RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2934
Majalah Kedokteran Bandung. 2023;55(2):98–105

Majalah Kedokteran Bandung (MKB)

Received: August 1, 2022
Accepted: October 31, 2022
Available online: June 30, 2023

Anxiety (DASS21) and the Quality of Life (FertiQol) of Infertile Women Underwent In Vitro Fertilization

Dian Tjahjadi,[1] Bayu Indrayana Irsyad,[1] Ahmad Yogi Pramatirta,[1] Santi Andayani[2]
[1]Department of Obstetrics and Gynecology Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
[2]Department of Psychiatry Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Infertility affects physical and mental health and has various consequences. In vitro fertilization (IVF) is one of the Assisted Reproductive Technology (ART) programs that include several phases, requiring longer time, more patience, and is expensive. This may make women who undergo the ART program to have higher stress level, especially anxiety. This study aimed to determine the relationship between anxiety and quality of life in infertile patients undergoing assisted reproductive technology programs. This was a cross-sectional observational analytic study performed at Aster Fertility Clinic, Dr. Hasan Sadikin General Hospital and Bandung Fertility Center, Limijati Hospital Bandung, Indonesia, from February until April 2020. The analysis was performed using the DASS21 scale and FertiQol count on 27 subjects undergoing the IVF program and 30 control subjects who were pregnant without IVF program. The statistical analyses used to determine the condition of anxiety and the condition of quality of life were the Chi-square and the Mann-Whitney tests, while the relationship between anxiety and quality of life as the main subject was measured using the Spearman correlation test. The anxiety level as measured with the DASS21 instrument in the subject and control groups was 6.2 vs 0.7 with p<0.001. From the analysis of the FertiQol instrument in the subject and control groups, the scores were 79.6 vs. 98.9 for the mind-body domain (p<0.001); 68.8 vs 98.5 for the emotional domain (p < 0.001); 83.2 vs 95.7 for the relational domain (p<0.001); and 77.6 vs 97.6 for the social domain (p<0.001). For the overall FertiQol core domains, the score was 77.3 vs 97.7 (p<0.001). The correlation between anxiety and the FertiQol total core domains were evident from the results of the Spearman correlation test, with an r-value of -0.479 (p<0.001). Therefore, there is a significant negative correlation between anxiety and quality of life.

Keywords: Anxiety, infertility, quality of life

Introduction

Reproduction is one of the most essential elements in life, and failure of this process can be a severe physical and mental health problem. Infertility is not easily seen, but it is common among reproductive age groups today. Infertility means the inability to have offspring after marriage for more than one year without using a contraceptive. Infertility is known to be a psychosocial problem in itself. It potentially has profound consequences that cover various aspects affecting socio-cultural, emotional, physical, and financial problems. The World Health Organization (WHO) evaluation of Demographic and Health Surveys (DHS) data found that more than 186 million married women of reproductive age in developing countries dream of a child. This means 1 in every four couples in developing countries.[1, 2] In Indonesia, according to the Central Agency of Statistics, in 2011, there are 10–15% cases of infertility from nearly 40 million women of childbearing age.[3] The factors that cause infertility are very diverse and complex; therefore, many fertility therapies are carried out following the cause of infertility.

In vitro fertilization (IVF) is one of the assisted reproductive technology, at this stage fertilization between sperm and ovum, is not carried out naturally but requires a lot of time. This procedure has many several phases. The first phase consists of taking the mature oocytes in the ovaries,

Corresponding Author:
Bayu Indrayana Irsyad
Department of Obstetrics and Gynecology Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: bayu_irsyad@yahoo.com

which will be brought together with sperm in the laboratory, and transferring the embryos into the uterine cavity. These oocytes are grown by stimulation of gonadotropin hormones. This stimulatory response can vary and sometimes takes more time. If the stimulation is successful, transvaginal ultrasound guidance of follicular oocyte retrieval after stimulation also takes time. After that, it will be examined under a microscope to assess its quality level before fertilization. The stages of in vitro fertilization are expensive, can be a long-time treatment, and need more patience with uncertain final results.[4] This makes the program so special because both before and after the program can put the patient under psychological pressure. Studies show that anxiety symptoms increase by almost 10% in patients undergoing this program.[5]

Many factors related to infertility make this a problem in itself which often causes stressors. It is not uncommon for a related partner to be prone to stress related to infertility such as stress when knowing the cause of infertility, disturbance or discomfort during intercourse with a partner or while undergoing a long series of fertility therapy.[6] A long series of fertility therapies is prone to placing patients in psychological conditions such as stress, and found that infertility therapy is the most stressor factor in infertile women.[7] Moreover, the National Health Insurance (JKN) program in Indonesia does not include fertility service as one that can be covered.

The number of people with mental disorders generally increases every year, especially in low-income countries, because population growth is taking place and most people are of reproductive age. Physical health is easier to recognize because it can be complained about, but mental health is still difficult to recognize. This encourages a person to take the necessary steps to prevent the threat or reduce its consequences. Anxiety is one of the most common psychiatric disorders. The population with anxiety disorders in 2015 was estimated to be 3.6% (264 million people) of the world's population. The National Comorbidity Study reports that one in four people will have at least one anxiety disorder. Its prevalence in Southeast Asia is as much as 23% of the world's total population with anxiety disorders. The majority of sufferers are women.[8]

Anxiety can be defined as a normal and adaptive response that has life-saving qualities and warns of threats of damage, pain, helplessness, frustration with physical and social needs; or separation from loved ones. This encourages a person to take the necessary steps to prevent the threat or reduce its consequences. Anxiety is one of the most common psychiatric disorders. The population with anxiety disorders in 2015 has been estimated to be 3.6% (264 million people) of the world's population. The National Comorbidity Study reports that one in four people will have at least 1 anxiety disorder. Its prevalence in Southeast Asia is as much as 23% of the total population with anxiety disorders in the world. The majority of sufferers are women.[8]

Emotional stress, such as frustration and aggressiveness, depression, and anxiety, impact pregnancy. Those who chose the ART programs tended to have higher distress when compared to infertile women who did not undergo any fertility program and did not try to conceive children.[9] Lazarus and Folkman[10] emphasize that stress can create positive and negative experiences. Another claimed that there are many negative associations between stress related to infertility. This stressor caused by infertility, also called infertility-related stress, can last quite a long time, even up to a lifetime, so that it can become a chronic stressor affecting various aspects of life such as relationships with partners and the surrounding community, sexual relations, even the desire to become parents.[11]

This study aims to determine the relationship between anxiety and quality of life in infertile patients undergoing assisted reproductive technology programs. This study can be beneficial as the basis of development for increasing the success of assisted reproductive technology.

Methods

This observational analytic study with a cross-sectional design aims to see the relationship between anxiety and the quality of life of women undergoing IVF in ART clinics. The subjects are the women participating in IVF Aster Clinic Dr. Hasan Sadikin Hospital Bandung and Bandung Fertility Center Limijati Hospital Bandung from February to April 2020. The subject's criteria are women who underwent IVF for the first time, had a history of primary and secondary infertility, and without a history of serious mental disorders or using psychotropic medication. Control subjects are women who have had a pregnancy without undergoing an IVF program and without a history of serious mental disorders or using psychotropic medication. The exclusion criteria are women who refused to follow the research procedure and had previously followed the IVF

program. The selection of subjects was obtained from all patients who came for the IVF program during the period from February until April 2020.

The measuring instrument used is the Depression, Anxiety, and Stress Scale (DASS). This questionnaire is designed to measure harmful emotional levels of depression, anxiety, and stress. Each rating scale consists of 14 points divided into 2–5 sections with the same content. Subjects will be asked to fill out a severity scale ranging from 1 to 4 points. The questionnaire consists of 21 questions. These numbers will be your depression, anxiety, and stress scores, which will be calculated by adding them up.[12]

Another measuring tool is Fertility Quality of Life (FertiQol). This FertiQol was created in 2002 by The European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) to identify areas affected by fertility problems. In this questionnaire, several domains can be assessed in a total of 36 questions, divided into two questions to evaluate overall personal physical health, 12 questions to assess the impact of fertility problems on the emotional domain (6 questions), and the mind-body domain (6 questions), 12 questions to measure the impact of fertility problems on relationship domain (6 questions) and social domain (6 questions) and ten questions to measure the quality of life during the program, both intervention and consultation based on environment (6 questions) and acceptance of the therapy given (4 questions).[13]

This study has received ethical approval from Health Research Ethics Committee Dr. Hasan Sadikin General Hospital with serial number LB.2.01/X.6.5/31/2020. The minimum sample of this study was 25 patients. The data was taken using a cross-sectional research design and analyzed with SPSS. The data collection was obtained through a questionnaire that was filled in and a score was calculated to determine the degree of anxiety and the quality of the research subject. Descriptions of female characteristics are presented in tabular form. Anxiety conditions obtained from the DASS research instrument and quality of life from the FertiQol instrument were processed and described by the number (n) and the percentage (%). The normality test shows that the distribution of study variables is normal. The analysis of this study was conducted using the Chi-square test for categoric criteria and the Mann-Whitney test for the numeric criteria, while the relationship between anxiety and quality of life in the main subject using the Spearman correlation test.

Result

Research has been conducted on 27 infertile women and 30 control women from February to April 2020. This study grouped the characteristics data by age, length of the marriage, and history of abortion.

Table 1 Characteristics of Both Groups

Characteristics	Group		p*)
	IVF Program (n=27)	Control (n=30)	
Age (year)			0.837
<35	21	24	
≥35	6	6	
Mean (SD)	32.0 (4.4)	28.3 (6.2)	
Range	24–41	19–44	
Duration of infertility (year)			0.161
≤5	17	25	
6–10	8	3	
>10	2	2	
Mean (SD)	5.7 (3.4)	4.0 (4.1)	
Range	1–15	1–18	
History of miscarriage			
Yes	7	8	0.949
No	20	22	

Note: *) Chi-square test

Table 2 Comparison between Anxiety of Both Groups

Anxiety	Group		p*)
	IVF Program (n=27)	Control (n=30)	
Anxiety score :			<0.001
Mean (SD)	6.2 (3.9)	0.7 (1.2)	
Median	8	0	
Range	0–18	0–4	
Anxiety level:			<0.001
Normal	13	30	
Mile	10	-	
Moderate	3	-	
Severe	1	-	

Note: *) Anxiety score using Mann-Whitney test; Anxiety level using Chi-square test

From the Table 1, both age groups, duration of infertility group, and history of miscarriage group, the results obtained with a p>0.05; this means that both groups have homogeneous data.

It appears that the median score of anxiety in women who attended the IVF program was more different than the control group (8 and 0), and this difference was statistically significant (p<0.001). In the IVF program group, 41.9% experienced mild to severe anxiety, whereas in the control group, all anxiety levels were classified as normal.

It found a significant difference (p<0.001) between the two study groups for each domain of quality of life. In the group of women who followed IVF, the median quality of life score was lower than the control group

In the IVF program group, the more age

Table 3 Comparison Between Quality of Life of Both Groups

Quality of Life (Domain)	Groups		p*)
	IVF Program (n=27)	Control (n=30)	
Mind-body			<0.001
Mean (SD)	79.6 (15.2)	98.9 (1.8)	
Median	79.2	100	
Range	58.33–100	95.8–100	
Emotional			<0.001
Mean (SD)	68.8 (19.4)	98.5 (2.0)	
Median	66.7	100	
Range	45.8–100	95.8–100	
Relational			<0.001
Mean (SD)	83.2 (13.3)	95.7 (4.0)	
Median	83.3	95.8	
Range	62.5–100	91.7–100	
Social			<0.001
Mean (SD)	77.6 (17.3)	97.6 (2.1)	
Median	79.2	95.8	
Range	25–100	95.8–100	
Total Core FertiQol			<0.001
Mean (SD)	77.3 (13.0)	97.7 (1.6)	
Median	82.3	97.9	
Range	55.2–100	94.8–100	

Note: *) using the Mann-Whitney test

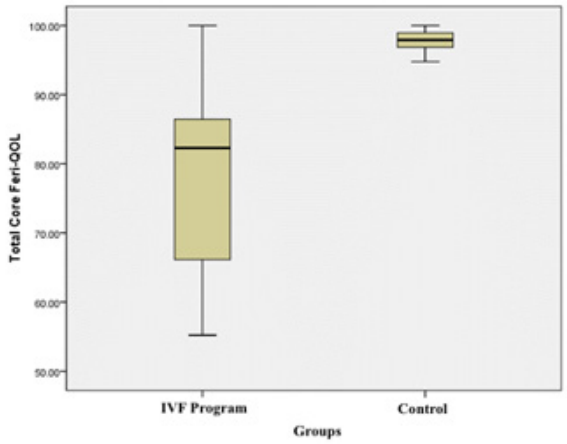

Figure 1 Comparison between the Quality of Life in Both Groups

increases, the more anxiety score increases. In the IVF program group, there was a relationship between age and quality of life, which is significant in mind, body, relational, social, and total core FertiQol; the more age increases, the lower the quality of life score. The correlation between the duration of infertility with anxiety scores and quality of life was not statistically significant (p>0.05). Analysis of the correlation between age and duration of infertility with anxiety scores and quality of life in the control group did not show a significant relationship (p>0.05). The correlation between anxiety scores and total core FertiQol in the IVF program group showed a negative correlation with significant differences (Table 4 and Figure 2); this illustrates the overall correlation between anxiety and quality of life. From this result, the higher the anxiety score, the lower the quality of life.

Discussion

The impact of infertility on the occurrence of psychological distress in a person who experiences it has long been known. The prevalence of anxiety disorders in infertile couples ranges from 25–60%.[14] A meta-analysis study said that anxiety occurs three times more in infertile couples than in fertile couples.[14] Anxiety can occur and is significantly associated with the duration of infertility and level of education. Generally, this anxiety occurs in women who have experienced infertility for 4–6 years.[15] History of miscarriage can be a form of post-traumatic stress disorder as anxiety and depression. Apart from comorbidities and financial factors, it was also found that psychological factors could cause the discontinuation of this IVF program.[16]

In Vitro Fertilization program is unique because this program might be a couple's last effort to get pregnant which involves feeling optimistic and full of hope while, as we know, there is always a failure rate. When starting therapy, usually the patient will undergo a series of tests to determine the cause of infertility. Patients who finally choose assisted reproductive technology programs face busy schedules that are time-consuming and long-term and live among hope, fear, and frustration.[17] This method also requires a lot of money, time, and a long and invasive procedure. Turner et al. found that these stress and anxiety levels increased with each stage of the IVF cycle. This can cause higher levels of distress in those undergoing the stages of the IVF program. This is what becomes the point that psychological distress will increase with time.[19] However, there are also other studies

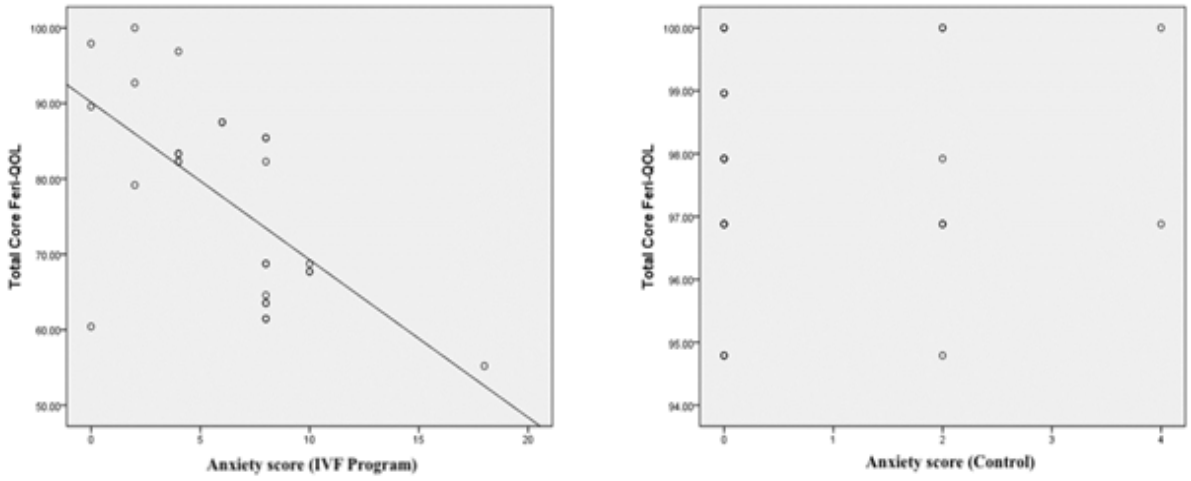

Figure 2 Correlation between Anxiety and Quality of Life in Both Groups

Table 4 Relationships between Age, Duration of Infertility, Anxiety, and Quality of Life in Both Groups

Correlation between	IVF Program		Control	
	r	p	r	p
Age and anxiety	**0.503**	**0.008**	0.098	0.607
Duration of infertility and anxiety	0.184	0.357	-0.112	0.555
Age and mind-body	**-0.401**	**0.038**	-0.026	0.891
Age and emotional	-0.335	0.087	0.036	0.850
Age and relational	**-0.521**	**0.008**	0.213	0.259
Age and social	**-0.605**	**0.001**	0.047	0.806
Age and total core FertiQol	**-0.547**	**0.003**	0.195	0.302
Duration of infertility and mind-body	-0.241	0.227	0.182	0.336
Duration of infertility and emotional	-0.209	0.296	0.179	0.344
Duration of infertility and relational	-0.263	0.185	0.192	0.309
Duration of infertility and social	-0.317	0.107	0.115	0.546
Duration of infertility and total core FertiQol	-0.295	0.135	0.304	0.103
Anxiety and mind-body	**-0.470**	**0.013**	-0.065	0.733
Anxiety and emotional	**-0.518**	**0.006**	0.075	0.695
Anxiety and relational	**-0.486**	**0.010**	0.092	0.631
Anxiety and social	**-0.622**	**0.001**	0.019	0.919
Anxiety and total core FertiQol	**-0.585**	**0.001**	0.027	0.886

Note: r = Spearman's rank correlation coefficient

with different results in which women who have been infertile for a long time have lower levels of anxiety and depression. This can occur due to psychological adaptation to infertility.[20]

Studies have shown that stress, anxiety, and other negative emotions affect pregnancy rates. High-stress levels and low quality of life are associated with low cortisol levels. Cortisol can be a biomarker of the hypothalamic-pituitary-adrenal (HPA) axis. Activating the hypothalamic-pituitary-adrenal (HPA) axis by stimulation of the gonadotropin-releasing hormone (GnRH) will impact a series of hormonal cycles involving reproductive functions.[21]

The World Health Organization defines the quality of life as an individual's perception of his position in life in the context of culture and place of residence concerning the goals, hopes, and concerns at hand. The FertiQol instrument was developed to assess the quality of life specifically for individuals with fertility disorders, regardless of the specifics of fecundity, gender, and social and cultural backgrounds.[22] FertiQol has two main components, namely core FertiQol, which includes the relationship between the subject and its surroundings, and treatment FertiQol (optional), which includes the relationship between the subject and fertility therapy. Core FertiQol consists of an individual part, namely the mind/body and emotional domain, and an interpersonal part, namely the relational and social domain. The mind/body domain assesses how far the experience is from the occurrence of negative physical symptoms (fatigue, pain) and cognitive and behavioral disorders (lack of concentration, disturbances in daily activities, hampered life plans) due to this infertility. The emotional domain assesses how far the individual experience a collection of negative emotions related to infertility (jealousy, hate, sadness, and depression). The relational domain assesses the extent of the impact of fertility problems related to marital or partner relationships (sexuality, communication, commitment). The social domain measures the level of social interaction affected by fertility problems (social inequality, expectations, stigma, and support).[13]

All the core components of FertiQol in this study showed significantly different results between infertile women who underwent the IVF program and control subjects. The difference in the core FertiQol and each component shows a lower value result than the control group. This is evident from previous studies, which found that women with children clearly show a higher quality of life, especially in the mind/body and emotional domains. The duration and causes of infertility are known to be low in the mind/body domain in this measurement.[23]

This study also found a significant relationship that the higher the level of anxiety in infertile women, the lower the quality of life. These results are similar to previous studies with different psychological measures and quality of life.[24]

Although unclear, knowing whether this psychological distress causes infertility is essential. Several studies link this relationship, especially in IVF programs. These studies found that the higher a person's distress, the lower the pregnancy rate,[25, 26] while some other studies say otherwise.[27]

Psychological interventions to reduce stress can be helpful for all causes of infertility, although the link is unclear. Re-counseling can be very useful in reducing negative effects such as anxiety and infertility-related stress even before starting fertility therapy to reduce tension and worry before fertility therapy is started. Another study even found that this intervention was associated with lower psychological distress and higher pregnancy rates.[28] The controversy over this matter is still unclear. Existing studies often have different results. Boivin et al. found that psychological intervention, although it has an impact on reducing adverse psychological effects, has no difference in pregnancy rates.[27] The Cochrane review found no evidence that higher pregnancy rates are associated with decreased anxiety.[29]

Despite their success in increasing pregnancy rates, almost all of these interventions have proved worthwhile as they have improved psychological outcomes and better marital relationships.[30] Therefore, researchers argue that by knowing the level of anxiety and quality of life of women who follow the IVF program, it is necessary to play an important role in thinking "how do we feel and what do we do" in challenging thoughts such as never having a baby, infertility as its fault, and husband's infidelity.

A limitation of this study is that the sample only comes from two fertility clinics. The FertiQol questionnaire has a treatment scale that assesses the tolerability of the therapy and the fertility clinic environment. However, in this study, no measurements were made on these domains because the control subjects were fertile women who did not get their pregnancies from the IVF program. Apart from this, it is necessary to identify confounding factors and explore the possibility of existing risk factors that can affect the research results. Also, this study wants to explain why the control group did not compare with the group of infertile patients who did not get an IVF program for some reason because it is challenging to find infertile patients who do not indicate IVF, as it is known that IVF is indicated mainly in tubal factor disorders, unexplained and male infertility.

This study concludes that there are significant differences in anxiety conditions and quality of life conditions between the patient groups who underwent IVF and controls. There was also a significant relationship between anxiety conditions and quality of life in the group of patients who underwent IVF, that the higher the level of anxiety, the lower the quality of life. Psychological measurement tools such as DASS and FertiQol in fertility clinics need to recommend to obtain psychological information and quality of life that can help overcome fertility problems and adapt better to psychological stress caused by infertility.

References

1. Rutstein SO, Shah IH. Infecundity, infertility, and childlessness in developing countries. dhs comparative reports No. 9. Calverton, Maryland, USA: ORC Macro and the World Health Organization; 2004.
2. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015;21(4):411–26.
3. Riset Kesehatan Dasar (Riskesdas). Badan Penelitian dan Pengembangan Kesehatan Kementerian Republik Indonesia tahun 2013. Available from: http://www.depkes.go.id/resources/download/general/hasil/riskesdas2013.pdf.
4. Wu MH, Su PF, Chu WY, Huey NG, Lin CW, Ou HT, dkk. Quality of life and pregnancy outcomes among women undergoing in vitro fertilization treatment: A longitudinal cohort study. J Formos Med Assoc. 2020;119(1 Pt 3):471–9.
5. Lok IH, Lee DT, Cheung LP, Chung WS, Lo WK, Haines CJ. Psychiatric morbidity amongst infertile Chinese women undergoing treatment with assisted reproductive technology and the impact of treatment failure. Gynecol Obstet Invest. 2002;53(4):195–9.
6. Peterson BD, Newton CR, Feingold T. Anxiety and sexual stress in men and women undergoing infertility treatment. Fertil Steril. 2007;88(4):911–4.
7. Rooney KL, Domar AD. The relationship between stress and infertility. Dialogues Clin Neurosci. 2018;20(1):41–7.
8. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva: World Health Organization; 2017.

9. Greil AL, Shreffler KM, Schmidt L, McQuillan J. Variation in distress among women with infertility: evidence from a population-based sample. Hum Reprod. 2011;26(8):2101–12.
10. Lazarus RS, Folkman S. Stress, appraisal, and coping. New York, NY: Springer Publishing. 1984.
11. Zurlo MC, Cattaneo Della Volta MF, Vallone F. The association between stressful life events and perceived quality of life among women attending infertility treatments: the moderating role of coping strategies and perceived couple's dyadic adjustment. BMC Public Health. 2019;19(1):1548.
12. Brown TA, Chorpita BF, Korotitsch W, Barlow DH. Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. Behav Res Ther. 1997;35(1):79–89.
13. FertiQoL Description.[Internet] 2019 [cited 2019 December 12]. Available from: http://sites.cardiff.ac.uk/fertiqol/background/
14. Fallahzadeh H, Zareei Mahmood Abadi H, Momayyezi M, Malaki Moghadam H, Keyghobadi N. The comparison of depression and anxiety between fertile and infertile couples: a meta-analysis study. Int J Reprod Biomed. 2019;17(3):153–62.
15. Ramezanzadeh F, Aghssa MM, Abedinia N, Zayeri F, Khanafshar N, Shariat M, et al. A survey of relationship between anxiety, depression and duration of infertility. BMC Womens Health. 2004;4(1):9.
16. Gameiro S, Boivin J, Peronace L, Verhaak CM. Why do patients discontinue fertility treatment? A systematic review of reasons and predictors of discontinuation in fertility treatment. Hum Reprod Update. 2012;18(6):652–69.
17. Gameiro S, Finnigan A. Long-term adjustment to unmet parenthood goals following ART: a systematic review and meta-analysis. Hum Reprod Update. 2017;23(3):322–37.
18. Turner K, Reynolds-May MF, Zitek EM, Tisdale RL, Carlisle AB, Westphal LM. Stress and anxiety scores in first and repeat IVF cycles: a pilot study. PLoS One. 2013;8(5):e63743.
19. Berg BJ, Wilson JF. Psychological functioning across stages of treatment for infertility. J Behav Med. 1991;14(1):11–26.
20. Kee BS, Jung BJ, Lee SH. A study on psychological strain in IVF patients. J Assist Reprod Genet. 2000;17(8):445–8.
21. Vincent K, Warnaby C, Stagg CJ, Moore J, Kennedy S, Tracey I. Dysmenorrhoea is associated with central changes in otherwise healthy women. Pain. 2011;152(9):1966–75.
22. Boivin J, Takefman J, Braverman A. The fertility quality of life (FertiQoL) tool: development and general psychometric properties. Fertil Steril. 2011;96(2):409–15 e3.
23. Sexty RE, Griesinger G, Kayser J, Lallinger M, Rosner S, Strowitzki T, et al. Psychometric characteristics of the FertiQoL questionnaire in a German sample of infertile individuals and couples. Health Qual Life Outcomes. 2018;16(1):233.
24. Xiaoli S, Mei L, Junjun B, Shu D, Zhaolian W, Jin W, et al. Assessing the quality of life of infertile Chinese women: a cross-sectional study. Taiwan J Obstet Gynecol. 2016;55(2):244–50.
25. Xu H, Ouyang N, Li R, Tuo P, Mai M, Wang W. The effects of anxiety and depression on in vitro fertilisation outcomes of infertile Chinese women. Psychol Health Med. 2017;22(1):37–43.
26. Terzioglu F, Turk R, Yucel C, Dilbaz S, Cinar O, Karahalil B. The effect of anxiety and depression scores of couples who underwent assisted reproductive techniques on the pregnancy outcomes. Afr Health Sci. 2016;16(2):441–50.
27. Boivin J, Griffiths E, Venetis CA. Emotional distress in infertile women and failure of assisted reproductive technologies: meta-analysis of prospective psychosocial studies. BMJ. 2011;342:d223.
28. Ying L, Wu LH, Loke AY. The effects of psychosocial interventions on the mental health, pregnancy rates, and marital function of infertile couples undergoing in vitro fertilization: a systematic review. J Assist Reprod Genet. 2016;33(6):689–701.
29. Verkuijlen J, Verhaak C, Nelen WL, Wilkinson J, Farquhar C. Psychological and educational interventions for subfertile men and women. Cochrane Database Syst Rev. 2016;3:CD011034.
30. Chow KM, Cheung MC, Cheung IK. Psychosocial interventions for infertile couples: a critical review. J Clin Nurs. 2016;25(15-16):2101–13.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2961
Majalah Kedokteran Bandung. 2023;55(2):92–97

Majalah Kedokteran Bandung (MKB)

Received: August 16, 2022
Accepted: February 13, 2023
Available online: June 30, 2023

Comparison of Several Indonesian Medicinal Plants Effects on LDL-C and IL-6 Levels in Wistar Rats After High Fat Feeding

Penny Setyawati Martioso,[1] Diana Krisanti Jasaputra,[2] Julia Windi Gunadi,[3] Ronny Lesmana,[4,5] Pinkan Al Shabrina[6]

[1]Department of Clinical Pathology, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
[2]Department of Pharmacology, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
[3]Department of Physiology, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
[4]Physiology Division, Department of Biomedical Sciences, Faculty of Medicine Universitas Padjadjaran, Indonesia
[5]Physiology Molecular Laboratory, Biological Activity Division, Central Laboratory, Universitas Padjadjaran, Indonesia
[6]Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

Abstract

High-fat diet habits lead to an increase in LDL-C levels that eventually influence the atherosclerotic plaque formation and progression, causing coronary heart disease. Atherosclerosis is a chronic inflammatory process that releases various pro-inflammatory cytokines, including IL-6. Indonesians often use medicinal plants to decrease cholesterol levels. This study aimed to compare the LDL-C and IL-6 levels after treatments of ethanol extracts from Java ginger (EEJG), turmeric (EET), garlic (EEG), and pomegranate flowers (EEPF) in a hypercholesterolemia animal model. This study was conducted at the Maranatha Biomedical Research Laboratory from June–December 2020. Male Wistar rats were divided into six groups (n=5 per group) and received high-fat feeding and 0.01% propylthiouracil. The following treatments were given for 28 days: oral carboxymethylcellulose 1% for negative control; 35 mg/200 g of oral EEJG, EET, EEG, EEPF for respective treatment groups (n=5 groups); and 0.36 mg/200 g of oral Rosuvastatin for positive control. It was demonstrated that the mean LDL-C levels were 65.75 mg/dL, 55.25 mg/dL, 56.75 mg/dL, and 59.60 mg/dL for EEJG, EET, EEG, EEPF groups, respectively, which were significantly different from that of the negative control (81.73 mg/dL). The IL-6 levels of the EEJG (27.55 pg./mL) and EEG (27.54 pg./mL) group were significantly different from the EEPF group (24.5 pg./mL) but not significantly different from the negative control (25.58 pg./mL), EET (25.60 pg./mL), and rosuvastatin (26.09 pg./mL) groups. The administration of ethanol extracts of Java ginger, turmeric, garlic, and pomegranate flower decreases the C-LDL levels; however, only the ethanol extract of pomegranate flowers administered for 28 days decreases the IL-6 levels of Wistar rat hypercholesterolemia model, albeit insignificantly.

Keywords: Garlic, Java ginger, high-fat feeding, IL-6, pomegranate flowers, turmeric

Introduction

High-fat diet habits cause an increase in LDL-C levels, which play a role in atherosclerotic plaque formation and progression, the cause of coronary heart disease. Atherosclerosis is a chronic inflammatory process that releases various pro-inflammatory cytokines, including IL-6. Interleukin 6 (IL-6) is one of the pro-inflammatory cytokines that mediate inflammatory reactions, which produce an acute response to tissue injuries that disrupt homeostasis.[1] A meta-analysis study reported the range of IL-6 in the blood circulation of healthy human donors between 0 and 43.5 pg./mL, with an estimation of the normal level at 5.186 pg./mL.[2] Inflammatory reaction accompanied by increased levels of IL-6 may occur in some circumstances such as hyperlipidemia.[1] Hyperlipidemia causes an inflammatory reaction in the endothelial blood vessels triggered by oxidized cholesterol-LDL. It causes the formation of foam cells between the basal endothelial membrane and smooth muscle cells, triggering the release of pro-inflammatory cytokines, including IL-6. Increased cholesterol levels will also increase C reactive protein via increased IL-6.[3]

Corresponding Author:
Diana Krisanti Jasaputra
Department of Pharmacology, Faculty of Medicine,
Maranatha Christian University, Bandung, Indonesia
Email: dianakjasaputra67@gmail.com

High-fat feeding (HFF) given to animal subjects will lead to increased expression of the IL-6 gene.[4] IL-6 is one of the mediators of inflammatory reactions, immune responses, and hematopoiesis.[1] As an inflammatory reaction, IL-6 might become a chosen parameter to reach a therapeutic target, any agent that reduces Il-6 levels or reduces the expression of the IL-6 gene has anti-inflammatory effects.[4] The HFF can increase LDL-cholesterol, a condition that can increase IL-6 levels, meaning that hyperlipidemia causes chronic inflammatory reactions, and lipid-lowering drugs that can lower IL-6 levels also have anti-inflammatory effects.[1]

Indonesians often use medicinal plants, such as Java ginger, turmeric, garlic, and pomegranate flowers, to lower lipid levels for metabolic syndrome management.[5] Java ginger (*Curcuma xanthorrhiza*) and turmeric (*Curcuma longa*) are parts of 15 species of *Curcuma* in Java.[6] Turmeric has more curcuminoid (curcumin, bisdemethoxycurcumin, and desmethoxycurcumin) than Java ginger.[5] In Vitro and in vivo studies have reported that curcumin showed a potent anti-inflammatory effect in hyperlipidemia.[7] Among the genus of *Allium*, garlic (*Allium sativum*) is a medicinal plant that is widely used as an anti-hyperlipidemia and anti-inflammatory agent.[8] Recent study reported that garlic could be suggested as a candidate for maintaining homeostasis by its immunomodulatory and immunotherapeutic activities, thus improving hyperlipidemia.[9] Another medicinal plant with antioxidant compounds (cyanidin, pelargonidin, delphinidin, and ellagic acid) is pomegranate (*Punica granatum*).[10]as a rule, is several times higher than the content of L-malic. The total acidity of pomegranate juice is high, on average 1.1 g of organic acids is present in 100 cm(3 Pomegranate flower significantly reduced tissue lipid peroxidation, increased HDL-C, and reduced vascular inflammatory markers and cytokines.[11] This study aimed to compare the anti-inflammatory effects of Java ginger, turmeric, garlic, and pomegranate flowers ethanol extract in lowering IL-6 in an HFF-induced animal model.

Methods

This research was conducted at Maranatha Biomedical Research Laboratory from June 2020 to December 2020. This study is an experimental laboratory study, using animal research subjects of male rat Wistar, aged 6–8 weeks, weighing 180 g –200 g. This research has been approved by the Research Ethics Commission of the Faculty of Medicine at Maranatha Christian University with the number: 149/KEP/X/2020. The study subjects of male Wistar rats to be used adapted first to the laboratory atmosphere and then induced by high-fat feeding (HFF) for 14 days, and continued until 28 days later and treated with Indonesian medicinal plants (Java ginger, turmeric, garlic, and pomegranate flowers) for 28 days.[12]

Making ethanol extract is by mixing medicinal plants with 70% ethanol for 24 hours, then the solid material is separated, and a thick extract is made using a rotatory evaporator.

Animal research subjects, after induction with HFF for 14 days, were then divided into six groups (n=5) and given the following treatment: (1) negative control: given CMC 1% and HFF in rats per oral for 28 days; (2) Java ginger: given Java ginger (*Curcuma xanthorrhiza*) ethanol extract dose of 35mg/200 g rats per oral and HFF for 28 days; (3) turmeric: given turmeric (*Curcuma* longa) ethanol extract dose of 35mg/200g rats per oral and HFF for 28 days; (4) garlic: given garlic (*Allium sativum*) ethanol extract dose of 35 mg/200g rats per oral and HFF for 28 days; (5) pomegranate flowers: given a dose of pomegranate (*Punica granatum*) flower ethanol extract dose of 35 mg/200 g rats per oral and HFF for 28 days; (6) positive control: Rosuvastatin was given to rats orally and HFF for 28 days.[13] At the end of the study, the rats were terminated, and the blood samples were stored in a -80°C refrigerator for further examination. The dose taken for all herbal ingredients was adapted from Huajing et al.[14]

The test principle for C-LDL examination was a Homogeneous enzymatic colorimetric assay using a spectrophotometer. he IL-6 examination is performed by ELISA Method using the ELISA Rat IL-6 kit (Elabscience, E-EL-R0015). Each group's data were analyzed using the Shapiro-Wilk Test, and all data were normally distributed. The data were analyzed using a one-way ANOVA, followed by the Tukey HSD test with alpha=0.05.

Results

The result of an examination of C-LDL Levels in Wistar rats was as follows: negative control (81.73+4.43 mg/dL), java ginger (65.75+2.59 mg/dL), turmeric (55.25+1.30 mg/dL), garlic (56.75+4.02 mg/dL), pomegranate flower (59.60+10.21 mg/dL), positive control/

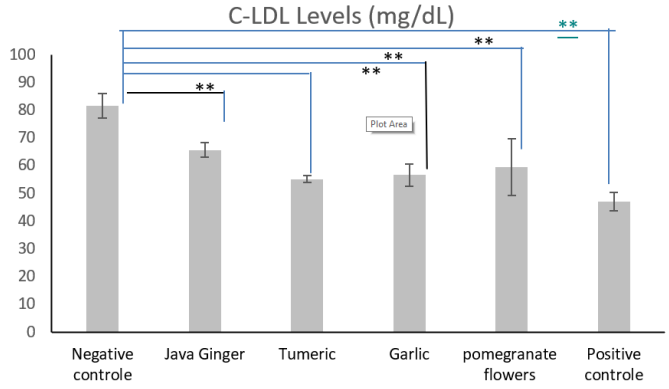

Figure 1 C-LDL Levels of Each Group

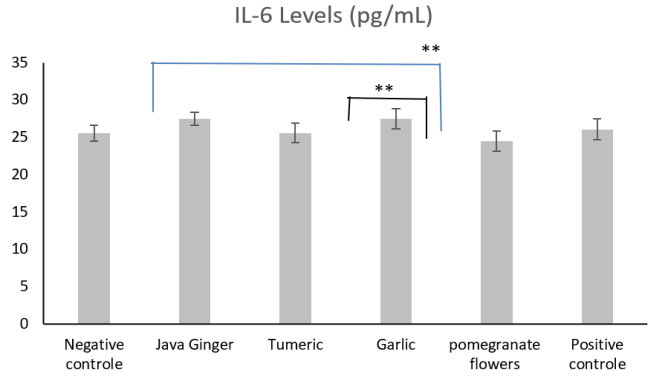

Figure 2 IL-6 Levels on 28 Days of Treatment

rosuvastatin (47.20+3.27 mg/dL). Examination of IL-6 levels in Wistar rats was conducted on the 28th day of treatment. The results were as follows: negative control (25.58±1.07 pg./mL), Java ginger (27.55±0.88 pg./mL), turmeric (25.60±1.30 pg./mL), garlic (27.54±1.34 pg./mL), pomegranate flower (24.56±1.35 pg./mL), and positive control/rosuvastatin (26.09±1.40 pg./mL). The result was shown in Figures 1 and 2.

Discussion

High-fat diet triggers hyperlipidemia, and hyperlipidemia causes endothelial dysfunction and increases pro-inflammatory cytokines such as IL-6.[3] Increased levels of IL-6 can have an impact on several organs, such as the onset of hepatocellular carcinoma, coronary heart disease, and high blood pressure.[1] IL-6 is also associated with the aging process, including atherosclerosis-related hypercholesterolemia.[15] Pro-inflammatory cytokines, including IL-6, play a role in the pathogenesis of aging-related diseases, such as atherosclerosis, type 2 diabetes, Alzheimer's, dementia, cancer, and arthritis.[16] Pro-inflammatory cytokines, including IL-6, are a low molecular weight regulatory protein secreted by various cell types.[1] Inflammation is a normal body reaction as a response to injury or infection for eliminating inflammatory microorganisms and can lead to cell death.[17]

Inflammation is generally divided into two types: acute and chronic. Acute inflammation has a short duration and a rapid onset. Acute inflammatory reactions occur in the recruitment of neutrophils and the discharge of blood plasma into the tissues. The main signs of inflammatory reactions are redness, swelling, pain, and warmth. Chronic inflammation has manifestations of persistent macrophages and lymphocytes with a long duration.[18]tumor, calor, and dolor, scientific investigations have revealed

chemical components, cells, and pathways involved in the process of inflammation. The body's initial defense in response to infection, trauma, or inflammation is through the acute-phase response (APR Acute inflammatory cytokines include IL-1, IL-6, IL-8, IL-11, TNF alpha (tumor necrosis alpha), and IL-16, IL-17, G-CSF (granulocyte-macrophage colony-stimulating factor). Chronic inflammatory cytokines are divided into two groups, groups that regulate hormones such as IL-4, IL-5, IL-6, IL-7, and IL-13, and groups that regulate cellular responses such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferons, and TNFα and β. Some cytokines play a role in both acute and chronic inflammation. The cytokines are IL-1, IL-6, IL-11, IL-17 and TNF-α. Pro-inflammatory cytokines play a role in the pathogenesis of cardiovascular disease and cancer.[19]

HFF-induced inflammation occurs in the central nervous system and peripheral tissues such as the liver, skeletal muscle, and intestine.[20,21]chronic overnutrition, such as consumption of a high-fat diet (HFD These two mechanisms lead to pro-inflammatory cytokines production such as IL-6, IL-1β, and tumornecrosis factor/TNF α) in the gut that is distributed to circulation, activating low-grade systemic inflammation.[20,22]Chronic overnutrition, such as consumption of a high-fat diet (HFD Besides that, increased levels of free fatty acid caused by HFF directly affect intestinal cells.[20]chronic overnutrition, such as consumption of a high-fat diet (HFD These two mechanisms lead to pro-inflammatory cytokines production such as IL-6, IL-1β, and tumor necrosis factor/TNF α) in the gut that are distributed to circulation, activating systemic low-grade inflammation.[20,22]chronic overnutrition, such as consumption of a high-fat diet (HFD Therefore, an anti-inflammatory agent is needed to counteract the effect of HFF on pro-inflammatory cytokine production.

Indonesian people like to use medicinal plants as a complementary therapy for many diseases and to boost their immune response.[5,23] Among them, Java ginger, turmeric, garlic, and pomegranate are known for their various health benefits, often used in Indonesian food recipes, and available in abundance. Those medicinal plants are known to have anti-hyperlipidemia and anti-inflammatory effects because of their compounds that exhibit potent antioxidant properties.[7,8,24] Java ginger and turmeric have curcumin that could decrease free fatty acids in plasma, inhibits pro-inflammatory cytokines (IL-6, IL-8, TNF-α, nuclear factor-Kappa B/NF-kB), lipid peroxidation, and protein carbonyl.[5]

This study compared the effect of various medicinal plants on lowering pro-inflammatory cytokine IL-6 after 28 days of induction of HFF. Before treatment, the rats were induced with HFF for 14 days, whose administration passed on during treatment for up to 28 days. The treatment given in this study is Java ginger, turmeric, garlic, and pomegranate flowers for another 14 days, along with the HFF This study found a very significant difference in the pomegranate group compared to the Java ginger and garlic groups (p=0.009). In contrast, we did not find any significant differences in other groups. Among 15 genus of *Curcuma* found in Indonesia, turmeric (*Curcuma longa*) has the highest compound of curcumin;[5] thus, the effect of Java ginger might not be as strong as turmeric in reducing IL-6 levels. The result of the study is somehow different from a study conducted by Jain et al that found a decrease of IL-6 in diabetic rats after curcumin supplementation.[25] However, the study was performed for seven weeks, while this study was conducted for four weeks. Garlic compounds such as alliin and *S-allyl cysteine* (SAC) were proven to have anti-inflammatory properties.[26] Recent studies showed that aged garlic extract has more health benefits than fresh garlic extract,[27]methylglyoxal or ribose this might explain why the IL-6 level in the garlic group is higher than in other groups. Triterpenoids oleanolic acid, and ursolic acid are bioactive compounds in pomegranate flowers.[10]as a rule, is several times higher than the content of L-malic. The total acidity of pomegranate juice is high, on average 1.1 g of organic acids is present in 100 cm. The reduction of IL-6 levels found in pomegranate group is in line with other studies using the pomegranate flower as an anti-inflammatory agent after HFF induction.[11,28]

This study found no significant difference in other groups, including in positive control, turmeric, and rosuvastatin groups; the short duration of HFF might cause this, and treatment has been given; also, time-modulation of IL-6 might have taken part in this study. The limitation of this study is that we did not measure IL-6 levels at some time points to investigate its modulation and did not measure other inflammatory cytokines that might also take part in low-grade systemic inflammation caused by HFF.

This study concluded that administering Java ginger, turmeric, garlic, and pomegranate flower extracts ethanol decreases C-LDL Levels compared to the negative control. The administration of extract ethanol pomegranate

flowers lowers IL-6 levels in Wistar rats induced with 28 days of a high-fat diet compared to the administration of extract ethanol Java ginger and garlic. Further studies that can be done include research linking total cholesterol, C-LDL, C-HDL, and triglyceride levels with IL-6 levels.

The induction with high-fat feeding to Wistar rats for 14 days has only succeeded in creating an experimental animal model of hypercholesterolemia or a model of dyslipidemia. Atherosclerosis is a chronic inflammatory process in which the inflammatory response is prolonged, so the increase in LDL-C levels within 14 days has not caused significant vascular endothelial injury and the accumulation of atherosclerotic plaques significantly for created animal atherosclerosis model, so the increase of Il-6 levels is not significant. This research needs to be continued by using experimental animal atherosclerosis models and a normal control group.

References

1. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295.
2. Said EA, Al-Reesi I, Al-Shizawi N, Jaju S, Al-Balushi MS, Koh CY, et al. Defining IL-6 levels in healthy individuals: a meta-analysis. J Med Virol. 2021;93(6):3915–24.
3. Oishi JC, Castro CA, Silva KA, Fabricio V, Cárnio EC, Phillips SA, et al. Endothelial dysfunction and inflammation precedes elevations in blood pressure induced by a high-fat diet. Arq Bras Cardiol. 2018;110(6):558–67.
4. Sukadana IM, Santi SR. The effect of antioxidant compounds in ethanol extract Inocarpus fagiferus fosb seed to expression TNF-α and IL-6 liver cells in hypercholesterolemia wistar rat. J Pharm Sci Res. 2017;9(5):690–4.
5. Arozal W, Louisa M, Soetikno V. Selected indonesian medicinal plants for the management of metabolic syndrome: molecular basis and recent studies. Front Cardiovasc Med. 2020;7:82.
6. Rahmat E, Lee J, Kang Y. Javanese Turmeric (Curcuma xanthorrhiza Roxb.): ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Zarrelli A, editor. Evidence-Based Complement Altern Med. 2021;2021:9960813.
7. Maithilikarpagaselvi N, Sridhar MG, Swaminathan RP, Sripradha R, Badhe B. Curcumin inhibits hyperlipidemia and hepatic fat accumulation in high-fructose-fed male Wistar rats. Pharm Biol. 2016;54(12):2857–63.
8. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L, et al. Immunomodulation and anti-inflammatory effects of garlic compounds. J Immunol Res. 2015;2015:401630.
9. G P S, Thomas S, K S, Sankar P, Bobby Z. Study the effect of s-methyl L-cysteine on lipid metabolism in an experimental model of diet induced obesity. J Clin Diagn Res. 2013;7(11):2449–51.
10. Khomich LM, Perova IB, Eller KI. [Pomegranate juice nutritional profile]. Vopr Pitan. 2019;88(5):80–92.
11. Harzallah A, Hammami M, Kępczyńska MA, Hislop DC, Arch JRS, Cawthorne MA, et al. Comparison of potential preventive effects of pomegranate flower, peel and seed oil on insulin resistance and inflammation in high-fat and high-sucrose diet-induced obesity mice model. Arch Physiol Biochem. 2016;122(2):75–87.
12. Oluwafemi AG, Ajayi OB, Aluko BT. Defensive potential and deleterious impact of turmeric (Curcuma longa) L rhizome powder supplemented diet on antioxidant status of indomethacin-induced ulcerated wistar rats. J Food Biochem. 2022 Jan;46(1):e14019.
13. Okada Y, Yamaguchi K, Nakajima T, Nishikawa T, Jo M, Mitsumoto Y, et al. Rosuvastatin ameliorates high-fat and high-cholesterol diet-induced nonalcoholic steatohepatitis in rats. Liver Int Off J Int Assoc Study Liver. 2013;33(2):301–11.
14. Huajing K, Florenly F, Ginting JB, Fioni F. Anti-Dislipidemia effectiveness test of turmeric ethanol extract (Curcuma longa) in male wistar mice given propylthiouracil (PTU). Budapest Int Res Exact Sci J. 2021;4(1):43–56.
15. Tyrrell DJ, Goldstein DR. Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. Nat Rev Cardiol [Internet]. 2021;18(1):58–68.
16. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and age-related diseases: role of inflammation triggers and cytokines. Front Immunol. 2018;9:586.
17. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and

inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204–18.
18. Ansar W, Ghosh S. Inflammation and inflammatory diseases, markers, and mediators: role of crp in some inflammatory diseases. Biology of C Reactive Protein in Health and Disease. 2016. p. 67–107.
19. Amin MN, Siddiqui SA, Ibrahim M, Hakim ML, Ahammed MS, Kabir A, et al. Inflammatory cytokines in the pathogenesis of cardiovascular disease and cancer. SAGE Open Med. 2020;8:205031212096575.
20. Duan Y, Zeng L, Zheng C, Song B, Li F, Kong X, et al. Inflammatory links between high fat diets and diseases. Front Immunol. 2018;9:2649.
21. Guillemot-Legris O, Masquelier J, Everard A, Cani PD, Alhouayek M, Muccioli GG. High-fat diet feeding differentially affects the development of inflammation in the central nervous system. J Neuroinflammation. 2016;13(1):206.
22. Bleau C, Karelis AD, St-Pierre DH, Lamontagne L. Crosstalk between intestinal microbiota, adipose tissue and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes. Diabetes Metab Res Rev. 2015;31(6):545–61.
23. Mustika M, Rivai H, Muna N, Voenna Z, Harnisah. Traditional medicines prescription and the potential of original Indonesian herbs as natural immunomodulators. Int J Pharm Sci Med. 2021;6:14–20.
24. Hou C, Zhang W, Li J, Du L, Lv O, Zhao S, et al. Beneficial effects of pomegranate on lipid metabolism in metabolic disorders. Mol Nutr Food Res. 2019;63(16):e1800773.
25. Jain SK, Rains J, Croad J, Larson B, Jones K. Curcumin supplementation lowers TNF-alpha, IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF-alpha, IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. Antioxid Redox Signal. 2009;11(2):241–9.
26. Quintero-Fabián S, Ortuño-Sahagún D, Vázquez-Carrera M, López-Roa RI. Alliin, a garlic (Allium sativum) compound, prevents LPS-induced inflammation in 3T3-L1 adipocytes. Mediators Inflamm. 2013;2013:381815.
27. Elosta A, Slevin M, Rahman K, Ahmed N. Aged garlic has more potent antiglycation and antioxidant properties compared to fresh garlic extract in vitro. Sci Rep. 2017;7:39613.
28. Neyrinck AM, Van Hée VF, Bindels LB, De Backer F, Cani PD, Delzenne NM. Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: potential implication of the gut microbiota. Br J Nutr. 2013;109(5):802–9.

CASE REPORT

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2842
Majalah Kedokteran Bandung. 2023;55(2):124–130

Majalah Kedokteran Bandung (MKB)

Received: June 2, 2022
Accepted: March 6, 2023
Available online: June 30, 2023

Diagnostic Challenge of Adult-onset Type 1 Diabetes Mellitus in a Remote Hospital

Bryan Arista Hartono,[1] Joshua Henrina,[2] Doddy M. Turmudzi[1,3]
[1]Majalengka General Hospital, Majalengka, Indonesia
[2]School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia
[3]Internal Medicine Department, Majalengka General Hospital, Majalengka, Indonesia

Abstract

Type 1 Diabetes Mellitus (T1DM) is a chronic endocrinological disease due to an autoimmune process. The prevalence of T1DM is 9.5% worldwide, with the incidence of 15 out of 100,000 people, ranging from childhood to 40 years of age. Autoimmunity-related late-onset Diabetes Mellitus (DM) patients could be diagnosed as classic T1DM or latent autoimmune diabetes in adults (LADA). A 30-year-old male patient with unremarkable previous medical history was admitted to the emergency room with dyspnea for the last three days that was worsened six-hour before admission. Physical examinations showed a body Mass Index (BMI) of 18.75 kg/m^2, irregular pulse, and Kussmaul breathing. The patient was diagnosed with diabetic ketoacidosis (DKA) on May 23, 2019. He was discharged with subcutaneous insulin pen injections. Two years later, he was readmitted with DKA due to discontinuing his treatment. He stated that the reason for stopping the insulin was because he was tired of injecting it. The patient was hospitalized and was discharged with oral antidiabetic agents to cope with his injection tiredness issue. One week later, the patient complained of dyspnea and was diagnosed with recurrent DKA. He was hospitalized and prescribed subcutaneous insulin. In this kind of situation, a diagnosis of LADA for patients presenting with DKA without prior history of DM in early adulthood needs to be considered. In contrast to the classic T1DM, the need for insulin occurs late in LADA. Affordable and widely available ancillary examinations are needed, including in remote hospitals. Finally, motivational support for patients is as important as the pharmacological treatment since lifelong insulin injections are needed.

Keywords: Adult onset, autoimmunity, diabetic ketoacidosis, type 1 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a complex chronic disease with both short- and long-term complications that can affect a person's quality of life. Therefore, it requires excellent glycemic control through medical treatment and appropriate multifactorial risk reduction strategies.[1,2] According to the American Diabetes Association, there are four diabetes classifications, i.e., type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), specific type diabetes due to other causes, and gestational diabetes.[3] T1DM is a chronic endocrinological disorder marked by an absolute insulin deficiency due to the destruction of pancreatic beta cells from an autoimmune process.[4,5]

Corresponding Author:
Doddy M. Turmudzi
Internal Medicine Department, Majalengka General Hospital, Indonesia
Email: dmturmudzi@gmail.com

Globally, the International Diabetes Federation (IDF) stated that as many as 463 million adults aged between 20 to 79 years suffer from DM, with 79% distributed in low- and middle-income countries (LMIC).[6] Globally, the prevalence of DM patients in Indonesia is 7th in rank, with a total of 10.7 million people in 2019.[7]

Based on a meta-analysis, the global prevalence of T1DM is 9.5% with an incidence of 15 out of 100,000 people.[8] T1DM can be seen in any age, including children to adults before the age of 40 years. Nonetheless, most T1DM occurred between the age of 4 to 14 years.[9]

Patients with adult-onset DM with an autoimmune disorder similar to T1DM may have classic type 1 DM or latent autoimmune diabetes of adults (LADA).[10] LADA cases are expected in Caucasian and Asian adult populations, with 40% of cases of T1DM occurring over the age of 30 years.[11]

Compared to T1DM, LADA has presentations

more similar to T2DM patients, due to slower pancreatic beta-cell failure than T1DM despite positive islet antibodies.[11,12] These presentations can lead to a misdiagnosis and will be managed as T2DM. The slower rate of beta-cell failure in LADA versus T1DM in adults is defined by the absence of insulin therapy during the first 6 to 12 months of diagnosis. However, the criteria are dependent on the clinical decision.[13] Alternatively, a fasting c-peptide level can indicate residual insulin secretion and predict late-onset presentations.[14] Finally, glutamic acid decarboxylase antibodies (GADA) are distinct antibodies that set LADA apart from other types of DM. This report aims to increase awareness of LADA, especially in patients with adult-onset DM, from diagnosis to holistic treatment.

Case

A 30-year-old man came to the ER of Majalengka General Hospital on April 23, 2019, with a primary complaint of shortness of breath in the last three days, which worsened six hours before admission. His complaint was accompanied by unbearable epigastric pain with a pounding quality. Previously, the patient has experienced symptoms of weakness, excessive thirst and hunger, and frequent urination. The patient's weight has decreased by 12 kg in the past two months. Other than dyspepsia, his past medical history was insignificant. None of the patient's family members have had similar complaints or a history of DM, hypertension, or autoimmune disease.

The physical examinations on admission were as follows: He has a weight of 48 kg and a height of 160 cm, with a body mass index (BMI) of 18.75 kg/m^2. Vital signs showed elevated blood pressure (150/90 mmHg), tachycardic (heart rate of 125 beats/minute dan irregular), a respiratory rate of 41 times/minute with a Kussmaul pattern, and a temperature of 36.8^{o}C. His oxygen saturation was 95% on ambient air. Other physical examinations were within normal limits. Laboratory findings were consistent for hyperglycemic crisis (Table 1). Accordingly, his electrocardiogram showed atrial fibrillation rapid ventricular response with peak-tall T waves at V5 and V6 leads.

Thus, the diagnosis of hyperglycemic crisis was made, with a differential diagnosis of diabetic ketoacidosis (DKA) or hyperglycemic hyperosmolar state accompanied by severe dehydration and electrolyte imbalances (hyponatremia and hyperkalemia).

Thus, the diagnosis of the hyperglycemic crisis was made, with a differential diagnosis of

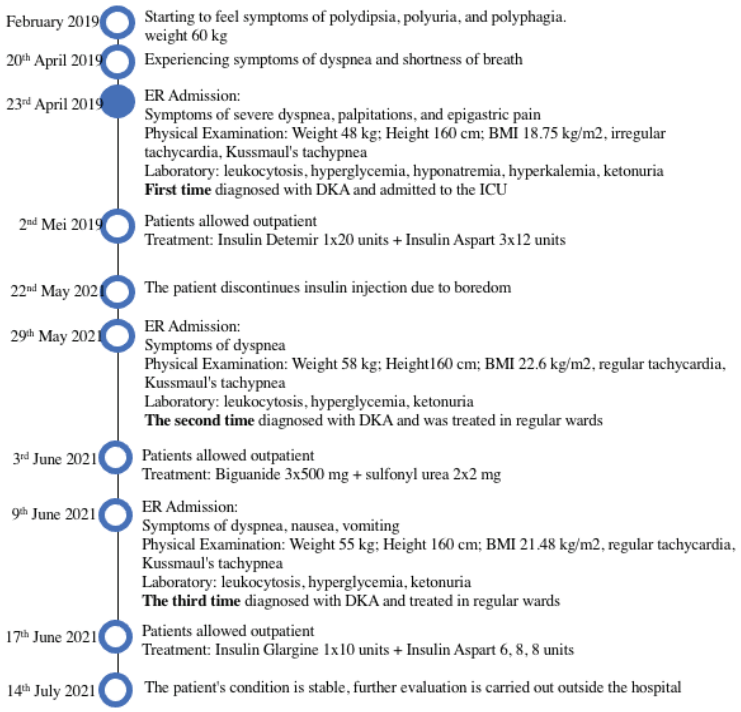

Figure 1 Patient Timeline

Table 1 Laboratory Findings on April 23 to 27, 2019

Examinations	Results		
	23rd April	**24th April**	**27th April**
Hematology			
Hemoglobin (g/dL)	15.3		14.2
Hematocrit (%)	44.5		41.0
Erythrocyte (10^3/uL)	5.32		4.96
Leucocyte (10^3/uL)	33.1		19.80
Thrombocytes (10^3/uL)	462		162
ESR (mm/hour)	5		
Blood chemical			
Random blood glucose level (mg/dL)	648		306
AST (U/L)	17		32
ALT (U/L)	24		12
Ureum (mg/dL)	86.2	42.7	106.7
Creatinine (mg/dL)	2.41	1.27	2.48
Electrolyte			
Sodium (mEq/L)	129.0	134.0	
Potassium (mEq/L)	8.3	5.1	
Chloride (mEq/L)	87.0	106.0	
Blood gas analysis			
pH			7.07
PO_2 (mmHg)			188
PCO_2 (mmHg)			14
SO_2 (%)			99
BE			-237
HCO_3^- (mEq/L)			4
Urinalysis			
Specific gravity	1.015		
pH	5.0		
Protein	Positive		
Glucose	Positive		
Ketone	Positive		
Erythrocyte	Positive		
Leucocyte	Negative		
Bacteria	Positive		

g=grams; dL=deciliter; uL=microliter; mm=millimeter; U=unit; L=liter; mEq=milliequivalent

diabetic ketoacidosis (DKA) or hyperglycemic hyperosmolar state accompanied by severe dehydration and electrolyte imbalances (hyponatremia and hyperkalemia).

The patient was fasted temporarily and was given supplemental oxygen via a non-rebreathing mask at a rate of 6–15 liters per minute, fluid boluses, insulin iv drip of five units per hour, bicarbonate iv drip of 100 mEq in D5% for three hours, and insertion of a urinary catheter.

Table 2 Laboratory Examinations on April 29, 2019

Examination	Result
Hematology	
Hemoglobin (g/dL)	16.4
Hematocrit (%)	50.5
Erythrocyte (10^3/uL)	5.38
Leucocyte (10^3/uL)	27.51
Thrombocytes (10^3/uL)	496
Blood chemical	
Current glucose level (mg/dL)	727
AST (U/L)	30
ALT (U/L)	20
Uremic (mg/dL)	40.9
Creatine (mg/dL)	1.2
Urinalysis	
Specific gravity	1.015
pH	5.0
Protein	Positive
Glucose	Positive
Ketone	Positive
Erythrocyte	Positive
Leucocyte	Negative
Bacteria	Positive

g=grams; dL=deciliter; uL= microliter; mm=millimeter; U=unit; L=liter; mEq=milliequivalent

The patient was admitted to the ICU for further monitoring and received additional therapy of iv calcium gluconate 4 gr in D5% 100 ccs for three hours and antibiotics. After one day of treatment, the patient's sodium and potassium levels improved, but his blood sugar was unstable. On the fourth day, laboratory examinations and blood gas analyses were carried out, which showed compensated metabolic acidosis. Furthermore, after seven days of treatment in the ICU, he was transferred to a regular ward. He was discharged on the ninth day of hospitalization and prescribed 20 units of daily subcutaneous detemir insulin injection in the morning and 12 units of aspart insulin given three times daily subcutaneously after each meal.

Two years later, on May 29, 2021, the patient was readmitted to the ER at Majalengka General Hospital, complaining of shortness of breath. He has been abruptly stopping insulin injections for one week. Physical examinations showed a weight of 58 kg, a height of 160 cm, and a body mass index (BMI) of 22.6 kg/m^2. His blood pressure was slightly elevated (140/97 mmHg), and he was tachycardic (HR: 134 beats/minute) and tachypneic (RR: 30 breaths/minute) with a Kussmaul pattern. The laboratory findings are listed in Table 2. The patient, again, was diagnosed with DKA. The DKA was precipitated due to sudden insulin discontinuation because of boredom due to the need to inject insulin daily. The patient was hospitalized for four days and was discharged with oral anti-diabetic agents (biguanides and sulfonylurea) to circumvent patient non-adherence.

One week later, on June 9, 2021, the patient again felt shortness of breath, accompanied by nausea and vomiting three times. The physical examinations showed a weight of 55 kg, a height of 160 cm, and a body mass BMI of 21.48 kg/m^2. His blood pressure was elevated (155/104 mmHg), with a rapid heart rate (145 beats/minute) and Kussmaul respiration of 30 breaths/minute. Other findings were within normal limits. Laboratory investigations were performed (Table 3) and were consistent with a diagnosis of DKA. The patient was then treated in the regular ward with fluid rehydration therapy, subcutaneous insulin detemir 1x10 unit, insulin aspart 3x10 unit, and bicarbonate iv drip 100 meq in D5% 200 ccs for three hours. It took eight days of treatment to stabilize the patient's condition. On the eighth day of treatment (June 17, 2021), the patient was discharged with subcutaneous insulin aspart injection, with doses of 6 units in the morning and eight units in the afternoon and evening given after meals, and ten units of subcutaneous insulin glargine in the evening, once daily.

In July 14, 2021, further evaluations were carried out to determine the cause of the patient's recurrent DKA and the onset of DM in a relatively young adult. More exhaustive laboratory investigations were carried out (Table 4). As a result of the treatment, the patient's weight returned to 58 kg and a BMI of 22.6 kg/m^2.

Discussion

The incidence of DKA in patients with no previous history of DM can be found in 1 of 3 cases that occur.[15,16] The Indonesian prevalence rate of DM in the productive age group who had not been previously diagnosed with DM, was higher than those who had been diagnosed, i.e.,

Table 3 Laboratory Examination in June 9 and July 14, 2021

Examinations	Results	
	9th June	14th July
Hematology		
Hemoglobin (g/dL)	16.7	13.9
Hematocrit (%)	50.7	42.6
Erythrocyte (10^3/uL)	5.49	4.62
Leucocyte (10^3/uL)	17.46	7.1
Thrombocytes (10^3/uL)	569	386
Blood Chemical		
Current glucose level (mg/dL)	832	192
AST (U/L)	12	18
ALT (U/L)	9	14
Uremic (mg/dL)	57.8	18
Creatine (mg/dL)	2.0	0.73
eGFR (mL/min/1,73m^2)	45	123
C-peptide (ug/mL)		<0.01
HbA1C (%)		10
Total Cholesterol (mg/dL)		221
LDL (mg/dL)		146
HDL (mg/dL)		56
Triglyceride (mg/dL)		77
Pancreatic amylase (U/L)		26
Pancreatic lipase (U/L)		27
Electrolyte		
Natrium (mEq/L)	129.0	
Potassium (mEq/L)	8.3	
Chloride (mEq/L)	87.0	
Urinalysis		
Density	1.015	1.005
pH	5.0	7.0
Protein	Positive	Negative
Glucose	Positive	Negative
Ketone	Positive	Negative
Erythrocyte	Positive	Negative
Leucocyte	Positive	Negative
Bacteria	Positive	Negative

g=grams; dL=deciliter; uL=microliter; mm=millimeter; U=unit; L=liter; mEq=milliequivalent

3.5% and 1.1%, respectively.[2] Thus, there is a possibility of encountering DKA cases in patients with no previous history of DM.

Shortly, DKA occurs when there is an absolute insulin deficiency or increased insulin requirements, as in infection, which stimulates

the secretion of counterregulatory hormones. Consequently, glucose utilization decreased, with simultaneous increased in liver lipase activation, causing the breakdown of adipose tissue into free fatty acids. These components are converted into acetyl coenzyme A, which enters the Krebs cycle to produce energy and the rest is broken down into ketones. The amalgamation of gluconeogenesis, lipolysis, ketogenesis, and decreased glycolysis are the hallmarks of DKA.[15,16]

Early symptoms of DM patients are weight loss accompanied by typical symptoms of polydipsia, polyuria, and polyphagia with a concomitant increase in blood sugar levels. T1DM is often diagnosed earlier with clinical presentations of either acute symptoms of diabetes or DKA. In contrast, patients with T2DM often do not feel sick, making an early diagnosis difficult. The physical appearance of T1DM patients with a normal or underweight BMI, in contrast to their T2DM counterparts who mainly were overweight/obese.

In this case, the patient has a normal BMI and before hospital admission, has been experiencing rapid weight loss for the last two months accompanied by typical symptoms of DM. Upon further examination, the patient was diagnosed with DKA without a previous history of DM.[17,18]

In young adults, autoimmune diabetes can be either classic type T1DM or LADA. According to research by Tang et al.,[19] the prevalence of T1DM in adults aged 30 years was 5.49% in men and 6.16% in women. The majority (65%) were LADA patients. In contrast, classic T1DM is associated with several autoantibodies, including anti-islet cell (anti-ICA), anti-insulin (anti-IAAs), anti-glutamic acid dicarboxylate (GADA), and anti-tyrosine phosphatase (anti-IA2), acute onset of insulin dependency pancreatic beta-cell failure, and susceptible to DKA,20 LADA is associated with GADA antibodies with or without anti-IA2. It does not require insulin during the disease's first six months due to the slower progression of pancreatic beta-cell damage.[3,10,19]

To illustrate the pancreatic beta-cell function, C-peptide levels can be used as a predictor of insulin secretion. They can be an alternative to the GADA examination to differentiate T1DM from LADA with a sensitivity of 90.5% and a specificity of 86.9%.[21] At the early phase of the disease, in LADA patients, C-peptide can be detectable at low levels, contrary to their T1DM counterparts, which are deficient or undetectable due to the inability to secrete insulin.

Furthermore, there is mild insulin resistance in LADA but not in T1DM.[11] In this case, the patient's C-peptide levels were deficient, indicating pancreatic beta-cell damage. Nonetheless, the examination was carried out two years after the onset of the diagnosis.

Importantly, the sensitivity and the specificity of a c-peptide assay to differentiate LADA from T1DM decrease with time, with their highest at the onset of DM. C-peptide assays far from the onset have decreased specificity and sensitivity compared to early onset in differentiating the type of T1DM. Therefore, we could not confidently enforce if it is T1DM. Moreover, we did not perform an autoantibody levels examination and insulin resistance, as they were unavailable at the peripheral health facilities.

In this case, the patient's second admission occurred due to the non-adherence to subcutaneous insulin injection. According to Maureen et al.,[22] T1DM sufferers, which mainly span from adolescence to young adulthood, coincide with a period of development that is prone to exacerbations due to treatment failure. Multiple factors, such as a strict schedule, difficulty in managing lifestyle, and the need for psychosocial and environmental support, need to be considered in this group.Increasing the patient's awareness and family support is equally essential to prevent non-adherence to insulin injections.

To conclude, further examination is necessary in cases of young adult-onset DKA without a previous history of DM. A complete medical history, consisting of risk factors and complete family history that can support the development of T1DM from birth until the present, should be asked. A complete physical examination, including the patient's BMI, should be considered. At remote healthcare facilities, the C-peptide level can be utilized as an alternative ancillary test that can be used as an alternative diagnostic tool to diagnose T1DM patients presented with symptoms.

Physicians should be cognizant and vigilant of T1DM cases in young adults from the diagnosis to its management pharmacologically and non-pharmacologically to prevent the risk of acute and chronic T1DM complications.

References

1. Association AD. Introduction: standards of medical care in diabetes—2021. Diabetes Care. 2021 1;44(Suppl 1):S1–2.
2. Mihardja L, Soetrisno U, Soegondo S.

Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. J Diabetes Investigation. 2014;5(5):507–12.
3. Association AD. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. Diabetes Care. 2021 1;44(Suppl 1):S15–33.
4. Okui T, Nojiri C, Kimura S, Abe K, Maeno S, Minami M, et al. Performance evaluation of case definitions of type 1 diabetes for health insurance claims data in Japan. BMC Med Informatics Decision Making. 2021;21:1–7.
5. Ortega M, Fraile-Martínez O, Naya I, García-Honduvilla N, Álvarez-Mon M, Buján J et al. Type 2 diabetes mellitus associated with obesity (diabesity). The central role of gut microbiota and its translational applications. Nutrients. 2020;12(9):2749.
6. IDF Diabetes Atlas. Tenth Ed. Diabetesatlas. org. 2022.
7. Demographic and geographic outline [Internet]. [cited 2021]. Available from: https://www.diabetesatlas.org/en/sections/demographic-and-geographic-outline.html
8. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. Health Promot Perspect. 2020 30;10(2):98–115.
9. What Is Type 1 Diabetes? [Internet]. EndocrineWeb. [cited 2021]. Available from: https://www.endocrineweb.com/conditions/type-1-diabetes/type-1-diabetes
10. Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R, et al. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype. Diabetes Care. 2013;36(4):908–13.
11. Pozzilli P, Pieralice S. Latent autoimmune diabetes in adults: current status and new horizons. Endocrinol Metab (Seoul). 2018;33(2):147–59.
12. Stenström G, Gottsäter A, Bakhtadze E, Berger B, Sundkvist G. Latent autoimmune diabetes in adults: definition, prevalence, β-cell function, and treatment. Diabetes. 2005 1;54(suppl 2):S68–72.
13. Brophy S, Yderstræde K, Mauricio D, Hunter S, Hawa M, Pozzilli P, et al. Time to insulin initiation cannot be used in defining latent autoimmune diabetes in adults. Diabetes Care. 2008 1;31(3):439–41.
14. Carlsson S. Etiology and pathogenesis of latent autoimmune diabetes in adults (LADA) compared to type 2 diabetes. Front Physiol. 2019;10:320.
15. Westerberg DP. Diabetic ketoacidosis: evaluation and treatment. AFP. 2013 1;87(5):337–46.
16. Eledrisi MS, Elzouki AN. Management of diabetic ketoacidosis in adults: a narrative review. Saudi J Med Med Sci. 2020;8(3):165–73.
17. Dabelea D, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, et al. Trends in the prevalence of ketoacidosis at diabetes diagnosis: the search for diabetes in youth study. Pediatrics. 2014 1;133(4):e938–45.
18. Lund-Blix NA, Sander DS, Størdal K, Nybo Andersen A-M, Rønningen KS, Joner G, et al. Infant feeding and risk of type 1 diabetes in two large Scandinavian birth cohorts. Diabetes Care. 2017;40(7):920–7.
19. Tang X, Yan X, Zhou H, Yang X, Niu X, Liu J, et al. Prevalence and identification of type 1 diabetes in Chinese adults with newly diagnosed diabetes. DMSO. 2019 28;12:1527–41.
20. Jacobsen L, Schatz D. Current and future efforts toward the prevention of type 1 diabetes. Pediatric Diabetes. 2016;17:78–86.
21. Li X, Huang G, Lin J, Yang L, Zhou Z. Variation of C peptide decay rate in diabetic patients with positive glutamic acid decarboxylase antibody: better discrimination with initial fasting C peptide. BMC Endocrine Disorders. 2013 1;13(1):10.
22. Monaghan M, Helgeson V, Wiebe D. Type 1 diabetes in young adulthood. Curr Diabetes Rev. 2015;11(4):239–50.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.3143
Majalah Kedokteran Bandung. 2023;55(2):85–91

Majalah Kedokteran Bandung (MKB)

Received: December 27, 2022
Accepted: February 3, 2023
Available online: June 30, 2023

Dose and Time-Dependent Lipopolysaccharide Exposure on A549 Cell Model Influences Pro-Inflammatory Cytokine Interleukin 8

Sarah Sumayyah,[1] Prayoga Suryadarma,[1,2] Rachmawati Noverina,[3] Wireni Ayuningtyas,[3,4] Firman F. Wirakusumah,[5] Ahmad Faried[6]

[1]Biotechnology Study Program, Postgraduate School, Bogor Agricultural Institute, Bogor, Indonesia
[2]Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, Bogor Agricultural Institute, Bogor, Indonesia
[3]Stem Cell Research and Development, Bio Farma, Bandung, Indonesia
[4]Doctoral Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[5]Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
[6]Department of Neurosurgery, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Hyperinflammation in COVID-19 patients is one of the causes of the high mortality rate of COVID-19. An in vitro model mimicking the inflammatory responses in COVID-19 patients is important in the efforts of finding new drug candidates for this disease. Lipopolysaccharide (LPS) can increase the proinflammatory cytokine interleukin 8 in response to the presence of foreign substances. This preliminary study sought to explore the use of the A549 cells as an in vitro inflammatory model. This study was conducted from August to November 2022 at the stem cell research and development laboratory of Bio Farma Indonesia. The exposure of 100, 500, and 1000 g/mL doses of LPS administered for 24, 72, and 120 hours on the A549 cells was analyzed for cell viability, population doubling time (PDT), and the presence of proinflammatory cytokine IL-8. The group differences were examined using one- and two-way analysis of variance in IBM SPSS Statistics Version 29, with a p-value of 0.05 considered significant. Cells exposed to a dose of 1,000 g/mL LPS had a lower viability and a higher proliferation rate ($p<0.05$) based on the viability and PDT. Viability, PDT, and pro-inflammatory cytokines showed concentration- and time-dependent responses. Therefore, increased levels of the proinflammatory cytokine IL-8 in cells exposed to LPS at a dose of 1000 g/mL for 24 hours can be used as a mimic to study hyperinflammation in COVID-19 patients.

Keywords: A549 cell, inflammation, interleukin 8, lipopolysaccharide exposure

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) refers to the respiratory disease triggered by the coronavirus related to SARS, which is of particular concern after the World Health Organization (WHO) declared it as a pandemic. Although the physiopathology of human coronaviruses (H-CoVs) such as SARS-CoV, MERS, and SARS-CoV-2 is not fully understood, their strong association occurs due to a disproportionately disturbed response of the immune system, especially to cytokine production.[1] Several studies have reported that very high levels of proinflammatory cytokines consumed during crosstalk between epithelial cells and immune cells in COVID-19 are associated with cytokine storms with severe complications.[2]

The involvement of LPS (also known as endotoxin) in ARDS pathology has been reported previously.[3] Toll-like receptor-4 (TLR4) activation with LPS during ARDS induces recruitment of leukocytes to the lung, activation of pro-inflammatory cytokine release, and consequent induction of lung injury, similar to SARS. LPS precisely activates TLRs, leading to activation of the nuclear factor-kappa B (NF-κB) signaling pathway and secretion of pro-inflammatory

Corresponding Author:
Rachmawati Noverina,
Stem Cell Research and Development, Bio Farma, Jl. Pasteur 28 Bandung, Indonesia,
Email: rnoverina@gmail.com

cytokines and chemokines, such as interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor α (TNF-α) and type 1 interferon.[4] Endotoxins have been shown to reduce alveolar epithelial cell viability in an animal model of LPS-induced acute lung injury (ALI)[5] and in vitro.[6]

Primary alveolar epithelial cell culture is currently considered the most representative in vitro model for alveolar studies. Still, the problem is tissue availability which requires ethical approval and patient consent to diagnose lung carcinoma.[7] In addition, using these cells has a short period because they will differentiate spontaneously within 1–2 weeks.[8] Therefore, cell lines such as A549 cells with lasting properties (provided they are correctly maintained) have their advantages. These continuous cells have the main advantages of ease of cultivation, reproducibility, and relatively unlimited supply.[9]

The critical parameter for successfully modeling hyperinflammatory reactions in vitro is the increased concentration of cytokines and chemokines in the conditioned culture medium. Intraperitoneal or intravenous injection of high doses of LPS into animals induces systemic inflammatory cytokine production, ultimately leading to tissue damage, body temperature dysregulation, and death.[10] Because of this, high-dose LPS injection has been used as an experimental model of septic shock. However, low-dose (sublethal) injection of LPS into animals can induce a state of "LPS tolerance" that alters the subsequent response to induction with LPS or other inflammatory stimuli. Long-term exposure to endotoxin at low doses has little or no effect on immune function, indicating that endotoxin can be rapidly eliminated by the immune system.[11]

The lack of effective target-specific therapies has been increasingly highlighted during the COVID-19 pandemic, resulting in severe acute respiratory failure and ARDS. Mortality and morbidity from this clinical condition remain high, so there is an urgent need to find new effective therapies to reduce mortality.[12] A549 cells, as an in vitro model exposed to LPS, will be the basis for making an inflammatory model so that later it will become the basis of targeted supporting therapy for COVID-19 patients.

Methods

This study was conducted from August to November 2022 at the stem cell research and development laboratory, Bio Farma. This research did not contain any studies involving animal or human participants because it uses the commercial cell line A549, nor did it take place on any private or protected areas. No specific permissions were required for corresponding locations.

The A549 human lung adenocarcinoma cell line (CLL-185™) were purchased from American Type Culture Collection (ATCC). A549 cells were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (Sigma Aldrich, Dorset, United Kingdom) supplemented with 10% (v/v) Fetal Bovine Serum (Gibco, Invitrogen, USA) and 1% (v/v) pen-strep (Gibco, Invitrogen, USA) seeded at an optimal cell density of 2×10^3 cells/cm^2 in a humidified atmosphere with 5% CO_2 at 37°C. The culture medium was renewed every third day. After the cells have 85% confluence, change the medium with the treatment medium.

Based on the analysis, LPS was dissolved in a culture medium and used for the cell treatment in a concentration and time-dependent manner. LPS-induced cultures with doses of 100, 500, and 1,000 µg/mL for 24 hours, and in the follow-up study, one dose was taken, which experienced a significant difference based on viability, PDT, and IL-8 using time intervals of 24, 72, and 120 hours.

To measure the viability and population doubling time of A549 cells, the A549 cells Passage 54 were plated at a density of $5x10^3$ cells/cm^2 and were seeded into four-well plates (Nunc, Thermo Scientific, Massachusetts, USA) supplemented with 2 mL of growth medium, grown to confluence, and incubated around 4 days in the growth medium at 37°, 5% CO_2. On the fourth day, media were replaced by culture media with LPS in varied concentrations in the presence of 10% FBS, and cells were incubated for 24, 72, and 120 h. A549 cells were harvested according to the time interval and dissociated with trypsin porcine for 3 minutes and centrifuged at 300 g for 4 minutes. The pellet was resuspended with a culture medium, and cells were counted with a hemocytometer. The viability cells were determined as a ratio of viable cells to the number of death cells and expressed as a percentage. The population doubling (PD) was calculated as described previously[13] using the equation of PD $=\log_2 / \log (C_H/C_S)$, where CH is the number of viable cells at harvest and CS is the number of cells seeded. The population doubling time (PDT) was calculated using the interval between cell seeding and harvest divided by the number of PDs. All experiments

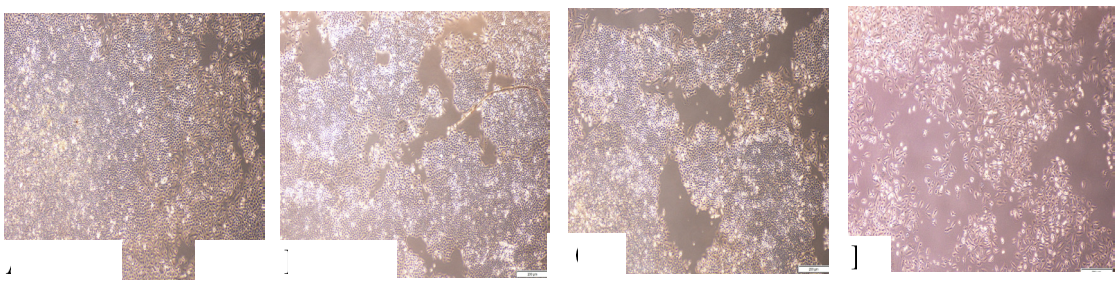

Figure 1 Morphology of A549 Cells After Exposure to LPS at Various Concentrations for 24 Hours. (A) 0 µg/mL LPS, (B) 100 µg/mL LPS, (C) 500 µg/mL LPS, (D) 1,000 µg/mL LPS

were performed in triplicate.

The IL-8 proinflammatory cytokine of A549 cells were evaluated by flow cytometry (BD FACSLyric, Becton, USA). A549 cells were seeded in four-well plates (2,000 cells/cm^2) and cultured in media with 10% FBS. On the fourth day, cells were treated with LPS of varied concentration of LPS in the presence of 10% FBS for 24, 72 and 120 h. The analysis phase was carried out by following the procedure of the FACS commercial kit. The secretome was transferred to a 1.5 mL tube, then 1µL/sample was given a cytokine bead capture. PE antibody was prepared with the same calculation as the capture cytokine bead and then stored in the dark. A 50 µL secretome sample was put into a 1.5 mL tube, then 50 µL of cytokine bead capture mix was added. Vortex briefly to make sure everything is homogeneous. Incubate in the dark, room temperature for 1 hour. The 50µL prepared PE antibody was added into the mixture of cytokine capture bead and secretome samples. Vortex at medium speed, then incubate in the dark, room temperature for 2 hours. 1 mL of wash buffer was added, then centrifuged at 1,600 rpm for 5 minutes. The supernatant was carefully removed. After that, it was vortexed, and 300 µL wash buffer was added and ended with another vortex.

Data of viability and PDT were presented as mean±standard deviation (SD) and analyzed using analysis of variance (ANOVA). If there were differences between the treatments, the Tukey Post Hoc Test was continued ($p<0.05$)data analysis using IBM SPSS statistics.

Results

The morphological observations showed a decrease in cell density with increasing LPS doses without changing the morphology of A549 cells (Figure 1). The phenomenon aligns with decreased cell viability (Figure 2A) and prolonged PDT (Figure 2B). Cell viability decreased to 96% after exposure with 100 µg/mL LPS, to 92% after exposure with 500 µg/mL LPS and to 84% after exposure with 1,000 µg/mL LPS for 24 hours. Proliferation in A549

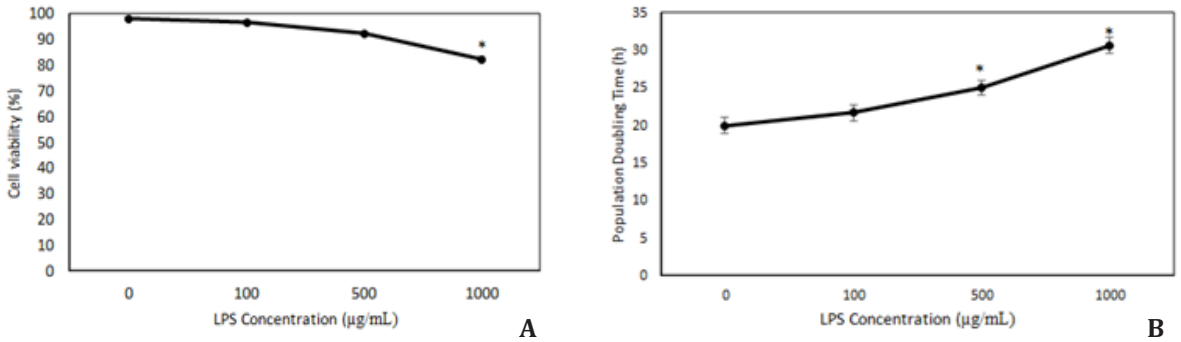

Figure 2 Viability and PDT After Exposure to LPS at Various Concentrations for 24 Hours. (A) Viability cell, (B) Population Doubling Time. Bars Represent the Mean±SD. Significant Differences were Considered when *p<0.05

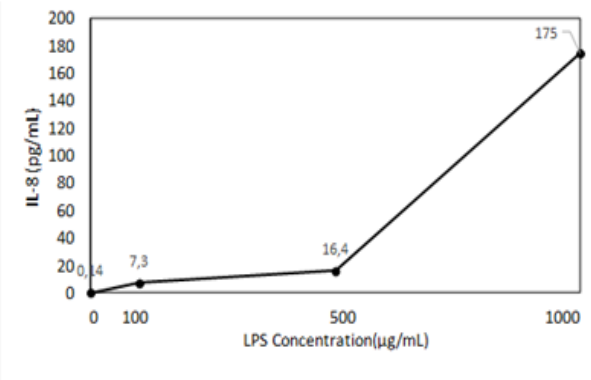

Figure 3 The Concentration of the Proinflammatory Cytokine IL-8 at LPS Concentrations Varied 24 Hours

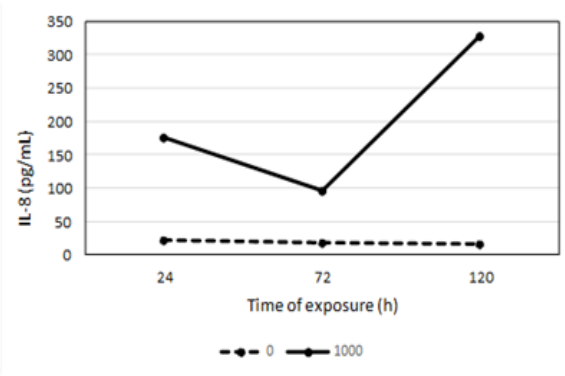

Figure 5 Concentration of Proinflammatory Cytokine IL-8 at LPS Concentrations of 1,000 µg/mL with Different Length of Exposure

cells exposed to LPS was significantly increased compared to cells not exposed to LPS.

A549 cells were analyzed for their secretome to determine the concentration of the pro-inflammatory cytokine IL-8. The increase in the pro-inflammatory cytokine IL-8 was marked when compared with cells that were not exposed (Figure 3). A sharp increase occurred with exposure to LPS with a 1,000 µg/mL concentration. So that in future studies, a dose of 1,000 µg/mL was used to see the effect of LPS exposure time.

Based on the morphology, viability, and PDT on the effect of LPS exposure time, there was a significant difference between cells exposed to LPS and those not exposed to LPS (Figure 4). The concentration of the pro-inflammatory cytokine IL-8 produced by cells exposed to LPS increased compared to those not exposed (Figure 5). Although the LPS exposure time of 72 hours decreased, the concentration of IL-8 produced was still much higher compared to cells that were not exposed to LPS.

Discussion

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects lung tissue, significantly accumulating immune cells and causing an inflammatory cytokine storm. The excess of inflammatory cytokines then leads to the infiltration of immune cells into the inflamed lung to induce alveolar damage and reduced lung function.[14] There was an increase in the pro-inflammatory cytokine IL-8 compared to cells that were not exposed to LPS. A sharp

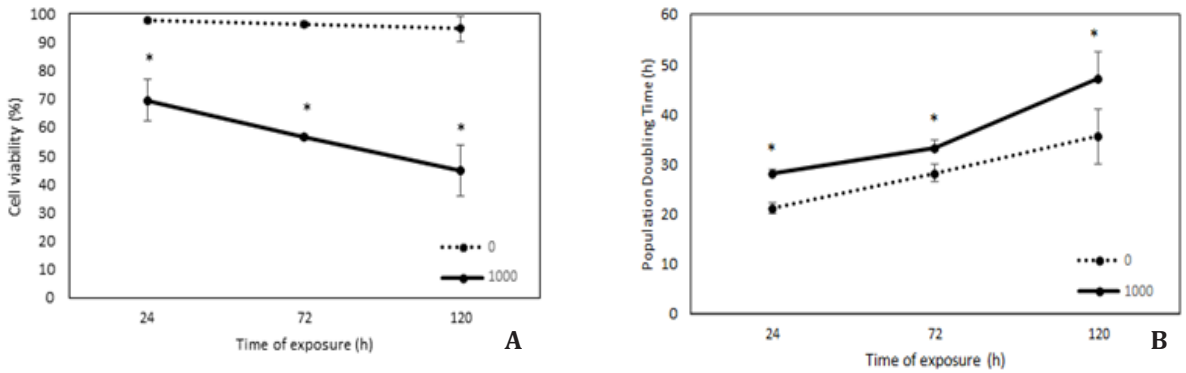

Figure 4 Viability and PDT of A549 Cells After Exposure to LPS at a Dose of 1000 µg/mL with Differences in Exposure Time. After Exposure to LPS at Various Concentrations for 24 Hours. (A) Viability cell, (B) Population Doubling Time. Bars Represent the mean±SD. Significant Differences were considered when *p<0.05

increase occurred with exposure to LPS with a concentration of 1000 µg/mL. IL-8 is one of the main mediators of the inflammatory response.[15] These results are in line with research conducted by Li et al. which showed an increase in IL-8 in COVID-19 patients,[16] which means that acute inflammation had occurred in A549 cells in the presence of LPS exposure. Lipopolysaccharide (LPS), the main component of the cell wall of Gram-negative bacteria, is the central stimulus for releasing inflammatory mediators. This is supported by changes in morphology, viability, and PDT with exposure to LPS at various concentrations.

Based on morphological observations, it was shown that there was a decrease in cell density with increasing exposure to LPS doses without changing the morphology of A549 cells. This is in line with the decrease in cell viability and prolonging PDT. Literature data regarding the responsiveness of A549 cells to LPS is highly controversial. Several studies have reported a significant decrease in A549 cell viability of around 50% at a low LPS concentration of 1 µg/mL after 24 hours.[17] Meanwhile, the minimal cytotoxic dose of endotoxin for A549 cells at a 50 µg/mL concentration.[18] However, cell viability did not fall below 80%. A potential reason for the different responses of A549 cells could be the type of LPS used for the experiment. Previous studies showed that the LPS substructure could modulate its endotoxic properties, possibly through different interactions of the LPS molecule with the TLR4 receptor complex, leading to different activation of subsequent inflammatory pathways.[19] This is supported by studies that found that structural differences in the O-antigen LPS molecule were able to modulate its recognition and phagocytosis by macrophages.[20]

The results of PDT showed that the longer the time needed to double the number of cells as the exposure to LPS dose increased. In cells that were not exposed to LPS, they showed a PDT of 21.9 hours; this is in line with the literature, which states that the PDT of A549 cells is 21.8 hours.[13] Based on analysis with one way ANOVA, it showed significance in cells exposed to LPS at a dose of 1000 µg/mL. This means that cells exposed to LPS experience disturbances in their proliferation process, so the time needed becomes longer. LPS with a concentration of 1000 µg/mL greatly affected the viability, PDT, and the concentration of the pro-inflammatory cytokine IL-8 in A549 cells. Therefore, an LPS concentration of 1000 µg/mL was set as the concentration to induce inflammation in the follow-up experiments.

Morphological observations showed a decrease in cell density with increasing exposure to LPS doses without changing the morphology of A549 cells. This is in line with the decrease in cell viability and prolonging PDT. The results of the PDT calculation also show an extension of the time needed for cells to double their cell number. PDT increases with increasing exposure time. Cells not exposed to LPS showed PDT 21 hours, then increased to 28 hours, and at the end, showed 35 hours. In control cells at 24 hours, the cells were still growing well with normal PDT. An increase in PDT in control cells indicates the occurrence of confluence, which causes the cells to experience abnormalities. Cells exposed to LPS showed abnormalities after exposure to LPS for 24 hours and then increased with the length of exposure, the difference in time needed for cells not exposed to LPS compared to cells exposed to LPS for 120 hours reached 11 hours.

Scoring cytokine storm by levels of MCP-3 and IL-8 can accurately stratify COVID-19 patients for high mortality risk.[21] The pro-inflammatory cytokine IL-8 showed a sharp increase during LPS exposure for 120 hours. Hyperinflammation of the lungs of severe COVID-19 patients is fueled by excessive production of chemokines Chemokines like CXCL1 (GROα) and IL-8 were found to be 30 times more abundant in BALF than in plasma and 200 times more abundant than IL-6 and TNF-α; consistent with the levels of these chemotactic molecules, BALF was rich in neutrophils, lymphocytes, and eosinophils.[22]

Neutrophils are essential effector cells in the innate immune defense against human infection. IL-8, secreted by macrophages and lung epithelial cells, is a neutrophil chemoattractant. IL-8 contributes to neutrophil activation and NET formation after binding to CXCR2 on neutrophils, causing hyperinflammation.[23] The results showed that the concentration of the pro-inflammatory cytokine IL-8 increased significantly after 1,000 µg/mL LPS exposure, indicating that the LPS-induced A549 cell inflammation model was successful.

References

1. Darif D, Hammi I, Kihel A, el Idrissi Saik I, Guessous F, Akarid K. The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong?. Microb Pathog. 2021;153:104799.

2. Henderson LA, Canna SW, Schulert GS, Volpi S, Lee PY, Kernan KF, et al. On the alert for cytokine storm: immunopathology in COVID-19. Arthritis and Rheumatology. 2020;72(7):1059–63.
3. Al-Ani B, ShamsEldeen AM, Kamar SS, Haidara MA, Al-Hashem F, Alshahrani MY. Lipopolysaccharide induces acute lung injury and alveolar haemorrhage in association with the cytokine storm, coagulopathy and AT1R/JAK/STAT augmentation in a rat model that mimics moderate and severe Covid-19 pathology. Clin Exp Pharmacol Physiol. 2022;49(4):483–91.
4. von Schéele I, Larsson K, Palmberg L. Interactions between alveolar epithelial cells and neutrophils under pro-inflammatory conditions. Eur Clin Respir J. 2014;1(1):24545.
5. Ogata-Suetsugu S, Yanagihara T, Hamada N, Ikeda-Harada C, Yokoyama T, Suzuki K, et al. Amphiregulin suppresses epithelial cell apoptosis in lipopolysaccharide-induced lung injury in mice. Biochem Biophys Res Commun. 2017;484(2):422–8.
6. Nova Z, Skovierova H, Strnadel J, Halasova E, Calkovska A. Short-term versus long-term culture of A549 cells for evaluating the effects of lipopolysaccharide on oxidative stress, surfactant proteins and cathelicidin LL-37. Int J Mol Sci. 2020;21(3):1148.
7. Thorley AJ, Ford PA, Giembycz MA, Goldstraw P, Young A, Tetley TD. Differential regulation of cytokine release and leukocyte migration by lipopolysaccharide-stimulated primary human lung alveolar type II epithelial cells and macrophages. J Immunol. 2007;178(1):463–73.
8. Fuchs S, Hollins AJ, Laue M, Schaefer UF, Roemer K, Gumbleton M, et al. Differentiation of human alveolar epithelial cells in primary culture: Morphological characterization and synthesis of caveolin-1 and surfactant protein-C. Cell Tissue Res. 2003;311(1):31–45.
9. Cooper JR, Abdullatif MB, Burnett EC, Kempsell KE, Conforti F, Tolley H, et al. Long term culture of the a549 cancer cell line promotes multilamellar body formation and differentiation towards an alveolar type II Pneumocyte phenotype. PLoS One. 2016;11(10):e0164438.
10. Nova Z, Skovierova H, Calkovska A. Alveolar-capillary membrane-related pulmonary cells as a target in endotoxin-induced acute lung injury. Int J Mol Sci. 2019;20(4):831.
11. Li C, Ma D, Zhang M, An L, Wu C, Zhou H. Effects of long-time exposure to lipopolysaccharide on intestinal lymph node immune cells and antibodies level in mice. Iranian J Immunol. 2020;17(3):175–84.
12. Cesta MC, Zippoli M, Marsiglia C, et al. The role of interleukin-8 in lung inflammation and injury: implications for the management of COVID-19 and hyperinflammatory acute respiratory distress syndrome. Front Pharmacol. 2022;12:808797.
13. Wiesmann N, Gieringer R, Viel M, Eckrich J, Tremel W, Brieger J. Zinc oxide nanoparticles can intervene in radiation-induced senescence and eradicate residual tumor cells. Cancers (Basel). 2021;13(12):2989.
14. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the inflammatory response to severe COVID-19 Illness. Am J Respir Crit Care Med. 2020;202(6):812–21.
15. Long X, Ye Y, Zhang L, Liu P, Yu W, Wei F, et al. IL-8, a novel messenger to cross-link inflammation and tumor EMT via autocrine and paracrine pathways (Review). Int J Oncol. 2016;48(1):5–12.
16. Li L, Li J, Gao M, Fan H, Wang Y, Xu X, et al. Interleukin-8 as a biomarker for disease prognosis of coronavirus disease-2019 Patients. Front Immunol. 2021;11:602395.
17. Zhao J, Li X, Zou M, He J, Han Y, Wu D, et al. MiR-135a inhibition protects A549 cells from LPS-induced apoptosis by targeting Bcl-2. Biochem Biophys Res Commun. 2014;452(4):951–7.
18. Li S, Guo L, Qian P, Zhao Y, Liu A, Ji F, Chen L, et al. Lipopolysaccharide induces autophagic cell death through the PERK-dependent branch of the unfolded protein response in human alveolar epithelial A549 cells. Cell Physiol Biochem. 2015;36(6):2403–17.
19. Migale R, Herbert BR, Lee YS, Sykes L, Waddington SN, Peebles D, et al. Specific lipopolysaccharide serotypes induce differential maternal and neonatal inflammatory responses in a murine model of preterm labor. Am J Pathol. 2015;185(9):2390–401.
20. Eder K, Vizler C, Kusz E, Karcagi I, Glavinas H, Balogh GE, et al. The role of lipopolysaccharide moieties in macrophage response to Escherichia coli. Biochem Biophys Res Commun. 2009;389(1):46–51.
21. Chen L, Wang G, Tan J, Cao Y, Long X, Luo H, et al. Scoring cytokine storm by the levels of MCP-3 and IL-8 accurately distinguished

COVID-19 patients with high mortality. Signal Transduct Target Ther. 2020;5(1):292.
22. Bendib I, Beldi-Ferchiou A, Schlemmer F, Surenaud M, Maitre B, Plonquet A, et al. Alveolar compartmentalization of inflammatory and immune cell biomarkers in pneumonia-related ARDS. Crit Care. 2021;25(1):23.
23. Chiang CC, Korinek M, Cheng WJ, Hwang TL. Targeting neutrophils to treat acute respiratory distress syndrome in coronavirus disease. Front Pharmacol. 2020;11:572009.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2982
Majalah Kedokteran Bandung. 2023;55(2):70–76

Majalah Kedokteran Bandung (MKB)

Received: August 25, 2022
Accepted: March 15, 2023
Available online: June 30, 2023

Drug Interactions in Diabetic Ulcer Patients in an Indonesian Private Hospital

Finny Alifiyatur Roosyidah,[1] Rika Yulia,[1] Fauna Herawati,[1] Heru Wijono[2]
[1]Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia
[2]Faculty of Medicine, University of Surabaya, Surabaya, Indonesia

Abstract

Diabetic ulcers can progress into tissue death, or gangrene, which create a risk for amputation. Measures for preventing other complications and accelerating wound healing in diabetic ulcers include blood sugar level control, diet adjustment, wound care, antidiabetic drug administration, and comorbid therapy. This leads to the use of various drugs that can potentially trigger drug interactions. This study aimed to identify possible drug interactions in the therapeutic management of diabetic ulcer patients treated in Husada Utama Hospital Surabaya, Indonesia, from January 2020–June 2022. This was a descriptive observational study using retrospective data from medical records. Results showed that 103 types of drugs were administered to 48 research samples with 41 of them experienced drug interactions (n=263 cases). Based on the severity of drug interactions, 31 cases were categorized as major cases (11.8%), with drug-class antibiotic-antiemetic interactions as the most frequent interactions. This study proves that it is essential for doctors and pharmacists.

Keywords: Diabetic ulcers, drug interaction, drug use, hospital

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels or hyperglycemia accompanied by disturbances in carbohydrate, lipid, and protein metabolism. Diabetes mellitus, whose blood glucose levels are poorly controlled, can cause complications, such as acute to chronic complications.[1] One of the complications that occur in patients with diabetes mellitus is diabetic ulcers. Ulcers occur in all layers of the skin with full depth and thickness due to peripheral neuropathy and/or peripheral arterial disease in people with diabetes mellitus.[2,3] Ulcers can be followed by bacterial invasion resulting in infection and decay.[3] These injuries can progress to gangrene or tissue death which is at risk of lower extremity amputation.[4]

The development of cases of diabetic ulcers has increased globally. Indonesia itself has a high prevalence of risk and incidence.[3] The annual incidence is 2% among all patients with diabetes mellitus, and 5–7.5% of patients with diabetes mellitus have peripheral neuropathy.[5] The mortality rate due to diabetic ulcers is between 17–32% and the amputation rate is around 15-32.5%.[6]

Efforts that can be made to prevent the emergence of other complications and accelerate the healing of diabetic ulcers are to provide appropriate therapy.[1] These efforts can be made by controlling blood sugar levels, eating arrangements, wound care, and giving OAD, insulin, and antibiotics for infection treatment of comorbid patients. The large variety of drugs given can potentially occur drug interactions.

Drug interactions are modifications of drug effects caused by other drugs so that the effectiveness and toxicity of these drugs change.[7] Clinically, a drug interaction is considered vital if it increases toxicity and decreases the effectiveness of the interacting drug, especially if the drug has a narrow therapeutic index. The category of severity of drug interactions is classified into three, namely minor, moderate, and major. Drug interactions are minor if the effects are mild and do not require a change in therapy. Drug interactions are moderate if the effects cause changes in the patient's clinical condition and require a change in therapy. Drug interactions are significant if the effects are potentially life-threatening and require

Corresponding Author:
Rika Yulia
Faculty of Pharmacy, University of Surabaya, Indonesia
Emai: rika_y@staff.ubaya.ac.id

intervention to minimize or prevent unwanted effects.[8]

Therapeutic failure due to drug interactions in the world is still relatively high. Data from the WHO Global Individual Case Safety Report database, over 20 years, as many as 3,766 cases were reported related to drug interactions. In Indonesia, the exact number of drug interactions has not been obtained because the documentation has not run optimally, so a thorough study of drug interactions cannot be carried out.[9] The impacts caused by drug interactions are increased costs, length of treatment time, decreased effectiveness of therapy, and increased unwanted drug reactions to the risk of death.[7] This study was conducted to identify drug interactions in diabetic ulcer patients at Husada Utama Hospital Surabaya. The results of this study support doctors and pharmacists to monitor drug administration in the therapeutic management of diabetic ulcer patients to avoid drug interactions.

Methods

Descriptive retrospective analysis of 48 research subjects received from medical records of diabetic ulcer patients for the period January 2020 - June 2022 at Husada Utama Hospital Surabaya with inclusion criteria, namely: 1) Diabetic ulcer patients with or without comorbidities, 2) Patients with ICD code -10 E11.5 Type 2 diabetes with complications of peripheral vascular circulation, 3) Patients older than 18 years, and 4) Patients treated during the study period at Husada Utama Hospital Surabaya and the exclusion criteria were: 1) The patient died, and 2) The patient is referred to another hospital or forced to go home. The method of taking the subject is done using total sampling. The analysis was carried out descriptively by screening using the online drug interaction checker medscape.com. Drug interaction data are grouped by severity level into three categories: minor, moderate, and major. Interaction if the effects are mild and do not require a change in therapy. Moderate interaction if the effect causes a change in the patient's clinical condition and requires a change in therapy. A major interaction is when the effect is potentially life-threatening and requires intervention to minimize or prevent the undesired effect. The study was conducted after obtaining a research permit from Husada Utama Hospital Surabaya and ethical clearance from the University of Surabaya Health Research Ethics Committee with No. 96/KE/VI/2022.

Results

Characteristics of the subjects showed 68 patients with a diagnosis of diabetic ulcer. After being grouped, 48 subjects met the inclusion and exclusion criteria. Table 1 shows the demographics of the subjects. Based on age, the age range is 41–81 years, with the dominance of subjects being male. Length of Stay (LOS) subjects were divided into three groups ranging from 3–21 days. Based on the medical treatment obtained, subjects who received debridement and amputation also received wound care. There were 21 comorbidities in diabetic ulcers. The most common comorbidities were anemia and hypertension. Drug use data shows 47 patients received more than five drugs during hospitalization.

Table 2 describes the drug use profile with acquiring 35 drug classes. The total types of drugs used in the research subjects were 103 drugs. The highest frequency of drug classes were antibiotics (100.97%), antidiabetic (84.47%), non-opioid analgesics (55.34%), and fluids and electrolytes (55.34%). The profile of drug use by research subjects can be observed in Table 2.

Table 3 and Table 4 shows that of the 48 research subjects who experienced diabetic ulcers at Husada Utama Hospital Surabaya, 41 subjects (85.42%) experienced drug interactions with a drug interaction case rate that occurred in as many as 263 cases. Cases of drug interactions that occur are divided into three categories, namely major (11.79%), moderate (67.30%), and minor (20.91%). Most interactions occur in the Antibiotic-antiemetic drug class, followed by the NSAID-non-opioid analgesic, anti-ulcer, and antiplatelet.

Discussion

Table 1 shows the characteristics of the subjects. Most cases of diabetic ulcers occurred in the category of the early elderly 46–55 years (33.33%). Age is one of the risk factors for diabetes mellitus and diabetic ulcers.[10] In older people, there are many changes in the body's physiological decline, including a decrease in the hormone insulin.[5] Another factor that can occur is insulin resistance due to a lack of physical activity that is not balanced with food intake.[5] The aging process can also reduce skin cells'

Table 1 Characteristics of Subjects

Variables	Amount (n=48)	Percentage (%)
Age (years)		
36–45	5	10
46–55	16	33
56–65	12	25
>65	15	31
Sex		
Male	26	54
Female	22	46
LOS (days)		
3–7	35	73
8–14	12	25
15–21	1	2
Medical treatment		
Wound care	48	100
Debridement	17	35
Amputation	17	35
Comorbid		
Anemia unspecified	10	21
Hypertension	9	19
Sepsis unspecified	8	17
Observation febrile	6	12
Chronic kidney disease	4	8
Hyponatremia	3	6
Cyst of kidney	2	4
Hypoglycemia	2	4
Diabetic nephropathy	2	4
Acute renal failure	1	2
Metabolic acidosis	1	2
Hypernatremia	1	2
Hypoalbuminemia	1	2
Hypokalemia post hyperkalemia	1	2
Hypothermia	1	2
Dyspnea	1	2
Osteomyelitis	1	2
Other and unspecified atrioventricular block	1	2
Parkinson	1	2
Pneumonia unspecified	1	2
Urinary tract infection	1	2
Drug use in patients		
≤5 drugs	1	2
>5 drugs	47	98

elasticity and decrease vascularized fluid in the skin and fat glands.[11] Decreased skin elasticity will reduce cell regeneration ability when an injury occurs, and wound healing becomes slower.[11]

This study shows that the highest gender is male, with as many as 26 subjects (54%). Based on a study, male patients tend to be less worried about their illness, so they rarely make hospital visits, check themselves, and perform wound care compared to female patients.[12] According to the Global Adult Tobacco Survey, the highest prevalence of active smokers mostly occurs in men in Indonesia. The nicotine content in cigarettes can damage the endothelium, which causes the attachment and aggregation of

Table 2 Profile of the Drug Use of Research Subjects

Drug Group	Frequency of Drug Use (n=103)	Percentage (%)
Antibiotics	104	100.97
Antidiabetic	87	84.5
Non-opioid Analgesics	57	55.3
Fluids and electrolytes	57	55.3
Antihypertensive	34	33.0
Anti-ulcer	33	32.0
Nausea and vertigo	26	25.2
Diuretics	21	20.4
Vitamins and supplements	18	17.4
Antiemetic	9	8.7
Opioid analgesics	7	6.8
Mucolytic	7	6.8
Antiepileptic	6	5.8
Gout and hyperuricemia	4	3.9
Statins	4	3.9
Hypnosis and anxiety	3	2.9
Corticosteroids	3	2.9
Anemia and other blood disorders	2	1.9
Antianginal	2	1.9
Antiarrhythmic	2	1.9
Anti-asthma and bronchodilator	2	1.9
Antiplatelet	2	1.9
Antithrombotic	2	1.9
Acute diarrhoea	2	1.9
Hormones	2	1.9
Minerals	2	1.9
Antifibrinolytic	1	0.9
Anti-inflammatory	1	0.9
Antifungal	1	0.9
Antiparkinsonian	1	0.9
Beta-blockers	1	0.9
Co-enzyme	1	0.9
Dementia	1	0.9
SSRI	1	0.9

SSRI= Selective Serotonin Reuptake Inhibitors

Table 3 Drug Interactions on Subject by Case

Interaction Incident	Patients (n=48)	Percentage (%)
There was interaction	41	85
No interaction	7	14

Table 4 Category of Drug Interaction Based on Severity Level

Category	Number of Cases (n=263)	Percentage (%)
Major	31	11.8
Moderate	177	67.3
Minor	55	20.9

platelets to leak and lipoprotein lipase, slowing down blood lipids and facilitating the onset of atherosclerosis. The presence of atherosclerosis results in decreased blood flow to the arteries.[13]

Research results based on LOS showed the highest length of stay was 3–7 days with 35 subjects (73%), 8–14 days with 12 subjects (25%), and 15–21 days with one subject (2%). The average length of stay is 6.35 days with an interval of 3–21 days. The length of hospitalization for diabetic ulcers depends on the type of ulcer, the infection's severity, and the treatment given.[10]

All study subjects received wound care or dressings, meaning that wound care was also given to subjects who underwent debridement and amputation. Wound care is one of the essential therapies in diabetic ulcers to keep the wound closed and clean or provide a moist healing environment to facilitate cell migration and prevent dry wounds. The choice of dressing depends on the amount and type of exudate present in the wound. Wound dressings that can be used, such as conventional dressings, are sterile gauze moistened with 0.9% NaCl or modern dressings available today, such as hydrocolloids, hydrogels, calcium alginate, etc.[3,14] The wound care used in this study were sterile gauze, hydrogel (Duoderm Gel), tensocrepe, cutimed sorbate, 0.9% NaCl, and antiseptic.

Another medical action given to research subjects is debridement. Debridement is done to remove dead tissue that can make wounds difficult to heal is debridement.[3] In this study, debridement was carried out on 17 subjects (35%). After debridement, irrigation should be

performed on the wound with normal saline or 0.9% NaCl followed by wound care or dressings to keep the wound in good moisture and protect it from bacterial contamination.

Amputation was performed on 17 subjects (35%). In another study, the incidence of amputation was found to be 37.5% and 39.5%.[12,15] Amputation in diabetic ulcers often occurs due to a history of diabetes of 10 years or more, history of previous amputation, inadequate glycemic control, history of hypertension, hyperlipidemia, peripheral arterial disease, history of peripheral neuropathy, history of osteomyelitis, and severity of the wound. Other factors contributing to the aggravation of having to be amputated include increasing age, smoking history, anemia, increased white blood cell count, hypoalbuminemia, and other microvascular and macrovascular complications.[15,16] Based on Wagner's classification, amputation occurs mainly in class 4 diabetic ulcers, namely gangrene in some leg tissue, and class 5, namely gangrene in the entire leg.[15]

Anemia and hypertension are the most common comorbidities in diabetic ulcer patients. Anemia was found in 10 of 48 subjects (21%). The results of this study are supported by a meta-analysis involving 15 studies with 2,895 diabetic ulcer patients, showing the prevalence rate of anemia in the severity of mild, moderate, and severe diabetic ulcers, respectively, was 69.7%, 49.5%, and 73One study in this meta-analysis reported that ulcers that did not heal occurred in 127 of 236 (53.8%) diabetic ulcer patients with anemia compared with 34 of 117 (29.1%) diabetic ulcer patients without anemia.[17] Chronic inflammation, diabetic nephropathy, and malnutrition are factors thought to cause anemia in diabetic ulcer patients. Of these factors, inflammation or chronic inflammation is most often associated with anemia in patients with diabetic ulcers. Several studies have shown that pro-inflammatory cytokines can suppress hematopoietic function and reduce serum iron levels, causing a shortage of hematopoietic raw materials.[18] Anemia is also associated with the degree of wound healing, the incidence of amputation, and increased mortality. Good healing of diabetic ulcers requires adequate blood flow to provide oxygen and other essential nutrients to damaged tissues. Increased deformability of red blood cells in diabetic ulcer patients can cause decreased blood flow and delay wound healing. Decreased oxygenation due to anemia can also exacerbate ulcer conditions.[19] The incidence of hypertension as a comorbid in this study reached a relatively high number, 9 out of 48 subjects (18%). These results follow a study that showed that 296 of 602 DM patients (49.17%) had comorbid hypertension.[20] In another research article, as many as 8 subjects (26.7%) had comorbid hypertension.[21] Hypertension in DM occurs because high blood viscosity decreases blood flow so vascular deficiency can occur. Hypertension with blood pressure >130/80 mmHg can cause damage to blood vessel lesions. Endothelial damage will affect macroangiopathy through platelet adhesion and aggregation, leading to vascular deficiency so that hypoxia can occur in tissues, which can increase the risk of ulcers.[22]

Table 2 shows that as many as 35 classes of drugs were used in 48 research subjects with the most frequently administered drugs being Antibiotics (100.97%), Antidiabetic (84.5%), Non-Opioid Analgesics (55.3%), and Fluids and electrolytes (55.3%). Antibiotics are one of the most widely used because of the high incidence of infection in diabetic ulcers. Antibiotics are the most effective drugs to fight infections, especially infections caused by bacteria. Antidiabetics such as insulin and oral antidiabetics are recommended therapies for controlling blood glucose during infection or controlling and eliminating the infection itself.[23] Non-opioid analgesics in diabetic ulcer patients are used as pain relievers. Non-opioid analgesics work well for pain when damage or injury to body tissues occurs.[24] Fluid and Electrolyte Groups are given as the main fluid choice to balance fluids and electrolytes in the body.

Table 3 shows that of the 48 study subjects who experienced drug interactions, 41 subjects (85%) and 7 subjects did not experience drug interactions (15%). Table 4 shows that drug interactions are grouped into three categories based on severity: major, moderate, and minor. Drug interactions that occurred in 41 study subjects found 263 cases based on the severity of the interaction, consisting of 31 major interactions (11.8%), 177 moderate interactions (67.3%), and 55 minor interactions (20.9%).

Drug interactions are essential things to be considered by doctors and pharmacists in monitoring drugs for patients, especially patients with drug use of more than five drugs. This effort is one way to minimize the risk of drug interactions. Collaboration between doctors and pharmacists needs to be carried out in considering the risks and benefits that will be received to minimize the incidence of drug interactions.[8]

Major interactions are interactions that cause permanent disability to be life-threatening.[9] If a significant interaction occurs, the patient needs intervention to minimize or prevent unwanted effects. Handling major interactions can also be done by avoiding using drugs that cause simultaneous interactions. In this study, there were 31 major interactions (11.8%), one of which was the interaction between Levofloxacin and Ondansetron. When combined with ondansetron, Levofloxacin can increase the risk of QT interval. The causative mechanism in this interaction is the additive elongation effect on the QT interval.[25] These two drugs should be avoided or replaced with other drug alternatives.[26]

There are limitations in this study, where it is necessary to conduct similar studies related to drug research in diabetic ulcer patients in other hospitals to obtain different data. In addition, conducting a similar study with a prospective data collection direction is necessary to obtain more complete data.

This study demonstrates that the large number of drugs used to treat diabetic ulcers triggers drug interactions. It has been shown that the use of 103 types of drugs in 48 study samples obtained 41 patients experiencing drug interactions with a total of 263 cases. The data from this study prove that it is important for doctors and pharmacists to monitor drugs for patients with polypharmacy to avoid or minimize the incidence of drug interactions.

References

1. Almasdy D, Sari DP, Suhatri S, Darwin D, Kurniasih N. Evaluasi penggunaan obat antidiabetik pada pasien diabetes melitus tipe-2 di suatu Rumah Sakit Pemerintah Kota Padang-Sumatera Barat. J Sains Farm Klin. 2015;2(1):104.
2. Agale SV. Chronic leg ulcers: epidemiology, aetiopathogenesis, and management. Hindawi Publ Corp. 2013;2013:9.
3. Rosyid FN. Etiology, pathophysiology, diagnosis, and management of diabetics foot ulcer. Int J Res Med Sci. 2017;5(10):4206–13.
4. Chaudhry WN, Badar R, Jamal M, Jeong J, Zafar J, Andleeb S. Clinico-microbiological study and antibiotic resistance profile of mecA and ESBL gene prevalence in patients with diabetic foot infections. Exp Ther Med. 2016;11(3):1031–8.
5. Detty AU, Fitriyani N, Prasetya T, Florentina B. Karakteristik ulkus diabetikum pada penderita diabetes melitus. J Ilm Kesehat Sandi Husada. 2020;11(1):258–64.
6. Sumarjo K, Waspadji S, Sosrosumihardjo R. Clinical picture and microbiological pattern in 3 rd and 4 th degrees of the pedis classification of diabetic foot infection. Majalah Kesehatan Pharma Medika. 2018;10(1):1–14.
7. Veryanti Pr, Safira I. Kajian interaksi obat pada pasien strok di Rumah Sakit Pusat Otak Nasional (Study of drug interaction in stroke patients at Rumah Sakit Pusat Otak Nasional). J Ilm Medicam. 2020;6(1):45–52.
8. Reyaan IBM, Kuning C, Adnyana IK. Studi potensi interaksi obat pada resep polifarmasi di dua apotek Kota Bandung. JMPF. 2021;11(3):145–52.
9. Kurniawati F, Yasin NM, Dina A, Atana S, Hakim SN. Kajian adverse drug reactions terkait interaksi obat di bangsal rawat inap Rumah Sakit Akademik UGM. J Manaj Pelayanan Farm (J Manag Pharm Pract). 2021;10(4):297–308.
10. Sukmana M, Sianturi R, Sholichin S, Aminuddin M. Pengkajian luka menurut meggit-wagner dan pedis pada pasien ulkus diabetikum. J Kesehat Pasak Bumi Kalimantan. 2020;2(2):79–88.
11. Sari YO, Almasdy D, Fatimah A. Evaluasi penggunaan antibiotik pada pasien ulkus diabetikum di Instalasi Rawat Inap (IRNA) Penyakit Dalam RSUP Dr.M.Djamil Padang. J Sains Farm Klin. 2018;5(2):102–11.
12. Agung G, Ira A, Dewi K, Wibisono S, Pawana IPA. Risk factors for lower extremity amputation in diabetic foot ulcer patients : a case-control study. J Ilmiah Mahasiswa Kedokteran Universitas Airlangga. 2020;(02):83–5.
13. Hidayatillah SA, Heri N, Adi MS. Hubungan status merokok dengan kejadian ulkus diabetikum pada laki-laki penderita diabetes melitus. J Epidemiol Kesehat Komunitas. 2020;5(1):32–7.
14. Langi YA. Penatalaksanaan ulkus kaki diabetes secara terpadu. J Biomedik. 2011;3(DM):95–101.
15. Andhika C, Ismiarto YD. Clinical profile of diabetic foot amputation in Hasan Sadikin Hospital, Bandung, Indonesia. J Endocrinol Trop Med Infect Dis. 2020;02(2):57–62.
16. Musa IR, Ahmed MON, Sabir EI, Alsheneber IF, Ibrahim EME, Mohamed GB, et al. Factors associated with amputation among patients with diabetic foot ulcers in a Saudi population. BMC Res Notes. 2018;1–5.

17. Yammine K. Is there an association between anemia and diabetic foot ulcers ? A systematic review and meta-analysis. Wound Repair Regen. 2021;29(3):432–42.
18. Chuan F, Zhang M, Yao Y, Tian W, He X, Zhou B. Anemia in patients with diabetic foot ulcer : prevalence, clinical characteristics, and outcome. Int J Low Extrem Wounds. 2016;15(3);220–6.
19. Gezawa ID, Ugwu ET, Ezeani I, Adeleye O, Okpe I, Enamino M. Anemia in patients with diabetic foot ulcer and its impact on disease outcome among Nigerians: Results from the MEDFUN study. PLoS One. 2019;14(12):e0226226..
20. Inayah, Hamidy MY, Sari M. Gambaran terapi diabetes melitus tipe 2 dengan komorbid hipertensi di Rumah Sakit X Pekanbaru. J Ilmu Kesehat. 2016;10(2):2016.
21. Anggriani Y, Restinia M, Mitakda VC. Clinical outcomes penggunaan antibiotik pada pasien infeksi kaki diabetik. J Sains Farmasi Klinis. 2015;01(02):111–21.
22. Rina, Setyawan H, Nugroho H, Hadisaputro S. Faktor-Faktor risiko kejadian kaki diabetik pada penderita diabetes melitus tipe 2 (Studi Kasus Kontrol di RSUP dr. M. Djamil Padang). J Epidemiol Kesehat Komunitas. 2016;1(2):48–60.
23. Samba, Sukarmini, Lestari, Sarasmita, Larasanty. Efek penggunaan obat antidiabetes terhadap luaran terapi pada pasien diabetes melitus tipe 2 dengan komplikasi ulkus kaki diabetik di RSUP Sanglah Denpasar. J Ilm Medicam. 2018;5(2):82–7.
24. Paschou SA, Stamou M, Vuagnat H, Tentolouris N, Jude E. Maturitas pain management of chronic wounds : diabetic ulcers and beyond. Maturitas. 2018;117:17–21.
25. Nyamagoud S, Jadhav C, Upadhyay K. QT Prolongation associated with multiple drug-drug interactions. Int J Adv Res Dev. 2018;3:98–101.
26. Drug Interactions Checker-Medscape Drug Reference Database [Internet]. 2022 [cited 2022 Aug 11]. Available from: https://reference.medscape.com/drug-interactionchecker.
27. Hikmawati F. Metodologi penelitian. Depok: PT Rajagrafinfo Persada;2017.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2986
Majalah Kedokteran Bandung. 2023;55(2):117–123

Majalah Kedokteran Bandung (MKB)

Received: August 27, 2022
Accepted: January 30, 2023
Available online: June 30, 2023

Effective Visual Media to Increase Knowledge and Comprehension of Multidrug Resistant Tuberculosis Among Patients and Their Caregivers

Lidya Chaidir,[1,2] Dyshelly Nurkartika Pascapurnama,[1] Claudia Selviyanti,[1] Cindy Natasha,[1] Mutiah Nurul Jihadah,[1] Prayudi Santoso[3]

[1]Research Center for Care and Control of Infectious Diseases (RC3ID), Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[2]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran/Hasan Sadikin Hospital, Bandung, Indonesia

Abstract

Indonesia ranks third among countries with the largest number of TB cases after India and China. Globally, more than 3-4% of all TB cases are Multidrug-Resistant Tuberculosis (MDR TB). MDR TB is a more complicated TB that needs extra treatments, which extend treatment time and increase adverse effects. Thus, MDR TB patients and their families often feel demotivated about completing treatment, leading to loss to follow up, which contributes to the never-ending transmission and greatly affects the success rate of the national TB control program. Thus, better knowledge and perception on MDR treatment for patients and families plays a crucial role in dealing with this issue. A cross-sectional study was performed from April to June 2019 to evaluate the effectiveness of visual educational media for TB patients and their caregivers. Participants consisted of 144 patients diagnosed with MDR TB and their caregivers or family members (n=22). A pre-test was administered before an education session by a nurse and visual media were used as the educational material. At the end of the session, a post-test was performed. The post-test score was then compared to the pre-test score to evaluate the session's effectiveness using the paired t-test. Result showed significant increase in the post-test score (t= 3.249, df=3, p=0.04), with the caregivers attained a higher score, showing better improvement in knowledge after the session compared to the patient group. Hence, the MDR TB educational intervention using visual media is considered effective to increase participants 'understanding of MDR TB. It is expected that with increased knowledge on MDR TB, the treatment success rate will increase and becomes the catalyst for the nationwide TB control strategy.

Keywords: Multidrug resistant tuberculosis, MDR TB knowledge, MDR TB education, visual educational media

Introduction

Tuberculosis is the leading infectious disease killer in the world and still a major public health concern in Indonesia, which ranks third worldwide (8.4% of the total population are infected) for high-burden TB nations, after India (26%) and China (8.5%). According to Global Report, 10 million individuals became ill with TB in 2021, with 3.3% being Multidrug-Resistant Tuberculosis (MDR TB).[1] MDR-TB usually occurs for one or two reasons. It can happen when strict medication regimens are not applied to a patient. This can be due to incorrectly prescribed regimens, ineffective medication, or interruption in medication (full medication regimen is not completed). These reasons are also known as treatment failure. MDR -TB can also happen when the disease is transmitted from person to person.[2] MDR TB usually rises resistance in first-line tuberculosis drugs, including Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol.[3] As substitutes, second-line drugs, include, for example, include levofloxacin, moxifloxacin, bedaquiline, delamanid and linezolid, are given during the treatment, which need longer duration, greater side effects, and higher treatment cost. This condition often demotivates patients to finish the treatment. It gives birth to dropout (DO) patients and various complications. It allows the disease to continue

Corresponding Author:
Lidya Chaidir,
Department of Biomedical Sciences,
Faculty of Medicine, Universitas Padjadjaran, Indonesia
Email: lidya.chaidir@unpad.ac.id

to be transmitted.[4] Moreover, the DO rate among MDR TB patients is much higher than in primary sensitive TB patients.

MDR-TB remains a public health crisis and a health security threat. Only about one in three people with drug-resistant TB accessed treatment in 2020. Worldwide in 2018, the treatment success rate of MDR/RR TB patients was 59%. Besides the patient's complete TB medication, a successful MDR TB treatment is also determined by TB national control strategy, quality and accessibility of health facilities, and TB drug stock and distribution.[3] Mental aspects such as knowledge and perception are crucial in infectious disease control and care.[4] They help igniting patient's positive behaviour management to achieve treatment success.[3,5].

Unfortunately, the approaches to successful treatment management, including education about the disease, are commonly misunderstood health topics among patients and their caretakers. Lack of knowledge of TB and MDR TB etiology, symptoms, transmission route, and treatment procedure inhibits inner motivation from the patients to finish the treatment properly.[2] Along with that, misconceptions about the disease often result in negative social stigma. This may lead to individual treatment failure and continuous disease transmission within community, as it hinders contact investigations.[6]

Therefore, to overcome the risks that may arise from a lack of knowledge of MDR TB among patients and their caretaker, there is a need for education about the disease to raise awareness among them.[7] Education must be given to escalate patients' and their families' understanding of MDR TB to help strengthen control in treatment management and prevent the disease.[8] Positive reinforcement from their caretaker, especially family member, influences patients to complete the treatment.[9] Well-motivated patients, medical staffs, and families play a great role in nationwide TB control strategy, especially in determining treatment success.[10,11] Effective education methods should be considered as interventions; one of them is educational visual media.

Visual media is considered one of the most effective methods in the learning process to deliver ideas and information. The use of visual media has been widespread in medical education. It provides multichannel inputs, as visuals can increase memory over reading alone.[12] Visuals can capture more attention which leads to efficient learning. It also promotes feasible applications with complex concepts. [12] Not only is the fun factor, but it also offers brief and concise explanations of MDR TB that are easy to remember and practical. There have been few studies on MDR TB understanding levels among people in Bandung and method effectivity for MDR TB education.[13] This study is aimed to evaluate the implementation and effectiveness of visual graphic media as an educational intervention in improving patient and caretaker understanding of MDR TB in Bandung.

Methods

This cross-sectional designated study began with a pre-test for several groups: pre-treatment MDR TB patients, in-treatment MDR TB patients, and MTB patients' caretakers (family members). A pre-test questionnaire is distributed by the research nurse to assess initial knowledge from each participant, followed by educational sessions using visual media for a certain amount of time. To minimize the possibility of bias, the research nurse describes one topic before moving forward to the next. This also helps interpret the data with more objectivity and nuance. The educational compositions of the graphic visual media are divided into pages, listed in Table 1. At the end of the session, a post-test is conducted using the same question. The intervention is successful when the post-test score is better than the pre-test.

The study population is patients or their caretakers (family members) who visits TB MDR Clinic Dr. Hasan Sadikin General Hospital Bandung as a research site. Participants must be older than 17 years old and able to complete the questionnaire independently. There were 136 participants, 7 patients on treatment, 107 pre-treatment patients, and 22 caretakers/ family members. From 7 patients on treatment, 4 patients have been in <10 months, 2 patients have been in treatment for 12 and 14 months, and 1 patient has been in 20 months.

Along with personal sociodemographic data (age, sex, treatment status), basic MDR TB knowledge questions (MDR TB definition, causes, diagnostics, and treatment) also exist in the questionnaire as a Yes/No question. Each correct answer scores 1, and the false answer scores 0. Questionnaires are given twice as pre and post-test. Both scores will be compared in order to evaluate visual media effectiveness.

The research nurse gave a person-to-person educational session using visual media in the form of graphic-packed cards. This instrument was designed by researchers based on the MDR

Table 1 Educational Content through Visual Graphic Media

MDR TB Educational Content (Visual Graphic Media)		
Page 1	What is MDR TB	Explaining the etiology of MDR TB to clear the misunderstanding of common negative stigma
Page 2	What caused MDR TB	Explaining how MDR TB happens (e.g., not completing an entire course of TB treatment, relapse case, or getting infected from the person with MDR TB)
Page 3	How MDR TB is transmitted	Explaining that MDR TB is transmitted through air droplets, cutlery, close contact with person with MDR TB and that MDR TB is not transmitted through blood
Page 4	What are the symptoms of MDR TB	Explaining the symptoms of MDR TB, including continuous cough, fever, heavy breathing, sweating at night without significant physical activity, extreme weight loss, loss of appetite
Page 5	How MDR TB is diagnosed	Explaining laboratory diagnosis for MDR TB
Page 6	Prevention of MDR TB transmission	Reminding patients to wear masks anywhere to prevent transmission Explaining the importance of good infection prevention behavior and health attitude
Page 7	How MDR TB is treated	Explaining two types of treatment (short- and long-term regiments) Explaining the role of medication supervisor in treatment
Page 8	Discussion of common MDR TB drug side effects	Reminding patients to notify their health provider if any adverse symptom develops during the treatment Explaining not to stop the treatment despite of the side effects
Page 9	The importance of patient motivation	Reemphasizing the importance of completing the entire treatment course Reminding the importance of visiting the MDR TB clinic to take medication and regular examination

fact sheet of the Centers for Disease Control and Prevention (CDC) (accessed at https://www.cdc.gov/tb/publications/factsheets/drtb/mdrtb.htm? Sort=Title%3A %3Aasc) and divided to several study groups, such as "Aetiology", "Cause and Transmission", "Diagnostic", and "Treatment". Each card contains graphics and information according to a specific study group.

GraphPad Prism 8.3.0 are used to acquire answers data and their frequency and percentage. Pre and post-test significant statistical analysis will be analyzed using paired t-test method with a p-value <0.05 and a confidence interval of 95%. The education is considered successful if the score is more significant than the median.

Written informed consent is distributed to eligible patients. Personal data will not be exposed and will be kept anonymous. This study is part of community service work from a fundamental research grant from Universitas Padjadjaran for MDR TB with validated ethic code document number 636/UN6.KEP/EC/2018.

Results

All eligible participants who answered pre- and post-tests are grouped into pre-treatment, in-treatment, and caretaker (family member). Table 2 shows that most participants are male (72 persons. 52.9%). The age median is 41.90 years old, with higher distribution in productive age (36–45 years old). Most participants come from the pre-treatment group, with 107 participants (78.7%).

Pre and post-test, each consisting of 13 questions, are distributed to evaluate their understanding of MDR TB. There are several questions based on study groups: MDR etiology, causes and transmission, diagnostics, and treatment. The frequency and percentage of total participants' answers show in Table 3.

Table 2 Participant Sociodemographic Characteristics (n=136)

Characteristics	n	(%)
Age		
17–25	18	13.2
26–35	26	19.1
36–45	41	30.1
46–55	29	21.3
56–65	18	13.2
>65	4	2.9
Sex		
Male	72	52.9
Female	64	47.1
Category		
Pre-treatment	107	78.7
In treatment	7	5.1
Caretaker/family	22	16.2

Most participants already understand MDR TB presence, causes, transmission, and diagnostics. They learned that MDR TB is curable, but only 20,6% believe that MDR TB symptoms are equal to regular TB. Although, according to pre-test answers, the majority understand that mask must be worn (98.5%) during two years of treatment (59.6%) that rises various side effects (65.4%), half of participants don't know that medication must be done under direct supervision by medication supervisor (nurse or health worker). Correct answers, as the indicator of participant's knowledge, rises to 100% in almost every question after MDR TB educational session was given.

Increasing test scores are observed in almost all post-test questions in comparison to the pre-test. These changes are significantly observed in caretaker and pre-treatment groups (p-value=0.02 and 0,04, CI 95%, p<0.05) but not in the in-treatment group (p-value=0.39) as they may have got an explanation about MDR TB from a physician, nurse, or health worker in MDR TB clinic during the treatment (Table 4).

Discussion

Many patients diagnosed with MDR TB have limited knowledge and understanding about the disease.[14] This also happens to their caretaker, especially family members.[7] The abundance of confusion about the diagnosis raises social misperceptions and negative stigma to patients and their families, which might delay in seeking medical care and treatment.[15, 16] Therefore, MDR TB education is advised to be delivered to patients and caretakers to provide an understanding of the disease and the importance of its treatment. Clear information about the treatment process, including the advantages and treatment of side effects, hopefully, will clear the misinformation, increase caretaker's support and boost patient optimism to complete the treatment. The lack of understanding about MDR TB, including its symptoms, treatment management, and how it is transmitted, contributes to demotivation, wrong perception, and increasing anxiety, which leads to unsuccessful treatment.[6, 14]

One of the basic approaches to providing a sufficient fundamental understanding of MDR TB is face-to-face discussions between physicians, nurses, or health workers with patients and family members.[7] In this study, MDR TB education is given personally to patients and caretakers using visual media. This study implements educational graphic cards with attractive illustrations and clear and concise information about MDR TB. The graphic card includes sections on the etiology, cause and transmission, diagnosis, and treatment.

Pre and post-test were given, resulting in significantly increasing scores (t=3.249, df=3, p=0,04), interpreted as increasing subjects' knowledge of MDR TB. Overall, the education of MDR TB through visual graphic media resulted in noticeable observations and improved inpatient and caretaker understanding of the disease. This model could assist physicians, nurses, and health workers in decreasing the misconception about MDR TB and raising awareness and knowledge for patients and their caretakers to increase treatment success rates and provide better TB control in the future.

All 13 questions are grouped based on 4 study groups: etiology, causes and transmission, diagnosis, and treatment. All categories besides 'diagnoses consist of 4 questions.

In the pre-test, only 28 (20.6%) of all participants answered question No. 2 ("Does the symptoms of MDR TB are equal to regular TB?" – answered "Yes") correctly. Possibly, most people think that there are visible symptoms that distinguish MDR TB from regular TB, whereas the differences can only be known through GeneXpert MTB/RIF examination and phenotypic DST (Drug Susceptibility Test). After

Table 3 Participant's Knowledge on MDR TB

Topics (Questions)	Correct Answer (n=136)				p-value (CI 95%)
	Pre-test		Post-test		
	n	(%)	n	(%)	
Aetiology					
MDR TB is equal to regular TB (No)	86	63.2	125	91.9	
The symptoms of MDR TB are equal to regular TB (Yes)	28	20.6	85	62.5	
TB release is possible (Yes)	105	77.2	133	97.8	
MDR TB can be cured (Yes)	129	94.9	135	99.3	
Causes/transmission					
MDR TB are caused by resistant mycobacteria (yes)	97	71.3	136	100	
MDR TB are caused by unfinished treatment (yes)	104	76.5	136	100	
MDR TB are caused by irregular drug consumption (yes)	102	75.0	136	100	
MDR TB can be spread to another patient (yes)	109	80.1	136	100	t (3.429). df (3). p-value 0.04
Diagnostics					
TB Diagnostics uses sputum as sample (yes)	117	86.0	136	100	
Treatment					
MDR TB Patients should wear mask to avoid transmission (yes)	134	98.5	136	100	
MDR TB treatment must be finished in 2 years (yes)	81	59.6	134	98.5	
MDR TB treatments side effects are nausea, vomiting, headache, diarrhea, malaise (yes)	89	65.4	136	100	
Everyday drug consumption must be under direct supervision by medication supervisor (yes)	55	40.4	127	93.4	

Table 4 Questionnaire Scores Based on Study Groups

Topic	Caretaker/ Family (n=22)				Pre-treatment patient (n=107)				In-treatment patient (n=7)			
	(Max. total score=286)				(Max. total score=1,391)				(Max. total score=91)			
	Pre-test		Post-test		Pre-test		Post-test		Pre-test		Post-test	
	Score	(%)	Score	(%)	Score	(%)	Score	(%)	Score	(%)	Score	(%)
Aetiology	55	62	77	88	270	63	375	88	23	82	26	93
Causes/ Transmission	68	77	88	100	316	74	428	100	28	100	28	100
Diagnosis	15	68	22	100	95	89	107	100	7	100	7	100
Treatment	54	61	86	98	277	65	419	98	28	100	28	100
Total Score	192	67	273	96	958	69	1,329	96	86	95	89	98
p-value (CI 95%)	t (3.941), df (3), p-value (0.02)				t (3.302), df (3), p-value (0.04)				t (1), df (3), p-value (0.39)			

the educational session, 85 (62.5%) subjects answer this question correctly. In the pre-test, most participants acknowledge that although there may be relapse (n=105, 77.2%), MDR TB is still curable (n=129, 94.9%). At these questions, 20.6% dan 5% score increments are observed in the *post-test.*

From pre-test answers, most people understand that MDR TB is caused by resistant TB mycobacteria (n=97, 71.3%) that rises after incomplete treatment (n=104, 76.5%), irregular treatment (n=102, 75%) or infection from another patient (n=109, 80.1%). All questions are answered correctly by all participants after the educational session. This study shows that visual educational media are impactful in increasing MDR TB knowledge (t= 13.29, df=3, p=<0.001). Better knowledge drives patients and their caretakers' motivation to complete the treatment.

Although GeneXpert MTB/RIF was used for MDR TB primary detection when this study was conducted, it is considered that patients and caretakers need to become more familiar with the name of the diagnostic test. Therefore, 'sampling material' is considered the only question in this section. Most participants know sputum as diagnostic material (n=117, 86%). Complementing this knowledge, information on correct sputum collection procedures, definitive diagnosis, and follow-up procedures are also informed in this educational session, raising participants' understanding to 100%.

Almost all participants know that masks must be worn during two years of treatment (pre-test n=134, 98.5%; post-test 100%), but only a few realize that drug consumption must be done under direct medication supervision (nurse or health worker) (n=55, 40.4%). This is a very important thing to remember to avoid patients' unattended and irregular drug consumption that might cause serious side effects such as nausea, vomiting, or tinnitus.[14]

After this educational intervention using graphic visual media, it is rewarding that the participants attained a high knowledge score (around 90%) in all group variables. The in-treatment patient group achieves the highest collective score, as they were currently doing the treatment. They likely acquired TB education during the treatment.[6] Higher significance of knowledge elevation, after educated using visual media, makes this method becomes more effective for families and pre-treatment patients (Caretaker/family: t (3,941), df (3), p-value (0.02); Pre-Treatment Patients Group: t (3.302), df (3), p-value (0.04)).

Few in-treatment patients come to the clinic due to limited transportation costs, tiredness of side effects, or not knowing that treatment should be supervised by a medication supervisor (usually a nurse or health worker). This resulted in the ratio balance of the number of participants in each group, which became our study limitation. Furthermore, there are few conducted studies on methods and learning media about TB and MDR TB knowledge for patients. This study needs more references and further research in the future.

This study reflects the need for education on MDR TB to raise awareness among patients and caretakers, as knowledge about MDR TB is crucial for patients and caretakers to support successful treatment. Therefore simple graphic visual media can be used to deliver education. Based on this study, this finding suggests that simple but effective graphic visual media resulted in satisfying improvement in all aspects of knowledge of MDR TB within the participants when implemented in the MDR TB clinic. This can provide an efficient and effective strategy for increasing patient and caretaker understanding of MDR TB. For further studies, there should be a comparison between this kind of visual media and another learning process and platform for further evaluation. Therefore, the most suitable method can be implemented to increase treatment success and obtain a better national TB control strategy.

References

1. WHO. Global Tuberculosis Report 2021. Geneva: WHO Library Cataloguing Data; 2021.
2. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med. 2015;5(9):a017863.
3. Yuni IDAMA. Hubungan fase pengobatan TB dan pengetahuan tentang MDR TB dengan kepatuhan pengobatan pasien TB (Studi di Puskesmas Perak Timur). J Berkala Epidemiologi. 2016;4(3):301–12
4. Isara AR, Akpodiete A. Concerns about the knowledge and attitude of multidrug-resistant tuberculosis among health care workers and patients in Delta State, Nigeria. Niger J Clin Pract. Published 2015 Jun 22;18(5):664–9.
5. Mbuthia GW, Olungah CO, Ondicho TG.

Knowledge and perceptions of tuberculosis among patients in a pastoralist community in Kenya: a qualitative study. Pan Afr Med J. 2018;30:287.
6. Wilson JW, Ramos JG, Castillo F, F Castellanos E, Escalante P. Tuberculosis patient and family education through videography in El Salvador. J Clin Tuberc Other Mycobact Dis. 2016;4:14–20.
7. Yousif K, Ei Maki M, Babikir RK, Abuaisha H. The effect of an educational intervention on awareness of various aspects of pulmonary tuberculosis in patients with the disease. East Mediterr Health J. 2021;27(3):287–92.
8. Miandad M, Nawaz-Ul-Huda S, Burke F, Hamza S, Azam M. Educational status and awareness among tuberculosis patients of Karachi. J Pak Med Assoc. Published 2016 Mar 1;66(3):265–9.
9. Huddart S, Bossuroy T, Pons V, Baral S, Pai M, Delavallade C. Knowledge about tuberculosis and infection prevention behavior: a nine city longitudinal study from India. PLoS One. 2018;13(10):e0206245.
10. Kigozi NG, Heunis JC, Engelbrecht MC, Janse van Rensburg AP, van Rensburg HCJD. Tuberculosis knowledge, attitudes and practices of patients at primary health care facilities in a South African metropolitan: research towards improved health education. BMC Public Health. 2017;17(1):795.
11. Esmael A, Ali I, Agonafir M, Desale A, Yaregal Z, Desta K. Assessment of patients' knowledge, attitude, and practice regarding pulmonary tuberculosis in eastern Amhara regional state, Ethiopia: cross-sectional study. Am J Trop Med Hyg. 2013;88(4):785–8.
12. Cooper AZ, Rodman A, Simpson D. Visual media in medical education. J Grad Med Educ. 2021;13(3):417-418.
13. Febriani UD, Ruslami R, Alisjahbana B. Level of knowledge about tuberculosis among type 2 diabetes mellitus patients at the Endocrine Clinic Dr. Hasan Sadikin General Hospital Bandung. AMJ. 2017;4(3):512–7.
14. Malangu N, Adebanjo OD. Knowledge and practices about multidrug-resistant tuberculosis amongst healthcare workers in Maseru. Afr J Prim Health Care Fam Med. 2015;7(1):774.
15. Esmael A, Ali I, Agonafir M, Desale A, Yaregal Z, Desta K. Assessment of patients' knowledge, attitude, and practice regarding pulmonary tuberculosis in eastern Amhara regional state, Ethiopia: cross-sectional study. Am J Trop Med Hyg. 2013;88(4):785–8.
16. Balakrishnan N, Md Monoto EM, Mohd Tohit N, Abdul Wahab A. Knowledge and perception of treatment among tuberculosis patients attending primary care clinics in Malaysia. J Infect Dev Ctries. 2021;15(8):1205–11.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2753
Majalah Kedokteran Bandung. 2023;55(2):77–84

Majalah Kedokteran Bandung (MKB)

Received: March 17, 2022
Accepted: March 12, 2023
Available online: June 30, 2023

Effectiveness of WHO Stress Management for Improving Insomnia Severity Index Score in Telegram's Self-Isolated Online Group Population

Ines Damayanti Octaviani, Gusti Ayu Maharatih, Adriesti Herdaetha
Department of Psychiatry, Faculty of Medicine, Universitas Sebelas Maret
RSUD Dr. Moewardi, Surakarta, Indonesia

Abstract

Insomnia is one of the various symptoms frequently found in patients with post-covid syndrome (PCS) (19.1%). The WHO Stress Management (WSM) is an eclectic psychotherapy that combines mindfulness and relaxation which, theoretically, can be effective in treating insomnia due to PCS. This study aimed to explore the effectiveness of WSM in improving the Insomnia Severity Index Score for people with PCS. This was a quasi-experimental pre-test-post-test control study on 18 participants in the population of online self-isolation group in the Telegram who experienced insomnia. The intervention applied was WSM. These participants were compared to 18 controls who only received psychoeducation. Clinical insomnia symptoms were examined using the Insomnia Severity Index (ISI) before the intervention, then the control group was given sleep hygiene psychoeducation, and the treatment group was given WSM in 5 Zoom on-cam meetings once a week for 30–60 minutes. A re-assessment using the ISI post-test was then performed, and data were analyzed statistically. There was a change in score for insomnia symptoms in the treatment group (delta score 27.50) compared to the control group (delta score 9.50, p=0.00; OR 0.00, p=0.99, 95% CI). Thus, WSM can improve the insomnia score in the population with PCS.

Keywords: Insomnia, post covid syndrome, WHO stress management

Introduction

After the second wave of Covid-19 hit Indonesia in the middle of 2021, a new problem was raised, namely, post covid syndrome (PCS). PCS is when residual symptoms persist for more than 20 days after the PCR shows negative result.[1] Symptoms that fall into the PCS category include chronic fatigue, anxiety, joint pain, headache, orthostatic hypotension, palpitations, impaired cognition, anxiety, depression, and insomnia. These manifestations are found in almost 90% of Covid-19 survivors and the severity of symptoms varies from mild to moderately severe depending on the severity of the Covid-19 infection experienced.

The symptoms experienced by Covid-19 survivors are dysautonomia symptoms. Dysautonomia is an imbalance between sympathetic and parasympathetic nervous system activation that occurs due to infection with the SARS-CoV-2 virus that interferes with the work of the RAS system. Some journals say that the spike of the SARS-CoV-2 virus when it binds to ACE2 can interfere with the work of ACE2, resulting in the shedding of ACE2 receptors and disrupting the body's hemodynamic regulation (Lo 2021). In addition to hemodynamic disorders, there are prominent psychiatric disorders in the post-Covid-19 syndrome, such as anxiety, depression, sleep disorders, memory disorders, impaired concentration, and chronic fatigue syndrome.[2]

Among the many physical and psychiatric disorders that accompany the PCS condition, insomnia is often found in Covid-19 survivors. Even though they are declared physically healthy and able to work normally, Covid survivors still complain of difficulties in returning their sleep quality to the way they were before suffering from Covid.[3] The prevalence of insomnia during the Covid-19 pandemic reached 19.1%, and loneliness during the pandemic was considered to have a role in increasing the prevalence of insomnia.[4] A preliminary study conducted in November 2021 in the online Telegram

Corresponding Author:
Ines Damayanti Octaviani
Department of Psychiatry, Faculty of Medicine, Universitas Sebelas Maret-RSUD Dr. Moewardi, Surakarta, Indonesia
Email: inesdamayantioct@gmail.com

application group found that the insomnia population was 29% of Covid-19 survivors who underwent self-isolation.

Hyperactivity of sympathetic neurons as a response to Covid-19 infection has a role in a cytokine storm. Dysautonomy correlated by Covid-19 mediated by viral infection itself[5], and post-infection stress is why this post covid syndrome phenomenon is ongoing even though SARS-CoV 2 PCR show a negative result. HPA axis activated on acute stress as a response of internal stressor namely infection, activated adrenal glands to produce cortisol as anti inflammation agent. Therefore, excessive glucocorticoid production makes resistency in the body. After this activation, adrenal glands showed hypoactivation because of HPA axis fatigue due to excessive neuroimmunology stimulation and cytokine storm.[6]

This hypocortisol state makes ongoing inflammation even in the real-time assessment, and no virus is shown. This condition correlated with various physical complaints that accompanied PCS. Persistent insomnia in PCS is caused by sympathetic overdrive. This condition has altered the neurotransmitter state in the brain. Inhibition of neurotransmitters such as GABA and melatonin has a role in interrupted circadian rhytm.[7,8]

Moreover, while proinflammatory cytokine is activated, so does the Mast cell, which will activate histamine. Histamine is a neurotransmitter that works in the state of wakefulness. Therefore its activation in an abundant amount resulted in difficulty in initiating sleep.[9,10] PCS is not only a problem caused by infection but a condition caused by stress mediated by sympathetic overdrive. Good stress management could result in the PCS's symptoms, including insomnia.

Beyond all the stress management methods recommended by various studies, relaxation and mindfulness are recommended for insomnia. In one meta-analysis study, there were 12 RCTs that recommended relaxation therapy for insomnia with an effect size of 0.99 (95% CI) compared to the control group; meanwhile, in a mindfulness study, there were 3 RCT that recommended this method to improve chronic insomnia symptoms with effect size 1.04 (95% CI)).[11] WHO Stress Management created by WHO to combine this two approachment so that this approachment therapy can be effective for insomnia.

When someone is influenced by negative emotion, there is interaction between sympathetic neurons and the HPA axis which can influence the immune system. Mindfulness can activate the parasympathetic nerve and decrease the peripheral stress response. Another study found that doing mindfulness meditation routinely can decrease TNF-α, IL-8, CRP and decrease NF-kB transcription to facilitate anti-inflammatory effects.[12] Mindfulness is known as a protective agent for aging because it can prevent telomere shortening, one of the chromosome components which has a role in the aging process.[13] That's why mindfulness is recommended to be given as a therapeutic modality in PCS because PCS is shown to accelerate the aging process in human cells due to inflammation action.[14]

Comprehensive management is needed to reduce morbidity and improve the quality of life of Covid-19 survivors so they can return to being productive as before. Because the PCR results have shown negative results, medical treatment for PCS is no longer needed so non-pharmacological approaches must be considered to manage complaints after Covid-19 infection. Non-pharmacological therapy based on relaxation therapy, such as progressive muscle relaxation, can be recommended as a non-psychopharmaca treatment because the intervention is easy, does not require special skills or special tools, and most importantly, can be done remotely, such as using video so that it can be given to patients who are undergoing treatment and undergoing isolation treatment.[5] Another non-pharmacological approach to mindfulness is also recommended because it is known to have a strong anti-inflammatory effect and slow down the inflammatory process that occurs in post-covid syndrome. Seeing that these two non-psychopharmaca therapy modalities promise good results for managing PCS symptoms, including insomnia, the authors obtained a stress management module issued by the World Health Organization WHO, which includes both non-psychopharmaca approaches. Hence, the authors are interested in using them to intervene in insomnia due to post-covid syndrome. This study aims to know the effectiveness of WHO Stress Management in improving insomnia scores in the population of post covid syndrome.

Methods

The research was conducted using a quasi-experimental, pre-test-post-test control group design. This study aimed to analyze the effect of giving the WHO stress management module to the population experiencing insomnia in the

online self-isolation group at Telegram. This research was conducted online in the period January–February 2022.

A preliminary study conducted online in November 2021 found that the insomnia rate was 29% in the PCS population. The sampling technique used was purposive sampling from the population screened in the preliminary study. The calculation of the research subjects was determined using the categorical comparative formula in pairs of two groups, and 18 subjects were found in each group. This calculation was determined by two groups paired comparative category formula by Sopiyudin Dahlan:

$$n1=n2 = 2\left[\frac{(Z\alpha+Z\beta)S}{X1-X2}\right]^2$$

Notes:
n1: the total amount of members in Group 1
n2: the total amount of members in Group 2
Zα: degree of freedom α (assigned by the researcher)
Zβ: degree of freedom β (assigned by the researcher)
S: standard deviation for insomnia score (assigned by the researcher)
X1-X2: meaningful difference in insomnia score between two groups (assigned by the researcher)

$$n1=n2 = 2\left[\frac{(1,96+0,84)\ 7,5}{7}\right]^2$$

n1=n2= 18

The subjects taken met the following inclusion criteria: 1. correspondent young to mid-adult (19–60 years), 2. able to read, write and have online access, 3. can understand and speak Indonesian, and 4. willing to be a research respondent and fill out a google form consent form. Meanwhile, the exclusion criteria are: 1. participants use sedative drugs (sleeping pills), 2. participants have life-threatening severe medical illnesses, and 3. patients refused to participate in this study.

The insomnia profile is assessed with Insomnia Severity Index (ISI), validated to use in Indonesia with a reliability score of Cronbach Alpha 0.989, online filled out by participants through Google Forms. ISI score is stated as a numeric scale. Clinical symptoms of insomnia stated on an ordinal scale stated as "not insomnia" (score 0–7), "subthreshold insomnia" (score 8–14), "moderate insomnia" (score 15–21), and "severe insomnia" (score 22–28).

WHO Stress Management is a module created by WHO on April 29th, 2020, uploaded online to WHO official site as a coping module to face stress and grief during the pandemic. It can use freely by the public and contains five introductory chapters: grounding, unhooking, acting on your value, being kind, and making room. This module combines mindfulness and relaxation therapy. No need to join any specific training to use this module. This module has audio guidance to help module application, either offline or online. This module is given online to participants through Zoom meetings five times once a week. During the online session, participants were urged to open the camera. This module was given 30–60 minutes according to chapter content.

The flow of this research is that individuals who meet the inclusion and exclusion criteria are motivated to participate in the study. Individuals who agree will be asked to fill out online consent (google informed consent forms), then divided into an exposure group and a control group, and then given the intervention according to the WHO stress management module. The intervention session was given 5 times in 5 weeks of online meetings via Zoom meetings for 30–60 minutes. The time selection is adjusted to the agreement of the author and the research subject. At the end of the study, the Insomnia Severity Index (ISI) Google form was distributed back to the two groups to be filled out as a post-test assessment. Then a comparison was made of the results of the two groups. The data obtained were tabulated and analyzed to determine the difference between scores and clinical insomnia. If the data is normally distributed, then the pre-and post-intervention scores are measured using a paired T-test. The Wilcoxon test is performed if the data is not normally distributed. In the measurement of control and treatment groups, if the data is normally distributed, then the measurement is carried out using the independent sample T-test. If the data is not normally distributed, then the measurement uses the Mann-Whitney test. The difference is significant when $p<0.05$. All statistical analyses used SPSS 17.0. This research has received permission from the Health Research Ethics Committee of Dr. Moewardi Hospital Surakarta with the number 27/I/HREC/2022.

Result

This research was conducted online by taking a sample of 36 people. Eighteen participants from the intervention group received psychoeducation

through sleep hygiene and Zoom meeting, guidelines, and training on WHO Stress Management for five sessions. In comparison, 18 participants from the control group were only given psychoeducation in the form of sleep hygiene. Both control and intervention groups had the most similarity in data except for age. The control group had a younger mean age than the treatment group (p=0.03). Both groups had more data on the female sex, with the highest data on higher education. The two majority groups did not have comorbid chronic diseases and did not take certain medicines from doctors.

A comparison of demographic data between the control and intervention groups was taken during first encounter of researcher and participant via Telegram, and the analysis was performed using the chi-square test, while the independent sample T-test was used for age. The results of the comparison of demographic

Table 1 Demographic Characteristics of Research Subjects

Characteristics	Psychoeducation (n=18)		WHO Stress Management (n=18)		P value*
	n	%	n	%	
Age (years)					0.03
Adult (19–44)	13	72.2	14	77.8	
Middle Adult (45–60)	5	27.8	4	22.2	
Gender					1.00
Man	6	33.3	8	44.4	
Woman	12	66.7	10	55.6	
Occupation					0.10
Not a Health Worker	7	38.9	10	55.5	
Health workers	11	61.1	8	44.4	
Co-morbidities					0.19
No co-morbidities	11	61.1	11	61.1	
With co-morbidities	7	38.9	7	38.9	
Diet and lifestyle					0.78
Sedentary lifestyle					
Living a sedentary lifestyle	4	22.2	3	16.7	
Not living a sedentary lifestyle	14	77.8	15	83.3	
Smoke					
Regular smoking	0	0	2	11.1	
Do not smoke	18	100	16	88.9	
Caffeine					
Regular caffeine consumption	2	11.1	2	11.1	
Do not consume caffeine	16	88.9	16	88.9	
Gadgets					
Using gadgets before bed	1	5.6	7	38.9	
Don't use gadgets before bed	17	94.4	11	61.1	
Routine drug history					0.09
Don't take regular medication	11	61.1	11	61.1	
Take regular medicine	7	38.9	7	38.9	

*chi-square

Table 2 Differences in Insomnia Scores in the Psychoeducational Group and the WHO Stress Management Group Pre and Post Treatment

	Pre-intervention	Post-intervention	*Delta score* (SD)	95% confidence interval		P value**
				Lower	Upper	
Psychoeducation (Mean±SD)	18.56±4.58	16.44±4.61	2.11	17.54	20.96	0.00
WHO Stress Management (Mean±SD)	19.94±5.51	9.61±5.03	10.33	11.04	15.02	0.00
P value*	0.00	0.00				

*Mann-Whitney test; ** Wilcoxon test

data for gender obtained a p-value of 1.00, education of 0.10, occupation of 0.10, a history of disease of 0.19, a history of routine drug use of 0.09, and diet and lifestyle data of 0.78. Overall demographic data can be seen in Table 1.

Clinical symptoms of insomnia are divided into three, namely subthreshold, moderate, and severe. Of the 18 people in the control group, 9 people experienced subthreshold insomnia, 8 people experienced moderate insomnia, and 1 person experienced severe insomnia; while in 18 people in the intervention group before exposure, 4 people experienced subthreshold insomnia, 8 people experienced moderate insomnia and 6 people who experienced severe insomnia.

The pre-intervention control group had a lower mean insomnia score of 18.56±4.58, while the treatment group had a higher average insomnia score of 19.94±5.51 with a p-value of 0.00 (95% CI: 20.96–17.56). After receiving psychoeducation, the control group had a mean insomnia score of 16.44±4.61. In contrast, after receiving psychoeducation and WHO Stress Management training, the treatment group had a mean insomnia score of 9.61±5.03 with p=0.00.

With p=0.00, there was no significant difference between insomnia scores in the control and treatment groups before the WHO Stress Management intervention was carried out. After that, a comparison was made between the control and treatment groups after the intervention, and the p-value =0.00. To see the effect of the WHO Stress Management intervention, pre-test and post-test measurements were made in the treatment group using the Wilcoxon test. There was a decrease in the clinical mean score in the control group from insomnia symptoms in the pre-test when compared with insomnia symptoms in the post-test results in the control group who were given psychoeducation in the form of sleep hygiene with p=0.00. There was a decrease in the clinical mean in the treatment group from the pre-test results compared to the post-test results in the treatment group with p=0.00 (95% CI) (Table 2).

The logistic regression analysis tests for the dominant risk factors of insomnia in COVID-19 survivors were gender (OR 1.49), occupation (OR 1.49), and diet and lifestyle (OR 1.49) with significant results (p<0.05) indicated by gender (p=0.04), occupation (p=0.04), diet and lifestyle (p=0.03) (Table 3).

Discussion

The different tests of demographic data between the control and treatment groups did not find any significant difference. Clinical conditions of insomnia in the control and treatment groups before the intervention showed p = 0.00, which showed significant results, indicating that in both groups before the intervention, there was clinical insomnia that was significantly different, where the insomnia score in the treatment group was higher than the control group (19.94±5.51). Then the control group received sleep hygiene psychoeducation, and the treatment group was given the WHO Stress Management intervention in addition to sleep hygiene psychoeducation. After five intervention sessions, repeated measurements were made. There was a change in the score on the Mann-Whitney test between the control and treatment groups after the WHO Stress Management intervention (p=0, 00). In the Wilcoxon test, there was a decrease in the clinical score of insomnia in the treatment group (9.61±5.03, p=0.00 95% CI) and the control group (16.44±4.61, p=0.00 95% CI). The improvement in insomnia symptoms in the control group is in line with a study conducted

Table 3 Logistic Regression Factors Affecting Insomnia

Characteristics	Psycho education (n=18)		WHO Stress Management (n=18)		p-value	Beta Coefficient	OR	95% Confidence Interval	
	n	%	n	%				Lower	Upper
Age					0.16	-0.25	0.77	-0.32	0.09
Early adulthood (19–44)	13	72.2	14	77.8					
Middle adult (45–60)	5	27.8	4	22.2					
Gender					0.04	0.41	1.49	0.01	0.46
Woman	12	66.7	10	55.6					
Man	6	33.3	8	44.4					
Occupation					0.04	0.38	1.49	0.05	0.42
Not a health worker	7	38.9	10	55.5					
Health workers	11	61.1	8	44.4					
Co-morbidities					0.82	-0.03	0.96	-0.24	0.19
No co-morbidities	11	61.1	11	61.1					
With co-morbidities	7	38.9	7	38.9					
Diet and lifestyle									
Sedentary lifestyle					0.28	0.53	1.65	-0.35	1.14
Living a sedentary lifestyle	4	22.2	3	16.7					
Not living a sedentary lifestyle	14	77.8	15	83.3					
Smoke					0.05	0.69	2.01	-0.02	1.16
Regular smoking	0	0	2	11.1					
Do not smoke	18	100	16	88.9					
Caffeine					0.65	-0.16	0.90	-0.93	0.59
Regular caffeine consumption	2	11.1	2	11.1					
Do not consume caffeine	16	88.9	16	88.9					
Gadgets					0.33	0.58	1.82	-0.41	1.15
Using gadgets before bed	1	5.6	7	38.9					
Don't use gadgets before bed	17	94.4	11	61.1					
Routine drug history					0.82	-0.04	0.96	-0.24	0.19
Don't take regular medication	11	61.1	11	61.1					
Take regular medicine	7	38.9	7	38.9					
WHO stress Management Interventions					0.26	-0.20	0.81	-0.32	0.09

by Chung et al., which showed that sleep hygiene can improve sleep efficiency in insomnia.[7] While the improvement of insomnia symptoms in the post-intervention treatment group was also in line with the meta-analysis conducted by Edinger et al.,[8] which showed that relaxation and mindfulness therapy had effect sizes of 0.99 and 1.04 so that the application of the WHO Stress Management Module which is a combination of the two provides effective results.

In logistic regression, which was conducted to look at the factors that influence insomnia, it was found that male gender and occupation as a health worker were influential factors, with ORs of 1.49 and p=0.04 (95% CI). The results on occupational and gender factors differ from the research conducted by Pappa and colleagues in 2020,[9] which stated that health workers were one of the risk factors for insomnia. This may be because the health workers in this study were health workers who worked as the front line in handling Covid-19, while the health workers in this study were mostly psychiatric residents who did not face Covid-19 directly in the emergency room or ICU and isolation rooms. For the female gender, a previous study by Pappa[9] found that women are generally more prone to insomnia because they are more prone to depression and anxiety. The results of diet and lifestyle factors are also in line with several previous studies that discussed the influence of certain lifestyles, such as a sedentary lifestyle that causes individuals to rarely move and interfere with light stimulation and circadian rhythms, increased time to use gadgets at night regularly before going to bed, as well as caffeine and alcohol consumption. Excessive alcohol can be a risk factor for increasing insomnia.[3,4,9,10]

In the logistic regression of the WHO Stress Management intervention, the OR was 0.81 with p=0.26. This shows that the intervention does not have a causative effect in influencing the improvement of insomnia symptoms clinically. This is possible due to the limitations of this study, including that there are confounding factors that are not taken into account, such as psychiatric disorders that can affect sleep quality such as anxiety disorders and depression which are commonly found during the pandemic.[11] In addition, the delivery of the WHO Stress Management module online can limit the engagement of participants, as shown by research conducted by Lally et al.,[13] which showed that performance in online classes is strongly influenced by participant engagement in class. Online to ensure that the material presented can be understood well and the homework can be done correctly so that the mastery and implementation of WHO Stress Management become more difficult to monitor because the delivery uses the online method. The mastery of the material from the WHO Stress Management intervention that was given was not carried out by post-test to test how deep the participants' understanding and mastery of the material was. Other factors that become limitations in this study are not being blinded at the time of sample selection and giving the intervention and the risk of the window effect.

This study is the first to examine the use of the Stress Management module issued by the WHO to be applied to symptoms of insomnia caused by post-covid syndrome. Research with offline methods is needed to see the effectiveness of WHO Stress Management interventions on other clinical symptoms of stress caused by post covid syndrome.

In conclusion, online WHO stress management can improve the average Insomnia Severity Index score in the population experiencing post-covid syndrome, but it is not clinically significant. This result is due to the online approach that can intervene in participants' focus and engagement. Further research is needed offline using the WHO Stress Management module to see the effectiveness of its use on other clinical symptoms of post-covid syndrome.

References

1. Kamal M, Abo Omirah M, Hussein A, Saeed H. Assessment and characterisation of post-COVID-19 manifestations. Int J Clin Pract. 2021;75(3):1–2.
2. El Sayed S, Shokry D, Gomaa SM. Post-COVID-19 fatigue and anhedonia: A cross-sectional study and their correlation to post-recovery period. Neuropsychopharmacol Reports. 2021;41(1):50–5.
3. Lin L yu, Wang J, Ou-yang X yong, Miao Q, Chen R, Liang F xia, et al. The immediate impact of the 2019 novel coronavirus (COVID-19) outbreak on subjective sleep status. Sleep Med. 2021;77:348-34.
4. Kokou-Kpoloua CK, Megalakakia O, Laimoua D, Kousourib M. Insomnia during COVID-19 pandemic and lockdown: Prevalence, severity, and associated risk factors in French population. Psychiatry Res. 2020;290:113128.
5. Tizenberg BN, Brenner LA, Lowry CA,

Okusaga OO, Benavides DR, Hoisington AJ, et al. Biological and psychological factors determining neuropsychiatric outcomes in COVID-19. Curr Psychiatry Rep. 2021;23(10):1–25.
6. Wang LA, Kloet AD de, Smeltzer MD, Cahill KM, Hiller H, Bruce EB, et al. Coupling corticotropin-releasing-hormone and angiotensin converting enzyme 2 dampens stress responsiveness in male mice. Physiol Behav. 2019;176(3):139–48.
7. Ortelli P, Ferrazzoli D, Sebastianelli L, Engl M, Romanello R, Nardone R, et al. Neuropsychological and neurophysiological correlates of fatigue in post-acute patients with neurological manifestations of covid-19: insights into a challenging symptom. J Neurol Sci. 2021;420:117271.
8. Anderson G, Reiter RJ. Melatonin: Roles in influenza, Covid-19, and other viral infections. Rev Med Virol. 2020;30(3):1–10.
9. Kempuraj D, Selvakumar GP, Ahmed ME, Raikwar SP, Thangavel R, Khan A, et al. COVID-19, mast cells, cytokine storm, psychological stress, and neuroinflammation. Neuroscientist. 2020;26(5–6):402–14.
10. Shimba A, Ikuta K. Glucocorticoids regulate circadian rhythm of innate and adaptive immunity. Front Immunol. 2020;11:2143.
11. Edinger JD, Arnedt T, Bertisch SM, Carney CE, Harrington JJ, Lichstein KL, et al. Behavioral and psychological treatments for chronic insomnia disorder in adults: an American Academy of Sleep Medicine systematic review, meta-analysis and GRADE assessment. J Clin Sleep Med. 2021;17(2):263-98.
12. aiswal S, Muggleton NG, Juan CH, Liang WK. Indices of association between anxiety and mindfulness: a guide for future mindfulness studies. Personal Neurosci. 2019;2:e9.
13. Needham BL, Mezuk B, Bareis N, Lin J, Blackburn EH, Epel ES. Depression, anxiety and telomere length in young adults: Evidence from the National Health and Nutrition Examination Survey. Mol Psychiatry. 2015;20(4):520–8.
14. Bektas A, Schurman SH, Franceschi C, Ferrucci L. A public health perspective of aging: Do hyper-inflammatory syndromes such as COVID-19, SARS, ARDS, cytokine storm syndrome, and post-ICU syndrome accelerate short- And long-term inflammaging?. Immun Ageing. 2020;17(1):1–10.
15. Liu K, Chen Y, Wu D, Lin R, Wang Z, Pan L. Effects of progressive muscle relaxation on anxiety and sleep quality in patients with COVID-19. Complement Ther Clin Pract. 2020;39:101132.
16. Chung KF, Lee CT, Yeung WF, Chan MS, Chung EWY, Lin WL. Sleep hygiene education as a treatment of insomnia: A systematic review and meta-analysis. Fam Pract. 2018;35(4):365–75.
17. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsi E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: A systematic review and meta-analysis. [published correction appears in Brain Behav Immun. 2021 Feb;92:247]. Brain Behav Immun. 2020;88:901-907
18. Clark I, Landolt HP. Coffee, caffeine, and sleep: a systematic review of epidemiological studies and randomized controlled trials. Sleep Med Rev. 2017;31:70–8.
19. Koob GF, Colrain IM. Alcohol use disorder and sleep disturbances: a feed-forward allostatic framework. Neuropsychopharmacology. 2020;45(1):141–65.
20. Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P, et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. The Lancet Psychiatry. 2020;7(7):611–27.
21. Lally RM, Kupzyk K, Mills A, Gallo S, Meneses K. Effects of social constraints and web-based psychoeducation on cancer-related psychological adjustment early-after breast cancer diagnosis. J Psychosoc Oncol. 2019;37(6):677–98.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2763
Majalah Kedokteran Bandung. 2023;55(2):106–110

Majalah Kedokteran Bandung (MKB)

Received: March 27, 2022
Accepted: December 28, 2022
Available online: June 30, 2023

Predictors of Urethral Stricture After Transurethral Resection of the Prostate Procedure

Daniel Saputra, Ahmad Agil, Akhmad Mustafa
Department of Urology, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

Transurethral resection of the prostate (TURP) is the most frequently used urology surgical method to manage benign prostate hyperplasia (BPH). Despite the relatively efficacious treatment, urethral stricture (US) may form after TURP. The prevalence of the urethral strictures (US) following TURP ranges from 2.2% to 9.8%. The study aimed to identify the predictors of urethral strictures in patients receiving TURP. This study was a retrospective cohort study on patients underwent TURP in Dr. Hasan Sadikin General Hospital Bandung, Indonesia, between 2015 and 2019. Data were obtained from medical records and urology registry of a minimum 12-month follow-up period. Data on patient demographics, estimated volume of the prostate, total resected prostate, and operating time were extracted. Multiple logistic regression was utilized to determine the odds ratio difference between groups. A total of 451 TURP cases were performed between 2015 and 2019, with 22 (4.87%) cases of post TURP US identified. The mean estimated prostate weight was 45.6 g and resected prostate weight was 20.4 g, with a 0.37 g/min resection rate. Prostate weight, operating time, and duration of catheterization after surgery were not significantly different statistically. Slower resection rate and smaller resected volume are the statistically significant predictors of increased occurrence of urethral stricture ($p<0.05$). Lower resection rate is also a predictor for urethral stricture after TURP procedure.

Keywords: Predictor factor, TURP, urethral stricture

Introduction

Over the years, transurethral resection of the prostate (TURP) has been the most common urology surgical technique to manage benign prostate hyperplasia (BPH). Monopolar transurethral resection of the prostate (M-TURP) is the gold standard for lower urinary tract symptoms due to benign prostatic obstruction (BPO). The TURP procedure is considered adequate, clinically and economically.[1] Tao H. et al. found that the earliest known case series in 1962 consisted of 2,015 patients receiving TURP and had a mortality rate of 2.5% compared to recent case series in the 2000s having 0.25% mortality rate with a similar number of patients.[2] Monopolar (M-TURP) and bipolar TURP (B-TURP) methods for the resections are available for use, with the latter was thought to have better safety profile compared to the former.[3] In the last decade, using normal saline irrigation, B-TURP emerged as an alternative to M-TURP with less perioperative morbidity.[1] TURP is still the primary choice due to its evident efficacy and persistent outcome in the long term.[3]

Despite being safe and effective in managing urological conditions, patients undergoing the TURP procedure were at risk of intraoperative and postoperativecomplications. Bleeding, transurethral resection syndrome, infection of the urinary tract/sepsis are the common early complications. Later, complications such as urethral stricture (US), bladder neck contracture, urinary incontinence, and retrograde ejaculation may occur. Urethral stricture reports incidence after M-TURP widely varies.[1,4-7] Rassweiler et al.[8] discovered that more extensive randomized clinical trial studies reported as many as 2.2–9.8% urethral stricture cases and 0.3–9.2% bladder neck contracture cases. A systematic review and meta-analysis by Tang and colleagues found 36 and 38 incidences of urethral strictures reported after M-TURP and B-TURP for BPH, respectively. These data resulted from eleven RCT studies or subgroups and eleven RCT researches or subgroups for the B-TURP group (948 subjects). Between M-TURP and B-TURP, no significant difference was shown from the pooled analysis in urethral stricture and contracture of bladder neck incidence.[9]

Corresponding Author:
Daniel Saputra
Department of Urology, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: danielsaputra.dr@gmail.com

The definitive cause of TURP related urethral stricture remains controversial. Some etiology factors include infection, mechanical trauma, extended catheter use, local anesthetic use, and electrical injury.[10] The meatus and fossa navicularis, penoscrotal junction, mid-bulbar region, and below urethral sphincter are the most prevalent location of urethral strictures related to TURP.[15] Surprisingly, the membranous urethra stricture is common after TURis.[11] Meatal stenosis in TURP usually occurs due to the unmatching size of the instrument and the urethral meatus diameter. Bulbar strictures arise because the lubricant lacks isolation, causing the monopolar current to leak.[5,6] The incidence of urethral strictures following TURP represents severe and late complications. It is also reported as the main etiology of iatrogenic urethral strictures.[1] Another factor is the lower resection speed. It has been correlated with undesirable procedure processes, including bleeding, poor vision, prolonged surgical duration, more fluid leakage/absorption, and impaired urethral mucosal; those are potential reasons for urethral stricture.[5,6] Investigation should be taken to identify various risk factors of scar formation in the urethra to decrease the occurrence of urethral stricture elicited by TURP.[12]

This study aims to identify predictor factors in developing urethral strictures in patients receiving TURP in Dr. Hasan Sadikin General Hospital Bandung, a tertiary hospital.

Methods

This study was a retrospective cohort of urethral stricture occurrence in a patient who had undergone TURP procedure in Dr. Hasan Sadikin General Hospital Bandung between 2015 and 2019. The procedure was performed in a Monopolar system with a 26 Fr continuous-flow resectoscope. The coagulation power was 80 W and the cutting power was 120 W. The irrigation fluid used was sterile water. All patients were given a lubricant gel (Cathejell) before the TURP procedure. Data of patients who had received TURP procedure in Hasan Sadikin General Hospital with 12 months follow-up period minimum were collected from the urology unit's (department) database and medical records. Incomplete or insufficient data and coexisting urethral stricture prior to surgery were excluded from the study. All of cases of urethral stricture cases were included. The presence of stricture was defined as narrowed segment of the anterior urethra (membranous or bulbosa) with urethral lumen less than 16 Fr which confirmed during urethrography or endoscopic examination.[12] Patient demographics, the estimation volume of the prostate, the total of resected prostate, operating time, resection rate, and catheter use duration following surgery were collected. The amount of prostate resected divided by the operating time was used to calculate the resection rate for each patient. The ethical clearance for this study was not required. All patients attended to Dr. Hasan Sadikin General Hospital Bandung already sign medical record disclosure consent form research purposes.

This study used IBM SPSS Statistics version 23.0 for data analysis. Data with abnormal distribution were reported as medians (interquartile range). Data comparison were carried out with the Mann-Whitney U-test. Multiple logistic regressions were carried out to determine factors associated with urethral stricture after TURP, comparing the groups. For analytical purposes, we randomly selected non-US patient as comparison with ratio 1:1 using simple random sampling method. A statistically significant result was considered in a p-value of <0.05.

Results

There were 451 patients who received TURP between 2015 and 2019 were documented. All data was taken from medical record and our Urology department patient records. No patient was considered loss to follow-up. The patient's characteristics are presented in Table 1. The mean age of these patients was 65 years old, ranging from 52 to 77 years. These subjects estimated prostate volume was varied, ranging from 30–69 g (mean volume was 46.86 g). As many as 378 patients had a history of urinary retention before undergoing surgery. The range of operation time was from 30 to 60 minutes (mean 55 minutes).

This cohort study revealed 22 cases of US after TURP procedure, therefore the rate of US incidence was 4.87%. The complication of these cases was acknowledged as Clavien- Dindo grade III. All of them had symptoms requiring management with surgery or endoscopic. Most of these patients (15 of 22, 68.2%) developed the US within 12 months after receiving the TURP, while 7 patients (31.8%) developed the US in the second year of the follow-up period. The most common sites of TURP-related urethral strictures

Table 1 Patient's Characteristics

Variables	Mean (Range)	n (%)
Age	65 (52–77)	
Estimated prostate volume (g)	46.86 (30–69)	
Operative time (min)	55 (30–60)	
Time of Development US		
12 months		15
2nd Year		7
Urethral Stricture		
Yes		22 (4.88%)
No		429 (95.12%)
Urethral stricture location		
Pendulous		2
Pendulobulbous		4
Bulbous		14
Membranous		2

Table 2 Univariate Analysis of Factors Affecting Urethral Stricture Occurrence

Variables	Stricture n = 22	No Stricture n = 22	P-value
Duration of catheterization after surgery (day)	3.00 (2.5–4.00)	2.5 (2.00–4.00)	0.523
Estimated prostate weight (g)	45.6 (35–65)	54.3 (42–69)	0.521
Operative time (min)	55 (30–60)	58 (40–60)	0.485
Prostate weight resected (g)	20.4 (12.2–30.5)	36.2 (28–40)	0.001*
Resection rate (g/min)	0.37 (0.40–0.50)	0.53 (0.46–0.56)	0.001*

*p<0.05, statistically significant difference; Data present as mean (range)

were bulbous urethra (63.6%), followed by pendulobulbous urethra, pendulous urethra, and membranous urethra (18.1%, 9.09% and 9.09%, respectively).

On univariate analysis (Table 2), resected prostate weight and resection rate were found to be significantly related to the occurrence of urethral stricture (p<0.05). In contrast, the occurrence of urethral stricture was not greatly influenced by the duration of catheterization after surgery, operative time and estimated prostate weight (p>0.05).

Multiple logistic regression analyses result shown in table 3 and discovered that resected volume weight and resection rate were associated significantly with US occurrence (p-value 0.022 and 0.042, respectively). Other variables such as duration of catheterization after surgery and estimated prostate weight volume were not significantly associated with US development (p-values>0.05).

Discussion

Transurethral resection of the prostate (TURP) is

Table 3 Analysis of the Variables Associated with the Occurrence of Urethral Stricture Using Multiple Logistic Regression

Variables	OR (95%CI)	P-Value
Duration of catheterization after surgery	0.953 (0.9–1)	0.128
Prostate volume	0.075 (0–1)	0.075
Resected volume	1.19 (1.02–1.38)	0.022*
Resection rate	0.420 (0–50.57)	0.042*

OR, odds ratio; CI, confidence interval. *p<0.05, statistically significant difference

the most well-known operative management for symptoms of lower urinary tract with a suspicion of benign prostatic obstruction (LUTS/BPO) since the 1970s.[4] TURP is still the primary treatment due to its evident efficacy and persistent outcomes in long term. TURP procedure also become a gold standard for symptomatic BPO in prostates between 30 and 80 cc.[5,7] Despite the advantages, TURP still has several complications. Complications that occur in patients who undergo TURP are urethral stricture, dysuria, and bladder neck contractures.[1,3] In our study, it was found that most patients who were included in the case group (15 of 22 patients) developed the US within 12 months after receiving the TURP. These findings are in accordance with a study conducted by Tan et al. in 2017, which reported 13 cases of US in patients receiving TURP, 61.5% of which developed within 12 months postoperation.[3]

Data from our study showed that the mean estimated prostate weight was 45,6 g. The mean operative time is 55 minutes. The mean prostate weight resection is 20.4 g, with a 0.37 gr/min resection rate. This result was similar to Tao Huang et al. research, which stated that lower resection speed was correlated with a higher risk of stricture in the urethra. Taking too much time in a resection of a small prostate gland, relatively, would be cautious.[2] Tan et al. revealed that the occurrence of urethral stricture was associated with slow resection rate, significantly. They found that the majority of strictures were at the bulbar urethra.[3] This phenomenon was thought to be caused by the TURP mechanism, which put a concentration of electrical Stream Energy to contact with the bulbar. Slow resection rate will prolong the exposure of a tremendous amount of electrical energy at that part of the urethra, resulting in a more considerable risk of thermal damage and, lastly, urethral stricture.[2,5,6] In this study, the results were in agreement with this research. It was documented that a slower resection rate was statistically significant in increasing the incidence of urethral stricture occurrence.

Aside from operation duration, another well-known risk factor for urethral stricture was the size of the resectoscopes. Previous studies revealed that an inappropriate relationship between the urethral meatus diameter and instrument diameter would cause meatus mucosa damage mechanically and then establish a stricture.[10] In China, several studies reported that many resectoscopes ordered from the West area are not adequately fit the Chinese population patients causing numbers of severe urethral mucosa damage.[1] Mamoulakis *et al*. detected the urethral mucosa was evolved at the end of the TURP Compression from the resectoscope causing the proximal bulbous urethra injury and penile urethra with several narrow rings held responsibility in stricture formation.[13] Other study from Gunes *et al*. compared the rate of urethral strictures after TURP with different resectoscope sizes discovered that a greater bulbar stricture incidence occurred in patients who underwent TURP with a 26F resectoscope than a 24F size (11.4% *vs.* 2.9%, p=0.018). Thus, the noncontinuous resectoscope shaft resulted in higher incidence of meatal stricture associated with the shaft's reciprocation in the axial.[7] According to those postulates, urethral stricture may occur due to inappropriate instrument diameters that would damage the urethral mucosa because of mechanical stress. This resulted in inflammatory and ischemic conditions.[1] In our study, we used a 26 Fr sheath continuous-flow resectoscope and the majority of strictures were located in the bulbar/bulbous urethra.

Electric current leakage can stimulate a stenosis formation. Conventional or B-TURP procedure can cause a formation of a high current urethral density that induced an electrothermal injury in the related urethral mucosa. This incidence occurs due to a short circuit formation between the metal or other parts integrated into the sheath (which is metal) and the active electrode.[1,14] Broken cutting loops, damaged insulation of the sheath, or trapped carbonized resection materials on the loop may induce a current conduction disturbance, causing contact of the resection loop and sheath directly.[14] Other than electrical power, electrothermal injury is also influenced by the lubricant gel quality and its conductivity. Lubricant with a conductivity lower than the mucosa may induce current leaks from the sheath's surface into nearby urethra with the relatively thin or totally displaced lubricant applied.[1,14,15]

It is worth noting that the study conducted by Wang et al.[1] has stated that despite the potential of life-long consequences, patients are mostly unaware of the risk from TURP-related urethral strictures. The meticulous indications of BPH surgery are the obvious and best complications preventive measures. The 20th-century technology depends on the resection or ablation of prostatic tissue using various laser or electric current energy. This usually requires an access sheath with large instrument, which

might lead to urethral trauma and subsequent stricture formation. Recently, more advanced and less invasive device technology innovations are emerged in clinical use purposely to gain smaller sheaths access and cut the procedure time. Hopefully, further functional complications prevent and the rate of urethral stricture decrease.[16] A relatively short follow-up duration limits this study. Another limitation that we recognize was multiple operators who performed the TURP surgeries. Authors also did not possess the exact data of time to stricture since several patient that further analyzed was not possible.

As conclusion, lower resection rate was the predictor factor associated with urethral stricture occurrence in the post-operative period of the TURP procedure. To reduce the stricture incidence, authors suggest reducing the time of the prostate resection, especially in a small-sized prostate.

References

1. Wang JW, Man LB. Transurethral resection of the prostate stricture management. Asian J Androl. 2020;22(2):140–4.
2. Tao H, Jiang YY, Jun Q, Ding X, Jian DL, Jie D, et al. Analysis of risk factors leading to postoperative urethral stricture and bladder neck contracture following transurethral resection of prostate. Int Braz J Urol. 2016;42(2):302–11.
3. Tan GH, Shah SA, Ali NM, Goh EH, Singam P, Ho CCK, et al. Urethral strictures after bipolar transurethral resection of prostate may be linked to slow resection rate. Investig Clin Urol. 2017;58(3):186–91.
4. Kumar BN, Srivastava A, Sinha T. Urethral stricture after bipolar transurethral resection of prostate - truth vs hype: A randomized controlled trial. Indian J Urol. 2019;35(1):41–7.
5. Chughtai B, Simma-Chiang V, Kaplan SA. Evaluation and management of post-transurethral resection of the prostate lower urinary tract symptoms. Curr Urol Rep. 2014;15(9):1–5.
6. Mayer EK, Kroeze SG, Chopra S, Bottle A, Patel A. Examining the 'gold standard': a comparative critical analysis of three consecutive decades of monopolar transurethral resection of the prostate (TURP) outcomes. BJU Int. 2012;110(11):1595–601.
7. Günes M, Keles MO, Kaya C, Koca O, Sertkaya Z, Akyüz M, et al. Does resectoscope size play a role in formation of urethral stricture following transurethral prostate resection?. International Braz J Urol. 2015;41:744–9.
8. Rassweiler J, Teber D, Kuntz R, Hofmann R. Complications of transurethral resectionof the prostate(TURP) – incidence, management, and prevention. Eur Urol 2006;50:969–79.
9. Tang Y, Li J, Pu C, Bai Y, Yuan H, Wei Q, et al. Bipolar transurethral resection versus monopolar transurethral resection for benign prostatic hypertrophy: a systematic review and meta-analysis. J Endourol. 2014;28(9):1107–14.
10. Tian Y, Wazir R, Yue X, Wang KJ, Li H. Prevention of stricture recurrence following urethral endoscopic management: what do we have?. J Endourol. 2014;28(5):502–8.
11. Komura K, Inamoto T, Takai T, Uchimoto T, Saito K, et al. Incidence of urethral stricture after bipolar transurethral resection of the prostate using TURis: results from a randomized trial. BJU Int. 2015;115:644–52.
12. Adi K, Alhajeri F, Satyagraha P. World changing scenario of urethral stricture management. In: Martins FE, Kulkarni SB, Köhler TS, editors. Textbook of male genitourethral reconstruction. Cham: Springer; 2020. p. 33–43.
13. Mamoulakis C, Skolarikos A, Schulze M, Scoffone CM, Rassweiler JJ, Alivizatos G, et al. Results from an international multicentre double-blind randomized controlled trial on the perioperative efficacy and safety of bipolar vs monopolar transurethral resection of the prostate. BJU Int. 2012;109(2):240–8.
14. Mamoulakis C, Schulze M, Skolarikos A, Alivizatos G, Scarpa RM, Rassweiler JJ, et al. Midterm results from an international multicentre randomised controlled trial comparing bipolar with monopolar transurethral resection of the prostate. Eur Urol. 2013;63(4):667–76.
15. Alhajeri F, Alwaal A, Soebadi MA. Etiology, epidemiology, demographic differences in urethral strictures: a worldwide perspective. In: Martins FE, Kulkarni SB, Köhler TS, editors. Textbook of male genitourethral reconstruction. Cham: Springer; 2020. p. 25–32.
16. Pham H, Sharma P. Emerging, newly-approved treatments for lower urinary tract symptoms secondary to benign prostatic hypertrophy. Can J Urol. 2018;25:9228–37.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2169
Majalah Kedokteran Bandung. 2023;55(2):65–69

Majalah Kedokteran Bandung (MKB)

Received: October 15, 2020
Accepted: March 10, 2023
Available online: June 30, 2023

Relationship between Age, Exercise Habits, Cigarette Smoke Duration Exposure, and Lung Vital Capacity in Passive Smokers

Saskia Oktaviani Puteri,[1] Ratnawati Ratnawati,[2] Nura Eky Vikawati[3]
[1]Faculty of Medicine, Sultan Agung Islamic University, Indonesia
[2]Department of Public Health, Faculty of Medicine, Sultan Agung Islamic University, Indonesia
[3]Department of Physiology, Faculty of Medicine, Sultan Agung Islamic University, Indonesia

Abstract

Lung vital capacity of is different for each individual depending on personal characteristics, such as age, body mass index (BMI), and exercise habits or environmental factors, such as exposure to cigarette smoke. This study aimed to determine the relationship between personal characteristics, duration of exposure to cigarette smoke, and lung vital capacity in passive smokers in one of the areas in Indonesia. This study was a cross-sectional observational analytic study conducted during the period of August to September 2019. Data were collected through interviews and measurements using the Contec SP10BT spirometer. Participants were sampled consecutively with only women who did not smoke but had at least one family member who actively smoked and was over 20 years old participated in this study. Women were selected as the population due to the fact that the proportion of passive smokers among women is higher than men, while the age group of above 20 years old was selected with a consideration of the average age for the optimum lung capacity. The relationship between personal characteristics and a history of exposure to cigarette smoke with lung vital capacity was analyzed using Pearson and Spearman correlation test. There is a significant relationship between age, exercise habits, duration of exposure to cigarette smoke, and vital lung capacity (p=0.000; p=0.018; p=0.000) among the participants of the study. However, further research is still needed to be able to generalize this finding into a broader community.

Keywords: Lung vital capacity, passive smokers, personal characteristics

Introduction

Lung vital capacity is the maximum volume of air that can be released after maximum inspiration in one breath.[1] The large volume and capacity of lung function can be one indication of pulmonary abnormalities or disorders, both obstructive and restrictive.[2] Spirometry is the basic lung function test with different measurements. One of these is vital capacity, the highest level of air volume a device can exhale or inspire during a forced vital capacity (FVC) or a slow vital capacity (VC) maneuver. Some studies have found that FVC is smaller than VC in asthma and COPD patients due to airflow limitation, small airway collapse, and gas trapping.[3] Lung vital capacity can be affected by various things, including exposure to cigarette smoke and personal characteristics. Some things that must be considered in assessing the lung's vital capacity include age, exercise habits, and nutritional status.[4]

As a person ages, the lungs' vital capacity will decrease.[5,6] Instead, the lungs' vital capacity will increase with higher exercise frequency.[7] Exercise can increase blood flow through the lung, causing oxygen to diffuse into the pulmonary capillaries at a greater volume. This is proved by the vital lung capacity of an athlete greater than those who have never exercised.[8] In obesity, compliance of the chest wall and abdomen decreases, resulting in an increased work of breathing, and a decrease in residual volume and vital lung capacity.[9,10]

The reduced lung function can also be affected by exposure to cigarette smoke. Previous studies stated a positive relationship between passive smokers and respiratory symptoms with lung vital capacity.[11] Irritation of the airways by cigarette smoke and other toxic substances would cause an inflammatory reaction, resulting in deposits of neutrophils and macrophages

Corresponding Author:
Ratnawati
Department of Public Health, Faculty of Medicine, Sultan Agung Islamic University, Indonesia
Email: ratnawati@unissula.ac.id

in these areas.[12] Passive smokers were more common in women than men.[13]

The Indonesian government has tried to reduce the number of passive smokers by issuing Law No. 36 of 2009 concerning Health in Article 115, about No Smoking Area (KTR). However, there are still no studies about the relationship between personal characteristics and exposure to cigarette smoke with lung vital capacity in the same region. Hence, this study aimed to know the relationship between personal characteristics and duration of exposure to cigarette smoke with lung vital capacity in passive smokers in RW 3 Penggaron Lor Semarang.

Methods

This was an observational study conducted in October 2019 at RW 3 Penggaron Lor, Semarang. Penggaron Lor was chosen because it is a fostered village of the Faculty of Medicine of Sultan Agung Islamic University, and RW 3 was chosen based on the location of previous research. The total population in there is about 3,200 people. The subject of this study were all residents of RW 3 Penggaron Lor Semarang who have at least one family member of an active smoker and live in one house. All samples were over 20 years old and had no complaints of pulmonary disease, such as shortness of breath and coughing. The exclusion criteria included having complaints of pulmonary diseases, such as shortness of breath and cough, a history of drug use, primarily asthma reliever or controller drugs, having an anatomical disorder, and the subject refused to participate. The minimum sample size is calculated using the formula below:

$$N = \left[\frac{Z\alpha + Z\beta}{0{,}5\mathrm{In}\left[\frac{1+r}{1-r}\right]}\right]^2 + 3 = \left[\frac{1{,}96 + 0{,}842}{0{,}5\mathrm{In}\left[\frac{1+0{,}39}{1-0{,}39}\right]}\right]^2 + 3 = 50$$

If the degree of relationship in previous research is the correlation coefficient= 0,39 (Fakhrullah,2016) α:0.05 (p=0.05), Zα: 1.96; (research power 80%) Zβ: 0.842, then the minimum sample size is 50 respondents. If there is a drop-out of 10%, the minimum sample size with drop-out correction is:

$$n=10\%\times N=10\%\times 50=5$$

Based on these calculations, the sample size in this study was 55 respondents. Sampling was done by consecutive sampling, by visiting people's homes, where all subjects who met the inclusion criteria were included in the study until the required number of subjects was met.

The age range of this study was 22–65 years old, with the median age being 43 years old. The BMI variable was measured using the formula:

The results are expressed in units of kg/m^2 based on Riskesdas 2013, were grouped as follows:[14]

$$\textbf{BMI} = \frac{\text{Weight } (kg)}{\text{Height } (m)^2}$$

In this study, the BMI was grouped into three groups, thin, normal, and obese, where the criteria for overweight and obesity on *Riskesdas* 2013 were included in one criterion. The weight was measured using a body scale, and a microtome measured the height. However, only participants who forgot their weight and height were measured due to inadequate space. The exercise habit variable was measured through direct interviews, including the one-week exercise frequency. The results are expressed in units of times/week, divided into three groups, 0 times per week, one time per week, and two times per week.

Length of exposure to cigarette smoke is the length of time the respondent is exposed to cigarette smoke in years. Variables were measured through direct interviews. The results are expressed in years. We did not use the Brinkman index because we focused on passive smokers, not active smokers. The lung vital capacity assessed in this study is the forced vital capacity (FVC), the maximum air that can be exhaled when blowing out as fast as possible.

FVC measurement is done by inhaling as deeply as possible, then exhaling quickly and forcefully. The results are expressed in percentage units and classified according to the degree of severity. The classification of FVC's degree can see in the following table:

Data on age, exercise habits, and duration of cigarette smoke exposure were obtained through direct interviews, while IMT data were obtained

Table 1 Body Mass Index Classification

	BMI*
Thin	≤18.5
Normal	18.5–25
Overweight	25.00–<27.0
Obesity	≥27

*Body mass index

Table 2 Degrees of Forced Vital Lung Capacity[15]

Degree	%pred FVC*
Normal	≥80
Mild	70–79
Moderate	60–69
Moderate-Severe	50–59
Severe	35–49
Very severe	<35

*forced vital capacity

through weight measurements using portable scales and height using microtoice. FVC values were obtained through measurements using the Contec SP10BT spirometer.

Data normality was tested with the Kolmogorov-Smirnov test, and data correlation was tested with the Pearson dan Spearman test. The normality test results on the independent variables obtained showed that age and duration of exposure to cigarette smoke were normally distributed, so both variables used the Mean value. While the variables of nutritional status and exercise habits are not normally distributed, so the two variables use the median value. This study has received Ethical Clearance from the Faculty of Medicine, Sultan Agung Islamic University with No. 716/X/2019/Bioethics Commission.

Results

Personal characteristics are independent variables of this study that consist of age, BMI, exercise habits, and the duration of cigarette smoke exposure. The personal characteristics and the duration of cigarette smoke exposure can see in Table 3.

From Table 3, it can be seen that the majority of the subjects of this study have a normal BMI, average age of 42.76 years, 78% of the people never exercised in one week, and were exposed to cigarette smoke for 19.38 years.

Lung vital capacity is a dependent variable in this study that is measured by forced vital capacity (FVC) value. All the subjects of this study had a mean FVC of 71.63% and were categorized as a mild degree. The normality test results based on Kolmogorov-Smirnov show that the FVC variable is normally distributed. The analysis of the relationship between personal characteristics and the duration of cigarette smoke exposure with lung vital capacity can see in Table 4.

Discussion

This study showed a relationship between age and lung vital capacity. A previous study conducted by Bintang et al. in 2017 found a negative correlation between age and vital

Table 3 Description of Personal Characteristics of All Subjects in RW 3 Penggaron Lor Semarang

Personal Characteristics Variable	n	Mean	SD*	Median	Min	Max
Age (years)	55	42.76	10.89			
The duration of cigarette smoke exposure (years)	55	19.38	11.00			
BMI** ($kg/m^{2)}$	55			23.20	17.3	35.4
Underweight	3 (5%)					
Normal weight	28 (51%)					
Overweight	24 (44%)					
Exercise Habits (times per week)	55			0	0	2
0 times per week	43 (78%)					
1 times per week	9 (17%)					
2 times per week	3 (5%)					

*standard deviation;** body mass index

Table 4 Relationship between Personal Characteristics and the Duration of Cigarette Smoke Exposure with Lung Vital Capacity in RW 3 Penggaron Lor Semarang

Variable	n	Mean	Median	Min	Max	p	r
Age	55	42.76	49	22	65	0.000	-0.496**
BMI	55	24.27	23.20	17.3	35.4	0.501	-0.093*
Exercise Habits	55	0.57	0	0	2	0.018	0.317*
The duration of cigarette smoke exposure	55	19.38	20	2	40	0.000	-0.563**

* Spearman's Rank Correlation Test; ** Pearson Product Moment Correlation Test

capacity, which means that vital capacity will decrease as age increases.[16] Another study in 2012 conducted on 30 parking attendants in Jalan Pandanaran, Semarang, stated there was a relationship between age and lung function of parking attendants who were generally healthy. This is in line with the theory, which states that as a person ages, organ function will decrease. [17] This study's results of BMI values showed no relationship between BMI and lung vital capacity. A cross-sectional study involving 2,617 students in all universities in China in 2015 found that body mass index was not related to vital capacity.[18] A meta-analysis conducted in 2018 stated that obesity will reduce a person's vital lung capacity.[19] The meta-analysis used the term "obesity," did not involve subjects with underweight nutritional status, and included children as subjects. This differs from this study because this study uses the term "fat," which involves subjects with underweight nutritional status and does not involve children.

This study showed a relationship between exercise habits and lung vital capacity. An experimental study involving female students in Iran in 2016 found that sports-type performance training would increase vital capacity.[20] Other studies in India have also shown similar results, that regular exercise will affect the vital capacity of the lungs, especially FVC value. This is due to the higher level of someone's physical activity and the higher level of fitness, too, especially in cardio-respiratory.[21]

The results of this study indicate a relationship between exposure to cigarette smoke with lung vital capacity. A cross-sectional study 2017 at the Faculty of Medicine James, University of Anguilla, Caribbean, found that active smokers, passive smokers, and ex-smokers will have lower lung vital capacity than people who are rarely or have never been exposed to cigarette smoke. This is because chronic exposure to cigarette smoke can lead to respiratory diseases such as chronic obstructive pulmonary disease (COPD), emphysema, chronic bronchitis, and lung cancer, thereby reducing the lung vital capacity.[22] This study showed that besides age, BMI, and exercise habits, exposure to cigarette smoke also affects lung capacity, which could impact the quality of life.

The limitation of this study is that other factors not analyzed in this study can affect lung vital capacity, such as work and exposure to dust or vehicle fumes. Data on the length of exposure to cigarette smoke studied in this study only smoked cigarettes from the home environment. At the same time, respondents could be exposed to cigarette smoke on the streets and at work. In conclusion, a significant relationship exists between age, exercise habits, and duration of exposure to cigarette smoke with lung vital capacity. Besides that, this study shows that smoking impacts smokers and the people around them.

References

1. Sherwood L. Introduction to human physiology. 8th ed. Jakarta: EGC; 2013.
2. Guyton AC, Hall JE. Guyton and hall textbook of medical physiology. 12th ed. Jakarta: Elsevier; 2011.
3. Yuan W, He X, Xu QF, Wang HY, Casaburi R. Increased difference between slow and forced vital capacity is associated with reduced exercise tolerance in copd patients. BMC Pulm Med. 2014;7:14–6.
4. Sirait M. Hubungan karakteristik pekerja dengan faal paru di Kilang Padi Kecamatan Porsea tahun 2010. Medan: Fakultas Kesehatan Masyarakat Universitas Sumatra Utara; 2010.
5. Badri H, Syahputra R., Pudia M. Relationship

of age on lung vital capacity. Advances in Health Sciences Research. 2021;35:226–7.
6. Talaminos BA, Marquez ME, Roa RLM., Ortega RF. Factors affecting lung function: a review of the literature. Archivos de Bronconeumología (English Edition); 2018;54(6):327–32.
7. Awad KM, Magzoub A, Elbedri O, Musa O. Effect of physical training on lung function and respiratory muscles strength in policewomen trainees. Int J Research Medical Sci. 2017;5(6):2516.
8. Kholifah N. Perbandingan tingkat kapasitats vital paru siswa yang mengikuti ekstrakurikuler bulutangkis dan ekstrakurikuer bola voli di SMA Negeri 1 Sedayu. Yogyakarta: Ilmu Keolahragaan Universitas Negeri Yogyakarta; 2016.
9. Dixon AE, Peters U. The effect of obesity on lung function. Expert Rev Respir Med. 2018;12(9):755–67.
10. Mafort TT, Rufino R, Costa CH, Lopes AJ. Obesity: systemic and pulmonary complications, biochemical abnormalities, and impairment of lung function. Multidiscip Respir Med. 2016;11:28.
11. Nurjanah. Efek paparan second hand smoke terhadap fungsi paru karyawan Universitas Dian Nuswantoro Semarang. J Visikes. 2013;12(1):16–26.
12. Haris A, Ikhsan M, Rogayah R. Asap rokok sebagai bahan pencemar dalam ruangan. Jakarta: Departemen Pulmonologi dan Ilmu Kedokteran Respirasi, Fakultas Kedokteran Universitas Indonesia-RS Persahabatan. Available from: https://repository.uinjkt.ac.id/dspace/handle/123456789/44041.
13. Salimzadeh H, Najafipour H, Mirzaiepour F, Navadeh S, Shadkam-Farrokhi M, Mirzazadeh A. Prevalence of active and passive smoking among adult population: findings of a population-based survey in Kerman (KERCADRS). Addict Health; 2016;8(1):16–24.
14. Riset Kesehatan Dasar. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan Republik Indonesia; 2013.
15. Haynes, J. Basic spirometry testing and interpretation for the primary care provider. Canadian J Respiratory Ther. 2018;54:92–8.
16. Bintang S, Yuli K. Hubungan usia, lama paparan debu, penggunaan APD, dan kebiasaan merokok dengan gangguan fungsi paru tenaga kerja mebel di Kecamatan Kalijambe Sragen. J Kesehatan; 2017;10(2):37–46.
17. Putra DP, Rahmatullah P, Novitasari A. Hubungan usia, lama kerja, dan kebiasaan merokok dengan fungsi paru pada juru parkir di Jalan Pandanaran Semarang. J Kedok Muhammadiyah. 2012;1(3):7–12.
18. Sun X, Chen X. The relationship between obesity and forced vital capacity among university students. Nutr Hospitalaria. 2015;31(5):2202–4.
19. Forno E, Han YY, Mullen J, Celedón JC. Overweight, obesity, and lung function in children and adults-a meta-analysis. J Allergy Clin Immunol Pract. 2018;6(2):570–81.e10.
20. Azhang M, Khayyam BK, Fazel AA, Bagheri L, Emamdoost S, Otadi K. Comparison effect of conventional corrective exercise and physioball exercise on improvement of hyperkyphosis and vital capacity in female students. J Modern Rehabilitation. 2016;9(6):168–76.
21. Sabaananth S, Gopinath V, Thevanthy T. Effect of different dance training on vital capacity among post-pubescent girls. Int J Multidisciplinary Studies; 2014;1(1):85–9.
22. Hammoudi D, Sanyaolu A, Adofo D, Antoine I. The effect of cigarette smoking on lung capacity in active, previous, and passive student smokers. GMJ Med. 2017;1(1):3–8.

RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n2.2875
Majalah Kedokteran Bandung. 2023;55(2):111–116

Majalah Kedokteran Bandung (MKB)

Received: July 5, 2022
Accepted: January 24, 2023
Available online: June 30, 2023

Therapeutic Outcome of High Flow Nasal Cannula (HFNC) for Severe COVID-19 Patients in Isolation Intensive Care Unit

Baginda Aflah, Erwin Pradian, Nurita Dian Kestriani
Departement of Anesthesiology and Intensive Care, Faculty of Medicine Universitas Padjadjaran
Dr. Hasan Sadikin General Hospital Bandung, Indonesia

Abstract

This retrospective descriptive study aimed to understand the outcomes of HFNC therapy in severe COVID-19 patients admitted to isolation ICU during the period of January to June 2021 in Dr. Hasan Sadikin General Hospital Bandung, Indonesia. A total of 134 patients with severe COVID-19 were admitted to the isolation ICU and received HFNC. Among them, 44 patients (32.8%, N:134) were successfully weaned from HFNC and 90 patients (67.2%, N:134) failing HFNC with 10 patients (7.5%, n:134) died on HFNC use, 72 patients (53.9%, n:134) died on ventilator use, 4 patients (2.9%, n:134) moved rooms under HFNC use, and 4 patients (2.9%, n:134) moved to non-ICU isolation with ventilator use as the outcome. Patients' median age was 60 years, most were male (52.3 %, n:134), median BMI was 25.4 kg/m^2, with hypertension and diabetes mellitus as the main comorbidities. There was an improvement in the SpO_2 on the first day after the use of HFNC. The ROX index had a median value of 3.6 on the first day, with the lowest ROX index of 3.2 and the highest of 4.4 during the treatment time. There was an improvement in the P/F Ratio in successful patients with a median initial P/F Ratio of 86.7 to 200.1 at the end of treatment. Overall, HFNC improves the hypoxemic conditions in early admission but does not correlate with general patient outcomes.

Keywords: High flow nasal cannula, intensive care, ROX index, severe COVID-19, therapeutic outcome

Introduction

COVID-19 has a degree of clinical manifestation ranging from asymptomatic, mild, moderate, to severe, with the assessment of the most severe case being pneumonia with acute respiratory distress syndrome (ARDS). COVID-19 degree is defined by clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one respiratory rate > 30 breaths/minute, severe respiratory distress, or SpO2 <93% on room air. Severe COVID-19 requires monitoring in the ICU because it can develop into ARDS and acute respiratory failure.[1,2,3]

In severe COVID-19, there is more severe lung damage, so supplementation modalities are needed that can provide fractional and higher oxygen flow. High flow nasal cannula (HFNC) is considered to be able to reduce *dead space*, protect the airway mucosa with *humidifier* technology that can maintain a temperature of 31–37 °C, provide oxygen supply with a constant flow and concentration with flow up to 60% and oxygen concentration 100%, HFNC also has a *positive end expiratory pressure* (PEEP) which can increase the residual functional capacity. HFNC, compared with traditional oxygen therapy has better comfort and therapeutic effect. [5,6]

Dr. Hasan Sadikin Hospital Bandung (RSHS), as a referral center for COVID-19 in West Java, is still relatively new to the use of HFNC, so RSHS, as the local setting of this research, is expected to provide a representative picture. The purpose of this study was to obtain an overview of the outcomes of HFNC therapy based on clinical SpO_2 before and after the use of HFNC, ROX index values on the first day, the highest and lowest during treatment, laboratory parameters of PaO_2, PCO_2 and P/F Ratio and demographic data.

Methods

This study is retrospective-descriptive research. The subjects of this study were medical records of COVID-19 patients who were admitted to ICU

Corresponding Author:
Baginda Aflah
Departement of Anesthesiology and Intensive Care, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia
Email: bagindaaflah@gmail.com

Dr. Hasan Sadikin General Hospital Bandung from January to June 2021. The inclusion criteria of this study were patients aged 18 years above diagnosed with severe COVID-19 and the exclusion criteria were in completed medical record data. The research obtained ethical approval from the Research Ethics Committee of Dr. Hasan Sadikin General Hospital, with ethical approval number LB.02.01/X.6.5/16/2022. The data collected including outcome; demographics; value of SpO_2 before HFNC use and on the first day of HFNC use; first-day ROX value, lowest ROX and highest ROX value during treatment; laboratory oxygenation profile (PaO_2, PCO_2, P/F Ratio) after the first day of HFNC use; and at the end before patient discharge from ICU. During the study period, 191 severe COVID-19 patients were admitted to the intensive care unit for COVID-19 isolation at RSUP dr. Hasan Sadikin Bandung. It was found that 57 patients were excluded from this study because they did not receive HFNC therapy. A total of 134 patients are eligible for this study. All these data will be displayed in a distributive table categorized into succeed group (weaning from HFNC) and failed group (went to ventilator use or die). The data is also displayed with statistical explanations accompanied by a discussion on the theoretical basis that has been found previously. Data processing and analysis were done using Statistical Product Service Solution (SPSS) version 25.0 for Windows.

Results

Of the 134 patients with severe COVID-19 who admitted the ICU isolation and received HFNC therapy, 44 patients (32.8%, n:134) succeeded in weaning HFNC, and 90 patients (67.2%, n:134) failed HFNC with an outcome of 10 patients (7.5%, n:134) died on HFNC use, 72 patients (53.9%, n:134) died on ventilator use, 4 patients

Table 1 Demographics of Severe COVID-19 Patients Receiving Therapy HFNC

Variable	Succeed (n=44)	Failed (n=90)
Age (years)		
Median	58.0	60.5
Range (Min.–Max.)	(21.0 –86.0)	(26.0–85.0)
Mode	58.0	65.0
BMI (kg/m^2)		
median	25.4	26.8
Range (Min.–Max.)	(17.6–40.0)	(19,5–34.0)
Gender, n (%)		
Male	21 (47.7%)	49 (54.4%)
Female	23 (52.3%)	41 (45.6%)
Comorbidities n (%)		
Hypertension	22 (50.0%)	38 (42.2%)
Diabetes mellitus	12 (27.2%)	21 (23.3%)
Lung disease	1 (2.2 %)	3 (3.3 %)
Heart disease	7 (15.9 %)	7 (7.7 %)
Kidney failure	4 (9.0 %)	7 (7.7 %)
Immunodeficiency	0 (0%)	3 (3.3 %)
Malignancy	0 (0%)	2 (2,2 %)
Pregnant	1 (2.2 %)	5 (5.5 %)
Hematological disease	3 (6.8%)	0 (0%)
Asthma	2 (4.5%)	3 (3.3 %)

Notes**:** n=frequency, %=percentage

Table 2 Clinical Overview of Severe COVID-19 Patients Receiving HFNC Therapy

Variable	Succeed (n=44)	Failed (n=90)
SpO_2 before HFNC		
Median	86.0	83.0
Range (Min - Max)	(68.0–90.0)	(50.0–90.0)
SpO_2 with HFNC		
Median	97.0	94.5
Range (Min - Max)	(80.0–99.0)	(59.0–100.0)
ROX index day 1		
Median	3.9	3.5
Range (Min.–Max.)	(2.1–8.1)	(1.8–4.1)
Lowest ROX index		
Median	3.6	3.1
Range (Min.–Max.)	(1.8–8.1)	(1.5–4.8)
Highest ROX index		
Median	7.8	4.0
Range (Min.–Max.)	(3.7–22.0)	(2.0–13.0)

Notes: n=frequency, %=percentage

(2.9%, n:134) changed rooms in use HFNC, and 4 patients (2.9%, n:134) moved to a non-isolated ICU with the use of a ventilator. Demographic data found that the median age was 60 years, most were male (52.3%, n:134), and the median body mass index was 25.4 kg/m^2, with the main comorbidities of hypertension and diabetes mellitus.

The clinical outcome was found an improvement in SpO_2 which was assessed on the first day after using HFNC with an increase in SpO_2 values after using HFNC with a median value of 97% in the successful group and 94.5% in the failed HFNC group. The clinical outcome of the ROX index had a median value of 3.6 on the first day. The lowest ROX index was 3.2, and the highest was 4.4 during treatment. The successful group had a higher ROX value than the HFNC failed group.

Improvements in the P/F ratio were found in patients who had successfully weaned HFNC with a median initial P/F ratio of 86.7 mmHg, an increase of 200.1 mmHg at the end of treatment, while in patients who died on a ventilator, hypercapnia was found with an increase in the median PCO_2 54.2.

Discussion

Table 3 Laboratory Overview of Severe COVID-19 Patients Receiving HFNC Therapy

Variable	Succeed (n=44)	Failed (n=90)
PCO_2 early		
Median	29.4	29.8
Range (Min.–Max.)	(13.9–44.1)	(12.7–107.8)
Initial PaO_2		
Median	80.1	68.2
Range (Min.–Max.)	(37.0–209.8)	(40.1–277.1)
Initial P/F Ratio		
Median	86.7	76.6
Range (Min.–Max.)	(37.0–288.0)	(17.8–278.1)
Final PCO_2		
Median	36.0	43.5
Range (Min.–Max.)	(22.3–49.6)	(22.8–148.1)
Final PaO_2		
Median	104.6	73.2
Range (Min.–Max.)	(38.3–189.0)	(35.4–182.1)
Final P/F Ratio		
Median	200.1	77.4
Range (Min.–Max.)	(38.3–189.0)	(28.8–263.2)

Notes: n=frequency, %=percentage

In this study, found a few discrepancies between gender populations. There were 70 male patients with 47.7% (n=44) in the success group, and 64 female patients with 52.3% (n=44) failed group. This finding also showed similar results from other meta-analysis studies involving 3,111,714 patients globally that there was no difference in the proportion between men and women who were infected with COVID-19. However, men had a 3-fold likelihood of needing intensive care in the intensive care unit (ICU). Previous literature has shed light on how gender differs in innate and adaptive immunity. Women have the advantage of having a higher number of CD4+ T cells, more active CD8+ T cell cytotoxic activity, and more production of immunoglobulins by B

cells than men. Females produce more interferon type-1 (IFN). This antiviral cytokine plays a role in the initial response to infection and works potently as a toll-like receptor for detecting viral RNA more than males. In addition, socio-cultural biases and behavioral attitudes contribute to the severity of COVID-19. To illustrate, men are likelier to smoke, rarely wash their hands, and are likelier to leave the house and be in crowds.[7,8]

In the successful group, the median age was 58, and the failed group was 60.5 years old. There were also few discrepancies in age population, but the older population was an important factor influencing the outcome in some literature. In a published quantitative meta-analysis of 2851 patients from 25 HFNC studies, the mean age was 61 years (±13 years). Similar data were also obtained in a quantitative and qualitative meta-analysis of 64,676 patients from 20 studies regarding gender, age, and comorbidities for COVID-19 mortality, which found that aged 50 years had 15.4 times the risk of death compared to patients aged <50 years. Old age correlates with decreased immunity, decreased organ function, more comorbidities, and higher ACE2 gene expression in old age. Some comorbidities, such as diabetes mellitus, are also associated with decreased immunity, inhibiting the body's ability to form antibodies and fight infection. Polypharmacy in old age also influences the body's immunity, where there is downregulation due to drug side effects. ACE inhibitors (ACEIs) and angiotensin II type-I receptor blockers (ARBs) are also associated with the upregulation of ACE-2 receptors in hypertensive and diabetic patients, which can exacerbate SARS-CoV-2 infection.[9]

Body mass index (BMI) in the overall patient population of this study showed that the majority were in the overweight category in both groups, with the successful group with a median value of 25.4 and slightly higher in the failed group with a median value of 26.8. A meta-analysis of 34,390 patients from 12 studies on body mass index and outcome in COVID-19 found that a higher body mass index was associated with a higher risk of mortality and disease severity. This is based on statistical tests based on a dose-response meta-analysis (DRMA) which found a 5% increase in the risk of a worse outcome for every 5 kg/mg2 increase in body mass index. A high body mass index has a higher risk of comorbid diseases, including hypertension, dyslipidemia, type 2 diabetes mellitus, cardiovascular disease, and cerebrovascular disease. A chronic increase in mild systemic inflammation makes it susceptible to infection. Obesity also affects innate and adaptive immunity, as happens in aging as well as in the presence of low physical activity, obese patients also make immunity and the body's defenses against the body weaken. A high body mass index is also associated with poor pulmonary function, including respiratory reserve volume, functional capacity, and low lung compliance, adding to the severity of COVID-19. In addition, intestinal dysbiosis and vitamin D deficiency in obese patients also contribute to a worse COVID-19 outcome.[9,10]

Comorbidity has a significant role in COVID-19 outcomes. In this study, as seen among patients in the failed group, hypertension (42.2%, n=90) was higher than that of the succeeded group. The second most dominant comorbidity among the failed group is diabetes Mellitus (23.3%, n=90). Each comorbidity has its own pathophysiology and mechanistic relationship to SARS-CoV-2 infection and outcome. For example, patients with hypertension and heart failure have a worse outcome risk associated with increased ACE2 expression in mRNA. Diabetes mellitus and chronic kidney disease (CKD) has been associated with inflammation and dysregulation of immune function, which may explain the increased risk of worse outcome and mortality.[9,11]

In this study, we found improvement in SpO2 and ROX index at day one admission after using HFNC. In the success group, the median SpO2 before HFNC was 86%, which improved to 97%. The increase was also seen in the failed group as before HFNC. The SpO2 median was 83% which was improved to 94.5% post-HFNC use. But unfortunately, in the long term, this finding does not correlate with general patient outcome, which was shown by the 60.5% of the patients were in the failed group.

The ROX index was used to monitor the need for mechanical ventilation requirements and a predictor of HFNC success. In this study, patients in the failed HFNC group had a median ROX index of 3.5, which was lower than the HFNC success group, with a median ROX index of 3.7. In the failed HFNC group, the highest ROX index was found on the first day with a median of 4.0, where this value is still at risk of requiring intubation as much as 80% at an index ROX value of <4.8.[12]

The higher number of failed groups in this study could be explained based on some literature studies about the pathophysiology of the COVID-19 pneumonia type. There were two types of pneumonia phenotype in COVID-19: L and H. In the L phenotype, hypoxemia occurs due to dysregulation of perfusion and loss of

the hypoxia pulmonary vasoconstriction (HPV) mechanism. In this type L phenotype, the lung compliance function is still good, the ventilation-perfusion ratio is low, the increase in lung weight is not too increased, the lung tissue with air is still a lot and the need for recruiting alveoli is small. In the L phenotype, average lung volumes were still obtained so the patient could not be short of breath and respond well to non-invasive oxygen therapy. The type H phenotype occurs in approximately 20–30% of patients with symptoms similar to severe ARDS with low lung compliance. There was a significant increase in lung weight, namely an increase in right-to-left shunt perfusion and a higher need for lung recruitment. In this type, H phenotype, lung volume is markedly reduced due to the resulting interstitial and alveolar edema. Type H phenotype requires more significant positive end-expiratory pressure (PEEP) for alveolar recruitment and reduced dead space. The use of HFNC in this type of H phenotype may be less successful because the PEEP provided by HFNC is minimal. Patients may present with one end of the spectrum or in a phase transition between the two spectrums. The transition from the L to the H phenotype is usually determined by disease progression. It is associated with patient-self-inflicted lung injury (P-SILI) caused by increased work of breathing. This pathophysiological course of type L and type H phenotypes may explain why patients with high ROX index scores eventually require a ventilator or experience mortality, as reflected in this study, which could be seen in the failed group.[13,14]

The median value of the initial laboratory output of blood gas analysis (BGA) was obtained with a mean PCO 29.4 mmHg, PaO2 80.1 mmHg, and P/F Ratio of 86.7 in patients with successful HFNC with improvement to PCO_2 36 mmHg, PaO2 104.6 mmHg and P/F Ratio 200.1 at the end of treatment. In the group of patients with HFNC failure, the PCO_2 29.8 mmHg, PaO_2 68.1 mmHg and P/F Ratio 76.6 with an increase in PCO_2 43.5 mmHg, a decrease in PaO2 to 73.2 mmHg and a P/F Ratio of 77.4 were not significantly different from the initial value. From these values, it was shown that at the beginning of the treatment period, all patients were included in severe ARDS according to the Berlin classification.[15]

A similar study in Bangladesh on 240 COVID-19 patients who were admitted to the ICU with HFNC therapy showed significant improvement in oxygenation status. Similar results showed an improvement in the SpO_2 value before HFNC from 83.71% (±6.61) to 93.11% (±2.53) after HFNC administration, a decrease in PaO2 value 56.99 mmHg (±13.89) in patients who died, which is lower than the patients who succeeded PaO_2 70.18 mmHg (±17.13). Also, the P/F Ratio value in the case of dead patients (64.07±17.47) was lower than the survivors (105.18±35.09).[14]

In the successful HFNC group, at the beginning of treatment, there was a hypocapnic condition with an initial PCO_2 of 29.5 than normal with a value of 36 at the end of treatment. In the HFNC failure group, at the end of HFNC administration, there was an increase in PCO_2 to 43.5. This condition of hypercapnia was more prevalent in patients who died on a ventilator with a median PCO_2 of 54.2. Several works of literature explain the possible causes of hypercapnia that occur in COVID-19 patients on mechanical ventilation, among others, related to pulmonary microvascular occlusion and thromboinflammation. Giving a low tidal volume increases the occurrence of dead space. Other investigations suggest a primary injury to the vascular endothelium that activates the clotting cascade in the presence of in situ thrombosis and ischemia of the arteries.[15,16]

This study is a pilot retrospective descriptive study to present the outcome of HFNC use in severe COVID-19 patients, finding that HFNC was improving the hypoxemic conditions in early admission but did not correlate with general patient outcomes. The limitation of this study is that other factors contribute directly to the patient outcome but were not concluded, such as medication given and other evaluations of the patient's systemic condition. This study was conducted during the peak case of the COVID-19 pandemic when all the resources were still limited. This research is meant to be the baseline for further research and evaluate the use of HFNC and managing severe COVID-19 patients in the ICU.

References

1. UNAIDS. Update on the HIV epidemic, 2011. Global HIV/AIDS response–progress report 2011. Geneva: World Health Organization; 2011
2. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Intens Care Med. 2020;46(5):854–87.

3. Liu J, Liu S. The management of coronavirus disease 2019 (COVID-19). J Med Virol. 2020;92(9):1484–90.
4. Lodeserto FJ, Lettich TM, Rezaie SR. High-flow nasal cannula: mechanisms of action and adult and pediatric indications. Cureus. 2018;10(11):1–12.
5. Helviz Y, Einav S. A Systematic Review of the High-flow nasal cannula for adult patients. Crit Care. 2018;22(1):1–9.
6. Patel M, Gangemi A, Marron R, Chowdhury J, Yousef I, Zheng M, et al. Retrospective analysis of high flow nasal therapy in COVID-19-related moderate-to-severe hypoxaemic respiratory failure. BMJ Open Respir Res. 2020;7(1):1–11.
7. Peckham H, de Gruijter NM, Raine C, Radziszewska A, Ciurtin C, Wedderburn LR, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. Nat Commun. 2020;11(1):1–10.
8. Mahayasa DPS, Putri AK. Maskulinitas dan pandemi Covid-19: studi kasus Pakistan. J Hub Int. 2020;13(2):163–84.
9. Biswas M, Rahaman S, Biswas TK, Haque Z, Ibrahim B. Association of sex, age, and comorbidities with mortality in COVID-19 patients: a systematic review and meta-analysis. Intervirology. 2021;64(1):36–47.
10. Pranata R, Lim MA, Yonas E, Vania R, Lukito AA, Siswanto BB, et al. Body mass index and outcome in patients with COVID-19: a dose–response meta-analysis. Diabetes Metab. 2021;47(2):1–10.
11. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: A systematic review and meta-analysis. Intensive Care Med. 2020;46(5):854–87.
12. Roca O, Caralt B, Messika J, Samper M, Sztrymf B, Hernández G, et al. An index combining respiratory rate and oxygenation to predict outcome of nasal high-flow therapy. Am J Respir Crit Care Med. 2019;199(11):1368–76.
13. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, et al. COVID-19 pneumonia: different respiratory treatments for different phenotypes?. Intensive Care Med. 2020;46(6):1099–102.
14. Das M, Das S, Bhuiyan AT, Nath SD, Biswas R, Gupta JD, et al. Factors affecting the outcome of the usage of high-flow nasal cannula on severe or critically ill COVID-19 patients: a multicentric study from a developing nation. Asian J Med Biol Res. 2021;7(3):260–72.
15. Gattinoni L, Marini JJ, Camporota L. The respiratory drive: an overlooked tile of COVID-19 pathophysiology. Am J Respir Crit Care Med. 2020;208(8):1079–80.
16. Oppenheimer BW, Bakker J, Goldring RM, Teter K, Green DL, Berger KI. Increased dead space ventilation and refractory hypercapnia in patients with coronavirus disease 2019: a potential marker of thrombosis in the pulmonary vasculature. Crit Care Explor. 2020;2(9):1–5.