintain placental homeostasis. PAPP-A serves as IGFBP-4 protease enzyme, that increased IGF availability, and useful to address nutrition; improve placental perfusion and tissue ischemia thereby lowering oxidative stress.

Along with increasing gestational age will increase oxidative stress in the placenta and will stimulate trophoblast cells to produce the protein PAPP-A as compensation for maintaining homeostasis placenta. In this study, there were significant negative correlation \( (p = 0.002, r = -0.362) \) between the ages of pregnancy with PAPP-A protein levels indicating the older the age of pregnancy it will be reduced levels of PAPP-A protein as a result of inadequate placenta to deliver more nutrients from placenta to the fetus, and the possibility of degeneration along with age of pregnancy.

5. CONCLUSION

This study shows that expression of carbonyl as marker protein damage tends to decrease in the group of pre-eclampsia, but not significantly different with control. Expression of PAPP-A mRNA relative expression reduced on pre-eclampsia group while the protein content of PAPP-A was higher than the normal pregnancy group. There was a significant weak negative relationship between levels of PAPP-A protein and carbonyl.

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References and Notes


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Cell Viability Inhibition Effect of Gallic Acid and Its Synthetic Derivative Forms on Primary Cultured Endometriosis Cells

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Introduction: There is an urgency to find a treatment for endometriosis, preferably of herbal nature, since currently there is no cure for endometriosis that guarantees no recurrence. Since gallic acid and its synthetic derivatives have been known to reduce cell viability in several cancer cells, this study intended to investigate whether these substances also inhibit the viability of endometriosis cells. Method: Endometriosis cells were isolated and cultured from patients who had undergone laparoscopy surgery using enzymatic technique. After cells were 70–80% confluent, they were harvested and divided into 10,000 cells per well. Cells were given treatment of gallic acid and two of its synthetic derivatives forms-heptyl gallate and octyl gallate-for 48 hours; each with three dosage of 25.6 µg/ml, 51.2 µg/ml and 102.4 µg/ml. All interventions were made in a set of triplo, along with control and blank. MTS assay was used to observe the viability of the endometriosis cells. Results: After the data was rationalised with control, all three substances showed inhibition of endometriosis cells viability. The highest inhibition for all treatments was at 102.4 µg/ml, with inhibition percentage of 56.481%, 65.028%, and 56.839% for gallic acid, heptyl gallate, and octyl gallate, respectively. Conclusion: The present study suggests that gallic acid, heptyl gallate and octyl gallate, have an inhibition effect towards the viability of endometriosis cell.

Keywords: Endometriosis, Gallic Acid, Heptyl Gallate, Octyl Gallate, Cell Viability, MTS Assay.

1. BACKGROUND

Endometriosis is a common gynecological disorder, in which endometrial-like tissue are present in abnormal locations, typically the abdominal cavity and its surrounding areas. It is estimated that endometriosis affects 6–10% of all women in their reproductive age, which is approximately 176 women worldwide. Furthermore, 20–50% women with infertility and 25–70% of women and adolescents with chronic pelvic pain or pelvic pain and dysmenorrhea are also affected by endometriosis. However, specific data on its incidence in Indonesia is still unavailable.

Endometriosis has been shown to have significant negative impacts on patients’ quality of life and overall functionality. Many women reported that the pain they suffer from endometriosis is severe enough to interfere with work productivity, therefore it is difficult to maintain employment, social activities, family responsibilities, and performing other daily activities. In addition, many studies have reported that sexual satisfaction is also compromised in endometriosis patients. In addition, it has also been associated with negative perception of health, thus decreasing ability to work, and may lead to mood and anxiety disorders.

Furthermore, endometriosis can also impose substantial economic burden to patients and the society. A study have shown that the economic burden to endometriosis is at least comparable to other chronic diseases, such as diabetes mellitus. A study in ten countries estimated that approximately £9579 is spent annually for every woman with endometriosis. The main direct cost-drivers for endometriosis include surgical procedures, monitoring tests, hospitalization, and physician visits; while indirect costs are associated with loss of productivity.

Currently, there is still no permanent cure for endometriosis. Instead, current approach to endometriosis is only to relieve its symptoms. Frequently used medical treatments are oral contraceptives, non-steroidal anti-inflammatory agents (NSAIDs), progestin, and gonadotropin-releasing hormone (GnRH) agonists.
While in severe cases, laparoscopic excision, laparotomy and hysterectomy may be performed, which aims to remove all endometriosis tissue and restore normal anatomy. However, there has been no management approach proven to be superior and prevent recurrence of symptoms. For instance, treatment with GnRH agonists and laparoscopy were reported to have 33–75% and 40–50% 5-year recurrence rates, respectively.

One emerging candidate for treatment option that has been a subject of research nowadays is gallic acid, or 3,4,5-trihydroxybenzoic acid. It is a naturally occurring phenolic acid that can be found in many plants, such as grapes, blueberries, tea leaves, oak bark, and a variety of other plants. Many research have shown that gallic acid can reduce cell viability especially on cancer cells. Through several pathways including inhibition of angiogenesis, induction of apoptosis, regulation of gene expressions, oxidative effects, and anti-inflammatory activities. Therefore, gallic acid has the potential to eliminate abnormal growing cells, including endometriosis cells. Furthermore, other derivative forms of gallic acid, has also shown anti-inflammatory effects in vascular endothelial cells.

Based on that knowledge, the aim of this research is to observe the effect of gallic acid and two of its synthetic derivative forms—octyl gallate and heptyl gallate, towards the viability of endometriosis cells.

2. METHOD

The research specimen were taken from endometriosis patients who had undergone laparoscopy surgery. Cells were isolated using enzymatic technique and cultured in complete medium consisted of DMEM, 1% Pen-strep, 1% fungizone, and 20% PBS for 2–5 ml until the desired amount of specimen has been reached.

Cultured cells were harvested and divided into 96 wells. Each well contained 10,000 cells. Treatment of gallic acid, heptyl gal late and octyl gallate were given at three dosage of 25.6 g/ml, 51.2 g/ml and 102.4 g/ml. There were also two sets of controls, namely blank and negative control. All interventions were made in a set of triplo. Cells were incubated for 48 hours in a 5% carbon dioxide incubator at 37 °Celsius. Optimum dosage was achieved from IC 50 by MTS test. After being incubated, MTS reagent was added 1.5 hour before spectrophotometry. MTS assay was performed at 490 nm to observe the viability of endometriosis cells.

Statistical analysis was made using SPSS version 11.5 by first assessing the data normality, then homogeneity, and One-Way Anova to test its significance.

Table I. Average absorbance of endometriosis cells after treated with gallic acid, heptyl gallate, and octyl gallate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MTS assay results (absorbance units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.413</td>
</tr>
<tr>
<td>Gallic acid 25.6</td>
<td>0.879</td>
</tr>
<tr>
<td>Gallic acid 51.2</td>
<td>0.863</td>
</tr>
<tr>
<td>Gallic acid 102.4</td>
<td>0.754</td>
</tr>
<tr>
<td>Heptyl Gallate 25.6</td>
<td>0.786</td>
</tr>
<tr>
<td>Heptyl Gallate 51.2</td>
<td>0.826</td>
</tr>
<tr>
<td>Heptyl Gallate 102.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Octyl Gallate 25.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Octyl Gallate 51.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Octyl Gallate 102.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

3. RESULTS

Two patient samples were isolated, cultured, and given the appropriate treatment as designed. Assessment of cell viability was performed using MTS assay. The average MTS assay results of both samples were calculated, rationalized with blank and the percentage of inhibition was calculated, as shown in Table I and Figure 1.

As pictured in Figure 1, the percentage of inhibitio for all three substances were the highest at 1 maximum dosage of 102.4 µg/ml, with hept gallate inhibiting cell viability the most at 65.028. Both heptyl gallate and octyl gallate had the lowe inhibition at the dosage of 51.2 µg/ml, whi increase in dosage of gallic acid increased i potency for cell inhibition. However, statistic analysis showed that the inhibitory effe differences between substances and dosage were not significant (significance is declared when p < 0.05). The morphological appearance of untreated cells and after treatment with 24.6 µg/ml, 51.2 µg/ml and 102.4 µg/ml of gallic acid, heptyl gallate and octyl gallate are shown in Figure 2. As cell viability decreases, we can observe several morphological changes such as decreased cell confluence and volume, cell shrinkage and decreased cell-to-cell contact. While in cells treated with gallic acid 51.2 µg/ml and 102.4 µg/ml, as well as octyl gallate 102.4 µg/ml; cells form web-like structures extending from dark-colored cells.

4. DISCUSSION

In this study, we have represented that gallic acid and its synthetic derivatives—heptyl gallate and octyl gallate—has cell viability inhibition effect towards endometriosis cells. This study is the first to assess the effects of gallic acid towards endometriosis cells. However, in the recent years, gallic acid has been a subject of research to treat other critical diseases with similar pathogenesis, including cancer.

Zhao et al. reported significant decrease in cell viability, proliferation and invasion of human cervical cancer cells after being treated with gallic acid. A proposed mechanism of cell viability
Extracellular O2 Level and pH Modulation Affected the Human Breast Cancer Stem Cells’ Survival and Stemness

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Introduction: It has been reported that cancer stem cells could maintain their viability and stemness under certain extracellular changes. Therefore, efforts that modulate tumor hypoxia and acidity should be compelled in order to impede the growth of cancer stem cells. This study was aimed to analyze the effect of extracellular pH and O2 level modulation on viability, apoptosis, and stemness of the human CD24−/CD44+ breast cancer stem cells (BCSCs). Methods: The primary BCSCs (CD24−/CD44+ cells) were cultured under hypoxia (1% O2) or under supplementation of sodium bicarbonate (100 mM) for various periods. After each incubation time, cell viability was determined by trypan blue exclusion assay and apoptosis was examined using flow cytometry with Annexin V/PI assay. Furthermore, total RNA was isolated for qRT-PCR analysis of HIF1α, ALDH1 and Klf4 mRNA expression. Results: This study demonstrated that hypoxia could suppress BCSC proliferation, but inhibit the cell apoptosis. Alkaline pH could also suppress BCSC proliferation and promote early apoptosis at the same time. Interestingly, the expressions of ALDH1 and KLF4 were downregulated in hypoxia-treated BCSCs which might be regulated through the increase of HIF1α. Conversely, ALDH1 and KLF4 expressions were upregulated in sodium bicarbonate-treated BCSCs under alkaline pH. Conclusion: The modulation of extracellular pH and the O2 level has diverse effects on the viability, apoptosis, and stemness of BCSCs. Therefore, we suggest that targeting tumor hypoxia and acidity may be a prospective therapeutic strategy to eradicate BCSCs.

Keywords: Breast Cancer Stem Cells, Tumor Hypoxia, Extracellular pH, Cell Viability, Stemness.

1. INTRODUCTION

The microenvironment is believed to have a significant role in tumor growth and progression.1 Likewise, its role in maintaining the unique properties of cancer stem cells (CSCs), the cancer cell subpopulation with stemness phenotype, such as self-renew and the ability to differentiate into multiple cell types comprising the tumor.2 One major characteristic of tumor microenvironment is hypoxia.3 Recent studies showed that the capability of the microenvironment to maintain the undifferentiated state of the stem cells, both embryonic, adult, or cancer, required the hypoxic condition.4,5

In the hypoxic condition, the cells would perform a various cellular adaptation to survive in the state of oxygen deficiency under the regulation of hypoxia-inducible factor (HIF).6 One of the dominant phenotype as the cellular response to hypoxia is the glycolytic metabolism.7 Several studies support the hypothesis that CSCs including the breast CSCs, are more glycolytic, while the differentiated progeny displayed a more oxidative phenotype.6,8 This metabolic preference, in turn, creates an acidic microenvironment as the results of a high lactate production as the end product of glycolysis.10 In consequence, the acidic extracellular pH (pHe) is also becoming the other unique characteristic of the tumor microenvironment.11,12

In contrast to the normal differentiated adult cells which pHe is generally ~7.4 and higher than the intracellular pH (pHi) of ~7.2, cancer cells have a lower pHe of 6.7–7.1 and a higher pHi of >7.4. This specific pH condition has significant roles toward cancer cell’s survival and malignancy.11,12 Recent evidence suggests that an increased pHi is necessary for cell proliferation, evading apoptosis, and cytoskeletal remodeling for cell migration. While a decreased pHe promotes cell invasion and cell-matrix remodeling, and stimulates the activation of acid-activated proteases to facilitate cancer cell dissemination.11–13 Seemingly, this cancer pH dysregulation provides a prospective therapeutic target to limit disease progression.

In this study, we cultivated the human breast CSCs in two different extracellular environments. First, the cells were grown in a hypoxic condition with 1% O2 that was expected to represent the hypoxic intratumor microenvironment of breast cancer tumors. Second, the cells were cultured in an alkaline pH supplemented...
2. METHODS

2.1. Patient Population and Characteristics

2.1.1. Culture of Human Breast Cancer Stem Cells (BCSCs)

In our previous study, primary culture of human breast cancer were sorted using magnetic-activated cell sorting (MACS) conjugated with anti CD24 and anti CD44 antibody resulting in CD24+/CD44+ cells for BCSCs and CD24−/CD44−cells for non-BCSCs (Patent registration from the General Directorate of Intellectual Property Right, Ministry of Law and Human Right, Republic of Indonesia; No. P0021300369).

BCSCs were cultured in DMEM-F12 medium containing HEPES buffer, 1% penicillin/streptomycin, 1% amphotericin B (250 μg/ml), 0.2% gentamycin sulfate (50 mg/ml) and 14.5 mM NaHCO₃ and incubated at 37 °C in a humidified atmosphere of 5% CO₂ and 20% O₂.

2.1.2. Hypoxia Exposure

The BCSCs were initially seeded at 5 × 10⁵ cells/well in the 6-well plate and cultured in 3 mL/well of DMEM-F12 medium containing HEPES buffer pH = 7.4 at 37 °C, 5% CO₂ and 20% O₂ for 24 hours. Afterwards, the hypoxia was performed by replacing the seeding medium with fresh medium. The cells were then incubated at 37 °C, 5% CO₂ and 20% O₂ (normoxia) as the group control and 1% O₂, 5% CO₂, and 94% N₂ as treated samples for 6-, 24-, and 48-hours, respectively. After each incubation period, pH of cell culture medium (pHe) was immediately measured using pH electrode with Micro Bulb for 96-well plate (Hanna)® connected with pH meter (HI 2210®, Hanna), and the BCSCs were harvested by centrifugation at 1000 rpm for 10 minutes to obtain cell pellet and culture supernatant for further analysis.

2.1.3. Alkalinization of the Culture Medium Using NaHCO₃

The BCSCs were initially seeded at 5 × 10⁵ cells/well in the 6-well plate and cultured in 3 mL/well of DMEM-F12 medium containing HEPES buffer pH = 7.4 at 37 °C, 5% CO₂ and 20% O₂ for 24 hours. Afterwards, the extracellular alkalinization was performed by replacing the initial BCSCs medium with DMEM-F12 supplemented with a volume of 8.4% NaHCO₃ (Meylon 84®, Otisuka) to generate a final concentration of 100 nM. The cells were then incubated in the alkalinized medium at 37 °C, 5% CO₂ and 20% O₂ for 6-, 24-, and 48-hours, respectively. After each incubation period, the cells were treated as well as the hypoxic cultures.

2.1.4. Cell Viability Assay

To determine cell viability, we applied trypan blue exclusion assay. Viable cells were counted using automated cell counter (Luna). Population doubling time (PDT) was determined using the following formula: PDT = 1/r where r = 3.32 (log NH/log NI)/(t₂−t₁). Where r = multiplication rate; NI = number of the inoculated cells; NH = number of the harvested cells; t₁ = inoculation time; t₂ = harvest time (hours). Cell viability was also examined using the calculation of percent LDH release obtained from the ratio between LDH activity in supernatant (extracellular) and in cell lysate (intracellular). Extracellular LDH activity represents the presence of this enzyme released by lysed cells, hence this method could be used to determine the cytotoxicity as previously described.¹⁴

2.1.5. Apoptosis Assay

Apoptosis test was performed using Annexin V-FITC Apoptosis Detection Kit (Abcam). The about 5 × 10⁵ BCSCs were harvested and rinsed with cold PBS twice. The next steps were carried out following the protocol instructions.

2.1.6. Quantitative Reverse Transcription-PCR

Total RNA was extracted from cell pellets using TriPure® RNA Isolation Kit (Roche) according to the manufacturer’s protocol. Total RNA concentration was quantified using spectrophotometer (Varioskan Flash). Samples with an A260/A280 ratio of 1.6–2.0 were considered to be free of DNA and proteins. Quantitative PCR was performed using KAPA SYBR FAST® qPCR (KAPA BIO SYSTEMS) in the ExicyclerTM 96 (Bioneer). The PCR primer sequences used in this study were HIF1α: 5′-GCCGC GAACGACAAGAAAAAG-3’ and 5′-GGGGCACTGATGAG CAAG-3’; ALDH1: 5′-TTGGAGATGGGCCTGCAC-3’ and 5′-GGAGGAACCTGCTTTCCTTTT-3’; KLF4: 5′-CCGCTCA TTACCAAGGT-3’ and 5′-TTTCTCACCTGTGTGGGTTC-3’; 18S RNA: 5′-AAACGCTACCACATCACCAG-3’ and 5′-CC TCAAATGATCTCGTTA-3’. Ct value for each gene was determined, and ΔΔCt was normalized to the designated refer- ence gene. Gene expression values were then relatively calculated using the Livak method (2−ΔΔCt).

2.1.7. HIF1α Protein Level

Total protein was extracted from 5.0 × 10⁵ cells. HIF1α protein level was assayed using HIF1α Human ELISA kit (Abcam) according to the manufacturer’s protocol. Data were presented per total protein.

2.1.8. Statistical Analysis

We present all data as mean ± standard error means (SEM) of at least triplicates. A p-value of at least < 0.05 in the independent t-test was statistically considered to be significant.

3. RESULT

3.1. Hypoxia Lowered While Alkalinization Increased the pHe

After 6-hour and the following incubation periods, the pHe of hypoxic BCSCs rapidly decreased compared to normoxia. Meanwhile, the alkalinization increased the pHe to 8.86 ± 0.03. After 6-hour and the following incubation periods, the pHe gradually decreased but still in the alkaline pHe range (Fig. 1).

3.2. Hypoxia and Alkaline pHe Induce HIF1α Expression of Breast CSCs

HIF1α mRNA expression and protein level were measured to evaluate the response of breast CSCs to low (1%) O₂ concentration exposure. The measurement also conducted to the
sodium bicarbonate-treated cells (alkalinized breast CSCs) to know whether a low pH could be a non-hypoxic activator of HIF1α. Breast CSCs gave a positive response to the pH that showed a significant increase (p < 0.01) of HIF1α mRNA expression from 6- and 24-hour incubation compared to control and alkalinized breast CSCs. After 48-hour incubation, there was no significant difference in the expression between control, hypoxic-, and alkalinized-breast CSCs. Surprisingly, HIF1α mRNA expression significantly (p < 0.01) increased after 24-hour alkalinization compared to control (Fig. 2(A)). At the protein level, HIF1α expressions of hypoxic breast CSCs were significantly higher (p < 0.01) than control and alkalinized breast CSCs at all incubation periods. There was also an increase of alkalinized-breast CSCs’ HIF1α protein level after 24-hour incubation (Fig. 2(B)).

3.3. The Alkaline pH Reduces Breast CSCs Survival But Not the Hypoxia
To evaluate the impact of hypoxia exposure and alkalinization on breast CSCs survival, the viability assay was conducted with the measurement of %LDH release that equal to the number of lysis cells. The population doubling time (PDT) was also counted to analyze the ability of breast CSC to resume proliferation after the exposures. Apoptosis was determined using Annexin V/PI flow cytometry method. Alkalinization induced breast CSCs lysis where the %LDH releases were significantly (p < 0.01) higher than control and hypoxic-breast CSCs at all incubation periods. Meanwhile, %LDH releases from the hypoxic-breast CSCs were lower (p < 0.05) compared to control and alkalinized-breast CSCs (Fig. 3(A)). These results indicated that hypoxic condition protected the cells to lyse. Both hypoxia and alkalinization could cause the extension of PDT (Table 1). This result showed that there was a delayed proliferation rate in this two conditions. It also was observed under inverted microscope that the cell counts of hypoxic and alkalinized BCSCs from one view were lesser than control (Fig. 3(B)). Moreover, the ability of BCSCs to form cell aggregates was inhibited under hypoxia and alkalinization. Apoptosis assay seemed in line with the %LDH release. The alkalinization promoted the breast CSCs entering the early apoptosis (68.1%) and late apoptosis (14.5%) state, but in contrast, the hypoxia did not, in fact, the percentage of viable cells (89.8%) was higher compared to control (59.1%) after 24-hour exposure (Fig. 3(C)).

4. DISCUSSION
The results presented here demonstrated that BCSCs responded the alteration of O2 level and pH toward their microenvironment. The hypoxia increased the HIF1α mRNA and protein expression of BCSCs. As widely recognized, HIF1α is the oxygen-responsive subunit of HIFs, the transcription factors that sense and coordinate cellular responses to hypoxia. Recent studies showed that multiple normal stem cells depend on HIF activity to maintain their undifferentiated phenotype. The roles of hypoxia and HIF in cancer stem cells were largely unknown. However, several studies demonstrated that hypoxia, mediated by HIFs, also played a critical role in regulating the self-renewal of BCSCs, and induced tumor cells de-differentiation by maintaining the stem cell properties such as Oct4, cMyc, and Nanog. Surprisingly, our study showed that BCSCs’ HIF1α mRNA expression increased after 24-hour incubation followed by a slight increase of HIF1α protein level. This results gave rise a question, and further studies are required to understand whether an alkaline pH could be an O2-independent activator for HIF1α. Several factors were proved as the non-hypoxic stimulators/activators for HIF1α such as some growth factors, cytokines, vascular hormones, and viral proteins.

Our results indicated that BCSCs were able to survive in the hypoxic condition shown by a low% LDH release and a high% viable cells compared to control (normoxia). Hypoxia has a dual role in determining the cell survival, both as a pro-apoptotic or an anti-apoptotic factor. A severe chronic hypoxia may initiate apoptosis. Meanwhile, the cells may adapt and survive in a moderate acute hypoxia. Apoptosis regulation toward hypoxia was mediated by HIF. This transcription factor could initiate the apoptosis via several mechanisms such as by upregulating the expression of anti-apoptotic binding protein like BNIP3 and NIX; inducing anti-apoptotic protein, Bel-2 and IAP-2; and down-regulating the expression of pro-apoptotic proteins Bid and Bax. In this study, 24-hour hypoxia exposure to BCSCs might be categorized as an acute hypoxia that seemed to induce the anti-apoptotic factors. However, the hypoxic BCSCs also showed an extended PDT until 101 hours that might indicate a halt of cell proliferation or quiescence. Previous studies revealed that hypoxic condition is necessary for maintaining dormancy phenotype (quiescence) both normal stem cells or CSCs in solid tumors such as neuroblastoma, breast, and cervical cancer.

Conversely, alkalinization seemed to increase the lysis BCSCs and the percentage of cells entering both the early and late apoptosis phase. Our unpublished data showed that alkalinization could promote a significant increase of anaerobic glycolysis phenotypes in BCSCs as a compensation mechanism toward the alkaline (high) pH. The main consequence of the anaerobic glycolysis is the high lactate production, and the accumulation of intracellular lactate might be the basic mechanism of the apoptosis by reducing the intracellular pH (pHl). pHl was known to induce apoptosis by altering the membrane potential of mitochondria, promoting DNA fragmentation, and activating the caspase-3. Another study demonstrated that alkaline culture could induce the mitochondrial instability due to subsequent Ca2+ overloads that lead to cell death. The alkalinized BCSCs also had an extension of the PDT (32 hours). It seemed that alkaline culture not only promoting apoptosis but also suppressing proliferation.

Unexpectedly, ALDH1 and KLF4 mRNA expressions in the hypoxic BCSCs were lower than their expressions in normoxia.

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Hypoxia did not seem to induce the increase expression of ALDH1 but suppressed it instead. The previous study revealed that the high ALDH activity through the activation of HIF2α enhanced stemness in the breast cancer cells. The inconsistency of our result might arise from the different type of cells. However, it is necessary to answer whether the low ALDH1 mRNA expression of hypoxic BCSCs correlates with its role as proliferation modulator whereas CSCs are likely in a dormant state. Furthermore, it is necessary to answer whether the low ALDH1 mRNA expression of hypoxic BCSCs correlates with its role as proliferation modulator whereas CSCs are likely in a dormant state under the hypoxic condition.

Hypoxia seemed to up-regulate the self-renewal properties of the BCSCs that represented here by the increase of KLF4 mRNA expression compared to the normoxia after 6-hour incubation. Further studies are necessary to confirm this result due to the decrease expression of this gene at the following incubation periods. In contrast to hypoxia, ALDH1 and KLF4 mRNA expressions of the alkalinized BCSCs were significantly higher than control at all incubation periods. These also need further studies especially for the protein level analysis to confirm the impact of alkaline culture on stemness properties of BCSCs.

5. CONCLUSION

In conclusion, extracellular modulation on the O2 level and pH could alter the BCSC survival and stemness. Low O2 level seemed to enhance BCSCs' survival. Conversely, alkaline culture decreases it. Further studies are required for a deeper understanding of the roles of hypoxia, and especially the pH on stemness since this study indicated potential therapeutic targets from these two parameters of the microenvironment. Hence, cancer therapy is not only focused on the cancer cells per se but also in conjunction with the microenvironment modulation which is expected to reduce the cancer cell survival, in particular, the cancer stem cells.

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The Apoptotic Effect of Gallic Acid and Its Derivatives on Primary Cultured Endometriosis Cells

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Background: Research into endometriosis falls behind despite increasing publication for the last 5 years, contributing to lack of non-invasive treatments and 20% decrease in quality of life. Since gallic acid usage as anti-inflammatory agent has been elucidated in cancer cells, this study serves to investigate the potential of gallic acid as an apoptotic inducer in endometriosis cells. Methods: Primary culture of endometriosis was derived from patients who had laparoscopy via enzymatic technique. In vitro endometriosis cells were treated with three dosages of 25.6 μg/ml, 51.2 μg/ml and 102.4 μg/ml of gallic acid, heptyl gallate, and octyl gallate for 48 hours. Determination of quality and quantification of early, late, viable, and necrotic cells was done using confocal fluorescence with acridine orange/ethidium bromide staining of at least 100 cells per sample. Results: Control samples showed 63.8% cell underwent apoptosis. Gallic acid, heptyl gallate, and octyl gallate showed different inhibition pattern. Apoptosis after gallic acid treatment decreased from 90.1% to 79.2% as the dose is increased. On the contrary, 51.2 μg/ml heptyl induce 92.5% apoptosis, while octyl show most apoptosis at 93.1%. Conclusion: This study exhibited apoptotic inducer effect of octyl gallate, followed by heptyl gallate and gallic acid and their potency as treatment for endometriosis.

Keywords: Endometrial Cells, Endometriosis, Gallic Acid, Heptyl Gallate, Octyl Gallate, Apoptosis, Confocal Fluorescence, AO/EB Staining.

1. INTRODUCTION
Endometriosis is a chronic inflammation disorder characterized by the presence of endometrium and endometrial stromal with ovaries, pelvic peritoneum and bowel as the predilection sites.1,2 It is found in 6–10% of general female population, costing about 124 trillion rupiahs nationally per annum. The co-morbidities of endometriosis negatively affect social functioning and mental health. Work affected by the associated symptoms results in lower quality of life. Inversely, lower quality of life also negatively affect work. It is essential to address this disease as study revealed that 50% of women still experience symptoms of endometriosis even after treatment and that they had significant decline in sexual function and quality of relationship.3–5

The study on the pathogenesis of endometriosis dates back to 1690 by Shroen and further elucidated by von Rokitansky over 100 years ago. However, until now, the exact pathogenesis or disease progression remains unknown. It is proposed that endometriosis may rise from endometrial origin or non-endometrial origin.6 There is currently no cure for endometriosis. The treatment given by medical services simply aims to decrease the pain and infertility. Surgery is also opted to remove the endometriosis. Healthcare providers usually suggest laparoscopy to remove endometriosis and scar tissue as well as to improve fertility in women with mild or minimal endometriosis.7,8 Researchers have investigated other means to induce apoptosis in endometriotic cells. One substance that possess apoptotic ability is gallic acid. Gallic acid is a naturally available plant phenol with antioxidant property found in almost all plants and particular high content in green tea, grapes, and red wine.9,10 In the recent years, gallic acid has shown its promising potential as an anti-inflammatory agent. Studies have found that gallic acid can induce cell apoptosis in some cancer types.11

Gallic acid or 3,4,5-trihydroxybenzoic acid is a polyhydroxy phenol compound found naturally in gall nuts, tea leaves, green
Exploration on Factors Affecting Adherence of Patient with Tuberculosis in Cakung Primary Health Care, 2016

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Introduction: Data from Cakung primary health care show that some indicators on pulmonary tuberculosis control has not yet achieved. Some indicators did not meet national target even declines, such as conversion rate (year 2015 65.64% compared to year 2012), cure rate (year 2015 45.17% compared to national target of 85%), and success rate (year 2015 category 1 45.17% and category 2 31.26%). Because patient adherence to TB medication is associated to TB medication success, hence factors that affect patient adherence in Cakung primary health care year 2016 need to be explored. Aim: To understand factors affecting successful TB treatment in Cakung primary health care, East Jakarta. Methods: Descriptive-analytic approach with primary data collected by performing in-depth interviews on one subject at a time and secondary data from Cakung primary health care 2015 annual reports file. Result: Ten informants are interviewed, composed of six patients with different characteristics and four medical personnel for data triangulation. Patient informants composed of 4 men and 2 women, aged from 21 to 75, four out of six informants do not work, two informants are considered inadherent, one informant is given category two medication and one informant is given MDR regimen. Data triangulation is achieved by health cadre, head of tuberculosis program, head of RW, and head of primary health care. Conclusion: The low cure and success rate is associated to patient adherence to medication, affected by knowledge and motivation, health access, administration and finance, socio-cultural, and other factors.

Keywords: Cure Rate, Success Rate, Adherence, Qualitative Study, Pulmonary TB, Cakung Primary Health Care.

1. INTRODUCTION
Pulmonary tuberculosis (pulmonary TB) is an infectious disease caused by Mycobacterium tuberculosis, with primary symptoms of cough for 2 weeks or more which may be accompanied by phlegm, blood mixed sputum, shortness of breath, weakness, loss of appetite, weight loss, malaise, night sweats, and fever for more than 1 month.1

Pulmonary TB is still a health problem in the world. Based on data from the WHO, there were 8.6 million cases of TB in 2012, with 1.1 million people (13%) TB-HIV patients and a mortality rate of 170,000 cases.2

In Indonesia, the prevalence of TB is still high. Based on data from Global Tuberculosis Control, Indonesia is now ranked fourth after India, China, and South Africa.3 The prevalence of tuberculosis in Indonesia is 660,000 cases with an estimated incidence of 430,000 cases in 2010.4 In addition, Indonesia has the highest HIV epidemic increase compared to another country in Asia5/To solve the problems of TB in Indonesia, Indonesian government has implemented a national TB strategy in 1995: DOTs (Directly Observed Treatment Short Course). It started to show result, as Indonesia has achieved several TB global indicators for case detection and treatment success in 2006, which were key to TB control. The backbone for this service is the primary health care (Puskesmas) which amounted to 7352 in total, with 98% of them have implemented DOTs strategy.4

Data from Cakung primary health care showed several indicators of pulmonary TB control that has not yet been achieved as it does not comply with national targets, even declined compared to the previous year. Among others, the conversion rate (year 2015 65.64% compared to year 2012), the cure rate (year 2015 45.17% compared to the national target of 85%) and the success rate (year 2015 category 1 45.17% and category 2 31.26%).4

Because of the low achievement in TB control program indicators, especially indicators related to TB treatment, it is necessary...
to do further study to understand factors affecting successful TB treatment in Cakung primary health care.

2. METHODS

Descriptive-analytic approach is used in this qualitative study, which take place in Cakung sub-district on 1–13 June 2016. For primary data collection, we use an in-depth interview to Cakung primary health care officers, head of RW or local village head, and tuberculosis patients; secondary data was obtained from Cakung primary health care 2015 annual reports file, as well as Cakung primary health care tuberculosis patients data year 2016 which amounted to 62 people. The subjects for in-depth interview were consecutively sampled based on variation of the following characteristics: adherence, sex, age, employment, and WHO standard tuberculosis regimen.

Inclusion criteria were subject who have been diagnosed with tuberculosis, listed as patient in Cakung primary health care, and agreed to be interviewed. Exclusion criteria were subject that is not in interview location when data collection was conducted. Sample size depended on the fulfillment of predetermined characteristic variations. After data retrieval, result from the interview will be made into table summarizing data. Next, data will be analysed qualitatively for in-depth understanding on reality studied.

3. RESULT

3.1. Secondary Descriptive Data

Cakung sub-district (kecamatan) is a part of East Jakarta, Indonesia. With an area of 4,282 hectares, Cakung sub-district is divided into 7 administrative village (kelurahan) which is further divided into 85 RW and 996 RT. Number of people living in Cakung sub-district in 2015 according to Central Bureau of Statistics (Badan Pusat Statistik) is 528,252 people composed from 103,331 family. Population in Cakung sub-district is the largest in East Jakarta, which is 18.37% of East Jakarta population. The average population growth rate of 2.63% per year. Details of the total population of each village in Cakung sub-district can be seen in Table I.

The amount of pulmonary TB patients in Cakung sub-district is 379 people. Detailed characteristics of patients with pulmonary TB in can be seen in Table II.

Table II. Pulmonary TB patients’ characteristics in Cakung sub-district.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age (yo)</th>
<th>Employment</th>
<th>Adherence</th>
<th>Regimen category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mr. W</td>
<td>59</td>
<td>Unemployed</td>
<td>Adhere</td>
<td>Category 2</td>
</tr>
<tr>
<td>2</td>
<td>Mrs. S</td>
<td>40</td>
<td>Unemployed</td>
<td>Adhere</td>
<td>MDR</td>
</tr>
<tr>
<td>3</td>
<td>Mr. AJ</td>
<td>30</td>
<td>Construction worker</td>
<td>Non-adhere</td>
<td>Category 1</td>
</tr>
<tr>
<td>4</td>
<td>Mr. AR</td>
<td>75</td>
<td>Unemployed</td>
<td>Adhere</td>
<td>Category 1</td>
</tr>
<tr>
<td>5</td>
<td>Ms. D</td>
<td>21</td>
<td>Student</td>
<td>Adhere</td>
<td>Category 1</td>
</tr>
<tr>
<td>6</td>
<td>Mr. S</td>
<td>54</td>
<td>Unemployed</td>
<td>Non-adhere</td>
<td>Category 1</td>
</tr>
</tbody>
</table>

3.2. Interview Result

Six respondents were interviewed corresponding the inclusion criteria. Patients were then selected based on several criteria such as age, sex, adherence, employment, and TB type.

Table VI. Interview result from health cadre, head of TB program, head of RW, and head of primary health care.
Triangulation of data is conducted by interviewing cadres, head of RW, head of the primary health care (Table IV). From interview, several subthemes have been obtained (Tables V and VI).

4. DISCUSSION
4.1 Knowledge and Motivation
Patients’ knowledge about Mycobacterium tuberculosis infection is strongly associated with their adherence to treatment. In this study, we found that almost every informant knows what TB is, its signs and symptoms, and also its route of transmission. However, information regarding the cause of TB still lacks. Majority of informants thought that TB is caused by cigarettes, dusts, or air pollutions; some even did not know what causes TB infection. When this information was crosschecked with inputs from community figures, the results remained the same; they also had no idea as to the real cause of TB.

Lack of TB knowledge is also correlated to patient’s interpretation about healthiness and sickness. In this study, majority of patients thought that if the signs and/or symptoms of TB are gone, then they are healed. This is in accordance to a study in Indonesia by Widjanarko et al., which said that most patients stop their treatment when they feel their symptoms are getting better. This false belief of healthiness and sickness is the main cause that prevents patients in our study from completing their treatment.

A decent knowledge about TB will build the correct interpretation about healthiness and sickness in patients, and later will improve adherence to treatment. A study in China suggested that patients often complete their treatment even when they were not experiencing symptoms of TB because they believe the “root” of the disease should be removed. A systematic review also reported that patients with the correct belief of healthiness and sickness tend to comply to treatment more than those who do not. This explains the association between knowledge of TB, interpretation of healthiness and sickness, and rate of adherence to treatment of TB patients in Cakung sub-district.

Patients who had a correct knowledge of TB tend to have higher motivation in complying their treatment. Study by Liu et al. suggested that strong motivation is associated with higher adherence to treatment. In our study, we found that patients’ motivation is to be cured or relieved of its symptoms. Patients’ motivation could also be improved through the attention given by close-related persons or health care workers. Study by Liu et al. also explains that by reminding patients to come to health care facilities by phone and also patients who did not show up for their treatment could help improve patient’s motivation. Information regarding support from close-related people and health care workers in Cakung will be discussed in later section.

4.2 Factors Affecting Adherence to Treatment
Based on results analysis, factors affecting adherence to treatment of TB patients can be illustrated as below.

Every single TB patient stated that family is a factor affecting adherence to treatment. This statement is confirmed by primary health care workers whom said that families affect patients’ compliance to treatment especially in patients whose family is their treatment observer. Family’s support to the patient could be as medication supervisor and reminder or as drug retriever when the patient could not come to the primary health care. However, from the interview, family could also be a barrier to treatment. There is a family that gave negative insight and this affect the patient’s decision in stopping TB treatment. This negative insight resulted from false knowledge about TB and its treatment. Therefore, knowledge and family’s attitude is of great importance because it affects the patient’s decision in taking TB treatment. This is in accordance to a study conducted by Munro et al., which suggested that family could affect patient’s knowledge, attitude, and belief towards TB and its treatment. Furthermore, family support could overcome structural and personality barrier of the patient.

Another factor which could affect patient’s adherence to treatment is the role of medication supervisor (Pengawas Minum Obat/PMO). According to our interview with the patients, community figures, and primary health care workers, medication supervisor is an important factor to support treatment. Supports from medication supervisor are crucial to ensure that the patient finish his/her treatment, given the long period of TB treatment could lead to treatment non-compliance. Medication supervisor ought to be the health care workers, but it could also be from health cadres, teachers, other community figures, or even family members. According to our interview, patients generally had family member as medication supervisor because living under the same roof made it easier for them to remind the patient. However, for patients that did not have any family member living in Jakarta, their medication supervisor is the health cadres. Medication supervisor act as supporting factor to treatment adherence, according to research done by Hayati et al.

Medication is another important factor that affects treatment adherence of TB patients. The number of drugs consumed and their side effects could be a barrier to treatment completion. According to our informants, side effects and size of the drugs act as a barrier to treatment, especially in TB-MDR patients. This is in accordance to a study which shown 31% of TB patients did not comply to treatment due to its side effects. However in our study, no patient stop their treatment due to its side effects. Community figures also stated that patients were always well-informed about medication side effects, thus no treatment withdrawal due to side effects.

Other factors which considered beneficial to patients’ adherence to treatment are patients’ motivation to be cured, easy access to retrieve medicines, and the use of social media by primary health care workers to remind the patients. As to another barrier to treatment is the need to go to primary health care every single day for category-2 and TB-MDR patients to retrieve medications. Furthermore, complicated administrations, the cost of treatment, and patients unwilling to treat are also a barrier to TB treatment in Cakung.

4.3 Health Access, Administration and Finance
Most of TB patients complained in financial aspect as one of the inhibiting factors affecting their adherence to TB treatment. Although TB treatment is free in Indonesia, there are other factors that aggravating financial burden from patients. Several factors were identified from this study, i.e., travel cost to the primary health care and many TB patients lost their job because of their disease and the treatment. This is even worse with most TB patients is in the middle to low socio-economy level. Financial aspect is very important in TB treatment as shown in a study by Prayogo in Pamulang subdistrict primary health care.
The Quality of RNA Isolation from Frozen Granulosa Cells

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Background: Granulosa cells express some important genes for oocyte development and fertilization which may give assessment biochemically to determine the optimal embryos. The stabilization of RNA from frozen cells would affect the quality of mRNA isolation because it is a prerequisite for any gene expression analysis. The purity and concentration from RNA isolation are the two key variable for successful RT-PCR experiment. Methods: The test was done on 28 patients who undergo IVF cycle in Yasmin IVF Clinic; Ciptomangunkusumo Hospital, Jakarta and Daya Medika Kedoya Clinic. The study was approved by the medical ethics committee of Faculty of Medicine, Universitas Indonesia. Informed consent was obtained from all the study participants. Frozen granulosa cells were collected during oocyte retrieval were preserved using RNAlater® Ambion and stored at −80°C, while fresh granulosa cells directly go through isolation. RNA isolation was conducted using QIAamp RNA Blood Mini Kit QIAGEN. Descriptive study was conducted based on purity and concentration as the result of RNA isolation. Gene expression analysis also conducted with Bax/Bcl2 ratio between two groups. Result: This study result showed the mean concentration of RNA isolation from frozen granulosa cells is 21.78 ± 13.09 ng/μl, from the highest (47.90 ng/μl) to the lowest (8.50 ng/μl). The mean purity of RNA isolation from frozen granulosa cells is 1.85 ± 0.37. In comparison, frozen granulosa cells have lower mean concentration of RNA isolation than from fresh RNA isolated granulosa cells (250.81 ± 223.67 ng/μl). However, for RT-PCR result, there is no significant different of Bax/Bcl2 ratio between two groups. Conclusion: These findings suggested that RNA quality of frozen granulosa cells is reliable for gene expression analysis. Nevertheless, further studies about the comparison between the RNA quality of frozen granulosa cells and fresh isolated granulosa cells are needed.

Keywords: Frozen Granulosa Cells, RNA Concentration, RNA Isolation, RNA Purity.

1. INTRODUCTION

Embryo selection is one of the most important step for in vitro fertilization (IVF) program because a high quality embryo has higher implantation and pregnancy rate.1 To date, the embryo is selected based mainly on morphological criteria by evaluate the presence of pronuclei in zygote then cells fragmentation and cleavage rates in cleavage embryo.2 The selection of embryo actually begins since oocyte quality assessment. It because a good quality oocyte will become a high quality embryo. The oocyte assessment is also based on morphological criteria including polar body, cytoplasm morphology, and zona pellucida.3 However, morphological appearance could not always accurately predict the quality of oocyte and embryo. Based on

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to the oocyte during follicular development and ovulation. Cumulus cells are also in turn regulated by oocyte factors. A study by Feuerstein et al. showed that several genes expression in cumulus cells was indicative of oocyte and embryo quality.

RNA quantity and quality are important for gene expression accuracy, and also critical elements for success RNA-based analyses. Using high quality intact RNA as a starting point in molecular biology is preferable, such as in RT-PCR, microarrays, CDNA library, and any other kind of array application. RNA quality analysis is also necessary in clinical application with limited or unique material. The assessment of RNA quality can be done by several methods: OD measurement, OD measurement with NanoDrop, gel electrophoresis or lab-on-chip technologies like Bioanalyzer 2100 (Agilent Technologies, USA) and Experion (Bio-Rad Laboratories, USA). A study by Vermeulen et al. has applied an analytical framework to evaluate RNA quality in relation to qPCR results using novel methods and demonstrated a measurable influence of RNA quality for gene expression results.

RNA quality can be influenced by several factors, including sample storage. A study showed that RNA quality may be impaired in samples stored for a long time or under sub-optimal conditions. The aim of this study was to assess the quality of RNA frozen granulosa cells after a certain time of storage time. We investigated the RNA quality from frozen granulosa cells with modern OD measurement using NanoDrop and RT-PCR for several genes. The results were compared to RNA quality from fresh granulosa cells.

2. METHODS

Granulosa cells were collected at In Vitro Fertilization (IVF) Laboratory Yasmin Kencana Clinic, Ciptomangunkusumo Kencana Hospital and Daya Medica Clinic, Kedoya. This study was approved and reviewed by Institutional review Board at Faculty of Medicine Universitas Indonesia. Granulosa cells has been collected from patients who had filled informed consent to participate in this study.

2.1. RNA Isolation

Granulosa cells were pooled by two groups before RNA isolation: those which were preserved by RNAlater® (Ambion, USA) and stored at −80 °C and those which were processed immediately to RNA extraction. QIAamp RNA Blood Mini Kit and Handbook was used as kit and protocol for RNA extraction with some modifications. Granulosa cells were thawed and centrifugated at 20 °C for 5 minutes at 1000× G. Reaction mix from Buffer RLT and β-Mercaptoethanol were added to samples after supernatant removal. Samples then were homogenated with up and down pipetting. Sample was transferred to QIAshredder spin column and centrifugated for 8 min at 20 °C at 8000 G. Ethanol 70% was added to sample for 600 μl after column filter was removed, after that 700 μl volume of sample was transferred to another spin column and were centrifugated in 15 s at 20 °C at 8000× G. Samples then transferred to 2 ml tube and then were centrifugated. Buffer RW1 (700 μl) was added to sample and centrifugated in 15 s at 20 °C at 8000× G. Sample were transferred again to new 2 ml tube and then 500 μl of RPE buffer was added to sample and was centrifugated with maximum speed for 3 minutes. After that, sample was transferred to 1.5 ml tube and was added 50 μl of Rnase-free water and were centrifugated with maximum speed at 13000× G.

2.2. RNA Yield and Purity

The concentration and purity (A260 nm/A280 nm and A260 nm/A230 nm) of the samples were assessed by spectrophotometry (Nanodrop 2000 Thermo Scientific).

2.3. cDNA Synthesis and RT PCR

For cDNA synthesis, RNA from each sample was added to reaction mix and reverse transcribed using QuantiTect reverse transcription kit (Qiagen Ltd.). RT PCR was performed using QuantiTec SYBR Green Kit (Qiagen Ltd.). For each sample, 25 μl reactions were set up in duplicate, which each reaction containing of 12.5 μl of QuantiTec SYBR Green, 10 μl of RNase-free water, 0.5 μl mix of primer forward and primer (Table I), and 2 μl cDNA template. The thermal cycling condition included an initial activation step at 95 °C for 15 minutes, followed by 40–50 cycles of denaturation (94 °C, 15 seconds), annealing, amplification (72 °C, 30 seconds), a final melting curve analysis. Negative control containing no RNA template were introduced to each run. Standard curve from gblock was used to determine the quantity expression for each target gene.

2.4. Statistical Analysis

Data were expressed as means and standard deviation (±SD). The statistical significance of any differences of the mean RNA yield between frozen and fresh sample was determined using t-test. The statistical significance of any differences of the mean mRNA levels between frozen and fresh sample was determined using Mann-Whitney test (two-tailed). Differences were considered statistically significant at P < 0.05. All statistical analysis using SPSS 22.0.

3. RESULTS

RNA yield and purity was assessed spectrophotometrically (Nanodrop 2000 Thermo Scientific) which showed that the RNA yields from frozen granulosa cells were lower than those from fresh granulosa cells: 21.78±13.09 versus 250.81±229.67 ng/μl (mean±SD) with significant difference (P < 0.05) (Fig. 1). RNA purity from both group produced RNA with OD 260/280 ratio.
Analysis of Socioeconomic Status and Personal Behavior on Hypertension in Jakarta, Indonesia: A Cross-Sectional Study

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Introduction: Hypertension as a leading cause of cardiovascular disease induces premature death with nearly one billion patients globally, two-thirds of which are in developing countries including Indonesia. Low socioeconomic status is an important determinant of health care access, but the relation with hypertension and personal behavior remain complicated and unclear. The objective of this study was to determine whether hypertension not only affected by personal behavior but also socioeconomic status. Methods: A cross-sectional study was performed with secondary data of Ketuk Pintu Layani dengan Hati (KPLDH) program in Kecamatan Matraman period of June 2016, which conducted by interviewing every households in RW02 Kayumanis. From total of 1320 data, 787 data of adults (>17 years old) were included in this study. The included data were socioeconomic status, personal behavior, and hypertension diagnosis. The bivariate analysis was conducted with chi-square test and each variable with \( p < 0.20 \) were included in multivariate analysis using logistic regression. Result: The study showed that the prevalence of hypertension was 11.2%. There were significant associations between marriage status (OR 4.74, 95% CI 2.22–10.13), house condition (OR 1.93, 95% CI 1.16–3.21), jimpitan or traditional peer-to-peer social donation (OR 1.75, 95% CI 1.09–2.81) and active smokers (OR 0.310, 95% CI 0.15–0.66) with hypertension according to the logistic regression analysis. Conclusion: Based on the result, the socioeconomic status and personal behavior could affect the level of hypertension. We expect that public health could focus on the socioeconomic status and personal behavior aspects as the basis of promotive and preventive programs.

Keywords: Community Medicine, Developing Countries, Hypertension, Personal Behavior, Socioeconomic Status.

1. INTRODUCTION

Hypertension, as a leading cause of cardiovascular disease, induces premature death with nearly one billion patients globally, two-third of which are in developing country, including Indonesia. In 2013, hypertension prevalence has reached 26.5% based on Riset Kesehatan Dasar 2013.¹ In fact, hypertension is more prevalent in less educated and unemployed citizen. Another study stated low socioeconomic status could affect hypertension, which related to low occupational income and low education level.² It is also assumed that low socioeconomic status may play important role on the funding of health and a healthy lifestyle or personal behavior. Based on those facts, other founding about low socioeconomic status can be an important determinant of health care access. However, the relation of which with hypertension and personal behavior remain complicated and unclear.

As a matter of fact, recently there were no significant difference about the impact of socioeconomic status or personal behavior on hypertension in either developed or developing country, aside from age related hypertension.² Hence, it is needed further study to acknowledge whether socioeconomic status or personal behavior are really an independent etiologic factor that may contribute to the outcome of hypertension. Therefore, the objective of this study was to determine whether hypertension not only affected by personal behavior but also socioeconomic status.

2. METHODS

2.1. Study Design, Setting, and Participant

A cross-sectional study was performed with secondary data of Ketuk Pintu Layani dengan Hati (KPLDH) program in Kecamatan Matraman period of June 2016 which conducted by...
interview to every households in this area. A standardized structured questionnaire and interview based on health department policies in DKI Jakarta was used to collect the data in KPLDH program. One community was selected randomly out of 62 RW in Kecamatan Matraman and total population sampling was conducted in that community. From total of 1320 data of individual in RW02 Kayumanis, 787 data were included in this study. People who aged over 17 years old was considered for this investigation based on The Eight Joint National Committee (JNC 8) on the age criteria of hypertension. The uncompleted or doubled data was excluded from this study. No personal identifier were recorded in this study to maintain the anonymity.

2.2. Variable Definition
Hypertension was defined as a condition of systolic blood pressure (SBP) exceeding 140 mmHg, or a diastolic blood pressure (DBP) exceeding 90 mmHg. Based on JNC 8, hypertension is classified into prehypertension (120 mmHg ≤ SBP ≤ 139 mmHg or 80 mmHg ≤ DBP ≤ 89 mmHg), stage I hypertension (140 mmHg ≤ SBP ≤ 159 mmHg or 90 mmHg ≤ DBP ≤ 99 mmHg), and stage II hypertension (SBP ≥ 160 mmHg or DBP ≥ 100 mmHg). In this study, the hypertension was divided into normal (normal blood pressure group and prehypertension group) and diagnosed hypertension (stage I and II hypertension group).

The socioeconomic status was defined as a measure of class standing influenced by social class, educational level, income and occupation. In the same way, the following gathered socioeconomic and demographic data was extracted for analyses: educational level based (bachelor or equivalent/upper secondary education/lower secondary education/primary education or below), marriage status (single/married/widowed), occupation (unemployment/government associate/entrepreneur/private sector worker/farmer/others), income (<1.5 million IDR/1.5–3 million IDR/>3 million IDR), house condition (ideal/not ideal), number of children (ideal based on the Indonesian family planning program/not ideal), rukan kematan or Indonesian traditional funeral ceremony (participate/not participate), jimpitan or traditional peer-to-peer social donation (participate/not participate), health insurance (BPJS-PBI/BPJS non-PBI/private/no insurance), routine social activities (yes/no).

The personal health behavior was defined as actions that are present in human relationship especially to their own body. The following data was included in this study: active smoker (yes/no), physical activity (≥1 time(s) in a week/<1 time in a week), and nutritional intake (normal/excessive).

2.3. Data Management
The quantitative data generated was entered into IBM SPSS 20 software for analysis. The bivariate analysis was conducted with chi-square test and each variable with p < 0.20 were included in multivariate analysis using logistic regression.

2.4. Ethics
The ethical approval to conduct the study was obtained from Faculty of Medicine Universitas Indonesia, Research and Ethics Committee. Permission to carry out the study on secondary data of KPLDH was obtained from the program officer in Puskesmas Matraman.

3. RESULT
3.1. Characteristic of Studied Individuals
We found that 88 out of 787 people (11.2%) in the population had hypertension (Table I). The hypertension could be further classified into: normal blood pressure (24.5%), prehypertension (64.3%), stage I hypertension (8.6%), and stage II hypertension (2.6%). Among the hypertension cases, 53 patients (60.2%) were female and 35 patients (39.8%) were male. Hypertension patients group was dominated by patients with age more than 60 years old (38.6%), upper secondary education level (39.7%), and income within the range of 1.5–3 million IDR (44.3%). We also found that majority of hypertension patients were non-smokers (90.9%) and had sedentary life style (60.2%).

The bivariate analysis between the socioeconomic and personal behavior factors associated with hypertension was conducted with chi-square test. In this study, we found several variable which has association with hypertension (P < 0.05), namely education level, gender, age group, occupation, marriage status, income, house condition, participation in rukan kematan, jimpitan, active smoker, physical activity and health insurance (Table II).

3.2. Social and Economic Factors
From the data, the socioeconomic factors associated with hypertension were educational level, occupation, marriage status, income, house condition, health insurance and participation in social events/traditions such as rukan kematan and jimpitan. Lower educational level, especially person with only primary education or below, had a significantly associated with hypertension (OR 3.36, 95%CI 1.47–7.65). Person with occupation had a tendency to have lower risk in developing hypertension than unemployment group, for example civil servant or governmental associate (OR 0.94, 95%CI 0.26–3.35) and private sector worker (0.27, 95%CI 0.14–0.54). Higher income has a lower risk to develop hypertension than the lower income (OR 0.54, 95%CI 0.29–0.98). We also found marriage status has a relation with hypertension, which described by widowed (OR 12.05, 95%CI 4.94–29.39) and married people (OR 4.52, 95%CI 2.13–9.61) had a higher risk than single person. People who do not participate in social events/traditions such as jimpitan has a higher risk to develop hypertension (OR 1.78, 95%CI 1.13–2.79).

3.3. Personal Behavior Factors
Active smokers and physical activity has attributed to hypertension cases. In this study, active smokers had a lower risk to gradually suffer hypertension (OR 0.29, 95%CI 0.14–0.60). In other hand, people who physically active has a lower risk compared to sedentary people (OR 1.46 95%CI 0.93–2.29).

Multivariate analysis was done by logistic regression model with included variables: occupation, marriage status, income, house condition, rukan kematan, jimpitan, active smoker, physical activity, and health insurance. On the final model, the result showed that variables which had a relation with hypertension were marriage status, house condition, jimpitan, and active smoker (Table II). There were three variables which belonged to risk factors (OR > 1), namely marriage status (OR 4.74, 95%CI 2.22–10.13), house condition (OR 1.93, 95%CI 1.16–3.21), and jimpitan (OR 1.75, 95%CI 1.09–2.81).
Relation Between Exposure of Rainforest Fire Smoke and Clinical Complaints During Indonesia Rainforest Fire in September–October 2015

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Background: Forest fires produce smoke pollutant that is hazardous to human health. The rainforest fire of Riau, South Sumatera, and Borneo/Kalimantan in September–October 2015 was a major disaster both economically, environmentally, and to human health. This research aims to seek the association between demographic and forest fire smoke exposure with a multitude of clinical symptoms ranging from respiratory, eye, etc. Methods: Research samples were taken by online questionnaire in October 2015 to residents of Borneo and Sumatra whose area were exposed and polluted by the smoke. Data were analyzed using multivariate and bivariate analysis. Results: Among the results shown that duration of direct smoke exposure to a person (in hour/day) are statistically significant to health problems such as eye irritation (p = 0.024; OR = 44), cough (p = 0.031; OR = 19), rhinorrhea (p = 0.002; OR = 65), sore throat (p = 0.064; OR = 7.9), dyspnea (p = 0.026; OR = 12). The usage of simpler, less-protective equipment like tissue showed a higher risk (p = 0.048; OR = 18.8) than simple disposable ordinary mask (p = 0.03; OR = 6.3) to get respiratory symptoms, especially cough, and sore throat. Conclusion: Duration of forest fire smoke exposure and type of breathing protective equipment was associated with the health problems.

Keywords: Rainforest Fire Smoke, Smoke Pollution, Clinical Complaint, Indonesia.

1. INTRODUCTION

The rainforest fire that occurs in 2015 at Riau, South Sumatera, and Borneo/Kalimantan caused a tremendous loss for Indonesia. Such events had occurred previously during 1997–1998, which burned 5.2 million hectares of rainforest in East Borneo/Kalimantan.1 Bearing a heavier disaster, however, the effect of fire in 2015 was felt until Malaysia, Singapore, Southern Philippines, and Thailand. The economic loss reaches 200 billion, causing the event to be one of the most notable disasters in 2015. One of the impact of such fire that spreads more than 1000 human settlements, is the fire smoke. Heavy smoke from rainforests fire results in health, weather and also economic loss. Hoffman et al., studied the cause of rainforest fire occurred in 1996. It has been suggested that the fire was not only caused by the natural process but also involved human work.1 In Borneo, land clearing done by farmers or field company, is the main reason for burning rainforests. Land clearing is usually done because it provides cheap alternatives to achieve new fields for farming. However, this alternative possesses risks to cause rainforests fire if left uncontrolled and monitored properly. Land clearing method has long been left in developed countries due to its heavy risks and impact towards the environment. However, it is still commonly practiced in Indonesia. Human involvement towards rainforests fire also caused Syam et al. Exposure of Rainforest Fire Smoke and Clinical Complaints by accident such as cigarette butts or remnants of active campfire. Environmental causes usually due to low humidity and dry air, especially during prolonged dry season. Drastic climate change also contributes to increased risks of rainforests fire, which is marked by increase of temperature on earth surface in the last few decades. Heavy smoke and fog caused by rainforests fire consists of Carbon Dioxide (CO2), suspended particle, vapor, Carbon Monoxide (CO), Organic component such as formaldehyde, acrolein, nitrogen oxide (NOx), etc. It is possible for these compounds to spread miles from its source if carried and suspended in the air. Some compounds (CO, NOx, and other organic compounds) are most commonly spread towards location with high population such as the city, urban area near fire location. It is not common for those compounds to spread to far distanced...
within the duration of the forest fire smoke pollution. Each health problems being sought in this research alongside their prevalence is described in the following Table II. Every respondent could submit to more than one clinical symptoms from all of the options being presented in the survey as they see fit. Sore throat and red eye were the most common symptoms complained by the respondents.

Multivariate analysis was then performed using backward logistic regression analysis for all the dependent variables above. Some analysis were performed and each result is shown in Table III above. Results shown from each of the variables derive from the last step of the regression analysis, hence showing only the statistically significant value of probability among the independent variables.

4. DISCUSSION

At the time the survey was taken, respondents’ area of living was quite diverse—covering a vast area of Kalimantan and Sumatra, as well as some from Singapore—and each of them reported to experience air pollution from the forest fire. This brings a degree of accuracy as the sample comes from a multitude of different areas. Respondents were mostly female (66.3%), comes from Sumatra (65%) and Kalimantan (about 25%), and of Melayu/Sumatranese descent (58%).

In our study, we found headache happened in 55.8% of population. Ho et al. also reported that headache was complained by 50.3% of their respondents. Of the headache complaints given in the survey, logistic regression showed a statistically significant association between headache and a low duration of direct smoke exposure and smoking ($p = 0.004; OR = 141$). Hemodynamic changes caused by various constituents of the haze could be responsible for symptoms like headache and dizziness. Smoking, as widely known to have a wide array of negative health effects, did have a meaningful risk towards headache. The notion of smoke exposure seems to have negligible association with headache complaints. 69.8% of our participants also complained red eye. Kolbe et al. also found that 52.8% of their participants post extreme bushfire event also complained eye discomfort. We also found that more than 8 hours smoke exposure can increase the risk of red eye ($p = 0.024; OR = 44.4$). It can be caused by the longer time exposure of haze particle components can be more irritative to the eyes.

According to one epidemiological literature review, forest fires and wildland fires results in increased number of particular matters which corresponded to an increased use of medical services (emergency room visits, medical consultations, and hospitalizations). Of those who complained, respiratory problems were the most prevalent. Respiratory symptoms such as nasal and airway irritation, wheezing, tightness in the chest, and difficulty breathing.

Ho et al. studied about the effect of Indonesia’s forest fires haze exposure in June 2013. They reported that the most common physical symptoms were the respiratory symptoms such as throat discomfort (68.8%), nose discomfort (64.1%), and breathing difficulty (40.3%). Aditama also stated that 81% of people who had no prior history of respiratory problem, had cough and 24% had dyspnea as the effect of forest fire smoke in Indonesia. Similar with their result, sore throat, cough, runny nose, difficulty in breathing are also the common symptoms that complained by our respondents. We found that sore throat, cough, runny nose, and difficulty in breathing were significantly associated with duration of smoke exposure per day. These are seen in the cough group ($p = 0.031; OR = 19$), runny nose ($p = 0.002; OR = 65$), sore throat ($p = 0.064; OR = 7.9$), and difficulty breathing ($p = 0.026; OR = 12$). The strongest association is seen in the runny nose/rhinorrhea, cough, and difficulty breathing group, whereas the ‘sore throat’ group holds the weakest association. Emmanuel reported that concentration of pollutant was associated with increases of 12% of upper respiratory tract illness and 26% of rhinitis as the impact of Indonesia’s forest fires in Singapore. Fire forest smoke, which contains high concentration of carbon dioxide, carbon mono-oxide, and other gases which are harmful component of air pollution, was inhaled then induced the inflammation in the respiratory track although the manifestation of symptoms could be different from person to person.

Moreover, the respiratory symptoms were also of significant result that the usage of simple protective equipment against smoke exposure pose a higher risk of health problems. The usage of simple handkerchief or tissue showed a statistically significant association between smoke exposure and respiratory symptoms, primarily cough, and sore throat. In the ‘cough’ group, the usage of even simpler, less-protective equipment i.e., tissue/handkerchief showed a statistically significant association between smoke exposure and respiratory symptoms, primarily cough, and sore throat. In the ‘cough’ group, the usage of even simpler, less-protective equipment i.e., tissue/handkerchief showed a higher risk ($p = 0.048; OR = 18.8$) than with simple disposable ordinary mask ($p = 0.03; OR = 6.3$).

The usage of breathing protective equipment such as masks has been studied in its effect to smoke exposure. In a study of bushfire smoke exposure, results found that differing characteristics of face masks affect the health problems smoke poses. In the randomized and blind study, the group with the better filtering capability mask showed a significant decrease in respiratory complaints such as wheezing, cough, and dyspnea. Filter capabilities were separated as P (particulate), POV (particulate/organic vapor), and POVF (particulate/organic vapor/formaldehyde) with increasing filtering capability respectively. This seems to suggest that the filter capabilities of masks influence its protective
Combination of Varicose Vein Ligation and Non-Selective β-Blockers as Secondary Prophylaxis for Recurrent Bleeding of Esophageal Varices in Liver Cirrhosis Patient: An Evidence-Based Case Report

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A patient 38 years old diagnosed with hematemesis melena ec recurrent bleeding from esophageal varices and liver cirrhosis. The purpose of this study was to determine whether administration of non-selective β-blockers combine with endoscopy vein ligation will reduce the incidence of recurrent varicose bleeding in liver cirrhosis patient. Literature searching was performed on PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), ProQuest, and EBSCOhost database. Two meta-analysis were obtained. The first meta-analysis study (Funakoshi et al.) shows no significant reduction in the incidence of recurrent esophageal bleeding after administration of the combination therapy (OR = 3.16; 95% CI = 1.76 to 5.34; P < 0.0001). From the second meta-analysis (Thiele et al.) reported a decreased risk of recurrent bleeding (RR = 0.65; 95% CI = 0.45 to 0.93; P = 0.02). As a conclusion, the combination of a non-selective β-blockers and vein ligation reduce the incidence risk of recurrent esophageal bleeding.

Keywords: Non-Selective β-Blockers, Secondary Prophylaxis, Esophageal Varices, Cirrhosis.

1. CASE ILLUSTRATION

A male, 38 years old, came to hospital with chief complaints of vomiting blood since 6 hours before admission. He vomited four times with a total volume of 600 ml. He also complained black stool. Patients have experienced this complaint since 3 years ago, when he was diagnosed with hepatitis C cirrhosis, and had been undergoing two times variccal ligation procedure. We performed a physical examination on the 7th day of treatment. Vital signs, including blood pressure, temperature, heartbeat, and respiratory rate, were normal. The conjunctiva were pallor in both eyes, palpable liver was negative, spleen was palpable in schuffner 2 point, jaundice was negative.

Lab examination showed anemia with hemoglobin 6.1 g/dL (normal 14–16 g/dL), low MCV (74.7 fL), low MCH (24.3 pg), increased AST (53 u/L), increased ALT (70 u/L), hypoalbuminemia (3.8 g/dL). The PT was prolonged to 15.0 (control 10.0), thrombocytopenia (48,000/uL), leukopenia (3.890/uL), and reactive anti-HCV. USG examination showed liver cirrhosis, cholecystitis, and ascites. From endoscopy procedure, it showed oesophageal varices grade 3, moderate portal hypertension gastrotyp.

Therefore, the patient was diagnosed with hematemesis melena ec esophageal variceal rupture, liver cirrhosis Child-Pugh A ec hepatitis C, DIC with splenomegaly, and anemia ec gastrointestinal bleeding.

2. INTRODUCTION

Esophageal varices bleeding is a fatal complication of liver cirrhosis.1 50% of cirrhotic patients will develop esophageal varices, 5–33% others develop gastric varices. When diagnosed, it is estimated that 30% of cirrhosis patients have esophageal varices. Bleeding from esophageal varices has a mortality of 20% within 6 weeks, even though the bleeding will spontaneously stop in 40% of patients.2 From the severity of cases, 40% of patients with Child-Pugh A have varices and 85% of patients with Child-Pugh B have varices.2

Liver cirrhosis is the end stage of progressive liver fibrosis which damage the architecture structure of liver and develop...
thrombocytopenia. The structural damage will increase the vascular resistance and blood flow and cause portal hypertension (normal pressure: 3–5 mmHg). The high pressure of portal vein will be compensated by collateral veins. If the pressure between the portal vein and systemic veins, it will develop esophageal varices. This varices can rupture, if the pressure is too high (>12 mmHg), and cause upper gastrointestinal tract bleeding. Patients with portal vein pressure below 12 mmHg has lowered the possibility of recurrent variceal bleeding.2

30% of patients with esophageal varies will rupture and bleed within 1 year after diagnosis. 60% of patients who spontaneously recover and left untreated, will become recurrent bleeding within 1–2 year.2 Patients with portal vein pressure >20 mmHg with variceal bleeding, is at high risk to experience recurrent bleeding within 24 hours.3 Risk factors for esophageal bleeding are INR score >1.5, portal vein diameter >13 mm, and thrombocytopenia.2

Principle treatment of variceal bleeding is to stop the bleeding. Treatment of choice that can be administered is splanchnic vaso-conststrictors (vasopressin, somatostatin, non-selective β-blockers, venodilators (nitrates), sclerotherapy/ligation of vein, and transjugular intraportal portosystemic shunt. In clinical practice, the use of non-selective β-blockers are not applied for bleeding in primary variceal cases. But in Child Pugh B/C cirrhosis patients, it is recommended to use non-selective β-blockers.1,1,3

The aim of this evidence-based research was to investigate the role of non-selective β-blockers in combination therapy with variceal ligation to prevent varicose recurrent bleeding.

3. METHODS

Searching strategy: we conducted a searching on Cochrane CENTRAL, ProQuest, EBSCO, and PubMed database on December 2nd, 2015. Keywords were “(β-blocker OR beta-blocker OR beta blocker OR beta-blocker) AND (banding ligation OR variceal band ligation OR VBL OR endoscopic variceal ligation OR EVL) AND (variceal rebreeding OR rebleeding)” Two articles were found and matched the searching criteria.

4. RESULTS

The first meta-analysis, found that there were three studies with total sample 256 patients, 253 of them were analyzed. 49 of 125 patients (39.5) who treated with monotherapy ligation had recurrent bleeding, while 22 of 126 patients (17.2%) who treated with combination therapy had recurrent bleeding. There was a significant decrease of recurrent bleeding between those two groups (OR = 3.16; 95% CI = 1.76–5.34; P < 0.0001). There was no heterogeneity between studies (I² = 0%; P = 0.85), no publication bias (Egger test, P = 0.46). For mortality rate, there was no significant correlation between monotherapy group and combination therapy group (OR = 1.78; I² = 0%; P = 0.09).27 of 125 (21.8%) patients who treated with monotherapy died, while 17 of 128 (13.3%) who treated with combination therapy died. No heterogeneity between studies (P = 0.09).3

The second meta-analysis, with a total of 724 patients, showed combination therapy statistically significant in decreasing the risk of recurrent bleeding than monotherapy (RR = 0.68; 95% CI = 0.54–0.85; I² = 0%). No heterogeneity between studies. Combination therapy was better than monotherapy of ligation (RR = 0.59; 95% CI = 0.41–0.85; P = 0.02) or medication monotherapy (RR = 0.76; 95% CI = 0.56–1.04; P = 0.003).27

For mortality rate, from 6 studies included in Thiele et al. meta-analysis with a total 879 patients, 59 of 405 patients died in group treated with combination therapy while 81 of 473 patient died in control group (RR = 0.90; 95% CI = 0.66–1.23). There was no heterogeneity (I² = 0%) and bias (Egger test, P = 0.588). This result was confirmed with fixed-effect model.4

5. DISCUSSION

Results from these two meta-analyses show that there are association between combination of endoscopic treatment with β-blockers as secondary prophylaxis for re-bleeding esophageal varices than endoscopic banding ligation alone or β-blockers medication alone. It is shown also that there is decrease in mortality rates for combination procedure than esophageal ligation or β-blockers alone. Banding ligation act locally on the esophageal bleeding, has fewer complication and lower mortality rates. It has synergistic action with β-blockers where β-blockers act by reducing the portal hypertension. However, the reduced risk of re-bleeding was not associated with mortality.

There are three meta-analyses have been previously published about this combination therapy. Meta-analyses of Gonzalez et al.5 and Ravipati et al.6 compared combination treatment with banding ligation alone. They found significant difference in re-bleeding rates but non-significant result for mortality. Cheung et al.7 also performed small meta-analysis including four trials comparing banding ligation alone with combination of banding and β-blockers, but did not find significant difference for either bleeding rates and mortality. Meta-analysis of Gonzalez et al.5 included 23 other trials. In these three meta-analyses included studies of β-Blockers with nitrates, but results are not shown in this study because we didn’t use nitrates for variceal rebleeding in this study.

Treatment adverse event and reaction is seriously concerned in this combination therapy. One observational study suggests that β-blockers have effects in refractory ascites.8 The proportion of patients with refractory ascites unfortunately was not reported in our two meta-analyses.

Overall, our findings support that combination therapy is superior. However, the frequency of adverse events still need to be assessed. Some questions were raised include the benefit of...
The Effect of Resistant Starch from Banana (musca spp) on Fatigue Response and Running Time During 10,000 m Endurance Running

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Introduction: Unripe Banana (musca spp) high carbohydrate fruit with high resistant starch influences banana glycemic index and glycemic response. Stable blood glucose level in running has a role in fatigue response and running time. Methods: This study conducted to determine effect of resistant starch in banana with different ripeness level and their influence to fatigue response measured by lactic acid levels and visual analog scale (VAS) scale also running time in 10,000 m endurance running. This is an advanced experimental study with crossover design, single blind and strata allocation, in 12 healthy male recreational runners aged 20–22 years old from previous study. Results: The results showed lower lactic acid level from three measurement (3.61 ± 0.44 vs. 5.20 ± 0.56; p < 0.001), VAS score (60.1 ± 0.38 vs. 66.2 ± 0.41; p < 0.001) and better running time (68.36 ± 0.85 vs. 65.28 ± 0.96; p < 0.001) between raw bananas compared to ripe bananas consumption. Conclusion: This study indicates the role from consumption raw banana before running as nutritional food that can affected to running performance and recovery.

Keywords: Raw Bananas, Ripe Bananas, Resistant Starch, Fatigue Response, Running Time, Endurance Running

1. INTRODUCTION

Fatigue is a physiological condition encountered during and after exercise. Some of fatigue theories related to glycogen depletion due to high energy expenditure in exercise such as endurance running.1 The most common method to maintain glycogen stores during endurance running is nutritional intervention.2 Certain type of carbohydrate and specific time of consumption were carefully arranged for endurance running. Consuming low glycemic index (GI) food before exercise provide slow absorption and influenced steady glucose release into blood circulation which affected blood glucose level and insulin activity, which allow glucagon metabolism to use fat as an energy source and preserved muscle glycogen availability during endurance exercise.3,4

Banana is one of carbohydrate foodstuff with resistant starch content which is usually consumed before sport and exercise as a source of nutrient. In our earlier study we found that there is a different composition of banana resistant starch in raw and ripe banana. We also found the higher fat content in raw banana rather than ripe banana. The high content of starch includes resistant starch affected to banana GI, and its related to blood glucose after raw and ripe banana consumption.3,5

Endurance run is one part of athletics sport, by definition running over certain distance and perform outside or outside athletic track.6 In endurance run the body need high energy requirement and obtainable from aerobic and anaerobic energy metabolism.8,10 There are three energy systems work simultaneously during endurance run, especially endurance, creatine phosphate, aerobic, anaerobic glycolysis and oxidative phosphorylation. Oxygen availability is a crucial factor that affects the aerobic energy system. Low intensity activities while resting affect the energy sources used. Before running or at rest, energy is obtained entirely from the oxidative phosphorylation system and most of the energy used derived from fat by 80–90%, while carbohydrate contributes 5–18% and protein 2–5% from total energy requirement.4,8–10 The energy sources play important role at early run which requires substantial energy in a short time. Blood glucose concentrations while resting also affected the glycemic responses after food ingestion influenced by IG food 11,12

At the beginning of run, the energy needs increased rapidly, the body compensated by increasing ATP production rapidly.
especially at the time of mid running and after finished running. Three subjects complained about breathing difficulty in last lap of the run (25%) and two subjects complained about stomach pain (16.67%). In raw banana group nine subjects complained about the taste (75%), seven subjects complained about bloating (58.3%) at the beginning until mid running. During both trial all subjects complained about pain in the muscles during and after running, but the intensity were lighter in raw banana trial.

3. RESULT

4. DISCUSSION

In subjects in this study corresponding with the phase 1 study, all subject serves as a control for himself. The subjects criteria, BMI and body composition have been described in phase one report earlier. In this study the running pace among subjects is same due to their running activity in group. To avoided fatigue due to dehydration from running, the subjects provided 150 ml mineral water every 15 minutes running which given in the same running duration. Fatigue response assessed by blood lactic acid showed no significant difference before raw and ripe banana consumption. The results showed that all the subjects have same activity which not affected the fatigue level. The lactic acid level show significant difference when blood sample taken shortly after the subjects finished running and 30 minutes resting after running.

The objective result for fatigue responses confirmed with subjective assessment using VAS. There was a significant different in vas score which is taken shortly after finished running. These results related to banana resistant starch which influenced the banana glycemic index and the effect on blood glucose level. Consumption of raw banana which has high RS affected with sufficient, lower and stable blood glucose level leads to lower insulin secretion and therefore not affected to glucagon secretion, which is allowed fat utilization as energy source for running. This condition can reduce glycojen store will delay glycojen depletion which is one of mechanism responsible for fatigue. This finding doesn’t correspond with studies from Wong et al. and Wu et al., which has shown no differences in lactate values in the low GI compared to high GI. This different results due to differences in the time of blood sampling. Wong et al. and Wu et al. conducted lactic acid sampling during running, while in this study the sample was collected immediately after running and 30 minutes after running. In other study, the researcher used a variable food with lower GI, but not to mention the glycemic load after consumption those variable low GI food. This condition can attribute to blood glucose level which can affected to fatigue responses. In this study we only uses single foodstuff with a different GI due to the ripening process, raw banana for low GI food and ripe banana for high GI food.

Running time was recorded for 10,000 m running and showed better running time of nearly 3 minute different which account into one lap faster for runners consuming raw bananas. Average running time required by the subject to complete the 10,000 m was considered as an average performance which were far below the professional athlete ability which is 6.5 m/sec or < 30 minutes to run 10,000 m. This finding is consistent with study from Wong et al., which has shown better performance in subjects given low GI food compared to subjects who received high GI food. In this study, raw banana containing higher RS2 contributes to lower GI which has been as shown in Wolever et al., and Lintas et al. studies and also confirmed in phase 1 of this study.

However 75% of the subjects complained about raw banana taste due to high starch and low glucose content which indicate poor reception taste by the subject. As mention in phase 1 study, the starch contents in ripe bananas have undergone a gelatinization process which has transformed the banana starch into glucose therefore provide sweeter taste than raw banana. All subject were well-received to consumed banana due to cultural habituation to consume banana once a day as a regular fruit consumption.

5. CONCLUSION

This study showed that food with low GI may have a role in endurance run. The different GI of banana influenced by their ripening process may have affect to running performance. Maintenance of blood glucose level may have a role in energy metabolism in endurance running which is affect to the energy source and system which has a role to reduced fatigue responses and therefore improve running performance. Therefore consuming raw banana as pre-exercise nutrition should be considered and recommended for recreational and endurance runners who are willing to accepts unusual banana taste.

References and Notes

Analysis of Body Mass Index, Waist Circumference, and Body Fat in Predicting Insulin Resistance of Polycystic Ovary Syndrome

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Introduction: Polycystic Ovary Syndrome (PCOS) can be found in 5–10% women in reproductive age. Insulin resistance plays role in 50–80% PCOS patients. Many methods of measurement has been established to determine insulin resistance. However, all method of measurements are invasive, expensive, and inaccessible in remote area. Objectives: To assess whether Body Mass Index (BMI), waist circumference and body fat can be used as predicting variables of insulin resistance in PCOS patients and to assess variables’ cut off point. Method: This was a cross-sectional study of BMI, waist circumference, and body fat to assess insulin resistance in PCOS patients. A diagnostic study with observations that can be a predictor of insulin resistance in PCOS patients. Samples of this study were 61 women with PCOS. Results: There were 47 subjects (77%) with insulin resistance and 14 subjects (23%) who were not insulin resistance. All of three variables were statistically significant, there were BMI (p = 0.001, cut-off point value = 24.75 kg/m², sensitivity = 72.9%, and specificity = 71.4%), waist circumference (p = 0.004, cut-off point value = 86.5 cm, sensitivity = 68.1%, and specificity = 71.4%), and body fat (p = 0.005, cut-off point value = 36.15%, sensitivity = 68.1%, and specificity = 64.3%). Conclusion: BMI, waist circumference and body fat can be used as predictors of insulin resistance in PCOS women.

Keywords: Body Mass Index, Waist Circumference, Body Fat, Insulin Resistance, PCOS.

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) can be found in 5–10% women in reproductive age. The diagnosis of PCOS is confirmed according to the revised Rotterdam 2003 criteria, in which the presence of any two out of the three following criteria were required: oligo-anovulation or chronic anovulation, hyper androgenism as defined by clinical symptoms or biochemistry sign and polycystic ovaries on ultrasonography.1 Consequences of PCOS do not only affect reproductive problems, women with PCOS also have risk of metabolic and cardiovascular disorders. Both the metabolic syndrome and PCOS related to insulin resistance as one of the important aspects of the pathogenesis.2 Insulin resistance can be found in 50–80% of women with PCOS.1 In obese patients with PCOS, insulin resistance can be found in 70–80% of patients and it can be found in 20–25% of lean patients with PCOS.1

Many methods of measurement that can be used to determine insulin resistance. Euglycaemic Hyperinsulinemic Clamp (EHIC) technique is the gold standard in assessing insulin sensitivity and is often combined with the Hyperglycaemic Clamp (HGC) to determine adequacy of compensation due to β cell hypersecretion. But this procedure is expensive, invasive and requires a long time.3 5 Other measurement, such as the Homeostasis Model Assessment (HOMA), can be an alternative to predict insulin resistance by calculating insulin and fasting glucose levels. Compared with EHIC, HOMA is widely used in research because it is simple, fast and relatively inexpensive, and has been validated against the gold standard.6 However, this measurement requires a blood sample, thus easier method would be helpful for daily practice.6 7 If we can predict insulin resistance in women with PCOS with an easier way, minimally invasive and cheaper, we can do the therapy quickly and accurately. Correlation between anthropometric indicators and insulin resistance has been widely studied and used in epidemiological studies and clinical practice.7 Through the examination of anthropometrics data and body fat also easier for practitioners to assess the progress of treatment and prevent complications.

2. METHODS

The study was conducted after approval by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia and to obtain samples of research based on inclusion and exclusion criteria from March 2014 until June 2016 in Yasmin Kencana Clinic.

*Author to whom correspondence should be addressed.

Keywords: Body Mass Index, Waist Circumference, Body Fat, Insulin Resistance, PCOS.
4. DISCUSSION

After the text edit has been completed, the paper is ready for Sixty-one subjects were enrolled in this study after screening based on inclusion and exclusion criteria. Subjects were taken consecutively from the Yasmin Kencana Clinic which diagnosed with polycystic ovarian syndrome. Data subjects were taken include BMI, waist circumference, body fat, and HOMA-IR. In this study, the incidence of resistance insulin was 77% of 61 subjects. This number was comparable to the results of research conducted by Nandi et al., that found the incidence of insulin resistance in PCOS patients was 50–80%.

In general, PCOS can be found in women of reproductive age, but PCOS itself is genetically related disorder that can be found in all women of various ages. Subjects in this study had age range of 20–35 years. While Michelmore et al. in UK got the prevalence of PCOS in age range of 18–25 years. Knochenhauer et al. research in US stated that PCOS in whites and black women were (29.4±7.1 and 31.1±7.8, respectively) and obtained mean age of PCOS women was (24.6±1.8) on research in Greece by Diamanti-Kandarakis et al. While the research conducted by Sunapraja et al. found the highest frequency in the age range of 26–30 years (45.7%).

In this study, mean of BMI was 27.21±5.41, which subjects with insulin resistance was 28.49±5.04 and the mean of BMI in subjects without insulin resistance was 23.07±4.64. The test results analysis by unpaired t test obtained by p value = 0.001, it implies significant relationship between BMI with HOMA-IR. Similar results stated by Wiweko et al. research which found 75% of patients with PCOS insulin resistance with mean of BMI 28.6 to the subjects with BMI ≥25 was 84% and subject BMI <25 was 50%. This also consistent with research by Skalba et al., which concluded that mean of HOMA-IR were higher in PCOS patients with excess BMI compared with normal weight (2.5±1.6 vs. 1.8±0.9; p < 0.01).

Approximately 30–70% of women with PCOS worldwide are overweight or obese, so the first treatment is weight loss with target BMI ≥25. Therefore, PCOS known to be associated with metabolic changes that may be exacerbated by the presence of obesity. Obesity can lead to polycystic ovary syndrome. This happens due to obesity can lead to insulin resistance through increased production of free fatty acids. The result is a decrease in insulin sensitivity resulting in hyperinsulinemia. High insulin levels are associated with insulin resistance may stimulate the ovaries to produce high levels of androgen excess. Obesity also increases blood cholesterol levels and stimulate the steroid pathway that will change cholesterol into androstenedione. After that, androstenedione will be converted to estrogen. High levels of androgens can result in disruption of androstenedione conversion process. As a result of androgen levels become higher and lead to polycystic ovary syndrome.

Mean of waist circumference was 90.08 ± 11.75, with mean of waist circumference in subjects with insulin resistance was 92.62 ± 10.60 and mean of waist circumference in subjects without insulin resistance was 81.57 ± 11.76. Test results analysis by unpaired t test obtained by p value = 0.001, it showed statistically significant association between waist circumference and HOMA-IR. This was similar with Jensterle et al. research in 50 women with PCOS that there was positive correlation between waist circumference and HOMA-IR. This was consistent with Luhas et al. study on the relationship between visceral fat, body fat metabolic parameters: fasting plasma glucose, fasting insulin, HOMA-IR, total cholesterol, HDL, LDL and triglycerides. There was a positive correlation between body fat with HOMA-IR (p < 0.05).

From this study, the cut-off point value for each variables are BMI (24.75 kg/m²), waist circumference (86.5 cm), and body fat (36.15%).

5. CONCLUSION

Based on bivariate analysis, all of three variables obtained statistical significance with p < 0.05, there are IMT (p = 0.001), waist circumference (p = 0.001), and body fat (p = 0.004). Based on the ROC curve, AUC value of IMT was 0.787, waist circumference was 0.753, and body fat was 0.74. AUC values indicate that BMI, waist circumference, and body fat can be a marker capable of distinguishing the group of insulin resistance and insulin resistance in PCOS with sensitivity were 72.3%, 68.1% and 68.1% respectively and specificity were 71.4%, 71.4% and 64.3% respectively. The cut-off point value for each variables are BMI (24.75 kg/m²), waist circumference (86.5 cm), and body fat (36.15%). Those variables can be used as a predictor of insulin resistance in PCOS women.

References and Notes

Effects of C. ramiflora Linn. Leaf Extract Against Dengue Virus Replication In Vitro on Huh7it-1 Cell

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Introduction: Dengue virus (DENV) causes an acute infection that may lead to death. Due to the morbidity and mortality produced, dengue infection is still a serious health problem especially in tropical countries. To this time, there is still no specific antiviral to overcome DENV infection. Therefore, a research to find a specific antiviral for DENV is necessary. This research is aimed to evaluate the effect of Cynometra ramiflora Linn. leaf extract in vitro on Huh7it-1 cell as a specific antiviral for DENV infection. Methods: An experimental research was conducted in August 2014–June 2015. The leaf extract of C. ramiflora Linn. was diluted to concentration 40 μg/ml, 20 μg/ml, 10 μg/ml, 5 μg/ml, 2.5 μg/ml, and 1.25 μg/ml. Next, DENV was exposed to each concentration. The inhibition of DENV replication (IC50) was observed using Focus Assay, while the toxicity of the extract to Huh7it-1 (CC50) was evaluated using MTT Assay. Data were analyzed using T-test and One Way ANOVA. Results: From the experiment, the value of CC50 and IC50 are 125 μg/ml and 20.1 μg/ml, respectively. Conclusion: From the research, it is concluded that C. ramiflora Linn. extract is not toxic and has a potency as antiviral for DENV.

Keywords: Cynometra Ramiflora Linn, DENV, CC50, IC50.

1. INTRODUCTION

Dengue is an acute infection caused by dengue virus (DENV). The infection is transmitted by Aedes aegypti or Aedes albopictus mosquito. DENV is an RNA virus origins from Flaviviridae family and is considered as the most important arboviral in human population based on the geographic distribution, morbidity, and mortality caused. There are various clinical manifestations resulting from the presence of DENV in human body, ranging from dengue fever (DF), dengue hemorrhagic fever (DHF), to dengue shock syndrome (DSS), which could result in death.1

DENV are distributed mainly in tropical and subtropical area such as Asian and African counties. The infection needs special concern since the people at risk reached 2.5 billion people in all countries. Globally, there are 50 million new cases of dengue every year, which 500,000 of the cases develop to DHF/DSS. The total mortality rate of dengue reaches 20,000 annually.2 In Indonesia, the cumulative incidence of dengue in June 2010–July 2011 reached 35,9/1000 people.3 The rate of dengue infection is predicted to increase every year along with the increase of travels, global climate, and population density.1, 2

DENV infection is also related to productivity and economic loss. In America, the annual economic loss related to DENV infection is estimated to reach 2.1 billion US$, while in South East Asia the number reaches 950 million US$. The economic loss is due to the lack of productivity while infected by DENV.3

Despite the serious problem caused, the management of DENV infection is still limited to the control of the vector breeding, supportive therapy, and recently vaccination. Regarding the high morbidity and mortality rate, there is an urgency to develop a specific therapy to treat DENV infection.4

One of the alternatives of DENV infection management is by utilizing medicinal plants, especially in the high-risk countries. Previously, some plants have been found to have antiviral effect, such as Carica papaya (papaya), Psidium guajava (guava), and Rhizophora apiculata (mangrove).5 The potency of other plants to have an antiviral effect is considered big due to the flora diversity in Asia and Africa. One of the potential medicinal plants to have antiviral effect is Cynometra ramiflora Linn., which is classified as one of the mangrove plants.5 The plant can be found in India, South East Asia countries (Indonesia, Malaysia, Thailand, and Philippines), to Australia.6

From the previous studies, C. ramiflora Linn. are found to have antibacterial, antidiabetic, and anticancer effect.7, 5 To this time, there has not been any research to evaluate the antiviral effect of C. ramiflora Linn. This research is intended to evaluate the antiviral effect of C. ramiflora Linn as well as its toxicity to the cell.
Fig. 1. Mean of cell infectivity in Huh7it-1 cells.

Table II

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<th>Extract concentration (µg/ml)</th>
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exposure. Result shows that in concentration of 12.5 µg/ml, the cell viability reached >90%. Statistical analysis using Kruskal-Wallis method and Post-Hoc test showed that in concentration of 100 µg/ml, 50 µg/ml, 25 µg/ml, dan 12.5 µg/ml significantly affected the cell viability. Figure 2 shows the linear regression analysis comparing the various extract concentration with cell viability, which the value of y is equal to 50. From the analysis, the value of CC50 is 125 µg/ml.

3.3. Selectivity Index

By comparing CC50 in uninfected cells and IC50 in infected cells, it is obtained that the selectivity index of C. ramiflora Linn. is 6.2. This showed that the extract has an antiviral potency.

4. DISCUSSION

Studies about the benefits of C. ramiflora Linn. are still very few. Until now, some studies of C. ramiflora Linn. are still limited to cytotoxicity effect (for anticancer alternative), antibiotic, antinociceptive, and antidiabetic effect. The research to study antiviral effect of C. ramiflora Linn. was still absent, so the extract concentration determination in this experiment did not refer to any previous studies.

From this research, it was obtained that the value of CC50 of C. ramiflora Linn. leaf extract against Huh7it-1 cells is 125 µg/ml. This is lower compared to other plants with anti-DENV effect. For example, Canistrocarpus cervicornis and Caulerpa racemosa CC50 value are consecutively 86.56 µg/ml and 121.8 µg/ml.

Some researches have indeed showed that C. ramiflora Linn. is cytotoxic. Haryoto et al, proved that the cytotoxic effect of the leaf extract is more abundant compared to the stem extract. In some cancer cells, the leaf extract was shown to have a very high potency for anticancer, such as in breast cancer cell (CC50 = 6.37 µg/ml), colon cancer cells CC50 = 0.41 µg/ml, and cervical cancer cells (CC50 = 1.92 µg/ml).

Same anticancer potency was shown in the stem extract of C. ramiflora Linn. Uddin et al used the methanol extract from the stem and showed that the CC50 value on colorectal cancer is 1.79 µg/ml, while in the duodenal carcinoma of the breast the CC50 value is 2.35 µg/ml.

This research showed that the selectivity index of C. ramiflora Linn. as anti-DENV candidate is 6.2, which is considered as low potential. Though in our experiment the CC50 value does not show high toxicity to the cells, we need higher concentration of the extract to inhibit the replication of 50% of the virus. Other plants with anti-DENV effect showed to have much lower IC50 compared to C. ramiflora Linn. leaf extract, such as Gymnogongrus griffithiae (IC50 = 0.9 µg/ml), Gymnogongrus torulosus (IC50 = 0.19–1.7 µg/ml), and Meristiella gelidum (IC50 = 0.14–1.6 µg/ml).

The mechanism of antiviral effect in this research still can not be explained since we did not test the compounds. Previous research by Afjalsus et al. mentioned that the plant contained flavonoid and saponin. Another research by Lee et al proved that saponin was effective in inhibiting the replication of Hepatitis C virus. Orham et al also mentioned the benefit of flavonoid in inhibiting the replication of ParaInfluenza-3 virus and herpes simplex virus (HSV-1). Other plants in the Leguminoseae group were found to have antiviral effect, such as Pseudopiptadenia concorta (on HSV-1) and Cassia odentalis (on HIV-1).

5. CONCLUSION

The C. ramiflora Linn. leaf extract has a potency as DENV antiviral and could be a candidate for further DENV antiviral development. Further researches are needed to discover the mechanism of the antiviral effect of the extract.

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References and Notes

Poor Oocytes Increase the Risk of Aneuploidy Embryos

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Introduction: Aneuploidy (trisomy or monosomy) is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies and the main genetic cause of miscarriages and congenital birth defects following both natural conception and in vitro fertilization (IVF). There is increasing evidence that the oocyte is the major contributor to embryo aneuploidy, especially in women with advanced reproductive age. The aim of the study is to evaluate the aneuploidy rate in embryos obtained from normal morphological oocytes and dysmorphism oocytes using Preimplantation Genetic Screening (PGS).

Methods: A prospective analysis was performed on 20 oocytes and 20 embryos who had undergone preimplantation genetic screening at Yasmin IVF Clinic. Oocyte and embryo quality were assessed based on morphological features. One blastomere from each embryo was analyzed for abnormalities by using array-Comparative Genomic Hybridization (a-CGH).

Results: The highest incidence of aneuploidy embryo was observed in poor oocyte quality (100%) followed by moderate oocyte (80%) and good oocyte quality (71.4%) while chaotic pattern oocyte obviously aneuploidy (100%). Conclusion: Women with poor oocyte quality are at risk for developing aneuploidy embryos compared with moderate and good oocyte quality. Therefore, genetic counseling is strongly recommended for woman with poor oocyte quality.

Keywords: Aneuploidy, Oocyte Dismorphism, Oocyte Quality, Preimplantation Genetic Screening.

1. INTRODUCTION
Aneuploidy (trisomy or monosomy) is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies and the main genetic cause of miscarriages and congenital birth defects following both natural conception and in vitro fertilization (IVF).1,2 One of the causes of embryonic aneuploidy is the aneuploidy in oocytes.3 The relationship between oocyte quality, embryo development, and in vitro fertilization (IVF) outcomes has been widely studied.3 Oocyte quality has been regarded as a variable that may influence the quality of embryo.5,6 Preimplantation Genetic Screening (PGS) can assess an embryo’s karyotype and has been used in screening chromosomal aneuploidy before transfer.5,10 There is increasing evidence that the oocyte is the major contributor to embryo aneuploidy, especially in women with advanced reproductive age.3,11,12 The aim of the study is to evaluate the aneuploidy rate in embryos obtained from normal morphological oocytes and dysmorphism oocytes using Preimplantation Genetic Screening (PGS).

2. METHODS
A prospective analysis was performed on 20 oocytes and 20 embryos who had undergone preimplantation genetic screening at Yasmin IVF Clinic. Oocyte and embryo quality were assessed based on morphological features. The oocyte quality was divided into several groups (good, moderate, poor oocyte quality and chaotic pattern) and embryo quality was divided into good and poor embryo quality. Oocyte quality was observed based on total oocyte score (TOS). The oocytes were classified as normal (score 1) and abnormal (score 0) according to their morphologic appearance. Total oocyte score for good oocyte quality was ≥4, moderate was 3 and poor was ≤2 (modification of Lazzaroni-Tealdi et al.).4 One blastomere from each embryo was analyzed for abnormalities by using array-Comparative Genomic Hybridization (a-CGH).

2.1. Controlled Ovarian Hyperstimulation
Standard protocol for controlled ovarian stimulation was short protocol which achieved by using recombinant follicle-stimulating hormone (FSH) 225–300 IU as a daily dose, starting on day 2 of the cycle. We used a gonadotropin-releasing
Knowledge and Attitude of Indonesian Obstetrician and Gynecologists Regarding Fertility Preservation in Cancer Patients


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**Introduction**: Long-term effects of cancer and its treatments may cause loss of fertility. Fertility has received significant attention in improving the quality of life for cancer patients of childbearing age. Therefore, efforts were made to maintain the reproductive function in cancer patients through fertility preservation (FP). This study aimed to explore knowledge and attitudes among obstetrician and gynecologists in Indonesia regarding FP in cancer patients. Methods: Data was collected using questionnaires from 80 obstetrician and gynecologists in Indonesia. The questionnaire measures knowledge and attitude of FP. Results: Most of participants knew about FP (88.3%) but only felt knowledgeable about pre-treatment with GnRH Agonists, not other methods of FP. 95% expressed a need for more information. About 92.5% agreed that FP is high priority to discuss with newly diagnosed cancer patients and 45% agreed that treating the primary cancer was more important than FP. Most reported checking how important future fertility was for the cancer patients, 80% suggested FP to their patients and 35% reported having referred patients to a fertility specialist, but only 15.1% providing patients with written information. Most of them had never consulted any FP guidelines (26.3%), but consulted local hospital guidelines only (30%). The main barriers to initiating discussions about FP were poor success rates of fertility preservation options (97.5%), patients could not afford FP treatment (93.8%), poor patient prognosis (92.6%), lack of obstetrician and gynecologists’ knowledge (91.3%), and lack of fertility services in their area (81.3%). Conclusion: This study discover lack of knowledge about FP options in Indonesian obstetrician and gynecologists and highlights that the provision of information to patients about FP may be sub-optimal.

**Keywords**: Knowledge, Attitude, Fertility Preservation, Obstetric and Gynecologist.

1. INTRODUCTION

A total of 1,399,790 new cancer cases are expected in the United States in 2006, of which 679,540 will be women. According to previous reports, 8% of female cancer cases occur under the age of 40 years.1,2 Developments of chemotherapy and radiotherapy in past ten years caused a five-year survival rate of cancer patients can reach 90%. The incidence and 5-year survival rates, respectively of most common cancers diagnosed in women under the age of 40 are breast cancer (0.48%; 88%), melanoma (0.21%; 91%), leukemia (0.13%; 46%); cervical cancer (0.16%; 73%); non-Hodgkin’s lymphoma (0.09%; 59%); and endometrial cancer (0.06%; 84%).3

As detection and treatments improve, patients with cancer live longer, but their lives are impacted by long-term effects of the cancer itself and treatments received.4,5 Loss of fertility is one such long-term effect. Treatment may lead to the loss of reproductive organs, premature ovarian failure or an inability to produce mature eggs for ovulation.6,7 Infertility is associated with significant psychological distress with levels of depression twice that of the normal population, and quality of life is reduced in areas of emotional well-being, relationships and sexuality. When infertility is superimposed on cancer, there can be great stress on the patient, partner and family.8 Surveys conducted by ethics committee of the American Society for Reproductive Medicine (ASRM) proved that cancer patients want to be informed about fertility preservation options before treatment. In fact, women who received this information reported better quality of life and less distress than women who did not discuss reproductive issues with a health care professional. It should be the duty of all physicians who handles cases of cancer in women of reproductive age.9

The American Society of Clinical Oncology (ASCO) and ASRM have recommended that the impact of cancer treatments on fertility be addressed with all cancer patients of reproductive age, and that options for fertility preservation should be

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*Author to whom correspondence should be addressed.*
discussed routinely. Those FP options are ovarian tissue, oocyte, embryo, sperm, and testicular tissue cryopreservation, and also pre-treatment with GnRH agonist. These options are highly dependent on the type of cancer, age of the patient, the protocol and timing of chemotherapy and marital status. Despite these strong recommendations, surveys have shown that many patients with cancer do not recall having had a discussion related to fertility before treatment. There are various barriers to FP referrals such as the treating physician’s knowledge and attitudes if it is outside their expertise, perceptions of the patient’s access to resources, or if it involves delaying treatment. This study aimed to explore current knowledge and attitudes of FP in cancer patients among Indonesian obstetrician and gynecologists.

2. METHODS

2.1. Measure
A 27-items questionnaire was developed to measure knowledge, attitude and also barriers of Indonesian obstetrician and gynecologists regarding fertility preservation in cancer patients.

2.2. Knowledge of FP
Participants were asked whether they knew about the fertility preservation or not. They were also asked to indicate their knowledge of six different FP options (cryopreservation of ovarian tissue, oocytes, embryo, sperm or testicular tissue and pre-treatment with gonadotropin-releasing hormones) on a four-point Likert scale from ‘not at all knowledgeable’ to ‘very knowledgeable’. They were also asked if they felt they needed more information about FP.

2.3. Attitude of FP
Attitude were evaluated using six statements (e.g., ‘I check with the patient how important their future fertility is for them’). They were also asked to indicated their agreement with the statements on a four-point Likert-scale (never to always) and on a five-point Likert-scale items (strongly disagree to strongly agree) (e.g., ‘Treating the primary cancer is more important than FP’). Additional questions asked whether they consulted any local/national guidelines for guidance on fertility issues, whether they did the counseling and whether they suggested FP to their patients.

2.4. Barrier of FP
Fourteen questions ascertained which factors participants thought might have a role, both in terms of characteristics of their practice or FP (six items, e.g., ‘Lack of fertility services in the area’) and characteristics of the patient (eight items, e.g., ‘The patient is too ill to delay treatment to pursue FP’). They were asked to indicate whether any of the items influenced their decision to initiate a discussion about FP on a three-point Likert-scale (‘not at all’ to ‘to a large extent’). They were also asked to describes their professional links with the reproductive medicine unit that was closest to them.

2.5. Procedure
Participants were obstetrician and gynecologists in Indonesia. In total, 100 obstetrician and gynecologists were asked to fill out all items of questionnaire. 7 of them did not agree to participate. Ninety-three of them were deemed eligible. After the questionnaires were collected back, 13 of them were excluded because they did not complete all items of questionnaire. Finally, 80 obstetrician and gynecologists were included in this study as participants. (See Fig. 1).

2.6. Statistical Analysis
Primary analysis was descriptive. Frequencies and proportions were summarised for characteristics and each questionnaire items. In cases where distributions of answers was very unequal, some items were turned into category items, for example, the answer options ‘never’, ‘rarely’, ‘usually’ and ‘always’ were divided into two categories by merging ‘never’ and ‘rarely’ and ‘usually’ and ‘always’. The statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL).

3. RESULT

3.1. Participants
All participants were obstetrician and gynecologists. Most of them were <40 years old (61.3%). Seven (8.8%) of them were consultant of obstetrics and gynecology and only 2.5% of them were oncologists. Characteristics of the obstetrician and gynecologists who took part in the survey are detailed in Table I.

3.2. Knowledge of FP
There were 86.3% of participants who knew about FP. The only questionnaires were collected back, 13 of them were excluded because they did not complete all items of questionnaire. Finally, 80 obstetrician and gynecologists were included in this study as participants. (See Fig. 1).

![Table I. Characteristics of participants.](image-url)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N = 80</th>
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Study of Cellular Immune Response from Intraperitoneal Post-Administration of Enterotoxigenic Staphylococcus aureus in Balb/c Mice

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Introduction: Bacterial infections commonly will activate the cellular immune response of the infected hosts. Our laboratory observations suggested the hypothesis that staphylococcal enterotoxin B (SEB) may have a prognostic differences in the cellular immune response. Methods: Two groups of male Balb/c mice were used. Each group consisted of 6 mice aged 8 weeks, of which labelled as the “Control” group and the “Test” group. Infection of SEB were carried out intraperitoneally with the volume of 200 μL on Day-0 and a subsequent infection of SEB with the volume of 400 μL on Day-13. Blood samples were collected through the retro-orbital plexus on Day-1, Day-4, Day-7, Day-14, and Day-22. Leucocytes differential count were summed manually and compared using SPSS. Results: Leucocytes differentials were counted and the results between “Control” group and “Test” group were compared. Increased of band neutrophils and lymphocytes were markedly seen. Day-1, Day-4, Day-7, and Day-14 showed significant differences of total leucocytes count (P = 0.050), (P = 0.003), (P = 0.001), and (P = 0.020) respectively. Histopathological examination showed infiltration of leucocytes in kidney and liver, as well as erosion of the epithelial mucosal membrane of the organs. Conclusion: There are significant increased on the production and circulation of peripheral band neutrophils and lymphocytes following bacterial infection. There are also infiltration of leucocytes and erosion of the epithelial mucosal membranes of the organs.

Keywords: Staphylococcus aureus, Enterotoxin B, Intraperitoneal, Histopathology, Haematology.

1. INTRODUCTION

Staphylococcus aureus has been linked to many human diseases and is the most common causes of food borne infections in most of the world.}\(^1\)\(^2\)\(^5\)\(^6\) S. aureus is a facultative anaerobic Gram-positive coccus with an average diameter of 0.8 to 1 μm, that tend to be arranged in pairs, tetrads, or more often, grouped in irregular clusters or ‘bunches of grapes.’ Colonies are usually white with regular edges. They are non-motile, catalase- and coagulase-positive, oxidase-negative, nonsporulating and are usually white with regular edges. They are non-motile, catalase- and coagulase-positive, oxidase-negative, nonsporulating and are usually not capsulated or have a limited amount of capsule.\(^1\)\(^2\)\(^5\)\(^6\) The various isolates of S. aureus show different characteristic features in their susceptibility/resistance towards antibiotics. Some S. aureus strains are able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisoning.\(^1\)\(^2\)\(^5\) S. aureus is able to grow in a wide range of temperatures (7° to 48.5 °C with an optimum of 30 to 37 °C), pH (4.2 to 9.3, with an optimum of 7 to 7.5), and sodium chloride (NaCl) concentrations up to 15% NaCl.\(^1\)\(^2\)\(^5\)\(^6\) There are about 30 species of staphylococci and most are found in animals but few are pathogenic. They are considered opportunistic pathogens. Infections with staphylococci are often acute and pyogenic.\(^6\) In addition to causing food poisoning, SEs have a nefarious potential for biological warfare and bioterrorism.\(^1\)\(^2\)\(^5\)\(^6\)

In the 1960s, the USA had an offensive biological warfare program and SEB was one of the agents studied as a biological agent that could be used to incapacitate soldiers in the battlefield. This was an attractive agent because low quantities were required to affect the desired incapacitation when compared chemicals synthesized in the laboratory. The SEB comprises of a large group of proteins and are 23- to 29 kDa polypeptides in the bacterial superantigen (SA) protein family.\(^1\)\(^2\)\(^5\)\(^6\)
Safety Profile and Cost Analysis of Reused Disposable Laparoscopic Instruments in RSCM

Randy Fauzani and Agus Rizal Hamid

Introduction: Studies regarding laparoscopic procedure has been focused on its cost, durability, upkeep, and repairmen cost, which will be important in the future. Reusing instruments aims to reduce cost without affecting procedure quality, even though reusable instruments are commonly used, but no sufficient data are available regarding to the effects of using reusable instrument to service quality. Methods: The data used on this study were retrospectively collected between January to December 2015. Total sampling method was used to collect data to all patients undergone laparoscopic procedures using re-sterilized laparoscopic instruments at urology operating rooms Cipto Mangunkusumo General Hospital (RSCM). The re-sterilized laparoscopic instruments used were harmonic scalpels, retroperitoneal balloons and pumps, laparoscopic trocars number 5, 10, and 11. Sterilization procedures were performed using plasma method in Sterilization Center RSCM. Results: The study was carried out to 63 patients undergone laparoscopic procedures using re-sterilized laparoscopic instruments at urology operating rooms in RSCM between January to December 2015. The specimens were swabbed from laparoscopic trocars, retroperitoneal balloons, and harmonic scalpels. All of the results came negative. Infection rates in trocar laparoscopic wounds was 1.6% and the rate of patients who experienced an increase in temperature greater than 37.5°C (Fever) after the surgery was 3.2%. We obtained a difference in cost as much as Rp. 2,074,000 (9.7 times less) in the reuse of disposable trocar laparoscopic instruments. The reuse of re-sterilized harmonic handpiece will enable us to save Rp. 8,945,000 (3.5 times less) and last every each of retroperitoneal balloon that thus been sterilized saved us Rp 862,500 (3,5 times less). Conclusion: The reuse of disposable laparoscopic instruments was able to reduce the cost of laparoscopic surgery with low complication rate thus can be safely performed.

Keywords: Laparoscopy, Reusable, Re-Sterilized Laparoscopic Instrument, Service Quality.

1. INTRODUCTION

It needs a large amount of cost to perform a surgical procedure in an operating room. The high cost will continue to develop as highly technological surgical tools have their cost constantly increases. Because of these reasons, surgical procedure has been one of main concerns on cost analysis; and studies regarding laparoscopic procedure in particular has been focused on its cost, durability, up keeping, and repairmen cost, which will be important in the future.

The implementation of minimally invasive laparoscopic surgical procedures and its developing technological advancement has reduced the total cost needed to afford laparoscopic instruments, although it remains higher than the cost of open surgical procedure. According to many studies, high surgical procedure cost could be compensated by short hospitalization duration, fewer medical tools needed, shorter recovery time, and shorter time needed to be back to daily normal activity.

Hospital staffs are able to choose to use disposable or reusable laparoscopic procedure instruments. The disposable instruments offer good sterility without instrument re-sterilization cost, but it needs higher space for instrument storage, good trash regulation, and creates problem on handling higher quantities of instruments. Reusing instruments aims to reduce cost without affecting procedure quality. Even though reusable instruments are commonly used, but no sufficient data are available regarding to the effects of using reusable instrument to service quality. The disposable instrument utilizations could reduce operational cost, but its procedural output quality remains unknown.

This study aimed to analyze cost and safety profiles of using disposable laparoscopic instruments on infection numbers, morbidity, post-surgical pain, length of hospitalization, and cost efficiency to patients performed laparoscopic procedures.

2. MATERIALS AND METHODS

The data used on this study were retrospectively collected between January to December 2015. Total sampling method was used to collect data to all patients undergone laparoscopic procedures using re-sterilized laparoscopic instruments at urology operating rooms Cipto Mangunkusumo General Hospital.

Keywords: Safety Profile, Cost Analysis, Reused Disposable Laparoscopic Instruments, RSCM.
Validating Indonesian Prostate Cancer Risk Calculator (IPCRC) for Predicting Prostate Cancer Risk in Cipto Mangunkusumo Hospital Jakarta

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Introduction: Prostate cancer is a progressive disease resulting in morbidity and mortality. The aim of this study is to assess the accuracy of Indonesian Prostate Cancer Risk Calculator (IPCRC) in predicting prostate cancer risk. Methods: Data were obtained retrospectively from medical records of suspected prostate cancer patients. Digital rectal examination, Prostate Specific Antigen (PSA), and prostate volume were used as predictive parameters in IPCRC. Prostate biopsy was used as the diagnostic gold standard. The accuracy of IPCRC was validated using the ROC analysis. Results: Our study included 127 subjects. Median age of BPH and prostate cancer patients were 66 (52–85) and 69.5 (50–100). Median of PSA of BPH and prostate cancer patients were 10.2 (1.6–203.1) and 74.06 (6.94–1412) respectively. The prostate volume of BPH patients 47.9 (13.74–108) compared to prostate cancer patients 50.25 (19.2–107). The largest AUC of IPCRC is 0.907 (95% CI 0.84–0.97). We determined three cutoff points (15%, 20%, and 25%) with the sensitivity (65.4% to 88.5%) and specificity (81.8% to 89.9%). Conclusion: IPCRC is accurate for predicting prostate cancer in our population. Further validation is needed in other population.

Keywords: Prostate Cancer, BPH, Prostate Biopsy, IPCRC.

1. INTRODUCTION
Prostate cancer considered as second most frequently diagnosed cancer in males that lead to high mortality rate.1,2 Earlier diagnosis and prompt treatment among those who undergo screening resulted in lower mortality rate.3 In the Indonesia, prostate cancer was revealed on 40.1% of 1957 patients underwent prostate biopsies.4

Prostate cancer early detection are needed by general practitioner (GP) and urologist, because prostate cancer is the most common cancer in elderly males (> 70 years of age) in Europe.11 It is a major health concern, especially in developed countries with their greater proportion of elderly men in the general population. Overall, during the last decade, the 5-year relative survival percentages for PCa steadily increased from 73.4% in 1999–2001 to 83.4% in 2005–2007.12

There were several attempts to predict the prostate cancer using either clinical or biochemical parameters.5–9 Recently, there is no absolute PSA value confirming prostate cancer, as this risk lies on a continuum of PSA values. Randomized studies that evaluate the efficacy of prostate-specific antigen (PSA) test to screen prostate cancer discovered the over diagnosis among patients with nonaggressive forms of prostate cancer. There were 10%–30% false negative biopsy rate according to PSA level compounded by incorrect prostate sampling.10,11 However, declining frequency of palpable prostate lesions lead the difficult decision to perform biopsy.12 Morbidity from PCa therapies and biopsy is significant, such that avoiding biopsy in men at lower risk would be ideal.13 However, the risk of having prostate cancer might be supported to decide a prostate biopsy. Several normograms have been developed for predicting prostate cancer risk, e.g., PCPT (Prostate Cancer Prevention Trial) in America, ERSPC risk calculator (European Randomized Study of Screening Prostate Cancer) and IPCRC (Indonesian Prostate Cancer Risk Calculator).5 In this study, we evaluate the accuracy of our calculator for predicting prostate cancer risk in Cipto Mangunkusumo Hospital, Jakarta.

2. METHODS
All benign prostate hypertrophy (BPH) and prostate cancer patients who had prostate biopsy and prostatectomy between August 2014 until December 2015 in Cipto Mangunkusumo Hospital Jakarta were included in this study. The data were collected from complete medical records. We excluded patients below 40 years old and volume below 10 ml. The variables used in this study were age, total PSA level, prostate volume and DRE.
fingrings. The measurement of PSA using PSA monoclonal antibody combined with reagenMaglumi 100 test 130201004 M. The prostate was measured by TRUS performed by certified physicians using the BK ultrasound. The estimation of prostate volume was used a modification of the prostate ellipsoid formula and recorded in cm$^3$ (0.523 [length (cm) × width (cm) × height (cm)]).

We use chi-square or Fischer’s test to find involvement of categorical variables to the outcome. In the model-building set, factors that were predictors of prostate cancer was analyzed as multiple logistic regression analysis. Prostate cancer calculator that we composed to predict the possibility of prostate cancer using chi-square analysis, Kolmogorov-Smirnov test, multiple logistic regression and receiver operating characteristic (ROC) curve regarded a $p$ value <0.05 as statistically significant. The logistic model was referred to the prior research. The probability of prostate cancer incidence stated as Odds. We analyze data with SPSS version 20.

3. RESULTS

127 subjects were included to our study. Table I shows characteristics of patients in this study. Median of age in BPH and prostate cancer is 66 (52–85) and 69.5 (50–100) respectively. The PSA level and prostate volume in BPH group is 10.2 (1.6–203.1) ng/ml and 47.9 (13.74–108) cm$^3$. Compared to prostate cancer group; PSA level is 74.06 (6.94–1412) ng/ml and 50.25 (19.2–107) cm$^3$. Eleven abnormal DRE examination found in prostate cancer group. In our center, total of BPH patients were 99, more than total of prostate cancer patients (26 patients).

According to logistic regression analysis,

\[
\ln(\text{odds}) = -1.883 + 0.621(l_{\text{psa}} - 4.25) + 0.041(l_{\text{vol}} - 5.47) - 1.199(l_{\text{dre}} - 6.05) + 3.999\text{(DRE abnormal)}
\]

The IPCRC score of BPH patients in our study were 8.57 (Median). Besides, the IPCRC score of prostate cancer patients were 45.79 (Median). The area under curve (AUC) of our calculator is 0.907. We found several cutoff point (15%, 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%) that if the probability of prostate cancer more than 15% using IPCRC, the sensitivity is 88.5% and specificity 20%, 25%)

4. DISCUSSION

The prediction of PCAs is important in early stage to gain better outcomes. Current evidence base shows that PSA has poor predictive value as a diagnostic tools to PCAs. In Indonesia, IPCRC has been developed as a prostate cancer that calculate risk of prostate cancer.\(^\text{11}\) Elevated values of PSA level had been found in many conditions, (prostate cancer, BPH, and prostatitis) because these conditions are organ specific. Characterizing risk based solely on serum PSA findings presents inherent difficulties. Conversely, a high body mass index erroneously lowers PSA values as a result of haemodilution. Thus, the interpretation of PSA values is prone to error arising from nonspecific sources. Furthermore, serum tPSA values are poor indicators of the aggressiveness of prostate cancer, regardless of the threshold chosen. Because PSA does not correlate well with aggressiveness, there is a trend in clinical practice toward overdiagnosis and consequent overtreatment of prostate cancer.\(^\text{16,17}\) Recently, a noninvasive urinary test for the prostate cancer gene 3 (PCA3) has been developed. PCA3 is an emerging gene-based marker that is highly specific for prostate cancer.

Therefore we have validated the Indonesian Prostate Cancer Risk Calculator in CiptoMangunkusumo general hospital, using the risk factor variables: age, PSA, DRE (consistency, nodule, exam), and prostate volume of patients. These variables were included in the calculator based on data from 127 patients in CiptoMangunkusumo General Hospital in Indonesia (Table I).

Nowadays, many calculators that have been developed e.g., PCPT (Prostate Cancer Prevention Trial) in America, ERSPC risk calculator (European Randomized Study of Screening Prostate Cancer) and IPCRC (Indonesian Prostate Cancer Risk Calculator). ERSPC trial reported a 20% reduction in prostate cancer mortality in their core age group (men aged 55–69 years) that largely occurred after 10 years of follow-up, although a non-statistically significant reduction of 1.5% was noted in all men (aged 50–74 years) randomly assigned. Improvements in prostate cancer treatment are probably at least in part responsible for declining prostate cancer mortality rates. Even if life is only prolonged by therapy, the opportunities for competing causes of death increase, especially among older men.\(^\text{18}\)

The PCPT risk calculator has been external validated, comparing its performance to PSA alone and to a novel, logistic regression-based model which included % fPSA and number of biopsy cores. The risk calculator gave a modest improvement in the performance characteristics of PSA alone in predicting an individual’s risk of prostate cancer or high grade disease.\(^\text{19}\) The current limitations of the PCPT risk calculator may also relate to the relative predictive value of its individual variables. While multivariate analysis confirmed that PCPT score component variables are significant predictors of cancer at biopsy, data also suggest that the current form of the calculator is not optimally

| Table I. Characteristics of patients. |
|-----------------|-----------------|-----------------|
|                 | BPH (n = 99)    | Prostate cancer (n = 26) |
| Age (year)      | 66 (52–85)      | 69.5 (50–100)    |
| PSA (ng/dl)     | 10.2 (1.6–203.1)| 74.06 (6.94–1412)|
| Prostate volume (cm$^3$) | 47.9 (13.74–108) | 50.25 (19.2–107) |
| DRE consistency | Hard 2 (2%)     | 11 (42.3%)       |
|                 | Rubbery 98 (98%)| 15 (57.6%)       |
| DRE nodule      | Positive 6 (6%)  | 7 (26.9%)        |
|                 | Negative 94 (94%)| 19 (73.1%)       |
| DRE examination | Normal 93 (93%)  | 15 (57.6%)       |
|                 | Abnormal 7 (7%)  | 11 (42.3%)       |

Table II. Distribution of patients based on prostate cancer.

<table>
<thead>
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<th>BPH median (min–max)</th>
<th>Cancer median (min–max)</th>
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<td>IPCRC score</td>
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modeled, at least for our contemporary population. Some predictors not currently included in the risk calculator, eg age and free PSA in the overall cancer model, and abnormal DRE and free PSA in the high grade model, had strong prognostic association with cancer in our data.\textsuperscript{10}

Nowadays, GP and urologist are confronted with request for early detection of prostate cancer. Several risk assessment tools have been developed to support decision making on which diagnostic tools should be conducted to screen suspected prostate cancer patient, a PSA test or a prostate biopsy.\textsuperscript{13} However, using PSA for screening purposes, may not be suitable since the prevalence may be related on individualized risk. A limited list of additional risk factors such as age, comorbidity, prostate volume, family history, ethnicity, digital rectal examination and previous biopsy status have been identified to modify risk and are important for consideration in routine practice.\textsuperscript{14,15}

Therefore we have validated the Indonesian Prostate Cancer Risk Calculator in CiptoMangunkusumo general hospital, using the risk factor variables: age, PSA, DRE (consistency, nodule, exam), and prostate volume of patients. These variables were included in the calculator based on data from 127 patients in CiptoMangunkusumo General Hospital in Indonesia (Table I).

From the logistic regression analysis as a model for IPCRC in CiptoMangunkusumo general hospital, there is no significant difference in mean of age and prostate volume between both group. However, there were difference between two groups in regard of PSA, DRE consistency, DRE nodule, and DRE examination. We found significant difference in IPCRC score between BPH patient and cancer patients in regards of IPCRC score in CiptoMangunkusumo hospital. The different details can be seen in Table II. From ROC curve analysis, we found excellent result from IPCRC in differentiating between patient with prostate cancer and patient without it. The details of this result can be seen in Table III and Figure 1.

This study had several limitations. First, the results may have been influenced by the heterogeneity of patients, tumours and biopsy techniques. Second, this validated study had fewer sample compared to the Indonesian prostate cancer risk calculator (IPCRC) was developed from 1957 men in Indonesian, PCPT (Prostate Cancer Prevention Trial) was developed from 18,882 men, while ERSPC (European Randomized Study of Screening Prostate Cancer) developed from 6,288 men.\textsuperscript{4} Third we did not consider race as one of the predictive variable in this calculators because of incomplete medical data. Four, Family history of prostate cancer was not accounted for its predictive factor. However, we believed that IPCRC can be useful and had a good predictive value for predicting prostate cancer risk in our population.

5. CONCLUSION
We have validated the Indonesian Prostate Cancer Risk Calculator, in CiptoMangunkusumo general hospital, which include age, PSA, prostate volume, DRE as its variables. Future studies to validate this risk calculator are still needed.

References and Notes

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