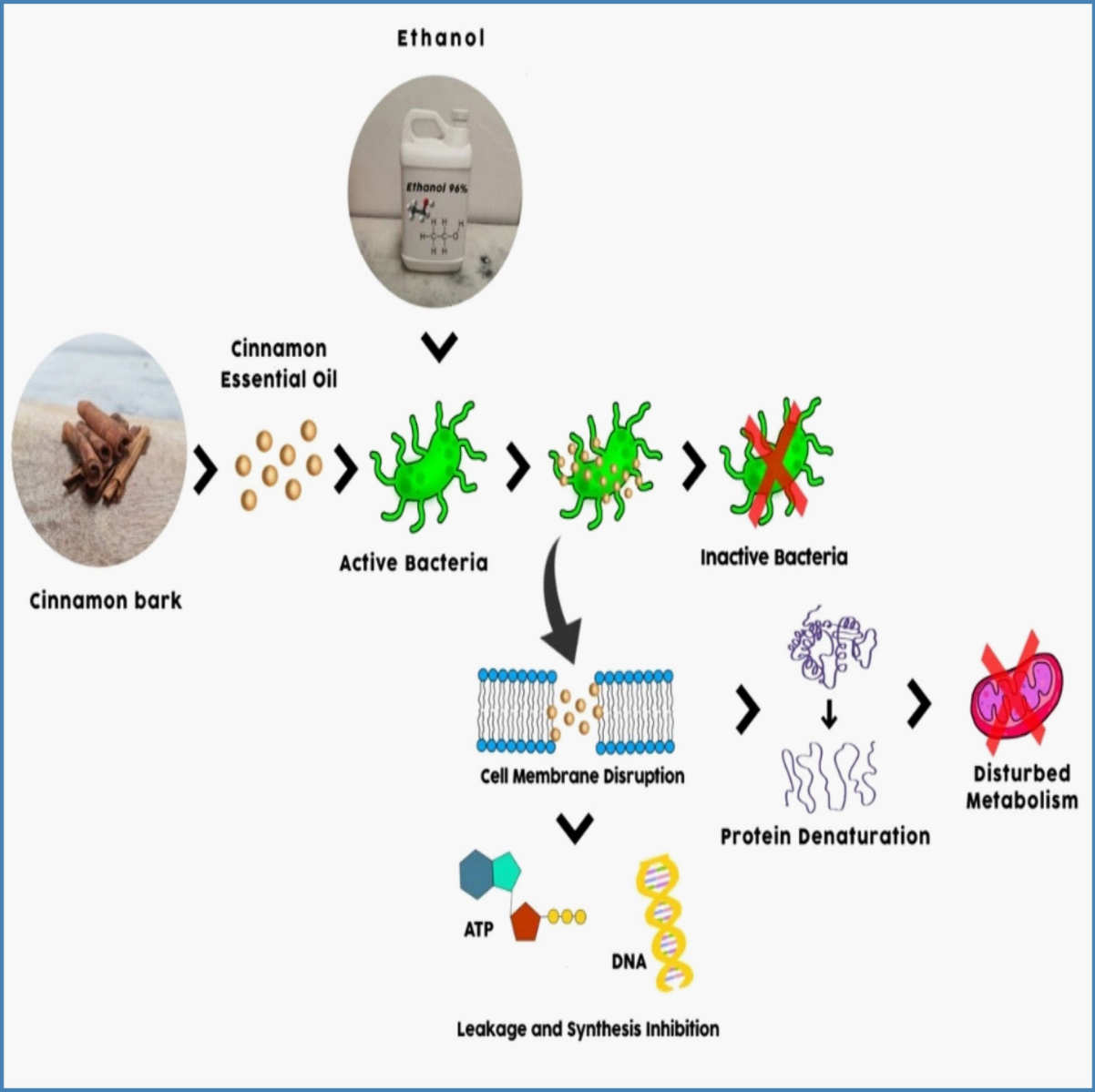
ISSN: 2356-3931 (Online)

Journal of the Medical Sciences

(Berkala Ilmu Kedokteran)

Gadjah Mada Journal of the Medical Sciences

Volume 55 No 1, 2023



Published by
Faculty of Medicine, Public Health, and Nursing,
Universitas Gadjah Mada, Yogyakarta, Indonesia

Table of contents

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 1-10
https://doi.org/10.19106/JMedSci005501202301

Unique truncated and non-synonymous mutations in functional domains of ORF3a SARS-CoV-2

Jeanne Elvia Christian*, Hartiyowidi Yuliawuri
Calvin Institute of Technology, Jakarta, Indonesia

Submitted: 2022-01-11
Accepted : 2022-05-23

ABSTRACT

Previous studies showed that mutations in the SARS-CoV-2 ORF3a protein can influence viral pathogenesis. Therefore, it is necessary to observe mutations, especially in the functional domain of the protein. We observed the presence of mutations in the ORF3a protein by analyzing 5,131 samples from the GISAID database since it was first discovered in March 2020 until November 2021. The sequence was aligned using Clustal Omega Multiple Sequence Alignment from EMBL-EBI and analyzed using BioEdit version 7.2.5 software using reference sequences NC045512. Samples having the letter N were omitted from the analysis. The effect of point mutations on proteins was analyzed using the Protein Variation Effect Analyzer (PROVEAN) v1.1.3 software. The functional domains of the ORF3a protein were visualized using RasWin software. We identified 312 mutations in the SARS-CoV-2 ORF3a protein. In addition, from 5,131 samples, 915 samples were found to be truncated in the C-terminal region of the protein. These non-synonymous mutations data in functional domains and truncated sequences indicate that amino acid changes in the ORF3a protein require further studies to determine the effect of viral pathogenicity in humans.

Keywords:
domains;
mutations;
ORF3a;
SARS-CoV-2;
truncated

ABSTRACT

Beberapa penelitian menunjukkan bahwa mutasi pada protein ORF3a SARS-CoV-2 dapat memengaruhi patogenesis virus. Oleh karena itu, pengamatan mutasi terutama pada domain fungsional protein perlu dilakukan. Kami mengamati keberadaan mutasi pada protein ORF3a dengan menganalisis 5,131 sampel dari database GISAID sejak virus SARS-CoV-2 pertama kali ditemukan pada bulan Maret 2020 hingga November 2021. Sekuens disejajarkan menggunakan *Clustal Omega Multiple Sequence Alignment from EMBL-EBI and analyzed using BioEdit version 7.2.5 software* menggunakan sekuens referensi NC045512. Sampel yang memiliki huruf N dihilangkan dari analisis. Efek mutasi titik pada protein dianalisis menggunakan *Protein Variation Effect Analyzer* (PROVEAN) v1.1.3 piranti lunak daring. Domain fungsional protein ORF3a divisualisasi menggunakan piranti lunak RasWin. Kami mengidentifikasi 312 mutasi pada protein ORF3a SARS-CoV-2. Selain itu, dari 5,131 sampel ditemukan sebanyak 915 sampel mengalami pemotongan pada daerah C-terminal protein. Data mutasi tidak identik pada domain fungsional dan sekuen terpotong ini menunjukkan bahwa perubahan asam amino pada protein ORF3a memerlukan penelitian yang lebih lanjut untuk menentukan efek patogenisitas virus pada manusia.

INTRODUCTION

The disease caused by SARS-CoV-2 is officially termed as COVID-19. The virus has a positive sense, single-stranded RNA virus, enveloped belonging to the genus Beta coronavirus in the family of Coronaviridae. The genome consists of 4 structural proteins (spike, envelope, membrane, and nucleocapsid), 16 non-structural proteins (nsp1-16) and 7 accessory proteins.[1,2]

ORF3a is the largest accessory protein of SARS-CoV-2 and has 275 amino acids in lengths. It is only present in SARS-CoV and SARS-CoV-2, and not found

*corresponding author: jeanne.christian@calvin.ac.id

in another Beta coronavirus.[3,4] Some analysis suggest that this protein might be derived originally from the M gene in the CoV lineage.[5,6] It has transmembrane proteins of the viroporin family that form ion channels in the host membrane and inhibit IFN-**α** signaling. So, it may be implicated in inducing apoptosis and virus release.[7,8] Some researchers showed that ORF3a protein has a role in cytokine storm by up-regulating fibrinogen secretion. SARS-CoV-2 ORF3a possesses six domains and interacts with the membrane (M) and envelope (E) protein during viral assembly. The presence of ORF3a protein is essential for viral reproduction when E protein is absent. It contains a PDZ-binding motif at C-termini (amino acid 209-264) which plays a role in viral pathogenesis.[4,9] Besides that, this accessory protein was found to be important in severity of COVID-19 and had the contribution to post-COVID conditions. Its mutations may be also correlated with mutations in the spike protein and could potentially affect the function of ORF3a.[1,4,10,11]

Based on some research, the ORF3a protein is indeed not conserved.[5,12] Several amino acid changes in ORF3a protein were likely associated with the characteristic's alteration of the virus variant. Previous Variants of Concerns (VOCs) like Beta (lineage B.1.351) and Gamma (lineage P.1) had amino acid changes in Q57H and S253P respectively. While the current VOCs like Delta (lineage B.1.617.2) and Omicron (lineage BA.1, BA.1.1, BA.2) had amino acid changes in S26L and T223I respectively. The previous Variants of Interest/VOIs (lineage B.1.427/1.429, P.2, B.1.525, P.3, B.1.526, B.1.617.1, C.37, B.1.621) had amino acid changes in S26L, P42L, Q57H, and V256del.[13] The variant that correlates with the designated VOC or VOI will be reclassified continuously through the assessment of global public health significance.

From our previous study, we found non-synonymous mutations in ORF3a SARS-CoV-2 protein and we focused on the highest frequency of the mutations from 3,791 samples. Those highest mutations did not occur in the functional domain.[14,15] So, the purpose of this study is to look for other non-synonymous mutations, which were found in Indonesia samples that occur in the functional domains of ORF3a SARS-CoV-2. Furthermore, we analyze the unique truncated protein of ORF3a SARS-CoV-2. This data can contribute to enhancing our understanding about the diversity of this virus and how these mutations could affect its functional role in viral pathogenesis.

MATERIAL AND METHODS

ORF3a sequences data retrieval

From our previous study, a total of 3,751 SARS-CoV-2 Indonesia samples retrieved from GISAID from March 2nd 2020 until July, 31st 2021 were analyzed and we found 203 non-synonymous mutations. We added more data and analyzed 1,380 Indonesia samples from August, 1st 2021 until November, 30th 2021. The data were retrieved from GISAID.[16] We aligned using Clustal Omega Multiple Sequence Alignment from EMBL-EBI (www.ebi.ac.uk/Tools/msa/ clustalo/) and analyzed using BioEdit version 7.2.5 software. The reference sequence was taken from GISAID database with isolate hCoV-19/Wuhan/WIV04/2019 (EPI_ISL_4021 24). We excluded the samples containing N letters due to inaccurate reading of amino acid.

Determine the biological effect prediction of ORF3 non-synonymous mutations

We predicted the protein sequences to Protein Variation Effect Analyzer (PROVEAN) v1.1.3. software online tools.[17] The software predicts if the amino acid substitution has an impact on the biological function of a protein with -2.5 as cut off value. Above -2.5 indicates that the substitution had no effect or neutral.

Protein 3D visualization

We used RasWin v2.7.5.2 software to visualize the six domains in ORF3a SARS-CoV-2. As the model, the 6XDC in PDB format was used.

RESULT

ORF3a sequences data retrieval

We analyzed 5,131 ORF3a SARS-CoV-2 from the GISAID database. Based on data alignment, we found total 312 non-synonymous mutations, and they were scattered in all six functional domains of ORF3a protein. Domain I, III, IV, and V proteins almost had mutations in their amino acid sequences. Whereas domain II and VI proteins had mutations in all amino acid sequences. The highest total frequency of mutations was found in VI domain with 83 non-synonymous mutations (TABLE 1).

TABLE 1. Non-synonymous mutations of ORF3a SRS-CoV-2 and their effect prediction based on PROVEAN Score.

Domains of ORF3a SARS-CoV-2	ORF3a amino acid locations	ORF3a amino acids		Total Frequency of mutations	PROVEAN Score	Variation effect on protein based on PROVEAN
		Wild type	Non-synonymous mutations			
I	1	M	N/A	0	N/A	N/A
	2	D	Y	1	-8.581	Deleterious
	3	L	N/A	0	N/A	N/A
	4	F	S	1	-7.257	Deleterious
	5	M	I	1	-1.257	Neutral
			V	11	-1.581	Neutral
	6	R	N/A	0	N/A	N/A
	7	I	N/A	0	N/A	N/A
	8	F	N/A	0	N/A	N/A
	9	T	K	1	-4.276	Deleterious
	10	I	N/A	0	N/A	N/A
	11	G	N/A	0	N/A	N/A
	12	T	I	1	-0.781	Neutral
	13	V	L	10	-1.648	Neutral
			A	1	-3.914	Deleterious
	14	T	I	4	-4.61	Deleterious
			N	1	-1.286	Neutral
	15	L	F	4	-1.314	Neutral
II	36	P	T	1	-5.924	Deleterious
	37	I	T	4	-0.305	Neutral
	38	Q	H	2	-2.286	Neutral
			K	4	-2.629	Deleterious
			R	2	-2.629	Deleterious
	39	A	T	3	-0.962	Neutral
			S	1	-1.638	Neutral
	40	S	L	9	-2.971	Deleterious
			P	1	0.276	Neutral

TABLE 1. Non-synonymous mutations of ORF3a SRS-CoV-2 and their effect prediction based on PROVEAN Score (cont.)

Domains of ORF3a SARS-CoV-2	ORF3a amino acid locations	ORF3a amino acids		Total Frequency of mutations	PROVEAN Score	Variation effect on protein based on PROVEAN
		Wild type	Non-synonymous mutations			
III	91	Y	N/A	0	N/A	N/A
	93	S	N/A	0	N/A	N/A
	109	Y	N/A	0	N/A	N/A
	127	L	F	2	-1.981	Neutral
	128	W	G	1	-7.419	Deleterious
			C	1	-7.419	Deleterious
			L	1	-7.752	Deleterious
	129	L	F	10	-3.829	Deleterious
	130	C	F	1	-7.79	Deleterious
	131	W	C	36	-7.752	Deleterious
			S	2	-8.733	Deleterious
			L	2	-6.752	Deleterious
	132	K	N/A	0	N/A	N/A
	133	C	S	1	-9.81	Deleterious
IV	141	Y	N/A	0	N/A	N/A
	14	D	H	8	-6.771	Deleterious
			Y	2	-8.733	Deleterious
	143	A	S	3	0.724	Neutral
			V	1	-2.59	Deleterious
	144	N	S	8	-3.571	Deleterious
			D	1	-1.571	Neutral
	145	Y	S	1	-5.495	Deleterious
	146	F	C	1	-7.848	Deleterious
	147	L	V	1	2.943	Neutral
			F	3	-1.962	Neutral
	148	C	N/A	0	N/A	N/A
	149	W	C	7	-9.752	Deleterious
			L	1	-9.419	Deleterious
V	160	Y	N/A	0	N/A	N/A
	161	N	S	4	-3.571	Deleterious
	162	S	N/A	0	N/A	N/A
	163	V	L	1	-1.238	Neutral
VI	171	S	L	83	-2.238	Neutral
			P	1	-2.419	Neutral
	172	G	C	46	-6.752	Deleterious
			V	3	-6.762	Deleterious
			R	3	-6.114	Deleterious
			D	2	-5.133	Deleterious
	173	D	G	12	-4.867	Deleterious

Out of 5,131 samples, we found 915 samples of ORF3a SARS-CoV-2 were truncated in C-termini. The amino acid position 212-218 were mutated and from 219-275 were deleted in these samples (FIGURE 1). Furthermore, we analyzed the lineage of these truncated samples and see if those samples were included in the Variants of Concerns (VOCs) based on WHO (FIGURE 2).

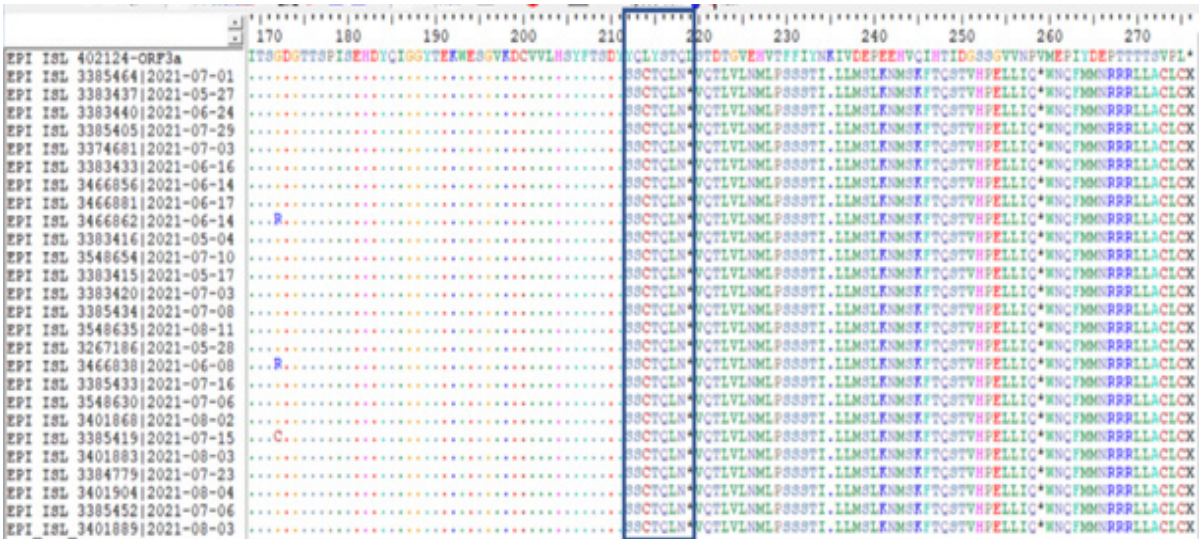


FIGURE 1. ORF3a SARS-CoV-2 truncated samples. Blue box indicates C-termini sequence was mutated and truncated protein at amino acid position 212-219. The dot indicates the similar amino acid compare with the reference sequence.

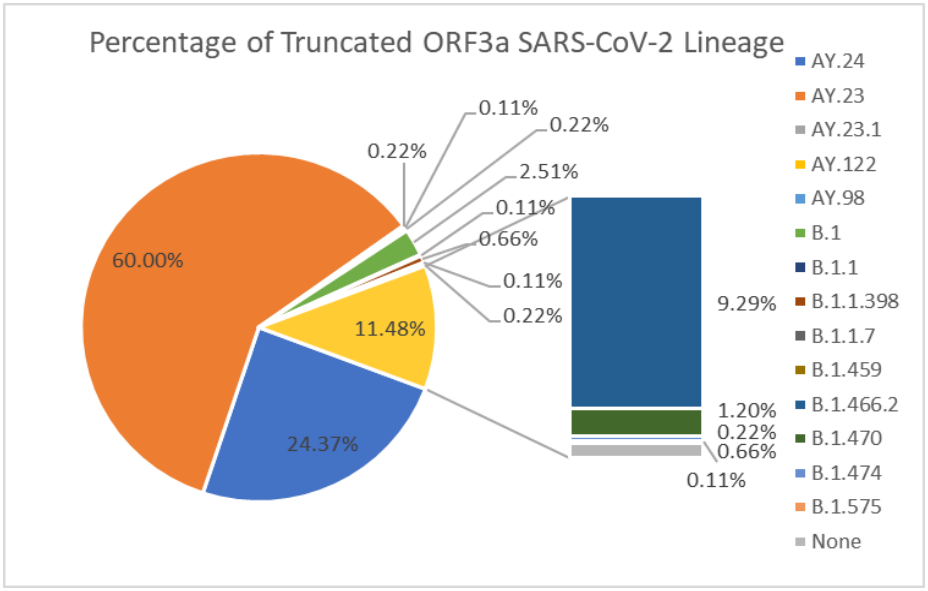


FIGURE 2. Lineage's percentage of ORF3a SARS-CoV-2 truncated samples in Indonesia.

The biological effect prediction of ORF3 non-synonymous mutations

Based on PROVEAN prediction, the non-synonymous mutations that found in six functional domains of ORF3a protein had neutral or deleterious effects. There were 32 deleterious effects and 19 neutral effects in six functional domains (TABLE 1).

Protein 3D visualization

The 3D visualization highlights the location of six functional domains in ORF3a proteins. Domain II, III, IV are located in alpha helices, while domain I and VI are located in loops and turns. The domain V could not be shown in this visualization (FIGURE 3).

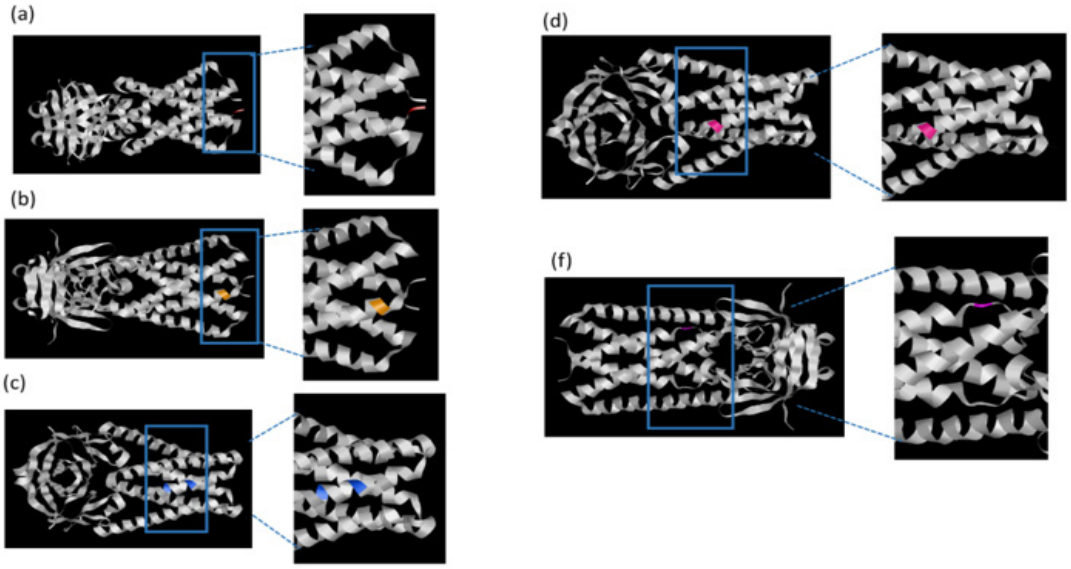


FIGURE 3. 3D Visualization of ORF3a SARS-CoV-2 functional domains: (a) domain I (amino acid 1-15), (b) domain II (amino acid 36-40), (c) domain III (amino acid 91, 93, 109, 127-133), (d) domain IV (amino acid 141-149), (e) domain V (amino acid 160-163, FIGURE not shown), (f) domain VI (amino acid 171-173).

DISCUSSION

We found a number of mutations in all six ORF3a protein domains (TABLE 1). Some mutations in the ORF3a domain show deleterious mutations based on Provean scoring. Even though deleterious mutations usually occur in nature, they tend to be fewer than neutral mutations. Mutation in structural protein might increase the stability of the protein, on contrary in non-structural protein might decrease its stability.[18]

Domain I (amino acids 1-15) is the N-terminal region of the signal peptide that plays a role in localizing the subcellular protein ORF3a SARS-CoV-2 (FIGURE 3).[1,9] In this area, a number of mutations in the amino acids were found. The D2Y, F4S, T9del, T9K mutations were deleterious which may eliminate the function of the signal peptide. The V13L mutation is neutral, but the valine arginine mutation at position 13 (V13R) is deleterious, even though it does not change the nature of the protein, but it might change the protein's function. Likewise, T14I is neutral, while T14N is deleterious.

Domain II (amino acids 36-40) has a TRAF-3 (TNF receptor-associated factor 3) binding motif. The presence of this

domain can activate inflammatory Nf-kB and NLRP3 by promoting TRAF-3-mediated ubiquitination.[1,19] P36T and S40L mutations were found which were deleterious. There were 3 types of mutations in amino acid position 38, where glutamine (Q) lysine (K) and glutamine (Q) arginine (R) are deleterious by changing the polarity of the amino acid to become positively charged. However, the change in the amino acid glutamine (Q) histidine (H) does not change the nature of the amino acid even though the amino acid at that position becomes positively charged as well.

Domain III functions as the SARS-CoV-2 viroporin. The ion channel activity was carried out in the amino acid domain 93-133.[8] The *in vitro* study showed the amino acid 70-133 in Domain III was responsible for increasing the suppressor of cytokine signaling 1 (SOC 1). As a result of this up-regulation, the JAK-STAT signaling activation was inhibited and therefore led to the JAK2 degradation. This study was conducted with HEK293T transfected with ORF3a plasmid to determine SOCS 1 in mRNA and protein levels.[20] Position 128 mutation of tryptophan (W) glutamine (G) changes the amino acid from non-polar to polar and deleterious. Changes in tryptophan (W) leucine (L) or cysteine (C) are also deleterious although they do not change the properties of amino acids. Likewise, mutations at position 131 of tryptophan (W) serine (S) change the amino acid to be polar and deleterious. Mutation in W131C and W131L that do not change the amino acid properties are also deleterious, where it is possible that these changes alter the protein's function. In addition, mutations L129F, C130F, C133S are also deleterious. Based on study, the C133S mutations in Domain III will reduce the level apoptosis.[4] The W131C mutations were the second highest frequency of mutations with deleterious mutations in our study. These amino acid changes might facilitate the process of tetramerization to form ion channels and support the infectivity of the virus.[21,22]

Domain IV regulates viral uptake and trafficking of proteins to the plasma membrane or intracellular membrane.[1,3] The mutation of position 143 of alanine (A) serine (S) which changes the polarity of the amino acid is neutral, while the change of alanine (A) valine (V) is deleterious although it does not change the polarity of the amino acid. Mutation of position 144 of asparagine (N) serine (S) is deleterious, but not for changes in asparagine (N) aspartic acid (D) although this change makes polar amino acids negatively charged. Position 142 mutations of aspartic acid (D) histidine (H) and tyrosine (Y) are deleterious and change amino acids into positively charged polar and neutral charged polar. The Y145S, F146C, W149C, and W149L mutations were deleterious although they did not change the amino acid polarity. The 147 position changes of leucine (L) valine (V) and phenylalanine (F) were neutral. These mutations in C148Y and A143S may increase the infectivity rate, even though these amino acid changes were neutral based on Provean score.[23]

Domain V is responsible for Golgi to plasma membrane transport, and mutations in this site made ORF3a protein to be aggregated. It has a bulky hydrophobic residue YNSV, 160-163.[3,9,23] Mutations at position 161 of asparagine (N) serine (S) and position 163 of valine (V) leusine (L) are deleterious although they do not change the polarity of amino acids.[3]

Domain VI is a di-acidic peptide that has an SGD motif that is not conserved in SARS-CoV-2.[1,5] In these samples, the three amino acids were found to be mutated. Changes at position 171 of serine (S) leucine (L) and proline (P) are neutral. Position 172 experienced with four kinds of changes, and the most changes from

glycine (G) cysteine (C), then from glycine (G) valine (V) followed by arginine (R), and the least from glycine (G) aspartic acid (D). Position 173 undergoes a deleterious change from aspartic acid (D) glycine (G). The highest frequency of mutations with deleterious mutations in our study was G172C with 46 frequencies of mutations. This effect mutation in SGD motif is unknown until now. The other mutations in SGD motif in Domain VI, S171L mutations show the highest frequency mutations with neutral mutations based on our data. More offer the G172V mutations in Domain VI in extracellular domain, could stabilize β-barrel to decrease the local flexibility.[24]

Domains V and VI are not conserved in ORF3a. These motifs are specific adaptations of the ORF3a family and they do not play a role in the structural integrity of the fold.[5] Another study showed that C terminus Domain III-VI were necessary for blocking autophagy. Using constructed truncated several proteins, in N-terminal, transmembrane, and C-terminal regions, they determine the function of autophagy inhibition. The N-terminal regions or Domain I and II did not show the function in blocking autophagy and had no influence in ORF3a localization.[25]

ORF3a-like viroporins contain two types of domains. A transmembrane domain (TM) in the position 33-141 and a cytosolic domain (CD) in position 145-237. We found 915 samples were truncated in the C-termini amino acid 220-275 C-termini (FIGURE 1).[26,27] Based on the lineage of truncated samples, we found the most lineage of SARS-CoV-2 was AY.23 (60%), but it does not belong to Variants of Concerns (FIGURE 2). Lineage B.1.1.7 that was found in one of 915 (0.11%) samples belonged to Alpha variants that designated previous Variants of Concern by WHO. Lineage B.1.466.2 (9.29%) was former Variants of Concerns, but had been reclassified by WHO because the variants are no longer circulating and impact significantly in public health.[13]

The wild type ORF3a protein is predicted to have six B cell epitopes which are located in six locations. This truncated position resulted in the loss of B cell epitope corresponding to 219-225, 237-243, 251-256 and 261-273 amino acid residues.[10] One study showed that the effect of mutation in ORF3a had a destabilizing effect for the protein.[18] The impact of the truncated proteins toward pathogenicity of virus needs further study. Other limitation in this study is we only used one software to predict the mutated proteins. Furthermore, the study needs to compare the prediction with other various tools to see the impact and functionality of the mutated proteins.

CONCLUSION

This study indicates 312 non-synonymous mutations in functional domains of ORF3a protein and showing their probable effects on protein. We also found 915 from 5,131 samples in Indonesia that truncated in the C-terminal of ORF3a protein. The data obtained here need validation to better understand the implications of these mutations on the function of ORF3a SARS-CoV-2 protein.

ACKNOWLEDGEMENT

We would like to acknowledge the infrastructural and financial support provided by Calvin Institute of Technology, Jakarta for this study.

REFERENCES

1. Hassan SS, Attrish D, Ghosh S, Choudhury PP, Roy B. Pathogenic perspective of missense mutations of ORF3a protein of SARS-CoV-2. Virus Res 2021; 300:198441.

https://doi.org/10.1016/j.virusres.2021.198441
2. Suryawanshi RK, Koganti R, Agelidis A, Patil CD, Shukla D. Dysregulation of cell signaling by SARS-CoV-2. Trends Microbiol 2021; 29(3):224-37. https://doi.org/10.1016/j.tim.2020.12.007
3. Issa E, Merhi G, Panossian B, Salloum T, Tokajian S. SARS-CoV-2 and ORF3a: nonsynonymous mutations, functional domains, and viral pathogenesis. mSystems 2020; 5(3):e00266-20. https://doi.org/10.1128/mSystems.00266-20
4. Zhang J, Ejikemeuwa A, Gerzanich V, Nasr M, Tang Q, Simard JM, *et al*. Understanding the role of SARS-CoV-2 ORF3a in viral pathogenesis and COVID-19. Front Microbiol 2022; 13:854567. https://doi.org/10.3389/fmicb.2022.854567
5. Ouzounis CA. A recent origin of Orf3a from M protein across the coronavirus lineage arising by sharp divergence. Comput Struct Biotechnol J 2020; 18:4093-102. https://doi.org/10.1016/j.csbj.2020.11.047
6. Tan Y, Schneider T, Shukla PK, Chandrasekharan MB, Aravind L, Zhang D. Unification and extensive diversification of M/Orf3-related ion channel proteins in coronaviruses and other nidoviruses. Virus Evol 2021; 7(1):veab014. https://doi.org/10.1093/ve/veab014
7. Chen IY, Moriyama M, Chang MF, Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front Microbiol 2019; 10:50. https://doi.org/10.3389/fmicb.2019.00050
8. Rodriguez CC, Honrubia JM, Gutiérrez-álvarez J, Dediego ML, Nieto-torres JL, Jimenez-guardeño JM, *et al*. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a, and 8a in replication and pathogenesis. mBio 2018; 9(3):e02325-17. https://doi.org/10.1128/mBio.02325-17
9. Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, *et al*. Structural insights into SARS-CoV-2 proteins. J Mol Biol 2021; 433(2):166725. https://doi.org/10.1016/j.jmb.2020.11.024
10. Majumdar P, Niyogi S. ORF3a mutation associated higher mortality rate in SARS-CoV-2 infection. Epidemiol Infect 2020; 148:e262. https://doi.org/10.1017/S0950268820002599
11. Zhang J, Li Q, Cruz Cosme RS, Gerzanich V, Tang Q, Simard JM, *et al*. Genome-wide characterization of SARS-CoV-2 cytopathogenic proteins in the search of antiviral targets. mBio 2022; 13(1):e016922. https://doi.org/10.1128/mbio.00169-22
12. Azad GK, Khan PK. Variations in Orf3a protein of SARS-CoV-2 alter its structure and function. Biochem Biophys Reports 2021; 26:100933. https://doi.org/10.1016/j.bbrep.2021.100933
13. WHO. Tracking SARS-CoV-2 variants [Internet]. 2022. [cited 2022 June 22] Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants
14. Yuliawuri H, Christian JE, Steven N. Non-synonymous mutation analysis of SARS-CoV-2 ORF3a in Indonesia. Mol Cell Biomed Sci 2022; 6(1):20. https://doi.org/10.21705/mcbs.v6i1.221
15. Sobhy H. The potential functions of protein domains during COVID infection: an analysis and a review. Covid 2021; 1(1):384-93. https://doi.org/10.3390/covid1010032
16. GISAID. GISAID Database [Internet]. 2021 [cited 2021 Dec 1]. Available

from: https://www.gisaid.org/
17. J. Craig Venter Institute. PROVEAN [Internet]. 2021 [cited 2021 Dec 1]. https://provean.jcvi.org/index.php
18. Fibriani A, Stephanie R, Alfiantie AA, Siregar ALF, Pradani GAP, Yamahoki N, *et al*. Analysis of sars-cov-2 genomes from West Java, Indonesia. Viruses 2021; 13(10):2097. https://doi.org/10.3390/v13102097
19. Siu KL, Yuen KS, Castano-Rodriguez C, Ye ZW, Yeung ML, Fung SY, *et al*. Severe acute respiratory syndrome Coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. FASEB J 2019; 33(8):8865-77. https://doi.org/10.1096/fj.201802418R
20. Wang R, Yang X, Chang M, Xue Z, Wang W, Bai L, *et al*. ORF3a protein of severe acute respiratory syndrome coronavirus 2 inhibits interferon-activated janus kinase/signal transducer and activator of transcription signaling *via* elevating suppressor of cytokine signaling 1. Front Microbiol 2021; 12:752597. https://doi.org/10.3389/fmicb.2021.752597
21. McClenaghan C, Hanson A, Lee SJ, Nichols CG. Coronavirus proteins as ion channels: current and potential research. Front Immunol 2020; 11:573339. https://doi.org/10.3389/fimmu.2020.573339
22. Kern DM, Sorum B, Mali SS, Hoel CM, Sridharan S, Remis JP, *et al*. Cryo-EM structure of the SARS-CoV-2 3a in lipid nanodiscs. 2021; 28(7):573-82. https://doi.org/10.1038/s41594-021-00619-0
23. Hassan SS, Basu P, Redwan EM, Lundstrom K, Choudhury PP, Aroca AS, *et al*. Periodically aperiodic pattern of SARS-CoV-2 mutations underpins the uncertainty of its origin and evolution. Environ Res 2021; 204(Pt B):112092. https://doi.org/10.1016/j.envres.2021.112092
24. Bianchi M, Borsetti A, Ciccozzi M, Pascarella S. SARS-Cov-2 ORF3a: mutability and function. Int J Biol Macromol 2020; 170:820-6. https://doi.org/10.1016/j.ijbiomac.2020.12.142
25. Zhang Y, Sun H, Pei R, Mao B, Zhao Z, Li H, *et al*. The SARS-CoV-2 protein ORF3a inhibits fusion of autophagosomes with lysosomes. Cell Discov 2021; 7(1):31. https://doi.org/10.1038/s41421-021-00268-z
26. Prosite. Coronavirus (CoV) 3a-like viroporin transmembrane (TM) and cytosolic (CD) domains profiles. https://prosite.expasy.org/PDOC51966
27. Rice AP, Kimata JT. SARS-CoV-2 likely targets cellular PDZ proteins: a common tactic of pathogenic viruses. Future Virol 2021; 10.2217/fvl-2020-0365. https://doi.org/10.2217/fvl-2020-0365

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 11-19
https://doi.org/10.19106/JMedSci005501202302

Effects of combination of alcohol and *Cinnamomum burmannii* essential oil against *Klebsiella pneumoniae* resistance

Jennifer Anggraini Sasangka[1]*, Handi Suyono[2], Gladdy Lysias Waworuntu[3]

[1]Undergraduate Program of Medical Doctor, the Faculty of Medicine, Widya Mandala Surabaya Catholic University, Surabaya, [2]Department of Physiology and Biochemistry, Faculty of Medicine, Widya Mandala Surabaya Catholic University, Surabaya, [3]Department of Microbiology and Parasitology, Faculty of Medicine, Widya Mandala Surabaya Catholic University, Surabaya, East Java, Indonesia

Submitted: 2022-01-10
Accepted : 2022-07-27

ABSTRACT

Alcohol-based antiseptics are widely used in the COVID-19 pandemic to prevent the transmission of infections, including bacterial infections. However, bacterial resistance to the alcohol-based antiseptics is begun reported. *Klebsiella pneumonia* resistance is one of the bacterial resistances that is prioritized by the WHO to be overcome. *Cinnamomum burmannii* essential oil, containing cinnamaldehyde and eugenol, was investigated for antimicrobial activity. This study aimed to evaluate the synergistic effect of the combination of alcohol and *C. burmannii* essential oil in inhibiting bacterial growth. Ethanol 80% in a combination with *C. burmannii* essential oil at concentrations of 1, 2, and 3% v/v were evaluated against *K. pneumoniae* using the Kirby-Bauer disc diffusion method. Test was repeated three times in independent experimental. Inhibition zone diameter (IZD, mm) and antimicrobial index (AI, %) were determined and analyzed using Kruskal-Wallis test continued the Mann-Whitney test. The combination of ethanol and *C. burmannii* essential oil was sensitive to *K. pneumoniae*, meanwhile, ethanol 80% was not more sensitive. The IZD of the combination solution at 1, 2, and 3% concentration were 6.7±0.19, 9.0±0.58, and 11.0±1.15mm, respectively ($p<0.05$). The AI of the combination solution at concentrations of 1, 2, and 3% v/v were 7.04±2.04, 30.53±6.79, and 51.64±12.91%, respectively ($p<0.05$). In conclusion, the combination of ethanol 80% and *C. burmannii* essential oil active against *K. pneumoniae* which resistant to the ethanol.

ABSTRAK

Antiseptik berbasis alkohol digunakan secara luas selama pandemic COVID-19 untuk pencegahan penyebaran infeksi, termasuk infeksi bakteri. Namun, terjadinya resistensi terhadap antiseptik berbasis alkohol tersebut mulai dilaporkan. Resistensi terhadap *Klebsiella pneumonia* merupakan salah satu resistensi bakteri yang diprioritaskan dicegah oleh WHO. Minyak atsiri *Cinnamomum burmannii,* yang mengandung sinamaldehida dan eugenol, telah diteliti aktivitas antimikrobanya. Penelitian ini bertujuan mengkaji efek sinergis kombinasi alkohol dan minyak atsiri dalam menghambat pertumbuhan bakteri. Kombinasi etanol 80% dan minyak atsiri *C. bumannii* pada konsentrasi 1, 2 dan 3% v/v dikaji aktivitasnya terhadap *K. pneumonia* menggunakan metode difusi cakram Kirby-Bauer. Uji diulangi tiga kali secara independent. Diameter zona hambatan (mm) dan indeks antimikroba (%) dihitung dan dianalisis dengan uji Kruskal-Wallis dilanjutkan dengan uji Mann-Whitney. Kombinasi etanol dan minyak atsiri *C. burmannii* sensitif terhadap *K. pneumoniae*, sedangkan etanol 80% tidak sensitif lagi. Diameter zona hambatan larutan kombinasi tersebut pada konsentrasi 1, 2, dan 3% berturut-turut adalah 6,7±0,19, 9,0±0,58, and 11,0±1,15mm ($p<0,05$). Indeks antimikroba larutan kombinasi tersebut pada konsentrasi 1, 2, dan 3% berturut turut adalah 7,04±2,04, 30,53±6,79, dan 51,64±12,91% ($p<0,05$). Dapat disimpulkan, kombinasi etanol 80% dan minyak atsiri *C. burmannii* aktif terhadap *K. pneumoniae* yang resisten terhadap etanol.

Keywords:
alcohol;
Cinnamomum burmannii;
Klebsiella pneumonia;
resistance;
antiseptics

*corresponding author: jennifer.anggraini@gmail.com

INTRODUCTION

Nosocomial infection is the most common adverse event during hospitalization that affects patient safety. It contributes to significant morbidity, mortality, and financial burden on patients and healthcare system.[1] *Klebsiella pneumoniae* is one of the Gram-negative bacteria that causes nosocomial infection. Nosocomial *K. pneumoniae* infection affect 46.6% of all hospitalized patients during their stay in ICU at Dr. Cipto Mangunkusumo General Hospital, Jakarta.[2] Nosocomial *K. pneumoniae* bloodstream infection was also associated with 47% of the mortality rate in Istanbul, Turkey.[3] *Klebsiella pneumoniae* is an opportunistic bacterium that often causes pneumonia due to the use of ventilators (ventilator-acquired pneumoniae) in hospitals.[4] During the COVID-19 pandemic, the use of a ventilator significantly increases to help breathing of patients lead to increase of *K. pneumoniae* infection risk.

Antimicrobial resistance due to misuse and overuse of antibiotics is a global health and development threat. More than 2.8 million microbial resistant infections were reported in the Unites States annually resulting more than 35,000 patients death.[5,6] World Health Organization (WHO) lists *K. pneumoniae* as one of the pathogens of high priority and promotes the research and development of new antibiotics due to the growing global problem of antimicrobial resistance.[7] Recently, *K. pneumoniae* is showing a high resistance to a broad spectrum of antibiotics including β-lactams antibiotics, fluoroquinolones and aminoglycosides.[8,9]

One way to control antibiotic resistance is by using antiseptics and disinfectants. They play an important role in the control of infection practices and in the avoidance of nosocomial infections.[9] Alcohol-based antiseptics are widely used in sterilization of medical devices and surgical instruments. However, massive use of the antiseptics might lead to the development of bacteria resistance that eventually causes they become ineffective.[10] Nosocomial bacteria, including *methicillin-resistant Staphylococcus aureus* (MRSA), *Acinetobacter baumannii, Escherichia coli, Klebsiella spp.,* and *Pseudomonas aeruginosa* have become resistant to the antiseptics in many health care centers.[10-12] The CDC recommends alcohol to be used as an antiseptic or disinfectant at a concentration of 70%.[13] An higher concentration, alcohol evaporate more quickly, even before it penetrates the microbial cell membrane and irritates the skin. Alcohol-based antiseptics resistance can be slowed by combination with another kind antiseptics. Some essential oils have been proven to have antibacterial activity and might be used in combination with alcohol as antiseptics.[14]

Essential oil is an oil derived from plant extracted from leaves, flowers, stems, bark, berries, roots, and other parts of plants.[14] The main constituents of cinnamon are cinnamaldehyde, trans-cinnamaldehyde, o-methoxy-cinnamaldehyde, cinnamyl acetate, benzaldehyde, phenylethanol, borneol, eucalyptol, eugenol, coumarin, and cinnamic acid. *Cinnamomum burmannii* essential oil was reported to have an antimicrobial effect. Cinnamaldehyde and eugenol in the essential oil of *C. burmannii* were proven active against *S. aureus, E. coli, A. baumannii,* and *P. aeruginosa*.[15,16] Essential oil from *C. burmannii* showed a better bacterial growth inhibition rate on respiratory tract pathogens than other types of essential oil.[17,18] This essential oil could be used in combination with another antiseptic and expected can slow the bacterial resistance to antiseptics. This study aimed to investigate the antibacterial effect of alcohol in combination with *C. burmannii* essential oil against *K. pneumoniae*.

MATERIALS AND METHODS

Bacterial strain

The study was performed against Gram-negative *K. pneumoniae* bacterium. Standardized *K. pneumoniae* ATCC – BAA 1706 was used in this study. The tested bacteria were cultured in the Clinical Microbiology Laboratory, Central Health Laboratory, Ministry of Health of Republic of Indonesia, Surabaya, East Java, Indonesia. This study was conducted from June to August 2021.

Essential oil preparation

Cinnamomum burmannii was purchased from a company, Purwakarta, Central Java, Indonesia. The essential oil was prepared by extraction the bark of the plant using the steam distillation method. The essential oil contents cinnamaldehyde at concentration of 67%.

Preparation of solution combination of ethanol 80% and *C. burmannii* essential oil

Five tested solutions were prepared against *K. pneumoniae* bacterium. They consisted of gentamycin 10 µg as antibiotic control group (C1), ethanol 80% control group (C2), and treatment group consisting ethanol 80% in combination with *C. burmannii* essential oil 1 (T1), 2 (T2), and 3% (T3). The ethanol 80% was prepared by diluting ethanol 96% with aquadest and glycerin 8% solution. Whereas the *C. burmannii* essential oils were prepared by diluting isolated essential oils with aquadest and glycerin 8% to obtained final concentration of 1, 2 and 3%.

Preparation of bacterial suspension

A standard McFarland suspension was prepared by mixing 0.5 mL of $BaCl_2$ with 99.5 mL of H_2SO_4. The bacterial suspension was prepared by mixing several bacterial colonies from cultured *K. pneumoniae* ATCC – BAA 1706 on Mac Conkey agar into a 0.9% NaCl solution. The bacterial turbidity is expected to be equal to the turbidity of the standard 0.5 McFarland suspension containing 1.5 x 10^8 CFU/mL.[19]

Preparation of the bacterial culture media

For the antibacterial susceptibility testing, the MHA (Muller–Hinton Agar) was used as bacterial culture media. The culture media were prepared in 1 L distilled water by dissolving 9.5 g of MHA. The obtained amber color solution was mixed thoroughly and boiled with frequent agitation to dissolve agar powder completely and a clear to slightly opalescent gel was obtained. The culture media were then sterilized by heating in an autoclave under pressure of 15 psi at 121 ^oC for 15 min. The sterilized culture media were then allowed to cool at room temperature in laminar flow hood.

Antibacterial susceptibility testing

The Kirby Bauer disc diffusion method was used for antibacterial susceptibility testing of different combination of ethanol 80% and *C. burmannii* essential oil. Twenty five mL of the cool sterilized culture media were poured into each Petri plate and were leaved for few minutes to allow the culture media to solidify. After solidification, the bacterial suspension were spread on the culture media by using cotton swab and cover the whole media with turn 90° degree rotation without leaving any gap. Five bores in diameter of 6 mm were made using a sterile cork borer in each Petri plate separated from each other by 2.5 cm distance. Thirty µL of each tested combination and control was poured in

the first three bores (T1-T3), gentamycin in the second last bore (C1), ethanol 80% in the third last bore (C2), and solvent in the last bore. All Petri plates were incubated in an incubator at 35 °C for 16-18 hr. Inhibition zone diameter (IZD) observed in the following day was measured to interpret antimicrobial susceptibility. This study has been approved by the Health Research Ethic Committee, Faculty of Medicine, Widya Mandala Surabaya Catholic University.

Data analysis

The inhibition zone diameter for each tested combination and control measured were presented as mean ± standard error of the mean (SEM). The tested combination or control is considered sensitive if the inhibition zone diameter > 6 mm, and considered resistant if the inhibition zone diameter ≤ 6 mm.[19-21] Furthermore, the antimicrobial index (AI, %) was calculated based on the following formula: $(1\text{-}Da/Dk) \times 100$ where Da is inhibition zone diameter in the experimental disc (cm) and Dk is inhibition zone diameter in the control disc (cm). The Kruskal-Wallis test continued the Mann-Whitney test were used to compare IZD and AI of each treatment and control. A p value < 0.05 was considered significant.

RESULTS

Antibacterial activity of various solutions tested against *K. pneumoniae* are presented in FIGURE 1 and TABLE 1 summarized the IZD of all solutions tested against *K. pneumoniae.*

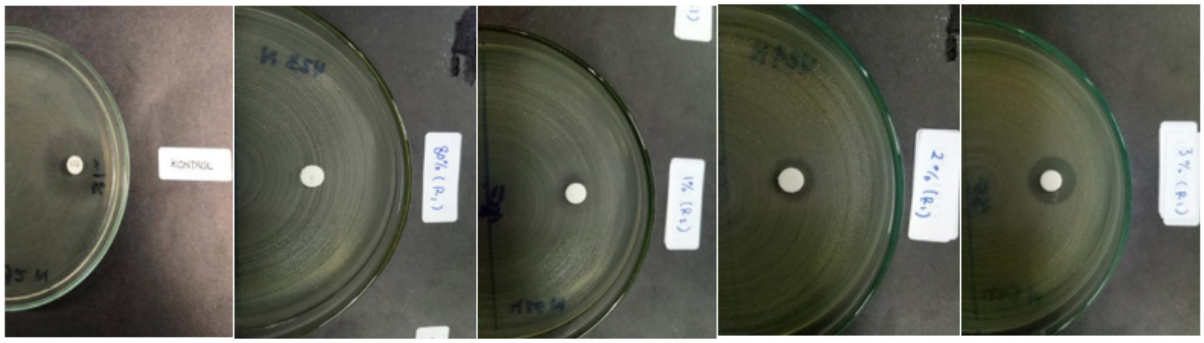


FIGURE 1. Antibacterial activity of various solutions tested against *K. pneumoniae.* C1:gentamycin; C2: ethanol; T1: ethanol + 1% essential oil; T2: ethanol + 2% essential oil; T3: ethanol + 3% essential oil.

TABLE 1. Results of disk diffusion test and AI

Groups	n	IZD (mean ± SEM mm)	Interpretation	AI (%)
C1	3	17.0 ± 0.88	Sensitive	100.0 ± 0.0
C2	3	< 6 ± 0	Resistant	0.0 ± 0.0
T1	3	6.7 ± 0.19	Sensitive	7.04 ± 2.04
T2	3	9.0 ± 0.58	Sensitive	30.53 ± 6.79
T3	3	11.0 ± 1.15	Sensitive	51.64 ± 12.91

n: replications; IZD: inhibition zone diameter; AI: antimicrobial index (%); C1:gentamycin; C2: ethanol; T1: ethanol + 1% ssential oil; T2: ethanol + 2% essential oil; T3: ethanol + 3% essential oil

Gentamycin 10 µg (C1) as antibiotic control and ethanol 80% (C2) as control had an IZD average of 17.0 ± 0.88 mm and < 6 ± 0.0 mm, respectively. Therefore, ethanol 80% was considered as had no antibacterial activity against *K. pneumoniae*. Furthermore, the ethanol 80% in combination with *C. burmannii* at concentrations of 1 (T1), (T2), and 3% (T3) had IZD average of 6.7 ± 0.19 mm, 9.0 ± 0.58 mm, and 11.0 ± 1.15 mm, respectively. Significantly different was observed between groups of this study ($p<0.05$). With a zone diameter < 0.6 mm, the ethanol 80% (C2) could not inhibit the *K. pneumoniae* growth. Therefore, it was considered that the *K. pneumoniae* is resistant to ethanol 80%. The combination of ethanol and *C. burmannii* essential oil could increase its sensitivity to *K. pneumonia* as indicated by the increase of the IZD. Furthermore, concentration-dependent in antibacterial activity of the combination of ethanol and *C. burmannii* essential oil was also observed. TABLE 1 also presented the AI of all solutions tested against *K. pneumoniae*. Gentamycin 10 µg (C1) as antibiotic control had an AI of 100%, whereas ethanol 80% did not have AI (0%). Furthermore, the ethanol 80% in combination with *C. burmannii* at concentrations of 1 (T1), (T2), and 3% (T3) had AI average of 7.04 ± 2.04%, 30.53 ± 6.79%, and 51.64 ± 12.91%, respectively. Significantly different was observed between groups of this study ($p<0.05$).

DISCUSSION

Alcohol-based antiseptic is widely used due to it is easy to find and does not require water for rinsing. It is designed as a hand antiseptic available in some formulations either in liquid, gel, or foam preparations to inactive microorganisms or temporarily inhibit their growth.[22] Antimicrobial activity of alcohol is well understood through its ability to denature and coagulate protein of microorganism.[23] Alcohol-based antiseptics have been used for cleaning routines in hospital such as hand rubs, positioned in and around hospital wards. However, due to its routine and massive use a number of bacteria species are already resistant to alcohol such as *S. aureus, A. baumannii, E. coli, Klebsiella spp.,* and *P. aeruginosa.*[9,10,24-26] In order to slow or stop bacterial resistance, new antiseptics or alcohol-based antiseptic combinations should be applied.

In this study, a combination of ethanol 80% with *C. burmannii* essential oil was evaluated against *K. penumoniae*. This combination can inhibit the *K. pneumoniae* growth which resistant to ethanol 80%. The IZD and AI of the combination significantly increased compared to that ethanol 80% alone indicating a synergic effect of both of them (TABLE 1).

The antibacterial activity of *C. burmannii* and other *Cinnamomun* sp. were reported by some authors. The *C. burmannii* essential oil had a high antifungal and antimicrobial activities against *A. flavus* and *K. pneumoniae*.[26] The methanol extract of *C. zeylanicum* was reported active against multidrug resistant (MDR) Gram-negative bacteria over expressing active efflux pumps including *K. pneumoniae* ATCC.[27] Another study reported that essential oils from *C. verum* and *C. camphora* actives against *A. flavus* and *K. pneumoniae* isolated from respiratory tract.[28] Zhang *et al.*,[21] have proven the antibacterial activity of cinnamon essential oils against *E.coli* and *S. aureus*, whereas Elcocks *et al.*[29] reported the antibacterial activity of cinnamon essential oils against *P. aeruginosa.* The active constituents of cinnamon essential oils as antibacterial dan antifungal have been also identified and isolated. The major constituents are found to be cinnamaldehyde (65-80%), cinnamyl acetate (2.5-16%), cinnamyl alcohol (2.25-4.6%), cinnamic acid (3-8%). Other abundant constituents are compounds containing endocyclic double bond

as α-thujene, α-terpineol, α-cubebene, unconjugated exocyclicdouble bond eugenol, β-caryophyllene, terpinolene and hydroxyl-substituted aliphatic compounds.[30-31]

The mechanism of actions as antibacterial both ethanol and cinnamon essential oil have been investigated. The antibacterial activity of ethanol is due to the ability to lyse cell membranes, denature and coagulate proteins from microorganisms.[23,32] Whereas, cinnamon essential oil acts by inhibit the ATPase lead to bacterial membranes damages as showed by irregular, invaginated, and abnormality of the bacteria membranes structure.[26,33-35] The combination of ethanol and cinnamon essential oil may result a synergistic effect as illustrated in FIGURE 2. This combination cause bacterial cell membrane disruption that lead to ethanol and active constituents facilitate enter into the bacterial cells and interact with ATPase. This interactions inhibit DNA synthesis and protein denaturation lead to bacterial metabolic disruption.

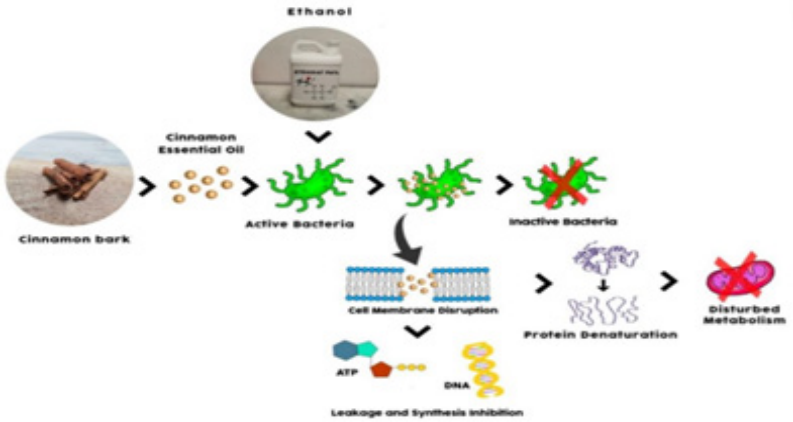


FIGURE 2. Illustration of mechanism of actions of the antibacterial activity of the combination of ethanol and cinnamon essential oils.

CONCLUSION

The combination of ethanol and *C. burmannii* essential oil has an antibacterial activity against *K. pneumonia* which resistant to the ethanol.

ACKNOWLEDGMENT

Authors would like to express massive gratitude to the Faculty of Medicine, Widya Mandala Surabaya Catholic University, for supporting this research and the organizer of InaSHG 2021 for allowing us to present our research at Ina-SGH conference.

REFERENCES

1. Sikora A and Zahra F. Nosocomial infections. [Updated 2022 Sep 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
2. Saharman YR, Karuniawati A, Sedono R, Aditianingsih D, Goessens WHF, Klaassen CHW, *et al*. Clinical impact of endemic NDM-producing *Klebsiella pneumoniae* in intensive care units of the national referral hospital in Jakarta, Indonesia. Antimicrob Resist Infect Control 2020; 9(1):61. https://doi.org/10.1186/s13756-020-

00716-7
3. Durdu B, Hakyemez IN, Bolukcu S, Okay G, Gultepe B, Aslan T. Mortality markers in nosocomial *Klebsiella pneumoniae* bloodstream infection. Springerplus 2016; 5(1):1892. https://doi.org/10.1186/s40064-016-3580-8
4. Hawley L, Ziegler RJ, Clarke Bl. Microbiology & immunology, 6th eds. Philadelphia: Lippincott Williams & Wilkins, 2014.
5. CDC. Antibiotic resistance threats in the United States. Centers Dis Control Prev. 2019; 65-77.
6. Eichenberger EM, Thaden JT. Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics 2019; 8(2):37. https://doi.org/10.3390/antibiotics8020037
7. World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO Press 2017: 1–7.
8. Ferreira RL, da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, *et al*. High prevalence of multidrug-resistant *Klebsiella pneumoniae* harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. Front Microbiol 2019; 9:3198. https://doi.org/10.3389/fmicb.2018.03198
9. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, *et al*. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg Infect Control 2017; 12:doc05. https://doi.org/10.3205/dgkh000290
10. Al-Talib H, Alkhateeb A, Ruzuki ASA, Zulkifli NF, Hamizi S, Muhamad NS, *et al*. Effectiveness of commonly used antiseptics on bacteria causing nosocomial infections in tertiary hospital in Malaysia. Afr J Microbiol Res 2019; 13(10):188-94. https://doi.org/10.5897/AJMR 2019.9058
11. Matthew EW, Lucy JB, Laura CB, Sutton JM. Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *Klebsiella pneumoniae* clinical isolates to chlorhexidine. Antimicrob Agents Chemother 2017; 61(1):1-12.
12. Mbajiuka C, Onuoha S, Ugah U. Comparative studies of the efficacy of some disinfectants on human pathogens. Researcher 2015; 7(1):39-45.
13. Centers for Disease Control and Prevention (CDC). Chemical disinfectants disinfection: Sterilization guidelines Guidelines Library Infection Control CDC [Internet]. Guideline for Disinfection and Sterilization in Healthcare Facilities. 2008. Available from: https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html
14. Mohsin T, Sarfaraz H, Gohar MUF. Antimicrobial applications of differnet plant essential oils. Eur J Pharm Med Res 2017; 4(3):58.
15. Winska K, Mączka W, Łyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential oils as antimicrobial agents—myth or real alternative? Molecules 2019; 24(11):2130. https://doi.org/10.3390/molecules24112130
16. Vangalapati M, Sree Satya N, Surya Prakash DV, Avanigadda S. A review on pharmacological activities and clinical effects of Cinnamon species. Res J Pharm Biol Chem Sci 2012; 31(1):653-63.
17. Ács K, Balázs VL, Kocsis B, Bencsik T, Böszörményi A, Horváth G. Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. BMC Complement Altern Med 2018; 18(1):227. https://doi.org/10.1186/s12906-018-2291-9
18. Julianti E, Rajah KK, Fidrianny I.

Antibacterial activity of ethanolic extract of cinnamon bark, honey, and their combination effects against acne-causing bacteria. Sci Pharm 2017; 85(2):19. https://doi.org/10.3390/scipharm85020019
19. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Sociaty for Microbiology, 2012.
20. CSLI. Performance standards for antimicrobial susceptibility testing, 32nd ed. CSLI supplement M100. Wayne PA: Clinical and Laboratory Standard Institute, 2020.
21. Zhang Y, Liu X, Wang Y, Jiang P, Quek SY. Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Food Control 2016; 59:282-9. https://doi.org/10.1016/j.foodcont.2015.05.032
22. Gold NA, Mirza TM, Avva U. Alcohol sanitizer. In: StatPearls [Internet]. Treasue Island (FL): StatPearls Publishing, 2022.
23. Aboualizadeh E, Bumah VV, Masson-Meyers DS, Eells JT, Hirschmugl CJ, Enwemeka CS. Understanding the antimicrobial activity of selected disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA). PLoS ONe 2017; 12(10):e0186375. https://doi.org/10.1371/journal.pone.0186375
24. Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, *et al*. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. 2019;13(Suppl 11):20. https://doi.org/10.1186/s12919-019-0176-7
25. Sauerbrei A. Bactericidal and virucidal activity of ethanol and povidone-iodine. Microbiologyopen 2020; 9(9):e1097. https://doi.org/10.1002/mbo3.1097
26. Akthar MS, Degaga B, Azam T. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: a review. Issues Biol Sci Pharm Res 2014; 2(1):001-7.
27. Seukep JA, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, Noumdem JA, *et al*. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. Springerplus 2013; 2:236. https://doi.org/10.1186/2193-1801-2-363
28. El-Shouny W, Abd El-Zaher E, Shabana S, Mohammed O. Antimicrobial activity of different essential oils against *Aspergillus flavus* and *Klebsiella pneumoniae* isolated from respiratory tract. Egypt J Bot 2017; 57:161-72. https://doi.org/10.21608/EJBO.2017.758.1044
29. Elcocks ER, Spencer-Phillips PTN, Adukwu EC. Rapid bactericidal effect of cinnamon bark essential oil against *Pseudomonas aeruginosa.* J Appl Microbiol 2020; 128(4):1025-37. https://doi.org/10.1111/jam.14538
30. Fajara A, Ammara GA, Hamzaha M, Manurunga R, Abduh MY. Effect of tree age on the yield, productivity, and chemical composition of essential oil from *Cinnamomum burmannii.* Curr Res Biosci Biotechnol 2019; 1(1):17-22.
31. Farias APP, Monteiro OS, da Silva JKR, Figueiredo PLB, Rodrigues AAC, Monteiro IN, *et al.* Chemical composition and biological activities of two chemotype-oils from *Cinnamomum verum* J. Presl growing in North Brazil. J Food Sci Technol 2020; 57(9):3176-83. https://doi.org/10.1007/s13197-020-04288-7
32. EL-Farmawi D, Olama Z, Holail H. The Antibacterial effect of some natural bioactive materials against

Klebsiella pneumoniae and MRSA. Int J Curr Microbiol App Scie 2014; 3(3):576-88.

33. Cui HY, Zhou H, Lin L, Zhao CT, Zhang XJ, Xiao ZH, *et al*. Antibacterial activity and mechanism of cinnamon essential oil and its application in milk. J Anim Plant Sci 2016; 26:532-41.
34. Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: a review. Microb Pathog 2018; 120:198-203. https://doi.org/10.1016/j.micpath.2018.04.036
35. Yang SK, Yusoff K, Ajat M, Thomas W, Abushelaibi A, Akseer R, *et al*. Disruption of KPC-producing *Klebsiella pneumoniae* membrane via induction of oxidative stress by cinnamon bark (*Cinnamomum verum* J. Presl) essential oil. PLoS One 2019; 14(4):e0214326 https://doi.org/10.1371/journal.pone.0214326

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 20-25
https://doi.org/10.19106/JMedSci005501202303

Atrial septostomy to prevent pulmonary hypertension crisis in children with ventricular septal defect (VSD) and pulmonary hypertension (PH) underwent cardiac surgery: a case series

Haryo Aribowo[1]*, Yoga Arditya[2], Gita Christy Gabriela[3]

[1]Division of Cardiac, Thoracic, and Vascular Surgery, Department of Surgery, Faculty of Medicine, Public Health, and Nursing/Dr. Sardjito General Hospital, Yogyakarta, [2]Division of Cardiac, Thoracic, and Vascular Surgery, Department of Surgery, Faculty of Medicine, Public Health, and Nursing/Dr. Sardjito General Hospital, Yogyakarta, [3]Department of Surgery, Faculty of Medicine, Public Health, and Nursing/Dr. Sardjito General Hospital, Yogyakarta 55281, Indonesia

Submitted: 2022-05-12
Accepted : 2022-07-24

ABSTRACT

Severe pulmonary hypertension (PH) was reported in 22.4% of ventricular septal defect (VSD) and it was mainly seen on a large VSD. Atrial septostomy (AS) could improve the hemodynamic condition and long-term survival of PH patients. Here, three VSD and PH cases in children who underwent AS surgery as their early treatment, concomitant with PH-specific pharmacological treatment were reported. Patient's hemodynamic and general condition improved with no further complications during the follow-up period. Atrial septostomy was usually conducted after all PH-specific pharmacological interventions failed. However, a study found that the survival benefit of AS was significantly increased if it was conducted before PH-specific pharmacotherapies. Most of the patients in this case received immediate hemodynamic and functional improvement. In this case series, it was reported that the AS procedure could lower the pulmonary arterial pressure and be safely conducted without further complications or death >24 hr post-surgery. Considering the clinical benefit, safety procedure, and improved pulmonary arterial pressure, performing AS procedure concomitant with PH-specific pharmacotherapy as an early treatment for PH patients is recommended.

ABSTRAK

Sebanyak 22,4% pasien ventrikular septal defek (VSD), terutama VSD berukuran besar, dilaporkan mengalami hipertensi pulmonal (HP) berat. Atrial septostomi (AS) dapat memperbaiki kondisi hemodinamik dan kelangsungan hidup jangka panjang pasien HP. Tiga kasus HP pada VSD anak yang menjalani AS bersamaan dengan terapi farmakologi khusus HP sebagai terapi awal disampaikan dalam laporan kasus ini. Paska prosedur AS, terdapat perbaikan hemodinamik dan kondisi umum tanpa disertai komplikasi. Pada umumnya, prosedur AS dilakukan setelah pemberian seluruh farmakoterapi khusus HP gagal. Namun, sebuah penelitian menunjukkan bahwa tindakan AS dapat meningkatkan angka kelangsungan hidup secara nyata bila dilakukan sebelum farmakoterapi khusus HP. Hampir semua pasien pada kasus ini mengalami perbaikan hemodinamik dan fungsional secara cepat. Pada kasus serial ini, dilaporkan bahwa prosedur AS dapat menurunkan tekanan arteri pulmonal dan aman dilakukan tanpa komplikasi lebih lanjut ataupun kematian >24 jam paska operasi. Dengan mempertimbangkan keuntungan klinis, keamanan prosedur, dan perbaikan tekanan arteri pulmoner, dilakukannya prosedur AS bersamaan dengan farmakoterapi khusus HP sebagai terapi awal pasien HP direkomendasikan.

Keywords:
atrial septostomy;
ventricular septal defect;
pulmonary hypertension;
children;
pharmacotherapy

*corresponding author: haryoaribowo@ugm.ac.id

INTRODUCTION

Ventricular septal defect (VSD) is one of the most typical congenital malformations of the heart (40% of all cardiac anomalies).[1] A large VSD is related to severe pulmonary hypertension and increases the risk of developing pulmonary vascular disease. Severe pulmonary hypertension was reported in 22.4% of VSD and it is mainly seen on a large VSD.[2]

Pulmonary hypertension (PH) treatment has undergone many changes in the last 30 years. Before vasodilator therapy, the mean survival age for children was one year.[3] New PH-specific medicine has improved children's five-year survival rates by at least 80%.[4-6] Improving survival rates in children with PH showed the development of PH treatment from pharmacological aspects, human resources, and understanding of PH pathophysiology.[4] PH treatments improve lung microvascular obstruction with anticoagulants, oral vasodilator, intravenous (IV) prostacyclin, or lung transplant as the last choice.[7] Less than 25% of PH showed an improvement with long-term oral vasodilator therapy, whereas 75% of patients with more severe PH needed long-term IV prostacyclin and lung transplant to receive an optimal condition.[8,9] The application of IV prostacyclin and lung transplant worldwide is limited due to technical and cost difficulties. Therefore, research related to a PH alternative therapy is really needed.

The death of PH patients is dominantly caused by right ventricle heart failure (RVHF).[10] Therefore, many interventions have been developed to fix the right ventricle (RV) without a lung transplant; one was pre-tricuspid valve shunt or Atrial Septostomy (AS), an endovascular intervention to make an artificial shunt between the right and left atrial.[5,10] The rationales for its use are: (1) the aggravating impact of RVHF on patient survival; (2) the unpredictable response to medical treatment; (3) the difference in treatment availability worldwide and limited access to lung transplantation.[11,12] If AS is successfully done, it could decompress RV and left ventricle (LV) 's failing and result in a significant clinical improvement, long-lasting hemodynamic effects at rest, and improved survival rate in patients with PH. Many studies showed that AS was usually done after all PH-specific pharmacological interventions failed,[10,13,14] however, Sandoval *et al.*,[13] found that the survival benefit of AS was significantly increased if it was first performed then, followed by PH-specific drug therapies.

Here, three different cases of VSD and PH in children who underwent AS surgery as their early treatment, concomitant with PH-specific pharmacological treatment were presented. One girl presented in doubly committed sub arterial (DCSA) VSD and PH, anther girl was shown in a perimembranous outlet (PMO) VSD and PH, the last girl showed PMO VSD and patent foramen ovale (PFO).

CASES

Case 1

A 6-y.o. girl was referred to Dr. Sardjito General Hospital, Yogyakarta with a history of repeated cough and difficulty gaining weight. The physical examination showed no signs of right heart failure, and her room air oxygen saturation was good. Transthoracic echocardiography (TTE) examination showed a significant DCSA VSD (subpulmonic) and PH. Heart catheterization examination showed a significant DCSA VSD and PH with high-flow low resistance and reactive

O_2 test. Furosemide 2 x 0.5-1 mg/kgBW/time, captopril 2 x 0.3-0.5 mg/kgBW/time, and sildenafil 3 x 0.5-1 mg/kgBW/time were given before surgery. Open-heart surgery was performed to close 2 cm VSD and create a 3 mm ASD. Firstly, we closed the VSD and then made a hole in the middle of the atrial septum using a puncher with a 3 mm diameter. Post-surgical TTE evaluation showed a reduction of pulmonary arterial systolic pressure from 60mmHg to 14mmHg (TABLE 1). Then, she received inotropic therapy (5-10 mg/kgBW) and was being follow-up in the ICU for the first 48 h. The inotropic therapy was stopped the following three days and moved her to the inpatient ward. She was sent home eight days post-surgery and consumed captopril 2 x 0.3-0.5 mg/kgBW/time, furosemide 2 x 0.5-1 mg/kgBW/time, and sildenafil 3 x 0.5-1 mg/kgBW/time.

Case 2

A 2.5-y.o. girl with a history of repeated cough since nine months old and pneumonia treatment was referred to Dr. Sardjito General Hospital in stable hemodynamic, well-room air oxygen saturation, and no signs of heart failure. TTE examination showed 11 mm PMO VSD and PH. Heart catheterization exam exhibited big PMO VSD and PH with high-flow high resistance and reactive O_2 test. She received captopril 2 x 0.3-0.5 mg/kg BW/time, furosemide 2 x 0.5-1 mg/kg BW/time, sildenafil 3 x 0.5-1 mg/kg BW/time, and surgery. Open-heart surgery was conducted to close 2 cm VSD and create an ASD. Firstly, the VSD was closed and then made a hole in the middle of the atrial septum using a puncher with a 3 mm diameter. Post-surgical evaluation with TTE showed decreasing arterial pulmonary systolic pressure from 47 to 23mmHg (TABLE 1). She was in the ICU for three days post-surgery and received inotropic therapy (5-10 mg/kg BW) for two days. There was an excellent hemodynamic improvement following the surgery. Therefore, she was moved to the inpatient ward on the 4th day. Eight days post-surgery, she was sent home and continued oral therapy with captopril 2 x 0.3-0.5 mg/kgBB/time, furosemide 2 x 0.5-1 mg/kgBW/time, and sildenafil 3 x 0.5-1 mg/kgBW/time.

Case 3

An 8-y.o. girl with a malnutrition history and repeated cough since six years old was referred to Dr. Sardjito General Hospital. Her hemodynamic condition was stable, her room-air oxygen saturation was adequate, and there were no signs of heart failure on the physical examination. TTE showed 8-10mm PMO VSD, PFO, and PH. Heart catheterization examination showed persistent left superior vena cava, big PMO VSD, and PH with high-flow low resistance and reactive O_2 test. She received captopril, furosemide, sildenafil, and surgery. Open-heart surgery was done to close 1.8mm VSD, foramen ovale, and create an ASD. Firstly, the VSD was closed and foramen ovale; after that, we made a hole in the middle of the atrial septum using a puncher. Post-surgical examination using TTE showed decreasing pulmonary arterial systolic pressure from 63 to 25mmHg. She was being followed up in ICU for the first 48h post-surgery, then moved to inpatient care for four days. She received inotropic therapy (5-10 mg/kgBW) for the first three days post-surgery. There were no complications following the surgery, Therefore, she was sent home and continued captopril 2 x 0.3-0.5 mg/kgBW/time, furosemide 2 x 0.5-1 mg/kgBW/time, and sildenafil therapy 3 x 0.5-1 mg/kgBW/time.

TABLE 1. Results of supporting examinations and patient hemodynamics.

Variable	Case 1	Case 2	Case 3
Heart catheterization examination			
• PAP (mmHg)	76/43 (60)	68/27 (47)	77/49 (63)
• MV (%)	77.6	71.53	85.27
• FR	2.51	1.75	3.77
• PARI (WU)	3.13	4.7	1.7
• RA pressure (mmHg)	-	18/14 (13)	14/9 (11)
• RV pressure (mmHg)	-	73/12 (18)	79/13 (14)
• LV pressure (mmHg)	79/5 (18)	79/15 (26)	93/1 (18)
Echocardiography			
• Type of defect	DCSA VSD	PMO VSD 11mm	PMO VSD 8-10mm PFO 2.2mm
TTE post-surgery			
• Ejection fraction (%)	62	59	59
• TAPSE	8	11	9,5
• Efusi pericardium	(-)	(+) 6-9 mm	(+)
• Systolic PA pressure (mmHg)	14	23	25
• TVG (mmHg)	4	15	17
SaO_2 Pre-Op/Post Op (%)	99/96	98/99	97/96

PAP: pulmonary artery pressure; MV: mixed vein saturation; FR: flow ratio; PARI: pulmonary artery resistance index; RA: right atrium; RV: right ventricle; LV: left ventricle; TAPSE: tricuspid annular plane systolic excursion; TVG: tricuspid valve gradient; PA: pulmonary artery.

DISCUSSION

Atrial septostomy is a procedure to create an intracardiac shunt in children with various congenital cardiac defects.[14] It was first conducted by Rich and Lam 20 years ago on a patient with severe PH.[7] In 1964, an animal study by Austin *et al.*[15] showed that inter-atrial communication could decompress the dilated and hypertension right ventricle and augment systemic blood flow, especially during exercise. The rationale for AS procedure was also supported by the fact that PH patients with patent foramen ovale (PFO) and Eisenmenger's syndrome patients have higher survival rates than PH patients without intracardiac shunting.[8,12,16,17] In this case, AS creation during open-heart surgery following the closure of VSD was performed. Firstly, the VSD was closed, then made a hole in the middle of the atrial septum with a certain-sized puncher (3 to 5 mm in diameter). The hole was made in the middle of the atrial septum to ease the hole closure by an amplatzer device.

Previous study reported that the combination of AS and pharmacotherapy could lower the prevalence of WHO functional class IV patients compared to AS alone, although it was not significantly different.[13] In most-reported studies, AS was usually conducted after all PH-specific pharmacotherapy failed. Therefore, the impact of the procedural intervention on long-term survival is lessened.[10-15] However, Sandoval *et al.*[13] reported that the survival benefit of AS is significantly increased if it is performed first, followed by PH-specific pharmacotherapies. Most of his patients who received AS procedure produced immediate hemodynamic and functional improvement. In this case, AS was also performed as an early treatment combined with pharmacotherapy. The

patient's hemodynamic and general condition improved with no further complications during the follow-up period. The difference between this case and the previous study was in the timing of pharmacotherapy and the AS technique. In this case, the pharmacotherapy was given before and after the surgery and performed ASD creation while performing open-heart surgery to close VSD and PFO, while in the previous study, the pharmacotherapy was given only after the surgery and AS was conducted using balloon dilatation technique.[13] After the ASD creation, all patients did not show severe limitations (WHO functional class IV).

All of three patients showed a notable decrease in pulmonary arterial pressure (TABLE 1). It was estimated post-surgical pulmonary arterial pressure count with TTE because heart catheterization was an invasive procedure. Previous studies also showed a decrease in mean pulmonary arterial pressure, though the drop was insignificant.[14,18]

The risk of AS procedure is high. Therefore, it is contraindicated in patients with (1) severe RVHF on cardiorespiratory support, (2) mean right atrial pressure (mRAP) >20mmHg, (3) room-air resting O_2 saturation <90%, (4) left ventricular end-diastolic pressure (LVEDP) >18mmHg because the most common cause of death in this procedure was resistant hypoxia.[10] The higher the right-sided pressure, the more likely the shunt to cause uncontrollable hypoxemia.[10] Our patient's general and hemodynamic condition was stable without cardiorespiratory support, and their room-air resting O_2 saturation was also >90%. Therefore, the AS procedure on the patients was performed.

In this case series showed that the AS procedure could be conducted safely without further complication or death 24 h post-surgery. The patients showed decent improvement in their hemodynamic and general condition with no complications during the follow-up period. Atrial septostomy was conducted to anticipate PH crisis or manifest PH because PH crisis can interrupt blood flow to the lung, which can be fatal. Atrial septostomy creation could decrease pulmonary arterial blood flow, so the obstructed blood flow could stream from right atrial to left atrial. Due to the study design, the number of patients was limited. Therefore, further research is still needed to confirm these findings.

CONCLUSION

Atrial septostomy procedure could lower the pulmonary arterial pressure and be safely conducted without further complications or death >24h post-surgery. Considering the clinical benefit, safety procedure, and improved pulmonary arterial pressure, performing AS procedure concomitant with PH-specific pharmacotherapy as an early treatment for PH patients is recommended.

ACKNOWLEDGMENTS

The authors affirm that informed consent was obtained from the patient who had undergone medical treatment in our hospital. The datasets used during the study are available from the corresponding author on reasonable request. The authors declare that they have no competing interests and funding sources. We thank all of the hospital staff involved in the patient's care.

REFERENCE

1. Penny DJ, Vick GW 3rd. Ventricular septal defect. Lancet 2011; 377(9771):1103-12. https://doi.org/10.1016/S0140-6736(10)61339-6
2. Chaudhry TA, Younas M, Baig A. Ventricular septal defect and associated complications. J Pak Med Assoc 2011; 61(10):1001-4.
3. Barst RJ, Maislin G, Fishman AP. Vasodilator therapy for primary

pulmonary hypertension in children. Circulation 1999; 99(9):1197-208. https://doi.org/10.1161/01.cir.99.9.1197
4. Grady RM. Beyond transplant: roles of atrial septostomy and Potts shunt in pediatric pulmonary hypertension. Pediatr Pulmonol 2021; 56(3):656-60. https://doi.org/10.1002/ppul.25049
5. Haworth SG, Hislop AA. Treatment and survival in children with pulmonary arterial hypertension: the UK Pulmonary Hypertension Service for Children 2001-2006. Heart 2009; 95(4):312-7. https://doi.org/10.1136/hrt.2008.150086
6. Zijlstra WMH, Douwes JM, Rosenzweig EB, Schokker S, Krishnan U, Roofthooft MTR, *et al*. Survival differences in pediatric pulmonary arterial hypertension: clues to a better understanding of outcome and optimal treatment strategies. J Am Coll Cardiol 2014; 63(20):2159-69. https://doi.org/10.1016/j.jacc.2014.02.575
7. Sandoval J, Rothman A, Pulido T. Atrial septostomy for pulmonary hypertension. Clin Chest Med 2001; 22(3):547-60. https://doi.org/10.1016/s0272-5231(05)70291-4
8. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. N Engl J Med 1992; 327(2):76-81. https://doi.org/10.1056/NEJM199207093270203
9. Rubin LJ. Primary pulmonary hypertension. N Engl J Med 1997; 336(2):111-7. https://doi.org/10.1056/NEJM199701093360207
10. Kurzyna M, Dabrowski M, Bielecki D, Fijalkowska A, Pruszczyk P, Opolski G, *et al*. Atrial septostomy in treatment of end-stage right heart failure in patients with pulmonary hypertension. Chest 2007; 131(4):977-83. https://doi.org/10.1378/chest.06-1227
11. Maluli HA, DeStephan CM, Alvarez RJ, Sandoval J. Atrial septostomy: a contemporary review. Clin Cardiol 2015; 38(6):395-400. https://doi.org/10.1002/clc.22398
12. Rozkovec A, Montanes P, Oakley CM. Factors that influence the outcome of primary pulmonary hypertension. Br Heart J 1986; 55(5):449-58. https://doi.org/10.1136/hrt.55.5.449
13. Sandoval J, Gaspar J, Peña H, Santos LE, Cordova J, del Valle K, *et al*. Effect of atrial septostomy on the survival of patients with severe pulmonary arterial hypertension. Eur Respir J 2011; 38(6):1343-8. https://doi.org/10.1183/09031936.00072210
14. Reichenberger F, Pepke-Zaba J, McNeil K, Parameshwar J, Shapiro LM. Atrial septostomy in the treatment of severe pulmonary arterial hypertension. Thorax 2003; 58(9):797-800. https://doi.org/10.1136/thorax.58.9.797
15. Austen WG, Morrow AG, Berry WB. Experimental studies of the surgical treatment of primary pulmonary hypertension. J Thorac Cardiovasc Surg 1964; 48:448-55. https://doi.org/10.1016/S0022-5223(19)33542-1
16. Glanville AR, Burke CM, Theodore J, Robin ED. Primary pulmonary hypertension. Length of survival in patients referred for heart-lung transplantation. Chest 1987; 91(5):675-81. https://doi.org/10.1378/chest.91.5.675
17. Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. J Heart Lung Transplant 1996; 15(1 Pt 1):100-5.
18. Law MA, Grifka RG, Mullins CE, Nihill MR. Atrial septostomy improves in select patients with pulmonary hypertension. Am Heart J 2007; 153(5):779-84. https://doi.org/10.1016/j.ahj.2007.02.019

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 26-33
https://doi.org/10.19106/JMedSci005501202304

Comparison of cardiac marker profiles in dengue myocarditis

Galang Tri Atmaja[1,2], Sarah Buntubatu[3], Conroy Surya Wijaya[1], Annisa Nurul Pratiwi Sudarmadi[1], Ida Safitri Laksono[2], Ratni Indrawanti[2], Eggi Arguni[2]*

[1]Bachelor in Medicine Program, Faculty of Medicine, Public Health and Nursing, [2]Department of Child Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada /RSUP Dr. Sardjito, Yogyakarta, [3]Karubaga District Hospital, Papua Pegunungan Province, Indonesia

Submitted: 2022-06-04
Accepted : 2022-10-15

ABSTRACT

Dengue patients may experience some grade of severity. Cardiac involvement is common in severe dengue, therefore cardiac markers could be used to ensure the diagnosis of dengue myocarditis. However, information of the cardiac marker profiles in patients with milder severity of dengue infection is limited. The study aimed to evaluate creatinine kinase (CK), creatinine kinase-MB (CK-MB) and troponin I (TnI) in dengue myocarditis against the spectrum severity of dengue infection in children. This cross-sectional study was conducted using secondary data from medical records of dengue myocarditis patients aged 1-18 yr in Dr. Sardjito General Hospital, Yogyakarta. Fisher's Exact tests were performed to compare the increase in cardiac markers to the dengue severity. The increase of CK was observed in dengue fever/DF (6 or 75% of patients), dengue hemorrhagic fever/DHF (6 or 67%) and dengue shock syndrome/DSS (16 or 73%). Furthermore, the increase of CK-MB was also observed in DF (6 or 75%), DHF (8 or 87%), and DSS (21 or 95%). No significant difference in the increase of CK and CK-MB proportions was observed in DF compared to DHF groups and in DF compared to DSS ($p>0.05$). The increase of Tn I was observed in DHF (2 or 22%) and DSS (10 or 45%) groups but not observed in DF group. Significant difference in the increase of Tn I proportion was observed in DF compared to DSS groups ($p=0.022$). In conclusion, cardiac involvement is common in all dengue severity level. The increment of Tn I corresponds to an increase in the dengue severity level. Further research by observing cardiac markers sequentially is needed.

ABSTRAK

Pasien dengue kemungkinan mengalami berbagai spektrum derajat keparahan. Keterlibatan jantung umumnya terjadi pada dengue berat sehingga petanda jantung dapat digunakan untuk menegakkan diagnosis. Namun demikian, informasi profil petanda jantung pada dengue dengan keparahan lebih ringan sangat terbatas. Penelitian ini bertujuan mengkaji kreatinin kinase (CK), kreatinin kinamse-MB (CK-MB) dan troponin I (TnI) pada mikokarditis dengue pada anak dengan dengan derajad keparahan infeksi dengue. Penelitian dengan rancangan potong lintang ini menggunakan data rekam medis pasien usia 1-18 tahun dengan miokarditis di RSUP Dr. Sardjito, Yogyakarta. Uji Fisher Exact digunakan untuk menganalisis perbedaan kenaikan penanda jantung dengan tingkat keparahan infeksi dengue. Kenaikan kadar CK teramati pada *dengue fever*/DF (6 atau 75% pasien), *dengue hemorrhagic fever*/DHF (6 atau 67%), *dengue shock syndrome*/DSS (16 atau 73%). Selanjutnya, kenaikan CK-MB juga teramati pada DF (6 atau 75%), DHF (8 atau 87%) dan DSS (21 atau 95%). Tidak terdapat perbedaan dalam proporsi kenaikan CK dan CK-MB antara kelompok DF dibandingkan dengan DHF dan DF dengan DSS ($p>0.05$). Kenaikan Tn I teramati pada DHF (2 atau 22%) dan DSS (10 atau 45 %), tetapi tidak teramati pada kelompok DF. Perbedaan nyata dalam kenaikan proporsi Tn I teramati pada kelompok DF dibandingkan dengan DSS ($p<0.022$). Dapat disimpulkan, keterlibatan jantung umumnya terjadi pada semua tingkat keparahan dengue. Peningakatan Tn I berhubungan dengan peningkatan tingkat keparahan dengue. Penelitian lanjutan untuk memantau petanda jantung secara berkala diperlukan.

Keywords:
cardiac marker;
dengue;
myocarditis;
creatinine kinase;
troponin I

*corresponding author: eggiarguni@ugm.ac.id

INTRODUCTION

Dengue is a viral infection caused by the dengue virus (DENV) which is transmitted to humans by infected mosquito bites. Around 390 million cases of dengue infection are reported annually, with 96 million of them have clinical manifestations. America, South-East Asia, and Western Pacific regions are the most severely affected regions, with Asia accounting for 70% of the global disease burden.[1] In Indonesia in 2020, 108,303 patients with dengue hemorrhagic fever (DHF) were reported with 747 of them died.[2] Children are particularly vulnerable to dengue infection in Indonesia with the incidence of cases aged 0-14 years was 53.08% in 2019.[3]

Dengue patients may experience a spectrum of clinical conditions, ranging from asymptomatic, mild dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which can be fathal.[4] Myocarditis is the most common manifestation of dengue.[5] A study in Brazil reported that 12 (15%) of 81 dengue patients have elevated cardiac biomarkers (troponin I/Tn I and pro-B-type natriuretic peptide).[6] Another study in Sri Lanka reported that 75 (62.5%) of 120 dengue patients diagnosed by serological testing show varying electrocardiography (ECG) abnormalities including T inversion, ST depression, and bundle branch block.[7] In Indonesia, the study of dengue myocarditis especially on pediatric patients is limited. A prospective cohort study in Indonesia reported that myocarditis dengue is diagnosed in 39 out of 50 pediatric dengue patients.[8]

Myocarditis is defined as inflammation of the myocardium. The pathophysiology of cardiac involvement in dengue cases is not clearly understand. Myocardial involvement in dengue can occur due to direct viral invasion of the heart muscle, damage caused by cytokines due to immune reactions, or both. Elevated levels of tumor necrosis factor alpha (TNF-α), interleukin (IL) 6, 13, and 18, and cytotoxic factors in patients with dengue may lead to increased vascular permeability and shock. Dengue virus antigen has been shown to play a role in inflammatory cells in the heart, and dengue virus has been shown to cause damage to the heart experimentally. The virus or viral RNA was detected in various tissues, including the kidneys, heart, lungs, and brain in cases of dengue death. A new hypothesis suggests that impaired calcium (Ca^{2+}) storage in infected cells contribute directly to myocarditis.[8]

The wide range of clinical manifestations and the difficulty of diagnosing myocarditis make the incidence difficult to be quantified. Signs of myocarditis can range from a subclinical rise in biomarkers or the presence of ECG abnormalities in asymptomatic cases, to more overt manifestations such as shortness of breath, chest pain, and sudden death. Testing of the levels of serum cardiac markers [creatine kinase (CK), Tn I and T] are routinely performed in suspected cases of myocarditis.[9] Endomyocardial biopsy and cardiac magnetic resonance imaging (MRI) can improve the accuracy of the diagnosis of myocarditis. However, these procedures are either considered too invasive or not widely available in dengue endemic areas.[10]

The aim of this study was to compare levels of the cardiac markers i.e. CK, CK-MB and Tn I in dengue myocarditis

with the severity of dengue infection in children. Appropriate information of cardiac inflammation marker relating dengue infection severity would increase the awareness of possibility myocarditis earlier.

MATERIALS AND METHODS

Design and subject

This cross-sectional study was conducted using secondary data of patients' medical records with diagnosis of dengue myocarditis at Dr. Sardjito General Hospital, Yogyakarta, Indonesia in the period July 2015 - May 2016. All patients aged 1-18 yr diagnosed with dengue myocarditis were included in this study.

Protocol of study

Dengue infection was diagnosed according to the World Health Organization (WHO) 2011 guidelines with a positive antidengue IgM/IgG serology test or positive nonstructural protein 1 (NS-1) test. Myocarditis was defined as patients with an increase of one or more serum cardiac markers (CK, CK-MB, or Tn I) and/or had ECG abnormalities. Laboratory examination of cardiac markers and ECG recording were conducted in the period of day 4-6 of fever, in order to get the most possible differences in cardiac function between patients with or without plasma leakage.[11] Patients with a history of previous heart disease, either congenital or acquired, were excluded from the study. Assuming a prevalence of 15% for pediatric dengue with abnormality of cardiac marker,[5] the Type-I error of 5%, and also based on the 95% confidence interval, the minimum required sample size of 50 patient was calculated.

Patient characteristics were collected from medical records including age, severity, outcome, days of fever, presence of bleeding manifestations, plasma leakage, hepatomegaly, routine peripheral blood test result, ECG characteristics, and levels of CK, CK-MB, and Tn I.

Statistical analysis

Fisher's Exact tests were performed to compare the increase in cardiac markers in dengue myocarditis to the severity. A p value < 0.05 was considered significant.

RESULTS

A total of 39 patients with dengue myocarditis were involved in this study. Characteristics of patients are presented in TABLE 1. Among 39 patients with dengue myocarditis, 8 (21%) were categorized as DF, 9 (23%) as DHF, and 22 (56%) as DSS. Dengue myocarditis patients mostly came to the hospital on days 3-5 of fever. Plasma leakage occurred in 9 (23%) cases, and pleural effusion was the most common form of plasma leakage. All of the patients survived during the study period.

TABLE 1. Clinical and laboratory (routine peripheral blood test) profile of dengue myocarditis patients (n=39)

Variables	Total
Clinical profile	
Age [n=39; n (%)]	
• < 5 y	15 (38)
• ≥5 y	24 (62)
Degree of severity [n=39; n (%)]	
• DF	8 (21)
• DHF	9 (23)
• DSS	22 (56)
Outcome [n=39; n (%)]	
• Died	0 (0)
• Survived	39 (100)
Day of fever [n=39; n (%)]	
• <72 hr	8 (21)
• 3-5 d	22 (56)
• ≥ 6 d	9 (23)
Hemorrhagic sign [n=14; n (%)]	
• Petechiae	5 (13)
• GIT bleeding	7 (18)
• Epistaxis	2 (5)
• Gum bleeding	2 (5)
Plasma leakage [n=9; n (%)]	
• Pleural effusion	6 (15)
• Ascites	4 (15)
Hepatomegaly [n=39; n (%)]	
• Hepatomegaly	26 (67)
• No hepatomegaly	13 (33)
Laboratory profile	
Hemoglobin (mean ± SD g/dL)	13.43 ± 2.02
Hematocrit (mean ± SD %)	38.52 ± 5.69
Platelet [med (min-max) cell/μL]	31 (4-501)
Leucocytes [med (min-max) cell/L]	4.9 (1.28-34)
NS-1 positive [n (%)]	16 (41)
IgM anti dengue positive [n (%)]	25 (64)
IgM & IgG anti dengue positive [n (%)]	18 (46)

TABLE 2. Characteristics of ECG abnormalities of dengue patients according to the disease severity (n=22)

Description	Degree of severity			Total
	DF [n (%)]	DHF [n (%)]	DSS [n (%)]	n (%)
Sinus tachycardia	2 (18)	4 (36)	5 (46)	11 (50)
Sinus bradycardia	1 (25)	0 (0)	3 (75)	4 (18)
Low voltage	0 (0)	1 (20)	4 (80)	5 (23)
Sinus tachycardia and low voltage	0 (0)	0 (0)	2 (100)	2 (9)
Total	3 (14)	5 (23)	14 (63)	22 (100)

DF=dengue fever; DHF=dengue hemorrhagic fever; DSS= dengue shock syndrome.

TABLE 3. Characteristics of cardiac marker abnormalities of dengue patients according to ECG abnormalities

Cardiac marker	ECG abnormalities			
	Sinus tachycardia	Sinus bradycardia	Low voltage	Sinus tachycardia & low Voltage
CK [n (%)]				
• Normal	1 (9)	0 (0)	1 (20)	0 (0)
• Increase	10 (91)	4 (100)	4 (80)	2 (100)
CK-MB [n (%)]				
• Normal	2 (18)	0 (0)	0 (0)	0 (0)
• Increase	9 (82)	4 (100)	5 (100)	2 (100)
Tn I [n (%)]				
• Normal	5 (45)	2 (50)	1 (20)	0 (0)
• Increase	6 (55)	2 (50)	4 (80)	2 (100)

ECG=electrocardiogram; CK=creatine kinase; CK-MB=creatine kinase – myocardial band.

Comparison of cardiac marker abnormalities to the dengue severity is presented in TABLE 4. The increase of CK was reported in DF (6 or 75% of patients), DHF (6 or 67%) and DSS (16 or 73%). Moreover, the increase of CK-MB was also observed in DF (6 or 75%), DHF (8 or 87%), and DSS (21 or 95%). There was no significant difference in the increase of CK and CK-MB proportions in DF compared to DHF groups and in DF compared to DSS ($p>0.05$). The increase of Tn I was observed in DHF (2 or 22%) and DSS (10 or 45%) groups but not observed in DF group (0 or 0%). Significant difference in the increase of Tn I proportion was observed in DF compared to DSS groups ($p=0.022$).

TABLE 4. Comparison of cardiac marker abnormalities to the severity of dengue (n=39)

Cardiac marker	Degree of severity			p*
	DF	DHF	DSS	
CK [n (%)]				
• Normal	2 (25)	3 (33)	6 (27)	0.563[a]
• Increase	6 (75)	6 (67)	16 (73)	0.645[b]
CK-MB [n (%)]				
• Normal	2 (25)	1 (13)	1 (5)	0.453[a]
• Increase	6 (75)	8 (87)	21 (95)	0.166[b]
Tn I [n (%)]				
• Normal	8 (100)	7 (78)	12 (55)	0.265[a]
• Increase	0 (0)	2 (22)	10 (45)	0.022[b]

*Fisher's exact test; DF=dengue fever; DHF=dengue hemorrhagic fever; DSS= dengue shock syndrome; [a]DF vs DHF; [b]DF vs DSS.

DISCUSSION

Characteristics of patients with dengue myocarditis in children in the Dr. Sardjito General Hospital, Yogyakarta was reported (TABLE 1). The profile of ECG abnormalities according to severity showed that the most ECG abnormalities occurred in the DSS group. Decreases in intravascular volume and in preload will affect the coronary microcirculation, causing damage to the myocardium which can interfere with contractility and heart rhythm.[10] The results of this study are in line with research conducted by Hussain *et al,*.[12] regarding the ECG profile in dengue infection in children in Indonesia.

This current study showed that there was no effect of the proportion of increased levels of CK and CK-MB on the severity of dengue (DF vs. DHF and DF vs. DSS). The insignificant result could be due to the low positive predictive value (PPV) of CK and CK-MB in assessing myocardium injury. The PPV of CK value is 40.5%, while the CK-MB is 64.9%. Therefore, it was prone to have false positive results. The results in this study are similar to the findings of Li *et al.*,[13] where the increase in CK-MB levels was higher in the non-severe dengue group with warning signs (34.29%) than non-severe dengue without warning signs (25.30%). However, it was not statistically significant (p=0.276).

The proportion of increased troponin I levels was not significant between the DHF group compared to the DF group (p=0.265). However, the proportion of increased Tn I levels was significant between the DSS group compared to the DF group (p=0.022). This finding showed that increased proportions of Tn I levels may be associated with the severity of dengue (DF vs. DSS). This may happen because in shock conditions there is a release of TNF-α in large quantities which causes a decrease in blood pressure or shock and hypoperfusion, whereas in the DF and DHF groups there is no hypoperfusion. Hypoperfusion conditions that occur in DSS will cause a decrease in the integrity of myocyte membranes and changes in coronary microcirculation, causing damage to the myocardium and the release of Tn I.[8,14,15]

Kularatne *et al.*,[7] reported that 29% of dengue patients with cardiac involvement had increased Tn T levels, where 12% of these occur in patients who had shock.[7] In addition, Iskandar *et*

al.,[15] reported that Tn T levels are higher in the DSS group compared to DHF. However, this study differs from the study conducted by Yacoub *et al.*,[16] of 17 patients who had Tn I levels checked, one patient with severe dengue had elevated Tn I levels, and 16 other patients with varying degrees of severity had normal Tn levels. The difference may occur due to the difference in the cut-off point of Tn I levels used. The study used a cut-off point of 0.3 ng/mL, while in our current study a cut-off point of 0.01 ng/mL was used with a sensitivity of 88.1% - 100% and a specificity of 79.9% - 96.3%.[7] Another marker such as amino-terminal pro-brain natriuretic peptide (NT-proBNP) can be used to detect myocardial injury, and this inflammatory activity can be confirmed by a higher leukocyte count and C-reactive protein levels.[6]

Even though cardiac troponins are the standard test used to diagnose acute myocardial infarction, however, those may be elevated in cases related to non-cardiac causes. Elevated levels of cardiac troponins can occur due to end-stage renal disease, strenuous exercise, sepsis, and rhabdomyolysis, acute pulmonary edema, chronic obstructive pulmonary disease, pulmonary hypertension, stroke, and subarachnoid hemorrhage.[17,18] These conditions may increase cardiac troponin concentration in the blood due to a mismatch between cardiac oxygen supply and demand even in the absence of coronary artery disease.[18]

Myocarditis can worsen the clinical outcome of patients with dengue infection with shock. Myocarditis causes a decrease in the left ventricular ejection fraction which will decrease the cardiac output and aggravate shock condition. This needs to be monitored closely since the initial management of dengue myocarditis in order not to further worsen the clinical course of the disease. Severe and prolonged shock conditions will also reduce the integrity of the myocyte membrane and affect the coronary microcirculation, causing the exacerbation of the myocarditis itself.[8-10]

Our study had a limitation in that the cardiac marker examination was only performed once during the hospitalization period, therefore it was not possible to detect changes in the cardiac marker levels during the dengue infection phase. The number of subjects did not meet the minimum calculated sample size; thus, the power of the study was not high. This condition was unavoidable since there was a limitation on the length of the study. Further research using a higher number of subjects and serial monitoring of cardiac enzymes are needed to confirm the role and kinetics of cardiac markers in dengue myocarditis.

CONCLUSION

In conclusion, Tn I level shows a significantly rise in the DSS spectrum of dengue infection parallel with increasing severity. A follow-up monitoring of the level of cardiac markers as well as ECG abnormality should be performed to understand more about dengue myocarditis characteristics in pediatric patients.

ACKNOWLEDGEMENT

Authors would like to thank the Director of Dr. Sardjito General Hospital, Yogyakarta for his support during the study.

REFERENCES

1. World Health Organization. Dengue and severe dengue. Published 2022. Accessed May 15, 2022. http://www.who.int/mediacentre/factsheets/fs117/en/
2. Kementerian Kesehatan Republik Indonesia. Profil kesehatan Indonesia tahun 2021. Jakarta: Kementerian Kesehatan Republik Indonesia, 2022.
3. Kementerian Kesehatan Republik Indonesia. Pedoman nasional pelayanan kedokteran tata laksana

infeksi dengue anak dan remaja. Jakarta: Kemesterian Kesehatan Republik Indonesia, 2021.
4. WHO. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Geneva: WHO, 2011.
5. Kadam DB, Salvi S, Chandanwale A. Expanded dengue. J Assoc Physicians India 2016; 64(7):59-63.
6. Miranda CH, Borges MC, Matsuno AK, Vilar FC, Gali LG, Volpe GJ, *et al*. Evaluation of cardiac involvement during dengue viral infection. Clin Infect Dis 2013; 57(6):812-9. https://doi.org/10.1093/cid/cit403
7. Kularatne SAM, Pathirage MMK, Kumarasiri PVR, Gunasena S, Mahindawanse SI. Cardiac complications of a dengue fever outbreak in Sri Lanka, 2005. Trans R Soc Trop Med Hyg 2007; 101(8):804-8. https://doi.org/10.1016/j.trstmh.2007.02.021
8. Buntubatu S, Prawirohartono EP, Arguni E. Myocarditis prevalence in paediatric dengue infection: a prospective study in tertiary hospital in Yogyakarta, Indonesia. J Trop Pediatr 2019; 65(6):603-8. https://doi.org/10.1093/tropej/fmz020
9. Magnani JW, Dec GW. Myocarditis: current trends in diagnosis and treatment. Circulation 2006; 113(6):876-90. https://doi.org/10.1161/CIRCULATIONAHA.105.584532
10. Yacoub S, Wertheim H, Simmons CP, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. Nat Rev Cardiol 2014; 11(6):335-45. https://doi.org/10.1038/nrcardio.2014.40
11. Kirawittaya T, Yoon IK, Wichit S, Green S, Ennis FA, Gibbons RV, *et al*. Evaluation of cardiac involvement in children with dengue by serial echocardiographic studies. PLoS Negl Trop Dis 2015; 9(7):e0003943. https://doi.org/10.1371/journal.pntd.0003943
12. Hussain SBS, Kuswiyanto RB, Iwan J. Electrocardiogram profile in children with dengue infection at Dr. Hasan Sadikin General Hospital and Bandung City Hospital. Althea Med J 2016; 3(4):629-32. https://doi.org/10.15850/amj.v3n4.950
13. Li Y, Hu Z, Huang Y, Huang Z, Li J, Hong W, *et al*. Characterization of the myocarditis during the worst outbreak of dengue infection in China. Medicine (Baltimore) 2016; 95(27):e4051. https://doi.org/10.1097/MD.0000000000004051
14. Bodor GS. Biochemical markers of myocardial damage. EJIFCC 2016; 27(2):95-111.
15. Iskandar B, Juherinah, Daud D, Febriani ADB. The levels of troponin T in patients with dengue hemorrhagic fever. Am J Clin Exp Med 2015; 3(4):149-53. https://doi.org/10.11648/j.ajcem.20150304.14
16. Yacoub S, Griffiths A, Hong Chau TT, Simmon CP, Wills B, Hien TT, *et al*. Cardiac function in Vietnamese patients with different dengue severity grades. Crit Care Med 2012; 40(2):477-83. https://doi.org/10.1097/CCM.0b013e318232d966
17. Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. Heart 2006; 92(7):987-93. https://doi.org/10.1136/hrt.2005.071282
18. Aydin S, Ugur K, Aydin S, Sahin I, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag 2019; 15:1-10. https://doi.org/10.2147/VHRM.S166157
19. Shivanthan MC, Navinan MR, Constantine GR, Rajapakse S. Cardiac involvement in dengue infection. J Infect Dev Ctries 2015; 9(04):338-46. https://doi.org/10.3855/jidc.6200

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 34-43
https://doi.org/10.19106/JMedSci005501202305

Pulmonary vascular resistance/systemic vascular resistance (PVR/SVR) ratio changes after sildenafil therapy in uncorrected congenital heart disease-associated pulmonary arterial hypertension

Evita Devi Noor Rahmawati*, Putrika Prastuti Ratna Gharini, Anggoro Budi Hartopo, Lucia Kris Dinarti, Dyah Wulan Anggrahini

Department of Cardiology and Vascular Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta

Submitted: 2022-02-28
Accepted : 2022-05-23

ABSTRACT

Pulmonary vascular resistance (PVR) to systemic vascular resistance (SVR) ratio is a prognostic predictor in congenital heart disease (CHD)-associated pulmonary arterial hypertension (PAH) after defect correction. Sildenafil, widely used as a PAH drug, can decrease PVR with minimal or without changes in SVR, resulting in decreased PVR/SVR ratio after treatment. However, there is limited evidence that PVR/SVR ratio reduced after sildenafil therapy in uncorrected CHD-associated PAH patients. This study aimed to investigate the decreasing of the PVR/SVR ratio after ≥ 1-year oral sildenafil therapy in adult uncorrected CHD-associated PAH. A total of 30 uncorrectable CHD-associated PAH subjects derived from the COHARD-PH registry were included in this study. Right heart catheterization (RHC) was performed during the first visit and further evaluations were conducted after ≥1-year oral sildenafil therapy. The PVR/SVR ratio at the baseline and after the evaluation was collected. The primary outcome of this study was the changes in PVR/SVR ratio from baseline to evaluated RHC. Characteristic analysis of subjects with decreased PVR or PVR/SVR ratio was perforemd as the secondary outcome. The mean PVR and SVR were not different from baseline and evaluated RHC (15.98± 10.67 vs. 18.38±13.93 WU, p=0.206 and 36.65±13.99 *vs.* 39.34±15.46 WU, p=0.262). There was no significant difference in the baseline PVR/SVR ratio and the evaluated PVR/SVR ratio after ≥1-year sildenafil therapy (0.48 ±0.32 vs. 0.49±0.36; p=0.882). As much as 15 subjects (50%) experienced decreased PVR/SVR ratio. However, there was no significant difference in the characteristics, including age, Eisenmenger syndrome, type of shunts, baseline PVR, PAH-specific treatment, and baseline NT-proBNP level (p>0.05). In conclusion, sildenafil therapy does not change PVR/SVR ratio in adults with uncorrected CHD-associated PAH.

Keywords:
congenital heart disease;
pulmonary arterial hypertension;
pulmonary vascular resistance;
sildenafil;
systemic vascular resistance

ABSTRACT

Rasio resistensi paru (Rp) terhadap resistensi sistemik (Rs) atau selanjutnya disebut Rp/Rs merupakan sebuah prediktor pada hipertensi arteri paru (HAP) yang berhubungan dengan penyakit jantung kongenital setelah koreksi defek. Sildenafil, obat HAP yang banyak digunakan, dapat menurunkan Rp dengan minimal atau tanpa perubahan pada Rs, yang menghasilkan penurunan rasio Rp/Rs setelah terapi. Namun belum terdapat bukti bahwa terapi sildenafil oral dapat menurunkan Rp/Rs pada pasien HAP yang berhubungan dengan penyakit jantung kongenital yang belum dikoreksi. Penelitian ini bertujuan untuk mengkaji penurunan Rp/Rs pada HAP yang berhubungan dengan penyakit jantung kongenital yang belum dikoreksi setelah terapi sildenafil ≥1 tahun. Sebanyak 30 subyek dengan HAP akibat penyakit jantung kongenital yang belum dikoreksi diambil dari data registrasi COHARD-PH. Kateterisasi jantung kanan (KJKa) dilakukan pada awal dan dilakukan evaluasi setelah

*corresponding author: evitadevi.dr@gmail.com

terapi sildenafil oral selama ≥ 1 tahun. Rasio resistensi paru terhadap resistensi sistemik awal dan evaluasi didapatkan dari KJKa tersebut. Luaran utama dari studi ini adalah perubahan Rp/Rs. Analisis karakter subyek dengan nilai Rp yang turun atau Rp/Rs yang turun dilakukan sebagai luaran sekunder. Hasil penelitian menunjukkan rerata Rp maupun Rs tidak berbeda dari awal dibandingkan dengan evaluasi (15,98±10,67 vs. 18,38±13,93 WU, p=0,206 dan 36,65±13,99 vs. 39,34±15,46 WU, p=0,262). Tidak terdapat perbedaan bermakna pada Rp/Rs awal dan evaluasi setelah terapi sildenafil oral selama ≥1 tahun (0,48 ±0,32 vs. 0,49±0,36; p=0,882). Sebanyak 15 subyek (50%) mengalami penurunan Rp/Rs, namun tidak didapatkan perbedaan signifikan dari karakteristik subyek, termasuk usia, sindrom Eisenmenger, tipe defek, Rp awal, terapi spesifik HAP dan level NT-proBNP awal. Simpulan, terapi sildenafil tidak mengubah Rp/Rs pada HAP yang berhubungan dengan penyakit jantung kongenital yang belum dikoreksi.

INTRODUCTION

Pulmonary hypertension (PH) is a pathological disorder in the cardio-pulmonary system that involves multiple clinical conditions. Distinct classifications based on clinical, pathological, and hemodynamic findings and treatment strategies are used to categorize the multiple clinical conditions found in PH. Congenital Heart Disease (CHD) is one condition that could lead to PH development over time.[1]

The incidence of CHD is approximately 8 over 1000 birth worldwide, and 30% of uncorrected CHD patients develop pulmonary arterial hypertension (PAH). The estimation of prevalence and incidence of PAH were 15.0 cases/million and 2.4 cases/million annually, respectively.[2] The spectrum of CHD that contributes to PAH development is Eisenmenger syndrome, PAH associated with prevalent systemic-to-pulmonary shunts, PAH with small/coincidental defects, and PAH after defect correction.[1] In less developed countries, such as Indonesia, a significant number of the adult with uncorrected CHD is looking for help because of the emerging symptoms and signs of complication.[3] Unfortunately, the availability of approved PAH targeted therapy in Indonesia is limited.

A previous study showed a positive correlation between mean pulmonary arterial pressure (mPAP) before correction and severity of pulmonary vascular morphology in lung tissue biopsy. However, due to the risk of bleeding and the need for specialized expertise for interpretation, the lung tissue biopsy was no longer used.[4] The indication of defect correction relies on hemodynamic parameters and vasoreactivity tests taken from right heart catheterization (RHC) in recent years. Hemodynamic parameters such as pulmonary vascular resistance (PVR), pulmonary vascular resistance to systemic vascular resistance ratio (PVR/SVR), and vasoreactivity test are used as predictors of surgical outcomes for CHD-associated PAH with shunt who undergoes defect correction. A progressive increase in PVR is known to be correlated with right heart failure and even death. Nonetheless, there was no proof whether these parameters could predict which patients develop PAH after the defect correction.[5]

Sildenafil, a phosphodiesterase inhibitor type 5 (PDE-5 inhibitor), is a widely used PAH drug that effectively induces smooth muscle relaxation and vasodilatation in pulmonary vascular.[6] The previous study has shown the effect of sildenafil in symptom improvement, functional capacity, and hemodynamics in PAH patients with any causes.[7] Sildenafil shows a decreasing PVR with minimal or without changes in SVR, resulting in decreased PVR/SVR ratio after treatment.

According to the European Society of Cardiology (ESC) guideline, right heart catheterization evaluation should be done in 6-12 months after first catheterization (based on hospital rules) or 3-6 months after treatment changes or when deterioration happens.[1] In this study, we investigated the changes in PVR/SVR ratio in uncorrected CHD-associated PAH patients with shunt after sildenafil therapy for 1-year in Dr. Sardjito General Hospital, Yogyakarta as well as the characteristics of patients who show a decrease in PVR/SVR ratio.

MATERIALS AND METHODS

Data collection

It was an observational analytic study with a self-controlled case study design. Data was collected from the PAH registry of Dr. Sardjito General Hospital (COHARD-PH registry) from January-May 2020. The PAH was defined as mPAP > 20 mmHg, PVR ≥ 3 WU, and pulmonary artery wedge pressure (PAWP) ≤ 15 mmHg from RHC.[8] The inclusion criteria of this study were: 1) male or female aged ≥ 18 years old PAH associated CHD with shunt patient; 2) undergo RHC evaluation with minimal separate 1 year after baseline and between RHC baseline and evaluation patient took sildenafil; 3) uncorrected defect; 4) hemodynamic data in RHC baseline and evaluation were available. Patients with complex CHD (more than one defect) were excluded. The minimal sample size of this study was 29 subjects. This sample size was yielded from the calculation below for the numeric analytic pair study:

$$n_1 = n_2 = \left\{ \frac{(Z_\alpha + Z_\beta)\ S}{X_1 - X_2} \right\}^2$$

$$n_1 = n_2 = \left\{ \frac{(1{,}96 + 1{,}28)\ x0{,}3}{0{,}2} \right\}^2$$

$$n_1 = n_2 = 29$$

where the type I error or α as 5% giving Z_α (alfa standard deviation) as 1.96; the type II error (β) we took was 10% providing Z_β (beta standard deviation) as 1.28. The total standard deviation (S) was calculated from the equation in the reference study[9]; and X_1-X_2 = differences between significant minimal mean in 2 groups.

Right heart catheterization was carried out by a cardiologist based on the standard operational procedure in Dr. Sardjito General Hospital, Yogyakarta. The measurement of PVR/SVR ratio was conducted by comparing PVR over SVR with the Fick equation. The calculation of PVR, SVR, and Fick equation are explained below:

PVR = (mPAP – PAWP) / CO[10]
SVR = (MAP – RAP) / CO[11]
CO (by Fick equation) = VO2 (ml/min) / A-V O^2 difference[12]

where mPAP is mean pulmonary artery pressure; PAWP is pulmonary arterial wedge pressure; CO is cardiac output; SVR is systemic vascular resistance; MAP is mean arterial pressure; RAP is right atrium pressure; VO2 is oxygen consumption, A-V O^2 difference is oxygen saturation difference between the pulmonary artery and venous.

The PVR/SVR ratio baseline was obtained from the first RHC followed by RHC evaluation >1 year after taking oral sildenafil. The primary outcome was the changes in PVR/SVR ratio from baseline to evaluated RHC. All subjects in this study had been informed and given their consent to this study. This study was approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, Public Health and Nursing/Dr. Sardjito General Hospital (ref. no. with Ethical KE/FK/0738/EC July 7, 2020).

Statistical Analysis

Data were expressed in mean ± SD (standard of deviation). The data normality was tested with the Shapiro Wilk test (p>0.05 means normal distribution). The PVR/SVR ratio changes were analyzed with paired t-test or Wilcoxon test.

RESULT

Primary outcome

A total of 72 subjects with CHD shunt were identified from the COHARD-PH registry in Dr. Sardjito General Hospital, who underwent twice RHC from March 2014-May 2020. Hoever, only 30 subjects were enrolled in this study (FIGURE 1). The subject enrollment was stopped afterward because the minimal sample size was achieved. The baseline characteristics of the subjects are shown in TABLE 1.

TABLE 1. The baseline characteristic of the subjects

Variable (n=30)	Value
Age [med (min-max) year]	35.13 (22-65)
Female [n (%)]	28 (93.33)
Hypertension [n (%)]	1 (3.33)
Other drugs	
• CCB [n (%)]	1 (3.33)
• ACEi/ARB [n (%)]	0
Peripheral oxygen saturation (%)	94.17± 4.62
6MWD (m)	337 ±79.09
WHO class functional (m)	1.8 ± 0.61
NT-proBNP [ng/L]	1226.86± 1017.52
Eisenmenger syndrome [n (%)]	12 (40)
PAH-specific therapy	
• Sildenafil monotherapy [n (%)]	14 (46.67)
• Combination therapy [n (%)]	16 (53.33)
Sildenafil dosage	
• < 120 mg/day [n (%)]	22 (73.33)
• ≥ 120 mg/day [n (%)]	8 (26.67)
Mean time baseline – evaluation RHC (mo)	23.3 (12-64)
Pre-tricuspid shunt [n (%)]	25 (83.33)
Right atrium diameter [mm]	45.90 ± 7.36
Right ventricle diameter [mm]	41.73 ± 9.88
TAPSE [mm]	21.40 ± 5.49
TR Vmax [m/s]	4.5 ± 0.73
Probability PH	
• Low [n (%)]	0
• Intermediate [n (%)]	4 (13.33)
• High [n (%)]	26 (86.67)
mPAP (mmHg)	58.07 ± 16.03
PVR (WU)	15.98 ± 10.67
SVR (WU)	36.65 ± 13.99
PVR/SVR (ratio)	0.48 ± 0.32

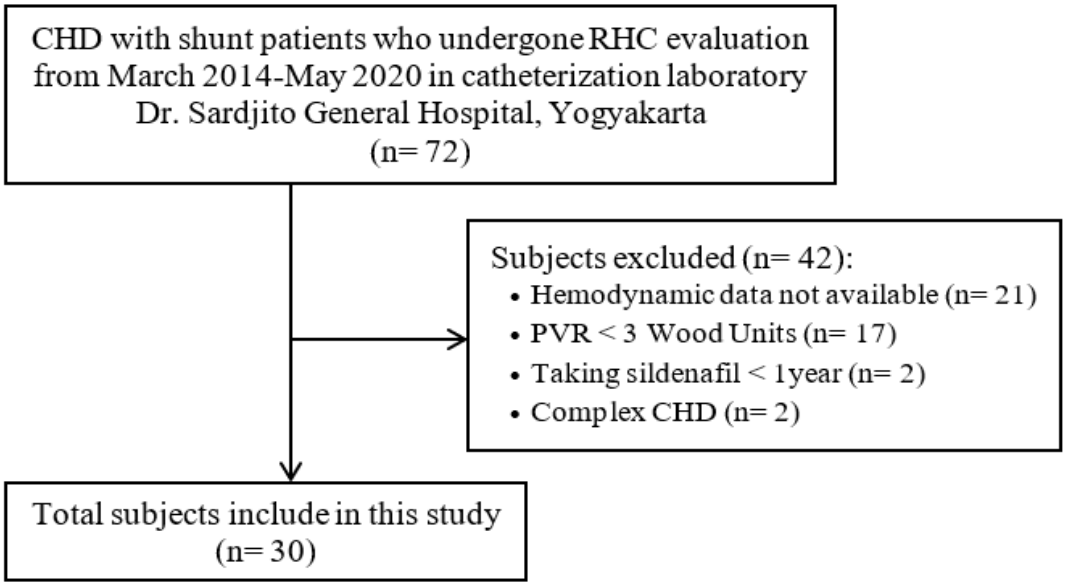


FIGURE 1. Subjects recruitment

Right heart catheterization evaluation was then performed to investigate hemodynamic parameters in subjects who received sildenafil for a minimal 1-year. Although we found changes in the mixed vein saturation and aortic saturation before and after sildenafil therapy, it was not statistically significant different (p>0.05). No significantly changes in the flow ratio, PVR and SVR were also observed (p>0.05), although there was a tendency toward decreasing in mPAP (TABLE 2). Furthermore, as much as 15 subjects (50%) were experiencing PVR/SVR ratio decreasing, even though they did not show any significantly difference before and after sildenafil therapy (0.48 ±0.32 to 0.49 ±0.36 p=0.882), as shown in TABLE 2 and FIGURE 2.

TABLE 2. Sildenafil effect in hemodynamic parameter (by RHC) before and after sildenafil therapy

Variable	Δ	p
Mixed vein saturation (%)	-1.6	0.288
Aortic saturation (%)	-0.73	0.777
Flow ratio	0.13	0.579
mPAP (mmHg)	-0.7	0.672
PVR (WU)	2.4	0.206
SVR (WU)	2.69	0.262
PVR/SVR ratio	0.01	0.882

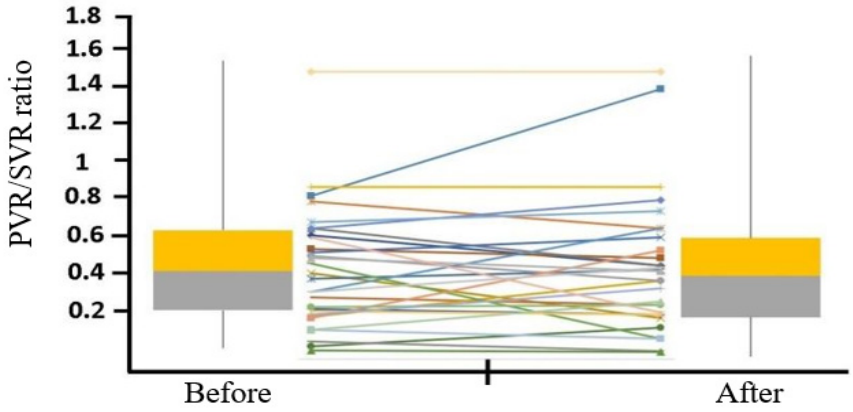


FIGURE 2. PVR/SVR ratio changes before and after sildenafil therapy

Secondary outcome

A further analysis was also performed to investigate the characteristics of subjects who showed decreased PVR and PVR/SVR ratios. The subjects were divided into 5 subgroups based on their clinical appearance (Eisenmenger syndrome vs. non-Eisenmenger syndrome), type of lesion (pre-tricuspid vs. post-tricuspid), baseline PVR (PVR < 8 WU *vs.* PVR > 8 WU), therapy (sildenafil monotherapy vs. combination therapy with beraprost 30 mcg twice to three times daily), and baseline NT-proBNP (NT-proBNP <1400 ng/L vs. NT-proBNP >1400 ng/L).

In the increased/unchanged and decreased PVR group, there was no significantly difference in the mean age (34.75 ± 9.83 and 36.29 ± 12.74 respectively; p=0.847). From the decreased PVR group, the proportion of non-Eisenmenger syndrome was higher than the proportion in Eisenmenger syndrome (64.3% vs. 35.7%; p=0.722), although it was not statistically significant. We found a higher proportion in pre-tricuspid shunt, PVR >8 WU, combination therapy, and NT-proBNP level < 1400 ng/L as well, however, the difference was not statistically significant (TABLE 3).

TABLE 3. Subgroups analysis in PVR changes and PVR/SVR ratio changes

Variable	Increased/ unchanged PVR [n (%)]	Decreased PVR [n (%)]	p	Increased/ unchanged PVR/ SVR ratio [n (%)]	Decreased PVR/SVR ratio [n (%)]	p
Age (mean ±SD years)	34.75 ±9.83	36.29 ±12.74	0.847	35.85 ±9.25	36.12 ±12.9	0.587
Eisenmenger syndrome						
• Yes	7 (43.7)	5 (35.7)	0.722	6 (40)	6 (40)	1.000
• No	9 (56.3)	9 (64.3)		9 (60)	9 (60)	
Shunt						
• Pre- tricuspid	13 (81.3)	12 (85.7)	1.000	13 (86.7)	12 (80)	1.000
• Post- tricuspid	3 (18.7)	2 (14.3)		2 (13.3)	3 (20)	
PVR						
• > 8 WU	14 (87.5)	10 (71.4)	0.378	13 (86.7)	11 (73.3)	0.651
• < 8 WU	2 (12.5)	4 (28.6)		2 (13.3)	4 (26.7)	
Therapy						
• Combination	8 (50)	8 (57.1)	0.730	10 (66.7)	6 (40)	0.272
• Mono-therapy	8 (50)	6 (42.9)		5 (33.3)	9 (60)	
NT-proBNP						
• > 1400 ng/L	7 (43.7)	4 (28.6)	0.466	8 (53.3)	3 (20)	0.128
• < 1400 ng/L	9 (56.3)	10 (71.4)		7 (46.7)	12 (80)	

In the increased/unchanged and decreased PVR/SVR ratio group, there was no significantly difference in the mean age (35.85 ±9.25 and 36.12 ±12.9; p=0.587). Although in the decreased PVR/SVR ratio group, the proportion of the non-Eisenmenger syndrome group was higher than the-Eisenmenger syndrome (60% *vs.* 40%; p=1.000), there was no significantly difference (p>0.05). Although the proportion in pre-tricuspid shunt, PVR >8WU, sildenafil monotherapy, and NT-proBNP level <1400 ng/L showed an increase, there was no significantly difference (p>0.05) (TABLE 3).

DISCUSSION

The PVR/SVR ratio has been known for a long time as a valuable parameter in CHD-associated PAH. This parameter could predict the surgical outcome in CHD-associated PAH. In this study, we found no changes in PVR/SVR ratio after minimal 1-year sildenafil therapy in CHD-associated PAH with the shunt. Sildenafil is a drug of choice for PAH. According to the SUPER-1 study, various doses of sildenafil could reduce mPAP, as well as improve clinical parameters such as exercise capacity and WHO functional class.[7] This study also revealed a decrease in mPAP as well, although it was no statistical significance (p>0.05). Moreover, there was no report on PVR/SVR ratio changes in the SUPER-1 study.

The discrepancy found in our study and previously published studies on sildenafil therapy might be due to the difference in the clinical condition of the subjects. In the SUPER-1 study, most study subjects were idiopathic PAH (IPAH) followed by connective tissue disease (CTD)-associated PAH, while CHD-associated PAH subjects were only less than 10% of all study subjects. In this study, all of the subjects were CHD-associated PAH; therefore, the subject response toward sildenafil therapy could be different.

The difference in the proportion of PAH patients was also described in other studies. This difference may be associated with PAH epidemiology in each country or region. In Western countries, IPAH is the most common type of PAH (30-50%), followed by CTD-associated PAH (15-30%), CHD-associated PAH (10-23%), and portopulmonary hypertension (5-10%). Meanwhile, in non-Western countries such as China, CHD-associated PAH is the most common PAH that contributes to 43% of all PAH cases.[13] A recent study in Sardjito General Hospital, Indonesia, demonstrated that the most common type of PAH is CHD-associated PAH. Most of these patients were undetected in earlier years, and 66.9% of them developed PAH later in life.[3]

Our secondary analysis in this study displayed characteristics of subjects with decreased PVR or PVR/SVR ratio after sildenafil therapy in specific sub-groups, including the presence of Eisenmenger syndrome, tricuspid lesion, the specific cut-off of PVR value, multi/monotherapy, and a certain NT-proBNP level. This study found that the proportion of non-Eisenmenger syndrome subjects was higher than Eisenmenger syndrome in decreasing PVR and PVR/SVR, although the difference was not statistically significant (p>0.05).

In the pathogenesis of PAH, there are 3 major processes of pulmonary artery constriction. First is vasoconstriction which is caused by a vasodilator/vasoconstrictor agent imbalanced in pulmonary circulation, followed by pulmonary vascular remodeling due to smooth muscle cells and endothelial cells proliferation. The coagulation abnormality results in thrombosis in situ, leading to an increase in PVR. The early stage of this process is known to be reversible.[14] However, the advanced stage is progressive, leading to obliteration of the pulmonary vascular bed (irreversible stage).[15] Progressive increased PVR could drive shunt reversal in CHD-associated PAH patients, from

the initial left-to-right shunt to right-to-left shunt, referred to as Eisenmenger syndrome. Most people perceived this syndrome as irreversible, although it is believed that not all Eisenmenger syndromes were irreversible PAH. A previous study showed that not all PAH with a negative oxygen response was irreversible PAH.[14] The same result was also reflected in our study in which we found Eisenmenger syndrome subjects with increased PVR, but the PVR/SVR ratio was decreased to the grey zone (0.3-0.5).

The characteristic of the tricuspid lesion in our study showed a higher proportion of pre-tricuspid lesion group in decreased PVR or decreased PVR/SVR ratio than the post-tricuspid lesion group, although the result was not statistically significant. This study result is consistent with Hascoët *et al.*[16] which showed no differences in Eisenmenger syndrome caused by pre-tricuspid shunt and post-tricuspid shunt in increasing PVR. This recent study revealed that 2 post-tricuspid shunt subjects with increased PVR/SVR ratio were Eisenmenger syndrome, while 3 post-tricuspid shunts with decreased PVR/SVR ratio were non-Eisenmenger syndrome.

The same result was also found in the PVR group. There was a higher proportion of the group with PVR > 8 WU compared to the group with PVR < 8 WU, although it was not statistically significant. European Society of Cardiology guideline for adult congenital heart disease management showed that CHD with shunt patients whose PVR ≥5 WU rarely improved the hemodynamic parameter. Therefore, these patients need PAH-specific therapy before undergoing RHC evaluation to get a prompt decision for the defect correction.[17]

In our study, although we could not find a significant difference in terms of therapy used by the subjects with a decreased PVR or PVR/SRV ratio, more than half of the subjects (57.1%) in this study with a decrease in PVR were in the combination therapy group. On the contrary, a higher proportion of the monotherapy group has been found in the decreased PVR/SVR ratio. This finding might be caused by the combination therapy used in this study. According to ESC guideline 2015, the recommendation for initial combination therapy is the PDE-5 inhibitor and Endothelin receptor antagonist (ERA). ERA plays role in decreasing pulmonary arterial vasoconstriction, which is the basic pathomechanism of PAH. However, due to the unavailability of ERA in our hospital, PDE-5 inhibitor and beraprost were used in our study. The previous study demonstrated that this combination therapy could improve functional capacity without affecting hemodynamic parameters, albeit using the PDE-5 inhibitor and prostacyclin analog combination was not recommended in ESC guideline 2015.[1]

In ALPHABHET study, beraprost initially improved 6MWD and WHO functional class, but the extended follow-up showed that the improvement was not sustained. Beraprost has been approved in some South-East Asian countries, including Indonesia that uses beraprost for treating PAH patients in WHO functional class III.[18] But unfortunately, in this study, we did not evaluate further clinical improvement, since we focused on hemodynamic improvement instead of clinical improvement.

The level of NT-proBNP cut-off used in this study referred to ESC guideline 2015, where NT-proBNP level >1400 ng/L is considered a high-risk feature of PH with more than 10% 1-year mortality estimation.[1] In our study, the proportion of subjects with NTproBNP level <1400 ng/L was higher than those with NTproBNP level > 1400 ng/L in both decreased PVR and decreased PVR/SVR ratio groups. Although the result was not statistically significant, this result was in contrast to a previous study showing that the degree of severity in PAH is proportional to the increased NT-

proBNP level.[19] However, another study suggested that the serial examination of NT-proBNP was superior to a single baseline examination.[20]

However, this study has several limitations. Our study design was an observational study with an abnormal distribution of subjects, namely in the pre-tricuspid lesion *vs.* post-tricuspid lesion, as well as in PVR <8 WU vs. PVR >8 WU. In addition, beraprost, a prostacyclin analog used in the combination therapy could enhance the vasodilatation effect in the pulmonary artery; however, there were no drug acts in decreasing pulmonary artery vasoconstriction. We believe the combination of sildenafil and ERA could carry a better result since ERA is one of the PAH-specific therapy that plays a direct role in the endothelin pathway to decrease vasoconstriction in the pulmonary artery.

CONCLUSION

This study does not find any significantly change in PVR/SVR ratio after ≥1-year sildenafil therapy in the adult with uncorrectable CHD-associated PAH. There is a tendency toward decreasing in PVR or PVR/SVR ratio occurred in subjects without Eisenmenger syndrome, pre-tricuspid shunt, and baseline NT-proBNP < 1400 ng/L.

ACKNOWLEDGEMENTS

This study was funded by Dana Masyarakat Research Grant 2019 under the contract number 1510/UN-1/FK-KMK/PP/PT/2019.

REFERENCES

1. Galiè N, Chairperson ESC, Humbert M, Chairperson ERS, Vachiery J, Gibbs S, *et al*. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Respir J 2015; 46(4):903-75. https://doi.org/10.1183/13993003.01032-2015
2. McLaughlin VV, Davis M, Cornwell W. Pulmonary arterial hypertension. Curr Probl Cardiol 2011; 36(12):461-517. https://doi.org/10.1016/j.cpcardiol.2011.08.002
3. Dinarti LK, Hartopo AB, Kusuma AD, Satwiko MG, Hadwiono MR, Pradana AD, *et al*. The COngenital HeARt Disease in adult and Pulmonary Hypertension (COHARD-PH) registry: a descriptive study from single-center hospital registry of adult congenital heart disease and pulmonary hypertension in Indonesia. BMC Cardiovasc Disord 2020; 20(1):163. https://doi.org/10.1186/s12872-020-01434-z
4. Schwerzmann M, Pfammatter JP. Approaching atrial septal defects in pulmonary hypertension. Expert Rev Cardiovasc Ther 2015; 13(6):693-701. https://doi.org/10.1586/14779072.2015.1047763
5. Myers PO, Tissot C, Beghetti M. Assessment of operability of patients with pulmonary arterial hypertension associated with congenital heart disease. Circ J 2014; 78(1):4-11. https://doi.org/10.1253/circj.cj-13-1263
6. Palma G, Giordano R, Russolillo V, Cioffi S, Palumbo S, Mucerino M, *et al*. Sildenafil therapy for pulmonary hypertension before and after pediatric congenital heart surgery. Texas Hear Inst J 2011; 38(3):238-42.

7. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, *et al*. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med 2005; 353(20):2148-57. https://doi.org/10.1056/NEJMoa050010
8. Galie N, McLaughlin VV, Rubin LJ, Simonneau G. An overview of the 6th World Symposium on Pulmonary Hypertension. Eur Respir J 2019; 53(1):1802148. https://doi.org/10.1183/13993003.02148-2018
9. Garg N, Sharma MK, Sinha N. Role of oral sildenafil in severe pulmonary arterial hypertension: clinical efficacy and dose response relationship. Int J Cardiol 2007; 120(3):306-13. https://doi.org/10.1016/j.ijcard.2006.10.017
10. Abbas AE, Fortuin FD, Schiller NB, Appleton CP, Moreno CA, Lester SJ. A simple method for noninvasive estimation of pulmonary vascular resistance. J Am Coll Cardiol 2003; 41(6):1021-7. https://doi.org/10.1016/s0735-1097(02)02973-x
11. Stefadouros MA, Dougherty MJ, Grossman W, Craige E. Determination of systemic vascular resistance by a noninvasive technic. Circulation 1973; 47(1):101-7. https://doi.org/10.1161/01.cir.47.1.101
12. Davies GG, Jebson PJ, Glasgow BM, Hess DR. Continuous Fick cardiac output compared to thermodilution cardiac output. Crit Care Med 1986; 14(10):881-5. https://doi.org/10.1097/00003246-198610000-00010
13. Lau EMT, Giannoulatou E, Celermajer DS, Humbert M. Epidemiology and treatment of pulmonary arterial hypertension. Nat Rev Cardiol 2017; 14(10):603-14. https://doi.org/10.1038/nrcardio.2017.84
14. Huang JB, Liang J, Zhou LY. Eisenmenger syndrome: not always inoperable. Respir Care 2012; 57(9):1488-95. https://doi.org/10.4187/respcare.01418
15. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, *et al*. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation 2010; 122(2):156-63. https://doi.org/10.1161/CIRCULATIONAHA.109.911818
16. Hascoët S, Baruteau AE, Humbert M, Simonneau G, Jais X, Petit J, *et al*. Long-term outcomes of pulmonary arterial hypertension under specific drug therapy in Eisenmenger syndrome. J Hear Lung Transplant 2017; 36(4):386-98. https://doi.org/10.1016/j.healun.2016.10.006
17. Baumgartner H, De Backer J, Babu-Narayan SV, Budts W, Chessa M, Diller GP, *et al*. 2020 ESC Guidelines for the management of adult congenital heart disease. Eur Hear J 2020;42(6):563-645. https://doi.org/10.1093/eurheartj/ehaa554
18. Humbert M, Ghofrani HA. The molecular targets of approved treatments for pulmonary arterial hypertension. Thorax 2016; 71(1):73-83. https://doi.org/10.1136/thoraxjnl-2015-207170
19. Souza R, Jardim C, Fernandes CJC, Lapa MS, Rabelo R, Humbert M. NT-proBNP as a tool to stratify disease severity in pulmonary arterial hypertension. Respir Med 2007; 101(1):69-75. https://doi.org/10.1016/j.rmed.2006.04.014
20. Wolfson AM, Maitland ML, Thomeas V, Glassner C, Gomberg-Maitland M. A serial NT-proBNP model to improve prognostication in patients with pulmonary arterial hypertension. J Clin Exp Cardiol 2017; 8(11):1000555. https://doi.org/10.4172/2155-9880.1000555

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 44-52
https://doi.org/10.19106/JMedSci005501202306

Evaluation of patients with suspected obstructive sleep apnea in a low-middle income country: Lagos experience

Oluwafemi Tunde Ojo[1,2]*, Adeola Olubunmi Ajibare[1,2], Akinola Olusola Dada[1,2], Ramon Moronkola[1], Ayoola Odeyemi[1], Temitope Fapohunda[1], Ikechukwu Adolphus Aganweze[1], Tracy Udoka Ossai[1], Ifeoluwa Owoeye[1], Olufunke Olayinka Adeyeye[1,2]

[1]Department of Medicine, Lagos State University Teaching Hospital, Ikeja, Lagos. [2]Department of Medicine, College of Medicine, Lagos State University Teaching Hospital, Nigeria

Submitted: 2022-11-10
Accepted : 2023-01-09

ABSTRACT

Obstructive sleep apnea (OSA) is a common chronic disorder that decreases the quality of life of patients. It is an underdiagnosed medical condition in Nigeria. This study aimed to describe the clinical presentations and validate the sleep apnea screening questionnaires with a home sleep study for the diagnosis of patients with suspected OSA seen in Lagos. This was a descriptive cross sectional study carried out on adult patients with suspicion of OSA referred to the Respiratory Clinic of Lagos State University Teaching Hospital. A proforma was used to obtain information and also data from home polysomnography was obtained for each patient. We selected 22 patients. The commonest presentations include snoring (20 or 90.9%), daytime somnolence (16 or 72.7%) and choking while sleeping (12 or 54.5%). The commonest comorbidities were hypertension (16 or 72.7%) and obesity (6 or 27.3%). The STOP-Bang score identified more patients with a high clinical probability for OSA than the Epworth score (20 and 12 patients respectively). Polysomnography showed evidence of sleep apnea in most suspected patients with severity ranging from mild, to moderate to severe disease (3 or 13.6%, 3 or 13.6%, and 10 or 45.5% respectively). The use of combined Epworth and STOP-Bang questionnaires combination is a great tool in identifying patients with suspected cases of OSA based on clinical presentations that will eventually benefit in a resource-limited environment like Lagos. There should be increased awareness of the use of this readily available and cheap questionnaire among physicians in Lagos for ease of OSAS diagnosis for many patients.

Keywords:
Obstructive sleep apnea;
polysomnography;
sleep study;
apnea;
sleep-disordered breathing

INTRODUCTION

Obstructive sleep apnea (OSA) is a respiratory sleep disorder characterized by intermittent upper-airway collapse during sleep.[1] The diagnosis of OSA requires the objective demonstration of abnormal breathing during sleep.[2] The apnea-hypopnea index (AHI) is widely used to define OSA in clinical and epidemiological studies.[3] The general prevalence of OSA defined by ≥5 apnea and hypopnea events per hour of sleep associated with excessive sleepiness is approximately 3-7% in men and 2-5% in women.[4] The risk factors for OSA include obesity, upper airway abnormalities, male gender, menopause, and increased age which peaks at approximately 55 years.[5]

Obstructive sleep apnea is associated with symptoms during sleep including

*corresponding author: ojofemi911@yahoo.com

snoring, choking and nocturia and wakefulness, excessive sleepiness, fatigue, lack of energy, and with sequelae such as psychological changes, and alterations in the quality of life.[4] The identification of OSA is low in Nigeria and this may be due to poor awareness among doctors about the disease presentations as well poor referral systems for sleep abnormalities evaluation and diagnosis. We hypothesized that sleep breathing disorders are common but rarely reported and referred to respiratory physicians in Nigeria.

This study aimed to evaluate the common presentations of OSA and also evaluate the use of common screening assessment tools as well as home polysomnography in the diagnosis of patients with suspected obstructive sleep apnea. The outcome of this study helps to highlight the importance of clinicians using the readily available and cheap sleep apnea screening questionnaire for early diagnosis of patients with OSA for timely treatment in a resource-limited setting like Lagos, Nigeria.

MATERIALS AND METHODS

Design and site of study

This was a cross-sectional descriptive study conducted at the Respiratory Clinic of Lagos State University Teaching Hospital, Nigeria. The study was carried out in Lagos State University Teaching Hospital (LASUTH) Ikeja, which is one of three tertiary centers that receive referrals from all parts of the Lagos metropolis and its environs. It has a bed capacity of about 774. Participants were recruited from the respiratory clinic of the hospital.

Participants of study

Participants aged > 18 yr who referred to the respiratory clinic on account of suspicion of obstructive sleep apnea were included in this study. Patients with low clinical probability for OSA with Epworth and stop bang scores of less than 10 and 3, respectively, and patients who were unable to do a sleep study were excluded. A convenient sampling method was used in this study. All the eligible patients were recruited after taking their inform consent. A proforma was used to obtained information from the case notes of the participants. This involves the socio-demographics, initial clinical presentations, Epworth score, Stopbang score, and the AHI result from the sleep study previously conducted for the patients.

Stopbang questionnaire

It is a reliable, concise, and easy-to-use screening tool for OSA.[6] It consists of eight dichotomous (yes/no) items related to the clinical features of sleep apnea. The total score ranges from 0 to 8. Patients can be classified for OSA risk based on their respective scores. The sensitivity of Stopbang score ≥ 3 to detect moderate to severe OSA (apnea-hypopnea index >15) and severe OSA (AHI > 30) is 93% and 100%, respectively.[6] Patients with a Stopbang score of 0 to 2 can be classified as low risk for moderate to severe OSA whereas those with a score of 5 to 8 can be classified as high risk for moderate to severe OSA.[6]

Epworth sleepiness scale (ESS)

It is a subjective measure of a patient's sleepiness with 8 items.[7] The test is a list of eight situations in which the tendency to become sleepy is rated on a scale of 0, no chance of dozing, to 3, high chance of dozing. The values of all the responses were added up. The total score is based on a scale of 0 to 24. Epworth score of > 10 indicates a high probability of OSA.[7]

Sleep study

Patients underwent a level 3 validated home sleep study with the polygraph system, ApneaLink device which is a pocket-sized, digital, multi-channel recording device that measures airflow through a nasal cannula connected to a pressure transducer, providing an AHI based on recording time.[8,9] It also can differentiate between obstructive and central events. Patients were instructed by nurses or physicians on how to operate the device for sleep recording. Respiratory events were scored when desaturations of at least 4% occurred in the absence of moving artifacts and irrespective of co-existing changes in snoring or heart rate. The ApneaLink default settings for apneas and hypopneas were used in this study. An apnea was defined as a decrease in the airflow by 80% of baseline for at least 10 s. Hypopnea was defined as a decrease in the airflow by 50% of baseline for at least 10 s. The AHI used for analysis was automatically analyzed by the ApneaLink software which was available for reviewing and rescoring by the clinician.[8,9]

Data analysis

The data were entered into excel and this was exported into SPSS version 26 for descriptive data analysis. The numerical demographic and clinical data e.g age was summarized with mean and standard deviation. Categorical variable e.g gender was summarized as frequencies and percentages. The Epworth score and Stopbang score were summarized on bar charts. The overall accuracy of Epworth and STOP-Bang was assessed by dividing summation of true positive and true negative by overall sample size.

Ethical approval

Ethical approval was obtained from the Ethics and Research Committee of LASUTH.

RESULTS

We selected 22 patients, including 15 (68.2%) males and 7 (31.8%) females, aged between 18 and 75 yr, with a mean age of 51.82±12.8 yr. The commonest presentations include snoring (20 or 90.9%), daytime somnolence (16 or 72.7%) and choking while sleeping (12 or 54.5%). The commonest co-morbidities were hypertension (16 or 72.7%) and obesity (6 or 27.3%) as shown in TABLE 1. STOP-Bang score (20 patients) indicated more patients with high clinical probability for OSA than Epworth score (12 patients) as shown in FIGURE1. Polysomnography showed evidence of sleep apnea in most of the patients with clinical suspicion ranging from mild to severe disease (3 or 13.6%, 3 or 13.6%, and 10 or 45.5%, respectively) as shown in FIGURE 2. The sensitivity of Epworth score and STOP-Bang score were 68.8% and 100%, respectively. While the specificity of Epworth score and STOP-Bang score were 83.3% and 33.3%, respectively. The overall accuracy of Epworth and STOP-Bang were 72.7% and 81.8% respectively as shown in TABLE 2. About 50% of the participants commenced weight loss while only nine people were able to commence CPAP treatment. Only four patients had sleep hygiene and mandibular advance device respectively.

TABLE 1. Socio-demographic and clinical characteristics of participants

Variable	Male	Female	Overall
Age (mean ± SD yr)	53.60 ± 13.4	48.00 ± 11.3	51.82 ± 12.8
BMI (mean ± SD kg/m^2)	34.58 ± 5.9	34.93 ± 5.9	33.33 ± 5.9
Symptoms [n (%)]			
• Snoring	14 (93.3)	6 (85.7)	20 (90.9)
• Daytime somnolence	11 (73.3)	5 (71.4)	16 (72.7)
• Choking while sleeping	8 (53.3)	4 (57.1)	12 (54.5)
• Tiredness	4 (26.7)	2 (28.6)	6 (27.3)
• Early morning headache	4 (26.7)	2 (28.6)	6 (27.3)
• Poor sleep	3 (20.0)	2 (28.6)	5 (22.7)
• Apnea	2 (13.3)	1 (14.3)	3 (13.6)
• Restlessness while sleeping	1 (6.7)	0 (0.0)	1 (4.5)
• Leg movement while sleeping	1 (6.7)	0 (0.0)	1 (4.5)
Comorbidity [n (%)]			
• Hypertension	11 (73.3)	5 (71.4)	16 (72.7)
• Diabetes	4 (26.7)	0 (0.0)	4 (18.2)
• Obesity	5 (33.3)	1 (14.3)	6 (27.3)

TABLE 2. Accuracy of Epworth score and Stopbang score in assessing OSA

Method	OSA status using AHI		Overall
	Absent	Present	
Epworth score			
• Low risk	5 (50.0)	5 (50.0)	72.7%
• High risk	1 (8.3)	11 (91.7)	
STOP-Bang score			
• Low risk	2 (100.0)	0 (0.0)	81.8%
• High risk	4 (20.0)	16 (80.0)	

*The accuracy of polysomnography for diagnosis of OSA was higher compared to Epworth and STOP-Bang questionnaires.

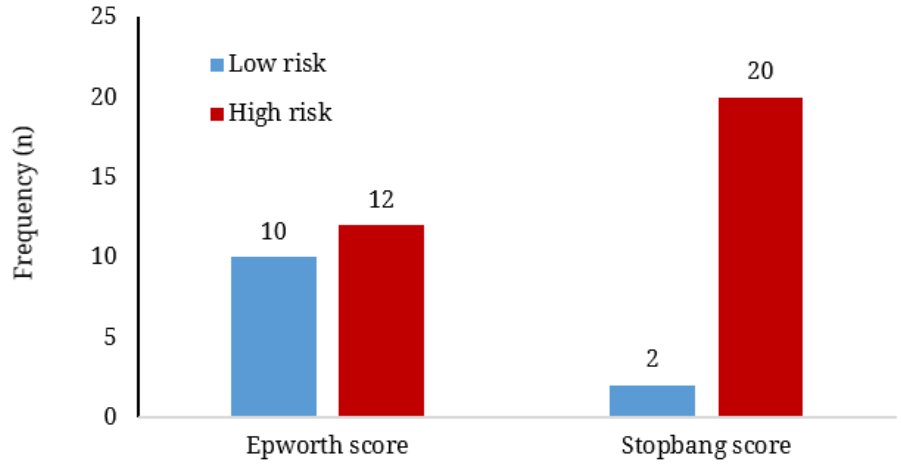


FIGURE 1. Sleep apnea assessment using Epworth and STOP-Bang questionnaires

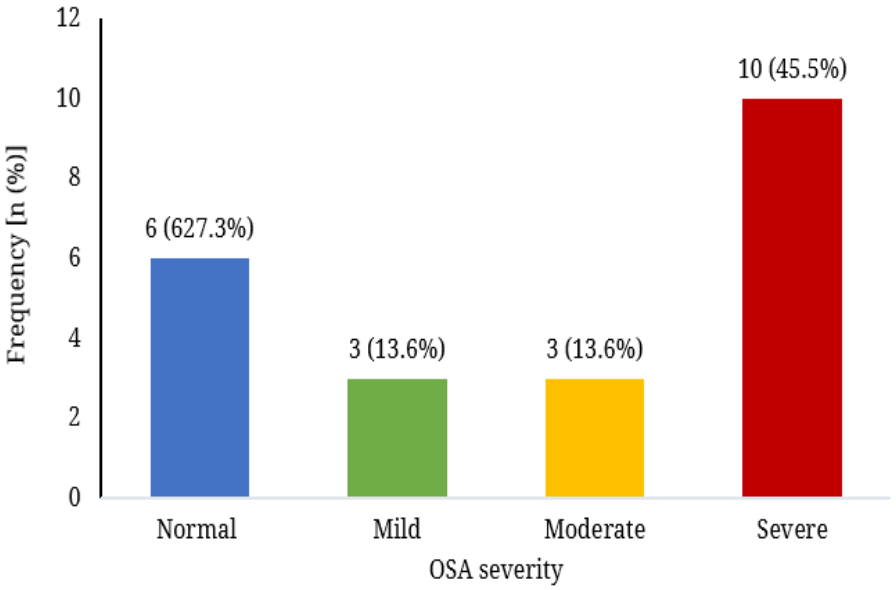


FIGURE 2. OSA classification among patients with suspected OSA

DISCUSSION

The objectives of this study were to describe the common presentations of OSA and to evaluate the usefulness of common screening assessment tools as well as home polysomnography in the diagnosis of patients with suspected OSA. The mean age of the participants was 51.82 ± 12.8 yr and majority were males. The commonest presentations include snoring, daytime somnolence, and choking while sleeping. The commonest comorbidities were hypertension and obesity. The STOP-Bang score identified more patients with a high clinical probability for OSA than the Epworth score. Polysomnography showed evidence of sleep apnea in most suspected patients. These findings imply that the use of combined Epworth and STOP-Bang questionnaires are a great tool in identifying patients with suspected cases of OSA, especially in a resource-limited environment like Lagos.

The high frequency of OSA among

middle-aged men in our study is similar to the report of Partinen *et al*.,[10] who noted that the prevalence of OSA is highest among men aged 40–65 yr. This is also corroborated by Al Lawati *et al*.,[11] who reported in a review that OSA is generally common, with moderate to severe disease present in approximately 9% of middle-aged men and 4% of women. Garvey *et al*.,[12] also reported that OSA is probably the most common respiratory disorder in the United States and Europe suggesting that between 14 and 49% of middle-aged men have clinically significant OSA. The prevalence of OSA in Nigeria was reported to be common among middle-aged people and ranges between 22% in men and 16% in women.[13] Desalu *et al*.,[14] reported that the frequency of high-risk for sleep apnoea increased with age and declined after 65 yr and also increased with the body mass index in a hospital-based study. This suggests that the epidemiology and prevalence of OSA are common in Nigeria and similar to what obtains in Europe and North America despite poor reporting.

Obstructive sleep apnea is associated with symptoms during sleep including snoring, choking, nocturia, wakefulness, excessive sleepiness, fatigue, and lack of energy.[15] Our findings suggest that the commonest presentations of patients with obstructive sleep apnea include snoring, daytime somnolence, and choking while sleeping. This is similar to the findings of Ohayon *et al*.,[16] who reported that snoring is very common in 35–45% of men and 15–28% of women with suspicion of OSA in a population-based study in the UK. Young *et al*.,[17] also reported that excessive daytime sleepiness is common and a poor discriminator in patients with OSAHS. The prevalence of snoring among patients assessed for OSA in Abuja was reported to be about 31% in a population-based study.[13] Akintunde *et al*.,[18] also reported a high prevalence of snoring in about 44.2% of university Community in South Western Nigeria. This suggests that common presentations of patients with OSA in Nigeria are not different from those reported elsewhere, and this should be appreciated by primary care clinicians to avoid underdiagnoses of this condition.

Our findings suggest that the commonest comorbidities were hypertension and obesity. This is similar to the report of Lacedonia *et al*.,[19] who reported that the prevalence of comorbidities was higher in patients affected by OSA, with arterial hypertension being the highest. Fusetti *et al*.,[20] in a descriptive study confirmed the existence of a statistically significant correlation between the severity of OSAS and BMI, ESS, average SO2, hypertension, diabetes mellitus, dyslipidemia, and metabolic syndrome. Desalu *et al*.,[14] in a multicenter observational hospital study showed that patients with systemic hypertension, obesity, excessive daytime sleepiness, history of smoking, snoring in a first-degree relative, and use of sedatives are at high risk of OSA. Other risk factors for OSA include heart failure among Africans.[18] Akanbi *et al*.,[21] reported that obesity and OSA may be more prevalent in Nigeria than previously predicted and obesity was found to independently increase OSA risk in a population-based study. The implication of this to practice in Nigeria is that patients seen in clinics with the comorbidities mentioned should be screened for OSA for early diagnosis and management. This may help to optimize the treatment of other background clinical conditions of such patients and improve their quality of life.

Our findings suggest that STOP-Bang score identified more patients with high clinical probability for OSA than the Epworth score. This is similar to the previous report that compared with the ESS, the SBQ is a more accurate tool for detecting mild, moderate, and severe

OSA.[22] Amra *et al*.,[23] in a cross-sectional study in Iran suggested that Berlin and STOP-Bang are more sensitive and accurate than ESS for OSA screening. A comparative study of the predictive values of OSA screening tools reported that the SB correctly identified more patients with OSA and SDB than the ESS alone.[24] The use of only ESS may not suffice for screening especially in patients who are not symptomatic for OSA that may have low clinical probability score. The use of STOP-Bang and ESS should be made readily available in the clinics for routine screening of patients with suspected risk factors.

Our findings revealed that polysomnography showed evidence of sleep apnea in most suspected patients. A study in France noted that Home-PSG is not feasible for about 33% of patients.[25] This figure most likely is much higher in our environment because of poor access and high cost of the device and test. Zou *et al*.,[26] reported that home sleep device is reasonably accurate for unattended home diagnosis of OSA. Su *et al*.,[27] also noted that home sleep monitoring device has good sensitivity, specificity, positive and negative predictive values for obstructive sleep apnea. Bilgin *et al*.[28] showed that the portable home sleep device can be used as an alternative diagnostic tool either at home or in sleep clinic for the diagnosis of OSA. The availability and affordability of sleep test is still a huge challenge in Nigeria. Currently, there are about 8 sleep centers in Lagos serving the population of about 20,000,000 Lagosians, of which 3 can perform level 1 (full polysomnography) sleep study. Only about 50% of patients with suspected OSA seen in the clinic are able to conduct sleep study due to the cost. It costs about 150-300 dollars depending on the center. There is a need for government and private health centers to invest in sleep medicine infrastructure to increase access and affordability for more Nigerians.

Our findings showed that only about nine patients with confirmed OSA were able to procure CPAP due to the high cost which ranges between 1000-1500 dollars. Majority of patients are not covered by insurance to cater for the treatment as in the case with other hospital services and would have to make out-of-pocket payments to use the device. This suggests that the treatment of diagnosed patients with OSA in Nigeria is still challenging dues to high cost of treatment. This calls for the attention of policy makers to capture the coverage of CPAP therapy under insurance for most Nigerians.

Limitations of this study

The study was performed using a small sample size and convenient sampling technique due to the limited number of patients available, hence the possibility of selection bias. Despite these limitations, this study highlights the importance of using the screening tools and polysomnography for evaluating patients with obstructive sleep apnea even in the absence of polysomnography especially in a resource limited setting like ours. This calls for more enlightenments of physicians in making use of the screening tools routinely in the clinics for assessing patients with risk factors for OSA.

CONCLUSION

These findings imply that the use of Epworth and STOP-Bang questionnaires combination is a great tool in identifying patients with suspected cases of OSA based on clinical presentations that will eventually benefit in a resource-limited environment from home sleep study like Lagos. There should be increased awareness on the use of this readily available and cheap questionnaire among physicians in Lagos for ease of OSAS diagnosis for many patients.

ACKNOWLEDGEMENT

Authors would like thank all participants were involved in this study.

REFERENCES

1. Kapur VK. Obstructive sleep apnea: diagnosis, epidemiology, and economics. Respir Care 2010; 55(9):1155-67.
2. McNicholas WT. Diagnosis of obstructive sleep apnea in adults. Proc Am Thorac Soc 2008; 5(2):154-60. https://doi.org/10.1513/pats.200708-118MG
3. Pevernagie DA, Gnidovec-Strazisar B, Grote L, Heinzer R, McNicholas WT, Penzel T, *et al*. On the rise and fall of the apnea− hypopnea index: A historical review and critical appraisal. J Sleep Res 2020; 29(4):e13066. https://doi.org/10.1111/jsr.13066
4. Anonym. Obstructive sleep apnea in adults: epidemiology, clinical presentation, and treatment options. Adv Cardiol 2011; 46:1-42. https://doi.org/10.1159/000327660
5. Punjabi NM. The epidemiology of adult obstructive sleep apnea. Proc Am Thorac Soc 2008; 5(2):136-43. https://doi.org/10.1513/pats.200709-155MG
6. Chung F, Abdullah HR, Liao P. STOP-Bang questionnaire: a practical approach to screen for obstructive sleep apnea. Chest 2016; 149(3):631-8. https://doi.org/10.1378/chest.15-0903
7. Johns MW. Sleepiness in different situations measured by the Epworth Sleepiness Scale. Sleep 1994; 17(8):703-10. https://doi.org/10.1093/sleep/17.8.703
8. Nigro CA, Dibur E, Malnis S, Grandval S, Nogueira F. Validation of ApneaLink Ox™ for the diagnosis of obstructive sleep apnea. Sleep Breath 2013; 17(1):259-66. https://doi.org/10.1007/s11325-012-0684-4
9. Nigro CA, Dibur E, Aimaretti S, González S, Rhodius E. Comparison of the automatic analysis versus the manual scoring from ApneaLink™ device for the diagnosis of obstructive sleep apnoea syndrome. Sleep Breath 2011; 15(4):679-86. https://doi.org/10.1007/s11325-010-0421-9
10. Partinen M, Telakivi T. Epidemiology of obstructive sleep apnea syndrome. Sleep 1992; 15(6 Suppl):S1-4. https://doi.org/10.1093/sleep/15.suppl_6.s1
11. Al Lawati NM, Patel SR, Ayas NT. Epidemiology, risk factors, and consequences of obstructive sleep apnea and short sleep duration. Prog Cardiovasc Dis 2009; 51(4):285-93. https://doi.org/10.1016/j.pcad.2008.08.001
12. Garvey JF, Pengo MF, Drakatos P, Kent BD. Epidemiological aspects of obstructive sleep apnea. J Thorac Dis 2015; 7(5):920-9. https://doi.org/10.3978/j.issn.2072-1439.2015.04.52
13. Adewole OO, Hakeem A, Fola A, Anteyi E, Ajuwon Z, Erhabor G. Obstructive sleep apnea among adults in Nigeria. J Natl Med Assoc 2009; 101(7):720-5. https://doi.org/10.1016/s0027-9684(15)30983-4
14. Desalu O, Onyedum C, Sanya E, Fadare J, Adeoti A, Salawu F, *et al*. Prevalence, awareness and reporting of symptoms of obstructive sleep apnoea among hospitalized adult patients in Nigeria: a multicenter study. Ethiop J Health Sci 2016; 26(4):321-30. https://doi.org/10.4314/ejhs.v26i4.4
15. Obstructive sleep apnea in adults: epidemiology, clinical presentation, and treatment options. Adv Cardiol 2011; 46:1-42. https://doi.org/10.1159/000327660
16. Ohayon MM, Guilleminault C, Priest RG, Caulet M. Snoring and breathing pauses during sleep: telephone interview survey of a United

Kingdom population sample. Bmj 1997; 314(7084):860-3. https://doi.org/10.1136/bmj.314.7084.860
17. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328(17):1230-5. https://doi.org/10.1056/NEJM199304293281704
18. Akintunde AA, Salawu AA, Opadijo OG. Assessment of Snoring and obstructive sleep apnoea in a Nigerian university: Association with cardiovascular risk factors. Niger Med J 2014; 55(6):469-73. https://doi.org/10.4103/0300-1652.144698
19. Lacedonia D, Carpagnano GE, Patricelli G, Carone M, Gallo C, Caccavo I, *et al*. Prevalence of comorbidities in patients with obstructive sleep apnea syndrome, overlap syndrome and obesity hypoventilation syndrome. Clin Respir J 2018; 12(5):1905-11. https://doi.org/10.1111/crj.12754
20. Fusetti M, Fioretti AB, Valenti M, Masedu F, Lauriello M, Pagliarella M. Cardiovascular and metabolic comorbidities in patients with obstructive sleep apnoea syndrome. Acta Otorhinolaryngol Ital 2012; 32(5):320-5.
21. Akanbi MO, Agaba PA, Ozoh OB, Ocheke AN, Gimba ZM, Ukoli CO, *et al*. Obesity and obstructive sleep apnea risk among Nigerians. J Med Trop 2017; 19(2):110-5. https://doi.org/10.4103/jomt.jomt_17_17
22. Chiu HY, Chen PY, Chuang LP, Chen NH, Tu YK, Hsieh YJ, *et al*. Diagnostic accuracy of the Berlin questionnaire, STOP-BANG, STOP, and Epworth Sleepiness Scale in detecting obstructive sleep apnea: A bivariate meta-analysis. Sleep Med Rev 2017; 36:57-70. https://doi.org/10.1016/j.smrv.2016.10.004
23. Amra B, Javani M, Soltaninejad F, Penzel T, Fietze I, Schoebel C, *et al*. Comparison of Berlin Questionnaire, STOP-Bang, and Epworth sleepiness scale for diagnosing obstructive sleep apnea in Persian patients. Int J Prev Med 2018; 9:28. https://doi.org/10.4103/ijpvm.IJPVM_131_17
24. Vana KD, Silva GE, Goldberg R. Predictive abilities of the STOP-Bang and Epworth Sleepiness Scale in identifying sleep clinic patients at high risk for obstructive sleep apnea. Res Nurs Health 2013; 36(1):84-94. https://doi.org/10.1002/nur.21512
25. Portier F, Portmann A, Czernichow P, Vascaut L, Devin E, Benhamou D, *et al*. Evaluation of Home versus Laboratory Polysomnography in the Diagnosis of Sleep Apnea Syndrome. Am J Respir Crit Care Med 2000; 162(3 Pt 1):814-8. https://doi.org/10.1164/ajrccm.162.3.9908002
26. Zou D, Grote L, Peker Y, Lindblad U, Hedner J. Validation a portable monitoring device for sleep apnea diagnosis in a population based cohort using synchronized home polysomnography. Sleep 2006; 29(3):367-74. https://doi.org/10.1093/sleep/29.3.367
27. Su S, Baroody FM, Kohrman M, Suskind D. A comparison of polysomnography and a portable home sleep study in the diagnosis of obstructive sleep apnea syndrome. Otolaryngol Head Neck Surg 2004; 131(6):844-50. https://doi.org/10.1016/j.otohns.2004.07.014
28. Bilgin C, Erkorkmaz U, Ucar MK, Akin N, Nalbant A, Annakkaya AN. Use of a portable monitoring device (Somnocheck Micro) for the investigation and diagnosis of obstructive sleep apnoea in comparison with polysomnography. Pak J Med Sci 2016; 32(2):471-5. https://doi.org/10.12669/pjms.322.9561

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 53-59
https://doi.org/10.19106/JMedSci005501202307

The difference in biofilms formations on duration less than 90 d and more than 90 d of tracheotomy cannula usage

Pradhana Fajar Wicaksana*, Dian Paramita Wulandari, Angga Kusuma, Siswanto Sastrowijoto

Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

Submitted: 2021-11-07
Accepted : 2022-11-28

ABSTRACT

Currently, prevention of local and systemic infections caused by implantable devices is increasingly improved. Tracheostomy is a surgical action followed by an implantable device called tracheotomy cannula into a trachea to maintain upper airway patenting. The incidence of biofilm-related complications and infections is associated with the length of duration of the attached tracheostomy. The formation and spread of biofilms from distal cannula increase the infection incidence in stoma, tracheitis, and even peripheral pneumonia. However, until now there has been no consensus on when the tracheostomy replacement supposedly conducted. Some manufacturers recommend that cannula replacement supposedly conducted within 30 d, but the data are not yet in agreement and need further study. This study aimed to determine the difference in biofilms formations in a duration of less than 90 d and more than 90 d of tracheotomy cannula usage. It was a cross-sectional study involving patients who underwent a tracheostomy at the Department of Otorhinolaryngology of Dr. Sardjito General Hospital, Yogyakarta. Fisher exact test was applied to analyze the biofilms formations of the two different duration of tracheostomy cannula usage. A total of 20 patients were involved in this study. Durations of more than 90 d had more biofilms formations compared to less than 90 d, although it was not significantly different ($p>0.05$). However, the PR value of 6 indicated that subjects who have attached cannula more than 90 d clinically have 6 times higher risk for developing biofilms formations than those less than 90. In conclusion, there is no significant differences in biofilms formations between the less than 90 d and more than 90 d of tracheostomy cannula usage. However, clinically subjects with longer duration of tracheostomy cannula usage have higher risk for developing biofilms formations.

Keywords:
bacterial biofilm;
duration of tracheotomy cannula;
complication;
risk factor;
tracheostomy care

ABSTRAK

Saat ini, pencegahan infeksi lokal dan sistemik yang disebabkan oleh alat implan semakin berkembang. Trakeostomi adalah tindakan pembedahan yang diikuti dengan implan kanula trakeostomi ke dalam trakea untuk mempertahankan patensi jalan napas atas. Insiden komplikasi dan infeksi terkait biofilm dikaitkan dengan lamanya durasi kanul trakeostomi yang terpasang. Pembentukan dan penyebaran biofilm dari kanula distal meningkatkan kejadian infeksi pada stoma, trakeitis, dan bahkan pneumonia perifer. Namun demikian, sampai saat ini belum ada konsensus mengenai kapan seharusnya penggantian trakeostomi dilakukan. Beberapa industri merekomendasikan agar penggantian kanula seharusnya dilakukan dalam 30 hari, namun data tersebut belum sesuai dan perlu kajian lebih lanjut. Penelitian ini bertujuan untuk mengetahui perbedaan pembentukan biofilm pada durasi kurang dari 90 hari dan lebih dari 90 hari penggunaan kanula trakeotomi. Penelitian ini merupakan studi potong lintang yang melibatkan pasien yang menjalani trakeostomi di Departemen THT RSUP Dr. Sardjito Yogyakarta. Uji eksak Fisher digunakan untuk menganalisis pembentukan biofilm dari dua durasi

*corresponding author: pradhanaf.mail.ugm.ac.id

penggunaan kanula trakeostomi yang berbeda. Sebanyak 20 pasien dilibatkan dalam penelitian ini. Durasi lebih dari 90 hari memiliki pembentukan biofilm yang lebih banyak dibandingkan kurang dari 90 hari, meskipun tidak berbeda nyata (p>0,05). Namun, nilai PR 6 menunjukkan bahwa subjek yang dipasang kanula lebih dari 90 hari secara klinis memiliki risiko 6 kali lebih tinggi untuk terbentuknya biofilm dibandingkan mereka yang kurang dari 90. Kesimpulannya, tidak ada perbedaan nyata dalam pembentukan biofilm antara kurang dari 90 d dan lebih dari 90 hari penggunaan kanula trakeostomi. Namun, secara klinik subjek dengan durasi penggunaan kanula trakeostomi yang lebih lama memiliki risiko lebih tinggi untuk terbentuknya biofilm.

INTRODUCTION

Historically, a tracheostomy represented the only treatment available for upper airway obstruction. Today, tracheotomy remains an important indication for tracheostomy, although numerous others intervention are available. A tracheostomy may be required in an emergent setting to bypass an obstructed airway, or more commonly, may be placed electively to facilitate mechanical ventilation, to wean from a ventilator, or to allow more efficient management of secretions referred to as pulmonary toilet, among other reasons. Although tracheostomy is mostly temporary, there are special conditions such as impaired airway function or unresolved conditions in which long-term or even permanent tracheostomy should be performed.[1,2] Due to long-term tracheostomy, it can cause some local or systemic infections. Infection can occur due to the accumulation of various kinds of microorganisms on the surface of the cannula which could form a biofilm.[2,3]

The biofilm formation on medical devices such as tracheostomy can lead to a chronic infection in the surrounding area as well as systemic infection. The biofilm formation has also been reported on other medical devices such as in venous catheters, urinary catheters, prosthetic heart valves, contact lenses, and intrauterine devices (IUDs). The Centers for Disease and Prevention estimated that more than 65% of chronic bacterial infections in humans are related to biofilms, and according to the National Institutes of Health it is as much as 80%.[4]

The duration of tracheostomy use has been related to tracheitis which is associated with the thickness of the biofilm that becomes attached to the inner cannula and can increase the risk of plugging, local lesions of the stoma including granulation, and peripheral pneumonia due to the release and spread of biofilms into the lungs.[5,6]

There is no consensus or universal guideline on when to replace a tracheostomy cannula. Most tracheostomy manufacturers mentioned that 30 d is the limit for replacing the cannula even though no agreement has been reached.[7] One author mentioned that degradation of the cannula will occur in 3 mo after the cannula has been inserted.[8] This event can be one of the indicators of the presence of biofilms.[9]

The purpose of this study was to determine the difference in bacterial biofilms with a duration of less than 90 d and more than 90 d of tracheostomy cannula usage at the Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

MATERIALS AND METHODS

Design of study

This study used a cross-sectional research design conducted at the Departement of Otorhinolaryngology (ENT), Dr. Sardjito General Hospital, Yogyakarta, from August 2020 to January 2021. The protocol of the study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (KE/FK/0843/EC/2020).

Procedure

Twenty patients who meet the inclusion and exclusion were involved in this study. The inclusion criteria were patients who underwent tracheostomy procedures at Dr. Sardjito General Hospital, Yogyakarta conducted by the Department of ENT and willing to participate in the study. Patients with an abscess around the stoma were excluded in this study.

The dependent variable was assessed by the presence or absence of a biofilm and the main independent variable was the duration of the insertion of the cannula divided by less than 90 d and more than 90 d. The course of the study was to collect patients who have undergone tracheostomy procedures by the Department of ENT at Dr. Sardjito General Hospital, Yogyakarta and sample selection was based on inclusion and exclusion criteria. Subjects' samples of tracheostomy were sent to the Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada for biofilm tests of culture isolates.

Biofilm testing

The cannula that has been removed from the patients would be put in a special container. The sampling was conducted on the part of the cannula that looks wet and dirty, and then it would be planted on blood agar and McConkey media for 48 h at 37 ^{o}C. The used cannula would be put on autoclave machine and it would be destroyed early. Each colony was taken with a sterile ose needle and inoculated with a microplate which added by 200 µL acid isopropanol 5%. The microplate formed the biofilm would be dark in color and the absorption was read at a λ 595 nm. To obtain quantitative data, readings were carried out with a microplate reader.

The results of biofilm formation were seen from the value of ODc and OD of growth control. The ODc value was obtained from the mean OD of negative control + 3x standard deviation (SD) of negative control. The results of biofilms obtained varying results: OD ≤ ODC means no biofilm, OD bacteria > ODc and ≤ 2x ODc values means a weak biofilm, OD bacteria > 2x ODc values and ≤ 4x ODC values means a moderate biofilm, OD bacteria > 4x ODc values means a strong biofilm. However, this study only aimed to see whether there was a biofilm or not, so the limitation of variation was not analyzed further.

Statistical analysis

The results of the study were presented in frequency distribution tables of the characteristics of the research subjects and an overview of each research variable with prevalence ratio (PR) using a 95% confidence interval (CI). Statistical analysis used Fisher exact tests with $p < 0.05$ was considered significant.

RESULTS

A total of 20 subjects were involved in this study. The sample size calculation obtained 20 subjects for each group with a minimum of 10 subjects in a duration of less than 90 d and 10 subjects in a duration of more than 90 d. The characteristics of subjects are presented in TABLE 1. There were 13 (65%) males compared to only 7 (35%) females. Adults with age 20 to >60 y were 11 (55%) subjects, and children aged 2 to 19 y were 9 (45%) subjects. For educational background, most of the subjects have attended school with as many as 17 (85%) subjects, compared to only 3 (15%) who have not attended school.

As many as 15 (75%) subjects performed routine daily washing of the cannula 3-4 times, while 5 (25%) subjects washed the cannula twice. The subjects with the duration of the tracheostomy cannula less than 90 d were 10 (50%), while those with more than 90 d were 10 (50%). Biofilms were obtained from

as many as 13 (65%) samples, and for those that did not contain biofilms were 7 (35%) samples.

There were 18 (100%) subjects with no complications of pneumonia, and 0 (0.0%) had no data from X-ray thoracic imaging. There were 7 (33.3%) subjects who had stoma granulation, and 13 (66.7%) without stoma granulation. Subjects with a history of active smoking were 10 (50%) subjects, while 10 (50%) subjects were not active smokers.

The most common indications for tracheostomy were due to airway obstruction in 19 (95%) subjects and prolonged endotracheal tube in 1 (5%) subject. Most subjects had gram-negative bacteria when the bacterial culture was performed in as many as 14 (70%) subjects compared to gram-positive bacteria in 6 (30%) subjects.

TABLE 1. Characteristics of research subjects

Characteristics	Frequency [n (%)]
Gender	
• Female	7 (35.0)
• Male	13 (65.0)
Age	
• Child (2 s/d 19 y.o.)	9 (45.0)
• Adult (20 s/d >60 y.o.)	11 (55.0)
Education	
• Not in school yet	3 (15.0)
• Elementary-High School	17 (85.0)
Wash times	
• 2x/day	5 (25.0)
• 3-4x/day	15 (75.0)
Duration	
• <90 day	10 (50.0)
• >90 day	10 (50.0)
Biofilm	
• No biofilm	7 (35.0)
• Yes biofilm	13 (65.0)
Pneumonia	
• No	18 (100.0)
• Yes	0 (0.0)
Stoma granulation	
• No	13 (65.0)
• Yes	7 (35.0)
Smoking	
• No	10 (10.0)
• Yes	10 (10.0)
Tracheotomy indication	
• Upper airway obstruction	19 (95.0)
• Prolonged endotracheal intubation	1 (5.0)
Bacteria type	
• Gram (-)	14 (70.0)
• Gram (+)	6 (30.0)

To evaluate the difference in biofilm formations in less than 90 d and more than 90 d of tracheostomy cannula usage, Chi-square tests was performed. However, due to it did not meet the feasibility of the Chi-square test, additionally performed Fisher's exact tests was performed (TABLE 2).

TABLE 2. Fisher's exact test to determine biofilm differences in several durations of tracheostomy cannula usage

Duration	No biofilm [n (%)]	Biofilm [n (%)]	p	PR	CI 95%
• <90 d	6 (60.0)	4 (40.0)	0.029*	6.0	1.07-41.21
• >90 d	1 (10.0)	9 (90.0)			

TABLE 3. Other variables tested for association with biofilm occurrence

Characteristics	Non biofilm [n (%)]	Biofilm [n (%)]	p	PR	95% CI
Gender					
• Female	2 (28.6)	5 (71.4)	0.526	0.74	0.19-2.89
• Male	5 (38.5)	8 (61.5)			
Age					
• Children (2 - 19 y.o.)	4 (44.4)	5 (55.6)	0.370	1.63	0.49-5.47
• Adult (20 - >60 y.o.)	3 (27.3)	8 (72.7)			
Education					
• Not in school yet	2 (66.7)	1 (33.3)	0.270	2.27	0.76-6.73
• Elementary – High School	5 (29.4)	12 (70.6)			
Wash time					
• 2x/day	2 (40.0)	3 (60.0)	0.594	1.20	0.33-4.36
• 3-4x/day	5 (33.3)	10 (66.7)			
Smoking					
• No	3 (30.0)	7 (70.0)	0.500	0.75	0.22-2.52
• Yes	4 (40.0)	6 (60.0)			
Tracheotomy indication					
• Upper airway obstruction	7 (36.8)	12 (63.2)	0.650	0.63	0.45-0.89
• Prolonged ET	0 (0.0)	1 (100.0)			
Bacterial type					
• Gram (-)	4 (28.6)	10 (71.4)	0.336	0.57	0.18-1.81
• Gram (+)	3 (50.0)	3 (50.0)			
Biofilm strength					
• Weak - moderate	2 (13.3)	13 (86.7)	-	-	-
• Strong	0 (0.0)	0 (0.0)			
Pneumonia					
• No	6 (31.6)	13 (68.4)	-	-	-
• Yes	1 (100.0)	0 (0.0)			
Stoma granulation					
• No	3 (25.0)	9 (75.0)	0.561	0.75	0.17-3.35
• Yes	2 (33.3)	4 (66.7)			

There were significant differences in the results of biofilm cultures in several durations of using a tracheostomy cannula (p=0.029). In the cannula duration of more than 90 d, more biofilm was found than in less than 90 d. Clinically, the PR value was 6, meaning that subjects who had a cannula for more than 90 d had a 6 times higher risk for developing biofilms formation than subjects with less than 90 d.

TABLE 3 shows the relationship of other variables as risk factors for the presence or absence of biofilm formation. The results showed that there were no significant differences in all tested groups (p> 0.05).

DISCUSSION

The processes of biofilm formation on the cannula occurs gradually over time. Shortly after the cannula is inserted into the body, the surface of the biomaterial is rapidly covered by a layer of protein, fibrin, platelets, and other elements known as a film, which changes the surface properties of the biomaterial.[10] Ravendraa *et al*.,[11] reported that biofilms began to appear on the tracheostomy cannula as early as 7 d after insertion.

At this initial stage, the actual attachment of microorganisms can easily be removed by routine cleaning. However, the cannula is always exposed to fluid from the stoma and trachea which is rich in bacteria causing significant changes in biomaterials. The changes of the surface of the cannula cause bacterial adhesion to be easier, so that the bacterial attachment will be irreversible. Costerton *et al*.,[12] reported that biomaterial change in the cannula caused a substratum effect that could increase the attachment of bacteria to a rougher surface. Backman *et al*.,[7] described the greatest severity of biomaterial degradation of the tracheostomy cannula occurring at 3 - 6 mo, when the cannula may become brittle and discolored.

This fragility and degradation can serve as indicators of the presence of a biofilm. The National Respiratory Center (NRC) reported that the cannula can still be used if there is no visible color and no broken cannula fragments are found. The NRC conducted a further study and determined that if the cannula is made of silicone it can be used for up to 85 d, while PVC can last up to 56 d and polyurethane (PU) for up to 51 d. Kumarasinghe *et al*.,[14] explained that the interval of cannula replacement should be 4 wk until 3 mo after insertion in order to prevent any further degradation and complications. Norkahfi *et al*.,[15] also recommended that the tracheostomy cannula should be replaced in less than 3 mo because the damage to the cannula reaches its peak within 3 mo due to various causes, especially damage to the surface properties of the cannula due to biofilm.

Some limitations of this study including this study was conducted not in serial form, but taking sample at one time only. Therefore, the independent variable that mostly influenced on biofilm formation could not be controlled. Furthermore, this study only described in general the presence or absence of a biofilm on the cannula because from the results of the study. Only one type of biofilm classified as strong biofilm was observed. Further study is needed to evaluate the microorganisms that involved in the biofilm formation.

CONCLUSION

In conclusion, there is no significant differences in bacterial biofilms formation between the less than 90 d and more than 90 d of tracheostomy cannula usage. More biofilms are found in cannula with durations of more than 90 d compared to cannula with durations of less than 90 d.

ACKNOWLEDGEMENT

Authors would like to thank our colleagues from the Department of

Microbiology, Faculty of Medicine, Public Health and Nursing who conducted the examination of biofilms formations.

REFERENCES

1. Raimonde AJ, Westhoven N, Winters R. (2021, December 29). Tracheostomy - StatPearls - NCBI Bookshelf. Tracheostomy. Retrieved July 3 2022. https://www.ncbi.nlm.nih.gov/books/NBK559124/
2. Wolcott R, Dowd S. The role of biofilms: are we hitting the right target? Plast Reconstr Surg 2011; 127(Suppl 1):28s-35. https://doi.org/10.1097/prs.0b013e3181fca244
3. Kinnari TJ. The role of biofilm in chronic laryngitis and in head and neck cancer. Curr Opin Otolaryngol Head Neck Surg 2015; 23(6):448-53. https://doi.org/10.1097/moo.0000000000000200
4. Roberts CG. The role of biofilms in reprocessing medical devices. Am J Infect Control 2013; 41(5 Suppl):77-8. https://doi.org/10.1016/j.ajic.2012.12.008
5. Rodney J, Ojano Dirain CP, Antoneli PJ, Silva RC. Effect of repeated tracheostomy tube reprocessing on biofilm formation. Laryngoscope 2016; 126(4):996-9. https://dx.doi.org/10.1002%2Flary.25473
6. David SE, Ho B, Kent SM, Hughes CA, Mattenburg D, Rueggeberg FA, *et al*. Changes in pediatric tracheostomy tubes exposed to home dishwashing. Int J Pediatr Otorhinolaryngol 2017; 100:96-102. https://doi.org/10.1016/j.ijporl.2017.06.020
7. Backman S, Bjorling G, Johansson UB, Lysdahl M, Markström A, Schedin U, *et al*. Material wear of polymeric tracheostomy tubes: a six-month study. Laryngoscope 2009; 119(4):657-64. https://doi.org/10.1002/lary.20048
8. Perkins J, Mouzakes J, Pereira R, Manning S. Bacterial biofilm presence in pediatric tracheostomy tubes. Arch Otolaryngol Head Neck Surg 2004; 130(3):339-43. https://doi.org/10.1001/archotol.130.3.339
9. Zhu H, Das P, Brereton J, Roberson D, Shah RK. Surveillance and management practices in tracheotomy patients. Laryngoscope 2012; 122(1):46-50. https://doi.org/10.1002/lary.22375
10. Cipriano A, Mao ML, Hon HH, Vazquez D, Stawicki SP, Sharpe RP, *et al*. An overview of complication associated with open and percutaneous tracheotomy procedures. Int J Crit IIIn Inj Sci 2015; 5(3):179-88. https://doi.org/10.4103/2229-5151.164994
11. Raveendra N, Rathnakara SH, Haswani N, Subramaniam V. Bacterial biofilms on tracheostomy tubes. Indian J Otolaryngol Head Neck Surg 2021; 6:1-5. htpps://doi.org/10.1007/s12070-021-02598-6
12. Costerton JW, Stewart PS. Battling biofilms. Sci Am 2001; 285(1):74-81. https://doi.org/10.1038/scientificamerican0701-74
13. Wood TK. Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling. Environ Microbiol 2009; 11(1):1-15. https://doi.org/10.1111/j.1462-2920.2008.01768.x
14. Kumarasinghe D, Wong EH, Duvnjak M, Smith MC, Palme CE, Riffat F. Colonization rates of tracheostomy tubes associated with the frequency of tube changes. ANZ J Surg 2020; 90(11):2310-4. https://doi.org/10.1111/ans.15970
15. Norkahfi MR, Marina MB, Wawaddah A, Abdullah SM. Prolonged placement of trachestomy tube causing unusual complication. Med & Health 2016; 11(2):298-302. https://doi.org/10.17576/MH.2016.1102.19

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 60-69
https://doi.org/10.19106/JMedSci005501202308

The effect of appropriate antibiotic use on the length of hospital stay in deep neck abscess (DNA) patients

Hesti Dyah Palupi*, Dian Paramita Wulandari, Feri Trihandoko

Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital Yogyakarta, Indonesia.

Submitted: 2021-12-27
Accepted : 2022-12-12

ABSTRACT

Deep neck abscess (DNA) is an emergency in the otorhinolaryngology head and neck surgery field due to the formation of abscesses in the potential space between the deep neck fasciae. It is typically caused by the expansion of infection from various sources, including the teeth, mouth, throat, paranasal sinuses, middle ear, and neck. The increase of DNA cases needs for improvement of patient management especially when the patients have comorbidities which lead to an extended length of treatment. The study aimed to evaluate the appropriateness of empirical antibiotics given according to culture results and any comorbid factors that affect the length of hospital stay (LOHS). It was case-control observational study involving 44 cases of DNA patients who treated at Dr. Sardjito General Hospital Yogyakarta in the period of January 2018 to December 2020. The patients were divided into two groups with 22 patients in each group. The first group was the DNA patients with > 7 d LOHS and the second one was those with ≤ 7 d. No significantly relationship was observed between variables evaluated included the appropriate antibiotic use (p=0.546). However, dental caries (DC) was significantly related with the LOHS (p=0.015). In conclusion, there is no relationship between the appropriate antibiotic use and the LOHS. However, the DC is risk factor that influence the LOHS in patients with DNA.

ABSTRAK

Abses leher dalam (ALD) merupakan kegawatan di Bidang THT-KL akibat terbentuknya abses di dalam ruang potensial di antara fasia leher. Hal ini umumnya disebabkan adanya perluasan infeksi dari berbagai sumber, seperti dari gigi, mulut, tenggorok, sinus paranasal, telinga tengah dan leher. Meningkatnya kasus ALD memerlukan perbaikan dalam penanganan pasien khususnya jika pasien mempunyai factor penyerta yang menyebabkan perpanjangan masa perawatan. Penelitian ini bertujuan untuk mengkaji kesesuaian pemberian antibiotik secara empiris berdasarkan hasil kultur dan faktor penyerta terhadap lama perawatan di rumah sakit (LPRS). Penelitian observasional dengana rancangan kasus kontrol ini melibatkan 44 kasus ALD yang menjalani perawatan di RSUP Dr. Sardjito, Yogyakarta periode Januari 2018 sampai Desember 2020. Pasien dibagi dalam dua kelompok dengan masing-masing kelompok terdiri dari 22 pasien. Kelompok pertama adalah pasien ALD dengan LPRS >7 hari dan kelompok kedua dengan LPRS ≤7 hari. Tidak terdapat hubungan nyata yang teramati antara variabel yang dievaluasi termasuk penggunaan antibiotik yang tepat (p=0,546). Namun demikian, karies gigi berkaitan nyata dengan LPRS (p=0,015). Dapat disimpulkan tidak ada hubungan antara penggunaan antibiotik yang tepat dan LPRS. Namun demikian karies gigi merupakan faktor risiko yang mempengaruhi LPRS pasien ALD.

Keywords:
deep neck abscess;
length of hospital stay;
appropriate antibiotic use;
empirical antibiotic;
dental caries

*corresponding author: hestitht@yahoo.com

INTRODUCTION

Deep neck abscess (DNA) is an emergency condition in the otorhinolaryngology head and neck surgery field. The formation of an abscess in the potential space of the deep neck fascia is typically due to the expansion of infection from various sources, such as teeth, mouth, throat, paranasal sinuses, middle ear, and neck. The DNA may lead to sepsis, which is a condition that triggers the patient's condition in multi-organ failure. These conditions are dreadful, so the management needs to be more comprehensive, and it must be taking more time than usual.[1-5]

The diagnosis and management of DNA is a difficult challenge in otolaryngology because of its complexity and depth of involvement of the abscess site, the incidence of multibacterial infection, and the compatibility of antibiotics given with the results of bacterial cultures in DNA. Changes in the pattern of bacteria and resistance to antibiotics have contributed to an increase in the incidence of DNA. Antibiotics play an important role in the hospital environment, especially inpatient rooms, and intensive care units where many patients are administered antibiotics as prophylaxis. Successful treatment and prevention of resistance depends on using antibiotics wisely. Inappropriate antibiotic use can have a negative impact on patients' outcomes.[6] In severe cases, incision and drainage procedure can be performed if the airway is stable by transcervical and transoral approach and the pus can be obtained by needle aspiration.[7] In Dr. Sardjito General Hospital, Yogyakarta the antibiotic for standard therapy derived from an empirical study was firstly stipulated since around end of 2018. It came up with combination of ceftriaxone and metronidazole based on the previous study.[8]

Study concerning evaluating the appropriateness of the use of antibiotics and risk factors related to length of hospital stay (LOHS) in patients with DNA at Dr. Sardjito General Hospital has never been conducted. Brito *et al.*[8] reported morbidity factors that can increase the LOHS of the inpatient with DNA such as diabetes mellitus (DM), and obesity, and identified that those comorbidities with the extending abscess, and the location of the potential space of the abscess. Other studies conducted by Kauffman *et al.*,[9] and O'Brien *et al.*,[10] also reported that there are some factors affecting the rise of LOHS stay, such as age, ASA class, repeat procedure, Charlson comorbidity index, cardiopulmonary diseases, patients with multiple space infections, and DM.

This study aimed to investigate the appropriate antibiotic use and the risk factors that may contribute to the LOHS. The results can provide the latest information on empirical antibiotics with the latest bacterial culture and inform about the risk factors related to the LOHS, therefore they can be used as a future guideline.

MATERIALS AND METHODS

Design

The study used a case control design to investigate the relationship between appropriate antibiotic use with LOHS in patients with DNA. The study was conducted by collecting data from the medical records of patients with DNA at Dr. Sardjito General Hospital, Yogyakarta in the period of January 2018 to December 2020.

Procedure

Patients with DNA who meet the inclusion and exclusion criteria were involved in this study. The inclusion

criteria were the patients with DNA who had received intravenous antibiotics in their first admission at once, had been performed culture and sensitivity test of bacteria from pus sample with detected bacteria growth and sensitivity of antibiotic to the bacteria, and had finished the treatment until patients leaving the hospital. Complete data of medical records must be complied with to meet the inclusion criteria. The exclusion criteria were patients with DNA without complete treatment, such as patients who had passed away before treatment finished or patients discharged.

In this study, the appropriate antibiotic use was when a sensitive value to bacterial culture and sensitivity test results were suitable to the regimen administered beforehand. The LOHS was described as the duration of a single episode of hospitalization. It was measured from the patient's admission to the patients discharge.

According to the calculation of the research sample obtained with minimal sample was 22 samples in each group. The case group was the group of patients who were diagnosed with DNA with LOHS for > 7 d and the control group was the group of patients with DNA who stayed for ≤ 7 d.

The protocol of study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta, Indonesia with the reference number KE/FK/0152/EC/2021.

Statistical analysis

After the data from the medical records were collected, then the results were tabulated with percentages and means with 95% confidence interval (CI), then analyzed by using Chi-square tests and logistic regression analysis for multivariate analysis with significance set as $p<0.05$.

RESULTS

The presentation of the results of the data analysis used descriptive and statistical analysis, which was divided into two main parts. The first part was the characteristics of the research subjects, and the second part was the main results which contained the analysis of the relationship of appropriate antibiotics to the LOHS in patients with DNA.

A total of 44 patients with DNA who met the inclusion and exclusion criteria were involved in this study. For the case and control groups, 22 patients were diagnosed as DNA with hospitalization of > 7 d, and 22 patients were diagnosed with DNA with hospitalization of ≤ 7 d. From the 44 patients diagnosed with abscess, the age range was between 6 to 79 y.o., and 5 of them experienced complications of upper airway obstruction so that a tracheostomy was needed (TABLE 1). Moreover, it was found that only dental caries (DC) was significantly correlated with the LOHS ($p = 0.015$), whereas other variables were not correlated with the LOHS ($p> 0.05$). This finding shows that the presence of DC in DNA patients is the only variable that significantly affected the LOHS (TABLE 1).

The location of the extension of DNA involved the submandibular space, parotid parapharynx, pretrachea, masticator, buccal, retropharynx, and peritonsillar. Bacterial culture results in DNA patients mainly found *Streptococcus* spp., *Staphylococcus* spp., and *Acinetobacter* spp. Bacteria (TABLE 2).

TABLE 1. Characteristics based on LOHS

Variable	LOHS >7 d	LOHS ≤ 7 d	Total	p
	n_1	n_2	n	
Gender				
• Male	11	18	29	0.056
• Female	11	4	15	
Age				
• Geriatric	9	6	15	0.525
• Non-geriatric	13	16	29	
DM				
• Yes	13	7	20	0.130
• No	9	15	24	
Hypertension				
• Yes	7	3	10	0.280
• No	15	19	34	
GIT bleeding				
• Yes	4	2	6	0.664
• No	18	20	38	
IDP				
• Yes	20	17	37	0.412
• No	2	5	7	
DC				
• Yes	17	8	25	0.015
• No	5	14	19	
IRF				
• Yes	6	4	10	0.719
• No	16	18	34	
Mediastinitis				
• Yes	1	2	3	1.000
• No	21	20	41	
Sepsis				
• Yes	2	2	4	1.000
• No	20	20	40	

DM: diabetes mellitus; IDP: incision and drainage procedure; CD: caries dentis; IRF: impaired renal function

TABLE 2. Culture and antibiotic sensitivity test results

Patient	Bacteria	Antibiotic	
		Sensitive	Resistence
53/M	*Klebsiella pneumoniae*	Gentamicin, amikacin, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole, aztreonam	Ampicillin
36/F	*Streptococcus viridans*	Gentamicin, ampicillin/sulbactam, cefuroxime, ceftriaxone, ciprofloxacin, levofloxacin	None
53/F	*Prevotella oralis*	Penicillin, erythromycin, ampicillin/sulbactam, cefuroxime, ceftriaxone, tetracycline, chloramphenicol, metronidazole, colistin	Cefazolin
72/F	*Acinetobacter baumannii*	Amikacin, trimethoprim/sulfamethoxazole	Gentamicin, Tigecycline
55/M	*Staphylococcus gallinarum*	Azithromycin, amikacin, ampicillin/sulbactam, cefuroxime, cefoxitin, ertapenem, imipenem, meropenem, tetracycline, trimethoprim/sulfamethoxazole	Clindamycin, Ciprofloxacin
43/M	*S. aureus*	Gentamicin, cefepime, trimethoprim/sulfamethoxazole, aztreonam	None
36/M	*Corynebacterium sp*	Imipenem, linezolid	Cefotaxime
21/M	*Micrococcus sp.*	Ampicillin, penicillin, clindamycin, oxacillin, ampicillin/sulbactam, cefuroxime, cefoxitin, ciprofloxacin, vancomycin, ceftazidime	Tetracycline
62/F	*A. baumannii*	Gentamicin, cefepime, ciprofloxacin, trimethoprim/sulfamethoxazole, aztreonam, ceftazidime	None
38/M	*S. viridans*	Ampicillin, erythromycin, clindamycin, ampicillin/sulbactam, cefuroxime, ciprofloxacin, imipenem, doxycycline, chloramphenicol	Cefoxitin, Ceftriaxone, Tetracycline
60/F	*A. baumannii*	Gentamicin, ampicillin/sulbactam, ciprofloxacin, meropenem, trimethoprim/ sulfamethoxazole, ceftazidime	Ceftriaxone
24/M	*S. anginosus*	Ampicillin, penicillin, azithromycin, erythromycin, clindamycin, oxacillin, cefuroxime, cefepime, ciprofloxacin, levofloxacin, imipenem, linezolid, trimethoprim/ sulfamethoxazole, chloramphenicol, moxifloxacin, trimethoprim/sulfamethoxazole, ceftazidime	Amikacin, Cefoxitin, Ceftriaxone, Tetracycline
17/F	*S. anginosus*	Ampicillin, penicillin g, azithromycin, erythromycin, clindamycin, oxacillin, cefuroxime, cefepime, ciprofloxacin, levofloxacin, imipenem, linezolid, trimethoprim/ sulfamethoxazole, chloramphenicol, moxifloxacin, trimethoprim/sulfamethoxazole, ceftazidime	Amikacin, Cefoxitin, Ceftriaxone, Tetracycline,
30/M	*S. viridans*	Flucytosine	None
65/M	*A. baumannii*	Oxacillin, ampicillin/sulbactam, cefoxitin, levofloxacin, linezolid, tetracycline, ofloxacin	Azithromycin, Erythromycin, Amikacin, Chloramphenicol
34/M	*A. baumannii*	Gentamicin, ampicillin/sulbactam, ciprofloxacin, trimethoprim/sulfamethoxazole, ceftazidime, tigecycline	Ceftriaxone
65/M	*S. viridans*	Ciprofloxacin, aztreonam, ceftazidime	None
29/M	*S. epidermidis*	Azithromycin, erythromycin, clindamycin, gentamicin, linezolid, vanco, tigecycline	Moxifloxacin
45/M	*Enterobacter cloacae ssp cloacae*	Gentamicin, ciprofloxacin, meropenem, aztreonam, ceftazidime	None
30/M	*Cryptococcus laurentii*	Ketoconazole, nystatin, fluconazole, voriconazole	Clotrimazole, Econazole
20/M	*Coagulase negative Staphylococcus*	Azithromycin, erythromycin, ciprofloxacin, levofloxacin, trimethoprim/ sulfamethoxazole, ofloxacin, moxifloxacin, rifampicin	None
6/M	*S. aureus*	Azithromycin, erythromycin, clindamycin, oxacillin, cefazolin, gentamicin, ampicillin/sulbactam, cefuroxime, ceftriaxone, ciprofloxacin, levofloxacin, linezolid, tetracycline, vancomycin, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime, amoxicillin/clavulanic acid, cephalothin, methicillin, quinupristin/dalfopristin, ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftizoxime,	None
51/F	*A. baumannii*	Amikacin, ampicillin/sulbactam, meropenem, trimethoprim/sulfamethoxazole, tigecycline	None

TABLE 2. Culture and antibiotic sensitivity test results (cont.)

Patient	Bacteria	Antibiotic	
		Sensitive	Resistence
79/M	*Prevotella oralis*	Ampicillin, penicillin, azithromycin, erythromycin, clindamycin, oxacillin, ampicillin/sulbactam, cefuroxime, ceftriaxone, ciprofloxacin, levofloxacin, doxycycline, tetracycline, trimethoprim/sulfamethoxazole, chloramphenicol, ofloxacin, cefotaxime, ceftazidime, metronidazole, colistin	None
60/M	*Pseudomonas aeruginosa*	Gentamicin, ceftriaxone, trimethoprim/sulfamethoxazole, aztreonam, ceftazidime	Cefotaxime
53/M	*K. pneumoniae*	Gentamicin, ampicillin/sulbactam, cefepime, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole	None
35/F	*S. epidermidis*	Ampicillin, penicillin, oxacillin, ampicillin/sulbactam, cefuroxime, cefoxitin, ceftriaxone, ciprofloxacin, levofloxacin, imipenem, vancomycin, chloramphenicol, ofloxacin, cefotaxime, ceftazidime	None
47/F	*Escherichia coli*	Ampicillin, penicillin g, azithromycin, amikacin, cefuroxime, cefoxitin, ceftriaxone, ciprofloxacin, levofloxacin, tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole, cefotaxime	None
32/M	*K. pneumoniae*	Gentamicin, ampicillin/sulbactam, ceftriaxone, ciprofloxacin, ertapenem, meropenem, trimethoprim/sulfamethoxazole, aztreonam	None
76/M	*A. junii*	Gentamicin, ampicillin/sulbactam, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole, ceftazidime	None
49/M	*K. pneumoniae*	Gentamicin, ampicillin/sulbactam, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole, ceftazidime	Cefotaxime
53/F	*S. hominis*	Gentamicin, ampicillin/sulbactam, cefuroxime, ceftriaxone, ciprofloxacin, levofloxacin, cefotaxime	None
32/M	*S. epidermidis*	Azithromycin, clindamycin, cefazolin, gentamicin, ampicillin/sulbactam, ciprofloxacin, meropenem, linezolid, tetracycline	None
42/F	*Salmonella sp*	Ampicillin, ampicillin/sulbactam, cefepime, ceftriaxone, ciprofloxacin, ertapenem, meropenem, trimethoprim/sulfamethoxazole, aztreonam, ceftazidime	None
49/M	*S. constellatus*	Ampicillin, penicillin g, azithromycin, ampicillin/sulbactam, cefoxitin, levofloxacin, ertapenem, meropenem, vancomycin, trimethoprim/sulfamethoxazole, ofloxacin, cefotaxime	None
68/M	*Enterococcus faecalis*	Ampicillin, penicillin g, ampicillin/sulbactam, cefuroxime, cefoxitin, ceftriaxone, levofloxacin	Doxycycline
31/F	*P. aeruginosa*	Gentamicin, cefepime, ciprofloxacin, meropenem, aztreonam, ceftazidime	Ceftriaxone
46/M	*S. viridans*	Ampicillin, penicillin g, azithromycin, clindamycin, erythromycin, clindamycin, oxacillin, ampicillin/sulbactam, cefuroxime, cefepime, cefoxitin, ceftriaxone, ciprofloxacin, imipenem, meropenem, trimethoprim/sulfamethoxazole, chloramphenicol, ofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime	Amikacin, Levofloxacin
65/M	*Salmonella sp*	Ampicillin/sulbactam, ceftriaxone, trimethoprim/sulfamethoxazole, ampicillin	Ciprofloxacin
64/M	*S. hominis*	Vancomycin, moxifloxacin, rifampicin,	Clindamycin, Gentamicin, Ciprofloxacin
62/F	*S. epidermidis*	Ampicillin, penicillin g, erythromycin, azithromycin, clindamycin, ampicillin/sulbactam, ertapenem, meropenem, tetracycline	Gentamicin, Chloramphenicol
35/F	*S. agalactiae*	Ampicillin, azithromycin, erythromycin, clindamycin, ampicillin/sulbactam, ceftriaxone, levofloxacin, imipenem, linezolid, tetracycline, vancomycin, trimethoprim/sulfamethoxazole, chloramphenicol, cefotaxime	Penicillin G
68/M	*S. viridans*	Gentamicin, cefepime, ciprofloxacin	None
40/F	*S. anginosus*	Ampicillin, azithromycin, clindamycin, ampicillin/sulbactam, cefuroxime, ciprofloxacin, trimethoprim/sulfamethoxazole	None

FIGURE 1 shows the sum of each type of potential space involved in the form of a bar chart. From the data, there were 29 cases with submandibular abscess, followed by parapharyngeal, pretracheal, parotid, masticator, buccal, retropharyngeal, and peritonsillar abscess cases.

A multivariate analysis of the variables with p< 0.200 on the main dependent variables to the LOHS was performed. Gender, DM, and dental caries were included (TABLE 3). After the logistic regression analysis conducted, it was found that only DC had a significant effect on the LOHS (p=0.029).

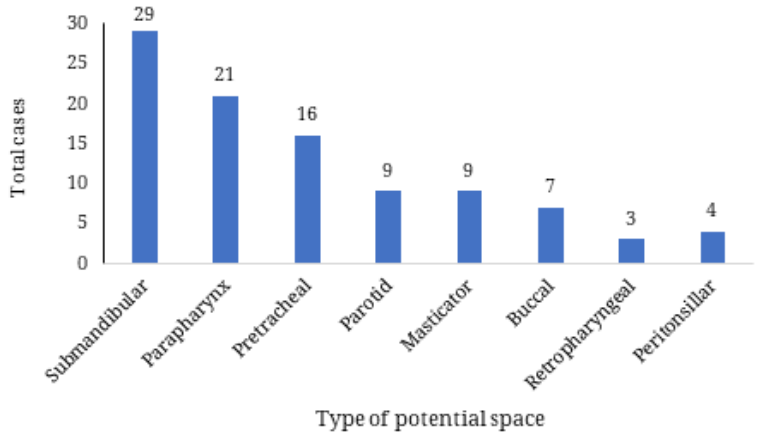


FIGURE 1. Type of potential neck space involved

TABLE 3. Multivariate analysis

Variable	Total	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	OR (95% CI)	p
Gender					
• Male	29	1	0.056	0.285 (0.064- 1.274)	0.100
• Female	15	0.222 (0.057-0.873)			
DM					
• Yes	20	1	0.130	2.782 (0.692- 11.180)	0.149
• No	24	3.095 (0.899-10.651)			
DC					
• Yes	25	1	0.015*	4.838 (1.180- 19.843)	0.029
• No	19	5.950 (1.586-22.328)			

DC: dental caries; DM: diabetes mellitus; CI: confidence interval; OR: odds ratio; *significant (p<0.05)

The main outcome of the study was the effect of the appropriate antibiotic use on the LOHS by comparing the sample LOHS > 7 d and ≤ 7 d in DNA patients with the appropriateness of the antibiotic given to the patients based on the culture and antibiotic sensitivity's results (TABLE 4). No significant relationship between the appropriate use of antibiotic on the DNA patients and the LOHS (p=0.546).

TABLE 4. Relationship of the appropriate use of antibiotic to LOHS

Antibiotic use	LOHS		Total (%)	OR (95% CI)	p
	> 7 d	≤7 d			
• Inappropriate	13	10	23	1	0.546
• Appropriate	9	12	21	0.577(0.175-1.905)	

CI: confidence interval; LOHS: length of hospital stay; OR: odds ratio.

DISCUSSION

This study showed that DC prolongs the LOHS of the patients with DNA (TABLE 1 and 3). Dental caries was the most common predisposing factor that caused the formation of odontogenic infections.[11] Poor oral hygiene and odontogenic infections could lead to lethal conditions such as descending necrotizing mediastinitis which required surgery. The cases of this condition had a mortality rate up to 40% although treatment had been conducted.[12] It was reported that the presence of DC in patients with DNA could deteriorate the condition requiring surgical treatment and prolong the treatment time. This condition was consistent with our study that found the DC was associated with an increase in the LOHS > 7 d. Bakir *et al.*,[13] also found that the DNA originating from odontogenic infection influenced a longer LOHS than non-odontogenic origin. Septic progressions in patient with dental infection can be aggravated by several predisposing factors such as DM, obesity, poor oral hygiene, and long-term nicotine or alcohol abuse so that these conditions can appear inclined inpatient stay.[14]

This study showed that no significantly relationship between the appropriate antibiotic use and the LOHS (TABLE 4). Marioni *et al.*,[15] reported that empirical antibiotic administration prior to culture and antibiotic sensitivity test obtained must cover Gram-positive, Gram-negative, and anaerobic bacteria. In addition, culture-appropriate antibiotic replacement could reduce the LOHS stay from 8.3 ± 6.2 d in 2000-2002 to 7.1 ± 5.3 d in 2003-2008. However, Nuryah *et al.*[16] reported that there was no correlation between the appropriate antibiotic use to clinical outcome in patients with MRSA infection. In this study, there was no significant correlation between the appropriate antibiotic use and LOHS. It was possible because the Department of Otolaryngology, Dr. Sardjito General Hospital, Yogyakarta had implemented an immediate antibiotic change according to culture.

In this study, each patient could contribute to more than one potential type (FIGURE 1). The number of submandibular abscesses was the highest number of abscess cases. This finding was similar to the results in the literature that the pre-antibiotic era of DNA came from pharyngeal or tonsil infections, but after the antibiotic era, DNA cases were caused by dental infections even in the antibiotic era. The number of cure rates also increased than the previous era.[17]

CONCLUSION

In conclusion, there is no relationship between the appropriate antibiotic use and the LOHS. However, the DC is risk factor the influence the LOHS in patients with DNA.

ACKNOWLEDGEMENT

We would like to thank the Director of Dr. Sardjito General Hospital, Yogyakarta for the financial support for this study and also the anonymous for their useful suggestion.

REFERENCES

1. Alias K, Colbert R, Devakumari S. Diagnosis and management of deeper neck infections: a review. IOSR J Dent Med Sci 2013; 9(5):36-41.
2. Dabirmoghaddam P, Mohseni A, Navvabi Z, Sharifi A, Bastaninezhad S, Safaei A. Is ultrasonography-guided drainage a safe and effective alternative to incision and drainage for deep neck space abscesses? J Laryngol Otol 2017; 131(3):259-263. https://doi.org/10.1017/S002221511700007X
3. Murray AD. Deep neck infections. Medscape, https://emedicine.medscape.com/ article/837048-overview, April 7, 2022.
4. Chan Y & Goddad JC. Lee, K.J. Lee's Otolaryngology: head and neck surgery, 12th ed. McGraw-Hill Company, 2019.
5. Lee YQ, Kanagalingam J. Deep neck abscesses: the Singapore experience. Eur Arch Otorhinolaryngol 2011; 268:609-14
6. Boyanova L, Kolarov R, Gergova G, Deliverska E, Madjarov J, Marinov M, *et al.* Anaerobic bacteria in 118 patients with deep space head and neck infections from the University of Hospital of Maxillofacial Surgery, Sofia Bulgaria. J Med microbial 2006; 55(9):1285-9. htpps://doi.org/10.1099/jmm.0.46512-0
7. McClay JE, Murray AD, Booth T. Intravenous antibiotic therapy for deep neck abscesses defined by computed tomography. Arch Otolaryngol Head Neck Surg 2003; 129(11):1207-12. https://doi.org/10.1001/archotol.129.11.1207
8. Brito TP, Hazboun IM, Fernandes FL, Bento LR, Zappelini CEM, Chone CT, *et al.* Deep neck abscess: study of 101 cases. Braz J Otorhinolaryngol 2017; 83(3):341-8. https://doi.org/10.1016/j.bjorl.2016.04.004
9. Kauffmann P, Cordesmeyer R, Tröltzsch M, Sömmer C, Laskawi R. Deep neck infections: A single-center analysis of 63 cases. Med Oral Patol Oral Cir Bucal 2017; 22(5):e536-e541. https://doi.org/10.4317/medoral.21799
10. O'Brien KJ, Snapp KR, Dugan AJ, Westgate PM, Gupta N. Risk factors affecting length of stay in patients with deep neck space infection. Laryngoscope 2020; 130(9):2133-7. https://doi.org/10.1002/lary.28367
11. Kityamuwesi R, Muwaz L, Kasangaki A, Kajumbula H, Rwenyonyi C. Characteristics of pyogenic odontogenic infection in patients attending Mulago Hospital, Uganda: a cross-sectional study. BMC Microbiol 2015; 15(1):1-10. https://doi.org/10.1186/s12866-015-0382-z
12. Diamantis S, Giannakopoulos H, Chou J, Foote J. Descending necrotizing mediastinitis as a complication of odontogenic infection. Int J Surg Case Rep 2011; 2:65-67.
13. Bakir S, Tanriverdi MH, Gün R, Yorgancilar AE, Yildirim M, Tekbaş G, *et al.* Deep neck space infections: a retrospective review of 173 cases. Am J Otolaryngol 2012; 33(1):56-63. https://doi.org/10.1016/j.amjoto.2011.01.003
14. Weise H, Naros A, Weise C, Reinert S, Hoefert S. Severe odontogenic infections with septic progress – a constant and increasing challenge: a retrospective analysis. BMC Oral Health 2019; 19(1):173. https://doi.org/10.1186/s12903-019-0866-6
15. Marioni G, Staffieri A, Parisi S, Marchese-Ragona R, Zuccon A, Staffieri C, *et al.* Rational diagnostic and therapeutic management of deep neck infections: Analysis of 233

consecutive cases. Ann Otol Rhinol Laryngol 2010; 119(3): 181-7. https://doi.org/10.1177/000348941011900306
16. Kamath MP, Shetty AB, Hegde MC, Sreedharan S, Bhojwani K, Padmanabhan K, *et al.* Presentation and management of deep neck space abscess. Indian J Otolaryngol Head Neck Surg 2003; 55(4):270-75. https://doi.org/10.1007/BF02992436
17. Nuryah A, Yuniarti N, Puspitasari I. Prevalence and evaluation of suitability of antibiotic use in patients with methicillin resistant *Staphylococcus aureus* in RSUP Dr. Soeradji Tirtonegoro Klaten. Majalah Farmaseutik 2019; 15(2):123-29. https://doi.org/10.22146/farmaseutik.v15i2.47911

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 70-78
https://doi.org/10.19106/JMedSci00550120239

Facial nerve paralysis in nasopharyngeal carcinoma: a case report

Jarod Wahyu Kristiyanto[1]*, Muyassaroh[2], Dwi Antono[2]

[1]Department of Ear Nose Throat-Head Neck Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, [2]Dr. Kariadi General Hospital, Semarang, Central Java, Indonesia

Submitted: 2021-11-11
Accepted : 2022-07-27

ABSTRACT

Nasopharyngeal carcinoma (NPC) is cancer originating from the mucosal lining of nasopharynx, with the highest predilection in the fossa of Rosenmüller. One-fifth of NPC cases have cranial nerve complications. The location of Rosenmüller's fossa which is adjacent to foramen lacerum and middle base of the cranium allows the tumor to extend directly into the cranium and surrounding cranial nerves. This paper reported a case of facial nerve paralysis in NPC. A 55-year-old man came to the Ear-Nose-Throat (ENT) Clinic at Dr. Kariadi General Hospital, Semarang with complaints of facial pain on the right side, headache, a lump on the left neck, fullness in ears, and nosebleed. However, the patient did not complain of double vision. After a thorough history taking, physical examination, radiology, and histopathology tests, the patient was diagnosed with WHO 3 ECOG I T3N2M0 stage III NPC with House Brackmann III facial nerve paresis at the level of Mastoid segment. The patient was treated using chemotherapy treatment by an ENT specialist with a chemotherapy regimen of paclitaxel-cisplatin for 6 cycles. In conclusion, although rare, NPC can cause facial nerve paralysis.

ABSTRAK

Karsinoma nasofaring (KNF) adalah kanker yang berasal dari lapisan mukosa nasofaring dengan predileksi terbanyak di fossa Rosenmuller. Seperlima kasus KNF menimbulkan komplikasi pada saraf kranial. Lokasi fossa rossenmuller yang berdekatan dengan foramen laserum dan dasar tengah kranium memberikan jalan bagi tumor untuk meluas langsung ke dalam kranium dan saraf kranial yang berdekatan. Makalah ini melaporkan sebuah kasus paralisis nervus facialis pada KNF. Seorang laki-laki usia 55 tahun datang ke poli THT-KL RSUP DR. Kariadi Semarang dengan keluhan wajah perot sebelah kanan, nyeri kepala, benjolan pada leher sebelah kiri, telinga gembrebeg, dan mimisan. Namun pasien tidak mengeluhkan adanya penglihatan ganda. Berdasarkan anamnesis, pemeriksaan fisik, radiologi dan histopatologis yang menyeluruh, pasien didiagnosis dengan KNF WHO 3 ECOG I T3N2M0 stadium III dengan Parese Nervus Fasialis (VII) *House Brackmann* III setinggi segmen Mastoid. Pasien kemudian ditangani oleh spesialis THT dengan regimen kemoterapi paclitaksel-cisplatin sebanyak 6 siklus. Dapat disimpulkan, meskipun jarang, KNF dapat menyebabkan paralisis nervus facialis.

Keywords:
nasopharyngeal carcinoma;
facial nerve paresis;
chemotherapy;
paclitaxel;
cisplatin

*corresponding author: jarodwahyu@gmail.com

INTRODUCTION

Facial nerve palsies are a common and significant presentation specifically to ear, nose, and throat (ENT) surgeons and also in general medical practice. Facial nerve is a fundamental structure both for communication and emotion, and as such, functional impairment can lead to significant deterioration in the quality of life. In most cases, the etiology for facial nerve palsy remains idiopathic and has the name 'Bell palsy' (70% cases), followed by trauma (10 to 23% cases), usually caused by fractures involving the petrous part of temporal bone, and facial wounds transecting the branches of the facial nerve thus causing facial nerve palsies. A viral Herpes-Zoster infection (4.5 to 7.0% cases) may lead to facial paralysis due to geniculate ganglionitis (also known as Ramsay Hunt syndrome/ RHS). Neoplasia, although uncommon (2.2 to 5.0% cases), can also cause facial paralysis. A slowly progressing onset of facial palsy should raise the suspicion of malignancy and prompt a full and thorough head and neck examination.[4]

Nasopharyngeal carcinoma (NPC), previously known as lymphoepithelioma, is a malignancy arising from the epithelium of the nasopharynx. An interplay of environmental factors, genetic structure, and Epstein-Barr virus (EBV) infection is involved in the etiology of disease. One-fifth of NPC patients have cranial nerve involvement at the time of diagnosis. In contrast to the frequently involved the V and VI cranial nerves, the I, VII and VIII cranial nerves are rarely affected in nasopharyngeal cancer. Symptoms of the neurological disorders include diplopia, loss of sensation in the cheeks, decreased corneal reflexes, and headaches involving the II, III, IV, V, and VI cranial nerves. Disorders of the IX, X, XI, and XII cranial nerves can cause dysphagia, soft palate hemiparesis, and tongue paralysis. Disorders of the VII and VIII cranial nerves are rare, accounting for less than 1% of all cases.[5-7]

Facial palsy due to NPC does not happen very often, with incidence of less than 1% of all cases. The facial nerve exits the brainstem, enters the cerebellopontine angle, the temporal bone, and finally to the parotid; after exiting the parotid it branches to supply the facial muscles. Tumor involvement anywhere along the nerve can cause facial paralysis. The nearby location of Rosenmüller's fossa to the foramen lacerum and middle base of the cranium provide way for tumor to extent directly to the cranium and adjacent cranial nerve.[8] The purpose of this paper is to report a rare case of facial nerve paralysis in NPC.[9]

CASE

A 55-year-old man came to the ENT clinic complaining of asymmetry of right facial muscle since 3 mo ago, accompanied by fullness in ears and nosebleeds, headache, and a lump on the left side of neck. The patient did not complain of double vision. He had 20 yr history of smoking and frequent consumption of salted fish.

Based on physical examination, this man had a good general condition (compos mentis), and normal vital signs. Physical examination of the facial nerve showed asymmetry due to weakness on the right side when the patient was asked to smile with showing teeth, raised his eyebrows, puffed his cheeks, and pouting lips with showing teeth (FIGURE 1). Further examination of the facial nerve is described in TABLE 1. We also found an enlarged lymph nodes on the right and left side of the neck, at level II and III with a size of 2x2x1 cm, and the same skin color as around.

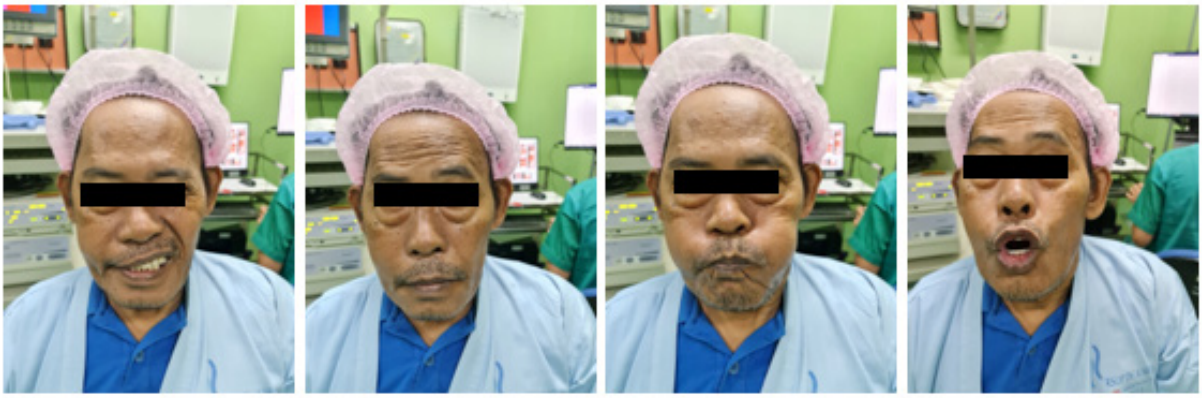


FIGURE 1. Facial nerve examination. The facial asymmetry is clearly visible when the patient smiled with showing teeth, raised his eyebrows, puffed his cheeks, and pouting lips with showing teeth.

TABLE 1. Facial nerve examination.

Examination	Right	Left
Raised eyebrows	Weak	+
Frown eyebrows	-	+
Lift and wrinkle nose up	Weak	+
Close your eyes tightly	Weak	+
Laughing out loud, showing teeth	Weak	+
Pouting mouth while showing teeth	Weak	+
Puffing cheeks	Weak	+
Whistling	Weak	+
Pull the corners of the lips down	Weak	+
Plunging the mouth forward	Weak	+
Gustatory test	-	+
Stapedius reflex	+	+
Scheimer test	10mm	5mm

The results of flexible nasopharyngoscopy examination showed a mass in front of the right torus tubarius extending to the right choana. The surface of the mass was lumpy, fragile and bleed easily (FIGURE 2). Biopsy was performed at three locations. Based on the morphological and immunohistochemical profile, it supports the diagnosis of non keratinizing squamous cell carcinoma, undifferentiated subtype.

The audiometric examination results showed a mixed degree of very severe hearing loss (PTA 81.25 dB) on the right ear while the left ear was still within normal (PTA 17.5 dB). The result of tympanometry are as follows: right ear B, left ear A, and right acoustic reflex positive (FIGURE 3). The facial nerve examination showed facial nerve paresis House Brackmann III at the level of Mastoid segment.

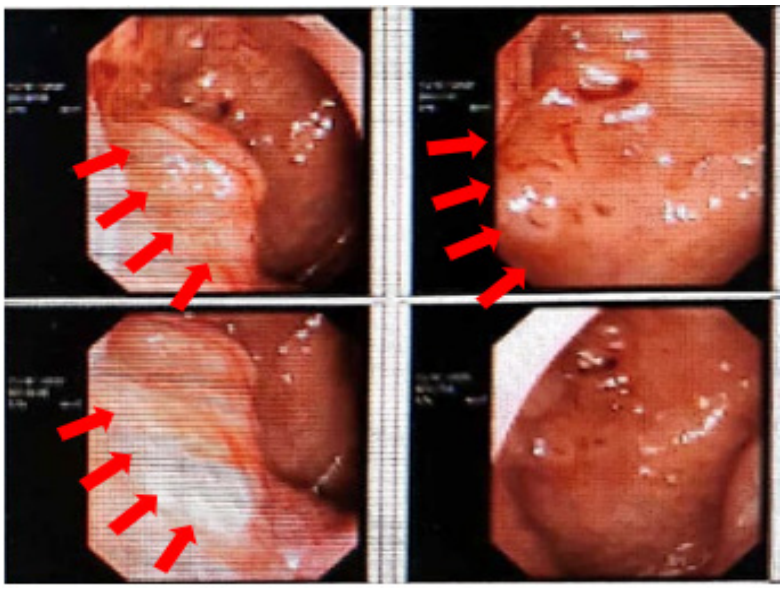


FIGURE 2. Nasopharyngoscopy examination. Red arrow: a mass in front of the right torus tubarius extending to the right choana.

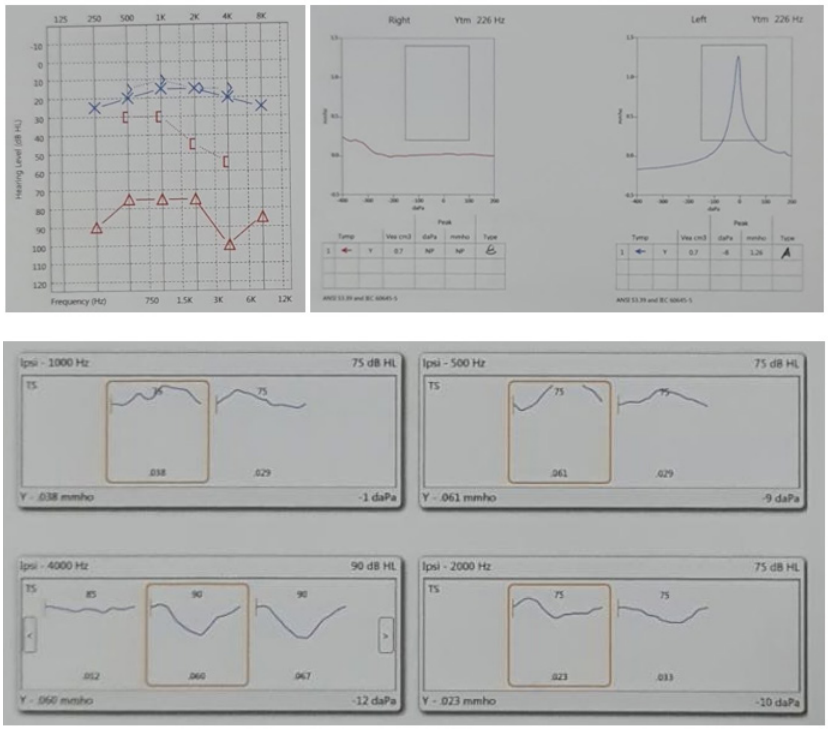


FIGURE 3. The audiometric and tympanometry results of both ears.

On a 1.5T MRI examination, a non-homogeneous solid mass appeared in the right nasopharyngeal mucosal space which seemed to extend to the right parapharyngeal space, right carotid space, right retropharyngeal space, right masticator space, right oropharyngeal mucosal space, right hard palate and seemed to expand, destroying posterior wall into the right maxillary sinus. Multiple lymphadenopathy were also observed in the right and left colli regions

of the level II, III with the largest size of ± 2.66 x 2.16 cm on the left level III, with a tendency to be a nasopharyngeal mass (T3N2Mx), no signs of increased intracranial pressure, and a right mastoiditis appearance was observed (FIGURE 4).

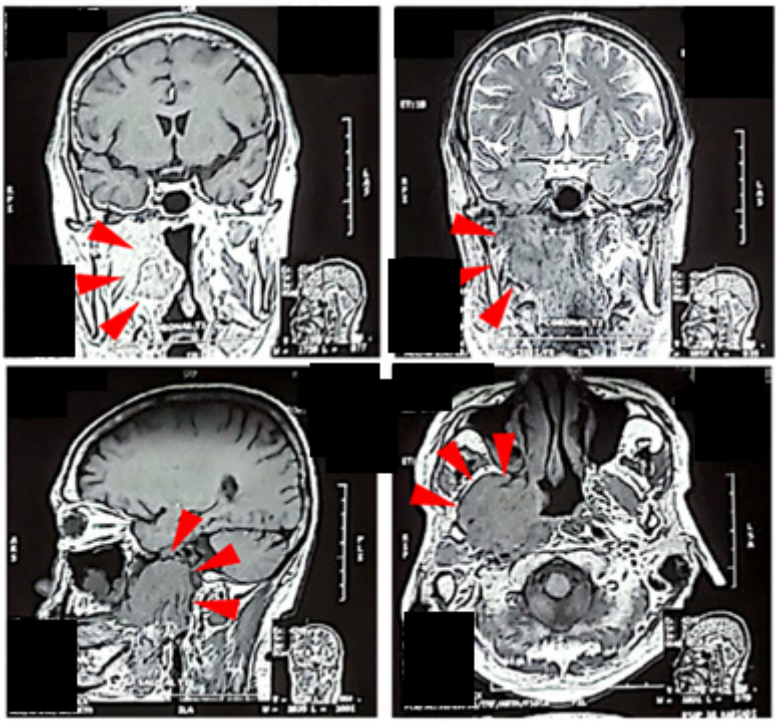


FIGURE 4. 1.5 T MRI results. The red arrow showed inhomogeneous solid mass appeared in the right nasopharyngeal mucosal space which extend to the right parapharyngeal space, right carotid space, right retropharyngeal space, right masticator space, right oropharyngeal mucosal space, right hard palate and destroy posterior wall to the right maxillary sinus.

Based on the history, physical examination, radiology, and histopathology exams, the patient was diagnosed with WHO 3 ECOG I T3N2M0 stage III NPC with House Brackmann III facial nerve paresis at the level of Mastoid segment. The was treated with paclitaxel-cisplatin chemotherapy regimen for 6 cycles.

DISCUSSION

Here we reported a case of NPC with a complication of facial nerve paresis. Among all facial nerve palsies, 5% of them are caused by tumor, one of which is NPC. Examinations that can be done to establish a facial nerve paresis includes facial nerve examination and in this case, it showed a House Brackmann III paresis of the VII nerve at the level of the Mastoid segment.

Nasopharyngeal carcinoma is a malignancy originating from the epithelial cells that cover the surface of the nasopharynx.[1,10,11] The local spread of NPC can be through the parapharyngeal space (FIGURE 5).[1,12,13]

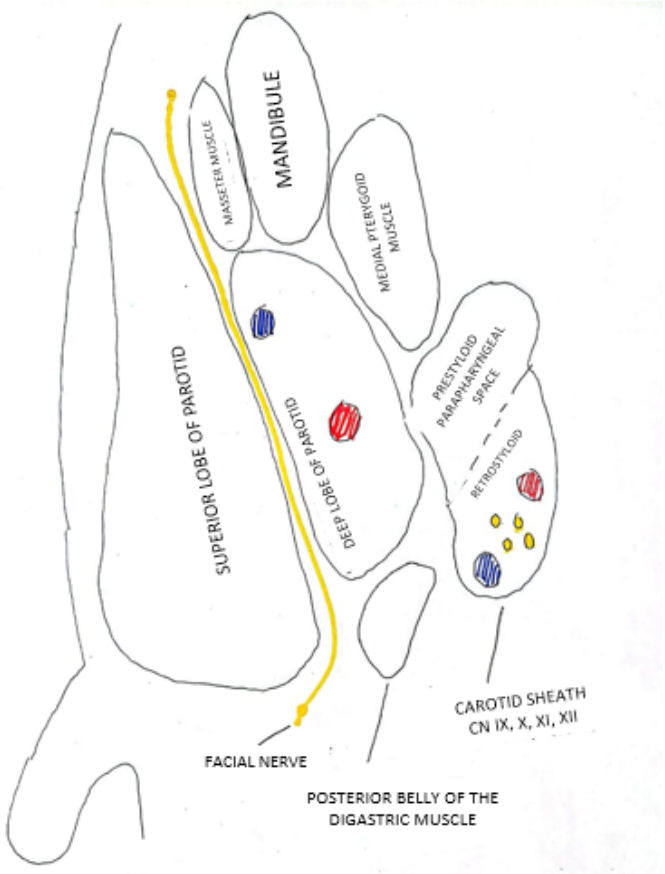


FIGURE 5. Axial anatomy of the parapharyngeal space.

Vertical upward spread

The tumor extends intracranially, spread along the medial fossa, this is called as petrosphenoid spread, usually through the foramen laserum, then into the cavernous sinus, middle cranial fossa and anterior cranial fossa involving the anterior cranial nerves (N. I and N. VI). Symptoms arising which caused by the damage of anterior cranial nerves due to tumor metastasis is called petrosphenoid syndrome. The most common symptoms are diplopia and trigeminal neuralgia (N. II - N.VI paresis).

Backward spread

The tumor extends extracranially through the pharyngobasilar fascia along the posterior fossa (including the foramen spinosum, foramen ovale and so on), wherein lies the IX and XII cranial nerves; this process is called retroparotidian spread. This spread affects the cranial nerves VII and XII along with the cervical sympathetic nerves. Symptoms caused by the damage of cranial nerves IX and XII is called retroparotidian syndrome/jugular jackson syndrome. Cranial nerves VII and VIII are rarely affected by tumors because they are located quite high in the anatomical system of the body.

Spread to lymph nodes

Spread to lymph nodes begins in the lymph nodes located lateral to the retropharynx, namely the nodes of Rouviere. Inside the gland, cancer cells grow and multiply making the gland to enlarge and appears as a lump on the lateral side of neck. This lump is often ignored by patients because it is painless. Afterwards, cancer cells will continue to grow, penetrating outside the glands and affecting the muscles below, making it difficult to move the muscle.

Distant metastases

Cancer cells can enter the blood or lymphatic flow, attacking organs that are located far from the nasopharynx (bones, liver, lungs). The facial nerve exits the skull base through the temporal bone and then passes through the parotid gland and supplies various extracranial structures in the head. This nerve is responsible for motor innervation to all muscles of facial expression, posterior to the digastric, stylohyoid and stapedius muscles. Facial nerve also supplies sensory parts to the anterior two-thirds of tongue and parasympathetic to facial glands including the submandibular, sublingual, nasal palatine, lacrimal and pharyngeal glands, but not the parotid glands (FIGURE 6).[7,14]

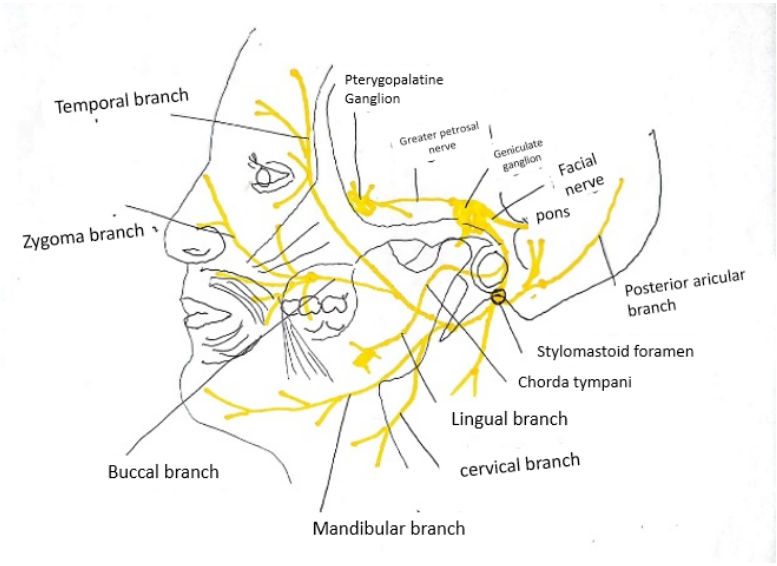


FIGURE 6. Facial nerve pathway

The facial nerve exits the brainstem to the cerebellopontine angle (CPA), the temporal bone (internal acoustic meatal, middle ear, and mastoid), the parotid gland and then branches to supply the facial muscles. The presence of inflammation, tumor infiltration along the path of the facial nerve can cause facial paralysis.[5-7] The most common intracranial tumors are CPA tumor, NPC (usually accompanied by other cranial nerve disorders) and parotid gland tumor.

Facial nerve involvement in CPA may be caused by the cancer metastasis (hematogenous, via cerebro-spinal fluid/CSF), or leptomeningeal spread), whereas involvement in the ear may result from direct invasion through the eustachian tube or parapharyngeal space. Indirect invasion may be due to tubal occlusion. The location of the eustachian tube which is adjacent to the fossa of Rosenmüller's can cause negative pressure in the tympanic cavity which results in otitis media and if not treated properly can cause mastoiditis and facial paresis. Involvement of the facial nerve due to parotid gland impairment should raise a suspicion of lymphatic spread of the tumor to the retropharyngeal lymph node group, which may drain into the parotid gland. From the parotid gland, the tumor has access to lymphatic plexus, parotid parenchyma, facial nerve, and even the parapharyngeal space.[5,12,15]

Facial paresis in this case occurred due to retroparotidian spread of the mass, toward the parapharyngeal, retropharyngeal, carotid, and masticator areas as seen on the MRI imaging. The prognostic for the facial palsy were good with chemotherapy due to mass reduction.

CONCLUSION

Although rare, facial nerve paralysis is one of the complications of NPC. In this case report we present a 55-year-old man who has been diagnosed with House Brackmann III facial nerve paresis at the level of the Mastoid segment due to retroparotidian spread of stage III NPC. The patient prognostic for facial nerve palsy are good due to the mass reduction in chemotherapy.

ACKNOWLEDGEMENT

All examination photos were original and under the patient consent. The identity of the patient was masked. We would like to thank all the staffs of Department of Otolaryngology-Head and Neck Surgery Dr. Kariadi General Hospital, Semarang for their full supports upon writing of this report.

REFERENCES

1. Wei W, Chua D. Nasopharyngeal cancer. In: Bailey BJ, Healey GB, Johnson JT, Rosen CA, *et al*. eds. Head and neck surgery otolaryngology. Philadelphia: Lippincott Williams & Wilkins. 187; 4: 97: 187.
2. Wu L, Li C, Pan L. Nasopharyngeal carcinoma: a review of current updates. Exp Ther Med 2018; 15(4):3687-92. https://doi.org/10.3892/etm.2018.5878
3. Adham M, Mat Lazim N, Carlos R. Clinical presentation of nasopharyngeal carcinoma. In: Abdullah B, Balasubramanian A, Mat Lazim N editors. An evidence-based approach to the management of nasopharyngeal cancer: from basic science to clinical presentation and treatment. London: Academic Press 2020; 93-109.
4. Walker NR, Mistry RK, Mazzoni T. Facial nerve palsy. StatPearls [Internet] Treasure Island (FL): StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK549815/
5. Pieter NAL, Prawira AM. Nasopharyngeal carcinoma with a complication of facial nerve paresis. IJNPC 2021; 03(01):13-6.
6. Bhardwaj S, Mukutawat P, Bhardwaj S, Kumar N, Kaushal V. Facial nerve palsy: an atypical presentation of carcinoma nasopharynx. IJRESM 2020; 3(6):576-77.
7. Chhabda S, Leger DS, Lingam RK. Imaging the facial nerve: a contemporary review of anatomy and pathology. Eur J Radiol 2020; 126:108920. https://doi.org/10.1016/j.ejrad.2020.108920
8. Shah AB, Zulfiqar H, Nagalli S. Nasopharyngeal carcinoma. In: StatPearls [Internet] Treasure Island (FL): StatPearls Publishing; 2022 Jan-, https://www.ncbi.nlm.nih.g ov/books/NBK554588/. 2021;
9. Mahajan M, Abishek R, Guleria TC, Thakur J, Azad RK. Facial nerve palsy as a presenting feature of nasopharyngeal carcinoma: a rare case report. ASOL 2022; 4(9):29-31. https://doi.org/10.31080/ASOL.2022.04.0491
10. Salehiniya H, Mohammadian M, Hafshejani AM, Mahdavifar N. Nasopharyngeal cancer in the world: epidemiology, incidence, mortality and risk factors. WCRJ 2018; 5(1):e1046.
11. Leung KKY, Tong MCF. Assessment and staging of nasopharyngeal carcinoma. In: Abdullah B,

Balasubramanian A, Mat Lazim N editors. An evidence-based approach to the management of nasopharyngeal cancer: from basic science to clinical presentation and treatment. London: Academic Press 2020; 111-39.
12. Aninditha T, Soebroto AD, Nurhayati E. Komplikasi neurologis pada karsinoma nasofaring. eJKI 2018; 6(2):123-7. https://doi.org/10.23886/ejki.6.9813
13. Aydın S, Demir MG, Başak K. Preauricular metastasis of nasopharyngeal carcinoma. Causa Pedia 2020; 9(1):10-14.
14. Davies J, Al-Hassani F, Kannan R. Facial nerve disorder: a review of the literature. IJS Oncol 2018; 3(7):e65. https://doi.org/10.1097/IJ9.0000000000000065
15. Amouzegar EY. Nasopharyngeal carcinoma with intracranial involved. IJNPC 2020; 2(02):50-3. https://doi.org/10.32734/ijnpc.v2i02.3900

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 79-85
https://doi.org/10.19106/JMedSci005501202310

Coinfection of COVID-19 and dengue: a case report

Fx. Wikan Indrarto*

Panti Rapih Hospitals, Yogyakarta/Medical Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia

Submitted: 2021-03-15
Accepted : 2021-06-05

ABSTRACT

Dengue hemorrhagic fever (DHF) and corona virus disease 2019 (COVID-19) are two viral infectious diseases that can occur simultaneously and have the potential to complicate each other. Therefore, sustained attention to this phenomenon is required. A 9 y.o. boy was diagnosed with dengue and COVID-19 at the Panti Rapih Hospital in Yogyakarta, Indonesia, in the early phase of the COVID-19 pandemic. Both viral infectious diseases have distinctive clinical and laboratory features. Acute fever, vomiting, weakness, thrombocytopenia, and hemoconcentration that improve from day 5 after infection are typical symptoms of DHF in children. Complaints of fever, runny nose, cough, and pain when swallowing that usually occurs in COVID-19 also appeared. Medical investigations with serological tests for DHF and COVID-19 at the beginning of the COVID-19 pandemic can be used to diagnose these two infections because standard diagnostic tools using the PCR swab test cannot yet be performed. Symptomatic therapy for moderate degrees of these two viral infections is appropriate.

ABSTRAK

Demam berdarah dengue (DBD) dan *corona virus disease* 2019 (COVID-19) merupakan dua infeksi virus yang dapat terjadi secara bersamaan dan berpotensi saling menyulitkan. Oleh karena itu diperlukan kewaspadaan akan fenomena ini. Seorang anak laki-laki berusia 9 tahun didiagnosis dengan DBD dan COVID-19 di RS Panti Rapih Yogyakarta, Indonesia, pada tahap awal pandemic COVID-19. Kedua penyakit infeksi virus tersebut memiliki gambaran klinis dan laboratoris yang khas. Demam akut, muntah, lemah, trombositopeni dan hemokonsentrasi yang membaik mulai hari ke 5, adalah khas untuk DBD pada anak. Keluhan demam, pilek, batuk dan nyeri telan pada COVID-19 juga muncul. Pemeriksaan penunjang medik dengan uji serologi untuk DBD dan COVID-19 pada awal pandemi COVID-19, dapat digunakan untuk penegakan diagnosis kedua infeksi ini, karena parasat diagnosis baku menggunakan uji usap PCR belum dapat dilakukan. Terapi simptomatis untuk derajad sedang kedua penyakit infeksi virus ini adalah memadai.

Keywords:
Dengue hemorrhagic fever;
COVID-19;
clinical picture;
laboratory results;
coinfection

INTRODUCTION

Dengue fever (DF) is a mosquito-borne disease caused by the dengue virus that belong to the Flaviviridae and present with symptoms of fever, nausea, headaches, myalgia, skin rashes, retro-orbital pains, and arthralgia. The severe form of DF also called dengue hemorrhagic fever (DHF) can cause serious bleeding, shock, and death.[1] Corona virus disease 2019 (COVID-19)

*corresponding author: wikan_indrarto@staff.ukdw.ac.id

is caused by a beta coronavirus called severe acute respiratory syndrome coronavirus (SARS-CoV-2) which mainly affects the lower respiratory tract and causes pneumonia in patients.[2]

Even though the pathophysiology of the two infections is different, DHF and COVID-19 share clinical symptoms and laboratory features. Thrombocytopenia and platelet dysfunction commonly occur in the both infections and are related to clinical outcomes. Coagulation and fibrinolytic pathways are activated during an acute dengue infection, and endothelial dysfunction is observed in DHF. On the other hand, COVID-19 is characterized by a high prevalence of thrombotic complications, where bleeding is rare and occurs only in advanced stages of critical illness; here thrombin is the central mediator that activates endothelial cells, and elicits a pro-inflammatory reaction followed by platelet aggregation.[3,4]

Some cases of coinfection by dengue and SAR-CoV-2 have been reported especially in the dengue-endemic regions.[3-6] This coinfection would undoubtedly be a dangerous combination disease if not managed cautiously. The physicians and health authorities should make decision accordingly to prevent the coinfection develops into ecoepidemic. In this case report a 9 y.o. boy diagnosed with coinfection of dengue and COVID-19 was presented.

CASE

On Wednesday, February 3rd, 2020, at 11.42 AM, a 9-year-old boy came to the emergency room at the Panti Rapih Hospital, Yogyakarta, Indonesia complaining of fever on the 4th day, redness on the skin, dizziness, and little nausea. On the examination found Compos Mentis consciousness, blood pressure of 95/83 mmHg, temperature of 38.90 ^{o}C, pulse rate of 140x /min, respiratory rate of 20x/min, pain scale of 1, body weight of 65 kg, and blood glucose level of 113 mg/dL. The physical examination found the head: anemia conjunctiva - / -, chest: C/S1-2 regular, pulmo: no basal wet crackles, stomach: supple, BU (+) N, examination: warm, strong pulse, petechiae (+). Managed by working diagnosis: febrile observation day-4 thrombocytopenia suspect DF dd DHF, by infusion, routine blood checks, Ro thorax RLD, IgM/G dengue, rapid COVID-19 antibodies.

On routine blood tests, HB: 15.4 g/dL (H), HCT: 44.7 g/dL (H), leukocytes: 4,300 x 10^{9}/L, neutrophils: 42.2% (L), lymphocytes: 45.5% (H), monocytes: 11.6% (H), platelets: 87,000 x 10^{6} L (L). On the RLD photo, there was no connection in the hemithorax, the right and left sinuses, and both diaphragms were good. By impression: no pleural effusion was observed (FIGURE 1).

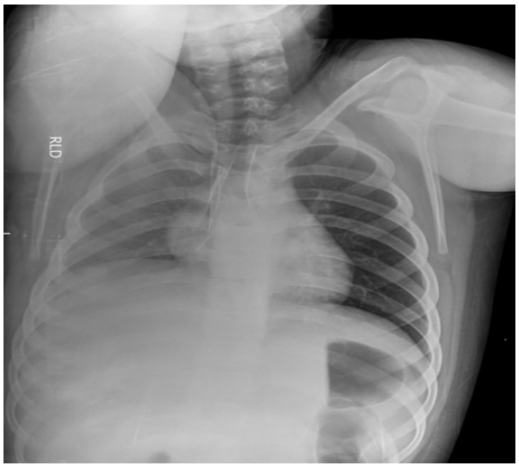


FIGURE 1. Chest X-ray

In the examination of dengue IgG (+), IgM dengue (-) with the immunochromatography (ICT) method, anti SARS CoV2 IgG (-) : 0.19 (<10U/mL), anti SARS CoV2 IgM (+): 1, 08 (<1) cut off index (COI) with the chemiluminescence immunoassay (CLIA) method.

Furthermore, the patient was managed in the particular ward for COVID-19 patients, while waiting for the results of the gold diagnostic test, the COVID-19 RTPCR nasopharyngeal swab. The Ringer's lactate solution infusion (RL) 120 cc /H, paracetamol 3 x 1 tablet in case of fever, vitamin C, 3 x 250 mg tablet, paracetamol 500 injection mg in the occurrence of high fever, and oral omeprazole 2 x 20 mg in the occurrence of abdominal pain were administered to the patient in the nursing ward.[7]

Fever, dizziness, vomiting, diarrhea, and weakness were found in this patient as the clinical symptoms of COVID-19 and DHF. Meanwhile, the patient was inexperienced with cough, runny nose, sore throat, tongue losing taste, nose losing smell, and nose bleeding. Improvement in fever, dizziness, vomiting, diarrhea, and weakness were found during observation in the ward. The redness of the skin on the arm where the blood sample was taken (FIGURE 2) and the legs' rashes (FIGURE 3 and 4) were observed in the patient.

Following increasing in the platelet count and decreasing in the hematocrit, the patient's condition was improved. The red patchy rash on the legs and arms (FIGURE 3a and 3b) which is known as convalescence rash also were observed during the recovery phase. The presence of this rash indicated that the dengue infection was resolved.

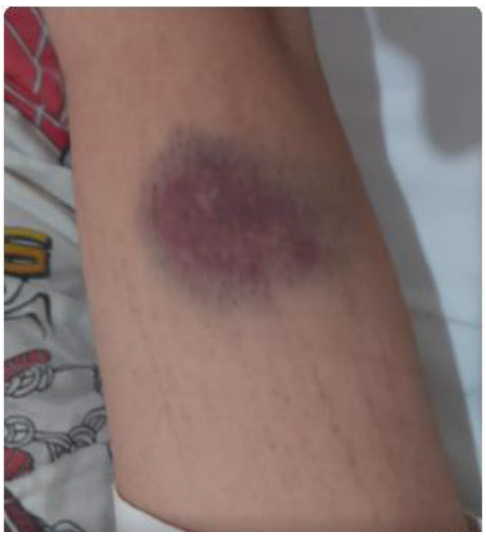
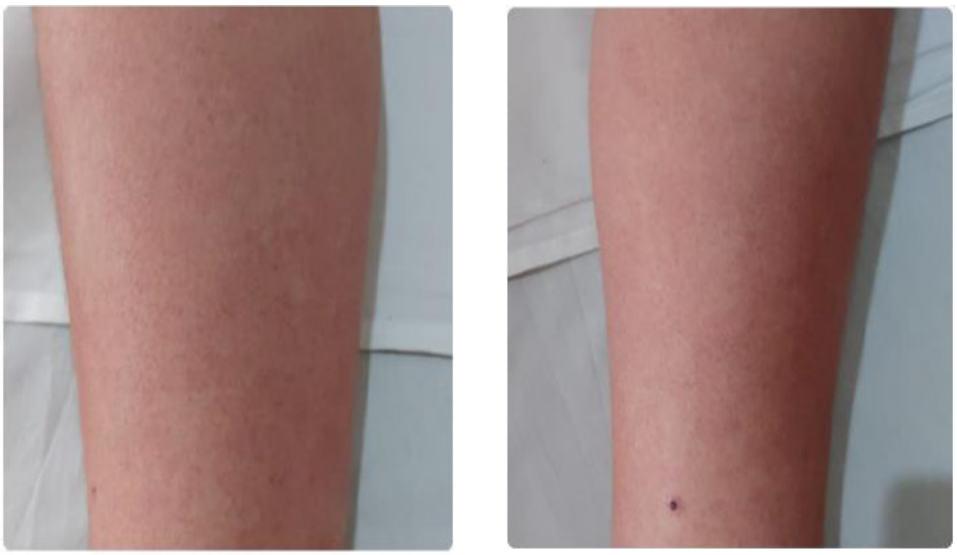


FIGURE 2. Hematoma on the left arm



A B

FIGURE 3. Convalescence rash of the left leg (A) and the right leg (B)

The results of daily platelet monitoring (FIGURE 4) showed that severe thrombocytopenia reached a low point of 11,000 on day 5 of fever and increased when conditions improved before discharge to 146,000/mm^3. There was also a decreasing process in hemoconcentration (FIGURE 5) from 44.7% on the 4th day of fever to 35.9%.

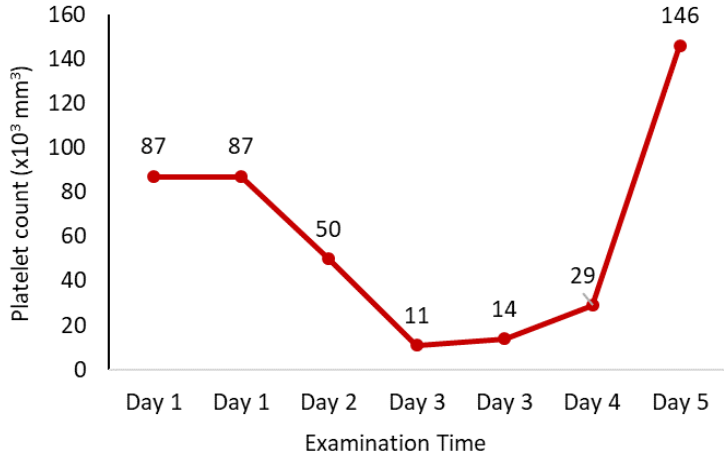


FIGURE 4. An improvement of platelet count during monitoring for 5 days examination

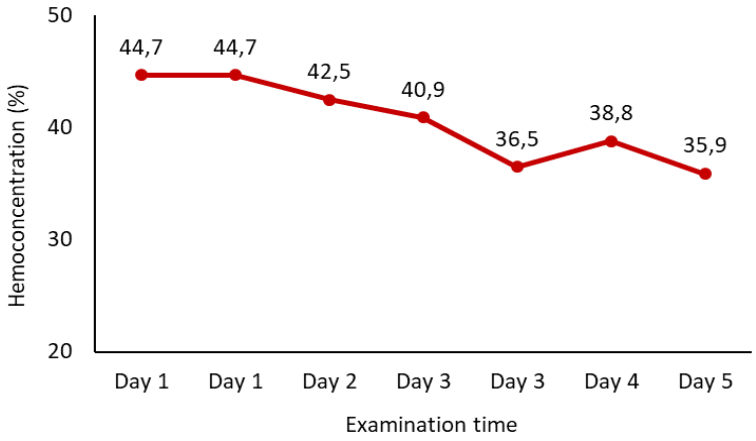


FIGURE 5. Hemoconcentration during monitoring from 44.7 to 35.9%.

On the 4th day of treatment, nasopharyngeal RT-qPCR swab examination results showed a positive result for 2019-nCoV twice. In this patient, NS1-Ag and RT-qPCR were not performed to detect DENV serotype (DENV), because the facilities were not available. The chest radiograph performed on the first day was not repeated, as there were no indications that the chest radiograph was required (FIGURE 1).

The patient was hospitalized for six days and discharged on Tuesday, February 9th, 2021. A further self-isolation was conducted in a private home, with the addition of the azithromycin tablet, 1 x 500 mg, for seven days. Three days after the self-isolation, the symptoms get cured. Further, the patient's parents agreed that their child's medical condition could be published for scientific purposes.

DISCUSSION

COVID-19 and DHF have a spectrum of diseases with overlapping clinical manifestations.[7,8] DHF is one of the most common tropical infectious diseases in tropical areas, including Indonesia and Thailand.[9] The first COVID-19 case was confirmed in China, spread over the countries, and became a national disaster in Indonesia on March 14th, 2020.[7]

On Wednesday, February 3rd, 2021, a 9 y.o. boy was diagnosed with both of them. The patient presented acute fever and nonspecific systemic symptoms, which made it very difficult to distinguish between COVID-19 and other tropical infections. In the early course of the disease, COVID-19 can appear as fever-like, but then chest X-rays usually show abnormalities similar to pneumonia.[6] Then, this case was eventually detected as mild COVID-19.

A more sensitive chest computed tomography (CT) scan was not carried out to detect pulmonary abnormalities related to COVID-19 in the patient, due to the absence of respiratory symptoms.[10] Leukopenia with lymphopenia is often found in either DHF or COVID-19 patients.[11] In this case, progressive leukopenia with relative lymphocytosis and the presence of atypical lymphocytes leads to decreased platelet count, and increased hematocrit. The results of serial laboratory examinations have unique characteristics according to the clinical course of dengue infection,[8,12] which also occurred in this case, namely improvements in hemoconcentration (FIGURE 4) and thrombocytopenia (FIGURE 5). In this case, historical records of close contact with COVID-19 survivors were not found. Then, only a rapid test serology for COVID-19 was carried out in the early stage.

This case demonstrated the difficulty of distinguishing COVID-19 from DHF in children, because of the non-specific clinical picture, which leads to a requirement confirmation of the gold standard diagnostic test, the COVID-19 RT PCR nasopharyngeal swab. In addition, some severe symptoms of these two diseases did not occur. However, hemoconcentration, plasma leakage, and severe thrombocytopenia are typical clinical manifestations of severe DHF, which can help differentiate dengue from COVID-19[8,12] were found in this case. On the other hand, some cases have raised concerns about becoming worse and fatal, due to the combination of COVID-19 infection with dengue.[13,14] However, to date this report was prepared, there were no fatal patients,[15,16] including this case.

Laboratory diagnosis for dengue during the COVID-19 pandemic is a challenge. Dengue antibody serologic tests can show false positives in COVID-19 patients and misdiagnose them with DHF.[17,18] Serological cross-reactivity between DHF and COVID-19 is also possible.[19] Therefore, an RT-PCR COVID-19 swab examination must be carried out to confirm a diagnosis of COVID-19. Checking the serology of DHF and COVID-19 antibodies thoroughly in an isolation room for suspected cases, while waiting for the results of the RT-PCR COVID-19 swab, is the best strategy. However, these may cause cost overburden in public health systems and hospitals in limited-resource tropical countries. The RT-PCR swab examination for SARS-CoV-2 diagnosis and serology DHF for this patient were free because they were guaranteed by the Social Security Agency of Health, and for COVID-19 suspected cases that meet the criteria, the funding is guaranteed by the Indonesian Ministry of Health, as in other countries.[13]

CONCLUSION

During the ongoing COVID-19 pandemic in tropical areas, COVID-19 must be established in a distinct diagnosis

from other children's fevers. To establish a correct diagnosis, understanding the clinical manifestations, exposure history, general initial laboratory results, and progression of the disease, together, is the best strategy. Furthermore, self-isolation for fever patients must be taken seriously to prevent the spreading of infection.

ACKNOWLEDGMENT

We would like to thank the patient and family for allowing us to present this case in a scientific paper.

REFERENCES

1. Tuiskunen Bäck A & Lundkvist A. Dengue viruses – an overview. Infect Ecol Epidemiol, 2013; 3:19839. https://doi.org/10.3402/iee.v3i0.19839
2. Islam MA, Kundu S, Alam SS, Hossan T, Kamal MA, Hassan R. Prevalence, and characteristics of fever in adult and paediatric patients with coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of 17515 patients. PLoS ONE, 2021; 16(4): e0249788. https://doi.org/10.1371/journal.pone.0249788
3. Harapan H, Ryan M, Yohan B, Abidin RS, Nainu F, *et al*. COVID-19 and dengue: double punches for dengue-endemic countries in Asia. Med Virol, 2021; 31(2):e2161. https://doi.org/10.1002/rmv.2161.
4. Islam A, Cockcroft C, Elshazly S, Ahmed J, Joyce K, Mahfuz H, *et al.* Coagulopathy of dengue and COVID-19: clinical considerations. Trop Med Infect Dis, 2022;7(9):210. https://doi.org/10.3390/tropicalmed7090210
5. Prapty CNBS, Ahmed N, Araf Y, Yang Z, Zhai J, Hosen MJ, *et al.* Coinfection of COVID-19 and dengue: a case report. Front Med, 2022; 9:872627. https://doi.org/10.3389/fmed.2022.872627
6. Nunthavichitra S, Prapaso S, Luvira V, Muangnoicharoen S, Leaungwutiwong P, Piyaphanee W. Case report: COVID-19 presenting as acute undifferentiated febrile illness-a tropical world threat. Am J Trop Med Hyg 2020; 103(1):83-85. https://doi.org/10.4269/ajtmh.20-0440
7. Gugus Tugas Percepatan Penanganan COVID-19 dan Kementerian Kesehatan Republik Indonesia, 2020 Pedoman Tata Laksana COVID-19. 2020; 1-38.
8. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al*. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382(18):1708-20. https://doi.org/10.1056/NEJMoa2002032
9. Luvira V, Silachamroon U, Piyaphanee W, Lawpoolsri S, Chierakul W, Leaungwutiwong P, *et al.* Etiologies of acute undifferentiated febrile illness in Bangkok, Thailand. Am J Trop Med Hyg 2019; 100(3):622-9. https:doi.org/10.4269/ajtmh.18-0407
10. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, *et al*. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020; 26(8):1200-4. https://doi.org/10.1038/s41591-020-0965-6
11. Huang C, Wang Y, Li X, Ren L, Zhao L, Hu Y. Clinical features of patients infected with the 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
12. Cozzi D, Albanesi M, Cavigli E, Moroni C, Bindi A, Luvarà S, *et al*. Chest X-ray in new coronavirus disease 2019 (COVID-19) infection: findings and correlation with clinical outcomes. Radiol Med 2020; 125(8):730-7. https://doi.org/10.1007/s11547-020-01232-9
13. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, *et al*. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a

descriptive study. Lancet Infect Dis 20(4):425-34.
https://doi.org/10.1016/S1473-3099(20)30086-4

14. Ospina JAC, Livias KA, Gomez WEV, Diaz CEP, Aldana DKB, Cardona AM, *et al*. Dengue and COVID-19, overlapping epidemics? An analysis from Colombia. J Med Virol 2020; 93(1):522-7.
https://doi.org/10.1002 / jmv.26194
15. Lorenz C, Azevedo TS, Neto FC. COVID-19 and dengue fever: a dangerous combination for the health system in Brazil. Trav Med Infect Dis 2020; 35:101659.
https://doi.org/10.1016/j.tmaid.2020.101659
16. Verduyn M, Allou N, Gazaille V, Andre M, Desroche T, Jaffar MC, *et al*. Co-infection of dengue and COVID-19: a case report. Plos Negl Trop Dis 2020; 14(8):e0008476.
https://doi.org/10.1371/journal.pntd.0008476
17. Ratnarathon AC, Pongpirul K, Pongpirul WA, Charoenpong L, Prasithsirikul W. Potential dual dengue and SARS-CoV-2 infection in Thailand: a case study. Heliyon 2020; 6(6):e04175.
https://doi.org/10.1016/j.heliyon.2020.e04175
18. Wee LE, Cherng BPZ, Conceicao EP, Goh KCM, Wan WY, Ko KKK, *et al*. Experience of a tertiary hospital in Singapore with management of a dual outbreak of COVID-19 and dengue. Am J Trop Med Hyg 2020; 103(5):2005-11.
https://doi.org/10.4269/ajtmh.20-0703
19. Epelboin L, Blondé R, Nacher M, Combe P, Collet L. COVID-19 and dengue co-infection in a returning traveler. J Trav Med 2020; 27(6):taaa114.
https://doi.org/10.1093/jtm/taaa114

JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 55, Number 1, 2023; 86-98
https://doi.org/10.19106/JMedSci005501202311

The effect of prophylactic negative pressure wound therapy on infection in obese women after C-section: a meta-analysis

Aris Widiyanto[1]*, Santy Irene Putri[2], Asruria Sani Fajriah[3], Yuly Peristiowati[3], Agusta Dian Ellina[3], Joko Triatmojo[1]

[1]School of Health Sciences Mamba'ul Ulum Surakarta, [2]University of Tribhuwana Tunggadewi Malang, [3]Institute of Health Sciences Strada

Submitted: 2021-08-02
Accepted : 2022-12-05

ABSTRACT

Most caesarean wounds resulted in infection. This become a huge burden on the health care system, considering the high number of women undergoing this type of surgery. Negative pressure wound therapy (NPWT) has been recommended for a wide variety of lesions including open abdominal wounds. The purpose of this study was to investigate the effect of prophylactic NPWT on infection in obese women after a C-section. This was a systematic review and meta-analysis study that used articles from online databases of EBSCO, Google Scholar, and PubMed which published until 2022. The dependent variable was infections post C-section, while the independent were NPWT and standard wound therapy. The data was analyzed by RevMan 5.3. This study showed that there is no difference in the outcome of superficial site infection (SSI), deep site infection (DSI), wound dehiscence, seroma, and hematoma between women with obesity after caesarean delivery who used NPWT and standard dressing.

Keywords:
prophylactic negative pressure;
wound therapy;
wound dressing;
obese women;
cesarean delivery

ABSTRACT

Luka operasi Caesar, sebagian besar mengalami infeksi. Hal ini menjadi beban besar bagi sistem pelayanan kesehatan, mengingat tingginya jumlah wanita yang menjalani jenis operasi ini. Terapi luka tekanan negatif (TLTN) telah direkomendasikan untuk berbagai macam lesi termasuk luka perut terbuka. Tujuan dari penelitian ini adalah untuk menginvestigasi pengaruh TLTN dengan pembalut luka standar terhadap kejadian infeksi pada wanita obesitas setelah persalinan caesar. Penelitian ini merupakan review sistematis dan meta-analisis dengan pencarian artikel yang diterbitkan di database online EBSCO, Google Scholar, dan PubMed yang dipublikasikan sampai tahun 2022. Variabel tergantung adalah kejadian infeksi pasca operasi Caesar dan variable bebas adalah TLTN dan terapi luka standar. Analisis data menggunakan software RevMan versi 5.3. Hasil analisis menunjukkan tidak ada perbedaan terhadap kejadian SSI (*superficial site infection*), DSI (*deep site infection*), *wound dehiscence*, *seroma* dan *hematoma* dalam TLTN dan *standard dressing* pada wanita obesitas yang menjalani operasi Caesar.

INTRODUCTION

Caesarean delivery rates are on average among obese women, with the risk of having an emergency caesarean delivery or elective delivery increase with the mount in body mass index (BMI).[1-6] Postoperative infection is one of a number of potential complications following caesarean section[7,8] with rates ranging from 1.2 to 5.0%, reported to occur in post-caesarean women during their hospital stay.[9-16] Postoperative wound complications such as surgical site infection (SSI), wound dehiscence (opening of closed surgical wounds), and hematoma and seroma formation are common complications of surgical

*corresponding author: widiyanto.aris99@gmail.com

procedures,[17] especially in women who are obese, diabetic, or both.[3]

Although the majority of caesarean section wound infections are superficial infections, this poses a huge burden to the health care system, given the high number of women undergoing this type of surgery.[18] Surgical site infection has been of concern because it contributes to intervention and re-treatment, increased length of hospital stays, delayed wound healing, and in some cases, death.[13,15]

Negative pressure wound therapy (NPWT) has been used since the late 1990s. It has been recommended for a wide variety of lesions including open abdominal wounds, open fractures, burns, ulcers, post-traumatic wounds, diabetic ulcers on the feet, skin grafts, sternal wounds, and postoperative cleansing in obese patients. It is also increasingly being used as a prophylactic in closed incision wounds to prevent surgical site complications, as well as being used in secondary wound healing such as chronic wounds or infected wounds.[16] Negative pressure prophylactic wound therapy consists of a closed wound management system by applying negative pressure (suction) to the wound surface. The wound is closed or packed with open cell foam or gauze dressing and sealed with an occlusive curtain. The continuous suction system is maintained by connecting the suction tube from the wound dressing to the vacuum pump and effluent collector. Standard negative pressure levels range from 50 to 125 mmHg.[19,20]

Surgical site infections (SSI) are infections of the skin after surgery. This type of infection consist of more significant kind of infection such as superficial site infection (SSI), deep site infection (DSI), wound dehiscence, seroma, and hematoma. Superficial site infection is an infection that occurs in the area where the surgical incision was made. Deep site infection is an infection that occurs under the incision area in the muscle and tissue around the muscle. Wound dehiscence is the re-opening of a surgical wound that has been primarily sutured. Seroma is a condition that occurs when sterile serum or body fluids collect under the surface of the skin causing swelling and sometimes pain. Hematoma is an abnormal collection of blood outside the blood vessels, this condition can occur when the walls of the arteries, veins, or capillaries are damaged so that blood flows out into tissues that are not where it belongs.[21]

However, NPWT is considered relatively expensive compared to standard postoperative wound care. Therefore, it should be considered judiciously in patients who are at high risk for complications of surgical site infections or if the consequences of complications of surgical site infections are high.[21]

Studies that discusses the effectiveness of NPWT as postoperative wound therapy are still limited, so further study is needed. Therefore, this study aimed to identify the effect of negative pressure prophylactic wound therapy on the incidence of infection in obese women after caesarean delivery.

MATERIALS AND METHODS

Study design

The design of this study was a systematic review and meta-analysis.

Inclusion criteria

Search articles using the EBSCO, Google Scholar, and PubMed online databases were performed. The articles used in this review were articles published from 2016 to 2021. In the process of searching for articles, the keywords “prophylactic negative pressure”, “wound therapy”, “wound dressing”, “obese women”, and “caesarean delivery” were used.

The inclusion criteria of this study were 1) an article describing the effect of negative pressure prophylactic wound therapy versus standard wound dressings on the incidence of infection in obese women after caesarean delivery; 2) articles consists 5 kinds of dependent variables including post-operative wound complications, namely: DSI, DSI, wound dehiscence, seroma and hematoma. 3) original research papers; 4) the study design was randomized controlled trials (RCT). The exclusion criteria for this study were 1) articles in languages other than English and Indonesian; 2) review papers; 3) research data is incomplete or not available.

Study instruments

Three online databases including EBSCO, PubMed, and Google Scholar were used. The Prism diagram guided to search the articles (FIGURE 1). Articles included in this study must meet the inclusion criteria and have been reviewed using a critical appraisal in accordance with the research design of each article. The data were analyzed by the software Review Manager version 5.3.

RESULTS

Characteristics of research articles

There are a total of 899 articles searched from the online database using the keywords "prophylactic negative pressure", "wound therapy", "wound dressing", "obese women", and "caesarean delivery" by choosing the year of publication until 2022.

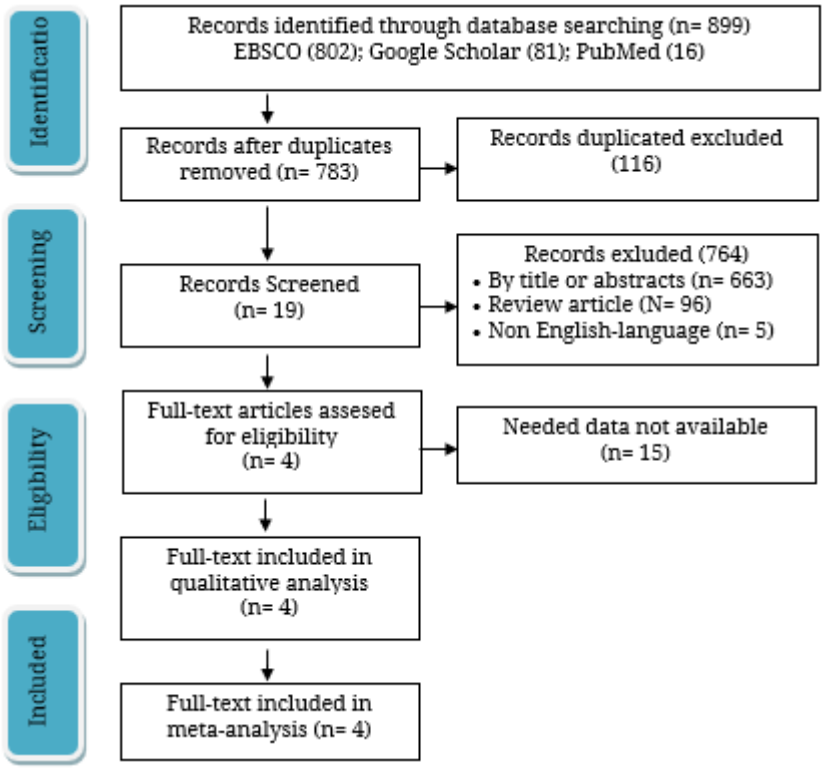


FIGURE 1. Prism diagram

A total of 4 articles that met the inclusion criteria were processed in qualitative and quantitative synthesis. The characteristics of each article included in this study are described in TABLE 1. Case and total of events extracted from each article included are summarized in TABLE 2. The numbers of cases and total in experiment group compared to the number of cases and total in control group in dichotomous data were analyzed. Furthermore, the effects with risk ratio were measured.

TABLE 1. Summary of characteristics from articles included

Authors (years)	Location	Design study	Age [mean (y)]	Intervention	Control	Total sample	Study period	Outcome
Gillespie *et al.*,[23]	Queensland, Australia	RCT	Cases: 31 Control: 31	Closed incisional NPWT	Standard wound dressing	Cases: 1017; Control: 1018	October 2015 - November 2019	SSI, DSI, wound dehiscence, seroma, hematoma
Hyldig *et al.*,[22]	Denmark	RCT	Cases: 32 Control: 32	Incisional NPWT	Standard wound dressing	Cases: 432; Control: 444	2013-2016	Wound dehiscence
Tuuli *et al.*,[24]	USA	RCT	Cases: 30.2 Control: 30.5	Prophylactic NPWT	Standard wound dressing	Cases: 816; Control: 808	February 8, 2017 - November 13, 2019	SSI, DSI, Seroma, Hematoma
Wihbey *et al.*,[25]	USA	RCT	Cases: 31 Control: 30	Vacuum-assisted closure dressing	Standard wound dressing	Cases: 80; Control: 86	January 5, 2015 - January 7, 2017	SSI, DSI, Wound dehiscence, Seroma, Hematoma

NPWT: negative pressure wound therapy; SSI: superficial site infection; DSI: deep site infection

TABLE 2. Data extraction for analysis from each article

Dependent variable	References	Experiment		Control	
		Cases	Total	Cases	Total
DSI	Gillespie *et al.*,[23]	70	75	93	99
	Tuuli *et al.*,[24]	18	806	16	802
	Wihbey *et al.*,[25]	12	80	8	81
DSI	Gillespie *et al.*,[23]	4	75	6	99
	Tuuli *et al.*,[24]	2	806	2	802
	Wihbey *et al.*,[25]	1	80	4	81
Wound dehiscence	Gillespie *et al.*,[23]	108	1017	103	1018
	Hyldig *et al.*,[22]	62	432	69	444
	Wihbey *et al.*,[25]	14	80	13	81
Seroma	Gillespie *et al.*,[23]	27	1017	26	1018
	Tuuli *et al.*,[24]	5	806	6	802
	Wihbey *et al.*,[25]	7	80	6	81
Hematoma	Gillespie *et al.*,[23]	11	1017	6	1018
	Tuuli *et al.*,[24]	4	806	8	802
	Wihbey *et al.*,[26]	2	80	4	81

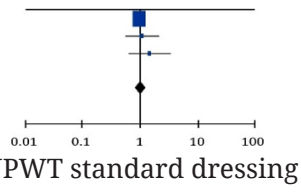
Effect of NPWT on SSI

The results of the meta-analysis showed that subjects using NPWT and those using standard wound care had almost the same risk of developing SSI (RR = 1.05; 95% CI = 0.91-1.22; p = 0.48). The heterogeneity in this analysis is low, so the authors use fixed effects to determine the results of the analysis (TABLE 3).

The author considers that there is no publication bias in the results of the analysis as indicated by the presence of a circle touching the center line and the positions of the circles in the symmetrical funnel plot (FIGURE 2).

TABLE 3. Forest plot of the SSI

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (%)	Risk ratio [M-H fixed (95%CI)]
Gillespie *et al.*,[23]	70	75	93	99	77	0.99 (0.92-1.07)
Tuuli *et al.*,[24]	18	806	16	802	15.4	1.12 (0.57-2.18)
Wihbey *et al.*,[25]	12	80	8	81	7.6	1.52 (0.66-3.52)
Total (95%CI)		961		982	100	1.05 (0.91-1.22)
Total events	100		117			



Heterogenity Chi^2 : 2.87; df: 2 (p=0.24); I^2: 30%; Test for overall effect Z: 0.71 (p=0.48)

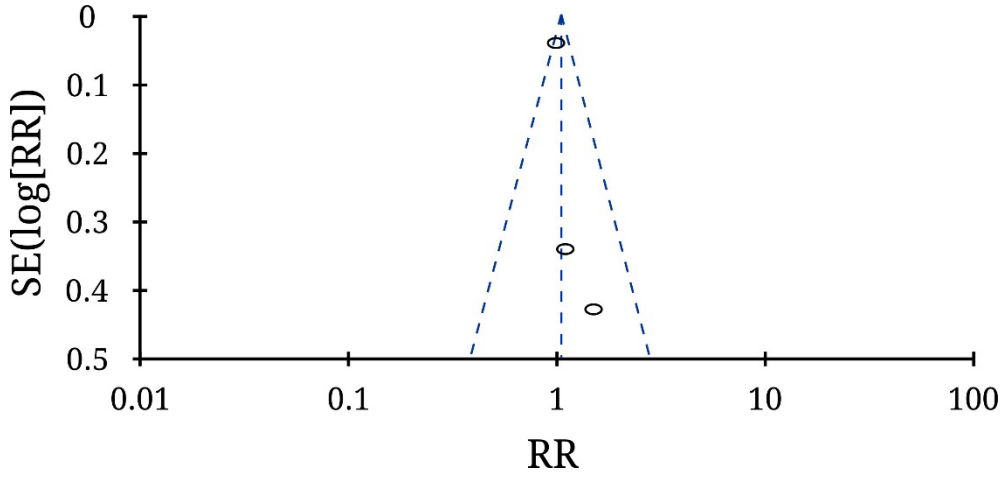


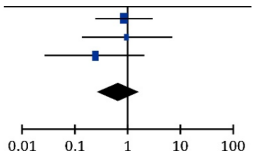
FIGURE 2. Funnel plot of SSI

Effect of NPWT on DSI

The results of the meta-analysis showed that subjects using NPWT had a lower risk of DSI than those using standard wound care. However, it was not significantly different (RR = 0.68; 95% CI = 0.27-1.68; p = 0.40). No difference between subjects using NPWT with those using standard wound care was reported (TABLE 4). The author considers that there is a publication bias in the results of the analysis, which is indicated by the asymmetrical position of the circle (FIGURE 3).

TABLE 4. Forest plot of the DSI

Study or subgroup	Experimental		Control		Weight (%)	Risk ratio [M-H fixed (95%CI)]
	Events	Total	Events	Total		
Gillespie *et al.*,[23]	4	75	6	99	46.4	0.88 (0.26-3.01)
Tuuli *et al.*,[24]	2	806	2	802	18.0	1.00 (0.14-7.05)
Wihbey *et al.*,[24]	1	80	4	81	35.6	0.25 (0.03-2.22)
Total (95%CI)		961		982	100%	0.68 (0.27-1.68)
Total events	7		12			



Heterogenity Chi^2 : 1.11; df: 2 (p=0.57); I^2: 0%; Test for overall effect Z: 0.84 (p=0.40)

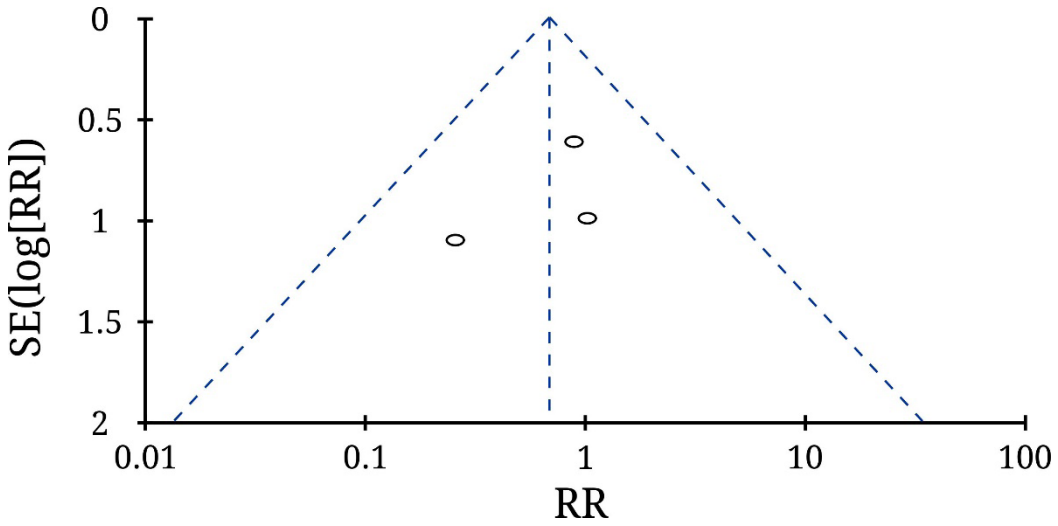


FIGURE 3. Funnel plot of DSI

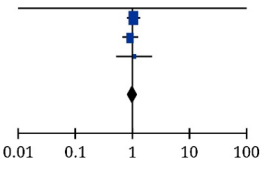
Effect of NPWT on wound dehiscence

The results of the meta-analysis showed that subjects using NPWT and those using standard wound care had almost the same risk of developing wound dehiscence (RR = 1.01; 95% CI = 0.83-1.22; p = 0.95). The heterogeneity in this analysis is low, so the authors use fixed effects to determine the results of the analysis (TABLE 5).

The author considers that there is no publication bias in the analysis results, which is indicated by the presence of a circle touching the center line and the positions of the circles in the symmetrical funnel plot (FIGURE 4).

TABLE 5. Forest plot of the wound dehiscence

Study or subgroup	Experimental		Control		Weight	Risk ratio
	Events	Total	Events	Total	(%)	[M-H fixed (95%CI)]
Gillespie *et al.*,[23]	108	1017	103	1018	56	1.05 (0.81-1.36)
Tuuli *et al.*,[24]	62	432	69	444	37	0.92 (0.67-1.27)
Wihbey *et al.*,[25]	14	80	13	81	7	1.09 (0.55-2.17)
Total (95%CI)		1529		1534	100%	1.01 (0.83-1.22)
Total events	184		185			



Heterogenity Chi^2 : 0.44; df: 2 (p=0.80); I^2: 0%; Test for overall effect Z: 0.06 (p=0.95)

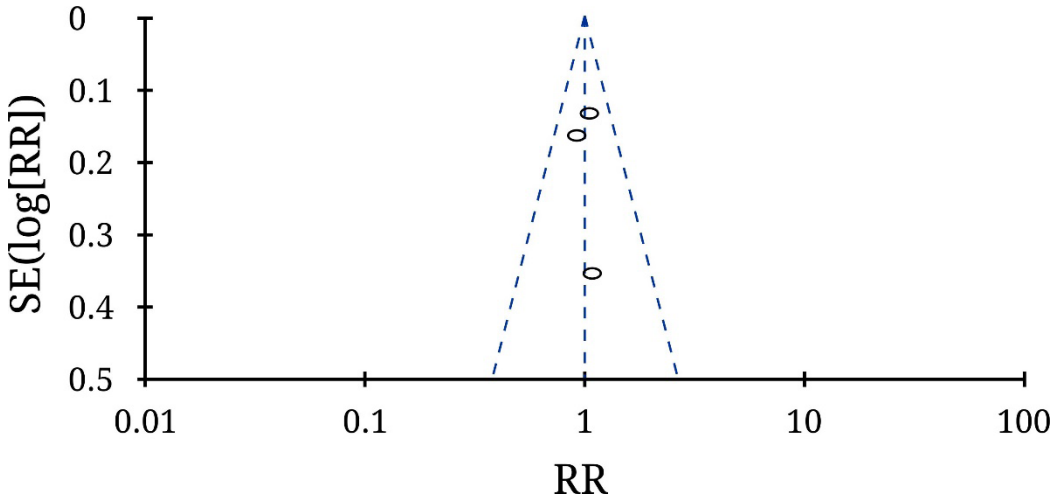


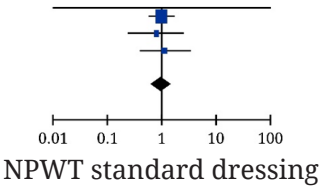
FIGURE 4. Funnel plot of wound dehiscence

Effect of NPWT on seroma

The results of the meta-analysis showed that subjects using NPWT and those using standard wound care had almost the same risk of developing seroma (RR = 1.03; 95% CI = 0.66-1.60; p = 0.90). The heterogeneity in this analysis is low, so the authors use fixed effects to determine the results of the analysis (TABLE 6). The author considers that there is no publication bias in the analysis results, which is indicated by the presence of a circle touching the center line and the positions of the circles on a symmetrical funnel plot (FIGURE 5).

TABLE 6. Forest plot of seroma

Study or subgroup	Experimental		Control		Weight	Risk ratio
	Events	Total	Events	Total	(%)	[M-H fixed (95%CI)]
Gillespie *et al.*,[23]	27	1017	26	1018	68.5	1.04 (0.61-1.77)
Tuuli *et al.*,[24]	5	806	6	802	15.8	0.83 (0.25-2.71)
Wihbey *et al.*,[25]	7	80	6	81	15.7	1.18 (0.42-3.36)
Total (95%CI)		1903		1901	100	1.03 (0.66-1.60)
Total events	39		38			



Heterogenity Chi² : 0.22; df: 2 (p=0.91); I²: 0%; Test for overall effect Z: 0.13 (p=0.90)

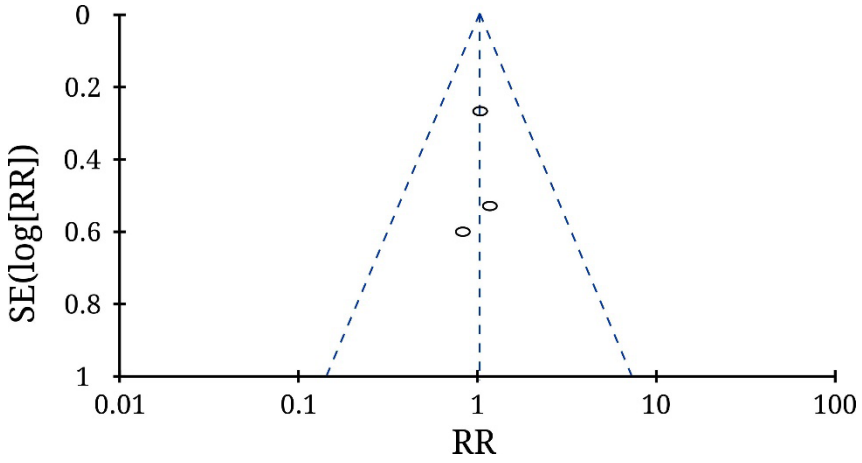


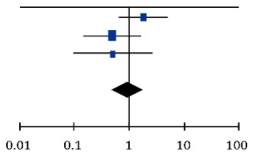
FIGURE 5. Funnel plot of seroma

Effect of NPWT on hematoma

The results of the meta-analysis showed that subjects using NPWT and those using standard wound care had almost the same risk of developing hematoma (RR = 0.95; 95% CI = 0.49-1.83; p = 0.87). The heterogeneity in this analysis is low, so the authors use fixed effects to determine the results of the analysis (TABLE 7). The author considers that there is a publication bias in the analysis results as indicated by the asymmetrical position of the circle (FIGURE 6).

TABLE 7. Forest plot of hematoma

Study or subgroup	Experimental		Control		Weight	Risk ratio
	Events	Total	Events	Total	(%)	[M-H fixed (95%CI)]
Gillespie *et al.*,[23]	11	1017	6	1018	33.3	1.84 (0.68-4.94)
Tuuli *et al.*,[24]	4	806	8	802	44.6	0.50 (0.15-1.65)
Wihbey *et al.*,[25]	2	80	4	81	22.1	0.51 (0.10-2.69)
Total (95%CI)		1903		1901	100	0.95 (0.49-1.83)
Total events	17		18			



Heterogenity Chi^2 : 3.37; df: 2 (p=0.19); I^2: 41%; Test for overall effect Z: 0.17 (p=0.87)

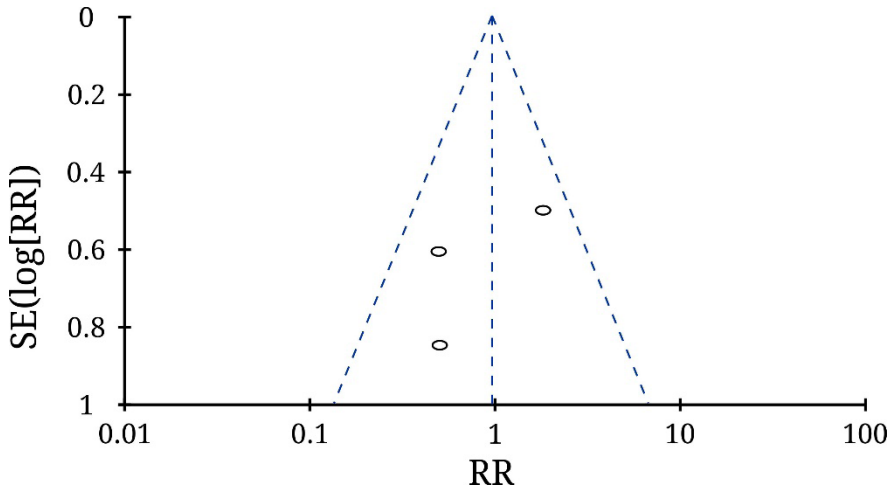


FIGURE 6. Funnel plot of hematoma

DISCUSSION

This study describes the comparison of the benefits of NPWT with standard wound care in obese mothers who gave birth by caesarean section. Previous studies reported that NPWT reduces bacterial contamination, edema, and exudate, as well as increases microvascular blood flow, and promotes granulation tissue by inducing mechanical stress that promotes cell growth.[26-29]

A total of 4 articles which met the inclusion criteria were analyzed. Five criteria of infection that occur in mothers who use NPWT and standard wound therapy including SSI, DSI, wound dehiscence, seroma, and hematoma were compared. Among 5 criteria for infection and wound complications analyzed, only DSI showed a moderate strength of measurement inclined towards benefits. The reduction of risk 32% lower on the incidence of DSI in the NPWT group compared to the standard dressing group was observed. However, it was not significantly different (RR = 0.68; 95% CI = 0.27-1.68; p = 0.40). Meanwhile, 4 other types of infection showed an inconsiderable value of risk ratio, which stated that there is no difference between the intervention and control group in terms of decreasing the SSI, wound dehiscence, seroma, and hematoma. The reduction in the incidence of DSI in the NPWT group may be explained by an increase in microvascular blood flow

during therapy, which leads to a decrease in the hypoxic response, thereby enhancing the oxidative bacterial killing mechanism in adipose tissue.[30,31]

As for the insignificant results in other complications, it may be due to the relatively small number of patients involving in the study, therefore further study is needed. Likewise, the limitations of each of the studies analyzed in this review and meta-analysis study. The results of this study are in accordance with the results of a study by Costa *et al*.,[32] which reported that there is no difference in patients using NPWT who underwent major trauma-related surgery for lower extremity fractures in reducing surgical site infection. Previous systematic review studies and meta-analyses also reported similar results, that there is insufficient evidence to support the use of NPWT among obese women for the prevention of caesarean wound complications.[33,34]

CONCLUSION

In conclusion, there is no difference in the benefits of NPWT with standard wound care to reduce SSI, DSI, wound dehiscence, seroma, and hematoma in obese women after caesarean section.

ACKNOWLEDGEMENTS

We would like to thanks the online database provider of EBSCO, Google Scholar, and PubMed for supporting the articles.

REFERENCES

1. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, *et al*. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes Relat Metab Disord 2001; 25(8):1175-82. https://doi.org/10.1038/sj.ijo.0801670
2. Poobalan AS, Aucott LS, Gurung T, Smith WC, Bhattacharya S. Obesity as an independent risk factor for elective and emergency caesarean delivery in nulliparous women--systematic review and meta-analysis of cohort studies. Obes Rev 2009; 10(1):28-35. https://doi.org/10.1111/j.1467-789X.2008.00537.x
3. Heslehurst N, Simpson H, Ells LJ, Rankin J, Wilkinson J, Lang R, *et al*. The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. Obes Rev 2008; 9(6):635-83. https://doi.org/10.1111/j.1467-789X.2008.00511.x
4. Denison FC, Price J, Graham C, Wild S, Liston WA. Maternal obesity, length of gestation, risk of postdates pregnancy and spontaneous onset of labour at term. BJOG 2008; 115(6):720-5. https://doi.org/10.1111/j.1471-0528.2008.01694.x
5. Denison FC, Norwood P, Bhattacharya S, Duffy A, Mahmood T, Morris C, *et al*. Association between maternal body mass index during pregnancy, short-term morbidity, and increased health service costs: a population-based study. BJOG 2014; 121(1):72-81; discussion 82. https://doi.org/10.1111/1471-0528.12443
6. Voinchet V, Magalon G. [Vacuum assisted closure. Wound healing by negative pressure. Ann Chir Plast Esthet 1996; 41(5):583-9.
7. Liu S, Liston RM, Joseph KS, Heaman M, Sauve R, Kramer MS, *et al*. Maternal mortality and severe morbidity associated with low-risk planned cesarean delivery versus planned vaginal delivery at term. CMAJ 2007; 176(4):455-60. https://doi.org/10.1503/cmaj.060870.
8. Bodner K, Wierrani F, Grünberger W, Bodner-Adler B. Influence of the mode of delivery on maternal and

neonatal outcomes: a comparison between elective cesarean section and planned vaginal delivery in a low-risk obstetric population. Arch Gynecol Obstet 2011; 283(6):1193-8. https://doi.org/10.1007/s00404-010-1525-y
9. Johnson A, Young D, Reilly J. Caesarean section surgical site infection surveillance. J Hosp Infect 2006; 64(1):30-5. https://doi.org/10.1016/j.jhin.2006.03.020
10. Vincent A, Ayzac L, Girard R, Caillat-Vallet E, Chapuis C, Depaix F, *et al*. Downward trends in surgical site and urinary tract infections after cesarean delivery in a French surveillance network, 1997-2003. Infect Control Hosp Epidemiol 2008; 29(3):227-33. https://doi.org/10.1086/527512
11. Ward VP, Charlett A, Fagan J, Crawshaw SC. Enhanced surgical site infection surveillance following caesarean section: experience of a multicentre collaborative post-discharge system. J Hosp Infect 2008; 70(2):166-73. https://doi.org/10.1016/j.jhin.2008.06.002
12. Szilágyi E, Füzi M, Böröcz K, Kurcz A, Tóth Á, Nagy K. Risk factors and outcomes for bloodstream infections with extended-spectrum β-lactamase-producing Klebsiella pneumoniae; Findings of the nosocomial surveillance system in Hungary. Acta Microbiol Immunol Hung 2009; 56(3):251-62. https://doi.org/10.1556/AMicr.56.2009.3.5
13. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, *et al*. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. JAMA Surg 2017; 152(8):784-91. https://doi.org/10.1001/jamasurg.2017.0904
14. Dias M, Dick A, Reynolds RM, Lahti-Pulkkinen M, Denison FC. Predictors of surgical site skin infection and clinical outcome at caesarean section in the very severely obese: A retrospective cohort study. PLoS One 2019; 14(6):e0216157. https://doi.org/10.1371/journal.pone.0216157
15. Ramírez-Wong FM, Atencio-Espinoza T, Rosenthal VD, Ramirez E, Torres-Zegarra SL, Díaz Tavera ZR, *et al*. Surgical Site Infections Rates in More Than 13,000 Surgical Procedures in Three Cities in Peru: Findings of the International Nosocomial Infection Control Consortium. Surg Infect (Larchmt) 2015; 16(5):572-6. https://doi.org/10.1089/sur.2014.201
16. Norman G, Goh EL, Dumville JC, Shi C, Liu Z, Chiverton L, *et al*. Negative pressure wound therapy for surgical wounds healing by primary closure. Cochrane database Syst Rev 2020; 5(5):CD009261. https://doi.org/10.1002/14651858.CD009261
17. Smyth ETM, McIlvenny G, Enstone JE, Emmerson AM, Humphreys H, Fitzpatrick F, *et al*. Four country healthcare associated infection prevalence survey 2006: overview of the results. J Hosp Infect 2008; 69(3):230-48. https://doi.org/10.1016/j.jhin.2008.04.020
18. Wloch C, Wilson J, Lamagni T, Harrington P, Charlett A, Sheridan E. Risk factors for surgical site infection following caesarean section in England: results from a multicentre cohort study. BJOG 2012; 119(11):1324-33. https://doi.org/10.1111/j.1471-0528.2012.03452.x
19. Ubbink DT, Westerbos SJ, Nelson EA, Vermeulen H. A systematic review of topical negative pressure therapy for acute and chronic wounds. Br J Surg 2008; 95(6):685-92. https://doi.org/10.1002/bjs.6238
20. Vikatmaa P, Juutilainen V, Kuukasjärvi P, Malmivaara A.

Negative pressure wound therapy: a systematic review on effectiveness and safety. Eur J Vasc Endovasc Surg 2008; 36(4):438-48. https://doi.org/0.1016/j.ejvs.2008.06.010
21. Medicine JH. Surgical Site Infections. Health. 2021.
22. Hyldig N, Vinter CA, Kruse M, Mogensen O, Bille C, Sorensen JA, *et al*. Prophylactic incisional negative pressure wound therapy reduces the risk of surgical site infection after caesarean section in obese women: a pragmatic randomised clinical trial. BJOG 2019; 126(5):628-35. https://doi.org/10.1111/1471-0528.15413
23. Gillespie BM, Webster J, Ellwood D, Thalib L, Whitty JA, Mahomed K, *et al*. Closed incision negative pressure wound therapy versus standard dressings in obese women undergoing caesarean section: multicentre parallel group randomised controlled trial. BMJ 2021; 373:n893. https://doi.org/10.1136/bmj.n893
24. Tuuli MG, Liu J, Tita ATN, Longo S, Trudell A, Carter EB, *et al*. Effect of prophylactic negative pressure wound therapy vs standard wound dressing on surgical-site infection in obese women after cesarean delivery: A randomized clinical trial. JAMA 2020; 324(12):1180-9. https://doi.org/10.1001/jama.2020.13361
25. Wihbey KA, Joyce EM, Spalding ZT, Jones HJ, MacKenzie TA, Evans RH, *et al*. Prophylactic Negative Pressure Wound Therapy and Wound Complication after Cesarean Delivery in Women with Class II or III Obesity: A Randomized Controlled Trial. Obstet Gynecol 2018; 132(2):377-84. https://doi.org/10.1097/AOG.0000000000002744
26. Gouttefangeas C, Eberle M, Ruck P, Stark M, Müller JE, Becker HD, *et al*. Functional T lymphocytes infiltrate implanted polyvinyl alcohol foams during surgical wound closure therapy. Clin Exp Immunol 2001; 124(3):398-405. https://doi.org/10.1046/j.1365-2249.2001.01547.x
27. Chen SZ, Li J, Li XY, Xu LS. Effects of vacuum-assisted closure on wound microcirculation: An experimental study. Asian J Surg 2005; 28(3):211-7. https://doi.org/10.1016/S1015-9584(09)60346-8
28. Malmsjö M, Gustafsson L, Lindstedt S, Gesslein B, Ingemansson R. The effects of variable, intermittent, and continuous negative pressure wound therapy, using foam or gauze, on wound contraction, granulation tissue formation, and ingrowth into the wound filler. Eplasty 2012; 12:e5.
29. Xia CY, Yu AX, Qi B, Zhou M, Li ZH, Wang WY. Analysis of blood flow and local expression of angiogenesis-associated growth factors in infected wounds treated with negative pressure wound therapy. Mol Med Rep 2014; 9(5):1749-54. https://doi.org/10.3892/mmr.2014.1997
30. Greif R, Akça O, Horn EP, Kurz A, Sessler DI. Supplemental perioperative oxygen to reduce the incidence of surgical-wound infection. N Engl J Med 2000; 342(3):161-7. https://doi.org/10.1056/NEJM200001203420303
31. Allen DB, Maguire JJ, Mahdavian M, Wicke C, Marcocci L, Scheuenstuhl H, *et al*. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg 1997; 132(9):991-6. https://doi.org/10.1001/archsurg.1997.01430330057009
32. Costa ML, Achten J, Knight R, Bruce J, Dutton SJ, Madan J, *et al.* Effect of incisional negative pressure wound therapy vs standard wound dressing on deep surgical site infection after surgery for lower limb fractures associated with major trauma: The

WHIST Randomized Clinical Trial. JAMA 2020; 323(6):519-26. https://doi.org/10.1001/jama.2020.0059

33. Smid MC, Dotters-Katz SK, Grace M, Wright ST, Villers MS, Hardy-Fairbanks A, *et al*. Prophylactic negative pressure wound therapy for obese women after cesarean delivery: a systematic review and meta-analysis. Obstet Gynecol 2017; 130(5):969-78. https://doi.org/10.1097/AOG.0000000000002259
34. Widiyanto A, Putri SI, Fajriah AS, Rejo R, Nurhayati I, Yuniarti T, *et al*. The implementation of family nursing care to patients with chronic diseases: a systematic review. STRADA: Jurnal Ilmiah Kesehatan 2021; 10(1):1225-33. https://doi.org/10.30994/sjik.v10i1.753