p-ISSN 2301-9123 | e-ISSN 2460-5441

GLOBAL MEDICAL & HEALTH COMMUNICATION

DEC 2019 VOL. 7 NO. 3

Global Medical & Health Communication

Editorial Team

Editorial Address
Jalan Hariangbanga No. 2, Tamansari, Bandung 40132, West Java, Indonesia
Phone/fax: (022) 4321213
E-mail: gmhc.unisba@gmail.com
Website: https://ejournal.unisba.ac.id/index.php/gmhc

Accredited by:
Ministry of Research, Technology and Higher Education of the Republic of Indonesia
(Kemenristekdikti) Number: 2/E/KPT/2015, 1st December 2015

This journal is indexed on:

Published by:
Faculty of Medicine Universitas Islam Bandung;
Pusat Penerbitan Universitas-Lembaga Penelitian dan Pengabdian kepada Masyarakat (P2U-LPPM)
Universitas Islam Bandung

Publish Every 4 Months
April, August, December

Global Medical & Health Communication

pISSN 2301-9123 | eISSN 2460-5441

Volume 7 Number 3, December 2019

TABLE OF CONTENTS

AUTHOR GUIDELINES

Global Medical & Health Communication (GMHC) is a journal that publishes medical and health scientific articles published every 4 (four) months. Articles are original research that needs to be disseminated and written in English.

The submitted manuscript must be the article that has never been published, and the author must ensure that all co-authors have agreed by signing a statement on the seal. Download template of the ethical statement (free plagiarism) here. The manuscript is an original article free from plagiarism. When the article published in another journal then in the next journal, the article will be disallowed.

All articles will be discussed by experts in the field of scholarly concerned (peer reviewer) and will be edited by the editor. The editor reserves the right to add or subtract sentences, both abstracts, and scripts without changing the meaning. Manuscripts that accepted for publication will become the property of the publisher. It is not allowed to be published in other media. The needed revised manuscripts will be returned to the author. Research articles must be approved by the health research ethics committee or consider the ethical aspects of research accounted for

Article Writing

Typed the article on an 80 gsm A4 (21.0 × 29.7 cm) white HVS paper with 4 cm left and top margin, 3 cm down and right, not back and forth. The maximum script length is 20 pages (including images, tables, and photos). Each page is numbered typed in the bottom right page, sequentially starting from the title page to the last page. The font is black Georgia with 12 pt size, typed justified except for a title with a spacing of 2 spaces in Microsoft Word 2007 format. Typing a new paragraph 6 taps from the left edge of the line, unless the first paragraph is not typed indented. In one manuscript only in English. Typed italic the untranslatable terms in a foreign language or regional language.

Table title is the typed center, font size 10 pt, bold, initial letter of each word written with capital letter, except conjunctions. The titles are numbered and written on top of the table. Example: Table 3 *Neisseria gonorrhoeae* Resistance to 8 Types of Antimicrobials in 20 Specimens. Table, no vertical dividing line, and there are only three horizontal borderlines. Created tables in sequence two spaces from the text. Table descriptions and abbreviations are placed in the table description, not on the table title.

Typed center figure title with 10 pt font size, bold, numbered according to the appearance in the text and typed under the image. The source of the cited image and or table should be added to references if it is not the author's work.

Pictures (graphs, diagrams, and photos) and tables besides written in its place, also created separately on other pages of texts with sufficient sharpness and blackness. A maximum number of tables and or images are six pieces. Photos are sent in black and white glossy, or colored format when required, minimum size 3R (9×13.5 cm). Images and photos can also be sent on CD.

Write correspondence as the footnote on the first page containing the full name of the author with degrees/academic degrees, institution, address, phone number, fax, mobile, and e-mail.

Content and Format Articles

The article contains results of original research in the field of basic medical or applied, and health. The article format consists of Title & Abstract (English) and *Judul* & *Abstrak* (Indonesian), Introduction, Methods, Results, Discussion, Conclusion(s), Conflict of Interest, Acknowledgments, and References.

Articles Title

Maximum article title consists of 12 words (choose words and terms that are dense meaning and able to characterize the entire contents of the script). Typed with bold fonts, size 12 pt, one space, the initial letter of each word is written in capital letters (except the conjunctive), and center. The ownership row consists of 2 elements, the author name and origin institution. Author's name written with the initial fonts are capital and bold, size 11 pt, one space, and center. Institution name written with the initial fonts are capital, size 10 pt, one space, and center.

Abstract

The abstract is typed using 12 pt font size and one spaces. The abstract is written in one paragraph, one space, maximum 250 words, and should describes the entire contents of the article. The abstract should be suitable for the format of introduction, methods (contain method, place, and time of study), results, and discussion. Abstract be equipped with key words consisting of 3–5 words.

Introduction

The introduction is written succinctly to stimulate the reader's interest include all the necessary information. At the end of the introduction was written the purpose of the study.

Methods

Methods contains the material under study, and the way described briefly by the order of operation as well as the location and time of the study. Explain statistical methods in detail. Consideration of ethical issues is included. If the protocol has been approved then the ethical clearance/approval letter number and the health research ethics committee must be written.

Results

The result is the core of scientific writing. This section

presents data and information that will be used as the basis of the conclusion and even expected to get a new theory. In results, listed the tables and or images, graphics, photos to explain and abbreviate the description should be given; numbered according to their appearance in the text. Results of the study and discussion should be written separately.

Discussion
Discussion of the article reveals, explains, and discusses the results of the study with an analysis by the research design, interpretation, and explanation of its synthesis. Also, the results obtained are compared with the results of previous research of others.

Conclusion(s)
The conclusion is submitted by the results obtained by the researcher and written briefly and clearly in two or three sentences.

Conflict of Interest
All authors must make a formal statement at the time of submission indicating any potential conflict of interest that might constitute an embarrassment to any of the authors if it were not to be declared and were to emerge after publication. Such conflicts might include, but are not limited to, shareholding in or receipt of a grant or consultancy fee from a company whose product features in the submitted manuscript or which manufactures a competing product.

Acknowledgment
Acknowledgments should be provided to research contributors without writing a degree.

References
References are written by the Vancouver system's writing rules, given the sequence number corresponding to appearing in the article. List all author names if no more than six people; when more than six authors write the first six authors followed by et al. The references cited in the article are the most important references. The minimum referral number of 25 (twenty-five) copies of the most recent 10 (ten) years of journal article/book publishing. Reference should be sought from 80% primary literature and 20% secondary literature. Avoid referral in the form of personal communication except for information that is not possible from a public source. Include source name, date of communication, written permission, and confirmation of the accuracy of the source of communication.

Example How to Write References
Journals
Theodoridou K, Vasilopoulou VA, Katsiaflaka A, Theodoridou MN, Roka V, Rachiotis G, et al. Association of treatment for bacterial meningitis with the development of sequelae. Intern J Infect Dis. 2013;17(9):e707–13.

Zhang B, Kunde D, Tristram S. Haemophilus haemolyticus is infrequently misidentified as Haemophilus influenzae in diagnostic specimens in Australia. Diagn Microbiol Infect Dis. 2014;80(4):272–3.

Books and Other Monographs
Editor as Author
Nriagu J, editor. Encyclopedia of enviromental health. Michigan: Elsevier BV; 2011.

Organization as Author
World Health Organization (WHO). Guideline: neonatal vitamin A supplementation. Geneva: WHO Press; 2011.

Chapter in Book
Miller LG. Community-associated methicillin resistant Staphylococcus aureus. In: Weber JT, editor. Antimicrobial resistance. Beyond the breakpoint. Basel: Karger; 2010. p. 1–20.

Conference Proceeding
Nicholai T. Homeopathy. Proceedings of the Workshop Alternative Medicines; 2011 November 30; Brussels Belgium. Belgium: ENVI; 2011.

Journal Article from Internet
King P. Haemophilus influenzae and the lung (Haemophilus and the lung). Clin Transl Med. 2012;1:10 [cited 2015 August 15]. Available from: https://clintransmed.springeropen.com/articles/10.1186/2001-1326-1-10.

Authors
Written equipped in the covering letter, containing the full name (with degrees/academic degrees), the area of expertise, institution, address, phone number, fax, mobile, and e-mail.

Article Submission
Submit article and correspondence with the editorial board online. Register at http://ejournal.unisba.ac.id/index.php/gmhc and follow the guidelines.

Editorial Board of
Global Medical and Health Communication
Faculty of Medicine, Universitas Islam Bandung
Jalan Hariangbanga No. 2, Tamansari,
Bandung 40132, West Java, Indonesia

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.5497

GMHC. 2019;7(3):157–61
pISSN 2301-9123 | eISSN 2460-5441

GUEST ARTICLE

Implementation of Sustainable Development Goals (SDGs) and Disaster Risk Reduction (DRR): a Case Study

Peter Davey
Director Centre of Excellence for Sustainable Development for Indonesia (CESDI),
Griffith University, Brisbane, Australia

Abstract

Climate change adaptation and mitigation measures a complicated process and community livelihoods are being seriously impacted. The current local community reality is that climate change and associated disasters are becoming more intense, unpredictable, frequent and costly impacting on rural and urban areas. Disaster Risk Reduction is very important. United Nations General Assembly already set the global policy with the hope that the impact of future disaster events on the community is substantially reduced. Solutions to internal refugee crises start at the local level and require that everyone plays a part: every city, every neighbourhood including farming areas, and every individual can contribute. Leaders must create spaces where everyone can live in safety, become self-reliant, and contribute to and participate in their local community, and not allow people to shift into slum areas after disasters strike. The UNISDR suggests community's use of the recovery, rehabilitation and reconstruction phases after a disaster to increase the resilience of nations and communities; through integrating disaster risk reduction measures into the restoration of physical infrastructure and societal systems, and into the revitalization of livelihoods, economies, and the environment.

Key words: Disaster risk reduction, climate change, community

Correspondence: Dr. Peter Davey, Ph.D. Director Centre of Excellence for Sustainable Development for Indonesia (CESDI), Griffith University, Brisbane, Australia. E-mail: peter.davey@griffith.edu.au

Introduction

The United Nations SDG Goals together with the UNDRR Disaster Risk Reduction Sendai Framework set the global policy action required by Nations to address local health issues and emerging risks.[1] The UNFCC - Cooperation of Parties fund research and guide climate change adaptation and mitigation measures.[2] It's a complicated process and community livelihoods are being seriously impacted. The current local community reality is that climate change and associated disasters are becoming more intense, unpredictable, frequent and costly impacting on rural and urban areas. Many rural communities particularly in low income countries and their local governments, residents and small to medium business enterprises (SMEs) find in the context of climate change adaptation that shifting towards Disaster Risk Reduction (DRR) difficult.[3] Local governments in rural areas particularly in developing countries and SMEs are often exposed and lack the capacity to better protect and prepare themselves. However, there are many resilient rural communities, farmers, businesses and good tools and examples where more public-private sector partnerships can help local government and business shift to DRR.[4] This case studies, based on research in this area at CESDI - Griffith University's' Centre for Sustainable Development for Indonesia with its main office in Brisbane, Australia.[5] This paper will discuss examples of climate change impacts effecting Australian and Indonesian community development. Communities, government and business all have an important role to play.

Methods

This is case study from 3 countries, Indonesia, Bangladesh and Australia regarding community participation for disaster risk reduction.[6,7] The brief explanation of each are as follows. A cross-sectional study in Bangladesh compares participants' present and retrospective information (before migration) in seven slums located in the 3 areas of Dhaka City; 74 participants who migrated from rural places of origin because of slow onset drought were interviewed about health status.[8–11] Second, traditional subsistence farming case study in West Timor, Indonesia is an important part of rural society in Indonesia, the seasonal yield is the main source of food to maintain health and livelihoods of these rural households, this yield is under threat from drought and then intensive rains.[12,13] Third, an Australian case study in the Lockyer Valley Region in South-East Queensland will be discussed. This rural setting experienced extreme flash flooding in January 2011 that resulted in significant impacts in the rural farming township of Grantham. In order to reduce future risks, the local government (Council) immediately committed to an innovative community resettlement project.[14]

Whatever the disaster impacts all communities need to 'Build Back Better'; this UNISDR concept will be discussed. In fact, in all communities collaboration between all sectors involved including community (farmers) members, governments and land-use planning, business and emergency management and public health practitioners was essential. This research highlights the importance of community participation and the need for ongoing assessments in the DRR planning, response, resettlement and recovery processes.

Results

A comparative understanding of access to basic services, disease issues and economic conditions of internal migrants living in slums in Dhaka was completed in 2017 by Mohammad Ehsanul Kabir, Ph.D. Candidate supervised by Dr. Peter Davey from Griffith University.[11] The purpose of this first case study was to make a comparative assessment of access to basic services, disease issues and economic condition of the disadvantaged internal migrants related to their places of origin and places of destinations. The analysis took place in seven slums located in Mohammadpur, Rayerbazaar and Jigatola areas of Dhaka City in Bangladesh. In brief the findings show some improvements in basic household infrastructure and hygiene practices after migrating from their droughty affected rural farm to these city slums compared to their previous status in places of origin. However, the frequency of diseases increased in the short to medium term after migration, as reported by the participants. The study argues that increased incidents of disease at places of destination can be associated with a limited access to free healthcare benefits and increased burden of living cost compared with the participants' places of origin. This study considered some key issues of internal migration with a temporal account before migration at places of origin and after migration at places of destinations. The feedback from the

disadvantaged migrants which compared their current living conditions with life at their place of origin has not previously been studied in low income countries. However, the most significant trend in human mobility continues to be and will likely remain internal migration or inter-regional migration as opposed to movement across international boundaries. To date, the majority of the research into the vulnerability of human systems has tended to focus on the bio-physical impacts of hazards. There is only limited understanding of how such groups are affected by various aspects of vulnerability.

Kabir and Davey[10] reportied that climate-migration research in general and noted that more recent studies have recognized the need to investigate how climate and environmental vulnerability could result in incremental or non-linear migration outcomes, depending on various contexts of natural hazards. In order to examine such complexities, the concept of 'drivers of vulnerability' offers a valuable analytical alternative.[9] Kabir and Davey[11] supports an approach that can explain how multiple drivers can influence the livelihoods of various diverse populations across the contexts of natural hazards, time and space, and between and within social groups. This study adopted a multi-method approach and answers the research questions by means of a structured interviews, focus group discussions and key informant interviews. Finally the results illustrate details of the underlying drivers of vulnerability which potentially influence involuntary internal migration from the affected areas, mostly to cities and to slum areas. A range of drivers of vulnerability were identified and classified into five broad thematic divides including economic, institutional, infrastructural, environmental and health-and-wellbeing.

This second case study[15] investigated food insecurity issues in Atoin Meto, a subsistence community in semi-arid parts of West Timor. It discussed the concept of subsistence living from the perspective of food insecurity in severe drought conditions. Yenny Tjoe a PhD graduate alos from Griffith University from 2014 collected data in Kupang and Timor Tengah Selatan Regencies in West Timor.[16] Data were analysed via mixed-methods of quantitative household surveys, and qualitative in-depth key informant interviews and participant observations assisting a way forward for subsistence farmers. In summary this case study looked impacts on at traditional subsistence farming as an important part of rural society. This community maintains food sovereignty without overly using the local resources: following seasonal cycles to grow staple food (being self-sufficient) and earn cash income via multiple activities within and outside the community to offset declining food stock in drought conditions. This study found that local knowledge and values of Atoin Meto is founded on their existing clan regime and emotionally-bonded moral values, which is a historical sustainable approach. However, the system has weaknesses and to support their adaptation to climate change and drought Tjoe[16] suggested three solutions - to enhance their food production; improve nutritious value of local diets and develop their ability to market produce, but keeping enough nutritious produce in the household for families to survive droughts.

Third, a case study in the Lockyer Valley Region in South-East Queensland, Australia will be discussed. This rural setting experienced extreme flash flooding in January 2011 that resulted in the loss of 19 lives including 12 in the rural farming township of Grantham. In order to reduce future risks, the local government (Council) immediately committed to an innovative community resettlement project despite an environment of political resistance and bureaucratic turmoil. During the summer of 2010–2011 Queensland experienced a series of natural disasters that will long be remembered. The floods that devastated central and southern Queensland coupled with the destruction by Severe Tropical Cyclone Yasi resulted in the entire State being declared disaster affected and the tragic death of 37 people. Grantham floods near Brisbane was a major disaster and the community working with local government built back better away from the flood plain and the low lying productive farming areas.

To monitor and coordinate the Government's program of reconstruction and recovery the Queensland Reconstruction Authority (the Authority) was established.[14] The Authority operates under the auspices of a comprehensive and integrated recovery and reconstruction plan for the State – Operation Queenslander, its mission is to 'reconnect, rebuild and improve Queensland, its communities and economy'. This is supported by four strategic objectives, two of which specifically focus on resilience: build a resilient Queensland and support resilient Queenslanders; and enhance preparedness and disaster mitigation. As part of Operational Plan

for Queenslander, six lines of reconstruction were also established to co-ordinate key aspects of the reconstruction and recovery effort: 1. Human and Social 2. Economic 3. Environment 4. Building Recovery 5. Roads and Transport and 6. Community Liaison and Communication.

In summary, disaster resilience is 'the capacity to prevent, mitigate, prepare for, respond to, and recover from the impacts of disasters'. Building resilience enhances our ability to minimise the effects of future disaster events on our communities, economy and environment. The Queensland Government Reconstruction Authority in 2011 commented that building disaster resilience is about improving the capabilities of individuals, families and communities, as well as that of businesses and governments.[14]

Integral to this is strengthening partnerships between communities, the not-for-profit sector, industry, the private sector and tiers of government. For many in the community an important aspect of this is planning ahead to reduce disaster risks and produce co-ordinated and effective efforts during disaster events. This approach is referred to as 'Betterment – Build Back Better'; building back better enhances a community's immunity to natural disasters.[14]

Conclusion

The research climate change impacts is important including building resilience towards disasters particularly drought and floods but embrace the concept of 'Build Back Better'. The UNISDR suggests community's use of the recovery, rehabilitation and reconstruction phases after a disaster to increase the resilience of nations and communities; through integrating disaster risk reduction measures into the restoration of physical infrastructure and societal systems, and into the revitalization of livelihoods, economies, and the environment.

References

1. Davey P. Lessons learnt integrating DRR and CCA into liveable Cassowary coast 'whole of community plan 2010–2020.' Paper presented at UNISDR ONEA-GETI Global Training Seminar; Incheon, Korea; 2016 April 15.
2. Davey P. UNISDR disaster risk reduction implementation. Paper presented at 41st EHA National Conference; Hobart, Australia; 2016 October 31–November 3.
3. Davey P. Global and local partnerships needed to implement Sendai Framework. Paper presented at Environmental Health in Disaster Management and Humanitarian Settings Seminar; Medan, Indonesia; 2015 September 14–15.
4. Davey P. Environment, humanitarian assistance and disaster initiative - Professional Short Course Training Examples from Indonesia. Paper presented at 40th EHA National Conference; Sydney, Australia; 2015 October 20–23.
5. Davey P. Disaster management and emergency response in Indonesia. Paper presented at 40th EHA National Conference; Sydney, Australia; 2015 October 20–23.
6. Ryan B, Davey P, Hatch T. Disaster risk reduction: environmental health science capacity building. The Interdisciplinarian: Newsmagazine of the Association of Interdisciplinary Doctors of Health Science. 2014;1(1).
7. Davey P. IFEH and CDC short course disaster training across the Asia Pacific region. Paper presented at 78th National Environmental Health Association (NEHA) Annual Educational Conference (AEC) and Exhibition; Las Vegas, USA; 2014 July 7–10.
8. Kabir ME, Serrao-Neumann S, Davey P, Hossain M, Alam MT. Drivers and temporality of internal migration in the context of slow-onset natural hazards: Insights from north-west rural Bangladesh. IJDRR. 2018;31:617–26.
9. Kabir ME, Davey P, Serrao-Neumann S, Hossain M. Seasonal drought thresholds and internal migration for adaptation: lessons from Northern Bangladesh. In: Hossain M, Hales R, Sarker T, editors. Pathways to a sustainable economy: bridging the gap between Paris climate change commitments and net zero emissions. Cham, Switzerland: Springer International Publishing AG; 2018. p. 167–89.
10. Kabir ME, Davey P. The trade-off in 'relocation': a comparative understanding of vulnerabilities of disadvantaged migrants moving from rural origins to urban areas in the context of Bangladesh. Paper presented at the Migration Conference 2017; Athens, Greece; 2017 August 23–26.
11. Kabir ME, Davey P. Seasonal drought

thresholds and internal migration for adaptation: lessons from northern Bangladesh. Paper presented at 42nd Environmental Health Australia National Conference; Brisbane, Australia; 2017 October 16–19.
12. King P, Davey P. Linking sustainable livelihoods and health risks in small scale fishing villages in SE Asia. Paper presented at 2nd International Conference Environmental Risks and Public Health (ICER-PH); Makassar; 2015 April 10–12.
13. Tjoe Y, Ratumakin PA, Hossain M, Davey P. Disadvantaged communities in Indonesian semi-arid regions: an investigation of food security issues in selected subsistence communities in West Timor. In: Sarkar A, Sensarma SR, vanLoon GW, editors. Sustainable solutions for food security: combating climate change by adaptation. Basel, Switzerland: Springer Nature Switzerland AG; 2018. p. 381–408.
14. Queensland Recontruction Authority, Queensland Government. Rebuilding a stronger, more resilient Queensland: the capacity to prepare for, withstand, respond to and recover from disasters. 2012 [cited 2019 October 10]. Available from: http://hardenup.org/umbraco/customContent/media/1367_QRA%20-%20Resilience.pdf.
15. Tjoe Y, Ratumakin PA, Hossain M, Davey P. Climate disruption: disadvantaged communities in the semi-arid regions of Indonesia. Presented at 42st EHA National Conference; Brisbane, Australia; 2017 October 16–19.
16. Tjoe Y. Sustaining livelihoods: an analysis of dryland communities in West Timor, Indonesia. PhD Doctorate [thesis]. Southport, Australia: Griffith Business School - Griffith University; 2017 [cited 2019 October 11]. Available from: https://research-repository.griffith.edu.au/handle/10072/366775.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3094

GMHC. 2019;7(3):162–9
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Relation between Vitamin D Level and Knowledge and Attitude Towards Sunlight Exposure among Asthma Outpatients in Surabaya

Amelia Lorensia,[1] Rivan Virlando Suryadinata,[2] Gebriella Ayuni Amir[3]

[1]Department of Clinical-Community Pharmacy, Faculty of Pharmacy, Universitas Surabaya, Surabaya, Indonesia,
[2]Department of Public Health, Faculty of Medicine, Universitas Surabaya, Surabaya, Indonesia,
[3]Pharmacist Study Program, Faculty of Pharmacy, Universitas Surabaya, Surabaya, Indonesia

Abstract

Asthma has become one of the health problems in the world. Asthma is characterized by chronic inflammation of the respiratory tract which leads to breathlessness, wheezing, and airflow limitation. Inflammatory reaction in asthma is related to inadequate vitamin D level. Vitamin D is a vitamin produced naturally by the body when exposed to sunlight that has immunomodulatory properties can reduce inflammation. Knowledge and positive attitude to sun exposure are necessary to prevent severe asthma attacks. This was a cross-sectional study involving 26 subjects in a private university in East Surabaya during January–June 2017 that was aimed to determine the relation between vitamin D and knowledge and attitude towards sunlight exposure. Data were collected by measuring the vitamin D level in blood serum and through the use of a questionnaire that consisted of two aspects, level of knowledge and attitude to sun exposure. Chi-square test was used to analyze the relationship between vitamin D level, knowledge, and attitude. No relationship was found between blood vitamin D level of asthma respondents and level of knowledge of sun exposure related to vitamin D ($p=0.444$, $p>0.05$). The same was also true for the relationship between blood vitamin D level of asthma respondents and attitude to sun exposure related to vitamin D ($p=0.768$, $p>0.05$). The closeness of the relationship between knolwedge and attitude was also relatively low (0.093). In conclusion, there is no correlation between vitamin D level, knowledge, and attitude. In addition, there is also no correlation between knowledge and attitude with low relationship between the two variables.

Key words: Asthma, attitude, knowledge, sun exposure, vitamin D level

Hubungan Kadar Vitamin D dengan Pengetahuan dan Sikap tentang Paparan Sinar Matahari pada Pasien Asma Rawat Jalan di Surabaya

Abstrak

Asma telah menjadi masalah kesehatan di dunia. Asma ditandai oleh peradangan kronis pada saluran pernapasan yang menyebabkan sesak napas, mengi, dan keterbatasan aliran udara. Reaksi peradangan pada asma terkait dengan kadar vitamin D yang tidak memadai. Vitamin D merupakan vitamin yang diproduksi secara alami oleh tubuh ketika terkena sinar matahari yang memiliki sifat imunomodulator dapat mengurangi peradangan. Pengetahuan dan sikap positif terhadap paparan sinar matahari diperlukan untuk mencegah risiko keparahan asma. Penelitian ini menggunakan metode *cross-sectional* yang melibatkan 26 subjek di sebuah universitas swasta di Surabaya Timur pada Januari–Juni 2017 yang bertujuan menentukan hubungan vitamin D dengan pengetahuan dan sikap terhadap paparan sinar matahari. Data dikumpulkan dengan mengukur kadar vitamin D dalam serum darah dan melalui penggunaan kuesioner yang terdiri atas dua aspek, tingkat pengetahuan dan sikap terhadap paparan sinar matahari. Uji *chi-square* digunakan untuk menganalisis hubungan antara kadar vitamin D, pengetahuan, dan sikap. Tidak ada hubungan yang ditemukan antara kadar vitamin D darah responden asma dan tingkat pengetahuan paparan sinar matahari yang terkait dengan vitamin D ($p=0,444$; $p>0,05$). Hal yang sama juga berlaku untuk hubungan antara kadar vitamin D darah responden asma dan sikap terhadap paparan sinar matahari yang terkait dengan vitamin D ($p=0,768$; $p>0,05$). Kedekatan hubungan antara pengetahuan dan sikap juga relatif rendah (0,093). Simpulan, tidak ada hubungan antara kadar vitamin D, pengetahuan, dan sikap. Selain itu, juga tidak ada hubungan yang rendah antara pengetahuan dan sikap.

Kata kunci: Asma, kadar vitamin D, paparan sinar matahari, pengetahuan, sikap

Received: 30 September 2017; Revised: 30 July 2019; Accepted: 31 July 2019; Published: 31 December 2019

Correspondence: Dr. Amelia Lorensia, S.Farm., M.Farm.Klin., Apt. Department of Clinical-Community Pharmacy, Faculty of Pharmacy, Universitas Surabaya. Jln. Raya Kalirungkut, Surabaya 60293, East Java, Indonesia. E-mail: amelia.lorensia@gmail.com

Introduction

Asthma has become a significant health problem in the world. It is estimated that the global prevalence for asthma has reached 235 million people and 24 thousands died because of this disease.[1] Asthma is characterized by chronic inflammation of respiratory tract with the symptoms of shortness of breath, wheezing, chest pressure, heavy coughing which constantly gets worse, and airflow limitation. This disease can specifically create burdens for the patient, and the family and society in general, because it decreases productivity.[2]

One of the factors affecting the high incidence of asthma is the level of vitamin D in the body. This is due to the fact that low vitamin D level in the body can trigger asthma attacks.[3] A previous study by Shebl et al.[4] stated that asthma patients generally have a low level of vitamin D in their body. Vitamin D has a strong relationship with inflammation reaction in asthma that vitamin D deficiency may worsen the disease.[5] According to a systematic review by Bozzetto et al.[6] vitamin D has been proven to have an immunomodulator nature by stimulating the immune system to prevent the production of cytokine in order to reduce inflammation. Vitamin D is considered to be a strong predictor of asthma. Increasing vitamin D level will reduce the exacerbation of asthma and may become the primary prevention of asthma.[7]

Vitamin D is the only vitamin that is naturally produced by the body when it is exposed to sunlight. In a study in England, around 72% of the participants know about vitamin D from television, internet, radio, newspaper, family, and friends.[8] Based on a study conducted by Arora et al.[9] to 599 participants in India on the level of knowledge and attitude towards sunlight exposure, almost all participants (99.5%) have heard about vitamin D and 53.3% of them know that sunlight is the primary source of vitamin D. Despite the knowledge on the main source of vitamin D, 385 participants (64.2%) do not like to be exposed to sunlight and almost half of the participants (49.7%) thought that sunlight is dangerous for their skin, and around 43% participants use umbrellas to protect them from sunlight.

Knowledge and attitude towards sunlight are essential because sunlight has a significant benefit in preventing vitamin D deficiency.[10] The level of knowledge and attitude towards exposure to sunlight can be measured using a questionnaire, which has been done by Arora et al.,[9] Zhou et al.,[11] and Al Bathi et al.[12] The purpose of this study was to identify relationship between the level of vitamin D and the level of knowledge and attitude towards the exposure to sunlight in its role as an important component of vitamin D formation among asthma patients.

Methods

This was a cross-sectional study on asthma patients, or respondents, where respondents were given a questionnaire to measure the level of knowledge and attitude towards sunlight exposure and had their blood vitamin D level measured. Sampling were carried out in a private university in Rungkut district, East Surabaya, Surabaya, East Java during January–June 2017 and analysis of vitamin D level was carried out at the Biochemistry Laboratory of Universitas Airlangga, Surabaya, East Java.

Variables in this study were vitamin D level and level of knowledge and attitude towards sunlight exposure related to the production of vitamin D. Measurement of vitamin D level in this study was performed by measuring serum vitamin D level [25(OH)D]. Knowledge was defined as the knowledge possessed by respondents on sunlight exposure as the primary source of vitamin D. The attitude was defined as the daily attitude towards sunlight exposure. Data collection for knowledge and attitude variables was performed using questionnaire from previous studies by Arora et al.,[9] Zhou et al.,[11] and Al Bathi et al.[12]

The population of this study was adult asthma patients (≥18 years) in the university. Samples (respondents) were those who met the inclusion and exclusion criteria. The inclusion criteria used in this study was willing to volunteer to participate in the study and signed a written informed consent form. Meanwhile, the exclusion criteria were: (1) other breathing-related diseases, (2) chronic and liver-kidney disorders, (3) smoking, (4) consumption of drugs that can affect vitamin D level. Purposive sampling method was used to obtain respondents using the following formula.

$$n = \frac{Z^2.P.Q}{d^2}$$

Description: n=sample size; Z=normal standard deviation, in which the value depends on the p value from distribution table Z=1.96; P=proportion for certain properties estimated to occur in the population (East

Java)=0.017; Q=1.0–P (Q is the proportion of properties that are not expected to occur in the population)=1–0.017=0.983; d=degree of deviation=0.05

Hence, the minimum sample size (n) for each group was 25.67≈26 people.

Two types of validation were conducted for the questionnaire, which was taken from other studies. The first validation was the internal validation where the questionnaire was translated into Indonesian and then validated by a peer group. The second validation was the external validation which was performed by trying out the questionnaire to 30 respondents to be filled and the results were analyzed using the SPSS software program. Reliability test was then carried out on a validated questionnaire using Cronbach's alpha method in SPSS software.

Measurement of 25-OH vitamin D level using blood serum. Blood sampling was performed by a health care worker drawing 3 mL venous blood. Blood sample was then kept in a vacutainer tube and centrifucation was done to get the serum. Serum was placed in an Eppendorf tube and transported to the Biochemical Laboratory of Universitas Airlangga for vitamin D level analysis. Ethical clearance for this study was obtained from the Institutional Ethical Committee, University of Surabaya with the issuance of an Ethical Clearance Certificate number: 021/KE/I/2017. Analysis of the results was performed using various tests as listed in Table 1.

Results

Female comprised the majority of the respondents and most of the respondents were in the age group of 20–22 years old with the range of 17 to 25 years old. Details on the demographics of respondents are shown in Table 2.

For knowledge aspect of the questionnaire, the validity and reliability tests revealed that each item in the knowledge section of the questionnaire was valid (r-count>0.361) and reliable (Cronbach's alpha=0.709, Table 3). Furthermore, the validity and reliability tests for attitude aspect of the questionnaire also showed that each item was valid (r-count>0.361 and realiable (Cronbach's alpha=0.704, Table 4).

The vitamin D level was divided into 3 categories: deficient (<20 ng/mL; <50 nmol/L); insufficient (20–32 ng/mL; 50–80 nmol/L), and normal (54–90 ng/mL; 135–225 nmol/L).[13] None of the respondents achieved the normal level of vitamin D, with 24 respondents experienced vitamin D insufficiency and 2 had vitamin D deficiency.

Level of knowledge was determined from the total score of individual item scores. Each correct response was scored 1. It was revealed that 19 of 26 of respondents had excellent knowledge (≥6 points) on sunlight exposure in relation to vitamin D and 7 of 26 respondents had low knowledge (<6 points) on sunlight exposure in relation to vitamin D (Table 5).

Of all respondents with vitamin D insufficiency, 18 had excellent knowledge of sunlight exposure in vitamin D production while 6 had low knowledge. Of the 2 respondents with vitamin D deficiency, 1 had excellent knowledge and 1 had low knowledge. Analysis using with the chi-square method showed a p value of 0.444 (p value>0.05); hence, no significant relationship was found between respondent's vitamin D level and level of knowledge regarding sunlight exposure related to vitamin D.

For the attitude aspect, 1 of 26 respondents

Table 1 Data Analysis

Purpose	Variables	Data Type (Scale)	Data Analysis
To identify relationship between vitamin D level and the level of knowledge towards sunlight exposure in asthma respondents	Vitamin D level	Ordinal	Chi-square
	Knowledge on sunlight exposure	Nominal	
To identify relationship between vitamin D level and the attitude of asthma respondents to sunlight exposure	Vitamin D level	Ordinal	Chi-square
	Attitude towards sunlight exposure	Nominal	
To identify relationship between the level of knowledge and attitude towards sunlight exposure	Knowledge on sunlight exposure	Nominal	Contingency coefficient
	Attitude towards	Nominal	

Table 2 Distribution of Frequency of Respondent Characteristics

Characteristics	Frequency
Gender	
Male	7
Female	19
Age (years)	
17–19	7
20–22	15
23–25	4
History of asthma treatment	
Oral short-acting β-2 agonist	7
Inhaled short-acting β-2 agonist	19

had a right attitude (≤9 points) to sunlight exposure related to vitamin D and 25 of 26 had a bad attitude (>9 points).

Among those with vitamin D insufficiency, 23 respondents had bad attitude towards sunlight exposure and one had the right attitude towards sunlight exposure. No respondent with vitamin D deficiency had the right attitude towards sunlight exposure (Table 6).

Chi-square test revealed a p value of 0.768 (p value>0.05), showing that there was no significant relationship between vitamin D level and with attitude towards sunlight exposure.

Analysis on the relationship between knowledge and attitude showed that 19 of 26 respondents had excellent knowledge but bad attitude towards sunlight exposure. None has good knowledge and attitude towards sunlight exposure. The approximate significance table presented a value of 0.093, showing low relationship between knowledge and attitude.

Discussion

More female involved as the respondents in this study. This might be due to the current prevalence of asthma that is higher in females. High levels of estrogen in women can inhibit the body's immune response to allergic reactions that may cause adverse effects on asthma. Estrogen also acts as a proinflammatory substance that can trigger inflammation, especially through its influence on mast cells to release histamine and other inflammatory mediators.[14] Based on a study conducted by Lee et al.,[15] women have better knowledge about sun protection when compared to men that they tend to use sunscreens more often to protect the skin from sun exposure than men.

Age can affect vitamin D level in the body

Table 3 Validity Test Results for Knowledge Items

Items		Corrected Item–Total Correlation
1	a. Are you familiar with vitamin D? b. In your opinion, what is vitamin D?	0.590
2	Where did you learn about vitamin D?	0.552
3	a. What is the greatest source of vitamin D? b. Other than the greatest source, mention other sources of vitamin D!	0.487
4	What is the benefit of vitamin D for your body?	0.517
5	What is the effect of vitamin D deficiency?	0.464
6	Which of the followings can cause your body to be vitamin D deficient?	0.431
7	a. Do you think sunlight help produce vitamin D? b. Why?	0.362
8	a. Is exposure to sunlight dangerous to your skin? b. Why?	0.433
9	In your opinion, the best time for the body to be exposed directly under sunlight is at ..... a.m./p.m. to ..... a.m./p.m. so that the body can obtain vitamin D.	0.413
10	How long does the body need to be exposed to direct sunlight in order to obtain vitamin D?	0.487
11	What is the sun protection factor (SPF) number which is best for your body?	0.556

Note: r_{table} value was 0.361. Declared as valid if $r_{count} > r_{table}$

Table 4 Validity Test Results for Attitude Items

	Items	Corrected Item–Total Correlation
1	Do you often travel or do outdoor activities under direct sunlight?	0.477
2	How long in a day are you exposed to direct sunlight?	0.418
3	a. Do you avoid exposure to direct sunlight? b. Why do you avoid or not avoid exposure to direct sunlight?	0.587
4	a. Do you use skin protection products (umbrella, hat, jacket, sunblock cream, etc.) when you are exposed to direct sunlight? b. What kind of skin protector do you use?	0.366
5	Why do you choose the skin protector?	0.621
6	a. Do you consume vitamin D supplements? b. What supplements do you consume? c. Why do you consume the supplements?	0.458
7	What is your purpose in consuming vitamin D supplements?	0.443
8	a. Do you think the vitamin D level in your body is sufficient/enough? b. Why?	0.433
9	a. Are you interested in knowing more about vitamin D? b. Why?	0.598

Note: r_{table} value was 0.361. Declared as valid if $r_{count} > r_{table}$

Table 5 Item Responses in Knowledge Aspect

	Items	Response	Respondents (n=26)
1	Information on vitamin D.	Have heard before	24
		Never heard before	2
2	Source of information on vitamin D.*	Pharmacist/nurse/doctor	5
		Family	7
		Book	6
		School	16
		Internet	5
		Don't know	2
3	Sun is the greatest source of vitamin D.	Agree	19
		Disagree	7
4	Vitamin D is beneficial for bone structure.	Agree	21
		Disagree	5
5	Effect of vitamin D deficiency.*	Cancer	8
		Bone disease	23
		Hypertension	4
6	Cause of vitamin D deficiency.*	Using an umbrella during the day	6
		Wearing clothes that cover the whole body	7
		Using sunblock	5
7	Sunlight can help produce vitamin D.	Agree	9
		Disagree	17
8	Sunlight is dangerous for your skin	Agree	24
		Disagree	2
9	Best time for the body to be exposed to sunlight is between 10.00 and 14.00	Agree	1
		Disagree	25
10	Length of time required by the body to obtain vitamin D from sunlight is 15–30 minutes	Agree	11
		Disagree	15
11	Best SPF for the body is ≥15.	Agree	11
		Disagree	15

Note: *respondents may choose more than one response

Table 6 Respondent Attitude towards Sunlight Exposure

Items		Response	Respondents (n=26)
1	Do you often travel or do outdoor activities under direct sunlight?	Yes	16
		No	10
2	The amount of time per day you are exposed to direct sunlight (in minutes).	5	2
		5–15	7
		20–30	6
		>30	11
3	Do you avoid direct sunlight?	Yes	23
		No	3
4	a. Do you use skin protection to avoid exposure to direct sunlight?	Yes	23
		No	3
	b. The type of skin protection used.*	Jacket	19
		Sunblock	4
		Jacket and sunblock	7
		No protection	3
5	The reason you use skin protection.	Based on religion	2
		Avoid dark skin	11
		Avoid heat	6
		Easier to apply	2
		Avoid wind	1
		Avoid dark skin and sweat	1
	No protection.		3
6	Consuming vitamin D supplements.	Yes (contain vitamin D)	0
		Yes (does not contain vitamin D)	10
		No	16
7	The purpose for consuming vitamin D supplements.		
8	The opinion on the needs of vitamin D in the body.	Enough	9
		Not enough	17
9	Interested in knowing more about vitamin D.	Yes	21
		No	5

Note: *the answer of the respondents may be more than one

because as we get older, vitamin D metabolism will also change, causing a decrease in calcium absorption, intestinal resistance to absorption of calcium to circulation 1,25(OH)D and reducing vitamin D production on the skin surface.[16] In older people, there is often an increased risk of vitamin D deficiency due to limited sun exposure and decreased capacity to synthesize vitamin D.[17] Adults tend to avoid sunlight exposure by using sunscreens and clothing that covers the skin to prevent early aging due to sunlight.[18] The majority of adults have heard about vitamin D; however, the knowledge regarding the sun as the most significant source of vitamin D is inadequate so they often use umbrella and sunscreens when exposed to sunlight.[19]

Vitamin D has a relationship with the severity and control of asthma. Adequate levels of vitamin D in the blood have proven effective in the treatment of asthma and can prevent exacerbation of asthma.[20] Vitamin D can be produced naturally by the body when exposed to sunlight but excellent knowledge of the danger of light the sun can affect the attitude that tends to avoid sun exposure that respondents who have a history of asthma and avoid sun exposure are at higher risk of experiencing exacerbations due to vitamin D deficiency.[21]

Most respondents experienced vitamin D insufficiency and some had vitamin D deficiency. This is similar to the results delivered by Shebl et al.[4] stating that asthma patients generally have low vitamin D level. The link between vitamin D and asthma patients is still unclear, but vitamin D has immunomodulatory properties that can reduce the inflammatory process[6] and has the function of modulating the function of various immune cells.[22] Vitamin D is the only vitamin that the

body can naturally produce when it is exposed to sunlight that the sun can be stated as the primary source of vitamin D. Sunlight ultraviolet B (UVB) radiation converts 7-dehydrocholesterol in the skin to previtamin D3, which is then converted into active vitamin D3.

This study has limitations due its limited sample size and non-customary distribution. It also does not consider other factors that can affect vitamin D levels including skin color, weight, residence, and nutritional intake; factors that can influence knowledge such as social factors, economic factors, environmental factors, and experience factors; and factors that can influence attitudes including experience factors, emotional factors, and cultural factors. The measurement of vitamin D level in this study is also still limited because it only used serum vitamin D level without considering vitamin D intake in the form of nutrients.

Conclusion

No relationship is found between serum vitamin D level and level of knowledge and between serum vitamin D level and attitude regarding sunlight exposure for vitamin production. The relationship between knowledge and attitude towards sunlight exposure related to vitamin D is relatively low.

Conflict of Interest

There is no conflict of interest at all authors.

Acknowledgments

The authors are grateful to the Faculty of Pharmacy, the University of Surabaya had supported this study, and The Research and Community Service Department (LPPM), University of Surabaya, and the Directorate of Higher Education of Indonesia who has funding for the implementation of this research.

References

1. World Health Organization (WHO). World health rankings. 2014 [cited 2017 September 29]. Available from: http://www.worldlifeexpectancy.com/country-health-profile/indonesia.
2. Global Initiative for Asthma. Global strategy for asthma management and prevention. Updated 2017 [cited 2017 September 29]. Available from: https://ginasthma.org/wp-content/uploads/2019/04/wmsGINA-2017-main-report-final_V2.pdf.
3. Niruban SJ, Alagiakrishnan K, Beach J, Senthilselvan A. Association between vitamin D and respiratory outcomes in Canadian adolescents and adults. J Asthma. 2015;52(7):653–61.
4. Shebl RE, Shehata SM, Elgabry M, Ali SAI, Elsaid HH. Vitamin D and phenotypes of bronchial asthma. Egypt J Chest Dis Tuberc. 2013;62(2):201–5.
5. Albanna EAM, Salah KM, Ahmed HS. Effects of vitamin D and the antimicrobial peptide in asthma. Egypt J Pediatr Allergy Immunol. 2012;10(2):101–7.
6. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy, and respiratory infections: the vitamin D hypothesis. Allergy. 2012;67(1):10–7.
7. Elnady HG, Fouda EM, Elsheikh OM, ElAlameey IR, Elshafie AI, Sherif LS, et al. Serum vitamin D level as predictor of bronchial asthma in Egyptian children. J Arab Soc Med Res. 2013;8(2):67–73.
8. Alemu E, Varnam R. Awareness of vitamin D deficiency among at-risk patients. BMC Res Notes. 2012;5:17.
9. Arora H, Dixit V, Srivastava N. Evaluation of knowledge, practices of vitamin D and attitude toward sunlight among Indian students. Asian J Pharm Clin Res. 2016;9(1):308–13.
10. Hartley M, Lucas R, Hoare S, Lithander F, King L, SEDS Investigator Team. The sun exposure and vitamin D study. Paper presented at NIWA UV Workshop; Auckland; 2014 April 15–17 [cited 2017 September 29]. Available from: https://www.niwa.co.nz/sites/niwa.co.nz/files/Hartley_UV%20Workshop_2014.pdf.
11. Zhou M, Zhuang W, Yuan Y, Li Z, Cai Y. Investigation on vitamin D knowledge, attitude and practice of university students in Nanjing, China. Public Health Nutr. 2016;19(1):78–82.
12. Al Bathi BA, Al Zayed KE, Al Qenai M, Makboul G, El-Shazly MK. Knowledge, attitude and practice of patients attending primary care centers toward vitamin D in Kuwait. Alexandria J Med. 2012;48(3):277–82.

13. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients. 2013;5(9):3605–16.
14. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. Curr Opin Allergy Clin Immunol. 2013;13(1):92–9.
15. Lee A, Garbutcheon-Singh KB, Dixit S, Brown P, Smith SD. The influence of age and gender in knowledge, behaviors and attitudes towards sun protection: a cross-sectional survey of Australian outpatient clinic attendees. Am J Clin Dermatol. 2015;16(1):47–54.
16. Gallagher JC. Vitamin D and aging. Endocrinol Metab Clin North Am. 2013;42(2):319–32.
17. Tsiaras WG, Weinstock MA. Factors influencing vitamin D status. Acta Derm Venereol. 2011;91(2):115–24.
18. Bourcher BJ. The problems of vitamin D insufficiency in older people. Aging Dis. 2012;3(4):313–29.
19. Ho-Pham LT, Nguyen MTT. Survey on knowledge and attitudes on vitamin D and sunlight exposure in an urban population in Vietnam. J ASEAN Fed Endocr Soc. 2012;27(2):191–5.
20. Korn S, Hübner M, Jung M, Blettner M, Buhl R. Severe and uncontrolled adult asthma in associated with vitamin D insufficiency and deficiency. Respir Res. 2013;14:25.
21. AlGhamdi KM, AlAklabi AS, AlQahtani AZ. Knowledge, attitudes and practices of the general public toward sun exposure and protection: a national survey in Saudi Arabia. Saudi Pharm J. 2016;24(6):652–7.
22. Herr C, Greulich T, Koczulla RA, Meyer S, Zakharkina T, Branscheidt M, et al. The role of vitamin D in pulmonary disease COPD, asthma, infection and cancer. Respir Res. 2011;12:31.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.4453

GMHC. 2019;7(3):170–6
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Antioxidant and Anti-tyrosinase Activities of *Aloe vera* Rind and Gel Extracts

Sonya Bonifacia Mahadi,[1] Rr. Anisa Siwianti Handayani,[2] Wahyu Widowati,[3] Wilsen,[4] Yunita Dewani,[4] Edy Fachrial,[4] I Nyoman Ehrich Lister[4]

[1]Master in Biomedical Science, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia,
[2]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia,
[3]Department of Biomolecular, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia,
[4]Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Abstract

Aging is a natural process in human that can be characterized by the appearance of black spot on the skin due to hyperpigmentation. Aging may occur due to an excessive amount of free radicals in the body. Antioxidants possess ability to capture free radicals and inhibit tyrosinase which induces skin aging. *Aloe vera* has been used in traditional medicine because it contains several bioactive compounds that act as antioxidant and prevent aging process. This study aims to determine phytochemical content, antioxidant activity and tyrosinase inhibition activity of *Aloe vera* rind (AVRE) and gel (AVGE) extract. This research was carried out at the laboratorium of Aretha Medika Utama-Biomolecular and Biomedical Research Center in Bandung city in September–November 2018. Phytochemical assay was determined using modified Farnsworth method. Antioxidant assay was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and antiaging assay was obtained using tyrosinase inhibition assay. AVRE contains flavonoid, phenol, steroid, and alkaloid. Meanwhile, AVGE contains steroid and alkaloid. IC_{50} DPPH scavenging activity of AVRE was 113.18 µg/mL followed by AVGE was 291.96 µg/mL. IC_{50} tyrosinase inhibition activity of AVRE was 65.04 µg/mL followed by AVGE was 111.89 µg/mL. AVRE had more active DPPH scavenging activity and tyrosinase inhibition activity than AVGE.

Key words: *Aloe vera*, anti-tyrosinase, antioxidants, DPPH

Aktivitas Antioksidan dan Antitirosinase Kulit dan Daging Lidah Buaya (*Aloe vera*)

Abstrak

Penuaan merupakan proses alamiah pada manusia. Penuaan dapat terjadi akibat kadar radikal bebas yang tinggi di dalam tubuh. Antioksidan memiliki kemampuan memerangkap radikal bebas dan menghambat kerja enzim yang berperan dalam proses penuaan. Lidah buaya (*Aloe vera*) telah digunakan dalam pengobatan tradisional karena diketahui mengandung senyawa bioaktif yang bermanfaat dalam menangkal radikal bebas dan menghambat penuaan. Penelitian ini bertujuan mengetahui kandungan fitokimia dalam ekstrak etanol kulit lidah buaya (EKLB) dan ekstrak etanol daging lidah buaya (EDLB), mengetahui aktivitas antioksidan, pemerangkapan 2,2-difenil-1-pikrilhidrazil (DPPH), serta aktivitas antitirosinase EKLB dan EDLB. Penelitian ini dilakukan pada September–November 2018 di laboratorium Aretha Medika Utama Biomolecular and Biomedical Research Center (BBRC). Analisis fitokimia dilakukan menggunakan metode Farnsworth yang dimodifikasi. Aktivitas antioksidan dianalisis menggunakan metode pemerangkapan DPPH, sementara uji antipenuaan dilakukan menggunakan metode uji aktivitas antitirosinase. Hasil uji fitokimia menunjukkan EKLB mengandung flavonoid, fenol, steroid, dan alkaloid; sedangkan EDLB mengandung senyawa steroid dan alkaloid. EKLB dan EDLB memiliki aktivitas pemerangkapan DPPH dengan nilai IC_{50} secara berurutan 113,18 µg/mL dan 291,96 µg/mL. Penghambatan tirosinase EKLB dan EDLB dengan nilai IC_{50} secara berurutan 65,04 µg/mL dan 111,89 µg/mL. EKLB memiliki aktivitas antioksidan dan penghambatan tirosinase yang lebih baik dibanding dengan EDLB.

Kata kunci: *Aloe vera*, antioksidan, antitirosinase, DPPH

Received: 18 February 2019; Revised: 1 November 2019; Accepted: 8 November 2019; Published: 31 December 2019

Correspondence: Dr. I Nyoman Ehrich Lister, dr., M.Kes., AIFM. Faculty of Medicine, Universitas Prima Indonesia. Jln. Belanga No. 1 Simp. Ayahanda, Medan 20118, North Sumatera, Indonesia. E-mail: yysunpri@gmail.com

Introduction

The skin is the outermost part of the body that is most visible making us aware of the aging process every time. Everyone's desire is to live longer, be younger, or at least look younger. According to Mukherjee et al.[1] there are two types of skin aging. The first one is physiological aging, which means chronological aging where aging is due to the passage of time with increasing age and the second is pathological aging, which means photoaging due to environmental factors, namely exposure to ultraviolet (UV) light. This illustrates clinical signs including dry skin, appearance of rough skin, skin pigmentation, deep wrinkles or severe atrophy, telangiectasia, and premalignant lesions.

Human skin colour is one of the most visible phenotypic variations between humans and is mainly determined by the type and amount of melanin synthesized in melanosomes and the pattern of distribution of melanosomes in melanocytes.[2,3] Dark skin occurs because there is a formation of melanin pigment in the skin through tyrosine oxidation by the tyrosinase enzyme to form 3,4-dihydroxy-L-phenylalanine (L-DOPA) which forms the melanin pigment in the final stage.[4] To inhibit the formation of melanin, the tyrosinase enzyme becomes one of the targets of whitening drugs.[5] Melanin production is induced after exposure to UV radiation and plays a major role in protecting skin cells from UV radiation. However, melanin pigmentation in the epidermis can cause skin changes such as darkening of skin color and pigmentation spots.

The need for skin care nowadays is a common thing for everyone, especially for women. Facial skin is a part of the body that describes an overall condition, so it is not surprising that today many kinds of beauty products are sold, especially lightening products that are sold freely from cheap to expensive. Many skin care products have evolved to those which cause mild side effects such as allergies and even those that endanger health to get instant and cheap results. The use of skin lightening products often occurs side effects, such as hydroquinone can cause ochronosis. Excessive use of steroids can cause side effects of skin thinning, hypertrichosis, and hormonal disorders, while mercury is toxic, as well as kidney and nerve damage.[5]

Due to the increasing circulation of various beauty products to watch out for, especially those that use chemicals that ultimately endanger the consumer, the medical world of the present era has studied again the plants that have antioxidant and antiaging effects scientifically. Some examples of plants that have been proven to be antioxidants and antiaging here are roselle flowers (*Hisbiscus sabdarifa*),[6] jasmine flowers (*Jasminum sambac*),[7] ripe sesoot (*Garcinia picrorrhiza* Miq.).[8] The source of antioxidants is mangosteen peel (*Garcinia mangostana* L.),[9] black tea, green tea, oolong tea (*Camellia sinensis* L.),[10] meniran (*Phyllanthus niruri*).[11] In addition to these plants, plants that are efficacious for the health of the body and skin are *Aloe vera*.[12] *Aloe vera* plants can be used for wounds healing and beauty treatments. Originally planted in Indonesia, *Aloe vera* is used for special skin care treatments.[13] One of the plants that is effective for body and skin health is the *Aloe vera* plant.

This is the background of the authors to examine natural ingredients that are safe and affordable, which can be one of the natural choice ingredients in skin care for skin antioxidants and skin lightening. In this case, the authors want to explore and examine the skin and gel of the *Aloe vera* plant in inhibiting the aging process of the skin and brightening the skin.

Methods

Plant determination is carried out to determine the real plant identity that will be used in the study and avoid sampling errors in the phytochemical test. The subjects of this study were the rind and gel extracts from *Aloe vera* (L.) Burm.f. from Semplak Kaum village, Semplak village, Semplak district, Bogor regency. The plants were identified by Herbarium Bogoriense, Botanical Field Research Center for Biology-Indonesian Institute of Sciences Bogor. Each rind and gel of *Aloe vera* (250 g) was mashed and extracted by using 1,000 mL destilled ethanol 70% with a maceration method. In every 24 h the ethanol filtrate was filtered and wastes were remacerated until the filtrate is colorless. Maceration was concentrated using 50°C evaporator to obtain the extract.

Phytochemical screening of *Aloe vera* rind extract (AVRE) and *Aloe vera* gel extract (AVGE) was evaluated with modified Farnsworth method to identify qualitatively presence of flavonoids, saponins, tannins, phenols, terpenoids, steroids/triterpenoids, and alkaloids.[6,14,15]

For flavonoids identification, about 10 mg of each extracts was inserted into the test tube and then Mg and 2N HCl were added. The sample mixture was heated for 5–10 minutes and then cooled and filtered. Then, the amyl alcohol solution was added to the filtrate. The reaction is positive if red/orange colour is formed.[6,14,15]

For tannins identification, about 10 mg of each extracts was added with 2 mL of 2N HCl in a test tube and then heated over a water bath for 30 minutes. The mixture was cooled and filtered. The filtrate was added to amyl alcohol. The reaction is positive if a purple colour is formed.[6,14,15]

For phenols identification, about 10 mg of each extracts was placed on a drip plate and then 1% of FeCl3 (Merck 1.03861.0250, USA) was added into the sample. The presence of phenols was indicated by green/red/purple/blue/black color.[6,14,15]

For steroids/triterpenoids identification, about 10 mg of each extracts was placed on the drip plate and then soaked with acetic acid until the sample was covered. After 10–15 minutes, one drop of absolute sulfuric acid (H_2SO_4) was added to the sample. The formation of green/blue colour showed the presence of steroids while the red/orange sediment showed the triterpenoid.[6,14,15]

For saponins identification, about 10 mg of each extracts was put into a test tube with water, boiled for 5 minutes and then shaken vigorously. The saponin content was indicated by the presence of foam on the surface of the solution.[6,14,15]

For terpenoids identification, about 10 mg of each extracts was placed on a drip plate and then vanillin and H_2SO_4 solution were added. The reaction is positive if the colour changes to purple.[6,14,15]

For alkaloids identification, about 10 mg of each extracts was put into the test tube and 10% of ammonia was added to the sample. Then, chloroform was added to the mixture of sample and two layers of liquid were formed. The lower layer was collected and then transferred to the test tube and inserted into 1N HCl until it formed two layers. The top layer was collected and then transferred to a new test tube. Then 1–2 drops of Draggendorf solution was added. The reaction is positive when yellow colour is formed.[6,14,15]

A total of 200 µL 2,2-difenil-1-pikrilhidrazil (DPPH) (Sigma Aldrich D9132, USA) 0.077 mmol in methanol was added with 50 µL of *Aloe vera* ethanol extract on the 96-well microplate. The mixture was incubated at room temperature for 30 minutes and then the absorbance value was read at 517 nm wavelength using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA). For the negative control, 250 µL of DPPH was used, while for blanks, 250 µL of an absolute DMSO was used.[6,7,10] The DPPH extracts and compounds generally fade purple colour into colourless when antioxidant molecules quench DPPH free radicals. The radical scavenging activity was measured using the following formula.

$$\text{Scavenging (\%)} = (Ac - As)/Ac \times 100$$

Description: Ac: negative control absorbance (without sample); As: sample absorbance

The median inhibitory concentration (IC_{50}) value of DPPH activity was calculated.

The inhibition of tyrosinase enzyme activity was measured based on the method described by Sigma Aldrich, Tocco et al.,[16] as well as Tu and Tawata,[17] and Widowati et al.[6,7,10] with minor modifications. The solution mixture consisted of 20 µL samples, 20 µL tyrosinase enzymes from the mushroom (125 U/mL, Sigma T3824), and 140 µL potassium phosphate buffers (20 mm, pH 6.8, Merck 104873, Merck 105104) incubated at room temperature for 15 minutes. In addition, it was also prepared for controls containing only 20 µL enzymes and 160 µL phosphate buffers and blanks containing only 160 µL phosphate buffers and 20 µL samples. Furthermore, a mixture of 20 µL of the L-DOPA substrate (1.5 mm, Sigma D9628) was added and incubated again at room temperature for 10 minutes. The absorbance was measured at 470 nm wavelength. The percentage of inhibitory activity was calculated using the formula.

$$\text{Tyrosinase inhibitory activity (\%)} = \frac{A - B}{A} \times 100$$

Description: A: control absorbance; B: sample absorbance

Statistical analysis was conducted using SPSS software (version 20.0). Value was presented as mean±standard deviation. Significant differences between the groups were determined using the analysis of variance (one-way ANOVA) followed by Tukey's HSD post-hoc test. The results of DPPH and anti-tyrosinase activity tests were continued by linear regression analysis. Then the value of Inhibitory Concentration 50 (IC_{50}) was determined.

Results

The percentage of AVRE yield was 2.71% and AVGE was 9.54%. These results indicate that AVRE extract is lower than AVGE. The quality of extract produced is usually inversely proportional to the amount of yield produced.

Phytochemical screening is a method to determine the class of chemical compounds contained in a plant qualitatively. The selection of solvents and extraction methods is the most important factor in conducting phytochemical screening. The compounds tested were flavonoids, saponins, phenols, tannins, terpenoids, alkaloids, and steroids/triterpenoids.

Based on Table 1, it is known that the levels of alkaloid content in both AVRE and AVGE were high. The content of flavonoids and phenols in the AVRE was low while in AVGE was undetected. Both extracts contain steroids, and there was no saponin, tannin, and terpenoids.

DPPH is a free radical that belongs to the hydrogen radical group. DPPH is sensitive to light, oxygen, and pH. However, it is stable in a radical form so it may be quite an accurate measurement of antioxidant activity. DPPH free radicals can capture hydrogen atoms from extract components which are mixed and then reacted to their reduced form and are characterized by reduced intensity of purple DPPH solution with maximum uptake at 517 nm.[10,18]

In the DPPH scavenging activity test, the final concentration of the sample used was 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. The DPPH scavenging activities by AVRE and AVGE samples can be seen in Figure 1. The DPPH assay is a colorimetric method to determine anti-free radical activity.[10] To find out whether there are differences in the various concentrations of AVRE and AVGE, statistical analysis was performed using one-way analysis of variance (ANOVA) with a degree of significance $p<0.05$. In Figure 1, it can be seen at all of concentration between AVRE and AVGE that the activities were different which AVGE was lower than AVRE. The IC_{50} value in the DPPH scavenging activity assay can be seen in Table 2.

Tyrosinase is an enzyme that plays a role in the formation of pigments such as melanin and other polyphenols. Tyrosinase inhibition activity from AVRE and AVGE is measured using L-DOPA as a substrate.[16] Tyrosinase catalyzes tyrosine oxidation reactions that produce chromophore and can be detected at wavelengths up to 470 nm. The following is the scheme of the reactions that occur (Figure 2).

This enzyme uses molecular oxygen to catalyze monophenol oxidation to the appropriate o-phenol and subsequent oxidation becomes o-quinone. The active center of tyrosinase, which is composed of dinuclear copper, is flexible during catalysis transfer.

In the tyrosinase inhibition activity test, the sample concentration used was 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.13 µg/mL. The results of the activity test can be seen in Figure 3.

In Figure 3, it can be observed that the higher the concentration of extract used, the higher the tyrosinase inhibition activity that occurs. There was a significant difference in tyrosinase inhibition activity with a concentration of 100 µg/mL between *Aloe vera* rind and gel extracts. IC_{50} values in tyrosinase inhibition activity test

Table 1 Qualitative Phytochemical Screening Results of Aloe vera Rind and Gel Extracts

Contents	AVRE	AVGE
Flavonoids	+	–
Saponins	–	–
Phenols	+	–
Tannins	–	–
Steroids/triterpenoids	+/–	+/–
Terpenoids	–	–
Alkaloids	+++	+++

++++: very high content, +++: high content, ++: medium content, +: low content, –: no content

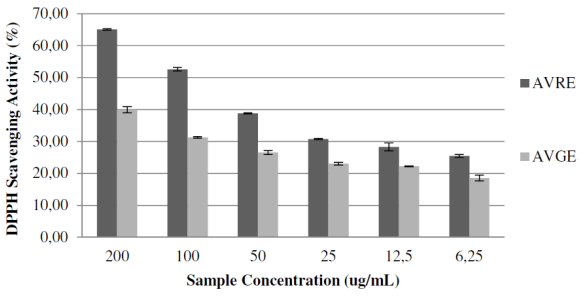

Figure 1 Comparison of DPPH Scavenging Activities by AVRE and AVGE

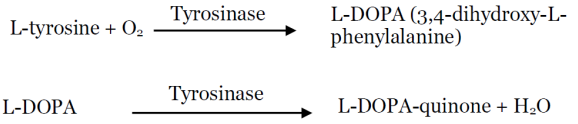

Figure 2 Role of Tyrosinase in Pigment Formation

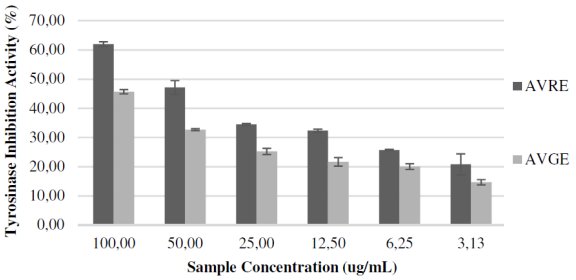

Figure 3 Comparison of Tyrosinase Inhibition Activities by AVRE and AVGE

can be seen in Table 3.

Discussion

Aloe vera contains enzymes, vitamins and minerals, natural sugars, amino acids, and antimicrobial, anti-inflammatory, and antioxidant agents. These plants are often used in beauty products for antiaging, anti-wrinkle and moisturizing creams. *Aloe vera* has the potential to cure skin diseases. *Aloe vera* is also commonly used to treat zits, black or white spots, stretch marks, and wrinkles.[19]

AVRE and AVGE were then tested for their phytochemical content qualitatively using the Farnsworth method with modification.[6,7,14,15] Phytochemical test results showed that AVRE contained a small amount of flavonoids, phenols, and steroids and a large number of alkaloids. Meanwhile, AVGE has only a small amount of steroids and a large number of alkaloids.

According to Furnawanthi,[20] one of the secondary metabolites contained in *Aloe vera* is saponin. However, in this study, no saponin

Table 2 IC_{50} Values of DPPH Scavenging Activities of AVRE and AVGE

Samples	Equation	R^2	IC_{50} (µg/mL)	
AVRE (replication 1)	y=0.2082x+26.331	0.97	113.68	113.18±0.49
AVRE (replication 2)	y=0.2077x+26.498	0.96	113.15	
AVRE (replication 3)	y=0.2043x+26.973	0.96	112.71	
AVRE (average)	y=0.2067x+26.601	0.96	113.20	
AVGE (replication 1)	y=0.1008x+20.634	0.98	291.33	291.96±11.85
AVGE (replication 2)	y=0.098x+20.197	0.96	304.11	
AVGE (replication 3)	y=0.1077x+19.797	0.97	280.44	
AVGE (average)	y=0.1022x+20.21	0.97	291.49	

Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated

Table 3 IC_{50} Values of Tyrosinase Inhibition Activities of AVRE and AVGE

Samples	Equation	R^2	IC_{50} (µg/mL)	
AVRE (replication 1)	y=0.3826x+25.274	0.96	64.63	65.04±1.63
AVRE (replication 2)	y=0.4111x+22.523	0.94	66.84	
AVRE (replication 3)	y=0.4069x+24.095	0.97	63.66	
AVRE (average)	y=0.4002x+23.964	0.96	65.06	
AVGE (replication 1)	y=0.2888x+17.269	0.95	113.33	111.89±1.25
AVGE (replication 2)	y=0.2948x+17.246	0.98	111.11	
AVGE (replication 3)	y=0.3012x+16.495	0.99	111.24	
AVGE (average)	y=0.2949x+17.003	0.98	111.89	

Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated

content was found in the AVRE or in the AVGE. This can be caused by various factors from environmental condition in which the *Aloe vera* was grown to the different solvents used in the maceration process.[20,21]

The antioxidant activity test by AVRE and AVGE in this study was carried out through DPPH scavenging test. The DPPH scavenging test is a fast and easy method. The DPPH free radical method is an antioxidant test based on electron transfer which produces a purple ethanol solution. These free radicals are stable at room temperature and will decrease in color intensity with the presence of antioxidant molecules.[22]

In the DPPH scavenging test, AVRE has better DPPH scavenging activity compared to AVGE (Figure 1). At the highest concentration (200 µg/mL) AVRE has a scavenging activity of 65.07±0.24% followed by AVGE of 39.93±1.00%. In addition, the IC_{50} value of AVRE (110.36 µg/mL) is smaller than the IC_{50} value of AVGE (303.06 µg/mL). This result is better compared to IC_{50} value of *Aloe vera* extract obtained by Prahesti et al.,[23] which is equal to 519.2 mg/L or equivalent to 519.2 µg/mL. According to Widowati et al.,[6] the smaller the IC_{50} value of a sample, the better the ability of the sample to trap free radicals. Meanwhile, according to Molyneux,[24] a substance has antioxidant activity if the IC_{50} obtained ranges from 200–1,000 µg/mL, including substances that are less active but are still considered as potential.

AVRE has a DPPH scavenging activity that is better than AVGE because of the content of flavonoids, phenols, and alkaloids. Phenols act as an antioxidant because their structure contains a hydroxyl (-OH) group which can donate hydrogen atoms (H^+) to free radicals. In addition, according to Gan et al.,[25] alkaloids have an antioxidant role even stronger than phenol

Antioxidant activity of AVRE and AVGE is also estimated to have a correlation with antiaging activity. In the tyrosinase inhibition test, AVRE has more tyrosinase inhibitory activity compared to AVGE (Figure 3). The highest activity was at the concentration of 100 µg/mL, AVRE was 62.02±0.79% and AVGE was 45.70±0.71%. IC_{50} values of AVRE 60.02 µg/mL and AVGE were 111.89 µg/mL.

Conclusions

Based on the research, it is shown that AVRE contains flavonoids, phenols, steroids and alkaloids. AVGE contains steroid compounds and alkaloids. Antioxidant activities in DPPH scavenging of AVRE and AVGE based on IC50 values are 113.18 µg/mL and 291.96 µg/mL. Meanwhile the antiaging activity in tyrosinase inhibition of AVRE and AVGE based on IC50 values are 65.04 µg/mL and 111.89 µg/mL.

Conflict of Interest

All authors state there was no conflict of interest.

Acknowledgment

Authors gratefully acknowledge Aretha Medika Utama-Biomolecular and Biomedical Research Center, Bandung, Indonesia for methodology and laboratory facilities, and their staffs for technical support.

References

1. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011;19(1):64–73.
2. Malathi M, Thappa DM. Systemic skin whitening/lightening agents: what is the evidence. Indian J Dermatol Venereol Leprol. 2013;79(6):842–6.
3. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci. 2013;14(6):12222–48.
4. Chang TS. An updated review of tyrosinase inhibitors. Int J Mol Sci. 2009;10(6):2440–75.
5. Arbab AHH, Eltahir MM. Review on skin whitening agents. Khartoum Pharm J. 2010;13(1):5–9.
6. Widowati W, Rani AP, Hamzah RA, Arumwardana S, Afifah E, Kusuma HSW, et al. Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. Nat Prod Sci. 2017;23(3):192–200.
7. Widowati W, Janeva BW, Nadya S, Amalia A, Arumwardana S, Kusuma HSW, et al. Antioxidant and antiaging activities of *Jasminum sambac* extract, and its compounds. J Rep Pharm Sci. 2018;7(3):270–85.
8. Utami S, Sachrowardi QR, Damayanti NA, Wardhana A, Syarif I, Nafik S, et

al. Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit as antiaging. J Herbmed Pharmacol. 2018;7(2):88–93.
9. Widowati W, Darsono L, Suherman J, Yelliantty Y, Maesaroh M. High performance lipid chromatography (HPLC) analysis, antioxidant, antiaggregation of mangosteen peel extract (*Garcinia mangostana* L.). Int J Biosci Biochem Bioinforma. 2014;4(6):458–66.
10. Widowati W, Herlina T, Ratnawati H, Constantia G, Deva IDGS, Maesaroh M. Antioxidant potential of black, green and oolong tea methanol extracts. Biol Med Nat Prod Chem. 2015;4(2):35–9.
11. Rusmana D, Wahyudianingsih R, Elisabeth M, Balqis B, Maesaroh M, Widowati W. Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin. Indones Biomed J. 2017;9(2):84–90.
12. Surjushe A, Vasani R, Saple DG. *Aloe vera*: a short review. Indian J Dermatol. 2008;53(4):163–6.
13. Mahandaru D, Dachlan I. The effect of *Aloe vera* on healing process of incision wound. J Plast Rekons. 2012;1(1):82–7.
14. Bera TK, Chatterjee K, Ghosh D. In vitro antioxidant properties of the hydro-methanol extract of the seeds of *Switenia mahagoni* (L.) Jacq. Biomark Genom Med. 2015;7(1):18–24.
15. Adnyana IK, Abuzaid AS, Iskandar EY, Kurniati NF. Pancreatic lipase and α-amylase inhibitory potential of mangosteen (*Garcinia mangostana* Linn.) pericarp extract. Int J Med Res Health Sci. 2016;5(1):23–8.
16. Tocco G, Fais A, Meli G, Begala M, Podda G, Fadda MB, et al. PEG-immobilization of cardol and soluble polymer-supported synthesis of some cardol-coumarin derivatives: preliminary evaluation of their inhibitory activity on mushroom tyrosinase. Bioorg Med Chem Lett. 2009;19(1):36–9.
17. Tu PTB, Tawata S. Anti-oxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of *Alpinia zerumbet*. Molecules. 2015;20(9):16723–40.
18. Widowati W, Fauziah N, Herdiman H, Afni M, Afifah E, Kusuma HSW, et al. Antioxidant and anti aging assays of *Oryza sativa* extracts, vanillin and coumaric acid. J Nat Remed. 2016;16(3):88–99.
19. Hooda R. Antiwrinkle herbal drugs-an update. J Pharmacogn Phytochem. 2015;4(4):277–81.
20. Furnawanthi I. Khasiat dan manfaat lidah buaya: si tanaman ajaib. Jakarta: AgroMedia Pustaka; 2003.
21. Patel DK, Patel K, Dhanabal SP. Phytochemical standardization of *Aloe vera* extract by HPTLC techniques. J Acute Dis. 2012;1(1):47–50.
22. Garcia EJ, Oldoni TLC, Alencar SMD, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Braz Dent J. 2012;23(1):22–7.
23. Prahesti NR, Suzery M, Cahyono B. The antioxidant activities, phenolic total and cytotoxicity of extract and fractions of *Aloe vera* Linn. JSM. 2015;23(2):50–4.
24. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. SJST. 2004;26(2):211–9.
25. Gan J, Feng Y, He Z, Li X, Zhang H. Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*). J Food Qual. 2017;2017:3185945.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3313

GMHC. 2019;7(3):177–83
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Health Conditions and Dangers Due to Work for Fishers in Pangandaran District, West Java

Neti Juniarti, Hartiah Haroen, Desy Indra Yani
Department of Community Health Nursing, Faculty of Nursing, Universitas Padjadjaran, Sumedang, Indonesia

Abstract

Work safety and health are essential for fishers in Pangandaran Beach because these fishers were not paying attention to their work health and safety. This attitude would pose a high risk for the fishers due to the weather conditions that can turn wild in a short period and can cause an elevated risk of an accident. This study aimed to identify the health conditions, hazards, and work accidents that were experienced by Pangandaran fishers. The methods used quantitative descriptive. The survey conducted in Pangandaran subdistrict, West Java in November 2017. The samples were recruited purposively, and a total of 17 fishers were willing to participate. The data were analyzed using descriptive univariate analysis. The results showed that 7 of 17 fishers have a health problem, and most of them have high blood pressure. All participants had experienced accident during their work, dan only 9 of 17 who wear safety equipment. Hazardous work hazard is a green jellyfish sting during fishing. Thus, it is recommended to the public health center, Regency Health Office, and Fisheries Office to establish the fishers' health post, which is close to the beach. And also provide training about first aid in the event of an accident of jellyfish stung.

Key words: Fishers' health, protection device, work safety

Kondisi Kesehatan dan Bahaya Akibat Kerja pada Nelayan di Kecamatan Pangandaran, Jawa Barat

Abstrak

Keselamatan dan kesehatan kerja sangat penting untuk para nelayan yang bekerja di sekitar pantai Pangandaran karena para nelayan di pantai Pangandaran masih kurang memperhatikan keselamatan dan kesehatan kerja. Hal ini sangat berisiko untuk keselamatan nelayan karena cuaca di pantai dan laut dapat berubah dengan cepat dan mengakibatkan risiko kecelakaan yang sangat tinggi. Penelitian ini bertujuan mengetahui kondisi kesehatan dan bahaya serta kecelakaan kerja yang dialami oleh nelayan di Kecamatan Pangandaran. Metode penelitian menggunakan deskriptif kuantitatif. Penelitian ini dilakukan di Kecamatan Pangandaran, Jawa Barat pada bulan November 2017. Penarikan sampel dilakukan secara *purposive* dan jumlah sampel sebanyak 17 orang. Data dianalisis menggunakan analisis univariat. Hasil penelitian menunjukkan bahwa 7 dari 17 nelayan memiliki masalah kesehatan dengan jumlah terbanyak menderita hipertensi. Semua responden pernah mengalami bahaya dan kecelakaan akibat kerja, serta hanya 9 dari 17 yang menggunakan pelampung. Bahaya kesehatan yang paling besar dikeluhkan oleh nelayan adalah sengatan ubur-ubur hijau. Oleh karena itu, disarankan pada pihak puskesmas, Dinas Kesehatan, serta Dinas Perikanan dan Kelautan setempat untuk membentuk pos usaha kesehatan kerja nelayan di Pangandaran, serta memberikan pelatihan tentang penanganan kecelakaan dan perawatan pasien yang terkena sengatan ubur-ubur hijau.

Kata kunci: Alat pelindung diri, kesehatan nelayan, keselamatan kerja

Received: 19 December 2017; Revised: 25 August 2019; Accepted: 20 December 2019; Published: 31 December 2019

Correspondence: Neti Juniarti, S.Kp., M.Kes., M.Nurs., Ph.D. Faculty of Nursing, Universitas Padjadjaran. Jln. Raya Bandung-Sumedang km 21, Jatinangor, Sumedang 45363, West Java, Indonesia. E-mail: neti.juniarti@unpad.ac.id

Introduction

A healthy paradigm approach is vital for fishers to improve their health conditions through occupational health and safety (OHS) efforts. Occupational health and safety is also essential to apply to the informal sector, such as fishers in Pangandaran subdistrict, Pangandaran regency is one of the regencies in West Java province, which has only been a Regency Government since 2012.[1]

This regency is strategically located, because it is situated on a provincial road, is on the coast with a length of 91 km, and has a variety of potential to be developed. Pangandaran regency is a district in West Java province whose capital is in Parigi. This regency is bordered by Ciamis regency and Banjar city in the north, Cilacap regency in the east, the Indian Ocean in the south, and Tasikmalaya Regency in the west.[1] The birth of the Pangandaran regency was based on Law Number 21 of 2012 as a new district (*daerah otonom baru*/DOB), which was signed by the President of Republic of Indonesia on 2012 November 16.[2] Then it was promulgated by the Minister of Law and Human Rights on 2012 November 17, later Pangandaran officially became a regency in West Java province. The Law Number: 21/2012 stated Pangandaran regency came from a part of the Ciamis regency.[2] The area of Pangandaran regency is 168,509 ha with a sea area of 67,340 ha.[1]

Pangandaran regency has a beach length of 91 km. The total population, according to sex in 2014, women numbered 212,022 people, and men totaled 210,564 people. The government covers ten subdistricts, namely Parigi subdistrict, Cijulang subdistrict, Cimerak subdistrict, Cigugur subdistrict, Langkaplancar subdistrict, Mangunjaya subdistrict, Padaherang subdistrict, Kalipucang subdistrict, Pangandaran subdistrict and Sidamulih subdistrict.[1]

The highest number of fishers is in the Pangandaran subdistrict, which is 2,935 people, with a total of 4,141 in 2015.[1] Considering the decreasing number of fishers in the Pangandaran subdistrict, it is necessary to study health conditions and work-related hazards for fishers to improve OHS.

Occupational health and safety is a condition and factors that affect the health and safety of employees or other workers (including temporary workers), visitors, or other people in the work area.[3] In the framework of ensuring occupational safety and health in Indonesia, Law Number 23 of 2003 concerning workforce article 86 states that every worker has the right to obtain protection for occupational safety and health.[4]

Occupational health and safety are needed to overcome risks that can arise in the scope of work, where the risk is a combination of the possibility of danger or exposure and the severity of injuries or health problems that can be caused by an event or exposure. The importance of occupational health and safety efforts because every work accident that occurs will cause economic losses, such as damage to machinery, equipment, building materials, medical costs, and accident compensation costs. Therefore, by taking steps to prevent accidents, in addition to preventing injury to workers, it can also save costs.[3]

Occupational safety and health are also crucial for fishers who work around Pangandaran beach. Based on the observation of researchers, the fishers on Pangandaran beach still pay less attention to occupational safety and health, such as not using proper footwear, not using personal protective equipment and safety buoys. It is very risky for the protection of fishers because the weather on the beach and sea can change quickly and result in a very high risk of accidents. The results also showed that fishers in the Thousand islands experienced hazards for worker safety including waves, slippery floors, fish spines, pinched, compressor engine fuel, corrosive fire hose, air pressure on the compressor engine tube, release levers, corals, bites of marine life, the tubing is bent, broken, or leaking and the body is stuck in the propeller of the ship. Health hazards include ergonomics, noise, extreme pressure, cold temperatures, hot temperatures, stinging fish and poisonous corals, CO gas, CO_2, and nitrogen.[5]

According to studies, efforts can be made to prevent health problems and work-related accidents there are five levels, namely health promotion, specific protection, early diagnosis and prompt treatment, disability limitation, and ehabilitation.[6]

Health promotion consists of health education for workers, improvement, and improvement of workers' nutrition, healthy development of workers' psychology, provision of robust worker housing, recreation for workers, provision of a healthy workplace and environment, inspection before work, attention to descent factors.[6]

The activities that can be carried out in

terms of specific protection include complete immunization, good work hygiene, sanitation of a healthy work environment, protection against work hazards, control of work-related dangers so that they are safe, protection against carcinogenic factors, avoid causes of allergies, human association (workers) with machines.[6]

Early diagnosis and prompt treatment encompass activities including looking for workers, both individuals or groups, to certain disease disorders, regular general check-ups for workers, screening.[6]

Disability limitation can be done to prevent disability is an adequate treatment to prevent and stop the disease process, excellent care, provision of facilities to limit disability and to avoid death. Rehabilitation can be done through training and education to train existing capabilities, community education to use disabled workers, selective placement of disabled workers, work therapy in hospitals, providing a protected workplace.[6]

Based on this background, a study was conducted on health conditions and hazards resulting from work for fishers in the Pangandaran subdistrict area. The general objective of this study is to improve primary health services in fishers groups through occupational health nursing care at Pangandaran beach in achieving optimal quality of life.

Methods

This research was a quantitative descriptive study. This research was conducted in Pangandaran subdistrict in November 2017. Sampling was done purposively, and the number of samples was 17 because of the difficulty of meeting fishers, and many fishers refused to be investigated on the grounds they were preparing nets for fishing. To respect participant rights, researchers only included data from fishers who voluntarily participated in this study. Fishers' health checks were carried out by three researchers with nursing education backgrounds. When checking, participants did not feel awkward, giving comments or answers. Before data collection, researchers had obtained ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran (letter number: 820/UN6.C10/PN/2017) and research permission from the Health Officer and the Office of the Indonesian Unity and Politic Pangandaran.

Data were analyzed using descriptive statistics to describe health conditions and occupational hazards experienced by fishers while working.

Results

The number of samples collected was 17 fishers. The results of physical health checks and occupational health counseling for these fishers obtained demographic data from 17 fishers along with several health problems and work accidents they have experienced. Table 1 shows demographic data and fishers health problems in Pangandaran.

Based on Table 2, the fishers in Pangandaran experienced various health problems. Looking at the fishers' health data, follow up on the efforts of primary health care following the existing issues is essential.

In addition to health problems experiencing health problems, the fishers also suffered work accidents while at sea. This data is presented in Table 3.

Table 3 shows that the two prominent work hazard was biological such as being bitten by a fish or being stung by green jellyfish and ergonomic problems. When asked about the use of personal protective equipment (PPE), the fishers answered varied as presented in Table 4.

From Table 4, it can be seen that awareness to use personal protective equipment, especially buoys, masks, and boots, was still quite low.

Table 1 Fishers Respondent Demographic Data

Demographic Data	f (n=17)
Age (year)	
<20	1
21–30	3
31–40	1
41–50	4
51–60	8
Gender	
Male	16
Female	1
Working period as a fishers (year)	
0–9	2
10–19	4
20–29	5
30–39	3
>40	3

Table 2 Health Problems Encountered by Fishers

Type	f (n=17)
Health condition	
Healthy	10
Unhealthy	7
Illness experienced	
Gout and rheumatism	3
Flu	2
Toothache	1
Back pain	1
High blood pressure	10

Discussion

These fishers work from early morning until morning, and they return home around 11 am. After that, they will get enough rest and sleep to get tired after working. It makes it difficult for the fishers to access services at the public health center (PHC) because PHC service hours are open from 08:00 to 14:00. By doing family nursing care, fishers can be visited at their homes at a time that suits their conditions after resting and taking a nap. Thus, occupational health nursing is needed to provide nursing care and health check-up for the traditional fishers. Occupational health nursing is a branch of community health care that provides services to workers or groups of workers. Services focus on health promotion, protection, and recovery of the workforce's health about safety and a healthy work environment.[7]

Occupational health nursing is the application of nursing and public health as well as skills related to workers for the prevention of illness and accident as well as health improvement in an optimal, productive and socially acceptable manner.[8]

Table 3 Work Hazards and Accidents Experienced by Fishers

Types of Work Accidents	f (n=17)
Work accidents	
Bitten by a fish or stung by a	9
jellyfish	7
Leaking boat or boat overturned	1
Fell from the boat	
Ergonomic problems	
Yes	12
No	5
Chemical hazard	
Yes	8
No	9

Table 4 Personal Protective Equipment Used

Type of Personal Protective Equipment	f (n=17)
Live vest	
Yes	9
No	8
Raincoat	
Yes	11
No	6
Gloves	
Yes	15
No	2
Mask	
Yes	1
No	16
Hat/helm	
Yes	13
No	3
Boots	
Yes	2
No	15

The results of this study indicate that almost half of fishers experience health problems due to work. Factors that can cause health problems include workload, the additional burden due to the environment, work capacity.[8]

The workload is a workload that must be borne by workers, which can be physical, mental, or social. If the workload exceeds work capacity, it can cause health problems. The additional burden due to the environment is the workload obtained due to the work environment that does not support the work process. Potential hazards consists of physical factors in the form of noise, temperature, vibration, pressure; chemical factors for example gas, dust, CO, insecticides; biological factors such as viruses, bacteria and other organisms; and psychological mental factors, in the form of demands, policies or management of companies that do not support, social relations that are not harmonious; and ergonomics.

Work capacity is the ability of a worker in carrying out his work. Work capacity depends on the skills mastered by workers, sex, age of

workers, body proportions, work nutrition, harmony in practice. The results of this study indicate that there are several burdens and hazards experienced by fishers. It is in line with research which states that there are many occupational hazards encountered by fishers.[5] This work hazard is also aggravated by fishers' health conditions, that are less than optimal. It is in line with the results of research that shows that the health of fishers in East Lombok regency was still relatively low even though the local Health Office has sought measures to improve the health of fishers' work.[9]

Occupational health care is carried out to protect the health of workers from potential threats emanating from the workplace and helping workers to create a work environment that is appropriate to their physical, mental, and emotional capacities. Thus, the efficiency levels can be achieved without conditions that endanger health and safety, guaranteeing service adequate health and rehabilitation from illness and injury, and encourage workers to maintain optimal health.[8] It is not only done by workers in large companies, but fishers groups also need to get optimal occupational health care.

Occupational health nurses can provide direct care which includes carrying out assessments according to the health needs of workers, formulating nursing diagnoses of nursing, planning, implementing actions and evaluating the impact of interventions; provide health services related to preventing, maintaining and correcting health problems.[10,11]

All respondents stated the need for Fishers Occupational Health Posts close to the beach, and they can use them if there are accidents and emergency conditions when they are work so that they don't have to go far to the PHC if they want to have their blood pressure or health condition checked. Nurses can play a role in coordinating administrative and service functions for the fishers to achieve cost-effective measures. As a manager/ administrator, nurses can create programs to improve worker health by applying management principles (using SWOT analysis in assessing, determining needs, planning, implementing the program, conducting monitoring and evaluating programs.[8] The other role of the nurse is as an educator to do health education for workers about risk management, prevention of illness and injury, the use of personal protective equipment, and others. The nurse is also a researcher to develop the program based on research results.[10,11]

Occupational health nursing care begins with the assessment until evaluation. Assessment in occupational health consists of assessing human biology, including characteristics of age and sex, genetic health problems of workers, physical function by identifying various body systems. Environmental assessment includes multiple potential hazards that can cause health problems due to work include physical, biological, chemical, psychosocial, ergonomic hazards. Lifestyle assessment includes patterns of food consumption, activity and rest, appearance at work, use of self-supporting devices. Assessment of the health system which consists of a health service system (referral), a monitoring program related to occupational safety, existing health promotion policies and programs, limitations, and promotion and protection efforts, a health care system for working families.[10,11]

In accordance with the results of another study, it is known that there are several factors associated with the disease occurrence in fishers, including that the length of service (p=0.001), personal protective equipment (PPE, p=0.001), work history (p=0.027), own health (p=0.027).[12] It is also in line with the results of this study, which shows that most fishers have more than ten years of service, inadequate use of personal protective equipment and are a matter of their health. Another factor that also affects health is the level of welfare of fishers. The results showed that 15% of fishers had a high level of welfare, while 85% had a medium level of welfare.[13]

After the assessment, a nursing diagnosis is carried out, which includes the client's health status, occupational illness, at-risk population, workplace hazards. This diagnosis is intervened by compiling a problem-solving plan in occupational health nursing that includes three levels of prevention consisting of After the assessment, a nursing diagnosis is carried out, which includes the client's health status, occupational illness, at-risk population, workplace hazards. This diagnosis is intervened by compiling a problem-solving plan in occupational health nursing that includes three levels of prevention consisting of primary prevention, secondary prevention, and tertiary prevention.[10]

Included in primary prevention activities are health promotion provides health education activities, improvement of nutrition, rest, and exercise for workers; prevention of

diseases includes reducing risk factors, giving immunizations, stress management; and injury prevention, which includes safety education, the use of personal protective equipment (PPE), handling dangerous substances, reducing hazards that threaten safety, improving ergonomic health.[10]

Included in secondary prevention efforts are screening workers, periodic health checks, examining environmental aspects that could pose a hazard to workers; case management; and emergency handling which includes physical, psychological, and work-related accidents.[10]

Included in secondary prevention activities include prevention of the spread of infectious diseases; prevention of recurrence; prevention of complications; and worker rehabilitation.[10]

Planning related to fisheries management and the fishers' health needs to be prepared by involving fishers as actors and also involving other stakeholders.[14] The fishers are partners of occupational health nurses to improve the degree of public health.[10]

Conclusions

It can be concluded that fishers have various health problems, have experienced work hazards and accidents, as well as paying less attention to the use of personal protective equipment. The most significant health hazard complained of by fishers is the sting of green jellyfish. Therefore it is suggested to the community health center, the Health Service, and the local Fisheries and Maritime Service to establish a Fishers Occupational Health Post in Pangandaran, as well as provide training on accident management and treatment of patients affected by green jellyfish stings.

Conclusions

It can be concluded that fishers have various health problems, have experienced work hazards and accidents, as well as paying less attention to the use of personal protective equipment. The most significant health hazard complained of by fishers is the sting of green jellyfish. Therefore it is suggested to the community health center, the Health Service, and the local Fisheries and Maritime Service to establish a Fishers Occupational Health Post in Pangandaran, as well as provide training on accident management and treatment of patients affected by green jellyfish stings.

Conflict of Interest

All authors state there was no conflict of interest.

References

1. Badan Perencanaan Pembangunan Daerah (Bappeda) Pemerintah Provinsi Jawa Barat. Laporan akhir penyusunan rencana kebutuhan investasi pusat pertumbuhan Pangandaran Raya [Internet]. Bandung: Bappeda Jawa Barat; 2016 [cited 2017 August 10]. Available from: http://bappeda.jabarprov.go.id/wp-content/uploads/2017/03/Laporan-Akhir-Kebutuhan-Inv-Pangandaran-Raya.pdf.
2. Undang-Undang Republik Indonesia Nomor 21 Tahun 2012 tentang Pembentukan Kabupaten Pangandaran di Provinsi Jawa Barat.
3. Suma'mur. Higiene perusahaan dan kesehatan kerja (Hiperkes). Jakarta: Sagung Seto; 2009.
4. Undang-Undang Republik Indonesia Nomor 23 Tahun 2003 tentang Ketenagakerjaan.
5. Dharmawirawan DA, Modjo R. Identifikasi bahaya keselamatan dan kesehatan kerja pada penangkapan ikan nelayan Muroami. Kesmas. 2012;6(4):185–92.
6. Efendi F, Makhfudli. Keperawatan kesehatan komunitas: teori dan praktik dalam keperawatan. Jakarta: Salemba Medika; 2009.
7. American Association of Occupational Health Nurses. What is occupational & environmental health nursing? [Internet]. AAOHN, Inc. 2017 [cited 2017 December 19]. Available from: http://aaohn.org/page/profession-of-occupational-and-environmental-health-nursing.
8. Oakley K. Occupational health nursing. 3rd Edition. Chichester, England: John Wiley & Sons; 2008.
9. Martiana T, Wilujeng LK. Upaya kesehatan kerja sektor informal dan lingkungan perumahan nelayan di Kabupaten Lombok Timur NTB. JKL. 2006;2(2):153–62.
10. Anderson ET. Community health nursing: essentials of practice. In: Anderson ET, McFarlane J, editors. Community as partner: theory and practice in nursing. 7th Edition.

Philadelphia: Wolters Kluwer; 2015. p. 19–28.
11. Kulbok PA, Thatcher E, Park E, Meszaros P. Evolving public health nursing roles: focus on community participatory health promotion and prevention. Online J Issues Nurs. 2012;17(2):1.
12. Cahyawati IN, Budiono I. Faktor yang berhubungan dengan kejadian dermatitis pada nelayan. Kemas. 2011;6(2):134–41.
13. Sugiharto E. Tingkat kesejahteraan masyarakat nelayan Desa Benua Baru Ilir berdasarkan indikator Badan Pusat Statistik. EPP. 2007;4(2):32–6.
14. Monintja D, Yusfiandayani R. Pemanfaatan sumberdaya pesisir dalam bidang perikanan tangkap. In: Bengen DG, editor. Prosiding Pelatihan Pengelolaan Wilayah Pesisir Terpadu; 2001 October 29–November 3; Bogor, Indonesia. Bogor: Pusat Kajian Sumberdaya Pesisir dan Lautan, Institut Pertanian Bogor; 2001 [cited 2017 December 28]. p. 56–65. Available from: https://www.crc.uri.edu/download/Proceeding_ToT_ICM.pdf.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.5341

GMHC. 2019;7(3):184–8
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

D-Dimer Level with Cerebral Venous Sinus Thrombosis (CVST) Occurrence Using Digital Subtraction Angiography (DSA)

Afdi Arahim Putra,[1] Eppy Buchori,[2] Hilman,[2] Lisda Amalia[3]

[1]Radiology Study Program, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, [2]Department of Radiology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, [3]Department of Neurology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

Abstract

Cerebral venous sinus thrombosis (CVST) is a cerebrovascular disease in the form of occlusion due to thrombus in the venous and cerebral sinuses. It rarely occurs and has varied clinical symptoms and radiological features and challenging to diagnose. D-dimer used as a diagnostic marker for cases of venous thromboembolism, with a sensitivity of around 90–92%. However, the specificity is not too high (70–73%) because it can also increase in other conditions. Digital subtraction angiography (DSA) is a gold standard examination to establish the diagnosis of CVST. The purpose of this study was to determine the relationship between the D-dimer level and CVST using DSA at Dr. Hasan Sadikin General Hospital in Bandung. This study used an observational analytic method with a case-control study design using retrospective data from medical records at Dr. Hasan Sadikin General Hospital in January 2017–August 2019. The research subjects divided into two groups, namely the high D-dimer levels and the normal/low D-dimer level. Forty people meet the inclusion criteria, ages averaging from 44.77±14.40 years, and consists of 9 male patients (22%) and 31 women patients (78%). For normal/low D-dimer levels 20 patients (50%) and high D-dimer levels 20 patients (50%). Statistical test results measuring D-dimer and CVST levels found a significant relationship ($p<0.05$). In conclusion, there is a relationship between D-dimer levels with CVST events that have been done by DSA. The higher the D-dimer level, the higher the suspicion of CVST.

Key words: CVST, D-dimer, DSA

Kadar D-Dimer dengan Kejadian *Cerebral Venous Sinus Thrombosis* (CVST) Menggunakan *Digital Subtraction Angiography* (DSA)

Abstrak

Penyakit *cerebral venous sinus thrombosis* (CVST) merupakan penyakit serebrovaskular berupa oklusi akibat trombus di saluran vena dan sinus serebral yang jarang terjadi dengan gejala klinis dan gambaran radiologis yang bervariasi, serta sangat sulit untuk didiagnosis. D-dimer dapat dijadikan sebagai penanda diagnostik bagi kasus-kasus tromboembolisme vena dengan sensitivitas 90–92%, namun spesifisitasnya tidak terlalu tinggi (70–73%) karena dapat juga meningkat pada kondisi lain. *Digital subtraction angiography* (DSA) merupakan pemeriksaan baku emas untuk menegakkan diagnosis CVST. Tujuan penelitian ini mengetahui hubungan antara kadar D-dimer dan CVST menggunakan DSA di RSUP Dr. Hasan Sadikin Bandung. Penelitian ini merupakan observasional analitik dengan rancangan kasus kontrol menggunakan data retrospektif dari rekam medis di RSUP Dr. Hasan Sadikin Bandung pada bulan Januari 2017–Agustus 2019. Subjek penelitian dibagi menjadi 2 kelompok, yaitu kelompok D-dimer tinggi dan kelompok D-dimer normal/rendah. Hasil penelitian didapat 40 orang yang memenuhi kriteria inklusi, usia rerata 44,77±14,40 tahun yang terdiri atas pasien laki-laki 9 orang (22%) dan perempuan 31 orang (78%). Untuk kadar D-dimer kategori normal/rendah 20 orang (50%) dan tinggi 20 orang (50%). Hasil uji statistik mengukur kadar D-dimer dan CVST didapatkan hubungan yang bermakna ($p<0,05$). Simpulan, terdapat hubungan antara kadar D-dimer dan kejadian CVST yang telah dilakukan DSA. Semakin tinggi kadar D-dimer, semakin tinggi kecurigaan kejadian CVST.

Kata kunci: CVST, D-dimer, DSA

Received: 12 November 2019; Revised: 17 December 2019; Accepted: 28 December 2019; Published: 31 December 2019

Correspondence: Afdi Arahim Putra. Department of Radiology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital. Jln. Pasteur No. 38, Bandung 40161, West Java, Indonesia. E-mail: putra.afdi@gmail.com

Introduction

Cerebral venous sinus thrombosis (CVST) is a rare cerebrovascular disease in the form of occlusion due to thrombus in the venous canal and cerebral sinuses. It has different clinical and radiological features that make it very difficult to diagnose. Underdiagnoses of CVST can cause severe complications, including infarction, and bleeding to death.[1]

Cerebral veins contain about 70% of the total brain blood volume. CVST occurs about a thousand times less than arterial strokes. Arterial and venous strokes cause different neurological deficits and affect all age groups. About half of arterial stroke patients are found at people of 75 years of age or older, while CVST is most common in people less than 40 years of age (young adults and children). It estimated that the incidence of CVST is around two to seven cases per one million people each year; three out of four people with CVST are women, with 61% of women aged between 20–35 years. This comparison may be related to pregnancy or the use of oral contraceptives. One in eight patients will die or ended up with disability.[2]

What distinguishes veins in the brain from other veins in the body is that veins in the brain do not have valves, allowing infection of the area around the veins and sinuses to spread in both directions, the venous system in the brain has many collaterals so that occlusion can arise without symptoms.[3,4]

Headache is the most common symptom of CVST. The symptom can be the only clinical manifestation or can be associated with other signs or symptoms of CVST. When symptoms of headache found, CVST is most often associated with lateral sinus thrombosis.[3]

Several studies have shown poor clinical results in about 10% of all CVST cases. A study reported that an initial diagnosis of CVST errors could occur in 73% of patients. Besides, delays in diagnosis for more than ten days can also occur in 40% of patients who hospitalized.[1]

In developing countries where most cases with CVST occur, biological markers accessible to performed and affordable are needed. D-dimer is a marker of endogenous fibrinolysis and must therefore be detected in patients with deep vein thrombosis. D-dimer levels in plasma which are cross-linked fibrin fragments that are degraded by plasmin are sensitive for the diagnosis of deep venous thrombosis and pulmonary thromboembolism.[5]

D-dimer recommended as an adjunct test. Since d-dimer is a sensitive test but has a poor specificity (70–73%), it should only use to rule out deep vein thrombosis (DVT), not to confirm a diagnosis. High plasma D-dimer level >2.0 mg/mL found in 68% of patients with DVT and 45% without DVT ($p<0.05$). Therefore, a high D-dimer level greater than 2.0 mg/mL showed 68% sensitivity, 55% specificity, 60% accuracy, 50% positive predictive rate, and 72% negative predictive rate in the detection of early DVT. However, it should also be noted that D-dimer levels could increase in certain pathological conditions besides thromboses such as cancer, liver disease, kidney disease, organ-graft repair, and thrombolysis treatment. Therefore, to increase the effectiveness of detecting thrombosis, a combination of D-dimer is required with a fibrinogen test.[6]

Although an increase in D-dimer has been shown to be associated with CVST, its predictive value in diagnosing CVST has not been clinically established. The aim of this study is to re-evaluate the D-dimer plasma value in CVST predictions with a greater number of cases from patients that are grouped according to acute CVST or suspect of CVST and their corresponding controls.[7]

Currently there are many radiological modalities that can be used to help with the diagnosis of CVST, ranging from computed tomography scan (CT-scan), magnetic resonance imaging (MRI), CT venography, MR venography to digital subtraction angiography (DSA) which clearly show veins in the brain and changes in brain parenchyma associated with thrombosis.[8]

Angiographic or DSA features in CVST patients will lack complete or partial filling. However, it is sometimes difficult to capture in certain areas such as one third anterior of the superior sagittal sinus or the lateral sinus. In the indirect sign, there is delayed emptying and dilation. Collateral veins (cortical, emissary, meningeal, diploic, and scalp veins) accompanied by the corkscrew appearance phenomenon.[9–11]

Digital subtraction angiography (DSA) is a minimally invasive diagnostic technique used to clearly depict arteries and veins.[2,12,13] DSA is still considered a gold standard test. When there is clinical suspicion in CVST patients, but the MRV or CTV examination shows no abnormalities, therefore DSA is the first choice.[14] The purpose

of this study was to determine the relationship between the D-dimer level and CVST using DSA at Dr. Hasan Sadikin General Hospital in Bandung.

Methods

This study was an analytic observational with a case-control design using retrospective data from medical records at Dr. Hasan Sadikin General Hospital Bandung from January 2017 to August 2019. The research subjects divided into two groups, the high D-dimer level group and the normal/low D-dimer level group with a total sample of 40 people who met the inclusion criteria.

Numerical-scale data such as patient age presented with the mean, standard, deviation, median, and range. For characteristics data in the form of categorical data such as the gender of the patient, code is given and presented as frequency and percentage distributions. Before statistical tests, the numerical data assessed by normality tests using the Shapiro-Wilk test. The significance test in this study uses the unpaired t test, while for statistical analysis for categorical data, it tested with the chi-square requirements.

The significance criteria used are the value of p, if $p \leq 0.05$ is significant or statistically significant, while $p > 0.05$ is insignificant or not statistically significant. The data obtained are recorded in a unique form and then processed through the SPSS version 24.0 for Windows.

This study conducted after obtaining the feasibility permit from the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung with letter number: LB.02.01/X.6.5/249/2019.

Results

Table 1 describes the characteristics of the study subjects based on age, gender, and D-dimer level. Age average is 44.77±14.40 years, and there is more female than male (78%:22%).

Table 2 explains the description of the results of DSA on the most research subjects is the left transverse sinus, 22 people (55%). To see the relationship between D-dimer levels with CVST events using DSA can be seen in Table 3.

In the DSA results group (+), the study subjects with normal or low D-dimer levels were eight people (30%) and subject with high 19 people (70%). In the DSA results group (−), research subjects with normal or low levels D-dimer are 12 people (12 of 13), and subject with a high level is one person (1 of 13).

Table 1 Characteristics of Research Subjects

Variables	n=40
Age	
Mean±Std	44.77±14.40
Median	45.00
Range (min–max)	20.00–80.00
Gender	
Male	9 (22%)
Female	31 (78%)
D-dimer level	
Normal/low	20 (50%)
High	20 (50%)

For the analysis of categorical data, the D-dimer level tested using the chi-square test. The value of p on the D-dimer content variable is less than 0.05 (p value<0.05), which means there is a statistically significant difference between the variable of D-dimer in the DSA (+) and (−) results groups.

Discussion

In this study, it inferred that the CVST incidence is higher in females, with 31 of 40. The result is consistent with the literature, which states that the highest incidence of CVST is in women with a ratio of 3:1 and in the age groups of 40 years or younger. The prevalence of CVST is common in women, perhaps due to conditions related to pregnancy, the puerperium and the use of oral contraceptives.[2]

The highest number of occlusions in DSA in the left transverse sinus was 55%. The results are not following the study of Galarza and Gazzeri,[15] who concluded CVST more often happened in the superior sagittal sinus, which is 62%.

Table 2 Overview of DSA Results

DSA Result	n=40
Right transverse sinus	4 (10%)
Left transverse sinus	22 (55%)
Bilateral transverse sinus	1 (2%)
Superior sagitalis sinus	0 (0%)
Normal	13 (33%)

Table 3 Comparison between D-dimer Levels and CVST Occurrence Using DSA

D-dimer Level	DSA Result Group		p Value
	(+) n=27	(−) n=13	
Normal/low	8	12	0.0001
High	19	1	

Thrombosis in the superior sagittal sinus will cause an increase in venous pressure, resulting in impaired cerebrospinal fluid absorption which will cause an increase in intracranial pressure with clinical signs and symptoms like headache, focal neurological deficits, seizures and decreased consciousness.[14,16,17]

There is a significant relationship between D-dimer levels with CVST events using DSA. CVST is common in the high D-dimer level group, which is 19 of 27. The study is consistent with research conducted by Wang et al.,[8] who found a significant relationship between high D-dimer levels and the incidence of CVST. D-dimers can also be used as diagnostic markers for cases like venous thrombosis, prediction of prognosis, as well as clinical monitoring and therapy in CVST.[18,19]

This research is retrospective so that it is investigated for the causes of prothrombotic and risk factors and does not assess the effect of D-dimer levels on clinical symptoms and clinical features.

Conclusion

Cerebral venous sinus thrombosis (CVST) is a rare cerebrovascular disease that varies in clinical symptoms and radiological features and very difficult to diagnose. DSA is a diagnostic examination of choice when there is clinical suspicion of CVST patients.

Conflict of Interest

The author has reported that no potential conflicts of interest exist with any companies/ organizations whose products or services may be discussed in this article.

References

1. Tatlisumak T, Jood K, Putaala J. Cerebral venous thrombosis: epidemiology in change. Stroke. 2016;47(9):2169–70.
2. Leach JL, Fortuna RB, Jones BV, Gaskill-Shipley MF. Imaging of cerebral venous thrombosis: current techniques, spectrum of findings, and diagnostic pitfalls. Radiographics. 2006;26(Suppl 1):S19–41.
3. Atanassova PA, Massaldjieva RI, Chalakova NT, Dimitrov BD. Cerebral venous sinus thrombosis-diagnostic strategies and prognostic models: a review. In: Okuyan E, editor. Venous thrombosis: principles and practice [e-book]. Rijeka, Croatia: InTech; 2012 [cited 28 August 2019]:129–58. Available from: https://www.intechopen.com/books/venous-thrombosis-principles-and-practice/cerebral-venous-sinus-thrombosis-diagnostic-strategies-and-prognostic-models-a-review.
4. Uflacker R. Atlas of vascular anatomy: an angiographic approach. 2nd Edition. Philadelphia: Lippincott Williams & Wilkins; 2007.
5. Crassard I, Soria C, Tzourio C, Woimant F, Drouet L, Ducros A, et al. A negative D-dimer assay does not rule out cerebral venous thrombosis: a series of seventy-three patients. Stroke. 2005;36(8):1716–9.
6. Raskob GE, Angchaisuksiri P, Blanco AN, Buller H, Gallus A, Hunt BJ, et al. Thrombosis: a major contributor to global disease burden. Arterioscler Thromb Vasc Biol. 2014;34(11):2363–71.
7. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. Blood Rev. 2015;29(1):17–24.
8. Wang HF, Pu CQ, Yin X, Tian CL, Chen T, Guo JH, et al. D-dimers (DD) in CVST. Int J Neurosci. 2017;127(6):524–30.
9. Saadatnia M, Fatehi F, Basiri K, Mousavi SA, Mehr GK. Cerebral venous sinus thrombosis risk factors. Int J Stroke. 2009;4(2):111–23.
10. Wasay M, Azeemuddin M. Neuroimaging of cerebral venous thrombosis. J Neuroimaging. 2005;15(2):118–28.
11. Sasidharan PK. Cerebral vein thrombosis misdiagnosed and mismanaged. Thrombosis.

2012;2012:210676.
12. Qu H, Yang M. Early imaging characteristics of 62 cases of cerebral venous sinus thrombosis. Exp Ther Med. 2013;5(1):233–6.
13. Zhang S, Hu Y, Li Z, Huang D, Zhang M, Wang C, et al. Endovascular treatment for hemorrhagic cerebral venous sinus thrombosis: experience with 9 cases for 3 years. Am J Transl Res. 2018 Jun 15;10(6):1611–9.
14. Piazza G. Cerebral venous thrombosis. Circulation. 2012;125(13):1704–9.
15. Galarza M, Gazzeri R. Cerebral venous sinus thrombosis associated with oral contraceptives: the case for neurosurgery. Neurosurg Focus. 2009;27(5):E5.
16. Saposnik G, Barinagarrementeria F, Brown RD Jr, Bushnell CD, Cucchiara B, Cushman M, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42(4):1158–92.
17. Stam J. Thrombosis of the cerebral veins and sinuses. N Engl J Med. 2005;352(17):1791–8.
18. Lip GY, Lowe GD. Fibrin D-dimer: a useful clinical marker of thrombogenesis? Clin Sci (Lond). 1995;89(3):205–14.
19. Wang J, Ning R, Wang Y. Plasma D-dimer level, the promising prognostic biomarker for the acute cerebral infarction patients. J Stroke Cerebrovasc Dis. 2016;25(8):2011–5.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.4051

GMHC. 2019;7(3):189–96
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Phylogenetic Analysis of *Culex tritaeniorhynchus* and *Culex vishnui* Vector of Japanese Encephalitis Virus

Raden Roro Upiek Ngesti Wibawaning Astuti,[1,2] Raden Wisnu Nurcahyo,[3] R.C. Hidayat Soesilohadi,[4] Suwarno Hadisusanto,[5] Budi Mulyaningsih[6]

[1]Doctoral Study Program in Biological Science, Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Division of Parasitology Laboratory of Animal Systematic, Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, [3]Department of Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, [4]Division of Laboratory of Entomology, Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, [5]Division of Laboratory of Ecology, Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, [6]Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

Culex tritaeniorhynchus and *Culex vishnui* are medically essential mosquitoes that transmit the Japanese encephalitis (JE) virus. There is less information about the recording data and research due to genetic character differences among them. The objective of this study was to examine the genetic variation of *Cx. tritaeniorhynchus* and *Cx. vishnui* in 3 sites of Central Java using polymerase chain reaction randomly amplified polymorphic DNA (PCR-RAPD). The study was done in January to November 2017 in Pekalongan city, Pekalongan regency, and Semarang regency. Adult female mosquitoes collected by human bite method. DNA of ten *Cx. tritaeniorhynchus* samples and fifteen samples of *Cx. vishnui* purified using DNA extraction kit. Furthermore, PCR amplification was conducted with 5 RAPD primers (OPA 11, 12, 15, 16, and 20) and would run into 2% gel electrophoresis for 45 minutes. Cluster analysis was using MVSPTM software (version 3.1). The results showed 213 genetic characters of *Cx. vishnui*, while 142 characters shown by *Cx. tritaeniorhynchus*. The dendrograms showed three distinct groups of *Cx. vishnui* from 2 sites of Pekalongan and one site of Semarang, while *Cx. tritaeniorhynchus* showed two distinct groups, which were 1 group from Pekalongan and 1 group from Semarang. Low genetic similarity (<10%) shown *Cx. vishnui* from Pekalongan city and Pekalongan district, and there was no genetic similarity in *Cx. tritaeniorhynchus* from Pekalongan and Semarang. It concluded that the polymorphism of *Cx. tritaeniorhynchus* and *Cx. vishnui* reached 100%.

Key words: *Culex tritaeniorhynchus*, *Culex vishnui*, JE-vector, PCR-RAPD, phylogenetic analysis

Analisis Filogenetik *Culex tritaeniorhynchus* dan *Culex vishnui* Vektor Virus *Japanese Encephalitis*

Abstrak

Nyamuk *Culex tritaeniorhynchus* dan *Culex vishnui* memiliki peran penting di bidang medis terutama dalam penularan virus *Japanese encephalitis* (JE). Sampai saat ini data dan riset tentang karakter genetik vektor JE masih sangat terbatas. Penelitian ini bertujuan menjelaskan variasi genetik *Cx. tritaeniorhynchus* dan *Cx. vishnui* di 3 lokasi di Jawa Tengah berdasar *polymerase chain reaction randomly amplified polymorphic DNA* (PCR-RAPD). Studi ini dilakukan dari bulan Januari sampai November 2017 di Kota Pekalongan, Kabupaten Pekalongan, dan Kabupaten Semarang. Metode *human bite* digunakan untuk koleksi nyamuk. Ekstraksi DNA nyamuk dilakukan pada 10 ekor *Cx. tritaeniorhynchus* dan 15 ekor *Cx. vishnui* menggunakan kit ekstraksi DNA. Selanjutnya, diamplifikasi dengan 5 macam primer RAPD (OPA 11, 12, 15, 16, dan 20), serta dielektroforesis pada 2% agar selama 45 menit. Analisis klaster dilakukan menggunakan program MVSPTM (versi 3.1). Ditemukan 213 dan 142 karakter genetik masing-masing pada *Cx. vishnui* dan *Cx. tritaeniorhynchus*. Analisis dendogram menunjukkan 3 grup yang berbeda untuk *Cx. vishnui*, sedangkan untuk *Cx. tritaeniorhynchus* terdapat 2 grup yang berbeda, yaitu 1 grup dari Pekalongan dan 1 grup dari Semarang. Similaritas genetik yang rendah (<10%) ditunjukkan *Cx. vishnui* dari Kota Pekalongan dan Kabupaten Pekalongan, bahkan tidak ada persamaan genetik pada *Cx. tritaeniorhynchus* dari Pekalongan dengan Semarang. Disimpulkan bahwa polimorfisme *Cx. tritaeniorhynchus* dan *Cx. vishnui* mencapai 100%.

Kata kunci: Analisis filogenetik, *Culex tritaeniorhynchus*, *Culex vishnui*, PCR-RAPD, vektor JE

Received: 5 September 2018; Revised: 25 August 2019; Accepted: 20 December 2019; Published: 31 December 2019

Correspondence: Dra. Raden Roro Upiek Ngesti Wibawaning Astuti, DAP&E., M.Biomed. Division of Parasitology Laboratory of Animal Systematic, Department of Biology, Faculty of Biology, Universitas Gadjah Mada. Jln. Teknika Selatan, Mlati, Sleman 55281, Special Region of Yogyakarta, Indonesia. E-mail: upiekastuti@ugm.ac.id

Introduction

Japanese encephalitis (JE) is a zoonotic viral disease and a health problem in Asia, including Indonesia. In Asia, there were about 68,000 cases annually, with 30% of the case-fatality rate for encephalitis.[1] In Indonesia, the first JE case reported in 1962[2] and the number rises since then. In 2001–2004, there were 163 of JE cases, and 94 cases were serologically JE infection.[3] In 2016, there were 326 cases of JE in 11 provinces mostly in Bali (69.3%).[4] JE virus infection also reported in West Sumatera, West Kalimantan, Yogyakarta, Central Java, East Java, West and East Nusa Tenggara, and Papua.[5]

JE is a severe disease that may cause death and spread by the mosquito bite. In India, there is a success story in preventing this disease by intensive vector surveillance and immunization.[6,7] The significant vectors for JEV transmission belong to *Culex vishnui* subgroup, which comprises of *Cx. pseudovishnui* Colles. Though JEV isolated from 16 species of mosquitoes, the majority of the isolations are from *Cx. vishnui* complex, which breeds extensively in the rice ecosystem.[6] In Hongkong, there were 30 species of mosquitoes identified positively infected with the JE virus, which belonged to 5 genera: *Anopheles*, *Culex*, *Aedes*, *Armigeres*, and *Mansonia*, species of *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. pseudovishnui*, *Cx. vishnui*, and *Cx. fuscocephala* becomes the vector.[8] In Indonesia, *Cx. tritaeniohynchus* is the primary vector of the JE and *Cx. vishnui* as a secondary or alternative vector of JE.[4]

The virus infects mainly in animals, pigs and wild birds. The viral agent of disease transmits to humans by the infected mosquito bite, from the genera of *Culex* mainly, *Cx. tritaeniorhynchus* that breeds in a rice field and also *Cx. vishnui* subgroup.[6] In Indonesia, there was limited information regarding recording data and research in terms of vector surveillance, and even less in the molecular study. The purpose of the study was to determine the genetic variation of *Cx. tritaeniorhynchus* and *Cx. vishnui* from Pekalongan city, Pekalongan regency, and Semarang regency using polymerase chain reaction rapid analysis polymorphism DNA (PCR-RAPD).

Methods

The method was a descriptive study conducted from January to November 2017 in the areas of Pekalongan city, Pekalongan regency, and Semarang regency in Central Java, Indonesia. Adult mosquitoes collected by human bite methods using an aspirator and identified using a manual book from the Ministry of Health.[9] Five mosquito from each site extracted for the DNA using the DNA extraction kit,[10] "gSYNCTM" (Geneaid, Cat. No. GS 100, PT. Genetika Science Indonesia). Nine of 10-mer RAPD primers (1st BASE; OPA 1, OPA 2, OPA 8, OPA 9, OPA 11, OPA 12, OPA 15, OPA 16, OPA 20) has selected for the subset of mosquito DNAs, and five of them (OPA 11, OPA 12, OPA 15, OPA 16, and OPA 20) produced clear bands, and it applied to samples.[11] The five primers of RAPD used for the DNA amplification shown in Table 1.

There were 35 cycles for the PCR (Thermal Cycler, Boeco), steps, and annealing modified from the previous works.[10,12] The samples were then electrophoresis (Mini Run Gel Electrophoresis System GE-100), run in 2% of gel agarose for 45 minutes. The marker was 100 bp DNA ladder (Geneaid). Samples finally observed by UV transilluminator (Biorad).

The data from each electrophoresis were manually counted and scored for the real baseband value compared with the marker. If there was a band, the score is 1 (one); however, if there was no band, the score was 0 (zero). Cluster analysis using Multi-Variate Statistical Package (MVSP) software (version 3.1) used for data analysis. Dendrogram of each mosquito from each site analyzed by synthesis-descriptive analysis as percent similarity. This study approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University-Dr. Sardjito General Hospital with number Ref: KE/FK/0612/EC/2017.

Results

Culex tritaeniorhynchus adult female was dominant and abundant in Semarang regency, while in Pekalongan city and Pekalongan regency *Cx. vishnui* was dominant and abundant. The optimum amplification DNA fragments obtained using five of nine OPA primers that produced numbers of *Cx. vishnui* and *Cx. tritaenirhynchus* DNA fragments.

There were, in total, 142 genetic characters of *Cx. Tritaeniorhynchus* from 2 sites of mosquito collection, while there were 213 genetic characters of *Cx. vishnui* from the three sites of mosquito

Table 1 Primers for DNA Amplification

Primers[12]	Sequences (5'–3')	Annealing[9] (t°C)
OPA-11	CAA TCG CCGT	35°C
OPA-12	TCG GCG ATAG	35°C
OPA-15	TTC CGA ACCC	35°C
OPA-16	AGC CAG CGAA	35°C
OPA-20	GTT GCG ATCC	35°C

collection (Table 2). The phylogenetic analysis was performed in percent of similarities and was showed in Figure 1–Figure 4.

Figure 2A showed two distinct groups of *Cx. vishnui* samples from Pekalongan city and Pekalongan regency, which show low similarities, less than 36%. The compilation of *Cx. vishnui* samples from Pekalongan city and regency (B2) separated in different group with the samples from Semarang regency (B1).

In Figure 4, *Cx. tritaeniorhynchus* from the two sites of collection, whereas 1 was Semarang regency and 2 was Pekalongan regency, both showed in two distinctive groups. The groups revealed that there was no (nul, 0%) similarities, it meant that *Cx. tritaeniorhynchus* from the two sites of collection had high genetic variation.

Discussion

The use of molecular techniques, particularly the PCR-based DNA techniques, significantly improves our knowledge and understanding of the mosquito population and dynamics.[13] The study of phylogenetic was essential to understand the relationship between transmission and epidemiology. The technic can be used as a control of disease also the genetic structure of mosquito vector-based in the genetic characterization,[14] especially for the field mosquito species of *Cx. tritaeniorhynchus* and *Cx. vishnui*.

As the above results, in Table 2, the genetic character differences between mosquitoes may be due to the geographical and ecological character differences between the three sites of mosquitoes collection. The sites of mosquitoes collection in Pekalongan regency were a combination of rural-urban area type, with rice fields in some areas and crowded housing and a lot of avian and other domestic animals. However, in Pekalongan city, the sites were urban type housing, where there was no rice field. Because of the lack of breeding sites, *Cx. tritaeniorhynchus* might not collect from this site. The ecological character in Semarang regency is the rural type with rice field and domestic animal, avian, and mammal (cow).

Individual genetic variation influenced by the reproduction pattern of the species in the population. The variation due to the randomly individual selection as its couple, and it will produce random mating in the population.[15] Besides, natural selection and the ability of many habitat-exploitation of mosquito may cause this fauna to become a cosmopolitan insect, and the environmental changing will support the gene flow and develop the high genetic variation.[16]

There were 104 characters between *Cx. vishnui* from Pekalongan regency (Figure 1A), while there were 96 characters from Pekalongan city (Figure 1B) and 48 characters of samples from Semarang regency (Figure 1C). Figure 1 showed that there were two different groups from each sample site. *Culex vishnui* from Pekalongan city showed the highest genetic similarities, 20–35% compared with the two other sites, and there was about less than 20–30% and 5–35% for Pekalongan regency and Semarang regency respectively. These genetic similarities showed the closer of kinship, so mosquitoes from Pekalongan city revealed to be closer in kinship if compared with mosquitoes from Pekalongan regency and Semarang regency. There were 165 characters in the compilation

Table 2 Genetic Characters of *Cx. tritaeniorhynchus* and *Cx. vishnui* from Pekalongan City, Pekalongan Regency, and Semarang Regency based on PCR-RAPD

Species	Number of Genetic Character from Sites of Collection			Total Character
	Pekalongan City	Pekalongan Regency	Semarang Regency	
Culex tritaeniorhynchus	–	80	63	142
Culex vishnui	96	104	48	213

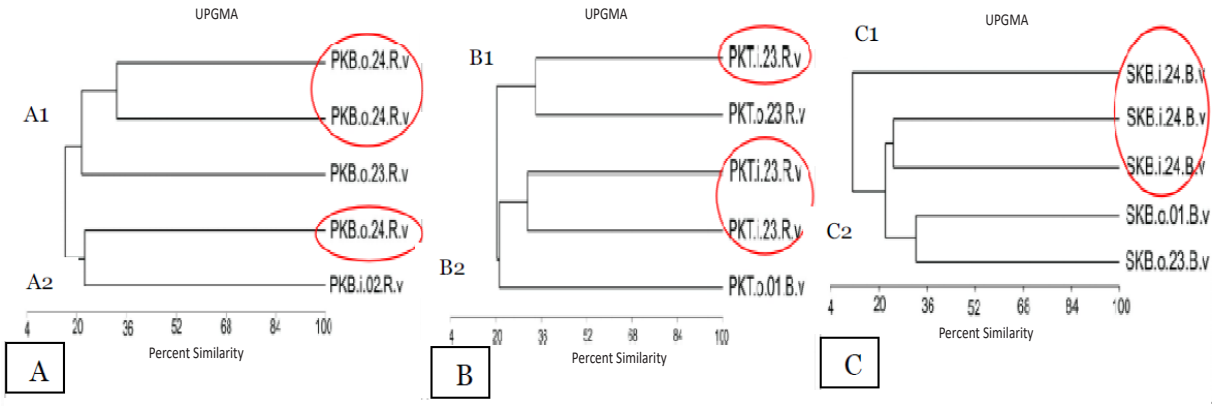

Figure 1 Dendrogram Percent Similarities of *Culex vishnui* Genetic Character from Pekalongan Regency (A), Pekalongan City (B), and Semarang Regency (C)

Axis refers to the distance (%) of genetic similarities of mosquito samples, PKB=Pekalongan regency, PKT=Pekalongan city, SKB=Semarang regency, o=outdoor, i=indoor, 24=24 hours of collection time, 23=23 hours of collection time, 02=2 hours of collection time, 01=1 hours of collection time, R=resting, B=biting, v=*Cx. vishnui*; Red circle in A=same code of mosquitoes (PKB.o.24.Rv) collected from Pekalongan regency in A1 and A2 groups, outdoor, at the 24 hours time, and catched at rest; Red circle in B=same code of mosquitoes (PKT.i.23.Rv) collected from Pekalongan city in B1 and B2 groups, indoor, at the 23 hours time, and at rest; Red circle in C=same code of mosquitoes (SKB.i.24.Bv) that collected from Semarang regency in C1 and C2 groups, indoor, at the 24 hours time, and cathed at bite

between *Cx. vishnui* samples from the two sites (Figure 2A), while there were 213 characters in compile data from the three collection sites (Figure 2B).

Three samples of *Cx. vishnui* from the same site and time of collection (code: PKBo.24.R.v) in Pekalongan regency also showed a different group of similarities. It could be understood that the mosquito was originally from different breeding sites.[17,18] *Culex vishnui* samples also showed a

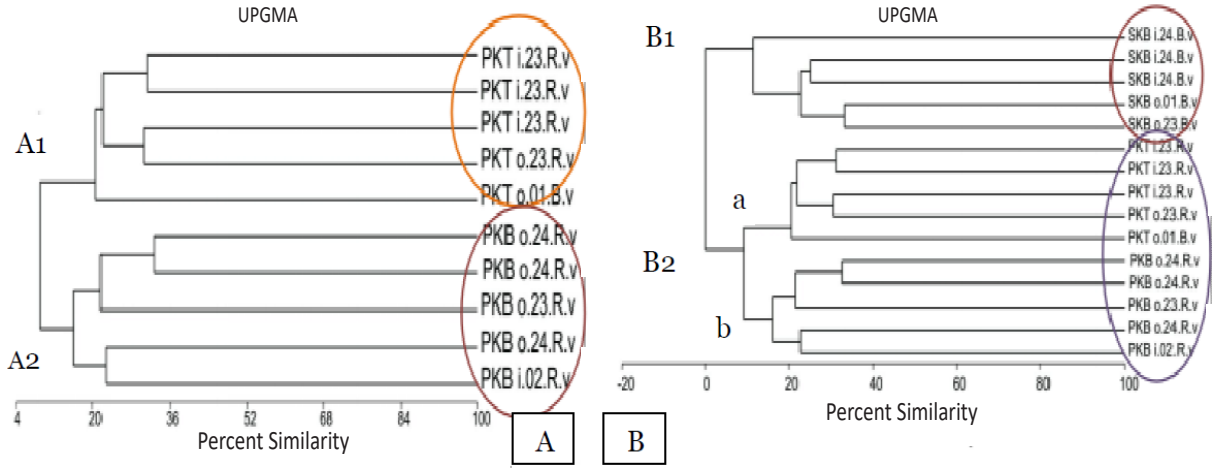

Figure 2 Dendrogram Percent Similarities Compilation of *Culex vishnui* Genetic Character from Two (A) and Three (B) Sites of Collection

Axis refers to the distance (%) of genetic similarities of mosquito samples, PKT=Pekalongan city, PKB=Pekalongan regency, SKB=Semarang regency, i=indoor, o=outdoor, 23=23 hours of collection time, 01=1 hours of collection time, 24=24 hours of collection time, 02=2 hours of collection time, R=resting, B=biting, v=*Cx. vishnui*; Red circle in A=same code of mosquitoes collected from Pekalongan city (A1, PKT.i.23.Rv) and Pekalongan regency (A2, PKB.o.24.Rv) groups, i=indoor, o=outdoor, at the 23 and 24 hours of collection time respectively and catched at rest; Brown circle in B=same code of mosquitoes collected from Semarang regency (B1, SKB.i.24.Bv); Blue circle in B=same code of mosquitoes collected from Pekalongan city (B2a, PKT.i.23.Rv), indoor, at 23 hours of collection time, at rest; and same code mosquitoes from Pekalongan regency (B2b, PKB.o.24.Rv), o=outdoor, 24=24 hours of collection time, R=resting, v=*Cx. vishnui*

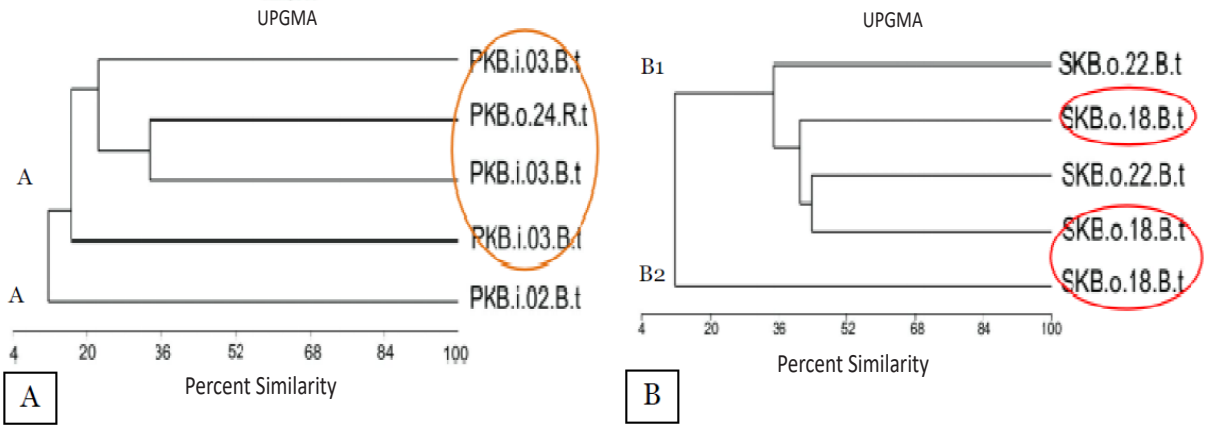

Figure 3 Dendrogram Percent Similarities of *Culex tritaeniorhynchus* Genetic Character from Pekalongan (A) and Semarang Regency (B)

Axis was the distance (%) of genetic similarities of mosquito samples; PKB=Pekalongan regency, SKB=Semarang regency, i=indoor, o=outdoor, 03=3 hours of collection time, 24=24 hours of collection time, 02=2 hours of collection time, 22=22 hours of collection time, 18=18 hours of collection time, B=biting, R=resting, t=*Cx. trytaeniorhynchus*; Red circle in A=same code of mosquitoes collected from Pekalongan regency (group A1 and A2), indoor, at the 03 hours of collection time and catched at bite; Red circle in B=same code of mosquitoes collected from Semarang regency (B1 and B2 groups), outdoor, at the 18 hours of collection time, and cathed at bite; It showed two distinct groups and had about 10% in similarity

significant distinct group and low similarities from Pekalongan city (PKT.i.23.R.v), two samples in one group, and 1 sample in the other group of similarities. Figure 2B showed that there were no character similarities at all of *Cx. vishnui* from Semarang and Pekalongan. This study revealed that there was up to 100% polymorphism of the genetic character of *Cx. vishnui* from the three sites of collection.

These findings showed there were significant genetic differences among the *Cx. vishnui* population. Kiliç et al.[18] said that the genetic

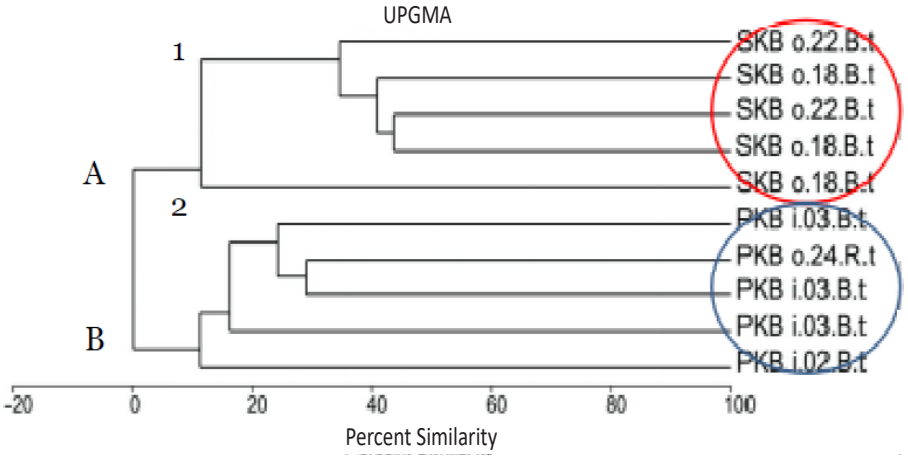

Figure 4 Dendrogram Percent Similarities Compilation of *Culex tritaeniorhynchus* Genetic Character from Pekalongan and Semarang Regency

Axis was the distance (%) of genetic similarities of mosquito samples; SKB=Semarang regency, PKB=Pekalongan regency, o=outdoor, i=indoor, 22=22 hours of collection time, 18=18 hours of collection time, 03=3 hours of collection time, 24=24 hours of collection time, 02=2 hours of collection time, B=biting, R=resting, t=*Cx. trytaeniorhynchus*; Red circle in group A (1 and 2)=same code of mosquitoes (SKB.o.18.Bt and SKB.o.22.Bt) collected from Semarang regency, outdoor, at the 18 and 22 hours of collection time and catched at bite; Blue circle=same code of mosquitoes collected from Pekalongan city in B (PKB.i.03.Bt), indoor, at the 03 hours of collection time, and at rest; It showed two distinct groups; The red and blue circle showed two distinct groups and there was no similarity (0%)

differentiation among *Cx. pipiens* in Aegean, Turkey indicated the high rate of gene flow among the population. Those findings suggest that *Cx. pipiens* are freely moving around the Aegean region in diverse habitat. Joice et al.[17] said that there was a significant finding of genetic divergence of *Cx. pipiens* population from five habitats in Merced in Central valley in California.

The genetic character of *Cx. tritaeniorhynchus* from Pekalongan regency were 80 characters and also showed high variation with two different groups. As shown in Figure 3A, there were three mosquito samples (PKBo.03.B) from the same time, behavior, and place that showed three different lines (group) of similarities. One of them separated and showed less than 20% of character similarities than the other two. These mosquitoes from Semarang regency (Figure 3B) showed 63 genetic characters and separated into two groups. Three samples of *Cx. triteniorhynchus* from the same collection time, behavior, and site had separated into three different lines in the dendrogram, with character similarity less than 10% (Figure 4). This condition may be due to the low genetic flow in Semarang regency rather than in Pekalongan regency. The low genetic flow might be due to the ecological character in Semarang regency that showed a close area with surrounding hardwood and rubber plantation. However, Pekalongan regency was an open area, rice field area. The *Cx. tritaeniorhynchus* samples from Pekalongan and Semarang regency showed almost dissimilarity of the genetic character, and it revealed that there was genetically 100% polymorphism.

In a significant population, naturally, random mating happens. The parental genetic combination will support to produce high genetic variation. The offspring individually may be the same in genotype but different in the phenotype. Otherwise, the offspring individually has the same phenotype but is genetically different.[16] This condition explains the genetic variation in the *Cx. vishnui* and *Cx. tritaeniorhynchus* population from the sites of collection.

There was minimal information regarding the surveillance and control of the JE vector in Indonesia; this might correlate with the variation of the geographic sites (topography, and annual rainfall), ecotype, and habitat of the vector in the areas of the city and regency. All this time, the vector surveillance, especially for *Cx. tritaeniorhynchus* was done in sporadic works if there were JE outbreaks. This obstacle might also correlate with supporting funding, as a researcher in Hongkong stated that the control program does not work because of time-consuming and expensive. It is challenging to cover all mosquito habitats, and it may cause environmental pollution. The JE vector control program may work by joining together with other mosquitoes controls.[19] In the future, the systematics of JE surveillance and standardized diagnosis should be established for better assessment and control program.[20]

In Indonesia, JE cases firstly reported from Lombok in 1960, and the virus was isolated from *Cx. trytaeniorhynchus* mosquito in 1972.[20] In 2018, there were 29 of 34 provinces reported as endemic areas for JE. The JE virus isolated from 10 species of mosquito: *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui*, *Cx. fuscocephala*, *Cx. bitaeniorhynchus*, *Cx. quinquefascaitus*, *An. vagus*, *An. kochi*, *An. annularis*, and *Armigeres subalbatus*.[20] In Jambi province they found the first evidence of the JE genotype 1 was in *Cx. gelidus*.[21]

In Cambodia, a study in peri-urban and a rural pig farming showed that there were seventeen of mosquito species was founded, and *Cx. gelidus* was to be the most abundant, followed by *Cx. vishnui* group and *Cx. trytaeniorhynchus*.[22]

It reported that there were 3 billion people within 24 countries in Southeast Asia and Western Pacific have transmission risk of the JE disease.[23] WHO announced that JE vaccination should be extended in endemic areas if JE became a public health problem. Comprehensive JE immunization program is done in Japan, South Korea, Taiwan, and Thailand. Furthermore, the development of immunization programs will continue in China, India, Nepal, Sri Lanka, Vietnam, and also in Indonesia.[24]

There was declined of JE incident in Taiwan, South Korea and Japan, this because of the change of pig farming management and declined the land used management.[25] The good manage of land for rice field and pig farming would be reduce the breeding sites of mosquitoes and followed by reducing the risk factor of JE infection.[25,26] Besides that, it was also applied of vaccination, data showed that vaccination program have significant impact to reduce JE cases.[23,25] In Indonesia, there has announced that the immunization program to reduce and prevent the JE cases will start from March 2018.[4]

As a result, that immunization itself gives cost-effective, prevent and reduce for new JE cases, and it will be followed by an appropriate control measured.[6,20] Also, the results will give benefits for the success of the JE control program, as in India.[6] The used of mosquito repellent, long-sleaved cloths, coil or vaporizers were good for personal preventive from mosquitoes biting that was infected with virus.[23] In recent year, the PCR-RAPD technique is abandoned, because of the time consuming, and the bias of the data. There were other molecular techniques proposed and give better results and analysis, such as RT-PCR.

Conclusions

There were low genetic similarities (less than 10%) of *Cx. vishnui* from Pekalongan city, Pekalongan regency, and Semarang regency. There were no genetic similarities of *Cx. tritaeniorhynchus* from Pekalongan and Semarang regency. This study revealed that there was up to 100% polymorphism of *Cx. tritaeniorhynchus* and also *Cx. vishnui* from Pekalongan city, Pekalongan regency, and Semarang regency.

Conflict of Interest

There is no conflict of interest among the authors.

Acknowledgments

Our sincere thanks was deliver to head and staff of Health Department, Local Government, and Local Center of Health Community Services in Pekalongan regency, Pekalongan city, and Semarang regency, and PATH's Japanese Encephalitis Project, for the supporting data and information.

References

1. World Health Organization (WHO). Japanese encephalitis [Internet]. 2015 December 31 [cited 2018 August 23]. Available from: http://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis.
2. Erlanger TE, Weiss S, Keiser J, Utzinger J, Weidenmayer K. Past, present, and future of Japanese encephalitis. Emerg Infect Dis. 2010;15(1):1–7.
3. Liu W, Gibbons RV, Kari K, Clemens JD, Nisalak A, Marks F, et al. Risk factors for Japanese encephalitis: a casecontrol study. Epidemiol Infect. 2010;138(9):1292–7.
4. Kementerian Kesehatan Republik Indonesia. Kemenkes canangkan imunisasi cegah radang otak Japanese encephalitis (JE) [Internet]. 2018 March 1 [cited 2018 March 30]. Available from: http://sehatnegeriku.kemkes.go.id/baca/rilis-media/20180301/2725083/kemenkes-canangkan-imunisasi-cegah-radang-otak-japanese-enchepalitis-je/.
5. Ompusunggu S, Hills SL, Maha MS, Moniaga VA, Susilarini NK, Wijaya A, et al. Confirmation of Japanese encephalitis as an endemic human disease through sentinel surveillance in Indonesia. Am J Trop Med Hyg. 2008;79(6):963–70.
6. Selvaraj I. Epidemiology of Japanese encephalitis and control measures [Internet]. [cited 2018 March 30]. Available from: https://www.powershow.com/view1/24a377-ZDc1Z/EPIDEMIOLOGY_OF_JAPANESE_ENCEPHALITIS_AND_CONTROL_MEASURES_powerpoint_ppt_presentation.
7. Centers for Disease Control and Prevention. Japanese encephalitis [Internet]. 2015 August 5 [cited 2018 March 30]. Available from: https://wwwnc.cdc.gov/travel/diseases/japanese-encephalitis.
8. Scientific Committee on Vector-borne Diseases, Centre for Health Protection, Department of Health of Hong Kong. Japanese encephalitis in Hong Kong [Internet]. December 2004 [cited 2018 March 30]. Available from: https://www.chp.gov.hk/files/pdf/vectors_of_japanese_encephalitis_in_hk_r.pdf.
9. Balai Besar Penelitian dan Pengembangan Vektor dan Reservoir Penyakit, Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia. Pedoman pengumpulan data vektor (nyamuk) di lapangan: riset khusus vektor dan reservoir penyakit di Indonesia [Internet]. Salatiga: B2P2VRP, Balitbangkes, Kemenkes RI; 2017 [cited 2017 March 30]. Available from: http://www.b2p2vrp.litbang.kemkes.go.id/publikasi/download/59.
10. Tiwari P, Arya R, Tripathi LM, Bhattacharya SM, Srivastava VLM. Genetic variation among filarial species as detected by random amplified polymorphic DNA (RAPD). J Parasit Dis. 2004;28(2):73–8.

11. Sharma AK, Mendki MJ, Tikar SN, Chandel K, Sukumaran D, Parashar BD, et al. Genetic variability in geographical populations of *Culex quinquefasciatus* Say (Diptera: Culicidae) from India based on random amplified polymorphic DNA analysis. Acta Trop. 2009;112(1):71–6.
12. Astuti RRUNW, Handayani NSN, Hadisusanto S, Poerwanto SH. Genetic variability in geographical population of *Culex quinquefasciatus* Say (Diptera: Culicidae) from lymphatic endemic areas based on random amplified polymorphic DNA analysis. In: Kusumawinahyu WM, Hartanto DP, Firdausi R, Atsomya MF, editors. Proceedings 2nd Basic Science International Conference; 2012 February 24–25; Malang, Indonesia. Malang: Mathematics Department, Faculty of Sciences, Brawijaya University; 2012 [cited 2018 March 30]. p. B-65. Available from: https://repository.ugm.ac.id/id/eprint/91950.
13. Beroiz B, Ortego F, Callejas C, Hernandez-Crespo P, Castañera P, Ochando MD. Genetic structure of Spanish populations of *Ceratitis capitata* revealed by RAPD and ISSR markers: implications for resistance management. Span J Agric Res. 2012;10(3):815–25.
14. Failloux AB, Rhodain F. Importance of mosquito population genetic studies in medical entomology. Ann Soc Entomol Fr. 1999;35(1):1–16.
15. Indrawan M, Primack RB, Supriatna J. Biologi konservasi. Revision Edition. Jakarta: Yayasan Obor Indonesia; 2007.
16. Frankham R, Ballou JD, Briscoe DA. Introduction to conservation genetics. Cambridge: Cambridge University Press; 2002.
17. Joyce AL, Melese E, Ha PT, Inman A. Population genetic structure of the *Culex pipiens* (Diptera: Culicidae) complex, vectors of West Nile virus, in five habitats. Parasit Vectors. 2018;11(1):10.
18. Kiliç S, Taşkin V, Doğaroğlu T, Doğac E, Taşkin BG. Genetic characterization of field population of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) sampled from Aegean region of Turkey. Turk J Zool. 2019;43(1):1–11.
19. Program for Appropriate Technology in Health (PATH). PATH's Japanese encephalitis project: collaboration and commitment to protect asia's children [Internet]. Seattle: PATH; 2009 [cited 2018 March 30]. Available from: https://path.azureedge.net/media/documents/VAD_je_rpt.pdf.
20. Garjito TA, Widiarti, Anggraeni YM, Alfiah S, Tunggul Satoto TB, Farchanny A, et al. Japanese encephalitis in Indonesia: an update on epidemiology and transmission ecology. Acta Trop. 2018;187:240–7.
21. Garjito TA, Prihatin MT, Susanti L, Prastowo D, Sa'adah SF, Taviv Y, et al. First evidence of the presence of genotype-1 of Japanese encephalitis virus in *Culex gelidus* in Indonesia. Parasit Vectors. 2019;12(1):19.
22. Peng B. Diversity and population dynamics of mosquito vectors of Japanese encephalitis virus in a peri-urban and rural pig farm setting in Cambodia. Cambodian J Nat Hist. 2017;2017(1):128–33.
23. World Health Organization (WHO). Japanese encephalitis. 2019 May 9 [cited 2020 January 5]. Available from: https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis.
24. Japanese encephalitis vaccines: WHO position paper. Wkly Epidemiol Rec. 1998;73(44):337–44.
25. Kass B. Japanese encephalitis reported in Bali. 2018 November 11 [cited 2020 January 21]. In: Globe Medical [Internet]. Available from: https://www.globemedical.com.au/adelaide/interact/blog/japanese-encephalitis-reported-in-bali.html.
26. SAGE Working Group on Japanese encephalitis vaccines. Background paper on Japanese encephalitis vaccines [Internet]. 2014 October 1 [cited 2020 January 21]. Available from: http://www.who.int/immunization/sage/meetings/2014/october/1_JE_Vaccine_Background_Paper.pdf.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3202

GMHC. 2019;7(3):197–205
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Impact of Integrated Reproductive Health Module Implementation on Junior High School Student Behavior Changes

Dini Saraswati Handayani,[1,2] Ariyati Mandiri,[1,2] Irna Kurnia Aprillani[1,2]
[1]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia,
[2]Diploma 4 Midwifery Study Program, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia

Abstract

Socio-cultural changes, low understanding of religion, development of information technology, and limitation of appropriate information sources, causes sexual and reproductive behavior problems in adolescents. The solutions are through the application of an integrated reproductive health module as a teaching guide for teachers by using student center learning approach. The objectives of this study are to analyze the implementation effect of an integrated reproductive health module on changes in student behavior. This study using a quasi-experimental pretest-posttest design with control groups, which conducted from March to May 2017. The subject in this research is the students of class VII in 5 (five) Bandung city areas by inclusion and exclusion criteria. A total of 36 students from the five junior secondary schools as the research group received an integrated learning module, while 38 students from different classes in the same school as the control group using the reproductive health module based on the 2013 Curriculum. The data collection used questionnaires to assess behavior based on self-assessment, friends, and parents. The results of this study indicate that the interpersonal communication behavior increased by 5.74%, reproductive health behavior increased by 18.65%, and sexual behavior increased by 9.07%, with a significant difference compared to students who received the 2013 Curriculum ($p<0.001$). In conclusion, the implementation of the integrated reproductive health module had affected the student behavior change in maintaining reproductive health, sexual, and interpersonal communication.

Key words: Adolescent, behavior, integrated, reproductive health

Pengaruh Penerapan Modul Pembelajaran Kesehatan Reproduksi Terintegrasi terhadap Perubahan Perilaku Siswa Sekolah Menengah Pertama

Abstrak

Perubahan sosial budaya, pemahaman agama yang rendah, perkembangan teknologi informasi, dan keterbatasan sumber informasi yang tepat menyebabkan permasalahan perilaku seksual dan kesehatan reproduksi pada remaja. Salah satu upaya yang dilakukan adalah melalui penerapan modul kesehatan reproduksi terintegrasi sebagai pedoman mengajar bagi guru dengan menggunakan pendekatan *student center learning*. Tujuan penelitian ini menganalisis pengaruh implementasi modul pembelajaran kesehatan reproduksi terintegrasi terhadap perubahan perilaku siswa. Penelitian ini menggunakan *quasi-experimental pretest-posttest design with control groups* yang dilakukan dari bulan Maret hingga Mei 2017. Subjek penelitian adalah siswa kelas VII di 5 (lima) wilayah Kota Bandung dengan kriteria inklusi dan eksklusi. Sebanyak 36 siswa dari lima sekolah menengah pertama sebagai kelompok perlakuan mendapatkan modul kesehatan reproduksi terintegrasi dan 38 siswa dari kelas yang berbeda di sekolah yang sama sebagai kelompok kontrol menggunakan materi kesehatan reproduksi berdasar atas Kurikulum 2013. Pengumpulan data menggunakan kuesioner penilaian perilaku oleh diri sendiri, teman, dan orangtua. Hasil menunjukkan bahwa perilaku komunikasi interpersonal siswa meningkat 5,74%, perilaku menjaga kesehatan reproduksi meningkat 18,65%, dan perilaku seksual sehat siswa meningkat 9,07% dengan perbedaan yang signifikan dibanding dengan siswa yang mendapatkan Kurikulum 2013 ($p<0,001$). Simpulan, penerapan modul pembelajaran kesehatan reproduksi terintegrasi berpengaruh terhadap perubahan perilaku kesehatan reproduksi siswa.

Kata kunci: Kesehatan reproduksi, perilaku, remaja, terintegrasi

Received: 16 November 2017; Revised: 18 December 2019; Accepted: 23 December 2019; Published: 31 December 2019

Correspondence: Dini Saraswati Handayani, S.S.T., M.K.M. Department of Public Health, Faculty of Medicine, Universitas Padjadjaran. Jln. Prof. Eyckman No. 38, Bandung 40161, West Java, Indonesia. E-mail: d_zsharaswaty@yahoo.com

Introduction

The behavior of adolescent reproductive health influences the quality of maternal and child health in Indonesia.[1–3] Reproductive health during adolescence is one of the ways for them to pursue healthy conditions in the next future. The fulfillment of balanced nutrition can help the process of growth and development of the organs and adolescent reproductive systems.

Besides that, good personal hygiene can also prevent them from problems that will disrupt the system and their reproductive functions. So that at the time of adulthood, system and reproductive functions can work better without any problems caused by the poor behavior of maintaining reproductive health during adolescence.[4,5] Besides, they are also faced with problems related to risky sexual behavior, dating at adolescence is one of the examples.[6]

Therefore, the education of reproductive health for children, adolescents, and young adults are needed more than just prevention of unwanted pregnancies, sexually transmitted infections, and human immunodeficiency virus.[6] According to Government Regulation of the Republic of Indonesia Number 61 Year 2014,[7] the services of adolescent reproductive health include communication, information and education, counseling, and medical services.

Reproductive education includes healthy life skills, mental resilience through social skills, systems, functions, and reproductive processes. It also discusses healthy and safe sexual behavior, risky sexual behavior and its consequences, family planning, and other risk behaviors or other health conditions which affect reproductive health.[7]

The interventions to overcome adolescent reproductive health problems are categorized based on program setting, namely, the school-based programs, mass media, communities, workplaces, and health facilities.[8] School-based interventions, although they have a slow impact, is one of the effective efforts because it has broad coverage and directed. The results concluded that students who received education became more responsible for sexual behavior, and they were able to postpone sexual relations.[9]

In the national education curriculum of Indonesian, reproductive health education is not given as a separate subject but integrated into several subjects. Based on Regulation of the Minister of Education and Culture of the Republic of Indonesia Number 24 Year 2016[10] concerning on core and essential competencies for learning in the 2013 Curriculum on primary and secondary education, it found that reproductive health materials specifically found in the Natural Sciences (*Ilmu Pengetahuan Alam*/IPA) and the Physical and Sports Education (*Pendidikan Jasmani, Olahraga, dan Kesehatan*/PJOK).

The results state that learning will be internalized and have a good impact on behavior change if it is constructed with integrated material and delivered from an early age.[11–13] Integrated learning of reproductive health delivers so that students can understand the phenomenon from all sides because it is viewed from other disciplines so that they can provide insight, knowledge, and understanding in dealing with direct experienced.[14–16] The integrated reproductive health education on adolescents expected to build the values, attitudes and adolescents behavior to be able to respect and protect their health and rights.[17,18]

The learning module of the integrated reproductive health contained material, methods, boundaries, and ways of evaluating systematically and interestingly. So, the module could help the teacher in delivering and understanding reproductive health learning material to students. Integrated reproductive health learning expected to provide an understanding of life skills, sexual and reproductive health, health behaviors and risk behaviors by integrating religious, socio-cultural and psychological by utilizing information and technological developments.[19]

The objectives of this study are to analyze the implementation effect of an integrated reproductive health module on changes in student behavior.

Methods

The method used in this study was the quasy experimental pretest-posttest with a group control design, which conducted from March to May 2017. In this research, there were two groups, namely the treatment and control group, which would be given a questionnaire before and after the intervention. The treatment group provided with the implementation of an integrated reproductive health learning module while the control group used reproductive health material based on the 2013 Curriculum.

The target population in this research were all junior high school at VII grader students

of Bandung city. In contrast, the reachable population is VII grader students in 5 public junior high schools (*sekolah menengah pertama negeri*/SMPN), which represent each region in Bandung city. This research used five junior high schools for the treatment and control groups, namely SMPN 12, SMPN 46, SMPN 31, SMPN 27, and SMPN 24. By using simple random sampling, it is obtained 40 students as a treatment group and 40 students as the control group. However, in the implementation processes, there were 4 students drop out of the treatment group and two students in the control group.

As a result, the research subjects were 74 students divided into treatment groups (36 students) and control groups (38 students). The character of the research included gender (male and female), age (12 and 13 years), and puberty status, which was characterized by menstruation on female and nocturnal orgasm/wet dreams on males. These characteristics are directly related to the dependent and independent variables. If the effect is statistically significant, the characteristics will change to confounding variables.[13]

Inclusion criteria are students in the VII grade of five SMPN who attend the reproductive health learning module class, living in one house with parents, and willing to participate in the research. While the exclusion criteria are all students who have received formal education about reproductive health and if parents and friends are not willing to provide behavioral assessments.

The subject is categorized to drop out if the attendance in integrated learning activities is less than 80%. The research starts with conducting an introductory survey, a literature study, and identifying the problems of reproductive health on adolescents. This module based on the results of a research of the syllabus in the 2013 Curriculum relating to reproductive health. The material of comprehensive sexuality education and the material of the great module.

In addition to the integrated reproductive health learning module that contains reproductive health material, there is also material on healthy living behavior, risky sexual behavior, and interpersonal communication that is associated with religious value, socio-cultural, and psychological. This module developed under the supervision of experts (reproductive health fields, obstetrics and gynecology/Obgyn, educational psychology, adolescent development psychology, communication, religion and socio-culture, nutrition, STIs and HIV/AIDS, and curriculum) to obtain validation, and curriculum parties of SMP of Bandung Education Office.

It is conducted the training on reproductive health learning modules by facilitators who are experts in their fields, which aim to increase the teachers' knowledge and self-efficacy about reproductive health. Afterward, the teacher implements the module for eight meetings with themes that are proper to the objectives in the module. Assessment of student reproductive health behavior conducted four times internally (herself) and externally (by parents and friends). Data analysis is used the chi-square test, paired t test, unpaired t ttest, and Mann-Whitney test.

This research was conducted after obtaining a feasibility permit from the Health Research Ethics Committee of Faculty of Medicine of Universitas Padjadjaran by applying the three basic principles of research, namely respect to person, beneficence and non-maleficence, and justice, with ethical approval letter number: 313/UN6.C10/PN/2017.

Results

Table 1 describes the character of students generally where the chi-square statistical test found that the characteristics in both groups homogeneous ($p>0.05$). Homogeneity of data can be used as a benchmark to compare between treatment and control groups.

Table 1 illustrated that the characteristics of gender, age and puberty in the treatment and control group in this research have p value>0.05 so that the treatment and control groups are categorized to be homogeneous, comparable and do not result in bias.

Table 2 shows that there were statistically significant differences in the increasing score of communication behaviors before and after the intervention was given in both groups ($p<0.05$), and there were significant differences in the percentage of the increasing score of communication behavior on treatment (5.74%), with p value<0.05.

Table 3 shows that there were statistically significant differences on the increasing score of reproductive health behavioral before and after intervention in both groups ($p<0.05$), and there were significant differences on the percentage of an increasing score of reproductive health behavior on intervention group (18.65%), with p value<0.05.

Table 4 shows that there were differences

Table 1 The Characteristic of Research Subject

Characteristics	Groups		p Value*
	Treatment (n=36)	Control (n=38)	
Gender			
Male	18	19	0.592
Female	18	19	
Age (year)			
12	17	17	0.507
13	19	21	
Puberty (wet dream/menstruation)			
Yes	30	32	0.583
No	6	6	
Having relationship			
Yes	16	18	0.493
No	20	20	

*Chi-square test, meaningful if p<0.05

Table 2 Comparison of Score Change in Communication Behavior Before and After the Implementation of the Integrated Reproductive Health Module

Communication Behavior Score	Groups				p Value**
	Intervention (n=36)	p Value*	Control (n=38)	p Value*	
Pretest					
Mean (SD)	67.57 (8,69)		66.61 (8.28)		
Median	67.59		66.84		0.628
Range	49.84–84.00		52.17–80.00		
		<0.001		0.005	
Posttest 1					
Mean (SD)	71.423 (6.3)		66.88 (8.1)		
Median	70.67		66.84		0.010
Range	60.50–84.00		52.17–80.00		
		0.283		0.535	
Posttest 2					
Mean (SD)	71.08 (6.4)		66.85 (8.16)		
Median	71.08		66.84		0.016
Range	56.67–83.17		52.17–80.00		
		0.092		0.142	
Posttest 3					
Mean (SD)	71.05 (6.8)		66.69 (6.82)		
Median	71.25		66.67		0.015
Range	56.67–83.17		52.17–80.00		
		0.07		0.62	
Posttest 4					
Mean (SD)	72.12 (6.57)		66.51 (7.97)		0.015
Median	72.91		67.5		
Range	60.50–83.17		50.50–80.00		
% change pretest to posttest 3	5.74		0.18		
p value***		<0.001			
% change pretest to posttest 4	7.44		−0.015		
p value***		<0.001			
% change posttest 3 to posttest 4	1.61		−0.202		
p value***		0.044			

*paired t test, **unpaired t test, ***Mann-Whitney test, posttest 1=direct after intervention, posttest 2=two weeks after intervention, posttest 3=two weeks after posttest 2, posttest 4=six month after intervention

Table 3 Comparison of Score Change in Reproductive Health Behavior Before and After the Implementation of the Integrated Reproductive Health Module

Reproductive Health Behavior Score	Groups				p Value**
	Intervention (n=36)	p Value*	Control (n=38)	p Value*	
Pretest					
Mean (SD)	62.11 (8.9)		63.61 (7.26)		
Median	61.07		63.60		0.463
Range	43.56–79.98		45.54–76.28		
		<0.001		0.001	
Posttest 1					
Mean (SD)	69.61 (6.2)		64.83 (6.8)		
Median	70.03		64.97		0.004
Range	58.39–83.95		46.24–75.26		
		<0.001		0.036	
Posttest 2					
Mean (SD)	71.91 (6.7)		65.184 (6.8)		
Median	72.37		65.2		<0.001
Range	58.39–84.99		50.67–75.55		
		<0.001		0.904	
Posttest 3					
Mean (SD)	72.96 (6.5)		65.19 (6.5)		<0.001
Median	73.80		64.55		
Range	58.39–84.99		50.67–75.55		
Posttest 4					
Mean (SD)	74.78 (5.7)		64.58 (6.11)		<0.001
Median	75.32		64.49		
Range	58.39–84.99		51.85–75.49		
% change pretest to posttest 3	18.65		2.89		
p value***		<0.001			
% change pretest to posttest 4	21.92		2.06		
p value***		<0.001			
% change posttest 3 to posttest 4	2.744		0.8		
p value***		0.02			

*paired t test, **unpaired t test, ***Mann-Whitney test, posttest 1=direct after intervention, posttest 2=two weeks after intervention, posttest 3=two weeks after posttest 2, posttest 4=six month after intervention

in the increased score of reproductive health behavioral scores before and after intervention in both groups ($p<0.05$), and there were significant differences in the percentage of an increasing score of reproductive health behavior on intervention group (9.07%), with p value<0.05.

Discussion

The application of integrated reproductive health learning modules that performed in this research has a significant influence on improving students' communication behavior. It is showed that the methods and material delivered by the teacher are quite capable of encouraging students to improve communication behavior. However, because communication is a behavior that must be strengthened with skills, the strengthening of communication skills material is needed to improve effective communication.[20]

Communication behavior is influenced by five elements of communication, according to Harold D. Lasswell. They are, for example, who? Says what? On which channel? To whom? With what effect?[21] That is, to change communication behavior, it requires the involvement of many factors. Not only learning in the classroom, but the repetition and stimulus from the environment also becomes an influences factor on the changes in communication behavior.

The results of the pre and posttest on communication behavior showed a significant increase only during the intervention. However, from posttest 1 to posttest 2 and posttest 2 to

Table 4 Comparison of Score Change in Sexual Behavior Before and After the Implementation of the Integrated Reproductive Health Module

Sexual Behavior Score	Groups				p Value**
	Intervention (n=36)	p Value*	Control (n=38)	p Value*	
Pretest					
Mean (SD)	79.38 (9.5)		80.04 (7.8)		
Median	79.23		80.87		0.745
Range	58.23–94.40		62.58–94.93		
		<0.001		0.014	
Posttest 1					
Mean (SD)	84.35 (6.6)		80.98 (7.1)		
Median	83.89		81.34		0.039
Range	71.61–97,47		65.30–97.01		
		<0.001		<0.001	
Posttest 2					
Mean (SD)	85.10 (6.5)		81.37 (7.1)		
Median	85.27		81.74		0.021
Range	71.61–97.39		65.97–97.01		
		<0.001		0.627	
Posttest 3					
Mean (SD)	85.97 (6.3)		81.44 (7.0)		
Median	86.05		81.76		0.005
Range	72.92–97.39		66.61–97.39		
		0.033		0.012	
Posttest 4					
Mean (SD)	87.12 (5.3)		80.76 (6.8)		<0.001
Median	86.85		80.81		
Range	72.92–97.39		66.61–93.58		
% change pretest to posttest 3	9.07		1.94		
p value***		<0.001			
% change pretest to posttest 4	10.78		1.12		
p value***		<0.001			
% change posttest 3 to posttest 4	9.07		1.94		
p value***		0.002			

*paired t test, **unpaired t test, ***Mann-Whitney test, posttest 1=direct after intervention, posttest 2=two weeks after intervention, posttest 3=two weeks after posttest 2, posttest 4=six month after intervention

posttest 3, both in the intervention and control group, their increases were not significant. Moreover, there was a decrease in the average score on communication behavior from posttest 2 to posttest 3 in the control group. In line with the theory, which states that skills strongly influence communication.[20] Although the difference in the percentage score gets a significant value in the treatment group, the value is lower when compared with the percentage of an increasing score of reproductive health behavior.

The results of in-depth interviews through focus group discussion (FGD) on parents and friends found that if compare to the material of reproductive health and sexuality, communication materials are a little more challenging to understand because it has not enough to explain only by the theory. Students who have shy characters will have little difficulty in performing the practices implemented in the communication theme learning.

However, according to several FGD participants, the game of Johari Window from the module provides a new experience to give and get ratings from friends, so they can be more open and understand themselves. On changes in reproductive health, this research provides significant results. It shows that the methods and material presented by the teacher can encourage students to reproduce better health behavior.

The main factor that underlying the formation of changes in reproductive health behavior is the provision of detail information and delivery of the right methods in the classroom.

During the implementation process, the teacher has been provided with core and supporting material by experts. At the time of implementation takes place, the teacher already has the knowledge and functional self-efficacy. In addition to understanding social and cultural-religious values and adolescent psychology becomes a strengthening factor in the presentation of material in the class.

At pretest time, students have inadequate knowledge and attitudes towards reproductive health behaviors, so that it can influence the behavior. Submitting the dangers of poor reproductive health behavior and explicit material on how to perform these behaviors makes students have better knowledge and attitudes at the end of learning, so they try to do these behaviors in their daily lives.

At the time after the implementation of the change in behavior exists, the value is not as significant as when the assessment conducted one month later. Behavior is a response to a stimulant that must be done repeatedly and is influenced by internal and external factors, so the assessment of 4-week, 8-week, and 6-month continue to very good improvement.

It is in line with the theory of models which developed to illustrate and explain that behavior change requires certain stages. This model is based on the statement that behavior change is a process, not an event and that individuals have varying degrees of motivation or readiness to change the behavior.[22] Reproductive health behavior in students after implementation is in the contemplation phase. It is where a person begins to respond to make changes to certain behaviors. One month later, students enter the preparation and action phase. It is where students have begun to make serious commitments to change and behavior. Then to make sure the behavior changes to the character, it will take six months or called by the maintenance phase.[23]

During the intervention, they only get an explanation of the nutrition fulfillment of adolescents, personal hygiene, and risky sexual behavior delivered by the lecture method in most schools.

In line with reproductive health behavior, the low of sexual behavior on adolescents often caused by ignorance of the impact that will occur.[24] For sexual behavior, the influence of external factors is more significant when compared to the behavior of maintaining reproductive health.[25]

By knowing the impact, students become more afraid to perform risky sexual behavior. Besides, the method of interactive presentation and student involvement will make them more understanding, not just only knowing. For the control group who did not get the module, they also experienced increasing scores on sexual behavior during the intervention (pretest to posttest 1). Besides that, the repeated questions in the questionnaire become a source of information for students to conduct good sexual behavior. However, this researcher cannot control the interactions between students in the control and the intervention group, so information exchange between the groups is likely to occur. In both groups, the score of the beginning assessment of sexual behavior before the intervention was good. It was because of the age of the respondents were still early adolescents and was still little influence from external factors that influenced sexual behavior. Therefore sexual health material can be used as a prevention effort, so when adolescence grows up, they have the self-concept and strong beliefs to avoid risky sexual behavior.

By using the Spearman rank correlation test between the three dependent variables, there is a significant relationship between them ($p<0.05$). Thus, it can be said that communication behavior, reproductive health, and sexual behavior have links with one to another. To achieve optimal reproductive and sexual health during adolescence to adulthood phase needs good behavior in communication, maintains reproductive health, and avoids risky behavior.

Conclusions

Implementation of the integrated reproductive health module had affected changes in students' reproductive health behavior.

Conflict of Interest

The authors have no conflict of interest to report.

Acknowledgments

Our thanks to teachers and students in five public junior high schools who participated

in this study. To all the experts who helped us solve the problems, Bandung City Department of Education, who gave research permission and especially to Universitas Padjadjaran for funding the research through an internal grant.

References

1. Fatmawati I. Perbandingan tingkat pengetahuan dan sikap remaja yang mendapat program DAKU! dan yang tidak terhadap kesehatan reproduksi remaja di Kota Singkawang [undergraduate thesis]. Depok: Fakultas Kesehatan Masyarakat, Universitas Indonesia; 2012 [cited 2017 February 20]. Available from: http://lib.ui.ac.id/file?file=digital/20320038-S-Ima%20Fatmawati.pdf.
2. Ramayanty D, Sanusi SR, Fitria M. Hubungan pengetahuan dan sikap remaja tentang kesehatan reproduksi dengan perilaku seksual di SMA Bayu Pertiwi Sunggal tahun 2015. GKRE. 2015;1(2):4553.
3. Farhanah, Ibrahim R, Ridwan R. Proceeding report: comperhensive sexuality education fair 2015 [Internet]. Jakarta: Rutgers WPF Indonesia; 2015 [cited 2017 February 20]. Available from: https://www.researchgate.net/publication/308103997_Proceeding_report_Comprehensive_Sexuality_Education_Fair_2015.
4. Ayu M, Rosmawar C. Hubungan pengetahuan dan sikap dengan perilaku kesehatan reproduksi pada remaja putri di SMA 5 Banda Aceh [undergraduate thesis]. Banda Aceh: STIKes U'Budiyah; 2013 [cited 2017 February 20]. Available from: https://adoc.tips/hubungan-pengetahuan-dan-sikap-dengan-perilaku-kesehatan-rep.html.
5. United Nations Educational, Scientific and Cultural Organization (UNESCO). Puberty education & menstrual hygiene management. Paris: UNESCO; 2014.
6. Hurlock EB, Istiwidayanti, Soedjarwo, Sijabat RM. Psikologi perkembangan: suatu pendekatan sepanjang rentang kehidupan. 5th Edition. Jakarta: Penerbit Erlangga; 1991.
7. Peraturan Pemerintah Republik Indonesia Nomor 61 Tahun 2014 tentang Kesehatan Reproduksi.
8. Speizer IS, Magnani RJ, Colvin CE. The effectiveness of adolescent reproductive health interventions in developing countries: a review of the evidence. J Adolesc Health. 2003;33(5):324–48.
9. Peraturan Menteri Pendidikan dan Kebudayaan Republik Indonesia Nomor 24 Tahun 2016 tentang Kompetensi Inti dan Kompetensi Dasar Pelajaran pada Kurikulum 2013 pada Pendidikan Dasar dan Pendidikan Menengah.
10. Breuner CC, Mattson G, AAP Committee on Adolescence, AAP Committee on Psychosocial Aspects of Child and Family Health. Sexuality education for children and adolescents. Pediatrics. 2016;138(2):e20161348.
11. Lally P, van Jaarsveld CHM, Potts HWW, Wardle J. How are habits formed: modelling habit formation in the real world. Eur J Soc Psychol. 2010;40(6):998–1009.
12. Creswell JW, Fawaid A, Pancasari RK. Research design: pendekatan metode kualitatif, kuantitatif, dan campuran. 4th Edition. Yogyakarta: Pustaka Pelajar; 2016.
13. Sastroasmoro S, Ismael S. Dasar-dasar metodologi penelitian klinis. 5th Edition. Jakarta: Sagung Seto; 2014.
14. International Youth Foundation. Integrating reproductive health into youth development programs: lessons for the future [Internet]. Field Notes. Volume 4. Number 18. December 2011 [cited 2017 March 30]. Available from: https://www.iyfnet.org/sites/default/files/FieldNotes18_Integrating_Reproductive_Health.pdf.
15. Wahba M, Roudi-Fahmi F. The need for reproductive health education in schools in Egypt [Internet]. Population Reference Bureau (PRB) Policy Brief. October 2012 [cited 2017 April 22]. Available from: https://www.prb.org/wp-content/uploads/2012/10/reproductivehealth-education-egypt.pdf.
16. United Nations Educational, Scientific and Cultural Organization (UNESCO). Emerging evidence, lessons and practice in comprehensive sexuality education: a global review. Paris: UNESCO; 2015.
17. World Health Organization (WHO). A standards-driven approach to improve the quality of health-care services for adolescents: policy brief [Internet]. Geneva: WHO; 2015 [cited 2017 April 30]. Available from: https://apps.who.int/iris/handle/10665/184035.
18. United Nations Population Fund (UNFPA). The evaluation of comprehensive sexuality education programmes: a focus on the

gender and empowerment outcomes [Internet]. New York: UNFPA; 2015 [cited 2017 April 31]. Available from: https://www.unfpa.org/sites/default/files/pub-pdf/UNFPAEvaluationWEB4.pdf.
19. Aarø LE, Flisher AJ, Kaaya S, Onya H, Fuglesang M, Klepp KI, et al. Promoting sexual and reproductive health in early adolescence in South Africa and Tanzania: development of a theory- and evidence-based intervention programme. Scand J Public Health. 2006;34(2):150–8.
20. Zoppi K, Epstein RM. Is communication a skill? Communication behaviors and being in relation. Fam Med. 2002 May;34(5):319–24.
21. Syam NW. Psikologi sebagai akar ilmu komunikasi. Bandung: Simbiosa Rekatama Media; 2011.
22. Fishbein M, Ajzen I. Predicting and changing behaviour: the reasoned action approach. New York: Psychology Press; 2010.
23. Parmar SM, Taylor R. Health promotion & behavioural change theory [Internet]. Calgary, Canada: Alberta Health Services; 2010 [cited 2017 April 29]. Available from: https://pdf4pro.com/cdn/health-promotion-amp-behavioural-change-theory-2d602.pdf.
24. World Health Organization (WHO). Developing sexual health programmes: a framework for action [Internet]. 2010 [cited 2017 May 10]. Available from: https://apps.who.int/iris/bitstream/handle/10665/70501/WHO_RHR_HRP_10.22_eng.pdf.
25. McCall D, McKay A; Society of Obstetricians and Gynaecologists of Canada. School-based and school-linked sexual health education and promotion in Canada. J Obstet Gynaecol Can. 2004;26(6):596–605.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.4202

GMHC. 2019;7(3):206–12
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

VEGF-A and PD-L1 Immunoexpression Association with Meningioma Histopathology Grade

Yunnica,[1,2] Afiati,[2] Hermin Aminah Usman,[2] Bethy Surjawathy Hernowo[2]

[1]Anatomical Pathology Study Program, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, [2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

Abstract

Histopathology grade of meningioma is one of the most common factors determining the prognosis and affects the risk of recurrence and aggressiveness of the tumor. Biological factors related to histopathological grade are vascular endothelial growth factor A (VEGF-A) and programmed death-ligand 1 (PD-L1). This research aimed to understand the association between VEGF-A and PD-L1 with meningioma histopathology grade. This is in vivo research on 60 paraffin blocks of meningioma cases at Dr. Hasan Sadikin General Hospital Bandung from April to November 2018. Paraffin block samples consist of grade I (30), grade II (15), and grade III (15) meningioma. Immunohistochemical staining of VEGF-A and PD-L1 performed to all samples. Data analyzed using the chi-square test with SPSS version 24.0 for Windows. The result shows a significant association between VEGF-A and PD-L1 immunoexpression with meningioma histopathology grade. PD-L1 is the most potent factor that influenced the histopathology grade of meningioma. The study concluded that the histopathology grade of meningiomas influenced by angiogenesis and immune checkpoints. VEGF-A and PD-L1 immunoexpression in meningioma considered as a factor that influences the aggressiveness of meningioma.

Key words: Histopathology grade, meningioma, PD-L1, VEGF-A

Hubungan Imunoekspresi VEGF-A dan PD-L1 dengan Derajat Histopatologi Meningioma

Abstrak

Derajat histopatologi meningioma merupakan salah satu faktor yang paling umum menentukan prognosis serta memengaruhi risiko rekurensi dan agresivitas tumor. Faktor biologi yang berhubungan dengan derajat histopatologi adalah *vascular endothelial growth factor A* (VEGF-A) dan *programmed death-ligand 1* (PD-L1). Penelitian ini bertujuan mengetahui hubungan imunoekspresi VEGF-A dan PD-L1 dengan derajat histopatologi meningioma. Penelitian *in vivo* dilakukan pada 60 blok parafin kasus meningioma di Departemen Patologi Anatomi RSUP Dr. Hasan Sadikin Bandung dari April hingga November 2018. Sampel blok parafin terdiri atas meningioma derajat I (30), derajat II (15), dan derajat III (15). Pulasan imunohistokimia VEGF-A dan PD-L1 dilakukan terhadap semua sampel. Data dianalisis menggunakan uji *chi-square* dengam SPSS versi 24.0 untuk Windows. Hasil penelitian menunjukkan bahwa terdapat hubungan yang signifikan antara VEGF-A dan PD-L1 dengan derajat histopatologi meningioma. PD-L1 merupakan faktor paling kuat yang memengaruhi derajat histopatologi meningioma. Simpulan penelitian ini adalah derajat histopatologi meningioma dipengaruhi oleh faktor angiogenesis dan *immune check point*. Imunoekspresi VEGF-A dan PD-L1 pada meningioma dapat dipertimbangkan sebagai faktor yang memengaruhi agresivitas meningioma.

Kata kunci: Derajat histopatologi, meningioma, PD-L1, VEGF-A

Received: 19 November 2018; Revised: 1 August 2019; Accepted: 5 September 2019; Published: 31 December 2019

Correspondence: Yunnica, dr. Department of Anatomical Pathology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital. Jln. Pasteur No. 38, Bandung 40161, West Java, Indonesia. E-mail: yunnicaicu@gmail.com

Introduction

Meningioma is the most common intracranial tumor from the meningothelial cells of the arachnoid layer.[1,2] Thirty-six percent of all primary central nervous tumor is meningioma.[2] Meningioma is the most common intracranial tumor in the USA and Korea. The incidence of meningioma in the USA is 36% and 37.3% in Korea.[1,3] The median age of patients with meningioma is 65 years. Females are at higher risk than male, with a female:male ratio of nearly 2:1.1.[4] Groups who have lower incidences of meningiomas, specifically men and children, tend to have more aggressive tumors when they do occur.[5,6]

The recurrence of meningioma is generally varies depending on the histopathology grade of meningioma, i.e., 12% in grade I, 29–40% in grade II, and 50–94% in grade III.[1,7] The recurrence of meningiomas is strongly associated with histopathological grade. The mortality rate of atypical meningioma grade II and anaplastic meningioma grade III in 5 years are 21% and 68%. The mean survival rate of anaplastic meningioma is 1.5 years.[8] Malignant meningiomas constitute about 1% to 3% of meningiomas.[9]

The histopathology grade is one of the most critical indicators in determining prognosis.[1] The histopathology grade of meningioma determined by tumor morphology, proliferation index, and invasion of brain tissue.[8] Variants of meningiomas are divided based on the risk of recurrence and aggressive of the tumor.[1] The aggressiveness of meningioma determined by mitotic index, other proliferation markers, cell differentiation, genetic in tumor cells, and morphological features of pathology. Morphological features of meningioma are essential in predicting the behavior meningioma.[10–12] Grade I is meningioma with a low risk of recurrence and is less aggressive. While grade II, III and any subtype of meningiomas with a high proliferation index are meningiomas with a high risk of recurrence and aggressive.[1]

Meningioma is a high vascularization tumor requiring neovascularization for its growth.[13] The secretion of angiogenesis factors—such as vascular endothelial growth factor A (VEGF-A)—can induce neovascularization as one of the necessary conditions for meningioma growth. Angiogenesis in meningiomas is associated with an aggressive tumor. Angiogenesis is significantly associated with considerable tumor growth, shorter recurrence, and survival rates in meningioma.[14–18]

VEGF-A affects the immunosuppressive tumor microenvironment by inhibiting the maturation of dendritic cells. It stimulates the immunoexpression of programmed death-ligand 1 (PD-L1) in tumor cells. VEGF-A interferes with the cancer-immunity cycle through stages, namely inhibiting the maturation of dendritic cells, disrupting infiltration of T cells into tumors, and promoting immunosuppressive tumor microenvironment.[19]

PD-L1 expressed in high-grade meningioma, suggested to have a significant role in tumor aggressivity.[8] Immunosuppressive effect tumor cells on immune surveillance caused by tumor cells expressing PD-L1 create the programmed death-1 (PD-1)/PD-L1 axis, which is the convergence of PD-1 in T cells with ligand namely PD-L1 in tumor cells. It is called an immune checkpoint. It causes cytotoxic T cells weakness.[20,21]

Currently, anti-VEGF target therapy usually used by bevacizumab. Bevacizumab is a monoclonal antibody at VEGF receptors interferes with binding and signal transduction needed for tumor vascularization. It resulting in a regression of blood supply to the tumor.[22,23] Bevacizumab has conducted phase II clinical trials in patients with recurrent or progressive meningiomas.[24] Targeted anti-VEGF therapy is a promising avenue for meningioma because of robust pharmaceutical development and VEGF expression.[25] Angiogenesis may represent a target for therapies aimed at reducing the growth of inoperable meningioma or recurrence risk of totally resected tumor.[26]

The intracranial lymphatic system is associated with dural sinus, which promises immunotherapy to meningioma. The Gelerstein et al.'s[27] case report study, show the use of immunotherapy (anti-PD-1/anti-PD-L1) reduces tumor size in meningioma.[28] At present the anti-PD-1 immunotherapy used is nivolumab. Phase II clinical trials in the evaluation of PD-1 checkpoint blockade with nivolumab carried out in grade II/III meningioma.[29]

This study aimed to describe the association between VEGF-A and PD-L1 immunoexpression with histopathological meningioma grade.

Methods

The time of study took place from April to November 2018. Samples were taken from

patients registered at Dr. Hasan Sadikin General Hospital Bandung and obtained 60 samples. They diagnosed histopathologically with meningioma from January 2013 to August 2018 and classified in the group of grade I (30 samples), grade II (15 samples), and grade III (15 samples). The tumor cells collected through operation and fixated in the paraffin block. This is an in vivo study using samples from paraffin blocks prepared for immunohistochemistry (IHC) analysis. IHC analysis was performed based on the protocol provided by the anatomical pathology laboratory. The slides visualized under the Olympus BX53F microscope.

Mouse monoclonal antibody VEGF-A SC-7269 (Santa Cruz Biotechnology, USA) with 1:100 dilution and rabbit monoclonal antibody clone 28-8 (ab205921) (Abcam, USA) with 1:100 dilution used in standard immunohistochemistry staining procedure. The positive result was shown/visualized as brown staining on the tumor cell. Analysis of VEGF-A and PD-L1 immunoexpression evaluated by brown staining assessed on the tumor cell membrane and or cytoplasm, respectively. The staining intensity measured under the microscope converted into a score. Intensity score explained as 0 (null) represents blank samples or no stained tumor cells; score 1+ for positive weak; score 2+ for moderate positive; and score 3+ for the strong positive. Distribution score explained as score 0 (null) represent no stained tumor cells; score 1+ for less than 20% colored/stained tumor cells; score 2+ for 20%–50% stained tumor cells; score 3+ for 51%–80% stained tumor cells, and lastly score 4+ represent >80% of stained tumor cells. Histoscore (distribution×intensity) was interpreted as ≥6=high and <6=low. IHC staining result was examined by two experts in the IHC technique using light microscope Olympus BX53F.[14,19]

This study used a retrospective analytic observational method with a cross-sectional study design. All data were analyzed using the chi-square test with a p value<0.05 of a significant level then proceed with SPSS version 24.0 for Windows.

Ethical clearance approved by Research Ethics Committee, Universitas Padjadjaran, with assessment number: 997/UN6.KEP/EC/2018.

Results

Table 1 showed the characteristics of subjects such as age and sex. The incidence of meningioma was more frequent among females (73%) than males (27%).

Table 2 showed no significant difference between each character of subjects on the histopathology grade of the meningioma group (p≥0.05).

Table 3 showed the significant association between the VEGF-A immunoexpression and histopathology grade of meningioma (p=0.0001). It also showed a significant association between the PD-L1 immunoexpression and histopathology grade of meningioma (p=0.0001).

Table 4 showed that there are association VEGF-A and PD-L1 with histopathology grade of meningioma, and PD-L1 is the strongest factor that influences the histopathology grade of meningioma (p=0.001).

Table 1 Characteristics of the Subjects

Age and Sex	n=60
Age (years)	
Mean	42
Sex	
Males	16 (27%)
Females	44 (73%)

Table 2 Homogeneity Test of the Subjects

Age and Sex	Histopathology Grade of Meningioma			p Value
	Grade I (n=30)	Grade II (n=15)	Grade III (n=15)	
Age (years)				0.614
Mean	43	41	40	
Sex				0.360
Males	6	4	6	
Females	24	11	9	

Note: for categorical data, the p value analyzed with the chi-square test, but for numeric data, the p value analyzed with the ANOVA test. The value of significance based on a p value<0.05

Table 3 VEGF-A and PD-L1 Histoscore Data on Histopathology Grade of Meningioma Group

Variables	Histopathology Grade of Meningioma			p Value
	Grade I (n=30)	Grade II (n=15)	Grade III (n=15)	
Histoscore VEGF-A				0.0001
Low	20	3	2	
High	10	12	13	
Histoscore PD-L1				0.0001
Low	23	4	2	
High	7	11	13	

Note: for categorical data, the p value analyzed with the chi-square test. The value of significance based on a p value<0.05

Table 4 Association VEGF-A and PD-L1 Immunoexpression with Histopathology Grade of Meningioma

Variables	Estimation Rate	Standard Error	p Value
VEGF-A	−1.8	0.6	0.005
PD-L1	−2.2	0.6	0.001

Note: for multivariate data, analysis using ordinal logistic regression analysis. The independent variable included in the logistic regression model is the independent variable, which in the bivariate analysis has a p value of less than 0.25. The value of significance based on a p value<0.05

Discussion

The sample's characteristics are homogeneous; both age and sex are not confounding variables in this study. Hence the data is qualified for further statistical analysis.

The VEGF-A plays an essential role in the regulation of tumor angiogenesis by promoting the migration, proliferation, and tube formation of endothelial cells.[13] Angiogenesis has evaluated as a mechanism which influences meningioma growth and recurrence.[30]

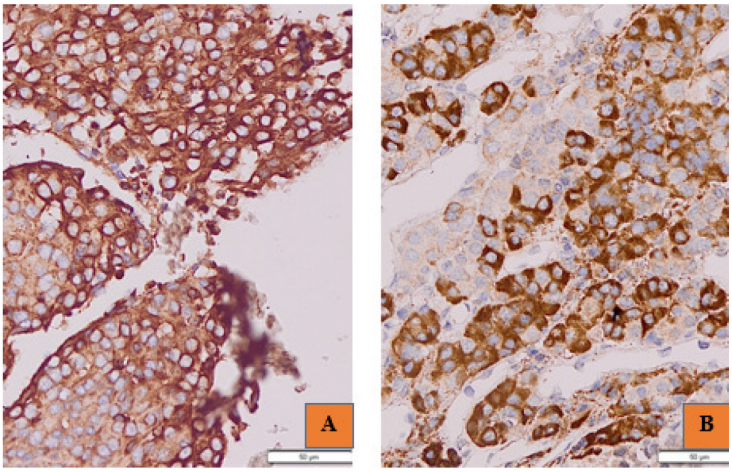

Figure VEGF-A and PD-L1 Imunoexpression of Meningioma with Strong Intensity (200× Magnification)

Note: A=VEGF-A strong intensity. Tumor cells stained with VEGF-A antibody in the membrane and or cytoplasm; B=PD-L1 strong intensity. Tumor cells stained with PD-L1 antibody in the membrane and or cytoplasm

This study performed a VEGF-A expression analysis on the meningioma histopathology grade. The result from IHC staining confirms that there were 12 of 15 with high VEGF-A immunoexpression in grade II dan 13 of 15 in grade III. There is a significant (p=0.0001) correlation between VEGF-A expression with meningioma histopathology grade. High immunoexpression VEGF-A is the result of high-grade meningioma. A study by Lee et al.[14] stated that VEGF is an angiogenesis activator, which correlated with increased tumor grade.

Meningioma that proliferates caused hypoxia, which will stimulate hypoxia-inducible factor-1α (HIF-1α) that stimulate VEGF-A. VEGF-A through the mitogen-activated protein kinase (MAPK) pathway cause endothelial cell proliferation. The phosphatidylinositol 3-kinase (PiK3)/Akt pathway will inhibit endothelial cell apoptosis resulting in neovascularization differentiation. Neovascularization differentiation provides more nutrition to the tumor so that it will cause the tumor to proliferate.[9] Neoangiogenesis shows that aggressive behavior is related to the high grade of histopathology.[14]

PD-L1 expressed in tumor cells binding to PD-1, which binds to T cells, and the engagement of PDL1 with PD-1 of T cell creates T cell dysfunction in tumor mass causes immunoresistence. The function of a tumor overexpressing PD-L1 is to protect itself from cytotoxic T cell (CD8+) mediated cell killing.[20,21] It has been suggested that the immunosuppressive effects of PD-L1 expression contribute to the aggressive behavior of meningiomas, especially those of higher grade.[8]

This research also performed the PD-L1 immunoexpression analysis on meningioma histopathology grade. The result from IHC staining confirms that there were 11 out of 15 with high immunoexpression PD-L1 in grade II and 13 of 15 in grade III. According to chi-square analysis, there is a significant (p=0.0001) between the PD-L1 immunoexpression and high-grade meningioma. Strong PD-L1 immunoexpression found in high-grade meningioma. The study by Du et al.[8] was similar to this study. Both showed PD-L1 expression increased in WHO anaplastic meningioma grade II and III. Du et al.[8] showed that high-grade meningioma harbor an immunosuppressive tumor microenvironment and that increased Treg cells and elevated PD-L1 might contribute to the aggressive phenotype of these tumors.

Hypoxia stimulates HIF-1α, which can upregulate PD-L1. PD-L1 can stimulate Tregs cells, which will inhibit cytotoxic T cells so that immunoresistance occurs.[31] The state of immunoresistance causes cytotoxic T cells to be unable to fight tumor cells so that they exhibit an aggressive phenotype related to high histopathological grade.[8]

VEGF-A not only promotes angiogenesis and vascular permeability, but it also contributes to an immunosuppressive tumor microenvironment. VEGF-A was reported to inhibit the maturation of dendritic cells, which cause differentiation of Treg cells and upregulate the immunoexpression of PD-L1 in tumor cells.[19]

There was an association between VEGF-A and PD-L1 with meningioma histopathology grade. PD-L1 is the most influential factor that influences the histopathology grade of meningioma (p=0.001). A study by Xue et al.,[19] confirmed that PD-L1 immunoexpression was significantly associated with the pathological grade, VEGF status, and KI-67 index in glioma.

Conclusions

There was a significant association between VEGF-A and PD-L1 with high-grade meningioma. Strong VEGF-A and PD-L1 immunoexpression positively associated with the likelihood of high-grade meningioma. Meningioma patients with II and III degrees recommended for VEGF-A and PD-L1 immunohistochemical examination to plan targeted therapy and immunotherapy.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Perry A, Louis DN, Budka H, von Deimling A, Sahm F, Rushing EJ, et al. Meningioma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, editors. WHO classification of tumours of the central nervous system. 4th Revised Edition. Geneva: WHO Press; 2016. p. 232–45.
2. Osborn AG. Tumors of the meninges. In: Osborn AG, Hedlund GL, Salzman KL, editors. Osborn's brain: imaging, pathology and anatomy. 2nd Edition. Salt Lake City: Elsevier Inc.; 2018. p. 659–94.

3. Dho YS, Jung KW, Ha J, Seo Y, Park CK, Won YJ, et al. An updated nationwide epidemiology of primary brain tumors in Republic of Korea, 2013. Brain Tumor Res Treat. 2017;5(1):16–23.
4. Perry A. Tumours of the meninges. In: Love S, Perry A, Ironside J, Budka H, editors. Greenfield's neuropathology. 9th Edition. Boca Raton: CRC Press; 2015. p. 1803–27.
5. Schniederjan MJ. Biopsy interpretation of the central nervous system. 2nd Edition. Philadelphia: Wolters Kluwer; 2018.
6. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
7. Johnson MD. PD-L1 expression in meningiomas. J Clin Neurosci. 2018;57:49–51.
8. Du Z, Abedalthagfi M, Aizer AA, McHenry AR, Sun HH, Bray MA, et al. Increased expression of the immune modulatory molecule PD-L1 (CD274) in anaplastic meningioma. Oncotarget. 2015;6(7):4704–16.
9. Jeung JA. Malignant (anaplastic) meningiomas. In: Yachnis A, Rivera-Zengotita M, editors. Neuropathology: a volume in the high-yield pathology series. 1st Edition. Philadelphia: Saunders; 2014. p. 144–5.
10. Velnar T. Meningiomas: pathology and clinical characteristics. In: Figueroa D, editor. Meningiomas: risk factors, treatment options and outcomes. New York: Nova Science Publishers, Inc.; 2016. p. 1–12.
11. Han MH, Kim CH. Risk factors of meningioma. In: Figueroa D, editor. Meningiomas: risk factors, treatment options and outcomes. New York: Nova Science Publishers, Inc.; 2016. p. 13–28.
12. Poon MTC, Leung GKK. Surgical treatment for intracranial meningioma in the elderly. In: Figueroa D, editor. Meningiomas: risk factors, treatment options and outcomes. New York: Nova Science Publishers, Inc.; 2016. p. 137–40.
13. Wu Y, Lucia K, Lange M, Kuhlen D, Stalla GK, Renner U. Hypoxia inducible factor-1 is involved in growth factor, glucocorticoid and hypoxia mediated regulation of vascular endothelial growth factor-A in human meningiomas. J Neurooncol. 2014;119(2):263–73.
14. Lee SH, Lee YS, Hong YG, Kang CS. Significance of COX-2 and VEGF expression in histopathologic grading and invasiveness of meningiomas. APMIS. 2014;122(1):16–24.
15. Huang MC, van Loveren HR. Anatomy and biology leptomeninges. In: DeMonte DF, McDermott MW, Al-Mefty O, editors. Al-Mefty's meningiomas. 2nd Edition. New York: Thieme Medical Publishers, Inc.; 2011. p. 25–34.
16. Claus EB, Morrison AL. Epidemiology of meningiomas. In: DeMonte DF, McDermott MW, Al-Mefty O, editors. Al-Mefty's meningiomas. 2nd Edition. New York: Thieme Medical Publishers, Inc.; 2011. p. 35–39.
17. Morrison AL, Rushing E. Pathology of meningiomas. In: DeMonte DF, McDermott MW, Al-Mefty O, editors. Al-Mefty's meningiomas. 2nd Edition. New York: Thieme Medical Publishers, Inc.; 2011. p. 40–50.
18. Ragel BT, Jensen RL. Molecular biology of meningiomas: tumorigenesis and growth. In: DeMonte DF, McDermott MW, Al-Mefty O, editors. Al-Mefty's meningiomas. 2nd Edition. New York: Thieme Medical Publishers, Inc.; 2011. p. 51–62.
19. Xue S, HU M, Li P, Ma J, Xie L, Teng F, et al. Relationship between expression of PD-L1 and tumor angiogenesis, proliferation, and invasion in glioma. Oncotarget. 2017;8(30):49702–12.
20. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol. 2017;8:561.
21. Momtaz P, Postow MA. Immunologic checkpoints in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmgenomics Pers Med. 2014;7:357–65.
22. Lou E, Sumrall AL, Turner S, Peters KB, Desjardins A, Vredenburgh JJ, et al. Bevacizumab therapy for adults with recurrent/progressive meningioma: a retrospective series. J Neurooncol. 2012; 109(1):63–70.
23. Nassehi D. Intracranial meningiomas, the VEGF-A pathway, and peritumoral brain oedema. Dan Med J. 2013;60(4):B4626.
24. Franke AJ, Skelton WP IV, Woody LE,

Bregy A, Shah AH, Vakharia K, et al. Role of bevacizumab for treatment-refractory meningiomas: a systematic analysis and literature review. Surg Neurol Int. 2018;9:133.
25. Gupta S, Bi WL, Dunn IF. Medical management of meningioma in the era of precision medicine. Neurosurg Focus. 2018;44(4):E3.
26. Barresi V. Angiogenesis in meningiomas. Brain Tumor Pathol. 2011;28(2):99–106.
27. Gelerstein E, Berger A, Jonas-Kimchi T, Strauss I, Kanner AA, Blumenthal DT, et al. Regression of intracranial meningioma following treatment with nivolumab: case report and review of the literature. J Clin Neurosci. 2017;37:51–3.
28. Imran H, Razia ET, Jawed HA, Nisar A, Choudry UK, Kumar A. Antibody targeted therapies in meningiomas: a critical review. J Surg Emerg Med. 2017;1(1):6.
29. Bi WL, Wu WW, Santagata S, Reardon DA, Dunn IF. Checkpoint inhibition in meningiomas. Immunotherapy. 2016;8(6): 721–31.
30. Dharmalingam P, Roopesh Kumar VR, Verma SK. Vascular endothelial growth factor expression and angiogenesis in various grades and subtypes of meningioma. Indian J Pathol Microbiol. 2013;56(4):349–54.
31. Westendorf AM, Skibbe K, Adamczyk A, Buer J, Geffersb R, Hansen W, et al. Hypoxia enhances immunosuppression by inhibiting CD4+ effector T cell function and promoting Treg activity. Cell Physiol Biochem. 2017;41(4):1271–84.

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3662

GMHC. 2019;7(3):213–7
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

The Resistance of *Aedes aegypti* to Permethrin 0.25% Insecticide, Malathion 0.8%, and Transfluthrin 25% in the Universitas Islam Bandung Tamansari Campus

Ratna Dewi Indi Astuti,[1] Ismawati,[1] Listya Hanum Siswanti[2]

[1]Department of Parasitology, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia,
[2]Department of Histology and Biomedical Sciences, Faculty of Medicine,
Universitas Islam Bandung, Bandung, Indonesia

Abstract

Massive and long term insecticide use causes resistance of mosquitos to insecticides. This research has a goal for assessing the resistance of *Aedes aegypti* to the insecticides of permethrin 0.25%, malathion 0.8%, and transfluthrin 25% in the Universitas Islam Bandung Tamansari campus. The *Aedes aegypti* resistance in the Universitas Islam Bandung Tamansari campus Bandung city to insecticides measured with the susceptibility test in September 2015. The susceptibility test to the permethrin 0.25% and malathion 0.8% insecticides implemented by using WHO standard instruments and methods. The susceptibility test to transfluthrin 25% implemented by using commercial insecticide according to the usage suggestion. The total mosquitos that died after the exposure of permethrin 0.25%, transfluthrin 25%, and malathion 0.8% for 60 minutes were 20%, 23%, and 80%. The WHO criteria state that mosquitos were still susceptible to insecticides if the death rate is 98–100%, tolerant if the death rate is 80–97%, and mosquitos are resistant if the death rate is less than 80%. In conclusion, the *Aedes aegypti* mosquitos in the Universitas Islam Bandung Tamansari campus are already resistant to the insecticides permethrin 0.25% and transfluthrin 25% and tolerant to malathion 0.8%.

Key words: *Aedes aegypti*, insecticide, resistance

Resistensi *Aedes aegypti* terhadap Insektisida *Permethrin* 0,25%, *Malathion* 0,8%, dan *Transfluthrin* 25% di Kampus Universitas Islam Bandung Tamansari

Abstrak

Penggunaan insektisida secara masif dan jangka panjang menimbulkan resistensi nyamuk terhadap insektisida. Penelitian ini bertujuan menilai resistensi resistensi *Aedes aegypti* terhadap insektisida *permethrin* 0,25%, *malathion* 0,8%, dan *transfluthrin* 25% di kampus Unversitas Islam Bandung Tamansari. Resistensi *Aedes aegypti* di kampus Unversitas Islam Bandung Tamansari Kota Bandung terhadap insektisida diukur dengan uji kerentanan pada bulan September 2015. Uji kerentanan terhadap insektisida *permethrin* 0,25% dan *malathion* 0,8% dilakukan menggunakan alat dan metode uji standar WHO. Uji kerentanan terhadap *transfluthrin* 25% dilakukan menggunakan insektisida komersial sesuai dengan anjuran penggunaan. Jumlah nyamuk yang mati dalam jangka waktu 60 menit setelah paparan *permethrin* 0,25%, *transfluthrin* 25%, dan *malathion* 0,8% berturut-turut adalah 20%, 23%, dan 80%. Kriteria WHO menyatakan nyamuk dikategorikan masih rentan terhadap insektisida jika tingkat kematiannya 98–100%, toleran jika kematiannya 80–97%, dan resisten apabila jumlah kematian nyamuk kurang dari 80%. Simpulan, nyamuk *Aedes aegypti* yang terdapat di kampus Universitas Islam Bandung Tamansari telah resisten terhadap insektisida *permethrin* 0,25% dan *transfluthrin* 25%, serta toleran terhadap *malathion* 0,8%.

Kata kunci: *Aedes aegypti*, insektisida, resistensi

Received: 6 April 2018; Revised: 19 August 2019; Accepted: 25 December 2019; Published: 31 December 2019

Correspondence: Ratna Dewi Indi Astuti, dr., M.Kes. Department of Parasitology, Faculty of Medicine, Universitas Islam Bandung. Jln. Tamansari No. 22, Bandung 40116, West Java, Indonesia. E-mail: ratnawidjajadi@gmail.com

Introduction

Aedes aegypti is a mosquito that is a vector of the dengue fever disease.[1] Other than spreading the dengue fever virus, *Aedes aegypti* also spreads viruses of other diseases such as chikungunya, Zika fever, and yellow fever.[1,2]

The incidence of dengue fever in Indonesia is 26.12 per 100,000 people, with a death rate of 0.72% in 2017.[3] Dengue cases in Bandung city showed an increasing trend in 2012 and 2013.[4] One of the means of reducing the incidence of disease spread by this *Aedes aegypti* mosquito, especially dengue fever, is by controlling the vector population.[5,6] Several means implemented to control the *Aedes aegypti* population, one of which is the use of insecticides to kill adult mosquitos.[7,8] Yet, the long term use of insecticides can cause resistance. This occurs because mosquitos mutate, so they become resistant to the insecticide.[9,10]

Insecticides that are often used in Indonesia are malathion, an organophosphate group, which is used for fogging by the government. The other insecticide is the pyrethroid group, which is used as domestic insecticides.[9] As of today, there are many reports of *Aedes aegypti* resistance to malathion and pyrethroid in several countries.[10–18] Several cities in Indonesia also have already shown the resistance tendency of *Aedes aegypti* to malathion and pyrethroid.[19–21]

This *Aedes aegypti* mosquito absorbs viruses that are present in the victim's blood and contaminates it to other persons when the female *Aedes aegypti* mosquito absorbs blood to develop her eggs.[22,23] The mosquito's character is to absorb blood in the daytime. If *Aedes aegypti* is disturbed when it is absorbing blood, this mosquito can bite more than one person, so there is a potential to move the virus from the victim to many persons at once.[22] The Universitas Islam Bandung campus is a place of activities for students, lecturers, and other employees who spend time in the day. There is also not a free area of *Aedes aegypty* adult female.[24] Therefore, the campus has a potential to be a place for dengue fever transmission.[25]

This research has a goal for finding the resistance of *Aedes aegypti* in the Universitas Islam Bandung Tamansari campus to permethrin 0.25% and transfluthrin 25% which are type 1 pyrethroid group insecticides that many people use, and malathion 0.8% which is an insecticide that is still used by the Bandung Health Agency in the eradication of *Aedes aegypti* adult mosquitos massively through fogging.

Methods

The resistance of *Aedes aegypti* mosquitos in the Universitas Islam Bandung Tamansari campus Bandung city to insecticides is measured with the susceptibility test in September 2015. The female *Aedes aegypti* mosquitos aged 5 days are obtained from the egg cultivation from the ovitrap which is spread in the Universitas Islam Bandung Tamansari campus environment. The susceptibility test of the *Aedes aegypti* mosquitos in the Universitas Islam Bandung Tamansari campus to the permethrin 0.25% and malathion 0.8% insecticides was implemented by using instruments and methods that correspond with the World Health Organization (WHO) standards. Twenty five mosquitos were contacted for 60 minutes in the insecticide contact tube, then moved into the collector tube and placed in fresh air for 24 hours (holding). For the holding period, the mosquitos are fed with sugar solvents in the cotton placed in the surface of the collector tube.[26]

The susceptibility test to transfluthrin 25% was implemented by using commercial insecticides in corresponding with the usage suggestion. A single spray commercial insecticide which consists of transfluthrin 25% was sprayed for one time in a room measured at 30 m^3 and spread for 60 minutes to 25 adult female *Aedes aegypti* mosquitos from the Universitas Islam Bandung campus, then the mosquitos are moved into the fresh air and provided food (holding). The transfluthrin spread was also provided to mosquitos farmed in the laboratory as a positive control.[27]

The mosquitos that died in the 24 hour holding period were counted and showed in the table form. The susceptibility tests to permethrin 0.25%, malathion 0.8%, and transfluthrin 25% were each implemented three times, so the total mosquitos which were contacted to insecticide are 75 mosquitos for each test.

Results

The results of the susceptibility test of the female *Aedes aegypti* mosquitos to the permethrin 0.25%, transfluthrin 25%, and malathion 0.8%

Table The Susceptibility Test of the Female *Aedes aegypti* Mosquitos to the Permethrin 0.25%, Transfluthrin 25%, and Malathion 0.8%

Insecticides	Mosquitos (n=75)
Permethrin 0.25%	
Died	15 (20%)
Not died	60 (80%)
Transfluthrin 25%	
Died	17 (23%)
Not died	58 (77%)
Malathion 0.8%	
Died	60 (80%)
Not died	15 (20%)
Control (–)	0

in the Universitas Islam Bandung Tamansari campus can see in the Table. The total mosquitos that died after the exposure of permethrin 0.25%, transfluthrin 25%, and malathion 0.8% for 60 minutes were 20%, 23%, and 80%.

The WHO criteria state that mosquitos are still susceptibility to insecticides if the death rate is 90–100%, tolerant if the death rate is 80–97%, and stated as resistant if the death rate is less than 80%. Therefore, the *Aedes aegypti* mosquitos in the Universitas Islam Bandung Tamansari campus are already resistant to the insecticides permethrin 0.25% and transfluthrin 25% and tolerant to malathion 0.8%.

Discussion

The resistance of *Aedes aegypti* mosquitos to pyrethroid insecticides has already occurred widely. Several pieces of research in Indonesia such as in Samarinda, Yogyakarta, Central Java province and also in several countries stated that local mosquitos are already resistant to permethrin 0.25%.[12–18,20,21] This has also happened in the environment of Universitas Islam Bandung, which is the *Aedes aegypti* mosquitos are already resistant to permethrin 0.25% and transfluthrin 25%.

This resistance occurs because of the long term use of permethrin, so there is a mutation in the sodium duct, which causes a reduction of nerve sensitivity to pyrethroid in what is known as knockdown resistance (kdr). This mutation occurs because of a single polymorph nucleotide, which produces the leucine substitution in the fenilalanin in the 1014 position (L1014F). This resistance to pyrethroid becomes higher if there is an additional mutation of M918T in the relation with L1014F (M918T and L1014F), this is known as the super-kdr phenotype.[9,17]

The reduction of *Aedes aegypti* susceptibility to malathion also has occurred widely. The mosquitos *Aedes aegypti* mosquitos in Central Java province and also in several countries are reported to be resistant to malathion 0.8%.1[3,15,17,18,20,21] Even though the *Aedes aegypti* mosquitos in the Universitas Islam Bandung environment has not been being resistant to malathion 0.8%, it has been already tolerant. This occurs because of the long term use of malathion as a mass insecticide in controlling the plague of the dengue fever disease in the long term, so mutation occurs which causes an improvement of the metabolism to malathion by α- and β-esterases and cytochrome oxidase.[17,18]

This resistance causes a new problem in vector control, which causes a constant amount of high dengue fever occurrence numbers, specifically in Bandung city. The flying range of these mosquitos are only about one hundred meters, yet advanced transportation means causes an increase of *Aedes aegypti* movement.[6,23,28] This causes the spread of mutated mosquitos and the widening of mosquito resistance to insecticides. Because of that, the control of the *Aedes aegypti* population needs to be more viable by an eradication movement of mosquito nests and not only with insecticide use.

Conclusions

Aedes aegypti mosquitos in the Universitas Islam Bandung Tamansari campus are already resistant to the insecticides of permethrin 0.25% and transfluthrin 25% and tolerant to the malathion 0.8% insecticide.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Hotez PJ, Murray KO. Dengue, West Nile virus, chikungunya, Zika-and now Mayaro?. PLoS Negl Trop Dis. 2017:11(8):e0005462.
2. Patterson J, Sammon M, Garg M. Dengue,

Zika, and chikungunya: emerging arboviruses in the new world. West J Emerg Med. 2016;17(6):671–9.
3. Kementerian Kesehatan Republik Indonesia. Profil kesehatan Indonesia tahun 2017. Jakarta: Kementerian Kesehatan Republik Indonesia; 2018.
4. Respati T, Feriandi Y, Ndoen E, Raksanagara A, Djuhaeni H, Sofyan A, et al. A qualitative ecohealth model of dengue fever (DF) in Bandung, Indonesia. Int J Trop Dis. 2018;1(1):008.
5. Respati T, Raksanagara A, Djuhaeni H, Sofyan A. Spatial distribution of dengue hemorrhagic fever (DHF) in urban setting of Bandung city. GMHC. 2017;5(3):212–8.
6. Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019;4(5):854–63.
7. Pusat Data dan Surveilans Epidemiologi, Kementerian Kesehatan Republik Indonesia. DBD di Indonesia tahun 1968–2009. Bul Jendela Epidemiol. 2010;2:1–14.
8. Marcombe S, Mathieu RB, Pocquet N, Riaz MA, Poupardin R, Sélior, S, et al. Insecticide resistance in the dengue vector *Aedes aegypti* from martinique: distribution, mechanisms and relations with environmental factors. PLoS One. 2012;7(2):e30989.
9. Kasai S, Komagata O, Itokawa K, Shono T, Ng LC, Kobayashi M, et al. Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: target site insensitivity, penetration, and metabolism. PLoS Negl Trop Dis. 2014;8(6):e2948.
10. Melo-Santos MAV, Varjal-Melo JJM, Araújo AP, Gomes TCS, Paiva MHS, Regis LN, et al. Resistance to the organophosphate temephos: mechanisms, evolution and reversion in an *Aedes aegypti* laboratory strain from Brazil. Acta Trop. 2010;113(2):180–9.
11. Stenhouse SA, Plernsub S, Yanola J, Lumjuan N, Dantrakool A, Choochote W, et al. Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribition and effect on deltamethrin resistance in Thailand. Parasit Vectors. 2013;6(1):253.
12. McAllister JC, Godsey MS, Scott ML. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* from Port-au-Prince, Haiti. J Vector Ecol. 2012;37(2) :325–32.
13. Ishak IH, Jaal Z, Ranson H, Wondji CS. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. Parasit Vectors. 2015;8:181.
14. Ponlawat A, Scott JG, Harrington LC. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. J Med Entomol. 2005;42(5):821–5.
15. Kandel Y, Vulcan J, Rodriguez SD, Moore E, Chung HN, Mitra S, et al. Widespread insecticide resistance in *Aedes aegypti* L. from New Mexico, U.S.A. PLoS One. 2019;14(2):e0212693.
16. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J, et al. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa. Parasit Vectors. 2011;4:79.
17. Alvarez LC, Ponce G, Oviedo M, Lopez B, Flores AE. Resistance to malathion and deltamethrin in *Aedes aegypti* (Diptera: Culicidae) from Western Venezuela. J Med Entomol. 2013;50(5):1031–9.
18. Goindin D, Delannay C, Gelasse A, Ramdini C, Gaude T, Faucon F, et al. Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in *Aedes aegypti* mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). Infect Dis Poverty. 2017;6(1):38.
19. Lidia K, Setianingrum ELS. Deteksi dini resistensi nyamuk *Aedes albopictus* terhadap insektisida organofosfat di daerah endemis demam berdarah dengue di Palu (Sulawesi Tengah). MKM. 2008;3(2):105–10.
20. Sunaryo, Ikawati B, Widiastuti B. Status resistensi vektor demam berdarah dengue (*Aedes aegypti*) terhadap malathion 0,8% dan permethrin 0,25% di Provinsi Jawa Tengah. J Ekol Kes. 2014;13(2):146–52.
21. Widiarti, Heriyanto B, Boewono DT, Widyastuti U, Mujiono, Lasmiati, et al. Peta resistensi vektor demam berdarah dengue *Aedes aegypti* terhadap insektisida kelompok organofosfat, karbamat, dan pyrethroid di propinsi Jawa Tengah dan Daerah Istimewa Yogyakarta. Bul Penelit Kesehat. 2011;39(4):176–89.
22. World Health Organization (WHO).

Dengue guidelines for diagnosis, treatment, prevention and control. New Edition. Geneva: WHO Press; 2009.
23. Powell JR, Tabachnick WJ. History of domestication and spread of *Aedes aegypti*-a review. Mem Inst Oswaldo Cruz. 2013;108(Suppl 1):11–7.
24. Astuti RDI, Ismawati, Siswanti LH, Suhartini A. Sebaran vektor penyakit demam berdarah (*Aedes aegypti*) di kampus Universitas Islam Bandung. GMHC. 2016;4(2):82–6.
25. García-Rejón JE, Loroño-Pino MA, Farfán-Ale JA, Flores-Flores LF, López-Uribe MP, Najera-Vazquez Mdel R, et al. Mosquito infestation and dengue virus infection in *Aedes aegypti* females in schools in Mérida, México. Am J Trop Med Hyg. 2011;84(3):489–96.
26. World Health Organization (WHO). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd Edition. Geneva: WHO Press; 2016.
27. Khadri MS, Kwok KL, Noor MI, Lee HL. Efficacy of commercial household insecticide aerosol sprays against *Aedes aegypti* (Linn) under simulated field conditions. Southeast Asian J Trop Med Public Health. 2009;40(6):1226–34.
28. Boewono DT, Ristiyanto, Widiarti, Widyastuti U. Distribusi spasial kasus demam berdarah dengue (DBD), analisis indeks jarak dan alternatif pengendalian vektor di Kota Samarinda, Provinsi Kalimantan Timur. Media Litbangkes. 2012;22(3):131–7.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3986

GMHC. 2019;7(3):218–23
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Effectiveness of Cadres Training in Improving Maternal and Neonatal Health in Soreang Subdistrict

Merry Wijaya,[1,2] Fardila Elba,[1,2] Ariyati Mandiri,[1,2] Wenny Friska,[1] Balkis Fitriani Faozi,[1] Danny Hilmanto[3]

[1]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia,
[2]Diploma 4 Midwifery Study Program, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia,
[3]Department of Child Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Abstract

The high maternal mortality rate (MMR) and neonatal mortality rate (NMR) are indicators of low health quality in society. Most maternal and neonatal mortality cases prevented through early detection and quick and accurate responses. Other factors that contribute to maternal and neonatal mortality are the quality of health care, the uneven distribution of health workers, and the inadequate health-supporting facilities. The government's efforts in improving fair health workers distribution and quality of healthcare services by involving integrated health service post (*pos pelayanan terpadu*/*posyandu*) cadres, as the front-liners of health care programs (especially concerning maternal and neonatal health), should be able to provide better healthcare services to the people. Hence, cadres training is crucial for improving the cadres' knowledge and skills in healthcare services. This study aims to analyze the effect of cadres training implementation on the development of cadres' knowledge and skills in giving maternal and neonatal (infant) health counseling. This study used a quasi-experimental pretest-posttest design conducted from July to November 2017. The sample consists of 32 active cadres from 12 villages in Soreang subdistrict who satisfy the inclusion criteria. Data is collected through questionnaires to measure knowledge improvement and checklists to measure cadres' skills development. The findings show that cadres training implementation can improve cadres' knowledge and skills in maternal and neonatal (infant) health counseling.

Key words: Maternal and neonatal healthcare, *posyandu* cadres, training

Efektivitas Pelatihan Kader dalam Meningkatkan Kesehatan Ibu dan Bayi di Kecamatan Soreang

Abstrak

Angka kematian ibu (AKI) dan angka kematian bayi (AKB) yang tinggi menjadi indikator status kesehatan masyarakat yang rendah. Sebagian besar kasus kematian ibu dan bayi dapat dicegah melalui deteksi dini dan penanganan yang cepat dan tepat. Faktor lain yang memengaruhi kematian ibu dan bayi adalah kualitas pelayanan kesehatan, distribusi tenaga kesehatan belum merata, dan sarana pendukung pelayanan kesehatan yang belum memadai. Upaya pemerintah dalam meningkatkan pemerataan tenaga kesehatan dan pelayanan kesehatan dengan melibatkan kader pos pelayanan terpadu (posyandu) sebagai ujung tombak program kesehatan, khususnya ibu dan anak, seyogianya mampu memberikan pelayanan kesehatan yang baik kepada masyarakat. Oleh karena itu, pelatihan kader untuk meningkatkan pengetahuan dan keterampilan dalam penanganan kesehatan mutlak diperlukan. Penelitian ini bertujuan menganalisis pengaruh implementasi pelatihan kader terhadap peningkatan pengetahuan dan keterampilan dalam melakukan penyuluhan kesehatan ibu dan anak. Penelitian ini menggunakan desain *quasi-experimental pretest-posttest* yang dilakukan dari Juli hingga November 2017. Sampel penelitian adalah 32 kader aktif dari 12 desa di Kecamatan Soreang yang memenuhi kriteria inklusi. Pengumpulan data menggunakan kuesioner untuk menilai perubahan pengetahuan dan daftar tilik untuk mengukur perubahan keterampilan kader. Hasil penelitian menunjukkan bahwa implementasi pelatihan kader mampu meningkatkan pengetahuan dan keterampilan kader dalam melakukan penyuluhan kesehatan ibu dan anak.

Kata kunci: Kader posyandu, kesehatan ibu dan anak, pelatihan

Received: 23 August 2018; Revised: 19 October 2019; Accepted: 25 December 2019; Published: 31 December 2019

Correspondence: Dr. Merry Wijaya, M.Kes. Department of Public Health, Faculty of Medicine, Universitas Padjadjaran. Jln. Prof. Eyckman No. 38, Bandung 40161, West Java, Indonesia. E-mail: merry_wijaya0605@yahoo.com

Introduction

The high maternal mortality rate (MMR) and infant mortality rate (IMR) were indicators of low health quality in a community.[1] Based on the Indonesia Demography and Health Survey, the MMR in Indonesia in 2017 was 305/100,000 live births, while the IMR was 24/1,000 live births.[2,3] West Java Province Health Profile reported 3,133 cases of neonatal deaths and 3,702 cases of infant deaths in the West Java province in 2016.[4]

The issue of maternal mortality and infant mortality rates required special attention and support from various parties to solve. One of the challenges was people's lack of knowledge concerning the importance of healthcare and prevention efforts, which included balanced nutrition, immunization, and monitoring of children's growth and development; all of which provided in integrated health service post (*pos pelayanan terpadu*/*posyandu*).[3,5–7]

The government had implemented many efforts to improve the minimum healthcare standard. However, these efforts had no positive correlation with the achievements of various programs.[5,8]

The main challenge was finding the most appropriate way to empower people to educate them about the importance of health and a healthy lifestyle. One approach is by involving *posyandu* cadres as the front-liners of maternal and infant health programs. *Posyandu* cadres were expected to provide healthcare services as well as health education anytime anyone needed them. Hence, a cadres' training concept with the practical design was necessary to implement ideas and frameworks of comprehensive training to improve cadres' quality.[6,9–11]

Methods

The method implemented in this study was the quasi-experimental pretest-posttest design in one group conducted from July to November 2017. In the pretest and posttest, the cadres had about one minute to answer each question. They seated with the 1-meter distance between each of them. The pretest conducted before the cadres provided the training module. The posttest administered at the end of the training after the participants received the intervention. The intervention consisted of training, case study, and lab-skills of handling umbilical cord of newborn baby, treatment for post-natal swollen breasts of mothers, and cleansing newborn baby. Training conducted in the Soreang Community Health Center meeting room within two days in 2017.

X1 (pretest) ⟶ Treatment ⟶ X2 (posttest)

Note: X1=objects before intervention, X2=objects after intervention

The population for this study was all *posyandu* cadres in Soreang subdistrict, Bandung regency. The sample was selected through random sampling, resulting in 32 *posyandu* cadres who met the inclusion and exclusion criteria. They represent 32 chosen citizens association (*rukun warga*/RW) from 10 villages that the model area of children's health improvement program in Soreang subdistrict, Bandung regency.

The obtained data recorded and processed using SPSS Version 21. The numerical scale data presented through averages, the standard deviation, median, and range. Data analysis in this study employed paired t test to see the comparison result of pretest and posttest scores.

This study conducted after the Health Research Ethics Committee of the Faculty of Medicine of Universitas Padjadjaran issued ethical approval with letter number: 07/UN6. C1.3.2/KEPK/PN/2017.

Results

Selected samples from each citizen association (RW) in Soreang subdistrict, Bandung regency shown in Table 1.

The evaluation of the training program was in Table 2. The table showed that the average pretest score was 71.85±6.301, and the average posttest score was 82.98±5.339. The result of statistical analysis using a non-paired t test showed a great significance (p value=0.000).

The comparison of each cadre's score showed in Figure 1.

Several clinical skills delivered in training included handling the umbilical cord of newborn baby, treatment of post-natal swollen breasts of the mother, and cleansing newborn baby. Before the treatment of providing materials and demonstrations, a pretest conducted to evaluate cadres' skills. Most of the cadres were in the 'bad' category; their skills were not as good as expected. After the treatment, each cadre asked to demonstrate their skills, and an evaluation was conducted based on the checklist for each competency.

Table 1 Total Selected Sample from Each RW in Soreang Subdistrict, Bandung Regency

Village	RW	Number	Village	RW	Number
Cingcin	3	1	Sadu	10	1
Cingcin	9	1	Sadu	4	1
Cingcin	7	1	Sadu	1	1
Cingcin	10	1	Sadu	11	1
Karamat Mulya	20	1	Sekarwangi	7	1
Karamat Mulya	6	1	Sekarwangi	5	1
Karamat Mulya	2	1	Sekarwangi	7	1
Karamat Mulya	15	1	Soreang	12	1
Karamat Mulya	7	1	Soreang	20	1
Pamekaran	13	1	Soreang	15	1
Pamekaran	2	1	Soreang	22	1
Pamekaran	6	1	Soreang	7	1
Panyirapan	13	1	Sukajadi	15	1
Panyirapan	14	1	Sukajadi	7	1
Parungserab	3	1	Sukanagara	1	1
Parungserab	16	1	Sukanagara	4	1
			Total		32

The competency scored in categories: A=very good, B=good, C=sufficient, and K=bad. The detailed scoring and definition for each category were as follow: A (79–100)=the cadre was able to perform very well; B (49–<79)=the cadre was able to perform well but still needed some refinement; C (<49–29)=the cadre was unable to perform well but had the willingness to try; and K (<29)=the cadre was unable to perform well and had no willingness to try. Figure 2 displayed the result of the clinical skills test.

Based on the graph in Figure 2, it could be seen that the average skill scores of the three competencies were in A category (very good). The skill of handling newborn baby's umbilical cord had an average score of 95, swollen breast treatment had an average score of 82.5, while the skill of cleansing baby had an average score of 93.7.

Discussion

Posyandu is one form of efforts carried out by, from, and with the community to empower and provide facilities for the community to obtain health services for mothers and children under five.[12–14] *Posyandu* is one of the most well-known forms of community-based health efforts today.[15–17] The management of *posyandu* was chosen from and by the community at the time of the formation of *posyandu*.[14]

Posyandu cadres are community members who are appointed to work voluntarily in carrying out activities related to simple health services in *posyandu*. The role and function of cadres are

Table 2 Pretest and Posttest Scores Results

Variables	Treatment (n=31)		p Value
	Pretest	Postest	
Mean±Std	71.85±6.301	82.98±5.339	
Median	72.41	82.76	0.000**
Range (min–max)	51.72–79.31	72.41–89.66	

Note: p value tested using a non-paired t test if the data distributed normally and the Mann-Whitney test if the data not distributed normally. Significance level based on p value<0.05. **p value<0.05 (statistically significant)

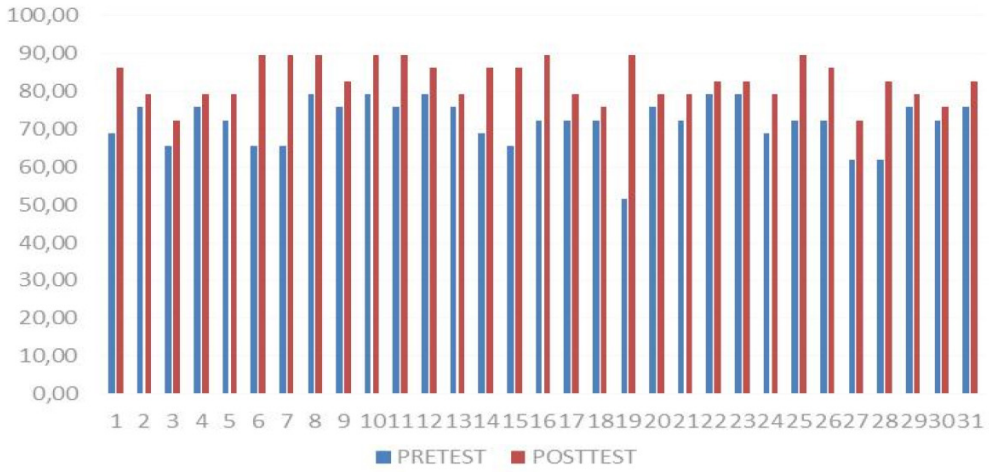

Figure 1 Comparison of Pretest and Posttest Scores

very important, starting before implementing *posyandu*, during, and after *posyandu*.[16,17]

A *posyandu* cadre is a person who, because of his skills or abilities, was appointed, selected, and or appointed to lead the development of a *posyandu* in a place or village. Every local male and female villager who can read and write latin letters, has free time, has the ability and is willing to work voluntarily, sincerely can be a cadre.[18,19]

Cadres expected to play an active role and be able to be a motivator and community instructor. Cadres expected to be able to bridge between health workers/experts and the community and help the community identify and deal with/ respond to their own health needs.[11,13,15,17] The results of the *posyandu* cadre knowledge and skill pretest on maternal and child health fall into the poor category. They do not understand the role of cadres in *posyandu* activities. Knowledge obtained by cadres so far only records and reports on the results of weighing under five. In the study conducted by Subagyo et al.[13] that there is a relationship between the role of cadres and the motivation of mothers visiting *posyandu*. The active role of *posyandu* cadres is also highly needed to support the improvement of the level of public health, especially the health of mothers and children under five.[19–21]

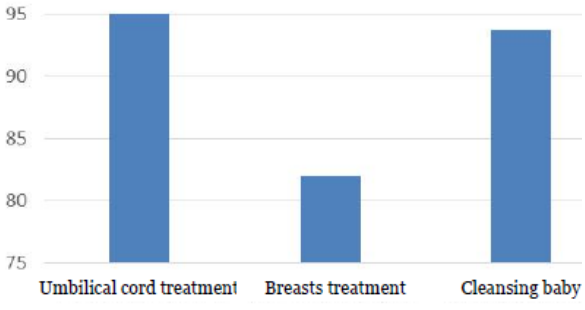

Figure 2 Result of Skill Test

In the results of the study, there was poor cadre knowledge and fewer skills before training. However, after the cadres were refreshed regarding knowledge and skills, there was an increase in knowledge and skills. This increase in knowledge is due to the existence of new information that is conveyed to the cadres through training, where the new information obtained is a substitute for the knowledge that has been obtained previously or is an improvement of the previous information.[20,22,23]

Knowledge enhancement can be done in various ways, one of which is training activities carried out by health workers. There are several things related to cadre knowledge about assessing the growth of children under five. Know is interpreted to remember a material or science related to assessing the growth of toddlers. In this case, the cadre recalls something specific from all the material learned or stimuli that have been received. Understanding is the ability of cadres to explain correctly about assessing the growth of toddlers.[17,22,23]

Increased knowledge of cadres after training can occur if the material presented is easily understood by the cadres. In this training,

the method used is the lecture and question and answer method. The cadres seemed very enthusiastic about the material presented, many of them asked about the growth of toddlers. In addition to the lecture and question and answer method, the increase in respondents' knowledge was also carried out by discussion, so that the cadres increasingly understood how to assess the growth of infants and their knowledge also increased.[11,24]

Components that can influence the success of training include the curriculum, instructors/ trainers, organizers, facilities used, methods, and characteristics of training participants such as age, occupation, education, and experience. The results of a study conducted by Lubis[14] showed that there was a significant effect of training on cadre actions. According to Notoatmodjo,[24] a person who has received training increases his knowledge and skills and can be measured by a questionnaire interview asking about the content of the material measured from study subjects or respondents in the knowledge that they want to know or adjust.

Factors that influence the performance of cadres are very complex and vary from one region to another. In addition to internal factors such as age, length of dedication, experience, social status, economic conditions, and family support; external factors such as the condition of the community and health agencies also influence the motivation and retention of cadres. Non-financial benefits are also very important for the success of a cadre program.[25–27] The results of focus group discussions (not published) conducted by the writer show that the cadres feel happy and proud of the tasks carried out because they have been considered as part of the health system.

Cadres who have completed the training expected to set an example and guide other cadres in performing their duties as cadres in *posyandu*.

Conclusion

The findings show that cadres training implementation can improve cadres' knowledge and skills in maternal and neonatal (infant) health counseling.

Conflict of Interest

The authors declare no conflicts of interest in this study.

Acknowledgment

This study has funded by Internal Grants from Universitas Padjadjaran and was a part of the Academic Leaderships Grant (ALG) Program 1-1-6.

References

1. Ruiz JI, Nuhu K, McDaniel JT, Popoff F, Izcovich A, Criniti JM. Inequality as a powerful predictor of infant and maternal mortality around the world. PLoS One. 2015;10(10):e0140796.
2. National Population and Family Planning Board (BKKBN), Statistics Indonesia (BPS), Ministry of Health (Kemenkes), ICF. Indonesia demographic and health survey 2017. Jakarta: BKKBN, BPS, Kemenkes, ICF; 2018.
3. Kementerian Kesehatan Republik Indonesia. Profil kesehatan Indonesia tahun 2017. Jakarta: Kementerian Kesehatan Republik Indonesia; 2018.
4. Dinas Kesehatan Provinsi Jawa Barat. Profil kesehatan Provinsi Jawa Barat tahun 2016 [Internet]. Bandung: Dinas Kesehatan Provinsi Jawa Barat; 2017 [cited 2018 December 10]. Available from: http://diskes.jabarprov.go.id/dmdocuments/9738b8d46840cc981f23c771c4187b6d.pdf.
5. Kementerian Kesehatan Republik Indonesia. Kurikulum dan modul pelatihan kader posyandu. Jakarta: Kementerian Kesehatan Republik Indonesia; 2012.
6. Green LW, Kreuter MW. Health program planning: an educational and ecological approach. 4th Edition. New York: McGraw-Hill; 2005.
7. Ononokpono DN, Odimegwu CO. Determinants of maternal health care utilization in Nigeria: a multilevel approach. Pan Afr Med J. 2014;17(Suppl 1):2.
8. Alcock G, Das S, Shah More N, Hate K, More S, Pantvaidya S, et al. Examining inequalities in uptake of maternal health care and choice of provider in underserved urban areas of Mumbai, India: a mixed methods study. BMC Pregnancy Childbirth. 2015;15:231.
9. Rosliza AM, Muhamad HJ. Knowledge, attitude and practice on antenatal care among Orang Asli women in Jempol, Negeri Sembilan. MJMHS. 2011;11(2):13–21.
10. Patel BB, Gurmeet P, Sinalkar DR, Pandya KH,

Mahen A, Singh N. A study on knowledge and practices of antenatal care among pregnant women attending antenatal clinic at a Tertiary Care Hospital of Pune, Maharashtra. Med J DY Patil Univ. 2016;9(3):354–62.
11. Kanu JS, Tang Y, Liu Y. Assessment on the knowledge and reported practices of women on maternal and child health in rural Sierra Leone: a cross-sectional survey. PLoS One. 2014;9(8):e105936.
12. Aminuddin, Zulkifli A, Djafar N. Peningkatan peran posyandu partisipatif melalui pendampingan dan pelatihan upaya pemantauan pertumbuhan dan masalah gizi balita di Bone, Sulawesi Selatan. Kesmas Natl Public Health J. 2011;5(5):201–5.
13. Subagyo W, Mukhadiono, Wahyuningsih D. Peran kader dalam memotivasi ibu balita berkunjung ke posyandu. JKS. 2015;10(3):158–66.
14. Lubis Z. Pengetahuan dan tindakan kader posyandu dalam pemantauan pertumbuhan anak balita. KEMAS. 2015;11(3):65–73.
15. Iswarawanti DN. Kader posyandu: peranan dan tantangan pemberdayaannya dalam usaha peningkatan gizi anak di Indonesia. JMPK. 2010;13(4):169–73.
16. Lukwan. Kontribusi pengetahuan kader terhadap kinerja kader posyandu di Puskesmas Matandahi Konawe Utara. JPPPK. 2018;2(1):17–22.
17. Pusat Promosi Kesehatan, Kementerian Kesehatan Republik Indonesia. Ayo ke posyandu setiap bulan: posyandu menjaga anak dan ibu tetap sehat [Internet]. Jakarta: Kementerian Kesehatan Republik Indonesia; 2012 [cited 2018 December 12]. Available from: http://promkes.kemkes.go.id/download/jrc/files5270buku_saku_Posyandu.pdf.
18. Kamil M. Model pendidikan dan pelatihan (konsep dan aplikasi). Cetakan ke-2. Bandung: Alfabeta; 2012.
19. Peraturan Menteri Tenaga Kerja dan Transmigrasi Republik Indonesia Nomor 8 Tahun 2014 tentang Pedoman Penyelenggaraan Pelatihan Berbasis Kompetensi.
20. Hamza M. Training material development guide. Karlstad: Swedish Civil Contingencies Agency (MSB); 2012.
21. Bhattacharyya S, Srivastava A, Roy R, Avan BI. Factors influencing women's preference for health facility deliveries in Jharkhand state, India: a cross sectional analysis. BMC Pregnancy Childbirth. 2016;16:50.
22. Direktorat Jenderal Bina Gizi dan Kesehatan Ibu dan Anak, Kementerian Kesehatan Republik Indonesia. Pedoman penyelenggaraan manajemen terpadu balita sakit berbasis masyarakat (MTBS-M). Jakarta: Kementerian Kesehatan Republik Indonesia; 2014.
23. Rostinah, Widajanti L, Wulan LRK. Evaluasi manajemen pelatihan kader pos pelayanan terpadu (posyandu) di Puskesmas Paruga Kota Bima Provinsi Nusa Tenggara Barat. JMKI. 2015;3(3):212–21.
24. Notoatmodjo S. Promosi kesehatan dan perilaku kesehatan. Revision Edition. Jakarta: Rineka Cipta: 2012.
25. Mpembeni RNM, Killewo JZ, Leshabari MT, Massawe SN, Jahn A, Mushi D, et al. Use pattern of maternal health services and determinants of skilled care during delivery in Southern Tanzania: implications for achievement of MDG-5 targets. BMC Pregnancy Childbirth. 2007;7:29.
26. Bulatao RA, Ross JA. Rating maternal and neonatal health services in developing countries. Bull World Health Organ. 2002;80(9):721–7.
27. Khemkratoke K, Thamsenanupap P, Noinumsai N. Community participatory training model for the conservation of medicinal plants biodiversity at Doijedee mountain tourist attraction, northeastern Thailand. Creative Educ. 2012;3(4):581–7.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.3673

GMHC. 2019;7(3):224–31
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Stunting Determinant on Toddler Age of 12–24 Months in Singaparna Public Health Center Tasikmalaya Regency

Erwina Sumartini,[1,2] Dida Akhmad Gurnida,[3] Eddy Fadlyana,[3] Hadi Susiarno,[4] Kusnandi Rusmil,[3] Jusuf Sulaeman Effendi[4]

[1]Midwifery Master Study Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia, [2]Diploma 3 Midwifery Study Program, STIKes Respati, Tasikmalaya, Indonesia, [3]Department of Child Health, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, [4]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran/ Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

Abstract

Stunting is a physical growth failure condition signed by height based on age under –2SD. The research goal is knowing the dominant factor associated with stunting on toddler age of 12–24 months in the working area of Singaparna Public Health Center Tasikmalaya regency. The research applies to the cross-sectional design of gender, weight, exclusive breastfeeding history, completeness immunization, and clinically healthy variables, while case-control is for nutrition intake variable. The sample was a total sampling of 376 toddlers, then 30 for case and control group with the simple random method from December 2017 to February 2018. The instrument is a questionnaire, food frequency questionnaire (FFQ), and infantometer. Data analyzed in several ways; univariable, bivariable with chi-square, and multivariable with logistic regression. Research result shows stunting prevalence was 22.5%, next pertain factor of stunting are gender (POR=0.564, 95% CI=0.339–0.937, p value=0.011), exclusive breastfeeding giving history (POR=1.46, 95% CI=1.00–2.14, p value=0.046), and clinically health (POR=1.47, 95% CI=1.00–2.16, p value=0.044). Moreover, dominant factor were gender (OR=0.56, 95% CI=0.339–0.937, p value=0.027) and clinically health (OR=1.68, 95% CI=1.022–2.771, p value=0.041). Thus, gender and clinical health are stunting determinant factors. Children's health should increase to create maximum growth.

Key words: Exclusive breastfeeding, gender, nutrition intake, stunting

Determinan *Stunting* pada Anak Usia 12–24 Bulan di Puskesmas Singaparna Kabupaten Tasikmalaya

Abstrak

Stunting merupakan kondisi kegagalan pertumbuhan fisik yang ditandai dengan tinggi badan menurut usia berada di bawah –2SD. Penelitian ini bertujuan mengetahui faktor determinan *stunting* pada anak usia 12–24 bulan di wilayah kerja Puskesmas Singaparna Kabupaten Tasikmalaya. Penelitian menggunakan desain *cross-sectional* untuk variabel jenis kelamin, berat badan lahir, riwayat ASI eksklusif, kelengkapan imunisasi, dan sehat secara klinis, sedangkan desain *case-control* untuk variabel asupan nutrisi. Pengambilan sampel secara total sampling sejumlah 376 anak, selanjutnya diambil 30 anak untuk kelompok kasus dan kontrol dengan metode random sederhana periode Desember 2017 hingga Februari 2018. Instrumen menggunakan kuesioner, *food frequency questionaire* (FFQ), dan infantometer. Analisis data dilakukan secara univariabel, bivariabel dengan *chi-square*, dan multivariabel dengan regresi logistik. Hasil penelitian menunjukkan prevalensi *stunting* sebesar 22,6%, faktor yang berhubungan dengan *stunting* di antaranya jenis kelamin (POR=0,564; IK95%=0,339–0,937; p=0,011), riwayat pemberian ASI eksklusif (POR=1,46; IK95%=1,00–2,14, p=0,046), dan sehat secara klinis (POR=1,47; IK95%=1,00–2,16; p=0,044). Faktor dominan yang berhubungan dengan *stunting* adalah jenis kelamin (OR=0,56; IK95%=0,339–0,937; p=0,027) dan sehat secara klinis (OR=1,68; IK95%=1,022–2,771; p=0,041). Jenis kelamin dan sehat secara klinis merupakan faktor determinan *stunting*. Kesehatan anak perlu ditingkatkan untuk menciptakan pertumbuhan anak yang maksimal.

Kata kunci: ASI eksklusif, asupan nutrisi, jenis kelamin, *stunting*

Received: 11 April 2018; Revised: 29 October 2019; Accepted: 26 December 2019; Published: 31 December 2019

Correspondence: Erwina Sumartini, S.S.T., M.Keb. Diploma 3 Midwifery Study Program, STIKes Respati. Jln. Singaparna km 11, Cikunir, Singaparna, Tasikmalaya 46181, West Java, Indonesia. E-mail: erwinasumartini5@gmail.com

Introduction

Growth is a continuous process that starts from conception until adult. Stunting is one of the physical growth failure condition signed by height based on age. Stunting occurs when height by age based on z score <−2SD and severe stunting if z score <−3SD.[1–3] Stunting occurs as an impact of chronic malnutrition during 1,000 first days of life. It is such a predictor of inadequate human resources, and then it decreases a nation's productive ability in the future. Besides that, they face some more significant possibilities to live as an adult with less education, weak, unhealthy, and more susceptible to getting a non-contagious disease such as obesity, hypertension, and diabetes.[4–6]

Gender, birth weight, breastfeeding history, nutrition intake, immunization, and clinical health are the immediate cause of a child's malnutrition.[7]

Tasikmalaya regency includes the four regions with bad nutrition status in the high category. Stunting toddler prevalence of Tasikmalaya regency in 2016 was 41.73%, wasting 15.6%, and obesity was 8.65%.[8–11] Singaparna Public Health Center Tasikmalaya regency has some problems associates with nutrition. In 2016, the stunting toddler was 672 people (17.2%) dan severely stunting was 245 people (6.3%) from all of the toddlers in the working area of Singaparna Public Health Center. Moreover, in Tasikmalaya regency, low birth weight places as first grade (7.15%) and has low exclusive breastfeeding with only (38.11%).[10,11] The research goal is knowing the dominant factor associates with stunting on toddler age of 12–24 months in the working area of Singaparna Public Health Center.

Methods

The research applies observational analytic using two research designs; the cross-sectional design used to review variables of gender, birth weight, exclusive breastfeeding history, complete immunization, and clinical health. Case-control design applies to review nutrition intake variable that involves protein and energy intake. Case-control design implemented after finding cases and control groups from the result of the cross-sectional design. The target population in this research is the entire toddler age of 12–24 months that live in the working area surrounding Singaparna Public Health Center of Tasikmalaya regency from December 2017 to February 2018.

A total sampling of 376 toddlers used for the first stage. While stage II with the case-control design, used 30 respondents in each group using simple random sampling. Inclusion criteria in this research were toddler age of 12–24 months (the preterm baby history age decided by age correction), healthy, no congenital abnormality, and get parent's agreement.

The research instrument was questionnaires, food frequency questionnaires (FFQ), and infantometer with an accuracy level of 0.1 cm. Data analyzed using the chi-square test and logistic regression analysis.

The research was conducted after obtaining ethical approval from the Health Research Ethics Committee of the Faculty of Medicine of Universitas Padjadjaran with letter number: 1112/UN6.C10/PN/2017. We also had permission from the National Unity and Community Protection Agency (*Kesatuan Bangsa dan Perlindungan Masyarakat*/*Kesbanglinmas*) of Tasikmalaya Regency, Tasikmalaya Regency Public Health Office, and Singaparna Public Health Center.

Results

Stunting prevalence on toddler age of 12–24 months had presented in the form of a frequency distribution table (Table 1).

Based on Table 2 can be seen that child's age factor had a significant correlation with stunting. It means the toddler age of 19–24 months has stunting risk 1.12 times bigger than toddler age of 12–18 months. A child's gender has a significant correlation with stunting. It means males had stunting risk 1.65 times bigger than females. Next, breastfeeding giving history has a significant correlation with stunting. It reflects non-exclusive breastfeeding toddlers may have a risk of 1.46 times bigger than the exclusive one.

Table 1 Stunting Case on Toddler Age of 12–24 Months in Working Area of Singaparna Public Health Center Tasikmalaya Regency

Stunting Case	n=376	Percentage
Severely stunting	18	4.8
Stunting	67	17.8
Non-stunting	291	77.4

Note: stunting prevalence (95%CI)=22.6% (18.7–27.1%)

Table 2 The Factor Associated with Stunting Case on Toddler Age of 12–24 Months in Working Area of Singaparna Public Health Center Tasikmalaya Regency

Studied Factor	Stunting Case (n=376)		p Value	POR (95%CI)
	Stunting	Non-stunting		
Child's age (months)			0.035*	
12–18	42	181		1.0
19–24	43	110		1.12 (1.00–1.27)
Father's age (years)			0.673*	
20–35	46	165		1.0
>35	39	126		0.92 (0.63–1.34)
Father's education			0.285*	
Elementary	33	97		1.93 (0.81–4.60)
Senior	47	161		1.72 (0.73–4.03)
University	5	33		1.0
Father's job			0.682*	
Employee	13	50		1.0
Freelancer	72	241		1.03 (0.89–1.18)
Mother's age (years)			0.172*	
<20	2	9		1.0
20–35	60	230		1.03 (0.78–1.37)
>35	23	52		1.18 (0.86–1.62)
Mother's education			0.397*	
Elementry	31	88		0.98 (0.52–1.86)
Senior	45	178		0.76 (0.41–1.41)
University	9	25		1.0
Mother's job			0.601**	
Employed	6	16		1.22 (0.60–2.48)
Unemployed	79	275		1.0
Gender			0.011*	
Male	54	139		1.65 (1.11–2.44)
Female	31	152		1.0
Birth weight			0.632*	
Low	10	29		0.95 (0.78–1.16)
Normal	75	262		1.0
Breastfeeding history			0.046*	
Exclusive	36	159		1.0
Non Exclusive	49	132		1.46 (1.00–2.14)
Immunization completeness			0.376*	
Complete	75	266		1.0
Incomplete	10	25		1.29 (0.74–2.27)
Clinically healthy			0.044*	
Yes	35	156		1.0
No	50	135		1.47 (1.00–2.16)

Note: *chi-square test in significance level, **Fisher's exact test in significance level 5%

Also, clinically health has a significant correlation with stunting. It showed the clinically unhealthy toddler possibly gets a risk 1.47 times to be stunting rather than the healthy one.

Table 3 shows a significant correlation between gender and stunting with OR value=0.56 (95%CI=0.339–0.937) and p value=0.027; this means males had a higher risk of 0.56 times than females, gender was the most dominant factor pertain to stunting. Moreover, there was another significant correlation on the variable of clinically healthy toddlers and stunting, pointed

Table 3 Stunting Determinant Factor on Toddler Age of 12–24 Months in Working Area of Singaparna Public Health Center Tasikmalaya Regency

Variables	β Coefficients	SE β	p Value	Adjusted Odds Ratio (95%CI)
First model				
Gender	−0.581	0.262	0.026	0.559 (0.335–0934)
Child's age	0.443	0.257	0.085	1.557 (0.940–2.578)
Breastfeeding history	0.414	0.258	0.109	1.513 (0.912–2.509)
Clinically healthy	0.547	0.257	0.033	1.729 (1.046–2.858)
Mother's age (1)	0.096	0.821	0.014	1.101 (0.220–5.497)
Mother's age (2)	0.613	0.849	0.470	1.846 (0.350–9.740)
Final model				
Gender (male)	−0.573	0.260	0.027	0.564 (0.339–0.937)
Child's age (19–24 months)	0.455	0.254	0.074	1.575 (0.957–2.592)
Breastfeeding (non-exclusive)	0.446	0.224	0.079	1.563 (0.949–2.573)
Clinically healthy (no)	0.520	0.255	0.041	1.683 (1.022–2.771)

Note: model accuracy=77.4%

Table 4 The Correlation between Nutrition Intake and Stunting on Toddler Age of 12–24 Months in Working Area of Singaparna Public Health Center Tasikmalaya Regency

Nutrition Intake	Stunting (n=30)	Non-stunting (n=30)	Total (n=60)	p Value*	OR (95% CI)
Energy				0.347	
Adequate (≥80% RDA)	25	22	47		0.55 (0.15–1.93)
Less (<80% RDA)	5	8	13		1.0
Protein				0.688	
Adequate (≥80% RDA)	27	26	53		0.72 (0.14–3.54)
Less (<80% RDA)	3	4	7		1.0

Note: *chi-square test in significant level 5%, RDA=recommended dietary allowance (*angka kecukupan gizi*/AKG)

by OR value=1.68 (95%CI=1.022–2.771) and p value<0.041.

Stage 2 analyzed between nutrition intake and stunting, energy, and protein intake presented in Table 4. Tabel 4 reflects there was no significant correlation between energy intake and stunting with p value=0.347, also no significant correlation between protein and stunting with p value=0.68.

Discussion

Stunting prevalence on toddler age of 12–24 months in the working area of Singaparna Public Health Center Tasikmalaya regency reaches 22.6%. This percentage exceeds the limit of WHO non-public health of 20%. Based on the z score, the sample that includes the stunting category was 67 children (17.8%), severely stunting was 18 children (4.8%), and the normal was 291 (77.4%).

Stunting prevalence gets higher in the age category of 19–24 months is 43 of 85 (28.1%) than 12–8 months category, 18.8%. Based on the statistical analysis result, the child's age of 19–24 months category has a more significant risk of experiencing stunting than the age of 12–18 months with POR=1.12 (95%CI=1.00–1.27) and p value=0.035, this means child's age correlates with stunting. Plus, age of 19–24 months technically experience stunting risk 1.12 times bigger than the age of 12–18 months; the older their age, the higher stunting risk will be.

There was a significant correlation between gender and stunting with p value=0.011. A boy had risk 1.65 much more than a girl. Previous research in Mozambique shows stunting on boy 0–59 months was higher than a girl; the boy has stunting chance 4.01 times higher than the girl.[12,13]

Gender becomes a stunting predictor on

toddler age of 6–23 months when a male has a bigger chance of experiencing stunting than female. Probably, this can happen because a tendency of a male to possess physical activity is higher than female so that he produces much energy that must use to increase the growth. Universal, culturally, the female is predicted less active, just staying at home with her mother, also closer to her meal preparation.[12,13]

The low birth weight (LBW) caused by prematurity and delayed fetal growth. In premature infants, the process of fetal growth in the uterus does not have interference, but low birth weight infants due to birth prematurely and weight according to gestational age. In infants with intrauterine growth restriction (IUGR), there was fetal failure to achieve full growth potential. Thus, infants in this group had a 2.9 percent higher risk of experiencing a slower rate of increment compared with premature infants.[14,15]

In this study found 39 research subjects who had a birth history of LBW, as many as 56.41% were born with premature and 43.59% with IUGR. Of the 39 subjects who had a history of LBW, 23 were in clinical health, 16 were in clinically unhealthy conditions. There was a difference in the proportion of unfortunate occurrences in the study subjects, mostly in clinical health. It concluded that the health condition of children is an essential factor to maximize the growth of children with a history of low birth weight.

The results of statistical analysis showed that there was a correlation between the history of exclusive breastfeeding and stunting with p value=0.046. Children with a non-exclusive breastfeeding history are 1.46 times more likely to have stunting compared with exclusively breastfed children.

Based on the results of the study, 49 of 181 (27.1%) of stunting children were not exclusively breastfed, the results of this study were in line with the results of a study conducted in Ecuador showing that 30% of stunting children were breastfed less than six months.[16]

Exclusive breastfeeding is breast milk given to infants from birth to six months of age, without adding and replacing with other foods or beverages (except drugs, vitamins, and minerals). Breast milk contains both macro and micronutrient components. Breast milk contains more whey protein that is more subtle and easily absorbed by the baby's intestine compared to cow milk that contains more casein proteins that are more difficult to digest by the baby's intestines. Breast milk has a complete type of amino acid than cow's milk. Breast milk also contains nucleotides compared to cow's milk that has these nutrients in small amounts. Nucleotides play a role in improving the growth and maturation of the intestines, stimulating the growth of good bacteria in the intestines, and increasing iron absorption and immunity to minimize the occurrence of illness in children. Besides, children with exclusive breastfeeding get colostrum which is the perfect food for newborns because of high nutritional value, and full of antibodies that protect infants against infection.[17,18]

The research results showed that there were several reasons for exclusive breastfeeding failure in the research subjects. Firstly, breast milk has not come out, so it was encouraging mothers and families to give formula milk because they are worried the baby feels hungry. This condition indicates that there is still a lack of understanding of mothers and families about the nutritional needs of newborns in the first three days of life that will cause the baby does not get colostrum from breast milk.

Secondly, mothers who give birth in a hospital with a caesarian section or para-vaginal will be hospitalized separately. It contributes to the failure of exclusive breastfeeding because as long as the infant treated in the perinatology room, the health-care provider will give milk formula. The practices indicate that there is no support from the hospital to facilitate the infant's breastfeeding exclusively.

Thirdly, supplementary feeding is too early and too late. It indicates that the mother and family have not understood the right time to provide supplementary feeding. Supplementary feeding (*pemberian makanan tambahan*/ PMT) in the research subjects start at the age of 2 months to the latest at the age of 12 months. Giving liquids and foods other than breast milk to infants aged 0–6 months will increase the risk of diarrhea and other diseases. Water and other liquids or foods may be contaminated, which can eventually cause diarrhea. Besides, the feeding process of babies fed or other foods at the age of 0–6 months disrupted that milk production will decline.[4] Supplementary feeding given at 12 months of age causes the baby to lack nutrients because, ideally, the breast milk supplementary administration begins at six months. In this research, some subjects get supplementary feeding of milk of more than six months and even

have supplementary feeding of milk at the age of 12 months. The condition is bad for the growth of children because from the age of 6 months, children need additional food in addition to breast milk to support growth. Exclusive breastfeeding for 12 months or longer does not provide enough energy for infant growth.[17]

Fourthly, health workers recommend using formula milk. From all respondents who gave formula milk, two people stated that the formula feeding because the health officer suggested it. Another proves that there is no support even from health workers toward exclusive breastfeeding.

Health workers need to facilitate children to achieve healthy growth by facilitating every newborn to get exclusive breastfeeding. In addition to health, education is not only for the mother alone, but husband and family. They should also know the baby's nutritional needs early in life and the importance of exclusive breastfeeding to support children's growth.

Based on Table 2, the results of statistical analysis showed that there was no significant correlation between the completeness of immunization with stunting p value=0.376. The results of this research are in line with the results of research which states that immunization status in children under five in rural and urban areas does not correlate with stunting with p>0.05, 0.279 for rural area and 0.086 in urban area.[19]

Other researches have shown that children without a history of immunization have a 1.98-fold higher chance of having stunting than a child with a history of immunization.[20]

Immunization is a way to increase a person's immunity actively against a disease so that when later exposed to the disease, they will not be sick or have only mild illness. Complete immunization has not guaranteed a child to avoid disease. To be able to provide protection is not only from the completeness of the provision of immunization but also from the quality of the vaccine and the way the vaccine storage.[19] Complete immunization is not enough to guarantee child health, proper nutrition, and environmental health are needed to support children's health.

Based on Table 2 showed that there was a significant correlation between clinically healthy and stunting p value=0.044. Children with unhealthy conditions are clinically at 1.47 times greater risk of stunting compared to clinically healthy children.

A health complaint defined as a disruption to the physical or mental condition, including accidents, or anything else that disrupts daily activities. Meanwhile, someone is said to be sick if they have health complaint and it disturbs their daily activities.[21]

Illness in children harms children's growth. Diseases affect food intake, absorption, and utilization of nutrients that affect child nutrition.[22] According to other studies, children who get sick a 0.6 chance of stunting compared with children who did get sick with a p value<0.001.[23,24]

In average, the toddler who suffers for 3–4 days of illness in the past month is one of the factors that keep their nutrition drained, so the growth becomes sluggish, and the low prevalence increases. Child disease increases the chances of malnutrition and stunting because when the child gets sick, usually the child's appetite will decrease so that it will affect the minimum intake of nutrition during illness. In addition to the condition of sick, the body is less useful in processing the food. If the child gets sick several times a year, the rate of growth will stop or slow down. The risk of malnutrition increases when diarrhea and other diseases further reduce the intake of protein, minerals and other nutrients that children need to stay healthy.[18]

The lack of nutrition occurs when the body does not get the amount of energy, protein, carbohydrates, fats, vitamins and minerals, and other nutrients in sufficient quantities. They are needed to keep organs and tissues staying healthy and functioning correctly. Protein-energy malnutrition (PEM) is still a significant public health problem.[25]

Based on Table 4, the stunting children, most had sufficient energy intake category that was 25 of 30 and only 5 of 30 with the category of less energy intake. The results of statistical analysis showed that there was no significant correlation between energy intake and stunting in aged 12–24 months children with p value>0.05. Protein intake in stunting children mostly has a sufficient protein intake category of 53 of 60 (88%) and only 12% with less protein intake category. The results of statistical analysis showed that there was no significant correlation between energy intake and stunting in children aged 12–24 months with p value>0.05.

The research results are in line with the results of the research in Sedayu subdistrict Bantul regency, which states that there was no significant correlation between the history of energy and protein intake with the incidence of stunting (p>0.05).

The subjects with adequate protein intake 63% had clinically unhealthy conditions, and the subjects with a sufficient energy intake 68% had clinically unhealthy conditions. In a sick condition, the process of absorption of food is less than the maximum. Abnormal absorption factors cause the nutrients that consumed cannot be absorbed entirely as in the condition of children having infectious diseases.[19]

Stunting in the working area of Singaparna Public Health Center is still a health problem. Children's health should increase to create maximum growth.

Conclusion

The determinant factors of stunting in children aged 12–24 months in the working area of Singaparna Public Health Center are gender and clinical health.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgment

The researchers would like to thank the respondents and research subjects who have been willing to participate in this research. Acknowledgments also researchers submitted to the Tasikmalaya Regency Public Health Office and Singaparna Public Health Center, who has given the research permission.

References

1. Soetjiningsih, Gde Ranuh IGN. Tumbuh kembang anak. 2nd Edition. Jakarta: EGC; 2016.
2. Indonesia Agency of Health Research and Development, Ministry of Health of Republic of Indonesia. Basic health research (Riskesdas) 2013 [Internet]. Jakarta: Indonesia Agency of Health Research and Development, Ministry of Health of Republic of Indonesia; 2013 [cited 2018 April 29]. Available from: http://labdata.litbang.kemkes.go.id/ccount/click.php?id=10.
3. Widanti YA. Prevalensi, faktor risiko, dan dampak stunting pada anak usia sekolah. JITIPARI. 2016;1(1):23–8.
4. UNICEF Indonesia. Gizi ibu dan anak. Ringkasan Kajian. Oktober 2012 [cited 2018 April 30]. Available form: https://bulelengkab.go.id/assets/instansikab/126/bankdata/ringkasan-kajian-gizi-ibu-dan-anak-12.pdf.
5. World Health Organization (WHO). WHA global nutrition targets 2025: stunting policy brief. 2014 [cited 2018 May 1]. Available from: https://www.who.int/nutrition/topics/globaltargets_stunting_policybrief.pdf.
6. Sandjaja, Poh BK, Rojroonwasinkul N, Le Nyugen BK, Budiman B, Ng LO, et al. Relationship between anthropometric indicators and cognitive performance in Southeast Asian school-aged children. Br J Nutr. 2013;110(Suppl 3):S57–64.
7. Rachmi CN, Agho KE, Li M, Baur LA. Stunting, underweight and overweight in children aged 2.0–4.9 years in Indonesia: prevalence trends and associated risk factors. PLoS One. 2016;11(5):e0154756.
8. Fuada N, Muljati S, Hidayat TS. Penentuan daerah rawan gizi berdasarkan analisis spatial. Media Litbangkes. 2012;22(1):18–29.
9. Dinas Kesehatan Kabupaten Tasikmalaya. Upaya peningkatan pelayanan kesehatan ibu dan bayi di Kabupaten Tasikmalaya. Paper presented at Seminar International STIKes Respati Tasikmalaya; 2017.
10. Dinas Kesehatan Kabupaten Tasikmalaya. Data bulan penimbangan balita berdasarkan indikator TB/U Kabupaten Tasikmalaya tahun 2016. Singaparna: Dinas Kesehatan Kabupaten Tasikmalaya; 2016.
11. Dinas Kesehatan Kabupaten Tasikmalaya. Cakupan ASI eksklusif dan BBLR Kabupaten Tasikmalaya tahun 2016. Singaparna: Dinas Kesehatan Kabupaten Tasikmalaya; 2017.
12. Nkurunziza S, Meessen B, Van Geertruyden JP, Korachais C. Determinants of stunting and severe stunting among Burundian children aged 6–23 months: evidence from a national cross-sectional household survey, 2014. BMC Pediatr. 2017;17(1):176.
13. Cruz LMG, Azpeitia GG, Súarez DR, Rodríguez AS, Ferrer JFL, Serra-Majem L. Factors associated with stunting among children aged 0 to 59 months from the central region of Mozambique. Nutrients. 2017;9(5):E491.
14. Maryunani A, Sari EP. Asuhan kegawatdaruratan maternal dan neonatal. Jakarta: Trans Info Media (TIM); 2013.

15. Wandita KHDIS. Prognostic factors for normal postnatal growth rate in low birth weight infants. J Med Sci. 2012;44(1):72–7.
16. Alemu ZA, Ahmed AA, Yalew AW, Birhanu BS, Zaitchik BF. Individual and community level factors with a significant role in determining child height-for-age Z score in East Gojjam Zone, Amhara Regional State, Ethiopia: a multilevel analysis. Arch Public Health. 2017;75:27.
17. Millennium Challenge Account (MCA) Indonesia. Stunting dan masa depan Indonesia [Internet]. Jakarta: MCA-Indonesia; 2017 [cited 2018 May 2]. Available from: http://www.mca-indonesia.go.id/assets/uploads/media/pdf/Backgrounder-Stunting-ID.pdf.
18. UNICEF, WHO, UNESCO, UNFPA, UNDP, UNAIDS, WFP, the World Bank, Kementerian Kesehatan Republik Indonesia. Penuntun hidup sehat. 4th Edition. Jakarta: UNICEF, WHO, UNESCO, UNFPA, UNDP, UNAIDS, WFP, the World Bank, Kemenkes RI; 2010.
19. Aridiyah FO, Rohmawati N, Ririanty M. Faktor-faktor yang mempengaruhi kejadian stunting pada anak balita di wilayah pedesaan dan perkotaan. JPK. 2015;3(1):163–70.
20. Picauly I, Toy SM. Analisis determinan dan pengaruh stunting terhadap prestasi belajar anak sekolah di Kupang dan Sumba Timur, NTT. J Gizi Pangan. 2013;8(1):55–62.
21. Kementerian Pemberdayaan Perempuan dan Perlindungan Anak Republik Indonesia, Badan Pusat Statistik. Profil anak Indonesia 2018 [Internet]. Jakarta: Kementerian Pemberdayaan Perempuan dan Perlindungan Anak Republik Indonesia, Badan Pusat Statistik; 2018 [cited 2018 June 20]. Available from: https://www.kemenpppa.go.id/lib/uploads/list/74d38-buku-pai-2018.pdf.
22. Mgongo M, Chotta NAS, Hashim TH, Uriyo JG, Damian DJ, Stray-Pedersen B, et al. Underweight, stunting and wasting among children in Kilimanjaro Region, Tanzania; a population-based cross-sectional study. Int J Environ Res Public Health. 2017;14(5):E509.
23. Fitri DI, Chundrayetti E, Semiarty R. Hubungan pemberian ASI dengan tumbuh kembang bayi umur 6 bulan di Puskesmas Nanggalo. JKA. 2014;3(2):136–40.
24. Darsene H, Geleto A, Gebeyehu A, Meseret S. Magnitude and predictors of undernutrition among children aged six to fifty nine months in Ethiopia: a cross sectional study. Arch Public Health. 2017;75:29.
25. Ubesie AC, Ibeziakor NS. High burden of protein-energy malnutrition in Nigeria: beyond the health care setting. Ann Med Health Sci Res. 2012;2(1):66–9.

Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v7i3.5276

GMHC. 2019;7(3):232–6
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Effects of Pseudoephedrine Administration in Early Gestation on Female Mouse Heart

Annisa Rahmah Furqaani,[1] Listya Hanum Siswanti,[1,2] Ajeng Kartika Sari[1,2]
[1]Department of Histology and Biomedical Sciences, Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia, [2]Graduate School of Biomedical Sciences Master Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Abstract

The pseudoephedrine in pregnant women associated with an increased risk of hypertension and increased heart rate. These conditions force the heart to work harder and cause changes in heart structure, such as left ventricular hypertrophy due to an increase in the number and size of muscle cells. This study aims to determine pseudoephedrine administration in early pregnancy on mice hearts histological features. This study was pure in vivo with a completely randomized design conducted at Medical Biology Laboratory, Faculty of Medicine, Universitas Islam Bandung, from January to August 2017. Subjects were 18 pregnant adult female mice randomly divided into four groups. One control group and three test groups were given oral pseudoephedrine every day at 0.312 mg/24 hours (P1); 0.624 mg/24 hours (P2); and 1.248 mg/24 hours (P3) for seven days starting from the age of pregnancy on day 1. On the 18th day of gestational age, mice sacrificed, then the heart organ was processed into microscopic preparations and stained by Harris' hematoxylin-eosin (HE) staining. Microscopic observations made using a microscope equipped with an optilab viewer with raster image 3. The results showed that the P3 group had a thicker left ventricular wall and significantly more heart muscle nuclei per mm^3 than the control group ($p<0.05$). The results show that the administration of high doses of pseudoephedrine in early pregnancy can affect the structure of the heart.

Key words: Gestation, heart histology, pseudoephedrine

Pengaruh Pemberian Pseudoefedrin pada Masa Awal Kebuntingan terhadap Gambaran Histologi Jantung Mencit Betina

Abstrak

Aktivitas vasokontriksi pseudoefedrin pada ibu hamil diduga kuat berkaitan dengan peningkatan risiko hipertensi dan denyut jantung. Kondisi tersebut memaksa jantung bekerja lebih berat dan dapat menyebabkan perubahan struktur jantung seperti hipertrofi ventrikel kiri akibat peningkatan jumlah dan ukuran sel-sel otot. Tujuan penelitian ini mengetahui pengaruh pemberian pseudoefedrin pada masa awal kebuntingan terhadap gambaran histologi jantung mencit betina. Penelitian ini merupakan eksperimental laboratorium murni *in vivo* menggunakan rancangan acak lengkap yang dilaksanakan di Laboratorium Biologi Medik, Fakultas Kedokteran, Universitas Islam Bandung dari bulan Januari hingga Agustus 2017. Subjek penelitian adalah 18 mencit betina dewasa bunting yang dibagi secara acak menjadi empat kelompok. Satu kelompok kontrol dan tiga kelompok uji yang diberi pseudoefedrin oral setiap hari dengan dosis 0,312 mg/24 jam (P1); 0,624 mg/24 jam (P2); dan 1,248 mg/24 jam (P3) selama 7 hari dimulai dari umur kebuntingan hari ke-1. Pada hari ke-18 umur kebuntingan, mencit dikorbankan kemudian organ jantung diproses menjadi sediaan mikroskopis dan dilakukan pewarnaan *Harris' hematoxylin-eosin* (HE). Pengamatan sediaan mikroskopik dilakukan dengan menggunakan mikroskop yang dilengkapi dengan *optilab viewer* dengan *image raster 3*. Hasil penelitian menunjukkan kelompok P3 memiliki dinding ventrikel kiri yang lebih tebal dan jumlah nuklei otot jantung yang lebih banyak per mm^3 secara signifikan dibanding dengan kelompok kontrol ($p<0,05$). Hasil menunjukkan bahwa pemberian pseudoefedrin dosis tinggi pada masa awal kehamilan dapat memengaruhi struktur jantung.

Kata kunci: Histologi jantung, kehamilan, pseudoefedrin

Received: 2 December 2019; Revised: 26 December 2019; Accepted: 28 December 2019; Published: 31 December 2019

Correspondence: Annisa Rahmah Furqaani. Department of Histology and Biomedical Sciences, Faculty of Medicine, Universitas Islam Bandung. Jln. Tamansari No. 22, Bandung 40116, West Java, Indonesia. E-mail: annisarahmahf@gmail.com

Introduction

Drug use in pregnant women has increased over the past 3–4 years in the United States and other countries. In the United States, drug use by pregnant women increased by 68% from 2.6 in 1976–1978 to 4.2 in 2006–2008. At present, almost half of pregnant women have used more than four types of drugs simultaneously.[1] In several developing countries, antenatal prescription drug is common. The majority of pregnant women who use one or more drugs recognized as having potential risks in pregnancy.[2] Lupattelli et al.[3] showed that more than 80% of pregnant women in Europe, Australia, and America use at least one drug during the pregnancy. During 2006–2008, more than 90% of women reported using one over-the-counter (OTC) drug during pregnancy.[4] The above results show that drug use in pregnant women is common.

One of the most common infection-related diseases in pregnant women is the common cold.[5,6] Various free drug brands that have limited functions to relieve the symptoms are widely available. Therefore pregnant women can easily access these drugs to relieve the symptoms.[3,7] Drug consumption not recommended for pregnant women, especially in the first 12 weeks which is an essential period of vital organ development.[4,6,8]

Pseudoephedrine is the most widely used decongestant during pregnancy because it can be obtained without a prescription and is widely considered a safe drug by pregnant women and healthcare providers.[9,10] This decongestant is an α- and β-adrenoceptor agonist, activation of α-adrenoceptors in the nasal mucosa causes vasoconstriction and reduces nasal obstruction.[11] Pseudoephedrine also plays a role in increasing NE release into the synaptic cleft and causing adrenoceptor activation in postsynaptic neurons.[12]

Vasoconstriction caused by pseudoephedrine suspected to associate with an increased risk of congenital defect due to vascular disorders in the first-trimester pregnancy. The activity also increases the risk of hypertension.[13] Physiologically, in pregnant women, there is an increase in heart work as a form of compensation to supply adequate nutrition and oxygen to the fetus.[13,14] Thus, pseudoephedrine use in pregnancy will cause the heart to work harder. The use of pseudoephedrine oral or nasal more than five days can cause a cardiovascular effect.[15] This condition can cause changes in heart structure, including left ventricular hypertrophy due to an increase in the number and size of muscle cells, thickening and enlargement of the left atrium, narrowing of the heart lumen, and enlargement of the heart valves.[16] This study conducted to describe the effect of pseudoephedrine administration in early pregnancy on the histological feature of the mice heart.

Methods

This study was in vivo laboratory experiments with a completely randomized design conducted at Medical Biology Laboratory, Faculty of Medicine, Universitas Islam Bandung, from January to August 2017. Twenty adult gestational mice (*Mus musculus*) divided into four groups: one control (C) and three treated groups (P1, P2, P3), but two mice in P2 and P3 groups died over the study. Based on the monthly index of medical specialties (MIMS) 2016, the maximum dose of pseudoephedrine used in adult humans is 240 mg/24 hours for seven days. Because the subjects in this study were mice, the dose equalization with a conversion factor of 0.0026 conducted to obtain a dose of 0.624 mg/24 hours (n). The effect of various pseudoephedrine doses on the general teratological feature of mice fetuses determined with two other doses. The doses were 0.312 mg/24 hours (1/2n) and 1.248 mg/24 hours (2n) and administered for seven days.

Mice adapted for two weeks before treatment. Unhealthy mice during and after this period not included in the study. During treatment, mice placed in a cage made of plastic tubs 28×34×14 cm covered with small wire mesh and given husk base to absorb urine and feces. Each tub filled with a maximum of five animals. Mice fed ad libitum, and tap water also provided ad libitum. Cage cleaning and husk replacement at least once a week.

Female mice mated naturally with male mice by uniting the two in one cage in a ratio of 5 females:1 male. Determination of gestational age by observing the absence/presence of vaginal plugs in the next morning. If vaginal plugs found, it counted as day-0 of gestation.

This study was part of a research project that also observes the teratological features of mice's offspring. The standard procedure for teratological observation was that the dissection performed on the 18th day of gestation to minimize bias related to gestational age.[17] On the 18th day of gestation, mice sacrificed by dislocation and dissected. The heart weighed, then immersed

in the 10% formalin solution, processed into microscopic preparations, and stained with the Harris' hematoxylin-eosin (HE) staining. The preparation was observed using a microscope equipped with an optilab viewer 2.2 and image raster three software for analysis. Morphology of the left ventricle and number of cardiocytes were analyzed. The cardiocytes' percentage calculation carried out in each field of view. Mean values from all fields calculated from all preparations of each group. Afterward, it followed by calculating the mean of each group.

Data obtained from this study were heart weight, left ventricular wall thickness, and the number of cardiocytes nuclei. The data analyzed using one-way ANOVA with post-hoc Bonferroni.

Results

The macroscopic and microscopic observation from this study presented in Table.

Based on the data in Table, it appears that cardiac wet weight tends to increase in the treatment group (P1, P2, and P3) who received pseudoephedrine doses of 0.312 mg/24 hours, 0.624 mg/24 hours, and 1.248 mg/24 hours, but the increase not statistically significant ($p>0.05$). The thickness of the left ventricle also increased, but only the P3 group showed a significant difference compared to the control group. The number of nuclei (cardiomyocytes) in the P3 group were significantly increased compared to the control group ($p<0.05$).

Discussion

This study shows that the number of cardiomyocyte nuclei increases in line with the dose of pseudoephedrine. The number of nuclei represents the number of cardiomyocytes. Therefore, the number of cardiomyocyte in treatment groups tend to increase along with the given dose. Based on these data, it concluded that the addition of left ventricular wall thickness in this study caused by hyperplasia or an increase in the number cardiomyocyte in that ventricular section. Farrell et al.[18] showed that in the hypertrophic cardiomyopathy mouse model, hyperplasia contributed to increasing the size of the heart. Hyperplasia is a process that stimulated by cytokines produced by cardiomyocytes and is a factor that plays a role in increasing left ventricular mass in rats with chronic overload volume.[19] During pregnancy, there is an increase in blood volume, hormonal changes, and a gradual increase in cardiac output until it reaches a peak in late pregnancy.[20] Simultaneously, there is an increase in heart rate and stroke volume or a decrease in vascular resistance.[21] As a result of hematological and hormonal changes in pregnancy, the heart forced to work harder so its structure must undergo adaptation, including mild hypertrophy in the left ventricle thus homeostasis to the fetus can be maintained.[20,22]

In this study, increasing in cardiomyocytes number and ventricular wall thickness has not contributed significantly to heart weight. Nevertheless, it tended to be more substantial in the treatment group. The high dose pseudoephedrine (1.248 mg/24 hours) in early pregnancy can affect the structure of the heart. Without drug interference, pregnancy itself is one of the conditions that cause remodeling of the heart structure toward hypertrophy.[23] In late pregnancy model, fibroblast growth factor 21 (FGF21) plays the leading role in cardiac hypertrophy.[24] FGF21 is cardiomyocyte produced by cardiomyocyte and works as autocrine,

Table Macroscopic and Microscopic Observation from Heart Preparation

Parameters	C (n=5)	P1 (n=5)	P2 (n=4)	P3 (n=4)	p Value
Heart weight (g)	0.19 (0.01)	0.19 (0.04)	0.24 (0.06)	0.21 (0.01)	0.401
Left ventricle thickness (μm)	1,540.96 (70.15)	1,533.00 (93.22)	1,591.20 (42.49)	1,714.78 (91.54)*	0.015
Number of nuclei ($10^{-6}/\mu m^3$)	35.56 (3.73)	49.70 (7.43)	61.98 (15.85)	80.29 (6.19)*	0.000

Note: data presented in mean (standard deviation); Statistical analysis for each parameter using the one-way ANOVA test with Bonferroni's advanced test at a 95%CI ($\alpha=0.05$); n=number of samples; C=control group; P1=treatment group received pseudoephedrine dose 0.312 mg/24 hours; P2=treatment group received pseudoephedrine dose of 0.624 mg/24 hours; P3=treatment group received pseudoephedrine dose and 1,248 mg/24 hours; *significantly different with C

paracrine, or endocrine. FGF21 in response to hypertrophy or other stress stimuli and is known for preventing cardiac hypertrophy, heart inflammation, and inhibiting oxidative stress activity.[25] FGF21 levels are relatively elevated during pregnancy and mediated by transcription factor peroxisome proliferator-activated receptor α (PPARα). The intracellular mechanism of action of FGF21 involves PPARα and Sirt1 which influence the regulation of transcription of FGF21 genes when exposed to exogenous stimuli.[24]

Cardiac hypertrophy is a physiological adaptation during pregnancy so that the survival of the fetus ensured.[20] Cardiovascular system adjustment during pregnancy can turn out to be pathological if accompanied by certain conditions, such as the consumption of certain drugs,[8,10] including pseudoephedrine. Nevertheless, based on the United States Food and Drug Administration (FDA) category, pseudoephedrine is in category C. It means that in animal and human studies, it shows side effects in the fetus (teratogenic or embryocidal). However, controlled studies in both are still not yet available or adequate.[8] This drug should only be given to pregnant women only if the potential benefits are more significant than the risk to the fetus. Unfortunately, lacking information received by pregnant women regarding the safety drugs during pregnancy and free circulation in the market makes it easily accessible to pregnant women.[26] More than 90% of pregnant women exposed to the OTC drug.[7]

Pseudoephedrine is a vasoconstrictor agonist α- and β-adrenoceptor.[11] Pseudoephedrine can bind the norepinephrine (NE) transporter and SLC6A2. Therefore it plays a role in releasing neurotransmitter NE.[12] NE is a sympathomimetic agent that causes an increase in heart rate, blood pressure, and cardiac output.[11] In pregnancy, pseudoephedrine potentially cause hypertension and increased work of the cardiovascular system. Hypertension can become a significant cause of left ventricle hypertrophy mediated by neuropeptide Y (NPY). It has vasoconstrictive and pro-angiogenic properties. It induced remodeling in response to hemodynamic overload. Long term hypertension can stimulate cardiomyocyte proliferation and decreased cardiac ventricular function.[27]

The cellular and molecular mechanism of pseudoephedrine in causing hypertrophy is not known certainly. Does it contribute to causing cardiac hypertrophy through enhancing NE release or it has the potential to influence the cardiomyocyte's proliferation pathway? It is necessary to further investigate the effect of pseudoephedrine utilization on factors related to cardiomyocytes hypertrophy and hyperplasia, including FGF21 and Sirt1 gene expression.

Conclusion

Administration of high doses of pseudoephedrine in early pregnancy can affect the structure of the mice's heart.

Conflict of Interest

There was no conflict of interest.

Acknowledgment

This study funded by Institute for Research and Community Service (Lembaga Penelitian dan Pengabdian kepada Masyarakat/LPPM) Universitas Islam Bandung.

References

1. Ayad M, Costantine MM. Epidemiology of medications use in pregnancy. Semin Perinatol. 2015;39(7):508–11.
2. Daw JR, Hanley GE, Greyson DL, Morgan SG. Prescription drug use during pregnancy in developed countries: a systematic review. Pharmacoepidemiol Drug Saf. 2011;20(9):895–902.
3. Lupattelli A, Spigset O, Twigg MJ, Zagorodnikova K, Mårdby AC, Moretti ME, et al. Medication use in pregnancy: a cross-sectional, multinational web-based study. BMJ Open. 2014;4(2):e004365.
4. Thorpe PG, Gilboa SM, Hernandez-Diaz S, Lind J, Cragan JD, Briggs G, et al. Medications in the first trimester of pregnancy: most common exposures and critical gaps in understanding fetal risk. Pharmacoepidemiol Drug Saf. 2013;22(9):1013–8.
5. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med. 2014;370(23):2211–8.
6. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol. 2010;63(6):425–33.
7. Sachdeva P, Patel BG, Patel BK. Drug use in pregnancy; a point to ponder! Indian J Pharm Sci. 2009;71(1):1–7.

8. Mosley AT, Witte AP. Drugs in pregnancy: do the benefits outweigh the risks? US Pharmacist. 2013;38(9):43–6.
9. Werler MM. Teratogen update: Pseudoephedrine. Birth Defects Res A Clin Mol Teratol. 2006;76(6):445–52.
10. Yau WP, Mitchell AA, Lin KJ, Werler MM, Hernández-Díaz S. Use of decongestants during pregnancy and the risk of birth defects. Am J Epidemiol. 2013;178(2):198–208.
11. Solanki P, Yadav PP, Kantharia ND. Ephedrine: direct, indirect or mixed acting sympathomimetic? Int J Basic Clin Pharmacol. 2014;3(3):431–6.
12. Schlessinger A, Geier E, Fan H, Irwin JJ, Shoichet BK, Giacomini KM, et al. Structure-based discovery of prescription drugs that interact with the norepinephrine transporter, NET. Proc Natl Acad Sci USA. 2011;108(38):15810–5.
13. van Gelder MMHJ, van Rooij IALM, Miller RK, Zielhuis GA, de Jong-van den Berg LTW, Roeleveld N. Teratogenic mechanisms of medical drugs. Hum Reprod Update. 2010;16(4):378–94.
14. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. Front Pharmacol. 2014;5:65.
15. Laccourreye O, Werner A, Giroud JP, Couloigner V, Bonfils P, Bondon-Guitton E. Benefits, limits and danger of ephedrine and pseudoephedrine as nasal decongestants. Eur Ann Otorhinolaryngol Head Neck Dis. 2015;132(1):31–4.
16. Lazzeroni D, Rimoldi O, Camici PG. From left ventricular hypertrophy to dysfunction and failure. Circ J. 2016;80(3):555–64.
17. Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 7 Tahun 2014 tentang Pedoman Uji Toksisitas Nonklinik secara In Vivo.
18. Farrell ET, Grimes AC, de Lange WJ, Armstrong AE, Ralphe JC. Increased postnatal cardiac hyperplasia precedes cardiomyocyte hypertrophy in a model of hypertrophic cardiomyopathy. Front Physiol. 2017;8:414.
19. Du Y, Plante E, Janicki JS, Brower GL. Temporal evaluation of cardiac myocyte hypertrophy and hyperplasia in male rats secondary to chronic volume overload. Am J Pathol. 2010;177(3):1155–63.
20. Xiao J, Li J, Xu T, Lv D, Shen B, Song Y, et al. Pregnancy-induced physiological hypertrophy protects against cardiac ischemia-reperfusion injury. Int J Clin Exp Pathol. 2014;7(1):229–35.
21. Yanamandra N, Chandraharan E. Anatomical and physiological changes in pregnancy and their implications in clinical practice. In: Chandraharan E, Arulkumaran SS, editors. Obstetric and intrapartum emergencies: a practical guide to management. Cambridge: Cambridge University Press; 2013. p. 1–8.
22. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr. 2016;27(2):89–94.
23. Maillet M, van Berlo JH, Molkentin JD. Molecular basis of physiological heart growth: fundamental concepts and new players. Nat Rev Mol Cell Biol. 2013;14(1):38–48.
24. Redondo-Angulo I, Mas-Stachurska A, Sitges M, Tinahones FJ, Giralt M, Villarroya F, et al. Fgf21 is required for cardiac remodeling in pregnancy. Cardiovasc Res. 2017;113(13):1574–84.
25. Planavila A, Redondo-Angulo I, Villarroya F. FGF21 and cardiac physiopathology. Front Endocrinol (Lausanne). 2015;6:133.
26. Clemow DB, Dewulf L, Koren G, Mikita JS, Nolan MR, Michaels DL, et al. Clinical data for informed medication use in pregnancy: strengths, limitations, gaps, and a need to continue moving forward. Ther Innov Regul Sci. 2014;48(2):134–44.
27. Tan CMJ, Green P, Tapoulal N, Lewandowski AJ, Leeson P, Herring N. The role of neuropeptide Y in cardiovascular health and disease. Front Physiol. 2018;9:1281.

Authors Index

Subjects Index

The Editor would like to thank you for the effort and expertise of all reviewers, without which it would be impossible to maintain the high standards of peer-reviewed journals.

Prof. Dr. Budi Setiabudiawan, dr., Sp.A.(K.), M.Kes.
Prof. Cordia Chu, B.Sc., M.A., Ph.D.
Ermi Ndoen, B.Sc.P.H., M.Sc.P.H., Ph.D.
Ferry Efendi, S.Kep.Ns., M.Sc., Ph.D.
Prof. Dr. Hendro Sudjono Yuwono, dr., Sp.B.V.(K.)
Prof. Dr. Herri S. Sastramihardja, dr., Sp.F.K.(K.)
Prof. Hidayat Wijayanegara, dr., Sp.O.G.(K.)
Prof. Dr. Ieva Baniasih Akbar, dr., A.I.F.
Dr. Lelly Yuniarti, S.Si., M.Kes.
Leri Septiani, dr., Sp.O.G., Ph.D.
Prof. Dr. M. Ahmad Djojosugito, dr., Sp.B., Sp.O.T.(K.), M.H.A.
Dr. Maya Tejasari, dr., M.Kes.
Mirasari Putri, dr., Ph.D.
Prof. Dr. Niniek Lely Pratiwi, drg., M.Kes.
Nunik Kusumawardani, S.K.M., M.P.H., Ph.D.
Prof. Nuzirwan Acang, dr., DTM&H, Sp.P.D.-K.H.O.M.
Prof. Dr. Thaufiq S. Boesoirie, dr., M.S., Sp.T.H.T.-K.L.(K.)
Prof. Dr. Tony S. Djajakusumah, dr., Sp.KK(K)
Prof. Umar Fahmi Achmadi, dr., M.P.H., Ph.D.
Dr. Wahyu Widowati, M.Si.
Dr. Wawang S. Sukarya, dr., Sp.O.G.(K.), M.A.R.S., M.H.Kes.
Dr. Yani Triyani, dr., Sp.P.K., M.Kes.

TABLE OF CONTENTS

p-ISSN 2301-9123
9 772301 912306

e-ISSN 2460-5441
9 772460 544103