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Original Research

LOW AND MODERATE INTENSITY EXERCISE DECREASED BODY FAT AND INCREASED FREE FATTY ACID IN OVERWEIGHT WOMEN

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ABSTRACT

Overweight is fat imbalances can affect the health. It is one of the problems in many countries, especially Indonesia recorded an increase case in 2007 (8.8%) to 2013 (13.5%). Overweight categorized by 25-30 kg/m^2 body mass index in units. Figures overweight can be pressed to provide some treatments, such as aerobics sports activities. This study aimed to determine the effect of Continuous Low Intensity Training (LICT) and Moderate Intensity Continuous Training (MICT) to decrease body fat content (FM) and increase in free fatty acids (FFA) in overweight women. 18 female subjects completed the study 4X/week exercise LICT or MICT for 5 weeks. LICT and MICT performed for 30 minutes with an additional 5 minutes warm-up and 5 minutes of cooling down with LICT intensity of 60%-70% and MICT 70%-80% of maximum HR where both types of exercise using ergo cycle while measurement FM and FFA were measured before and after practice. LICT occurred decreasing in body fat and increasing in free fatty acids that significant pre and post LICT ($p < 0.05$). MICT occurred decreasing in body fat and increasing in free fatty acids that significant pre and post MICT ($p<0.05$). In the second comparison group (LICT and MICT) were decline against body fat and an increased in free fatty acids between workouts Low-Intensity Continuous Training (LICT) greater tendency than Moderate-Intensity Continuous Training (MICT) with delta FM ($p = 0.120$) and delta FFA ($p=0.131$) in which the value is >0.05. LICT and MICT was increase while body fat was decreased. The results of a comparison test occur list downward trend in body fat while in free fatty acids was increased.

Keywords: *Exercise; intensity; fat; overweight; human & health*

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Highlights:

1. Aerobic exercise was reduced body fat and increased free fatty acids.
2. LICT and MICT decreased body fat and increased free fatty acids.

INTRODUCTION

According to the World Health Organization in 2018, being overweight is a fat imbalance that can affect health. Overweight categorized by 25-30 kg/m^2 body mass index in units. Based on the data of the Ministry of Health in 2007 and 2013, Indonesia is a country that experienced an increase in the overweight number from 2007 (8.8%) to 2013 (13.5%).

Overweight can be pressed to provide some treatments, such as doing aerobics sports activities. Aerobic exercises can be lipolysis fat or triglyceride affecting the use of body fat and free fatty acids into energy by the oxygen (Guyton & Hall 2014). This happens, because the triglyceride hydrolysis that will produce fatty acids and sent to an active network will be oxidized (Wolinsky & Driskell 2008).

Triglycerides cannot immediately turn into free fatty acids and glycerol, but requires some hormones and enzymes. It can be stimulated due to sports activities with a long duration (Jeppesen & Kiens 2012). Cortisol, catecholamine, growth hormone and other hormone that assist fat metabolism will increase triglycerides lipolysis by stimulating the β androgenic hormone receptor with the addition of sensitive lipase (HSL), but for the growth of hormone work during night when the body break (Wolinsky & Driskell 2008).

Fisher et al (2015) in their study which used ergo cycle to determine body composition, fat in the blood, insulin sensitivity, and cardiovascular fitness using High Intensity Interval Training (HIIT) and ContinuousModerate Intensity Training (MICT), stated that the increase results of both practices certainly decreased body fat. Another study examines fat metabolism. especially abdominal visceral fat and body composition in obese women with metabolic disorders exercise Low-Intensity Exercise Training (LIET) and High- Intensity Exercise Training (HIET), resulted that the data were the effective changes in body composition and exercise intensity of HIET (Irving et al. 2009).

In the study of Lazer et al (2011) on body composition and substrate of metabolism in men obese unknown physical activity with low intensity in week-3 of body mass and fat mass, decreased significantly in all groups of Low Intensity (LI) and High Intensity (HI), while the most significant decreased in LI compared to HI. In addition, Iwayama et al (2015) on lipid oxidation using the method of measurement before breakfast, indicated an increase in fat oxidation before breakfast.The bias in the study was that people were trying to do the exercises with their own choice (Iwayama et al. 2015).

Several studies mostly used interval training, but a study on body composition and free fatty acids were compared between Low Intensity Continuous Training (LICT) and Moderate Intensity Continuous Training (MICT) has never been done.

MATERIALS AND METHODS

This study manifold experimental pretest-posttest design. The subjects were 18 females with age range 19-32 years old. Exercises were divided into 2 groups, the LICT and MICT. LICT used an intensity of 60%-70% of maximum HR with a duration of 30 minutes exercise time as well as additional 5 minutes to warm up and 5 minutes for cooling, doing 4X/week for 5 weeks. The procedure was similar to the LICT and MICT, while the difference was the maximum HR intensity of 70%-80%. The exercises were using ergo cycles and monitored using a polar heart rate monitor. The variables were body fat and free fatty acids. Body fat was measured using Body impedance Analyzer (BIA), and free fatty acids were measured using ELISA Human Free Fatty Acid KIT.

RESULTS

The respondents were 18 females aged 19-31 with a dominating amount less than 22 years old as many as 11 females.

Table 1. The mean of age in each group

Age group	Frequency	Percent
19 – 22	11	61.1
23 – 24	5	27.8
25 – 26	1	5.6
32	1	5.6
Total	18	100.0

Table 2. The mean of BMI in each group

BMI group	Frequency	Percent
25 – 27	5	27.8
27.01 - 28.00	6	33.3
28.01 - 29.00	6	33.3
29.01 - 30.00	1	5.6
Total	18	100.0

The overweight group most in groups 2 and 3 on the percentage reached 33.33%. The data normality test results in this study. The study results showed significant with values >0.05 which value indicates data from this study normal distribution.

Table 3. Descriptive analysis

	n ·	Minimum	Maximum	The mean ± SD
Pre-Body fat	18	31.10	48.89	39.8483 ± 4.95831
Post Body fat	18	30.00	47.89	38.3361 ± 4.91210
Pre-Free Fatty Acids	18	710 057	897 133	831.23133 ± 52.525617
Post Free Fatty Acids	18	801 473	994 551	940.92439 ± 47.970949

Table 4. Results of normality test data by Shapi-Wilk

Group		Shapiro-Wilk sig.
Pre-Body fat	low Intensity	0.062
	moderate Intensity	.451
Post Body fat	low Intensity	0.134
	moderate Intensity	0.337
Pre-Free Fatty Acids	low Intensity	0.998
	moderate Intensity	0.076
Post Free Fatty Acids	low Intensity	0.174
	moderate Intensity	0.185

Table 4 shows the data normality test results in this study. The results of this study showed significant with values >0.05 which value indicates data from this study normal distribution.

Table 5. Results of data analysis on decreased body fat and increase in fatty acids using pair t-test

Variables	The mean ± SD	Sig.
Pretest Body Fat - posttest Body Fat	1.863 ± 1.193	0.002
Pretest Free Fatty Acid - posttest Free Fatty Acid	-126.102 ± 47.054	0.000

Table 5 shows the test results data on the effect of exercise on body fat reduction and an increase in free fatty acids from the second exercise shows that there is a difference between before and after exercise which analyzes test results indicate significant value <0.05 between body fat pre-post body fat (p = 0.002) and a free fatty acid pre-post free fatty acid (p = 0.000).

Table 7 Results of data analysis on decreased body fat and increase in fatty acids using pair t-test

Variables	The mean difference ± SD	Sig.
Pretest Body Fat- posttest Body Fat	1.863 ± 1.193	0.002
Pretest Free Fatty Acid - posttest Free Fatty Acid	-126.102 ± 47.054	0.000

The test results data on the exercise effect on body fat reduction and an increase in free fatty acids from the second exercise shows that there was a difference between before and after exercise which analyzes test results indicate significant value <0.05 between body fat pre-post body fat (p= 0.002) and a free fatty acid pre-post free fatty acid (p = 0.000).

Table 8. Results of comparative analysis using independent t-test

	Group	The mean difference ± SD	Sig.
Δ BT	low intensity	-1.863 ± 1.193	0.120
	moderate intensity	-1.161 ± 0.473	
Δ FFA	low intensity	123.102 ± 27.054	0.131
	moderate Intensity	93.283 ± 40.025	

Note: BT: Body Fat, FFA: Free Fatty Acid

The levels of body fat and free fatty acids pretest and posttest with low and moderate intensity exercises had resulted that posttest results of the two variables showed the influence of low and moderate intensity exercises with the interpretation of body fat significantly (p=0.000) and free fatty acids (p=0.010). the results of independent t-test to determine the effective exercise between low and moderate intensity exercises showed no significant difference between the results of body fat (p=0.120) and free fatty acids (p=0.131) with a significance value >0.05, although it was descriptive of both exercises which showed an increase in body fat and a decrease in free fatty acids. These results can be seen in Table 8.

DISCUSSION

In this study, the population was dominated by the age less than or equal to 22 years with a total of 11 people. The samples in this study were females with the highest rate of overweight category, namely in the IMT group 2 and group 3 consisting of 6 each group.

The normality data was normal distribution with a significance value >0.05 in pre-body fat with low intensity (p=0.062), pre-moderate intensity body fat (p=0.451), post-fat body with low intensity (p=0.134), post-body fat with moderate intensity (p = 0.337), pre-free fatty acids with low intensity (p=0.988), pre-free fatty acids with moderate intensity (p=0.076), post-acid low intensity body fat (p=0.174), and post-free fatty acids with moderate intensity (p=0.185).

The significant value in the data analysis between body fat pre-post in body fat (p=0.002) and a free fatty acid in pre-post free fatty acids (p=0.000) with the increasing value <0.05. These studies supported the theory by Wolinsky and Driskell (2018) which stated carbohydrates. Fat was the main provider of energy during endurance exercise. Fat was a major provider of energy during rest, activity, and low intensity exercise. It was due to the increased free fatty acids resulting from the exercise and stimulation hormone cortisol, catecholamines, and increased growth hormones and stimulated androgen receptor ϐ that increased resulting an increased lipolysis of triglycerides through the assistance of HSL (hormone sensitive lipase) (Wolinsky & Driskell 2008), This study supports the theory of Purdom et al (2018) which stated that when the exercise was less than 60%, it could stimulate endocrine to release epinephrine which increased lipolysis and epinephrine concentration that enabled to increase by 2-3 times when it broke, as well as stimulating HSL to produce more to lipolysis TG into FFA and glycerol (You et al. 2012).

Similarly, Lazer et al (2011) also revealed oxidation low intensity that supported the fat and advised for people with overweight or obese to be more feasible and acceptable. Other studies also proved similar results and found the effect of aerobic exercise on body fat, blood, and fitness in overweight and obesity (Powell 2011)

Analysis on body fat and free fatty acids pre-post exercise showed p values of 0.000 each, indicating an increase as the significance value was <0.05. It was in line with Ogasawara et al (2015) that moderate intensity exercise could cause lipolysis acceleration response in humans. During moderate intensity

exercise, the free fatty acids group was bound to carnitine that would bring out of the mitochondrial membrane in acyl-carnitine form. It occurred in low to moderate intensity exercise.

The previous result was in line with the findings of Wewege et al (2017) study which revealed body fat and waist circumference changes during the exercise MICT. In line with Horowitz and Klein (2000), aerobic exercise also increased fat oxidation during submaximal exercise resulting from the body's adaptive response mitochondria thickness that increased in skeletal muscle and increased fat oxidation capacity. Aerobic exercise with duration of 30 minutes could be increased to lipolysis fat (Hargreaves & Spriet 2020). It showed that in 30 minutes of exercise, there was an increase in lipolysis of fat which also balanced with the increased use of fatty acids (Horowitz & Klein 2000).

The changes in body fat and free fatty acids by using low and moderate intensity exercise of prior exercise up to the post-test. The results of this study showed no significant difference in ΔFM ($p = 0.120$), and ΔFFA ($p = 0131$) with the significance value was > 0.05. It was also in line with a study by Kong et al (2016) which compared HIIT workout and MICT with the result that changes in body composition, but the views from the higher used MICT of HIIT (Kong et al. 2016), Lazer et al (2011) compared the exercise LI and HI and resulted no significant difference in fat between the LI and H. In addition, Marra et al (2005) also found the same result that no significant difference in body fat of moderate intensity group (Marra et al. 2005), Descriptively, there was a difference between exercise and MICT with LICT greater value on LICT. Similar result was also relevant to the theory in the study of Wolinsky et al (2008) that to know decreased fat, the most prevalent in low intensity were caused by the increased lipolysis in adipose tissue triglycerides (Wolinsky & Driskell 2008).

Strength and limitation

The study has a clear objective to determine the effect of two types of exercise on overweight women's body fat content and free fatty acids. It does not include a control group, which could have helped to determine whether the observed changes were solely due to exercise or other factors such as diet or lifestyle changes.The study only includes female subjects, which limits the generalizability of the results to other populations.Also, It does not provide information on the intensity of physical activity or diet of the subjects outside of the exercise program.

CONCLUSION

Aerobic exercise can reduce the amount of signaling body fat and increase free fatty acids found in LICT and MICT which indicate a change after a work-out. In this study, there were some decreases in body fat and an increase in free fatty acids in LICT and MICT, but the results of a comparison test obtained a list of downward trends in body fat and an increase in free fatty acids. By suppressing the number of overweight figures with LICT exercise and MICT usage with ergo cycle, the application of exercise on LICT and MICT required a work-out to reschedule.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

All the authors contributed in design of the research, data analysis, and interpretation of the obtained results and collected the specimens and wrote the manuscript.

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Original Research

THE IMPLICATION OF MASTECTOMY FLAP FIXATION IN DECREASING THE INCIDENCE OF SEROMA ON BREAST CANCER PATIENT

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ABSTRACT

Seroma is the most common complication after mastectomy procedure. This study was conducted to analyse the treatment of flap fixation in seroma production after modified radical mastectomy in breast cancer patients. An interventional prospective clinical study in 35 female patients with breast cancer who underwent modified radical mastectomy with flap fixation in the period August-December 2018 and 35 patients without flap fixation through historical data of patient who underwent modified radical mastectomy in the period 2016-2017 at RSUD Dr. Soetomo Surabaya. Data of characteristic patients will be presented descriptively and analyzed statistically using the appropriate test. The result of the statistical test using Chi Square with a 95% significance level obtained a value of p = 0.000 (p <0.05), it was said statistically that there was a significant relationship between the treatment of flap fixation and the time of releasing drain in this study. The treatment of flap fixation will reduce drainage time by <10 days. The results of the odds ratio (OR) obtained OR = 16 and CI: 95% (4.094 – 62.528) which means that the treatment of flap fixation will reduce the releasing time of the drain 16 times more effectively than not performing flap fixation. Correlation between the treatment of flap fixation and the time of releasing drain was considered to be moderately positive (Contingency Coefficient Chi Square = 0.476). There was a significant reduction in the number of seroma assessed from the time of releasing drain faster in patients who underwent modified radical mastectomy with flap fixation.

Keywords: *Seroma; flap fixation; modified radical mastectomy; health risk*

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Highlights:

1. Modification of radical mastectomy with flap fixation treatment in seroma production in breast cancer patients was analyzed.
2. The number of seromas from the drain removal time in patients undergoing modified radical mastectomy with flap fixation was reduced.

INTRODUCTION

Breast cancer in Indonesia is a cancer with the highest percentage of new cases currently, at 30.5% of all types of cancer in Indonesian women (GLOBOCAN). Modified radical mastectomy is still one of the main therapies used to treat breast cancer in Indonesia (Purwanto et al. 2015, van Bastelaar et al. 2016, Sobri et al. 2017). Seroma is the most common complication after mastectomy. Seroma in patients after mastectomy will inhibit wound healing through infection (10%), wound dehiscence (1.3%), flap necrosis (2.6%), and the incidence of flap necrosis is as high as 17.8% (Bland 2011, Yildiz & Sulu 2013).

In the end, these complications cause discomfort to the patient and delays the administration of adjuvant therapy. Seroma formation is predicted when we minimize production by mechanical closure to reduce dead space by sewing flap mastectomy on the base (Bland 2011, Sakkary 2012, Brunicardi 2014). As a result of minimal seroma formation, it is expected that post mastectomy complications should also be significantly reduced. The purpose of this study was to analyze the relationship of flap fixation techniques in reducing seroma production after modified radical mastectomy in breast cancer patients and analyzed other factors associated with seroma formation after mastectomy.

MATERIALS AND METHODS

This study was an interventional prospective clinical study conducted at Dr. Soetomo General Academic Hospital, Surabaya, by involving women with breast cancer undergoing modified radical mastectomy with primary closure on the period between August-December 2018 taken from random sampling. Subcutaneous flap mastectomy suturing technique in the pectoralis major muscle and serratus anterior. Suturing is made between the subcutis part of the flap with the pectoralis muscle and serratus anterior muscle with an inside simple interrupted technique.

The material used in this study was 3.0 absorbable monofilament suture using 3 knots. The control group used medical record data taken from medical record data of women with breast cancer who underwent modified radical mastectomy with primary closure for 2017-2018 period. The drain was removed after the drain fluid production was ≤ 30 cc/24 hours. Patient data included age, BMI, tumor size, anatomical pathologic grading, staging, tumor location, chemotherapy cycle, length of operation, and drainage time were tabulated and analyzed using statistical tests.

RESULTS

This study involves 70 patients with modified radical mastectomy. Using 35 patients as a sample size in each group. It was found that the mean age of the subjects undergoing modified radical mastectomy with flap fixation was 50.74±9.36 and patients undergoing modified radical mastectomy without flap fixation were 48.57±8.78. The characteristics of the study subjects were based on Body Mass Index in patients with flap fixation 25.25±3.48 kg/m2 and those without flap fixation 25.98±4.90 kg/m2 (Table 1).

From tumor size, the tumor volume of patients undergoing modified radical mastectomy with flap fixation was 37.75±26.85 cm^3, while the tumor volume of patients undergoing modified radical mastectomy without flap fixation was 26.55±22.21 cm^3. Based on histopathological grading in the flap fixation group, grade III consisted of 25 patients (71.4%), while in the group without flap fixation with grade III was 22 patients (62.9%). Based on staging, the most flap and non-fixation fixation groups, 29 patients were in advanced stage (82.9%) with flap fixation and 23 (65.7%) without flap fixation. Most of tumor locations

Table 1. Subject's characteristics

Characteristics	Total Sample		p (<0,05)
	Flap Fixation (n=35)	Without Flap Fixation (n=35)	
Age (years)	50.74	48.57	0.320
	SD: 9.36	SD: 8,78	
Body Weight (kg)	58.49	58.91	0.634
	SD: 8.79	SD: 11,82	
Body Height (cm)	152.51	150.46	0.072
	SD: 3.58	SD: 5.61	
BMI (kg/m2)	25.25	25.98	0.618
	SD: 3.48	SD: 4.90	
Tumor Size (Volume: cm^3)	37.75	26.55	0.049
	SD: 26.85	SD: 22.21	
PA Grading PA	No Data: 1 (2.9%)	No Data: 3 (8,6%)	
	I: 3 (8.6%)	I: 5 (14.3%)	0.333
	II: 6 (17.1%)	II: 5 (14.3%)	
	III: 25 (7.4%)	III: 22 (62.9%)	
Staging	Early Stage: 6 (17.1%)	Early Stage: 12 (34.3%)	0.103
	Advance Stage: 29 (82.9%)	Advance Stage: 23 (65.7%)	
Tumor Location:	No Data: 0 (0%)	No Data: 4 (11.4%)	
1: Medial Superior Quadrant	1: 4 (11.4%)	1: 4 (11.4%)	
2: Lateral Superior Quadrant	2: 6 (17.1%)	2: 5 (14.3%)	0.310
3: Lateral Inferior Quadrant	3: 5 (14.3%)	3: 4 (11.4%)	
4: Medial Inferior Quadrant	4: 2 (5.7%)	4: 3 (8.6%)	
5: Central	5: 18 (51.4%)	5: 15 (42.9%)	
Chemotherapy Cycle (times)	3.00	3.23	0.891
	SD:2.95	SD: 3.1	
Operation Time (hours)	2.57	2.66	0.668
	SD: 0.66	SD: 0.73	

in the central quadrant in each group were 18 (51.4%) in the flap fixation group, and 15 (42.9%) in the without flap fixation group.

Based on the history of neoadjuvant chemotherapy administration, the flap fixation group neoadjuvant chemotherapy administration was 3±2.95 cycles and 3.23±3.1 cycles in group without flap fixation. The mean length of surgery in the flap fixation group was 2.57±0.66 hours and 2.66±0.73 in group without fixation.

In Table 1, p values have been found using Chi Square analysis to show the relationship between risk factors and flap fixation in patients undergoing modified radical mastectomy. It appeared that only the size of the tumor volume had a significant difference between the flap fixation group with without flap fixation group with p value = 0.049 (p <0.05).

Furthermore, the overall drainage time in patients undergoing modified radical mastectomy performed with flap fixation and without flap fixation group had resulted that the statistical test using Chi Square with a 95% significance level obtained p-value of = 0.000 (p < 0.05) that there was a statistically significant relationship between the treatment of flap fixation and drainage release time in this study. The treatment of flap fixation will reduce drainage time by < 10 days.

Table 2. Relationship between flap fixation intervention and drain removal time

Flap Fixation	Drain Removal Time (Day)				P (p<0,05)	OR
	≤ 10		> 10			
	f	%	F	%		
Yes	32	69.6	3	12.5		
No	14	30.4	21	87.5	0.000	16
Total	46	100	24	100		

(CI 95% = 4.094– 62.528) (Chi Square Contingence Coefficient: 0.476)

The odds ratio results were OR=16 and CI: 95% (4,094–62.528), and showed that the action of flap fixation could reduce the drain removal time 16 times more effectively than without flap fixation. Relationship strength (correlation) between the treatment of flap fixation and the discharge time was considered to be moderately positive (Contingency Coefficient Chi Square = 0.476).

Table 3 shows variables with significant correlation (p<0.05): BMI (p=0.019), tumor size (p=0.017) with the drain removal time apart from the flap fixation intervention (p=0.000). However, there was a strong correlation (correlation coefficient >0.5) between flap fixation intervention and drain removal time (Correlation Coefficient 0.665). BMI had a weak correlation (Correlation Coefficient 0.1-0.3) with drain removal time (Correlation Coefficient = 0.280), and Tumor Size had a weak correlation with drain removal time (Correlation Coefficient = 0.285).

Table 3. Multivariate correlation analysis: Spearman's rho test

Variable	Drain Removal Time (p<0,05)	Correlation Coefficient
Age	0.486	0.085
BMI (kg/m2)	0.019	0.280
Tumor Size (cm2)	0.017	0.285
Hemotherapy Cycle	0.639	0.057
Grading	0.050	0.236
Staging	0.289	0.129
Tumor Location	0.205	0.153
Operation Time	0.803	0.030
Flap Fixation	0.000	0.665

DISCUSSION

There are several factors that can influence the formation of post-mastectomy seroma including age, breast volume, tumor size, positive lymph node counts, intraoperative cautery, tissue damage, axillary dissection extension, tumor histopathology, length of operation, obesity, and hypertension history (Dahri et al. 2011, Zielinski et al. 2013, Faisal et al. 2016). In this study, several of these factors might influence seroma production (DeVita et al. 2014). However, some parameters in this study had no significant differences between without flap fixation group and the flap fixation group, except for tumor volume. In accordance with Table 1, only the size of tumor volume had a significant difference between the flap fixation group with without flap fixation group with p=0.049 (p<0.05).

The formation of seroma as a complication of a mastectomy surgery can cause some problems in patients. These include sensations of discomfort, wound healing delay, flap necrosis, wound dehiscence, extended length of hospital stay, increased risk of infection, more frequent visits to clinics, increased rates of surgical intervention, poor cosmetics, higher overall costs, and delay in adjuvant therapy administration (Dahri et al. 2011, Sakkary 2012).

Various efforts were made to reduce seroma production to minimize complications due to excessive seroma formation. Obliteration of dead space through fixation of skin flaps on the structure of the chest wall is an attempt to reduce seroma formation (Zielinski et al. 2013, DeVita et al. 2014, Faisal et al. 2016). Variety of flap fixation

techniques were developed and researched as an alternative method that can be supplemented post-surgical procedures that might leave extensive defects, such as post mastectomy. In this study, the reduction in seroma production was measured by calculating the drain removal time when seroma production was <30 cc seroma/day. Using faster drain removal, a smaller number of seroma production is expected (Yilmaz et al. 2011, Kurnia et al. 2016).

Dead space post modification of radical mastectomy has the potential to cause a build-up of serous fluid that occupies the cavity. This is the most common complication and occurs immediately after mastectomy. Although not life-threatening, this can cause morbidity in patients after mastectomy. Usually, seroma will be absorbed in a few weeks. If the seroma fluid is formed excessively, the skin will stretch that can cause discomfort on patients. In some patients, this build-up of fluid will present several problems, such as prolonging the treatment period, and it will increase the cost of treatment. In addition, it will also be aspirated repeatedly, so that the patient will feel uncomfortable (Mackay-Wiggan 2016, Ouldamer et al. 2016, Ridha 2017).

In seroma fluid analysis, several inflammatory mediators, such as TNFα and IL6 activate monocytes and macrophages. This shows that seroma formation results from an acute reaction to the inflammatory process during the first phase of the wound healing process. With the basis of the inflammatory process, the conditions that can inhibit or reduce the severity of the inflammatory process will be able to reduce the occurrence of seroma (Sampathraju & Rodrigues 2010, Yilmaz et al. 2011, Suyatno & Pasaribu 2014, Mackay-Wiggan 2016).

In this study, flap fixation intervention in patients after modified radical mastectomy was proven to have reduced seroma assessed from faster drain removal than the without flap fixation intervention group (p = 0,000) (Table 2). Flap fixing will reduce the drain removal time sixteen times more effectively than without flap fixation. Reduction of dead space due to flaps suturing on the muscle base has been effective in reducing seroma production.

In the reduction of seroma production through flap suturing, a study also found that a reduction in the amount of drained fluid in patients with flap fixation in the chest wall muscle compared to conventional flap closure (Cong et al. 2020). A decrease in the incidence of seroma production, reduction in drain removal time and the amount of drainage production were achieved by fixing skin flaps in the chest wall muscles to obliterate dead space (Sakkary 2012). It was also found that there was a difference between seroma formation in mastectomy patients and skin flap fixation (van Bastelaar et al. 2016, Ridha et al. 2017). In addition, there was a difference between seroma formation in mastectomy patients and skin flap fixation with p=0.002 (p<0.05) (Sakkary 2012, van Bastelaar et al. 2016).

Another study also found fewer seroma in patients who had modified radical mastectomy with flap fixation compared with patients without flap fixation with p=0.003 (p<0.05) (Skandalakis & Skandalakis 2014, Ridha 2017). However, they used different technique made by interrupted suture flap fixation to the wound base using 3.0 unabsorbable polyfilament suture with vertical mattress technique, i.e., the needle was passed from curtis to the subcutaneous and took the muscle underneath. Then, the needle was returned from subcutis to the cutis and tied the knot from outside, so that the skin flap was fixed with the chest wall (Zollinger & Ellison 2011, Zielinski et al. 2013, Audrina 2014).

(a) (b)

Figure 1. (a) Subcuticular suturing of superior flap on the pectoralis major muscle, (b) Subcuticular suturing of inferior flap on the pectoralis major muscle

The suturing technique used in this study was the subcutis flap mastectomy in the pectoralis major muscle and the serratus anterior muscle (Figure 1). Suturing is carried out between the subcutis flap and the pectoralis muscle, and the serratus anterior muscle with an inside simple interrupted knot technique. The suture material used was 3.0 absorbable monofilament using 3 knots. This suturing technique was chosen, because it had good cosmetics and did not need to be removed later after intervention. Subcuticular sutures did not damage the flap and were cosmetically good and relatively safe. Complications due to flap suturing in the chest muscles, such as bleeding and pain were not found in patients who underwent flap suturing after modified radical mastectomy.

Analysis on other factors in Table 3 which also influenced seroma production after modification of radical mastectomy were age, BMI, tumor size, history of neoadjuvant chemotherapy, histopathological grade, histopathology type, staging, tumor location, and duration of operation. It was found that there was an influence of BMI (p=0.019) and tumor size (p=0.017) on the number of postoperative modified radical mastectomy seroma production apart from the flap fixation suture factor. However, the correlation of BMI and tumor size to the reduction in seroma was not as strong as the correlation between the intervention of flap fixation on the reduction of seroma production (Correlation Coefficient 0.665).

Strength and limitation

The study is conducted at a single center, which may limit the generalizability of the results to other populations or institutions. This study only evaluates the effect of flap fixation on seroma production and does not assess other complications or outcomes. The study provides clinically relevant information on reducing the time of releasing the drain and reducing the number of seromas but, does not the severity or volume of seromas or the clinical significance of reducing the time of releasing chute.

This study was non-homogeneous data between fixation groups and without fixation groups in terms of tumor size and BMI. In the flap fixation group, the tumor size was greater than group without flap fixation, whereas BMI was influenced by the height of the patient, and the flap fixation group had a greater average BMI than group without flap fixation. The use of medical record data in the control group might also have influenced the result of this study.

CONCLUSION

Seroma is a build-up of serous fluid in a postoperative cavity clinically proven due to dead space formation under the skin flap and in axilla in post mastectomy patients. The formation of seroma as a complication of a mastectomy surgery can cause problems in patients. Various efforts were made to reduce seroma production in the hope of minimizing complications due to excessive seroma formation. Obliteration of dead space through fixation of skin flaps on the structure of the chest wall is one of the attempts to reduce seroma formation.

Flap fixation has been shown to reduce seroma formation seen from faster time of drain removal than without flap fixation. There was a significant reduction in the number of seromas assessed from the time of drain removal in patients undergoing modified radical mastectomy with flap fixation. The recommended flap fixation is subcuticular sutures because it has better cosmetics. However, further research is needed to gather data with fixation groups and control other risk factors that have the potential to influence the amount of seroma production.

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Author contribution

P CAK contributed in design of the research, data analysis, and collected data.HP wrote the manuscript and final revision.

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Original Research

THE CARRIER RATE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING BACTERIA IN COCKROACHES (PERIPLANETA AMERICANA) IN HOSPITAL AND COMMUNITY

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ABSTRACT

Cockroach (Periplaneta americana) is one of the vectors in the environment that can transmit disease. Cockroaches can act as potential mechanical vectors of antibiotic resistant bacteria. Enterobacteriaceae is a gram-negative bacteria that has natural habitats in the digestive tract of humans and animals. Enterobacteriaceae that produce Extended Spectrum β-lactamases (ESBLs) have emerged as major pathogens in hospitals. The study analyzed the prevalence of ESBL producing bacteria in cockroaches that lived in hospitals and residential homes. In this study, a total of 200 cockroaches consisting of 100 cockroaches from the hospital environment and 100 cockroaches from the residential environment were analyzed bacteriologically for colonization of ESBL producing Enterobacteriaceae. The specimen of the alimentary tract was taken and sub-cultured in MacConkey agar supplemented with cefotaxime 2 ug/ml. Growth colonies were suggested as an ESBL-producing bacteria, then were confirmed as ESBL producers by the Double Disk Synergy Test (DDST). The ESBL gene was detected by Polymerase Chain Reaction (PCR). Among 100 household cockroach samples, 14 (14%) were identified as ESBL producers, while 100 hospital cockroaches were 26 (26%) positive ESBL. The ESBL gene, in hospital cockroach were identified of CTXM 19 (19%), SHV 7 (7%), and not any TEM gene, while among household cockroaches were identified CTXM 2 (2%), SHV 11 (11%), and also not detected TEM ESBL gene. Among ESBL genes, only the CTXM gene was significantly different between household and hospital cockroaches.

***Keywords**: ESBL; E coli; cockroach; disease*

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Highlights:

1. Bacteriologically for colonization of ESBL producing Enterobacteriaceae in cockroaches (*Periplaneta americana*) were analyzed.
2. The prevalence of ESBL producing bacteria among cockroaches in hospitals is bigger than in households.

INTRODUCTION

Cockroaches (*Periplaneta americana*) are known as an insect living in dirty environments at housing, restaurants and even hospitals, where they eat a variety of waste substances that possibly inhabited by any bacteria. They can passively transmit microbial pathogens including Salmonella, Campylobacter, Shigella, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae to humans when they eat food scraps. However, cockroaches that colonize this environment can also act as potential mechanical vectors of antibiotic resistant bacteria (Loucif et al. 2016, Gwenzi et al. 2021).

Enterobacteriaceae is the bacteria that most often causes diseases, such as urinary tract infections (UTI), pneumonia, septicemia, cholecystitis, cholangitis, peritonitis, gastroenteritis and meningitis. Enterobacteriaceae is a gram-negative bacterium that has natural habitats in the digestive tract of humans and animals, and also the environment. Enterobacteriaceae that produces Extended Spectruom β-lactamases (ESBLs) has emerged as major pathogens in hospitals. The gene was first reported in the mid-1980s, mainly found in Klebsiella pneumoniae and Escherichia coli (Bradfrod 2011, Kuntaman et al. 2011). ESBL-

producing Enterobacteriaceae is also present in animals, in patients and populations in the community, with and without any diseases or chronic conditions (Mirelis et al. 2013).

ESBL producing, especially Klebsiella pneumoniae and Escherichia coli, are mostly resistance against any antibiotics, mainly the third generation cephalosporins, and aztreonam, where the enzyme activity can be inhibited by β-lactamase inhibitors, such as clavulanic acid. The ESBL encoding genes are in a plasmid that is easily transmitted to the other germs resulting in the spread of resistance (Paterson et al. 2010). The third-generation cephalosporin, which was marketed in the 1980s, was originally intended to overcome the β-lactamase producing germ, and widely used due to its less toxic effect. Nevertheless, its wide use impacted the increasing and spread of ESBL resistant genes among Gram negative (Park 2014, Ruppé et al. 2015).

ESBL-producing bacteria was also reported by Reich et al (2013) in chicken carcasses of about 88.6% cases and in cloaca of chicken of about 72.5% samples. Most of these bacteria were E. coli. The study of Salviati et al (2014) showed a high prevalence of ESBL-producing E. coli from the environment faecal waste storage of about 47.6%, and 5.9% of pig cage swabs.

Resistant bacteria in the environment can spread to humans through contaminated food and water or through direct contact with animals or humans. In the study of Mesa (2006), ESBL-producing bacteria in food samples of salads (tomatoes and lettuce) and cooked foods by 0.4%, and in waste disposal system is almost 100%. Aycan et al (2013) also reported that hospital waste was found in 89% of ESBL-producing E. coli strains.

Organisms that produce ESBL also carry genes that provide resistance to non-beta lactams including quinolones, aminoglycosides, tetracyclines and sulfonamides. This is an antibiotic resistance enzyme that is often found in members of Enterobacteriaceae including Escherichia coli and Klebsiella pneumoniae as well as non-enteric bacteria, such as Pseudomonas aeruginosa and Acinetobacter baumannii. The selection pressure that drives the evolution of ESBL has been associated to the intensive use of oxyimino-beta lactam, broad spectrum antibiotics, prolonged hospitalization, medical devices and severe illness (Lin et al. 2010).

These organisms can also transmit to humans through fomites and other non-human hosts, such as cockroaches, and in such circumstances, they cause infection. Since arthropods (i.e., cockroaches) can contaminate human food by carrying agents of various diseases with the risk of human infection in hospitals and in the community, it is therefore important to take cockroaches seriously into consideration for detecting bacteria that contain drug-resistant genes, such as ESBL resistant gene.

MATERIALS AND METHODS

The design of this study was observational analytic-comparative with cross sectional study. The sampling technique used was consecutive sampling which required the inclusion criteria of the research sample. In this study, a total of 200 cockroaches consisted of 100 cockroaches from the hospital environment, and 100 cockroaches from the residential environment. The cockroaches were picked randomly from different parts of the house, like the kitchen, bathroom, guest room, storage, gutter, and exhaust of the house. Cockroaches (*Periplaneta americana*) were picked by using insecticide-spray and taken using sterile gloves. Then, every cockroach is put into a sterile pot.

Furthermore, the cockroaches were soaked in 70% alcohol for 5 minutes to clean the contaminant in the cockroach surface. After that, the alcohol was thrown away, and the sterile 0.9% physiological saline was added for 5 ml then vortexed. The next step was that the cockroaches were picked using sterile pincers and put into a glass plate for dissecting the cockroach visceral. The cockroach alimentary tract was dissected, then put out the inside organ and kept in the 5 ml of 0.9% physiological saline, and finally let it for 10-20 minutes for a homogenate bacterial test.

The identification of ESBL producing bacteria

The homogenate sample was inoculated in MacConkey Agar plate supplemented with Cefotaxime (CTX) 2 mg/L. and incubated 37 o C for 18 – 24 hours. The growth colonies were suspected as ESBL producers, and the ESBL confirmation was conducted by using DDST. Then, if ESBL bacterial production was proven, the biochemical test for bacterial identification was applied (Nakayama et al. 2012).

The growth colonies were taken 4-5 colonies, and suspended in peptone water, diluted until the turbidity equal to McFarland 0.5, then inoculated spread evenly on MuellerHinton agar; keeping it dry for 15 minutes, then put Amoxicillin-ClavulanicAcid (AMC) disc (30/10 ug) in the center of the plate. Other cephalosporin disc was located around the AMC in distance at 15 mm from AMC. The increasing inhibition zone in area between cephalosporin disc and clavulanic acid were interpreted as ESBL producing bacteria.

Bacterial identification

The biochemical test was applied to identify bacterial species of ESBL producers. The biochemical tests were oxidation, sulfide indole motility (SIM), Methyl-red Voges-Proskauer (MR-VP), citrate, urea, and triple sugar iron agar (TSIA). All the biochemical tests and identification according to Bailey and Scotts Diagnostic Microbiology 13th Edition (Tille 2014).

ESBL gene detection

The Polimerase Chain Reaction (PCR) assay to the detection gene of TEM, SHV and CTX-M. The DNA extraction was done by boiling method as a procedure (Ferreira et al. 2011). One bacterial colony was suspended into a 100 ul sterile TE buffer. The suspension was then heated at 100oC temperature for 5 minutes, and centrifuged at 10,000 rpm for 5 minutes. Supernatant was taken as aDNA template, stored at-20^{o} C until used.

PCR was run at each of ESBL gene with total 25µl PCR mix, consisted of 12.5 µl GoTaq Green Master Mix 2x, 1 µl primer forward (blaCTX-M, blaSHV, blaTEM), 1 µl primer reverse (blaCTX-M, blaSHV, blaTEM), 5 µl DNA template and 5.5 µl distilled water (Ferreira et al. 2011).

BlaTEM gene amplification: denaturalised under 96^{o}C for 5 minutes followed by 35 cycles in 96 C for 5 minutes, annealing in 58^{o}C for one minutes and extension in 72^{o}C for 1 minutes and final extension in 72^{o}C for 10 minutes. The used primer TEM-F 5'-ATGAGTATTCAACATTTCCG-3' and TEM-R 5'-CTGACAGTTACCAATGCTTA-3'. The amplicon result was 867 bp.

Amplification of gene blaSHV: denatured in 96^{o}C temperatures for 5 minutes followed by 35 cycles in 96^{o}C for one minute, annealing in 60^{o}C for one minute, and extension in 72^{o}C for one minute and final extensionin 72^{o}C for 10 minutes. Primer used were SHV-F5'-GGTTATGCGTTATATTCGCC-3' and SHV-R 5'-TTAGGTTGCCAGTGCTC-3'. The amplicon result was 867 bp.

Amplification of geneblaCTX-M: denaturated in 94^{o}C for 7 minutes followed by 35 cycles in 94^{o}C for 50 second, annealing in 50^{o}C for 40 second, and extension in 72^{o}C for one minute and final extension in 72^{o}C for 5 minutes. Primer used were CTX-M-F 5'-ATGTGCAGYACCAGTAARGT-3' and CTX-M-R 5'-TGGGTRAARTARCT SACCAGA -3'. The product of amplicon results was 593 bp. The PCR product was visualized in 1.5 % agarose gel. Electrophoresis was conducted on 100 volts for ± 60 minutes, then stained Ethidium Bromida (0.5 µg/ml).

Data analysis

The result of the research was presented in the table. Data analysis was done by using Chi Square test (if it was eligible), if it was not, Fisher Exact test was applied. The statistic test was SPSS version 22.

RESULTS

Among 100 samples of house cockroaches, 14 (14%) were identified as the ESBL producing bacteria, while hospital cockroaches 26 (26%). The total 14 ESBL producing bacteria from household cockroach, consisting of *Escherichia coli* 8 (8%), *Klebsiella spp* 1 (1%), *Pseudomonas aeruginosa* 1 (1%), *Acinetobacter Spp* 1 (1%), *Klebsiella pneumoniae* 3 (3%), while 26 hospital ESBL producing bacteria were consisting of *Escherichia coli* 9 (9%), *Citrobacter spp* 5 (5%), *Acinetobacter spp* 8 (8%), and *Klebsiella pneumoniae* 4 (4%) (Table 1).

Table 1. Distribution of bacteria ESBL-producing on household cockroaches' samples and hospital samples

No.	Bacterial Types	Numbers n (%)		Total of bacterial types
		Household cockroaches	Hospital cockroaches	
1.	Escherichia coli	8 (8%)	9 (9%)	17
2.	Klebsiella spp	1 (1%)	0 (0%)	1
3.	Citrobacterspp	0 (0%)	5 (5%)	5
4.	Pseudomonas aeruginosa	1 (1%)	0 (0%)	1
5.	Acinetobacterspp	1 (1%)	8 (8%)	9
6.	Klebsiella pneumoniae	3 (3%)	4 (4%)	7
7.	Non ESBL bacterial	86 (8%)	74 (74%)	160
	Total samples	100 (100%)	100 (100%)	200

Table 2. The distribution of ESBL genes of house and hospital cockroaches

Location	CTX-M		SHV		TEM	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Household (n=100)	2 (2%)	98 (98%)	11 (11%)	89 (89%)	0 (0%)	100 (100%)
Hospital (n=100)	19 (19%)	81 (81%)	7 (7%)	93 (93%)	0 (0%)	100 (100%)
Total gene	21	179	18	182	0	200 genes

The result of ESBL gene showed that TEM gene was not found in household cockroaches and hospital cockroaches. From 14 of house ESBL producers, 7 (7%) SHV genes and 19 (19%) CTXM were identified. In this study, the SHV gene was mostly found in household samples. It was possible, because the SHV gene was infected through food (Greko et al. 2017). SHV variants were detected for the first time in Switzerland with blaSHV–12 (Table 2).

DISCUSSION

ESBL-producing bacteria in cockroaches (Periplaneta americana) on residential environment were mostly found in cockroach of gutter 11 (11%) and 3(3%) were found inside of the house, such as kitchen and bedroom, while the hospital cockroaches with ESBL were found in the gutter 20 (20%) and in hospital environment 6 (6%).

The study of Salviati et al (2014) showed that ESBL producers *E.coli* were isolated from the environment of about 47.6 % from feces waste and boots swab, and 5.9% from the swab of swine farming. Bacteria resistance of the environment were spread through food and contaminated water and direct contact with the animals.

In Mesa's study (2006), ESBL producing bacterial were found in food sample like salad (tomato and lettuce), 0.4% in cooked food, and in waste exhaust was almost 100%. Aycan (2013) also reported that in hospital waste was found 89% *E.coli* that were ESBL producer. This study also found the combination of CTX-M+SHV gene. This was similar to the study by Dagi et al (2015) which found that 8% had CTX-M gene and 77.4% had the combination of TEM and CTX-M. This was because the plasmid coded CTX gene of plasmid IncFII plasmid type categorized into big plasmid. The common type of plasmid was F2:A-:B-. This plasmid type was found in blaCTXM in Enterobacteriaceae isolated from other country. CTX-M gene found in other isolated were in plasmid which had high spreading ability (highly transmissible plasmids), so that the spreading was fast and efficient. The bacterial which expressed CTX-M was mostly co-resistant or multi resistant (Ramos et al. 2020).

The appearance and the wide spreading of ESBL among *E.coli* isolate clinic in the hospital became the main concern in some countries which human infected. This infection brought a great impact, because it could lead to the failure medication and the level of serious condition. ESBL consists of TEM, SHV, dan CTX-M. Among those, the number of highest variants was CTX-M. The existence of CTX-M made *E.coli* was resistant to any type of beta-lactam and transferred trough plasmid including unconnected microbes (Canton 2012).

The use of third generation of cephalosporin antibiotic, beta lactam type of antibiotic, and fluoroquinolone type of antibiotic in the hospital was suspected as the factor of the ESBL producer bacterial appearance. On the other hand, the utilization of antibiotic in the community also had a role in the spreading of resistant gene among bacterial species (Adelyap 2011). The higher incorrect antibiotic used, the higher evolution process selection and microorganism strain resistant proliferation (Pratiwi 2008).

In the last ten years, it was revealed that CTX-M almost changed other ESBL enzyme on Enterobacteriaceae, including TEM and SHV variants. The changes did not only happen as the result of the spreading of blaCTX-M gene on genetic incredible transfer mobilization including plasmid and transposon, but also the success of cloning (Rogers 2011). The increase of resistant phenomena which happened to the CTX-M producer organism to aminoglycoside, and fluoroquinolone also facilitated the selection process inn resistance.

Strength and limitation

The study is important public health concern, which is the potential transmission of antibiotic resistant bacteria by cockroaches in hospital and residential environments but, it only analyzes the presence of ESBL-producing Enterobacteriaceae in cockroaches and does not investigate the potential for transmission of these bacteria to humans or other organisms and the factors that contribute to the presence of ESBL-producing bacteria in cockroaches, such as the use of antibiotics in the environment or the presence of other potential vectors. This study uses a large sample size of 200

cockroaches, with 100 from each environment, which increases the validity and generalizability of the results and also, it combination of bacteriological culture and molecular methods to identify ESBL-producing bacteria,

CONCLUSION

The prevalence of ESBL producing bacteria among cockroach in hospital was 26 (26%), while in household cockroach was 14 (14%). It was significantly different among cockroaches in hospital and residential.

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Author contribution

AP and RY contributed to the conceptualization, the study design and methodology, and data collection. AP contributedto write and revise the manascrupt. EBW and K validation the data analysis, grammar and final content.

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Original Research

IL-17 AND DISEASE ACTIVITY IN SPONDYLOARTHRITIS

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ABSTRACT

IL-17 is a new cytokine involved in the pathogenesis of Spondyloarthritis (SpA). Recent studies show that IL-17 level correlates to disease activity, and it is used as a basis in treating SpA patients who do not respond to anti-TNF-α. This study identified the correlation of IL-17 to disease activity measured by The Ankylosing Spondylitis Disease Activity Score C-Reactive Protein (ASDAS-CRP). This study was a cross-sectional study involving SpA patients according to the 2009 ASAS criteria in Dr. Soetomo General Academic Hospital, Surabaya. Disease activity and IL-17 level were analyzed using Spearman correlation test to see the strength of correlation. Forty SpA patients showed mean age of 53.58 ± 9.28 years with a body mass index of 24.36 ± 3.23 kg/m2, ESR of 39.50 ± 18.76 mm/hour, clinically obtained Schober Test of 13.11 ± 1.22 cm, chest extension test of 1.45 ± 0.77 cm, and tragus-to-wall test 13.53 ± 1.99 cm. The median CRP and IL-17 were 0.3 (0.10-5.70) mg/dL and 9.30 (7.70-13.60) pg/dL, respectively. Based on the ASDAS-CRP system, the patients showed disease activities that fall into the category was high (62.5%), moderate (35%), and inactivity (2.5%). IL-17 level is strongly correlated to disease activity in SpA patients (p=0.000, r=0.711).

Keywords: *IL-17; spondyloarthritis; health risks; disease*

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Highlights:

1. The correlation of IL-17 to disease activity by The Ankylosing Spondylitis Disease Activity Score C-Reactive Protein (ASDAS-CRP) was identified.
2. IL-17 level is strongly correlated to disease activity in SpA patients.

INTRODUCTION

Spondyloarthritis (SpA) is a chronic inflammatory arthritis group that primarily affects the axial and peripheral bones. The characteristics of the SpA are sacroiliitis, inflammatory back pain, oligoarthritis, enthesitis, and negative rheumatoid factors (Ehrenfeld 2012, McGuckin et al. 2010) In general, the symptoms are rarely well described by the patient and frequently overlooked and undiagnosed by physicians, which then lead to increased morbidity and high economic burden due to disability (Dincer et al. 2008). Until recently, the pathogenesis of SpA had not been fully discovered. The pathogenesis of SpA is allegedly related to Human Leukocyte Antigen-B27 (HLA-B27) gene. The HLA-B27 misfolding hypothesis is currently the most widely accepted pathogenesis of SpA which also explains the presence of Interleukin (IL)-17 in SpA.

The HLA-B27 misfolding hypothesis explains the emergence of endoplasmic reticulum (ER) stress and Unfolded Protein Response (UPR). UPR activation triggers the Toll-Like Receptor (TLR) influenced by Interferon (IFN)-β to induce IL-23 secretion through UPR Target Gene that has a correlation with HLA-B27 and Th17 Cell-Driven Disease. (Colbert et al. 2009, Dincer et al. 2008). Triggered by unfolded proteins, the malfunctioning ER or ER-stress causes interference in signal transduction. The function of UPR is to strictly regulate protein translation, degradation of misfolded proteins, and activation of signalling to increase the synthesis of chaperone molecules involved in protein folding. If UPR function is disrupted, apoptosis will occur.

ER stress further leads to the production of pro-inflammatory cytokines, such as IL-23, IL-6, and Transforming Growth Factor (TGF)-β. IL-23 is the largest cytokine in UPR, and it will stimulate the conversion of naive CD4 T cells to T-helper-17 (Th17) that secretes IL-17 (Chabaud et al. 2000). There is currently no full explanation about the mechanism of IL-17 in its correlation to disease activity in SpA. However, several studies have found that IL-17 increases the expression of Receptor Activator of Nuclear factor-κB Ligand (RANKL), expression of molecular adhesion, chemokine secretion, and MMP enzyme secretion. The increased pro-inflammatory cytokines can cause synovitis, cartilage degradation, and enthesitis which lead to arthritis and joint stiffness (Braun & Zwerina 2011, Machado et al. 2011).

Assessing the actual disease activity in SpA is not easy. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI) are among other scoring systems to determine disease activity in SpA. Assessment of the Spondyloarthritis International Society (ASAS) in 2009 proposed a new system in the form of ASDAS (Ankylosing Spondylitis Disease Activity Score) which adopted several points in BASDAI and added several points, such as CRP and the Blood Depth (ESR). Demir (2013) compared some parameters about disease activity in SpA and found that ASDAS was significantly more appropriate in describing actual activity of SpA compared to BASDAI and BASFI. Until recently, research on the correlation of IL-17 with ASDAS scores was still lacking. This study investigates the correlation of IL-17 serum with parameters of disease activity in SpA using ASDAS-CRP.

MATERIALS AND METHODS

This study involved 40 patients from Javanese ethnicity in Dr. Soetomo General Academic Hospital, Surabaya who were diagnosed with SpA based on 2009 ASAS criteria. The patients were sampled using consecutive sampling methods. Patients with BMI >30kg/m2, diabetes mellitus, liver cirrhosis, asthma, tuberculosis, and smoking history were all excluded. Every patient enrolled in this study had voluntarily signed an informed consent form before participating as a study subject. All study subjects were aware that they were involved in this study, and their rights and confidentiality were ultimately respected. This study was approved by the Ethical Committee of Dr. Soetomo General Academic Hospital, Surabaya and conducted according to Good Clinical Practice (GCP).

This was a cross-sectional study, where clinical and laboratory assessments of the patients were performed on the same day. We evaluated the disease activity using the ASDAS- CRP whilst physical examinations were performed to determine physical mobility, including tragus-to-wall (TWD) distance and lumbar flexion (Schober index).

Serum IL-17 level is measured by Enzyme-Linked Immunosorbent Assay (ELISA), using Human IL-17 (Quantikine® ELISA Human IL-17 Immunoassay, R&D System, IncCatalog D1700) as a reagent. The minimum detectable level was 7.8 pg/ml. A blood sample was drawn in a serum separator tube for 30 minutes, then centrifuged for 15 minutes and stored in <-20 C fridge. 100 µL of each standard, control, and sample were added into tubes containing 100 µL of Assay Diluent RD1-19. Tubes were sealed with adhesives and incubated at room temperature for 2 hours before aspirated and washed four times. Aspiration and washing were repeated after adding 200 µL of IL-17 Conjugate and a one-hour incubation period. Furthermore, 200 µL of substrate solution was added, and tubes were stored in light-proof storage for a 30-minute incubation period. Then, 50 µL of stop solution was added, ELISA reader: 450 nm (620nm, reference wave).

Disease activity is a condition that describes the severity of SpA assessed by the ASDAS (Ankylosing Spondylitis Disease Activity Score) system (Table 1). The components of the ASDAS scoring system consisted of back pain, morning stiff duration, peripheral pain or swelling, and global assessment of disease activity, in the scale of zero to ten, and CRP level or Erythrocyte Sedimentation Rate (ESR). Each item was inserted in the ASDAS formula to generate an assessment score and its category.

Table 1. Ankylosing spondylitis disease activity score with C-reactive protein (ASDAS-CRP)

ASDAS CRP = (0.12 × back pain score) + (0.06 × morning stiffness score) + (0.11 × global assessment score) + (0.07 × peripheral pain /swelling score) + [0.58 × ln (CRP + 1)]
Total score is classified as:
1. Inactive (score < 1.3)
2. Moderate disease activity (score: 1.3-2.1)
3. High disease activity (score: 2.1-3.5)
4. Very high disease activity (score > 3.5)

All data was entered into a computer through a statistical progra (SPSS edition 17) and delivered in descriptive statistics. Kolmogorov-Smirnov tested the data distribution which appeared to be abnormally distributed. Hence, Spearman's rank correlation test (for non-parametric) analyzed the correlation of IL-17 level and ASDAS-CRP score. The strength of correlation (r) with a value of 0.00-0.199 was interpreted as ővery

weak'. Values 0.20-0.399 were interpreted as weak, values 0.40-0.599 as moderate correlation, values 0.60-0.799 as strong correlation, and 0.80-1.00 as very strong correlation. The significance level was 5%.

RESULTS

In Table 2, forty patients with SpA aged 53.58±9.28-year-old showed mean body mass index of 24.36±3.23 kg/m2, ESR of 39.50±18.76 mm/hour, Schober test of 13.11±1.22 cm, chest expansion test of 1.45±0.77 cm, and tragus-to-wall test of 13.53 ± 1.99 cm. Medians of CRP and IL-17 were 0.3(0.10-5.70) mg/dL and 9.30 (7.70-13.60) pg/dL respectively. ASDAS-CRP scores showed 2.5% inactive, 35% moderate, and 62.5% high disease activities. None had very high disease activity.

Table 2. Characteristics of research subjects

General Characteristics		Result
Gender	Man	11 (27,5%)
	Women	29 (72,5%)
SpA subtypes	Axial SpA	24 (60%)
	Peripheral SpA	16 (40%)
Old (year)	Mean ± SD	53.58 ± 9.28
Body Mass Index (BMI)	Mean ± SD	24.36 ± 3.23
Schober Test (cm)	Mean ± SD	13.11 ± 1.22
Chest Expansion Test	Mean ± SD	1.45 ± 0.77
Targus-to-Wall (cm)	Mean ± SD	13.53 ± 1.99
ESR (mm/hour)	Mean ± SD	39.50 ± 18.76
CRP (mg/dl)	Median (min-max)	0.3 (0.10-5.70)
Albumin (g/dL)	Mean ± SD	3.86 ± 0.32
IL-17 level (pg/dL)	Median (Min-Max)	9.30 (7.70-13.60)
Axial SpA	Mean ± SD	10.01 ± 1.49
Peripheral SpA	Median (Min-Max)	9.10 (7.70-11.50)

Gender distribution varied in numerous studies. Some reports showed more significant male incidents than women, but other researchers claimed that there was no difference. In this study, the proportion of women and men was 3:1 classified as 29 patients (72.5%) were women, and 11 (27.5%) were men. SpA generally occurs at a young age ranging from 20 to 30. In this study, the average age of the sample was 53±9 years. Patients' average age might be different because, in developing countries, patients are usually constrained by economic problems that result in patients coming late to health services. Moreover, diagnosis takes about 8-10 years after the earliest manifestation of SpA symptoms, because they were often unnoticed (insidious).

Twenty-four patients (60%) were categorized as Axial SpA group and 16 patients (40%) as Peripheral SpA group. The average Body Mass Index (BMI) of patients was 24.36 kg/m^2 and categorized as normal. The average Schober test was 13.11±1.22 centimetres which indicated the measurement of the Schober test was also relatively homogeneous. The average value of the ESR was 39.50±18.76 mm/hour.

The CRP level did not follow a normal distribution and had a median of 0.3 (0.10-5.70). The inflammation in RA was different from that of SpA. The SpA was characterized by a low-grade inflammation with elevated cytokines concentration and acute-phase proteins of two to three times normal level (Anne et al. 2005). In this study, the mean ESR and median CRP level were 39.50±18.76 and 0.3 (0.10-5.70) mg/dl respectively. Woloshin and Schwartz (2005) reported that in the United States, the normal value of CRP was <0.3 mg/dl, but the range 0.1-1.0 mg/dl was a normal variant in individuals without pathological abnormalities, so that the inflammatory condition in the SpA patient in this study was categorized as a low-grade inflammation. Besides, the CRP level and ESR were lower than those in Yildirim et al. (2004) and Arthur et al. (2010) which reported Rheumatoid Arthritis (RA) patients. In contrast, Kay et al. (2014) reported that most of the patients with active RA were not followed by elevated CRP levels, but CRP was reported to be related to the prognosis.

Table 3. Clinical Characteristics According to ASAS criteria

ASAS Criteria	n	%
Inflammatory Back Pain	26	65
Arthritis	18	45
Enthesitis (heel)	16	40
Uveitis	1	2.5
Dactylitis	3	7.5
Psoriasis	12	30
Inflammatory Bowel Disease	Not examined	-
Good Response to NSAID	35	87.5
Family History for SpA	2	5
Elevated CRP	13	32.5
HLA-B27	Not examined	-
Sacroiliitis by Radiograph	28	70

In general (Table 3), complaints of patients with SpA were back pain (inflammatory in nature), oligoarthritis, especially in the lower legs, dactylitis (sausage-like digits), enthesitis in the heel or other places, and extra-articular manifestations, such as uveitis, inflammatory bowel disease and psoriasis (Rudwaleit 2010). In this study, Table 3 indicated the main complaints of patients were low back pain (inflammatory back pain) in as many as 26 patients (65%) patients followed by arthritis

(45%), symptoms of Frank's arthritis—stiff joints and heel pain (enthesitis)—in 16 people (40%), and acute uveitis in only 1 person (2.5%). Sacroiliitis by plain radiograph were apparent in 28 patients (70 %). Finally, as the subjects visited the clinic not in the early stage of disease, 87.5 % of patients responded to NSAIDs.

Table 4. Levels of IL-17 in patients with SpA

IL-17 Level		Result (pg/dl)
IL-17 level (pg/dL)	Median (Min-Max)	9.30 (7.70-13.60)
Axial SpA	Mean ± SD	10.01 ± 1.49
Peripheral SpA	Median (Min-Max)	9.10 (7.70-11.50)

In Table 4, the median result of IL-17 in this study was 9.30 (7.70-13.60) pg/dL. The lowest IL-17 level was 7.70 pg/dl whilst the highest was 13.60 pg/dl. The mean IL-17 level in the Axial SpA group was 10.01±0.30 pg/dL, while the peripheral SpA group showed a median value of 9.10(7.70-11.50) pg/dL.

Table 5. Disease activity according to ASDAS-CRP and corresponding IL-17 level

ASDAS-CRP Median 2.20(1.20-3.50)		IL-17 level (pg/dl)
Inactive Disease (skor < 1.3)	1 subject (2.5%)	7.7
Moderate Disease (1.3≤ skor <2.1)	14 subject (35.0%)	8.79±0.53
High Disease (2.1≤ skor <3.5)	25 subject (62.5%)	10.36±1.35
Very High Disease (skor >3.5)	0 subject (0.0%)	-

Furthermore, the median value of the disease activity score of 40 SpA patients measured by the ASDAS-CRP score was 2.20 (1.20-3.50) pg/dL (Table 5). The number of SpA patients who are classified according to ASDAS-CRP score is one person (2.5%) for inactive disease-based, 14 people (35.0%) for moderate disease activity, 25 people (62.5%) for high disease activity, and none for the very high disease.

Hypothesis testing of the association between serum IL-17 levels and disease activity in patients with SpA was carried out by association analysis. The Kolmogorov-Smirnov data normality test should conclude that the data distribution was normal when the p-value was above the significance level of 5%. The results for the IL-17 and ASDAS-CRP distribution test resulted in a p-value of 0.003 and 0.013 respectively, which concluded that the obtained data were not distributed normally. Thus, the Spearman's rank test for non-parametric association was used to analyze the association between IL-17 levels and ASDAS-CRP scores.

Table 6. Spearman's rank for the correlation of IL-17 level and ASDAS-CRP

Variable 1	Variable 2	r-value	p-value
IL-17	ASDAS-CRP	0.711	0.000

Analysis of the association between IL-17 levels and the ASDAS-CRP score with the Spearman's rank resulted in a Spearman r-value of 0.711 with a p-value of 0.000 (Table 6). These results concluded a significant correlation between IL-17 level and the ASDAS-CRP score in the study subjects. The correlation was positive or unidirectional, which indicated that the more elevated the IL-17 level in SpA patient was, the higher ASDAS-CRP score would be, the more severe disease activity could be. The association between IL-17 levels and ASDAS-CRP scores was shown in Figure 1.

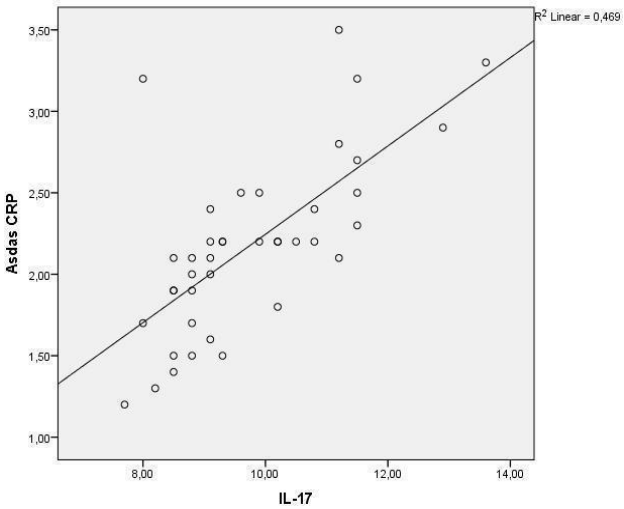

Figure 1. Correlation of IL-17 level and ASDAS-CRP (disease activity) in SpA patients

DISCUSSION

Spondyloarthritis. (SpA) is caused by genetic factors that are triggered by environmental factors originating from gastrointestinal and genitourinary infections. Various hypotheses of the pathogenesis of SpA arise, because the exact pathogenesis is not particularly clear at this time. The hypotheses that have been developed to date are the arthritogenic-peptide hypothesis, misfolding, and the HLAB27 homodimer or monomer hypothesis. The discovery of Th17 cells and the resulting cytokines raised questions on autoimmune disease induced by auto-reactive cells in an early study by Wendling et al. (2007) who evaluated IL-12/IL-23 levels in serum and synovial fluid of SpA patients

reported a significant association. However, many studies showed a significantly associated relationship between IL-17 in the pathogenesis of SPA. Wang et al. (2009) reported a correlation between the significantly elevated IL-17 and IL-23 levels in SpA patients with BASDAI scores higher than 4. Furthermore, they also found that IL-17 level correlated with ASDAS–CRP axis.

The normal range of IL-17 levels varies in healthy individuals. Ciprandi et al. (2008) reported IL-17 level of 0.28–5.47 pg/dL in healthy individuals and 31.37 ± 8.4 pg/dL in healthy controls, while Hussein et al. (2008) and Arican et al. (2005) mentioned 0.01±0.0 pg/dl. Arthur et al. (2010) reported that IL-17 level in RA patients elevated by an average of 17.28 pg/ml, while Hussein et al. (2008) reported a mean IL-17 level 0.2±0.1 pg/dl in RA patients. Arican et al. (2005) and Petersen and Pedersen (2005) in studies with psoriatic arthritis population reported serum IL-17 levels were 8.3±3.8 pg/dl, while Chen et al. (2012) reported in patients with Ankylosing spondylitis IL-17 levels of 66.03±17.8 pg/dl. Julija et al. (2013) reported average IL-17 level of 18.9±39.6 pg/dl in patients with ankylosing spondylitis with control of 5.4±26.0 pg/dl. These various results might be correlated with diseases activity or different ethnicity related to genetic factor. In this study, the median serum IL-17 level for SpA patients was 9.30 pg/ml, and the lowest serum IL-17 level was 7.70 pg/ml, while the highest serum IL-17 level was 13.60 pg/ml. These results were lower than average IL-17 levels in healthy individuals that have been reported but lower than some other studies.

Baraliakos et al. (2014) reported that ASDAS has a better ability to determine disease activity than BASDAI in axial SpA patients receiving treatment with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). They reported a mean ASDAS-CRP score in SpA patients of 2.5±0.6. Madej et al. (2015) reported on SpA patients in Poland the ASDAS-CRP value of 3.0±1.2. In this study, the median ASDAS-CRP score was 2.20 (1.2-3.5). The axial and peripheral SpA groups' scores showed no significant difference, considering the study subjects in each group were mostly in high disease activity condition. In this study, the majority of subjects showed high disease activity. The therapeutic modality which only consisted of a combination of NSAIDs, sulfasalazine (SSZ), and methotrexate (MTX) as well as patients' compliance in taking medication might contribute to disease activity. Dougados et al (1992) reported in a retrospective study that out of 372 SpA patients who received SSZ, there were only 59% who showed proper clinical development (Singh et al. 2007).

In this study, the correlation between serum IL-17 levels with ASDAS-CRP scores resulted in a p-value of 0.000 (α=5%). It is concluded that there was an association between IL-17 level and disease activity in SpA patients in Dr. Soetomo General Academic Hospital, Surabaya. The association between IL-17 levels and disease activity in patients with SpA in Dr. Soetomo General Academic Hospital, Surabaya, was strong (r=0.711). The results of this study were consistent with those reported by Chen et al (2012) in Taiwan on levels of IL-17 in patients with Ankylosing Spondylitis (AS), reported that IL-17 had an association with disease activity measured by BASDAI. However, Julija et al. (2013) study in Latvia, IL-17 had no association with disease activity in AS patients (Baraliakos et al. 2014).

According to the RESPONDIA study (Gallinaro et al. 2010), the most common clinical manifestations were inflammatory back pain (58.7%) and peripheral arthritis (37.7%). The RESPONDIA study involved 4,405 SpA patients from Ibero-American ethnicity in 10 countries. Among 1,168 patients with SpA in Spain, the most common clinical manifestations were inflammatory back pain (55.2%) and psoriatic arthritis (22.2%). Tayel et al. (2012) in Egypt reported that the dominant clinical manifestations in patients with SpA were inflammatory back pain (34%) and enthesitis (29.3%). Zhang et al. (2011) reported that the clinical picture of SpA in Asia was not much different from in various other parts of the world. In this study, the most common clinical manifestations were inflammatory back pain 65% and peripheral arthritis 45%. Thus, this study shows similar reports from various geographical areas worldwide (Europe, Latin America, the Middle East, and Asia).

Inflammatory arthritis is currently distinguished according to the degree of inflammation. In arthritis with obvious inflammatory symptoms in clinical examination (warm, painful, joint effusion) are classified as arthritis with high-grade inflammation (e.g., Rheumatoid Arthritis or RA). RA is characterized by severe inflammation characterized by high levels of inflammatory cytokines and acute-phase proteins (Siloşi et al. 2016). A study by Yildirim et al. (2004) in RA subjects described CRP levels of 2.4±1.9 (mg/dl) and LED values of 36.0±23.5 (mm/hour), while Arthur et al. (2010) mentioned that the average ESR in RA patients at Cipto Mangunkusumo Hospital in Jakarta was 58.50±32.10 (mm/hour).

The CRP and LED levels in this study were lower than those reports. Inflammation in RA was different from what happened in SpA. The SpA had the characteristics of low-grade inflammation. Low-grade inflammation is defined as inflammation with an increase in the concentration of cytokines and acute-phase proteins by 2-3 times the normal level. In this study, the mean value

of ESR and median CRP levels were 39.50±18.76 and 0.3(0.10-5.70) mg/dl. Woloshin and Schwartz (2005) also reported the normal CRP value was <0.3 mg/dl, but the range 0.1-1.0 mg/dl was a normal variant in individuals without pathological abnormalities. Therefore, the inflammatory conditions in SpA patients in this study were categorized as low-grade inflammation.

Albumin is known as a negative acute-phase reactant or an acute phase protein and its synthesis decreases when inflammation occurs (Ballantyne et al. 1971). In high-grade inflammation like RA, most patients experienced hypoalbuminemia (Ciprandi et al. 2008). In this study, the mean albumin level was within a normal range (3.86±0.32 g/dl). It was possibly due to the inflammation, where it was not severe enough to suppress albumin synthesis and cause hypoalbuminemia.

As measured by the ASDAS-CRP score, SpA disease activity has several measurement components, namely pain in the axial joints, joint stiffness in the morning, and pain or swelling in the peripheral joints. IL-17 increases the activity of SpA disease by causing axial and peripheral joint pain, causing joint stiffness and joint edema. Singh et al. (2007) reported that, in 51 patients with Reactive arthritis (ReA) and Undifferentiated SpA (uSpA), IL-17 levels in synovial were found to be higher than in RA patients, so that IL-17 was also thought to be a cytokine that had a dominant role in ReA and uSpA. Appel et al. (2011) reported that IL-17 levels in the vertebral joint facet area of patients with ankylosing spondylitis increased. Appel also mentioned that the number of cells producing IL-17 was more in the inflamed joints. The increased IL-17 in joints could cause joint pain and stiffness (Pinto et al. 2010).

Pain in patients with SpA is deemed to be related to IL-17. In animal (rate) study, Pinto et al. (2010) described that IL-17 could cause pain in arthritis (RA) due to increased nociceptive excitations resulting in hypernociception. Richter et al. (2012) reported a trial, where mice were given IL-17A injections in the knee joint area. In these mice, prolonged nociceptive sensitization did not disappear by giving anti-TNF-α and IL-6. IL-17A was found to cause rapid phosphorylation of protein kinase B and extracellular-signal-regulated kinase (ERK), and this increased excitability quickly. Richter et al. (2012) raised a suspicion that IL-17 acted as a pain mediator that caused hyperalgesia. The increased levels of IL-17 in the blood and joints of patients with SpA were thought to exacerbate disease activity, because they caused joint pain and joint stiffness.

IL-17 caused bone remodelling and new bone formation in SpA through its effects on osteoclasts and osteoblasts. IL-17 stimulated the production of PGE2, nitric oxide (NO), and receptor activator of NFκB ligand (RANKL) by osteoblasts. This could cause osteoclast activation and differentiation, which ended with increased bone destruction. IL-17 upregulated fibroblasts, epithelial cells, endothelial cells, monocytes, and osteoblasts to increase pro-inflammatory cytokines, such as IL-6 and granulocyte monocyte colony-stimulating factor (GM-CSF), IFN-γ, and TNF-α. These pro-inflammatory cytokines acted as mediators of bone destruction. Ruddy et al. (2004) found that IL-17 stimulate osteoblasts to increase the expression of chemotactic factors, such as CCL2 and CXCL5 that stimulated the recruitment of leukocytes, such as neutrophils and T cells. Mobilization of inflammatory cells caused an increase in inflammatory mediators, such as IL-6, IL-1, TNF-α, and RANKL, that could cause bone destruction and new bone formation (Onishi & Gaffen, 2010).

Synovial inflammation in SpA patients is not as significant as in RA. Synoviocytes play a role in causing bone and cartilage damage due to stimulation by IL-17. IL-17 stimulates synovial macrocytes and macrophages to produce chemokines (e.g., IL-8, CXCL2, CCL20, CCL2, CXC5, and CCL5), the mobilization of neutrophils, lymphocytes, and macrophages to synovium and further increases the inflammatory process. Park et al. (2011) reported their studies in experimental animals in mice, where IL-17 increased cadherin-11, an adhesion molecule that played a role in synovial inflammation and cartilage degradation. IL-17 stimulates synoviocytes to increase pro-inflammatory cytokines and chemokines, such as IL-6, IL-8, CCL20, TNF-α, and p19 subunit IL-23.

Chowdhury et al (2013) found that IL-17 inhibited mRNA degradation that encoded TNF-α cytokines. The final result of this process caused mRNA to be translated longer, so that the levels increased. Hot and Miossec (2011) stated that IL-17 was also responsible for increasing the production of matrix metalloproteinases (MMP), such as MMP3, MMP9, and MMP13. Moz et al (2017) reported that MMP in the synovium was associated with parameters of arthritis activity, such as cell infiltration. A cohort study by Yang et al. (2004) showed that MMP-3 levels correlated with disease activity in ankylosing spondylitis.

Cartilage is a target organ attacked in SpA. In SpA, cartilage will experience degradation. From various research reports, IL-17 was said to mediate the pathological process of cartilage degradation. Honorati et al. (2002) experimented on human chondrocyte cells which were given IL-17. They found that IL-17 stimulated chondrocytes to produce NO and increase gene expression associated with joint inflammation and cartilage degradation (NO synthase, cyclooxygenase 2, IL-1β, IL-6, IL-8, CCL2, and MMP). Dudler et al (2000) reported intra-articular injection of IL-17 in mice causing inhibition of proteoglycan synthesis by cartilage and causing destruction of cartilage. The effect of cartilage degradation was further investigated by Koenders et al. (2005) in in-vivo mouse experiments. Koenders et al. (2005) found that the IL-17 receptor blockade reduced the occurrence of cartilage degradation.

From the data of animal studies, in vitro studies, and clinical studies, it is reported that IL-17 was a cytokine that was highly potent as a pro-inflammatory cytokine (Miossec & Kolls 2012). Besides, it also played a role in osteoclastogenesis and had the ability to induce the formation of blood vessels as the characteristic of inflammation. It is suspected that IL-17 was a highly strong cytokine in inducing inflammation responsible for the immunopathogenesis of SpA (Yago et al. 2009). The formation of Th17 is induced by antigen stimulation on naïve CD4 + T cells which caused differentiation into Th1, Th2, and Th17. In mice, TGF-β and IL-6 had a role in the differentiation of CD4 T cells to Th17. Along with IL-1 and TNF-α, triggered this process. TGF-β and IL-6 regulated retinoic acid orphan receptors (RORγt and RORα) on naïve T cells resulting in upregulation of IR-23 receptors that caused cells to respond to IL-23. IL-23 is a family of IL-12 consisting of IL-12p40 and IL-23. IL-23 plays a role in triggering the secretion of IL-17. TGF-β and IL-6 regulate RORγt in humans (Miossec & Kolls 2012).

A recent study examining macrophages derived from PBMCs of AS patients also found defects in IFN-γ expression. From these data, it is concluded that IFN-γ expression was very low in SpA compared to other immune-mediated inflammatory diseases. The low level of IFN-γ in infection could increase the organism's survival which could lead to reactive arthritis. IFN-γ expression was low, but Th17 expression was increased, and it was suspected that cytokine had a role in the protection of SpA patients. The decrease in IFN-γ levels in SpA might have triggered the increase in Th17 (Taams et al. 2018). IFN-γ can play a dual role, increasing the expression of HLA-B27, and ultimately increasing the Unfolded Protein Response (UPR). UPR triggers the production of several cytokines against bacterial products captured by TLRs, and Pattern Recognition Receptors, such as IFN-β that induces IL-23 overexpression. This is very interesting, because the increase in IL-23 will stimulate Th17 to secrete IL-17. Th17 and its cytokines affect several immune-mediated inflammatory diseases whilst the IL-23/IL-17 axis is activated in the AS and the SpA (Colbert et al. 2009).

Various research reports on IL-17 further reinforced the alleged role of IL-17 in the pathogenesis of SpA. Chen et al. (2012) found a significant association between IL-17 and SpA disease activity in their study. IL-17 causes clinical manifestations and symptoms that are characteristic of SpA, such as pain, axial and peripheral joint inflammation, enthesitis, cartilage degradation, and new bone formation. Therefore, the hypothesis that IL-17 correlates to SpA disease activity was progressively being proven.

Strength and limitation

In this study, as the correlation between IL-17 level and SpA disease activity appeared to be strong, this study further supported the notion of IL-17 being a highly favourable biomarker for disease activity in SpA in the future. However, this study was conducted using cross-sectional design due to timeline and resource limitations. Therefore, the results could not describe the fluctuation in IL-17 level and its correlation with the course of the SpA as the subjects were not followed up prospectively. Finally, as the study was conducted at the Outpatient Clinics, Dr. Soetomo General Academic Hospital, Surabaya, that was relatively isolated considering the limited sample numbers, the report was unable to represent the general community's situation.

CONCLUSION

IL-17 level strongly correlated to the disease activity in SpA patients evaluated using the ASDAS-CRP system. As the correlation goes unidirectionally, the elevated IL-17 level in patients with SpA resulted in higher ASDAS-CRP score reflected through worsening disease activity in SpA patients. However, the result of this study was unable to fully represent the condition of the more general population conducted in an isolated setting with limited samples.

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Conflict of interest

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Author contribution

All authors were contributed to the conceptualization, study design and methodology, and data collection. YL wrote and revised the manusript. Y was checked the final manusript

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Original Research

DECREASE OF LDL CHOLESTEROL THROUGH THE INCREASE OF HDL CHOLESTEROL BY ADMINISTERING *Garcinia mangostana L.* PEEL EXTRACT IN WHITE MICE

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ABSTRACT

Atherosclerosis contributes to coronary heart disease which may lead to fatality. High cholesterol consumption, stress, and smoking can increase LDL cholesterol in the blood. Consumption of unsaturated fats, high fiber foods, exercise, quitting smoking, losing weight, and giving hypolipidemic drugs, especially herbs, can increase HDL cholesterol and decrease LDL cholesterol. Garcinia mangostana L. peel extract can decrease LDL cholesterol by increasing reverse HDL cholesterol transport to the liver. The study used post test control group design. This study was experimental laboratory research with population of hypercholesterolemic male white mice aged 3-4 weeks with 100-200 grams weight. The HDL and LDL cholesterol data were collected through an enzymatic method by spectrophotometer. This study used analysis of variance (Anova) with significance level of α <0.05. The experiment divided the subjects into positive and negative control groups with dosage variations of 50, 150, 250, and 350 mg/kgBW. Examination of hypercholesterolemia in white mice was conducted on the 8th day. The examination of HDL and LDL cholesterol given peel extract of Garcinia mangostana L. was conducted on the 22nd day. The analysis showed that giving Garcinia mangostana L. peel extract for various dosages could significantly decrease LDL cholesterol and increase HDL cholesterol (p <0.05). Peel extract of Garcinia mangostana L. that contained mangosteen could increase non-radical products that could prevent the transfer of ester cholesterol from HDL to VLDL which impact in increasing HDL cholesterol and decreasing LDL cholesterol.

***Keywords**: Garcinia mangostana L; peel extract; LDL cholesterol; HDL cholesterol; hypercholesterolemia; health risk*

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Highlights:

1. Ethanol extract of *Garcinia mangostana* L. peel reduce malondialdehyde.
2. *Garcinia mangostana* L. peel extract can increase non-radical products which impact in decreasing LDL cholesterol and increasing HDL cholesterol.

INTRODUCTION

Hypercholesterolemia has a role in the etiology of atherosclerosis. Atherosclerosis contributes to coronary heart disease as the main cause of mortality. WHO data in 2012 stated that 31% of mortality was due to heart disease and 38.8% was due to coronary heart disease that will increase to more than 20 million in 2030. In 2013, the data displayed that 1.5% of diseases in Indonesia were coronary heart disease with hypertension, hypercholesterolemia, and diabetes mellitus as the main risk factors (Ghani et al. 2016, Hussain et al. 2016).

Atherosclerosis occurs due to saturation of LDL receptors. The saturation of LDL receptors converts to oxidized LD*, which is partially absorbed by scavenger receptors and partially taken up by macrophages. If LDL is excessively oxidized, macrophages will transform into foam cells found in early atherosclerotic lesions (Stephen et al. 2012). High consumption of cholesterol, stress, and smoking can increase the secretion of VLDL by the liver which impacts in increasing IDL that forms LDL (Trajkovska & Topuzovska 2017, Macho-González et al. 2019, Sinulingga et al. 2019), and increasing cholesterol in cells (Maisaroh et al. 2020).

Consuming unsaturated fats can lower blood cholesterol by inhibiting the conversion of cholesterol to be bile acids (Liu et al. 2017). Exercise, stop smoking, reducing body weight, and providing hypolipidemic drugs, especially herbs can increase HDL cholesterol, decrease LDL cholesterol, and increase the expression of lipoprotein lipases. *Garcinia mangostana L.* peel extract can increase HDL (Raharjo & Monica 2015), and decrease LDL through transporting back to the liver (Arozal et al. 2020).

If diet modification and lifestyle management fails, hypolipidemic drugs can be alternative to be given. Hypolipidemic drugs have the effect of gastrointestinal disorders, skin rashes, and even liver dysfunction. Besides, there are contraindications which make not all people can consume the drug due to a high potential-risk (Wirasuta et al. 2014). Herbal hypolipidemic treatment is active to be conducted, because ethanol extract of *Garcinia mangostana L.* Peel that contains antioxidant mangosteen can increase HDL and decrease LDL through transporting back to the liver by increasing non-radical products (Arozal et al. 2020).

MATERIALS AND METHODS

The design of this study was a post test control group between *Garcinia mangostana L.* peel extract and HDL and LDL. This study was experimental laboratory research with a population of hypercholeterolemic male mice aged 3-4 weeks with 100-200 grams. This study used an enzymatic method by spectrophotometer to collect HDL and LDL cholesterol data, and analyzed using analysis of variance (Anova) with significance level of $\alpha < 0.05$.

Hypercholesterolemia in white mice was made by feeding a high-fat diet. Experiment of giving ethanol extract of *Garcinia mangostana L.* peel was suspended in 1% carboxymethyl cellulose in hypercholesterolemic white mice which was grouped into 7 groups with 6 mice in each group. Negative control group, positive control group, and treatment group were in accordance with the dosage variations of 50, 150, 250, and 350 mg/kgBW. The examination of hypercholesterolemia of white mice was conducted on the 8th day. Meanwhile, the examination of HDL and LDL cholesterol given ethanol extract of *Garcinia mangostana L.* peel was conducted on the 22nd day.

RESULTS

The results showed that LDL cholesterol after being induced by MDTL increased significantly with a significance level of p=0.021 rather than before being induced. Meanwhile, HDL cholesterol after being induced did not increase significantly with a significance level of p=0.620.

Table 1. Different HDL and LDL cholesterol tests in experiment group before and after being induced by high fat diet foods (*makanan diet tinggi lemak (MDTL))*

Dependent variable	Group		Sig. (p)
	Before (Group 1) N = 6	After (Group 2) N = 6	
HDL cholesterol (Mean ±SD)	21.67 (± 2.21)	20.00 (± 6.19)	0.620
LDL cholesterol (Mean ±SD)	7.50 (± 0.83)	14.67(± 2.06)	0.021

Table 2. Different test with ANOVA variable based on control and treatment group of *Garcinia mangostana L.* peel extract

Dependent variable	F count	Sig.
HDL cholesterol	3.410	0.023
LDL cholesterol	5.482	0.003

The results showed that there was a significant difference of reduction in LDL cholesterol between control group and treatment group by giving ethanol extract of *Garcinia mangostana L.* peel in accordance with dosage variations of 50mg, 150mg, 250mg, and 350 mg/kgBW with significance level of p=0.003 and significant increase in HDL cholesterol with significance level of p=0.023 (<0.05).

Table 3. Least significant differences (LSD) test against HDL cholesterol variable

Group	Control	Dose 50 mg	Dose 150 mg	Dose 250 mg
Dose 50 mg (Sig.)	0.311	-	-	-
Dose 150 mg (Sig.)	0.560	0.662	-	-
Dose 250 mg (Sig.)	0.249	0.036	0.089	-
Dose 350 mg (Sig.)	0.042	0.004	0.011	0.346
Standard of Error	= 2.257			

The results showed that there was a significant increase between control group and group of 350 mg dose (p=0.036) and between group of 50 mg dose, group of 250 mg dose (p=0.036), and group of 350 mg dose (p=0.004). However, there was a significant increase between group of 150 mg dose and group of 350 mg dose (p=0.011)

The results showed that there was a significant decrease between groups with 50 mg dose (p=0.003), 150 mg dose (p=0.036), 250 mg dose (p=0.001), and 350 mg dose (p=0.002).

Table 4. Least significant differences (LSD) test against LDL cholesterol variable

Group	Control	Dose 50 mg	Dose 150 mg	Dose 250 mg
Dose 50 mg (Sig.)	0.003	-	-	-
Dose 150 mg (Sig.)	0.000	0.427	-	-
Dose 250 mg (Sig.)	0.001	0.569	0.819	-
Dose 350 mg (Sig.)	0.002	0.819	0.569	0.732
Standard of Error	= 1.444			

DISCUSSION

Induction of high-fat diet foods (MDTL) to make hypercholeterolemic mice

The results of the study showed that after MDTL induction, LDL cholesterol increased significantly with p=0.021, while HDL cholesterol did not increase with p=0.620. MDTL was made from palm oil which contained triacylglycerol saturated fat and beef fat that contained sterols, and it was induced into mice. Before being absorbed by the intestine, triglycerides were firstly emulated by bile acids (Sagar et al. 2016). In epithelial cells of intestine, triacylglycerols together with proteins, phospholipids, and cholesterol esters, combine to form chylomicrons.Chylomicrons are hydrolyzed into fatty acids and glycerol which then enters tissue cells to be converted into energy. Sterols are the components of cholesterol.

Mechanism in increasing cholesterol due to induction of high-fat diet foods (MDTL) is estimated to cause an increase in saturated fatty acids in the blood. It can impact in reducing LDL receptors and increasing to form VLDL particles which are smaller precursors of LDL and smaller VLDL that contains a lot of cholesterol (Carson et al. 2019).Furthermore, a decrease in LDL receptors and an increase in VLDL particles impacts in amassing of LDL cholesterol in the blood (hypercholesterolemia).

The increase of VLDL particles impacts the transfer of triacylglycerols from VLDL to HDL and cholesterol esters from HDL to VLDL mediated by CETP (cholesteryl ester transfer protein). This transfer impacts the formation of HDL core that is richer in triacylglycerol instead of cholesterol esters (known as small dense HDL). The increase in the formation of small dense HDL impacts in reducing HDL formation (Ahn & Kim 2016).

The increase of HDL cholesterol after given ethanol extract of *Garcinia mangostana L.* peel

The result of the study was also found through different Anova test found in treatment group that was given ethanol extract of *Garcinia mangostana L*. peel in accordance with dosage variation which showed that there was a significant increase for HDL cholesterol with significance level of p=0.023.LSD test was conducted between control group and 350 mg dose (p=0.036), between group of 50 mg dose and group of 250 mg dose (p=0.036) as well as group of 350 mg dose (p=0.004). Furthermore, there was a significant increase between the group of 150 mg dose and the group of 350 mg dose (p= 0.011).

The ethanol extract of *Garcinia mangostana L*. peel which contains mangosteen (Pratiwi et al. 2016), where it can effectively save α-tocopherol (Nakatomi 2004). α-tocopherol is a chain-breaking antioxidant and donor of hydrogen phenolic (Akbari et al. 2016, Viglianisi & Menichetti 2019, Polumbryk et al. 2013), as well as a substitute for tocopheryl radicals which are less reactive (α-TO•). α-tocopherol is also a direct reagent with radical initiation to prevent the formation of LOO in non-radical production, and one of which is Malondialdehyde decreases (damaged).

The decreased or broken Malondialdehyde can reduce adducts with amino acid side chains from apolipoprotein B-100 (Murray et al. 2009), and it impacts on the decrease of interaction and absorption of oxidized LDL. This decrease impacts on the decrease of oxidized LDL taken by macrophages, so that the co-substrate of fatty acyl by enzyme Acyl-CoA cholesterol transferase (ACAT) in macrophages containing oxidized LDL is fulfilled. This adequacy can inhibit the action of HMG-CoA reductase enzyme in cell membrane (Brown et al. 2001, Snaebjornsson et al. 2020). Hence, VLDL blood decreases (Murray et al. 2009).

The decrease of VLDL will prevent the transfer of triacylglycerol from VLDL to HDL and cholesterol ester from HDL to VLDL mediated by CETP (cholesteryl ester transfer protein) that impacts on forming HDL core which is richer in triacylglycerol instead of cholesterol ester (known as small dense HDL) that decreases. The decrease in forming small dense HDL impacts on the increase of forming HDL (Murray et al. 2009). Providing pure mangosteen suspension showed a significant increase in serum of HDL cholesterol (Raharjo & Monica 2015 stephen).

Decrease of LDL cholesterol after given ethanol extract of *Garcinia mangostana L.*peel

The result of the different Anova test was found in the treatment group given ethanol extract of *Garcinia mangostana L*. peel in accordance with dosage variations which showed that there was a highly

significant decrease in LDL cholesterol with significance level of p=0.003. Meanwhile, the LSD test showed a significant decrease between control group and groups of 50 mg dose (p=0.003), 150 mg dose (p=0.036), 250 mg dose (p=0.001), and 350 mg dose (p=0.002).

The ethanol extract of *Garcinia mangostana L.* peel that contains antioxidant mangosteen can increase HDL. HDL plays a role in Reverse Cholesterol Transport. HDL absorbs cholesterol from the tissues and lecithin cholesterol acyltransferase (LCAT) esterified and precipitates it in the middle of the particles. Cholesteryl ester in HDL is absorbed in the liver either directly or after moving to VLDL, IDL or LDL through the cholesteryl ester transfer protein. Excess cholesterol is excreted from the liver into the bile as cholesterol or bile salts (Murray et al. 2009). The increase in HDL impacts the increase in Reverse Cholesterol Transport through the liver to the bile which impacts the decrease of LDL cholesterol (Murray et al. 2009).

The decrease of LDL also occurs due to the inhibition of malondialdehyde (MDA) formation by extract of *Garcinia mangostana L.* peel (Williams et al. 1995). Decrease or broken malondialdehyde impacts in reducing the adduct between malondialdehyde and amino acid side chain from apolipoprotein B-100 (Murray et al. 2009), and reducing the interaction and absorption of oxidized LDL by lower affinity systems (known as scavenger receptors) (Murray et al. 2009). Due to the reduced interaction and absorption of oxidized LDL, oxidized LDL is also less which is taken by the macrophages. Thus, co-substrate of fatty acyl by Acyl-CoA enzyme; cholesterol transferase (ACAT) in macrophages that is contained oxidized LDL is fulfilled and it will be able to inhibit the enzyme HMG-CoA reductase in cell membrane (Murray et al. 2009) as well as impacting on the decrease of LDL cholesterol synthesis (Murray et al. 2009).

Strength and limitation

The study employed different dosages of the extract, which helps in identifying the optimal dose and dose-response relationship. This study used an experimental design with a post-test control group, which is a rigorous approach to test the efficacy of the intervention.The study did not investigate the long-term effects of Garcinia mangostana L. peel extract on cholesterol levels and cardiovascular outcomes,and control for other potential confounding factors, such as diet and physical activity, which could influence the results, and examine the mechanism by which the peel extract reduces LDL and increases HDL cholesterol levels.

CONCLUSION

Ethanol extract of *Garcinia mangostana L.* peel that contains mangosteen can increase Non-Radical Products (NRP) and reduce or damage radical products, such as malondialdehyde. Decreased or broken malondialdehyde can prevent the transfer of triacylglycerol from VLDL to HDL and ester cholesterol from HDL to VLDL which impacts the increase of HDL cholesterol. Besides, the decreased or broken malondialdehyde can reduce the interaction and absorption of oxidized LDL by the scavenger receptor as well as the increase of reverse cholesterol transport through the liver to bile by HDL that impacts to reduce LDL cholesterol.

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Conflict of interest

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Author contribution

The author was contributed to the all conceptualization, study design and methodology. data collection, write, and final revision.

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Original Research

COMPLAINTS OF MUSCULOSKELETAL DISORDERS ON SALES PROMOTION GIRL AT COSMETIC STORES IN SURABAYA, INDONESIA

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ABSTRACT

One of the occupations that have a risk of Musculoskeletal Disorders (MSDs) is sales promotion girl (SPG), especially the SPG at a cosmetic store, because their job position is mostly done by standing and wearing high heels. This study aimed to analyze the correlation between duration of standing position and shoe characteristics to complaints of MSDs on sales promotion girl at cosmetic stores in Surabaya. This study was quantitative research with observational analytic research, where the data collection was carried out by cross-sectional. This research was conducted at 21 cosmetic stores from three shopping centers in Central Surabaya. The population in this study was 64 SPG. The sample was taken using simple random sampling with exclusion criteria, so that 47 people were obtained. The independent variables consisted of duration of standing position and shoe characteristics included shoe type and the height of heels. The dependent variable was complaint of MSDs. The data analysis was carried out using the Spearman correlation test. The result showed that there was a correlation between the duration of standing position and complaints of MSDs (Pvalue=0.011; r=0.369; positive direction), no correlation between shoe type and complaints of MSDs (Pvalue=0.735; r=0.051; positive direction), and no correlation between the height of heels and complaints of MSDs (Pvalue=0.256; r=0.169; positive direction). It was concluded that there was a correlation between the duration of standing position and complaints of MSDs, and there was no correlation between shoe type and the height of heels with complaints of MSDs.

***Keywords**: Health risk; musculoskeletal disorders; sales promotion girl*

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Highlights:

1. Standing position duration and shoe characteristics to MSDs's complaints on sales promotion girls at cosmetic stores in Surabaya were analyzed.
2. There was no correlation between height of heels with complaints of MSDs and shoe type, while, the duration of standing position and complaints of MSDs were correlated.

INTRODUCTION

Occupational health is one of the efforts to keep the workforce physically, mentally, and socially healthy while working, whereas occupational diseases is a health problem that is physically and psychologically caused by work activity or condition that is related to the occupation (Winoto 2018). One of the factors that causes occupational disease is the ergonomic factor. The ergonomic factor is related to posture or inappropriate body movement while working (Suma'mur 2020). The proper application of ergonomics will increase the effectivity and reliability of the work system, reduce health care cost of workers, increase the workers benefit, increase the quality of work processes, products, and productivity (Tarwaka 2014). If ergonomic is not applied appropriately, it will lead to work-related musculoskeletal disorders or MSDs.

MSDs is disorder on body's structures, such as muscles, joints, ligaments, tendons, bones, cartilages, nerves, local blood circulation system (de Kok et al. 2019). According to Kuswana (2014), some signals that show MSDs are pins and needles, pain, anxiety, burning, numbness, stiffness, swelling, cramps, grip strength in moving hands, changes in body balance, short range of motion, breathlessness, or loss of flexibility. According

to Basic Health Research in 2013, the prevalence magnitude of joint disease experienced by Indonesian based on diagnosis or symptoms is 24.7%, and 70% of these cases are experienced by workers. The MSDs that are experienced by workers have some impacts on the workers themselves and their works as well as producing impacts, such as the limitation of workers' daily activities and the workers' absence (de Kok et al. 2019). Referring to Labor Force Survey from HSE (Health and Safety Executive) in 2018-2019, there are 1,4 million workers in England that get occupational diseases and in the amount of 37%; or it is about 498,000 workers get MSDs. Musculoskeletal disorders have resulted in a reduction of 6,9 million working days.

Three factors can cause MSDs, namely individual factors, biomechanical factors, and psychosocial factors. The individual factor is a factor that comes up from the workers, such as genders, ages, smoking habits, exercise habits, body mass index, and working period. The biomechanical factor is also the factor that comes from the workers including body posture while working, duration, frequency, load, and also exposure to vibrations. Meanwhile, the psychosocial factor is the interaction that occurs among the workers, work environment, work condition, culture, capacity, and fulfillment of works, as well as personal considerations about works that affect performance, satisfaction, and occupational health (Mayasari & Saftarina 2016).

One of the occupations that have a risk of MSDs is sales promotion girl (SPG) at a cosmetic store. According to Tarwaka (2014), complaints of MSDs can occur when the muscles get load statically and repeatedly for a long time. Working with standing position for a long time can cause health problem. SPG's job description requires to work by standing and moving longer causes the muscles to get load statically. SPG is an occupation that relates to product promotions and marketing. This occupation is mostly occupied by women that physically good-looking in attracting the customer (Agow 2017). SPG needs to have persuasive speaking, recognize the product being promoted, and have an attractive appearance (Dewi & Duana 2013).

One of the supporting factors for an attractive appearance at SPG at cosmetic store is the use of high heels. For SPG, the use of high heels while working is the risk factor of MSDs. High heel is a type of footwear that forces the proximal part to be higher than the forefoot or distal during standing (Sysbania et al. 2018). According to Mika et al. (2012), muscle activity will increase and may exacerbate muscle fatigue when wearing high heels, and permanent wearing of high heels could excessive muscle use and repetitive strain injuries. The height of heels that are safe for health is about 3 to 4 cm (Purba et al 2015).

Based on Dewi and Duana (2013), 97% from 92 SPG mall in Denpasar that wear high heels while working get complaints of MSDs and the higher complaint occurs on the lower extremities, especially on the right and left feet, right and left calves. This complaint increases along with the increase of age, the height of the heels, and the duration of wearing high heels. However, Destiana et al. (2015), showed that a shopkeeper at X Department Store in Semarang wears ≥5 cm high heels and medium narrow heels and has a higher risk of getting pain in the lower back. Besides, a work position that is at risk of causing MSDs to SPG is the standing while working and the length of standing position is carried out.

One of the SPG fields that are at risk of MSDs is cosmetics SPG. Based on Sakale and Bhalekar (2019) on the SPG department store in India stated that cosmetics SPG has a low risk of MSDs on, the knee, moderate risk on the arms, and high risk on the back and neck. While, according to Sinta et al. (2014) in Manado, their research gained 97% from 30 people cosmetics SPG gets MSDs complaints on the mild to severity level.

Based on the previous research that was done on SPG cosmetic store in Surabaya, it was revealed that 5 of 8 people said that they ever had complaint of MSDs, while working and most of their job was done by standing up. These 8 SPGs wore a various type of shoes namely flat shoes, high heels until it had a pointed right with a heel height of between 3 cm to 7 cm. Based on that background explained, the purpose of this research was to analyze the correlation between the duration of standing work position and shoe characteristics with complaints of musculoskeletal disorders on sales promotion girl at cosmetic stores in Surabaya, Indonesia.

MATERIALS AND METHODS

This study was a quantitative research with analytic observational research, where the data were collected by cross-sectional method. This study was conducted on SPG at twenty-one cosmetics stores from three shopping centers in Central Surabaya from November 2020–March 2021. The cosmetic stores were specific to boutique stores, where they sold only one brand of cosmetic products. The population in this research was 64 SPG. The research sample was 55 SPG and taken by simple random sampling. The exclusion criteria in this study were pregnant women and/or people with a history of injury, disease, and surgery to the musculoskeletal system. There were 8 people from the sample who were not further included in this study, because they were in the exclusion criteria, so that the sample of this study was 47 people.

The independent variables were the duration of standing work position and shoe characteristics. Shoe characteristics included shoe type and the height of heels. Meanwhile, the dependent variable was the complaints of musculoskeletal disorders felt by the workers. The data were divided into primary and secondary data. The primary data included the data about duration of standing work position, shoe characteristics of respondents, and complaints of MSDs obtained through an online questionnaire by Google Form and observation. The secondary data were obtained from journal articles and books (included e-book) that were relevant to the object being studied.

Complaints of MSDs variables assessed using the Nordic Body Map (NBM) questionnaire as a method to measure the level of the severity of any disorders or injuries that occurred in the musculoskeletal system (Tarwaka 2014). The respondent measured subjectively their level of severity on their 28 body parts by rating 1 to 4, 1 meant no pain, 2 meant less painful, 3 meant painful, and 4 meant severe pain. The score of each body part was summed up into an NBM score ranging from 28 to 112. The NBM score was categorized into 4 (four) categories, namely 28-49 (low), 50-70 (moderate), 71-91 (high), and 92-112 (very high).

The data obtained were then inputted and processed using SPSS version 25. The data were analyzed by univariate and bivariate analysis. The univariate analysis described the data result through a table and graphic. Meanwhile, bivariate analysis was used to see the correlation among variables using Spearman Correlation Test.

The data obtained were then inputted and processed using SPSS version 25. The data were analyzed by univariate and bivariate analysis. The univariate analysis described the data result through a table and graphic. Meanwhile, bivariate analysis was used to see the correlation among variables using Spearman Correlation Test. This study had received an approval from the Ethics Commission of the Faculty of Dentistry, Universitas Airlangga, Surabaya under a decree No. Ethics 046/HRECC.FODM/II/2021.

RESULTS

The activities that were done by the respondents during working were standing up to welcome and serve customers, arrange stock items on shelves with various shapes and heights, and record sales. The respondent's working hours were 48 hours per week. The respondents' ages ranged from 18 years to 41 years old.

position category was 5-7 hours in one shift, 16 respondents (34.0%) in 3-4 hour category, 8 respondents (17%) in 8 - 9 hours, 2 respondents (4.3%) in ≤ 2 hours' category, and 1 respondent (2.1%) in > 9-hour category. Based on shoe type that was worn while working, there were 21 respondents wearing flat shoes (44.7%), 16 respondents with pointy high heel shoes (34.0%), and 10 respondents with high heel wedges shoes (21.3%). Based on the height of heels, it showed that the most of respondents wore high heels <5 cm with a total of 26 people (55.3%), and those who wore high heels ≥ 5 cm were 21 people (44.7%).

Table 1. Respondent characteristics based on standing duration and shoe characteristics

Variables	n	%
Standing duration		
≤ 2 hours	2	4.3
3 – 4 hours	16	34.0
5 – 7 hours	20	42.6
8 – 9 hours	8	17.0
> 9 hours	1	2.1
Total	47	100
Shoe types		
Flat shoes	21	44.7
High heels wedges shoes	10	21.3
Pointy high heels shoes	16	34.0
Total	47	100
The height of heels		
< 5 cm	26	55.3
≥ 5 cm	21	44.7
Total	47	100

Table 2. The score of Nordic body map

NBM Score Category	n	%
Low	41	87.2
Moderate	6	12.8
Total	47	100

Based on Table 2, it showed that complaints of MSDs felt by the respondents through the NBM score were in the low to moderate catagory. The majority of respondents were in the low category with a total of 41 people (87.2%), and respondents who were in the moderate category were 6 people (12.8%). Furthermore, complaints of MSDs felt by respondents based on each body part in the NBM questionnaire, obtained five higher scores on the waist with a score of 93, left calf with a score of 90, back with a score of 86, right calf with a score of 83, and left knee with a score of 72. This result indicated that the most severe complaints felt by respondents were on the waist, back, left and right calf, and left foot.

The Spearman's test result on the duration of standing position and complaints of MSDs obtained a p-value of 0.011, the p-value α(0.05) indicated that two variables had a significant correlation with a correlation level of 0.369. This showed that the correlation was weak, and the direction of the correlation was positive. It showed that the longer the duration of standing work position, the higher was the NBM score. It also meant that the more severe the MSDs complaints were felt (Table 3).

The Spearman's test result between shoe type and complaints of MSDs obtained p-value of 0.735. The p-value α(0.05) indicated that there was no correlation between shoe type and complaints of MSDs. The coefficient correlation was 0.051, which showed that the correlation between two variables were very weak and the direction was positive. It showed that the higher of respondents' shoe type category, the higher was the NBM score. It meant that the complaints of MSDs were worse.

Then, the Spearman's test result on variable of the height of heels and complaints of MSDs obtained p-value of 0.256. The p-value α(0.05) indicated that there was no correlation between the height of heels and complaints of MSDs. The coefficient correlation was 0.169, which showed that the correlation between two variables were very weak and positive. It showed that the higher the category of respondents' shoe heels, the higher was the NBM score and the more severe the complaints of MSDs were felt.

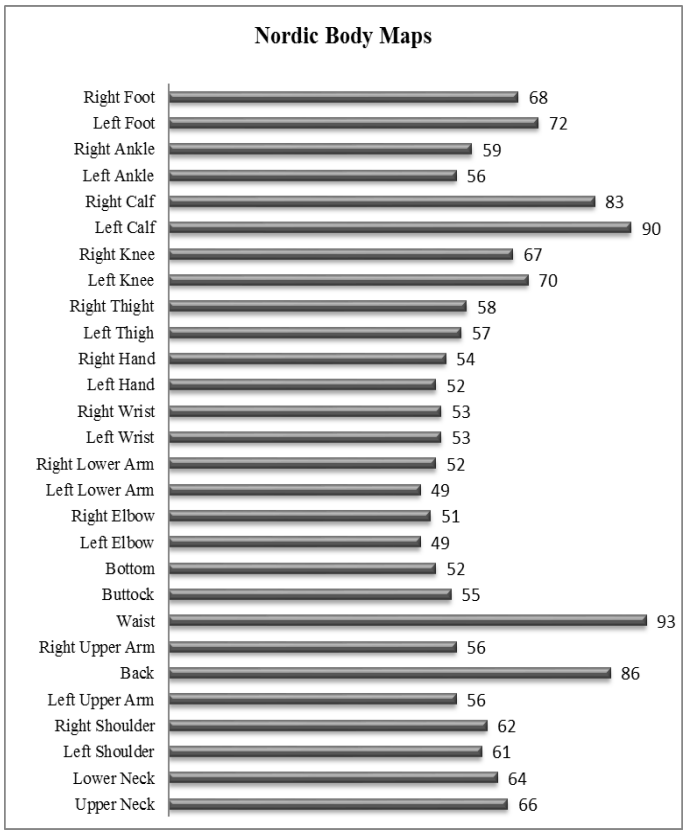

Figure 1. Complaint distribution graph according to body parts

Table 3. Spearman correlation test results

Variable			
Standing duration	Correlation Coefficient	1.000	.369*
	Sig. (2-tailed)	.	.011
Shoe type	Correlation Coefficient	1.000	.051
	Sig. (2-tailed)	.	.735
The height of heels	Correlation Coefficient	1.000	.169
	Sig. (2-tailed)	.	.256

DISCUSSION

The duration of standing work position with complaints of MSDs has a significant correlation and the strength of correlation is weak and positive. The higher standing work position category is experienced by respondents, the higher is the level of complaints of MSDs severity. The longer the duration of standing work position, the more severe complaints of MSDs are felt. Some activities that are done by standing are such waiting and serving the customers, checking and arranging products on the shelves.

Most of respondents felt that most of their working time was done by standing position. This was also in line with a study by Lestari (2012) that mostly 87.5% of SPG working time was spent on standing and actively moving. The assessment was done by using one instrument to assess body posture, namely REBA (Rapid Entire Body Assessment) when SPG tidied the product in a standing position, which resulted in a high level of ergonomic risk or high-risk category.

The result was also in line with a study by Arih et al. (2013) that varicose veins or abnormal blood vessels often attacked in people who stood too long, especially when wearing high heels. People with varicose veins usually felt pain and cramps in the legs. Meanwhile, Ramdan and Laksmono (2012) stated that standing up was more tiring than sitting, because the energy expended was 10-15% more than sitting. Standing is a good alert attitude both physically and mentally, so that work activities are carried out stronger, faster, and more thoroughly.

According to a study by Nadrah et al. (2018), there was also poor standing position and good standing position found in their study. The MSDs symptoms among sales woman were different among sales woman with poor and good standing position. Sales woman with poor standing position had higher MSDs symptoms than those with good standing position. Good or natural standing position is the straight position of back and head with opened feet. Poor standing position is leaning against a display window or a wall repeatedly and standing with one leg.

According to a study by Anggrianti et al. (2017), there was relationship of standing work posture with complaints of leg pain among mechanical workers in the welding section. There were changes in the body system when standing for a long time. There were three effects when a person stood for a long time without stretching or resting. These impacts were infusion of back flow of blood to the legs, pressure on the joints, and fatigue in the muscles. According to Tarwaka (2014), working with standing position for a long time was hard and resulted in low back pain, tension in the calves and legs, fatigue in the musculoskeletal system, and other health problems.

The shoe type with complaints of MSDs has no correlation. The degree of correlation strength is very weak and the direction is positive. The higher the respondent's shoe type category, the more severe of complaints of MSDs are felt. Shoes that have pointy, narrow, and wedges heels are riskier than flat shoes or shoes that do not have heels. The smaller width of the heels worn by the respondents, the higher of musculoskeletal complaints they felt. This result correlated to a study by Yohana and Winata (2017), that there was no correlation between shoe type and musculoskeletal complaint on the lower back (low back pain), the strength correlation between shoe type and low back pain are very weakly. This finding correlated with a study by Amaliyah et al. (2020), that smaller the surface area of the bottom of respondent's shoes, the greater complaints of musculoskeletal foot and ankle experienced.

According to Destiana et al. (2015), salespeople who wore shoes with narrow and pointy heels and medium heels have 8 times greater risk of experiencing complaints than those who wore flat-heeled types. A study conducted by Mustafa et al. (2020) had showed that even medium heels shoe type was more comfortable than narrow heels shoe type, however, both type of shoes could cause pain in the leg and heels. It was different when wearing flat shoes, where the pressure was given and rested on a larger surface, so that the pressure became smaller. When the type of heel shrinked and the area of the footing decreased, it could cause an imbalance of the ankle due to changes in the tibiotarsal angle. This change resulted in elevation and forward displacement of the gravity center resulting in a postural imbalance.

The height of heels with complaints of MSDs had no correlation. The degree of correlation strength was very weak and the direction was positive. The higher the category of respondents' shoe heels, the higher was the severity of the complaints of MSDs they felt. The higher the heel of the shoe, the more severe complaints of MSDs were felt. The result was in line with a study by Yohana and Winata (2017), that there was no correlation between the height of shoes and musculoskeletal complaint on the lower back or low back pain, while the correlation between two variables were weak.

According to Dewi and Duana (2013), musculoskeletal complaints increased along with the height of the heels.

The SPG that did not experience musculoskeletal complaints was SPG who wore shoes with a heel height of 1 cm - 5 cm, while the high-level musculoskeletal complaints were mostly experienced by SPG who wore shoes with heels >5 cm. According to Purba et al. (2015), the SPG with a higher heel 7 cm height had more musculoskeletal complaints in the lower extremity muscles compared to SPG who wore shoes with shorter heels, namely 5 cm and 6 cm.

The normal human body load when standing and walking was distributed by 90% on the heel and 10% on the front foot. However, when using high heels, the distribution pattern changed to 50% on the front foot and 50% on the heel (Rao et al. 2012). Due to this distribution, the body made any adjustment. The normal adjustments were to firstly raise the head, bend the back causing curvature of the spine, and bend the knees. Secondly, pulled the upper part of the body which caused a large flexion angle of the knee joint and bend of the spine. Thirdly, an excessive extension of the knee and pulled the body back caused the spinal curve to increase (Dewi & Duana 2013).

The use of high heels causes complaints of MSDs mainly on the legs, waist, and back. In this study, it resulted in the five highest scores for the severity of respondents that were on the waist, back, left and right calf, and left foot. This result was in line with a study by Lestari (2012) that the parts of body that experienced the most symptoms of CTDs or Cumulative Trauma Disorders on SPG were the right and left calves, buttocks, waist, back, right upper arms, left and right knees, and left and right ankles. In addition, Dewi and Duana (2013) also stated that most of musculoskeletal complaints in SPG occurred in the lower lamb muscles, especially the legs and calves, and the trunk muscle, especially the back and waist. Shoes can support women's activities and benefit for their health and fashion. Good shoes are shoes that fulfill those two functions. In terms of fashion, shoes can support appearance, while in terms of health, shoes can maintain cleanliness, protect the feet, and help the feet to support the body. The use of high heels does fulfilling a function in terms of fashion, but has a high enough risk to health. The use of high heels causes negative impact for health, such as varicose vens, hallux vagus, and plantar fasciitis (Wulan & Rahayu 2016).

Based on the observation, the choice of shoes worn by the respondents was also related to the regulations for each cosmetic brand in each outlet. These regulations included the mandatory use of flat shoes, such as loafers or sneakers, so that some of them wore shoes with at least 5 cm heels. However, it was also found that respondents chose to wear shoes with higher heels than required by the company due to their preference for high heels. Besides, some companies did not apply special regulations regarding the type and height of heels; they just recommended to wear shoes according to the working conditions, but workers prefer to wear shoes with high heels.

Strength and limittaion

The limitation of the study was the small population size. Further studies were recommended to conduct similar research with larger population size. It was also recommended to conduct similar research by considering and assessing other possible factors, such as psychosocial factors and individual factors including ages, exercise habit, work period and body mass index. Besides, there was a necessary to use other empirical viewpoints that could assess the severity of complaints of MSDs by focusing on the waist, back, and leg areas.

CONCLUSION

Complaints of MSDs that were felt by the respondents ranged from low to moderate category. The mostly felt complaints were on the waist, back, left and right calf, and left foot. The variable standing work duration had a significant correlation with complaints of MSDs, while the direction of correlation was positive. Type of shoes and the height of heels variable had no significant correlation with complaints of MSDs.

Sales promotion girls were suggested to sit down when there was no task that required to take a standing position. They were also suggested to standing with a good posture and not resting on one leg, bring and wear flat footwear, such as sandals or flat shoes when heading to work, resting, and returning from work to reduce exposure time to high heels. It was also suggested to stretch or warm-up before and after work to reduce the risk of pain in the musculoskeletal system.

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Conflict of interest

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Author contribution

SDM wrote the manusript. SDM and NW contributed to conceptualization, collected and analysis data. NW was final authorization of the completed work.

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Original Research

POEDJI ROCHJATI SCORE CARD AS MEDIA FOR PRECONCEPTION COUNSELING

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ABSTRACT

Preconception care (PCC) is a health approach that includes promotive and preventive activities to detect risk factors and interventions carried out to expectant mothers by considering biological, behavioral, and social aspects that affect their health. This study studied the effect of PCC counseling using Poedji Rochjati Score Card (PRSC) on premarital knowledge about high-risk pregnancies. This study was quasi-experimental with 52 respondents divided into 4 groups, namely intervention and control groups, 2 groups with partners, and 2 groups without partners that were chosen through total sampling by applying inclusion and exclusion criteria. Knowledge was measured using pretest and posttest questionnaires. The intervention given was in the form of counseling using PRSC for ± 20 minutes. There were differences in knowledge between the intervention and control group among respondents without partners. Preconception counseling using PRSC was effective for women attending premarital health checks. Applying PRSC as media for counseling could improve knowledge in high-risk pregnancy that could be avoided.

Keywords*: Preconception counseling; poedji rochjati score card; maternal health*

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Highlights:

1. The PCC counseling effect using Poedji Rochjati Score Card (PRSC) on premarital knowledge about high-risk pregnancies was studied.
2. PRSC for preconception counseling is effective for women to improve knowledge about high-isk pregnancy.

INTRODUCTION

Maternal and Child Health (MCH) is an indicator in determining the health of the community, even the success of the public health care system. According to the Department of Health, Surabaya in 2018, as the nationwide case, the major causes of maternal death were preeclampsia/eclampsia, postpartum bleeding, and infection, while other causes are minor. It has shown that the lack of knowledge of mothers about pregnancy was one of the factors that influence maternal mortality (Sari et al. 2014). Another study found that a mother's antenatal care (ANC) regularity had no relation with early detection of maternal mortality causes (Dewi & Sulistiyono 2015), thus suggesting the implementation of preconception care. Preconception care (PCC) is a medical approach that includes promotion and prevention activities to detect risk factors and interventions to consider in terms of biological, social behavior, and maternal health (Steel et al. 2016). Meanwhile, antenatal care (ANC) given to mothers during pregnancy aims to improve the mother and baby's health (Alemu & Aragaw 2018).

In other words, PCC can be defined as a series of interventions that aim to improve the quality of women's health and conception outcomes through prevention and management of risk factors (King et al. 2019). Moreover, PCC aims to improve a partner's ability to make decisions related to their reproductive health (Zee et al. 2013). Optimizing health before conception was important for men and women in reproductive age because of maternal and paternal factors that affect preconception health. However, the understanding of the PCC importance was still low (Lan et al. 2017).

Various complications in pregnancy and childbirth are associated with risk factors during organogenesis, and can actually be modified. However, most of the complications actually appeared before the pregnancy occurred, which is known as the preconception period (Poels et al. 2018). Thus, preconception counseling was needed as a preventive health promotion activity from biomedical side, health behavior, and social behavior of a person before pregnancy to improve health conditions in preparing healthy mothers and babies (WHO 2012).

The Regulation of the Ministry of Health Number 97 of 2014, had provided reproductive health services for teenagers as well as female adults prior to their pregnancies, and required that premarital health check and counseling could be undertaken before a marriage certificate was issued. Midwives, especially those working in public health service providing preconception care within pre-marital health check, can take active roles as counselors.

The counselling target was huge as pre-marital health-check was required nationally. Additionally, based on the Center of Statistics for Indonesia in 2018, adolescence reproductive health has not been integrated in schools and non-health related college's curricula, while the percentage of married women aged <21 years was 58.65% contributing to pregnancies among women aged <21 years which reached 45.99%. A 75-page pocket book on reproductive health for bride and groom has been issued by the Ministry of Health, but its use in pre-marital counseling and effectiveness have not been evaluated. With the tight schedule, there are worries on whether midwives can provide enough time to discuss the book with the target. A simpler education media for counseling highlighting the risks for developing high-risk pregnancy needed to raise the awareness of bride-and-groom-to-be on the matter to minimize their own risks. This study aimed to improve the knowledge of the bride-groom-to-be on high-risk pregnancy through counseling using PRSC.

MATERIALS AND METHODS

This was a quasi-experimental study designed to examine the effect of counselling on bride's with and without groom's knowledge on high-risk pregnancies. Using Federer's formula, sample size was determined, and 52 respondents were recruited, 13 respondents were non-randomly assigned to each of four groups that were 2 intervention and 2 control groups. Consecutive sampling was applied. Evaluation was done based on pre- and post-tests later after intervention. Variables in this study were counselling with the use of PRSC and knowledge about high-risk pregnancy.

PRSC is a score card that is used as a family-based antenatal screening tool to find risk factors for pregnant women. Furthermore, the card makes it easier to recognize conditions experienced by pregnant women to prevent obstetric complications during childbirth. This card has been used especially in East Java to group of pregnant women at risk according to the score and early detection of high-risk pregnancy. The counselling technique used was that the researchers and respondents sat facing one another. 1 PRSC was given to respondents who came with or without their partners and 1 for the researchers. According to risk factors for pregnant women, the counselling materials were described respectively all 20 items, namely 1) too young to be pregnant (≤ 16 years); 2) too old for the first pregnancy (≥ 35 years); 3) pregnancy interval ≥ 10 years; 4) pregnancy interval ≤ 2 years; 5) having > 4 children; 6) too old to be pregnant (≥ 35 years); 7) too short (≤ 145 cm); 8) history of miscarriage; 9) previous labour with forceps/ vacuum, manual placenta, infusion/ transfusion; 10) history of caesarean section; 11) disease in pregnancy (anaemia, malaria, TBC, cardiac disease, diabetes, STI); 12) edema in pregnancy (face and feet) and high blood pressure; 13) twin pregnancy; 14) hydramnion; 15) intra uterine fatal death; 16) postdate; 17) breech position; 18) transverse lie position; 19) bleeding during pregnancy; 20) preeclampsia/eclampsia.

Counseling was delivered for 20 minutes. Knowledge was assessed prior and after interventions using questionnaires in pre- and post-tests. To evaluate the difference before and after counseling knowledge, this study used paired-t test for normally distributed data, while Wilcoxon signed-rank test was applied when data were not normally distributed. Meanwhile, to determine the effect of counselling that increased knowledge, paired-t test was applied for normally distributed data and Mann-Whitney test was run when data distribution was not normal. This study was approved by the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Universitas Airlangga under a decree No.230/E/KEP /FKUA/2019.

Table 1. Baseline characteristics of respondents

Characteristics	Counseling				Non counseling				Total		P value
	without partners		with partners		Without partners		with partners				
	n	%	n	%	N	%	n	%	N	%	
Age											
<20 years	2	15.4	0	0	0	0	1	7.7	3	5.77	0.243
20-35 years	10	76.9	13	100	13	100	11	84.6	47	90.38	
> 35 years	1	7.7	0	0	0	0	1	7.7	2	3.85	
Total									52	100	
Profession											
Work	10	76.9	12	92.3	11	84.6	11	84.6	44	84.61	0.951
Does not work	3	23.1	1	7.7	2	15.4	2	15.5	8	15.39	
Total									52	100	
Education											
Basic	1	7.7	1	7.7	1	7.7	2	15.4	5	9.62	.994
Secondary	6	46.2	6	46.2	7	53.8	5	38.5	24	46.15	
High	6	46.2	6	46.2	5	38.5	6	46.2	23	44.23	
Total									52	100	
Income											
< minimum	8	61.5	11	84.6	6	46.2	7	53.8	32	61.54	0.283
≥ minimum wage	5	38.5	2	15.4	7	53.8	6	46.2	20	38.46	
Total									52	100	

Data collection was conducted in the Public Health Center, Keputih, Surabaya in October-November 2019. Prior to this study, respondents who met the inclusion and exclusion criteria before had been given an explanation on the research that would be carried out and informed-consent was taken. The intervention groups (with and without partners) were given a pre-test questionnaire, counseling and post-tests respectively, whereas the control groups (with and without partners) were counseled after pre- and immediate post-tests. Data of knowledge were then categorized as well and enough. The intervention used in this study was PRSC as the media of PCC to within ±20 minutes. Each respondent was allowed to ask questions after the counseling material was delivered.

RESULTS

Characteristics of respondents

In this study, the characteristics of respondents were categorized based on their age, education, employment and income status (Table 1).

Knowledge scores on pre- and post-tests

Intervention group

There was an increase in the average score of knowledge held by the intervention, and knowledge of the respondents had quite a good category (Table 2).

Table 2. Knowledge scores within the intervention groups

Statistical	Intervention group			
	without partners		with partners	
	Pre-test	Post-test	Pre-test	Post-test
Average	70.77	85.38	73.85	89.23
Standard deviation	22.9	9.67	19.8	6.4
Median	80	80	80	90
Minimum-Maximum*	30-90	70-100	40-100	80-100
Category Knowledge	Enough	Well	Enough	Well

*) Min-max range of knowledge scores of 0-100

Control group

Table 3. Knowledge scores within control groups

Statistical	Control group			
	without partners		with partners	
	Pre-test	Post-test	Pre-test	Post-test
Average	73.07	70	72.3	70.77
Standard deviation	13.77	10.8	14.23	11.15
Median	70	70	70	70
Minimum-Maximum*	50-100	50-90	40-90	50-90
Category Knowledge	Enough	Enough	Enough	Enough

*) Min-max range of knowledge scores of 0-100

In the control group that did not get the intervention before the posttest, there was no increase in the average score of knowledge and were in the same category between pre-test and post-test.

Effect of counseling on knowledge

In this study, counseling using PRSC significantly increased the knowledge of respondents. It was found that there were significant differences in the average knowledge scores of the respondents in the intervention group.

Table 4. Differences of knowledge scores on pre-test and post-test

Group name	Knowledge score		
	Pre-test	Pre-test	p value
Intervention without partners	70.77	85.38	0.011
Intervention with partners	73.84	89.23	0.032
Control without partners	73.07	70	0.264
Control with partners	72.3	70.77	0.549

Table 5. Differences of knowledge scores between pretest and posttest of intervention and control groups

0.004

Group name	Knowledge score		
	Pre-test	Pre-test	p value
Intervention without partners	70.77	85.38	
Control without partners	73.07	70	
Intervention with partners	73.84	89.23	0.018
Control with partners	72.3	70.77	

Based on Table 4 and Table 5, there was a significant difference between the pre-test and post-test knowledge of the pre-married women without partners and with partners in the intervention and control groups. There was an effect of counseling on increasing knowledge in each group.

Differences of knowledge between with and without partners intervention groups

Table 6. Differences of knowledge scores on pre- and post-tests between groups with and without partners

Group name	Knowledge score		p value
	Pre-test	Post-test	
Intervention without partners	70.77	85.38	.920
Intervention with partners	73.84	89.23	
Control without partners	73.07	70	0.719
Control with partners	72.3	70.77	

The independent-t test in the intervention group with a result of p>0.05 showed that there was no significant difference in increase of the knowledge of prospective brides who were neither with their partners nor with their partners after being given counseling. The Mann-Whitney test in the control group with results p>0.05 showed that there was no significant difference in the increase of knowledge of the prospective bride and groom who were not accompanied by their partners or accompanied by their partners who were not given counseling.

DISCUSSION

Mother's age will determine maternal and new-born health with regard to the condition of pregnancy, childbirth, postpartum and breastfeeding and caring for the baby. It is also in line with the relevant government programs on the Maturation Age of Marriage in women up to 21 years, while data on Demographic and Health Survey in 2017 showed that the median age at first marriage of Indonesian women was in the range of 20-24 years old. Similarly, in accordance with the Indonesian Constitution No. 16 2019 permitted the marriage age when men and women have reached 19 years, as it was expected to reduce the birth rate and decrease the risk of maternal and child mortality.

These findings illustrate that the average bride began to marry after completing secondary school and even through college. Based on Indonesian Ministry of National Development Planning in 2017, these data also indicate that most respondents had met their target, namely to increase the participation of women at school and delay marriage age. Education level helps to determine a person's ability to absorb and understand science (Notoatmodjo 2010). There are external factors that can affect a person's knowledge, namely education, mass media exposure, economic, social relationships and experience (He et al. 2016, Rezaei et al. 2021).

The difference of pre-married knowledge before and after counseling using PRSC may come from clients, counselors, materials and the media given counselors. Media and booklets are important tools in improving clients' knowledge (Farudin 2011), namely the PRSC. Counseling is a method of delivering information provided face-to-face, to assist clients in improving their ability in dealing with problems (Wong et al. 2018). Factors that may affect counseling is a the source, message, the media used, and the factor of the recipient of the information (individual characteristics, social and cultural conditions, the values that exist in the community and experience) (Nasir & Muhith 2009).

The counseling process could also affect the information obtained by the respondent, where this study set the counseling process for a conducive area to an enclosed space according to the respondents and the researchers' deal (agreement).

In this study, the role of counseling significantly increased the knowledge of respondents. It was found that there were significant differences in the average rise of the knowledge possessed by respondents in the intervention group, and the group that was given counseling. Counseling is basically a method that is intended to help a person recognize the condition and the problem today, as well as to find alternatives to resolve the issue (Runcan et al. 2013). In this study, counseling provision was not to find out the problems experienced by the bride and groom as respondents, but for the purposes of prevention (Gibson & Mitchell 2016). This study was in line with Garthwaite and Wilkes's study in 2018 on preconception counseling theory, namely (1) building up awareness of women about problems that can affect pregnancy, so that they can make decisions; (2) managing and modifying risk factors; (3) optimizing management of chronic diseases suffered; and (4) identifying women who are at high risk of having babies with genetic disorders (Garthwaite & Wilkes 2018).

The limitation of this study was not ideal compared to the theory that the ideal interval for measuring the scores of post-test after being given health education was 15-30 days to anticipate the respondents still remember about the pre-test and the possibility that the respondents obtained information from sources other than counseling that had been given (Notoatmodjo 2010). In this study, post-test was administered immediately after the counseling, because knowledge of the respondent was the improvement that occurred purely due to counseling without being affected by other things. In addition, anticipation was also done to anticipate whether the respondents did not come back or not to fill in the post-test questionnaire. Moreover, to determine the effect of counseling on knowledge, this study also found out that there were differences in knowledge between women who came without and with partners, both in the intervention and the control groups. The respondents who came with their partners, allowed to discuss before answering the questionnaires. The results showed no difference in knowledge between women given counselling with and without partners.

This study showed that counselling increased knowledge of brides who came with or without their partners. This finding was in contrast to a study which provided counseling to pregnant women in the 3rd trimester with or without partners about postpartum (Khotimah et al. 2016). Counseling with a companion has the advantage of providing a good additional knowledge to partners or family respondents (Khotimah et al. 2016). Counselings are tailored to client's needs. Therefore, although the authors delivered the intervention as uniform as possible for all clients, there were some unavoidable minor adjustments from client to client. Nevertheless, the main topic of counseling was still about PRSC. During the study, there was no specific schedule of premarital check-up in the public health center, so that it could not be ascertained that every day, there were premarital persons/couples who came, thus taking more time to collect the respondents needed.

In this study, we noticed that when answering the pre-test and post-test questions, the pre-married women who came with their partners and had a discussion to determine the answer options, but basically the women were able to answer the questions presented in the questionnaire herself.

Through this research, it can be concluded that the provision of counseling only to the bride-to-be is sufficient to increase knowledge about high-risk pregnancies. There had been no other studies on premarital women that differ knowledge with or without their partners. Based on the instruction by the Department of Health, for Public Health Centers in Surabaya, health check-up along with reproductive health education should be provided in pre-married couples, unless the bride and groom couples are in the region of different health centers.

A study found that premarital relationships education effectively improved 50-60 % the communication between partner (Fawcet et al. 2010). Similarly, Halford et al. (2008) found that it was important to provide education in premarital phase on the bride and groom to produce a pair of mutual support in terms of health and producing healthy off-springs. Counseling using PRSC as a premarital preparation in knowledge had provided good understanding to the participants about risk factors that could affect maternal health to be avoided.

Strength and limitation

The use of Poedji Rochjati Score Card (PRSC) as a tool for counseling is a practical and comprehensive approach that considers multiple aspects of preconception care. The study focused on an important aspect of reproductive health, preconception care, which can have a significant impact on maternal and fetal outcomes but, did not include a long-term follow-up to assess the

impact of the counseling on actual pregnancy outcomes and control for other potential confounding factors that could influence knowledge about high-risk pregnancies, such as education level or access to information.

CONCLUSION

Counseling using PRSC has a positive impact on the improvement of brides' knowledge on high-risk pregnancy. There was no significant difference in brides' knowledge who came with or without partners. Brides-to-be who get counseling should be able to plan her pregnancy to produce the next generation of healthy and support each other and get used to maintain a healthy life. Midwives can improve premarital knowledge and skills through counseling in preparing for pregnancy and to identify risk factors that could reduce MMR. This study recommended, especially in East Java, where the PRSC was found that PRSC could be used not only to measure and classify the risk in pregnancy, but also to prevent the risks. Developing a premarital counseling program in public health center could also provide positive impact in creating a healthy marriage and improving the pregnancy outcome by managing the risks.

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Conflict of interest

None.

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Author contribution

NH collected, analyzed, and interpreted the data, drafted, revised, and approved the final manuscript. MIAA conceptualized the design of the study, analyzed and interpreted the data, drafted, revised, and approved the final manuscript. SH conceptualized the design of the study, interpreted the data, revised, and approved

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Original Research

HIGH CALORIE DIET WITH A COMBINATION OF INTERMITTENT RESTRICTION AFFECTS THE REPRODUCTIVE CYCLE AND THE UTERINE WEIGHT OF MICE *(Mus musculus)*

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ABSTRACT

Balanced calories affected for body health and daily activities. Lose of energy can disturb the health and daily activities, while over calorie for diets also causes disorders such as metabolic. When there are excess calories in the body will be involved. It can be an obesity risk, diabetes mellitus, fertility disorder, cancer, and cardiovascular disease. Furthermore, in women there can be an increase in menstrual disorders and fertility disorder risk (Silvestris et al., 2018). This study aims to analyze high-calorie diet influence with interval restrictions combination on the reproductive cycle and weight of the uterus in mice. Experimental laboratory is a mouse (mus musculus) female's balb/c as object population. Sampling techniques using probability sampling with simple random sampling type. The study results on fisher's exact test gave a result of $p > 0.05$ so there was no significant difference between control group, high calorie diet group, and high calorie diet with interval restrictions group on the results of vaginal swabs /post-treatment reproductive cycle. The study outcome on robust tests between control, high calorie diet, and high calorie diet with interval restrictions groups. Obtained results ($p < 0.05$) that mean there are some significant discrepancies in the weight of uterine organ between groups. Calories balance in the body can impact female reproductive health.

***Keywords**: High-calorie diet; restriction; health risk; reproductive health; dextrose*

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Highlights:

1. High-calorie diet influence with interval restrictions combination on the reproductive cycle and weight of the uterus in mice were analyzed.
2. Calories balance impact for female reproductive health.

INTRODUCTION

Balanced calories intake affects body health and daily activities. Less and over of calorie affected in the body so it requires a healthy diet with balanced calorie to keep good physical health. According to the Department of Public Health, Ministry of Health of the Republic of Indonesia in 2018, a healthy and balanced diet is a pattern of consumption of foods containing nutrients in the type and amount that suits the needs of the body. Energy and nutrients were depending on age, gender, weight and high, climate, and physical activity. Less of energy can disturb physical health and daily activity, the opposite over of diet calorie could be infected disorder in the body like metabolic disorders. If there is overload calorie in the body, it will cause over weight. When there were excess calories in the body, it will involve overweight. It can be obesity risk, diabetes mellitus, fertility disorder, cancer, and cardiovascular disease. Furthermore, in women, a risk of menstrual disorders and fertility disorder can increase (Silvestris et al. 2018).

The prevalence of obesity in Indonesia is 15,4% until the last 2019. Based on the Ministry of Health in the East Java province in 2018, there was 16% or 1,163,118 people less than 5 years old were obese. Obesity can cause many disorders, so that the morbidity and morality of the obese people were quite high. Therefore, obesity can impact on direct and indirect health cost. It was estimated in developed countries that the obesity cost is 2-10% of the national health cost in each country every year. Therefore, a daily calorie reduction was needed under mortgage without malnutrition and other important nutrients called restrictions. In 2012, the American College of Obstetricians and Gynecologists reported that the obesity women will have hyperplasia endometrium premenopausal risk, where estrogen levels are higher than progesterone hormones are low until none. Restriction is calorie limitation with a consistent pattern to reduce the average daily caloric intake.

Some previous studies showed that calorie limitation has health benefits. Energy intake and obesity incidence in health inspectors on Pidie sub-district in Aceh indicated significant correlation (Ramadhaniah et al. 2014). The study on hormonal estrogen effects in estradiol and the histology of mice's uterus (*mus musculus*) concludes that estrus cycle age seen in the phases of diestrus and estrus mice, the treatment was six times longer than control. It could happen, because estrogen induced in treated mice affects the pituitary's performance in secreting LH and FSH, so that there is chaos in the estrus cycle age and in the estrus cycle constituent phase on the thickness of the control endometrium and the estrogen treatment has a significant effect that is equal to 0.04 ($p<0$ 0.05) (Narulita et al. 2017). A full nutrition macro-Nutritional status and stress can affect a normal menstrual cycle in teenagers (Sitoayu et al. 2017).

A study concluded that low carbohydrate diets can reduce insulin levels in the blood, correct hormone imbalance, and produce a normal ovulation return to pregnancy (McGrice & Porter, 2017). However, the research of a high-calorie diet combined with a sequential restriction to prevent excess calories from healthy female reproduction, where one of which was seen in the weight of the uterus and the reproductive cycle, was unknown clearly. Therefore, it is recommended to analyze the high-caloric diet impact with a three-time a week combination of restriction on the reproductive and weight of the uterus.

The study aimed at seeing high-calorie effect diet without a regular combination of restriction cycles on the inverse cycle of reproduction and the weight of the uterus in mice, knowing high-calorie diet effect with an interval of the reproductive and weight of the uterus in inducing, the difference between a high-calorie diet and the thinning weight mice, the difference between a high-calorie diet with and without the interval of reproductive cycle and uterus mice weights.

MATERIALS AND METHODS

We had carried out an experimental laboratory to investigate and search for the influence of certain treatments under controlled conditions. The research design used was the randomized post-test only control design. The samples given treatment, as well as a control group, were taken randomly from the population (Sugiono 2012). The studied period in April 2021. The research population was mice (*Mus musculus*) female balb/c. The sample technique used was probability sampling involved with random sampling (Herawati et al. 2016). The independent variables were restriction calories interval and a high-calorie diet, while the dependent variables were reproduction cycle and the weight of the uterus.

The study was initiated with research ethics and declared worthy of ethics by the Ethics Commission of the Faculty of Medicine, Universitas Airlangga, under a decree no.83/ ec/kepk/fkua/2021. Before given treatment, the mice were acclimatized (adjusted to the environment) for a week in experimental animal cages at the Faculty of Veterinary Medicine, Universitas Airlangga. During acclimatization, they were fed and watered according to the standard amount and nutrition. Then, the mice swam and cooled to introduce the mice to the water. The mice were weighed at the beginning before treatment and every week to determine the weight and dose of 40% dextrose sonde in each mouse. After acclimatization, a vaginal swab was performed on the mice to determine the reproductive cycle, with the estrus mice phase could be treated for 4 weeks.

The mice were randomly divided into three treatment groups, namely K1 (control) was mice group that were not treated, K2 was mice group treated with a high-calorie diet with 40% dextrose sonde as much as 0.0325 ml/1 gBW mice (Herawati et al. 2016) (± 10.00 am), and K3 was mice group treated with a high-calorie diet with 40% dextrose sonde as much as 0.0325 ml/1 gBW mice (Herawati et al. 2016) (±10.00 pm) and Calorie restriction (-50% feed) (Pósa et al. 2015) from standard feed is 20g/mice every three times per-week (Tuesday, Thursday, Saturday at ±10.00 am) (Herawati et al. 2016).

After treatment for 4 weeks, a vaginal swab was undertaken to determine the reproductive cycle experienced by mice (not waiting for a certain phase in mice) for surgery, then taking blood and organs from the uterus, liver, ovaries, brain, heart, visceral fat of mice at the Embryology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga. The surgery was performed by anesthetizing the mice using 70% ether which was placed in a closed container. Surgery was performed on the abdomen to take the uterine organs in the mice and weighed to determine uterus weights. After surgery, the mice organs that could still be used were stored and preserved for further research, while unused organs of mice were destroyed or cremated by the laboratory as part of the research waste treatment.

The processing and data analysis used descriptive statistic test, the normality test using kolmogorov-smirnov, the homogeneity test using levene's test, acquired variant of a homogeneous nature if p> 0.05, the different was that if normal distribution data used anova one way analysis, and data were not normally distributed, so that kruskal Wallis could be used.

RESULTS

Characteristics of the mice

Statistical tests of descriptive, normality, and comparative homogeneity were conducted on uterine weight data in the control group (K1, with n=12), high-caloric diets group (K2, with n=9), high-calorie dieters with restriction intervals (K3, with n=11), and the significance of the average difference in body weight between groups (α=0,05). The results of the body weight statistics test were displayed (Table 1).

The previous table indicated that there was no significant difference in average weight before and after treatment between groups, indicated by a p-value >0.05. In the control group (K1), the p-value was < 0.05 which indicated a significant difference between the weight before and after the treatment, the high-calorie diet group (K2) with a p-value of >0.05 indicated no significant difference between weight before and after the treatment, while the high-calorie diet group with interval restriction (K3) with p-value <0.05 indicated a significant difference between weight before and after treatment. The vaginal swab calculations of mice in the control group, high-calorie diet, and high-calorie diet with interval restriction were using Fisher's exact test analysis, because the samples were less than twenty.

According to Table 2, it declared that there were discrepancies in reproductive cycles based on treatment. At the most encountered by proestrus in negative control groups (K1) with a value of 42.9%, diestrus is most likely to be in high-caloric diets (K2) with a value of 60%, estrus was most likely to be in high-calorie dieters with restriction intervals (K3) with a value of 28.6%, and metestrus was most encountered in high-calorie diets (K2) with a value of 20%.The fisher's exact test results, p>0.05 made no significant difference between negative control groups with a high-calorie diet and a high-calorie diet with a restriction interval against the vaginal swab results.

Descriptive statistic tests, normality, and homogeneity of comparison were carried out on uterine weight data obtained in the control group (K1, n=12), the high-calorie diet group (K2, n= 9), and the high-calorie diet group with interval restriction (K3, n). = 11) to look at the significant difference in the mean between groups (α = 0.05). The post-treatment uterine weight statistic tests results were described in Table 2.

Table 1. Weight distribution, differences between groups, and statistical test results

	K1(n=12) Avarage±SD	K2(n=9) Avarage ± SD	K3(n=11) Avarage± SD	Comparation test (p value)
BB(pre)	20.25±3.251	21.89 ± 5.159	22.18 ±3.311	0.266(Kruskal wallis)
BB(post)	24.75±3.166	25.22±3.801	24.73±1.902	0.920 (anova)
(p value)	0,002 Wilcoxon	0,084 Pair t test	0.001 Pair t test	[redacted]
Delta	4.50±2.195	3.33±5.074	2.55±1.809	0.416 (Robust Tests)

Table 2. Vaginal swab in mice

Group	n	(%) Swab Vagina Percentage				Comparison test (p value)
		Proestrus	Estrus	Metestrus	Diestrus	
K1	7	42,9%	0%	0%	57,1%	0,38
K2	5	0%	20%	20%	60%	
K3	7	14,3%	28,6%	0%	57,1%	

Table 3. Statistic data test for mice uterus weight post-treatment

Group	n	Uterus weight (g)(avarage ± SD)	Comparative test (p value)
K1	12	0,11 ± 0,445	0.032
K2	9	0,19 ± 0,101	
K3	11	0.12±0.053	

The results from the pre, can be seen that the high-calorie diet group (K2) has a higher average uterine weight than the other groups, they had an average value 0.19. Normality test with Shapiro-Wilk test and homogeneity test with Levene's test showed that uterine weight data were normally distributed ($p>0.05$) and were not homogeneous ($p<0.05$), so that the Robust test was carried out to see the differences between groups. The results obtained ($p<0.05$) indicated that there was a significant difference in the weight of uterine organs between groups.

DISCUSSION

The effect of a high-calorie diet without restriction on the cycle of reproduction and the weight of the uterus in mice

A study on the effect of feeding a high-fat diet of caloric intake function of diet resulted in that 5 mice were kept on a standard diet of animal pellets to serve as a control group (a) and 15 are fed a high-fat diet of 9 weeks to induce obesity (Hussain et al. 2016). Animals fed a high diet of fat are divided into four groups, namely (a) the high-fat diet of *ad libitum*, (b) the isokal, (c) high-fat diet groups, and (d) the hypoglycaemic diet. These could conclude that *ad libitum* high-fat diet dominated the normal estrus cycle and increased the luteal cell apoptosis in fat mice.

High-fat dietary restraint disrupts normal estrus cycles and causes functional deficiency of corpuscles in fat females. A result of a study suggested that dietary high fat definitely affected women's reproductive function regardless of their caloric intake. In addition, a study by Cheng et al. (2018) concluded that transgenic profile changes that were affected by e2 and/or HFD could interfere with the homeostasis endometrium and contribute to hyperplasia endometrium development. The uterus tissue changed its dynamic histology structure according to the hormone estrogen changing levels eihua et al. coloniet et al. conducted a study on macronutrient balance reproductive unction and liespan in human mice leads the largest uterus mass on a highprotein and lo carbohydrate diet hereas the biggest ovarian ollicles number on a highprotein and low-carbohydrate diet. Otherwise, the estrus cycle was more likely in mice with lower protein and carbohydrates, and corpora luteal numbers, which showed the most recent ovulation on high-protein and low-carbohydrate diets similar to those favoring the greatest longevity. The study noted that a high-fat diet could cause hyper cholesterol that could increase oxidative stress, and resulted in apoptosis of the nuclear arkuata neuron. This arcuate nucleus neuron apoptosis could affect the hypothalamus in producing GnRh, and reduce its production of anterior pituitary in producing FSH and HHLS.

Hormones played a role in affecting corpus luteum secretes progesterone and estrogen. FSH worked on granulosa cells to increase the androgen conversion into estrogen. Production of estrogen for endometrial proliferation and progesterone to prepare for implantation of the embryo in the endometrium (Young 2013). Therefore, if FSH and LH levels decreased, it could cause a decrease in the hormones estrogen and progesterone, so that the uterine tissue underwent dynamic histological changes in accordance with changes in estrogen hormone levels (Weihua et al. 2000). Lenert et al. (2021) study on homeostatic regulation of estrus cycle of young female mice on western diet, concluded that there was no effect on the cycle of diet, measured by the percentage of mice that complete one full cycle of estrus during each sample study period.

Based on the results of this study, the uterine weight analyses used 12 samples in the control group (K1), 9 samples in the high-calorie diet group without interval restriction (K2), and 11 samples in the high-calorie diet group with interval restriction (K3). There was a significant difference in the uterine weight of mice in the control group (K1) with the effect of a high-calorie diet without a combination of interval restriction group (K2), with the largest uterine weight in the high-calorie diet without restriction (K2) group. In vaginal swab analysis or reproductive cycle with a total of 7 samples in the control group (K1), 5 samples in the high-calorie diet group without interval restriction (K2), and 7 samples in the high-calorie diet group with interval restriction (K3), found no significant difference. Between negative control group (K1) and high-calorie diet group (K2) on the results of the vaginal swab or reproductive cycle with the most reproductive cycles were the diestrus phase among all groups. This would not coincide with the calculation for four weeks that the proestrus or estrus cycle would correspond to the 5-day cycle. The circumstances were due to external factors, environmental and internal, physical, and addict psychological abuse.

Effect of high calorie diet with interval restriction combination on reproductive cycle and uterine weight in mice

Xie et al. (2020) concluded that restriction saves female mice from weight gain, glucose intolerance, ovarian follicle loss, and estrous cycle dysfunction caused by a high-fat diet. A study by Markowiak and Slizewska (2017) on caloric restriction increases ratio of estrogen to androgen receptors expression in murine ovaries - potential therapeutic implications, concluded that prolonged calorie restriction (9 months) in female rats led to increased expression of estrogen receptors, while at the same time not affecting the expression of androgen receptor on ovarian cells. Calorie restriction resulted in estrogen lower levels in the blood circulation, leading to a higher sensitivity of cells to this hormone.

In the proliferative phase, the main hormone during this phase was estrogen, particularly 17-beta estradiol. The phase goal was to grow the uterus endometrial lining. 17-beta-estradiol achieved this by increasing the growth of the uterus endometrial lining, stimulating an increase in the number of stroma and glands, and increasing the depth of the artery supplying the endometrium, the spiral arteries (Thiyagarajan et al. 2019). Uterine tissue experienced dynamic changes in histologic structure according to changes in estrogen hormone levels (Weihua et al. 2000), so that if the estrogen level was low, it could reduce the uterus weight and the reproductive cycle affects. For example, calorie restriction was a "stressor" processed in the brain and could modify the release of sex hormones through the hypothalamic-pituitary-gonadal axis. Reproduction and fertility were regulated through the hypothalamic-pituitary-gonadal axis hormones.

With regard to intermittent fasting, it was possible that individuals who did not get sufficient caloric intake to support the hypotamater-pituitary-gonadal axis might experience irregular periods. The result of this study also resulted that the uterine weights analyzed were 12 samples in the control group (K1), 9 samples in the high-calorie diet without interval restriction group (K2), and 11 samples in the high-calorie diet with interval restriction group (K3). There were significant differences in uterine weight mice in the control group (K1) with the high-calorie diet effect with an interval restriction group (K3) combination.

In vaginal swab analysis or reproductive cycle with a total of 7 samples in the control group (K1), 5 samples in the high-calorie diet without interval restriction group (K2), and 7 samples in the high-calorie diet with interval restriction group (K3), there were no significant differences between the control group (K1) and the high-calorie diet with interval restriction group (K3) on the results of the vaginal swab or reproductive cycle with the most estrus phase compared to other groups, but the most diestrus phase occurred in the high-calorie diet with interval restriction group (K3). This situation occurred, because it was influenced by external factors, namely the environment and internal (physical and psychological) in mice that could not be avoided.

The difference between the effect of high calorie diet with and without a combination of interval restriction on the reproductive cycle and uterine weight in mice

Previous studies conducted on the effect of a high-calorie diet without a combination of interval restriction on the reproductive cycle and uterine weight in mice, on the effect of a high-calorie diet with a combination of interval restriction on the reproductive cycle and uterine weight in mice, had resulted in different results. This was reinforced by a significant study on the effect of a high-calorie diet without a combination of interval restrictions on the reproductive cycle and uterine weight in mice, which showed feeding a high-fat diet definitely affects female reproductive function regardless of caloric intake (Hussain et al. 2016).

A study by Cheng et al. (2018) on estrogen and a high-fat diet causing changes in the endometrial transcriptome profile of C57BL/6 mice, concluded that changes in the endometrial transcriptome profile influenced by E2 and/or HFD could disrupt endometrial homeostasis and contribute to endometrial hyperplasia development. Uterine tissue experienced dynamic histological structural changes in accordance with changes in estrogen hormone levels (Weihua et al. 2000). A study on the effect of a high-calorie diet with a combination of interval restriction on the reproductive cycle and uterine weight mice, concluded that restriction saved female rats from weight gain, glucose intolerance, ovarian follicle loss and estrus cycle dysfunction caused by a high-fat diet (Hua et al. 2020).

A study by Słuczanowska-Głąbowska et al. (2015) on caloric restriction increases the ratio of estrogen to androgen receptors expression in murine ovaries - potential therapeutic implications, concluded that prolonged calorie restriction (9 months) in female rats causes increasing estrogen receptor expression, while at the same time not affecting the expression of androgen receptors on ovarian cells. Calorie restriction resulted in lower levels of estrogen in the blood circulation leading to a higher sensitivity of cell to this hormone. Uterine tissue experienced dynamic histological structural changes in accordance with changes in estrogen

hormone levels (Weihua et al. 2000), so that if the level of estrogen was low, it could reduce the weight of the uterus and affect the reproductive cycle.

Based on uterine weight analysis, the high-calorie diet group (K2) had a higher average uterine weight than the other groups, there is 0.19 ± 0.101. Robust tes t was conducted to see differences between groups. The results were obtained ($p < 0.05$), which meant that there was a significant difference in the weight of the uterine organ between the control group (K1), high-calorie diet without interval restriction group (K2), and high-calorie diet with interval restriction group (K3). In the post-treatment vaginal swab analysis or the reproductive cycle of mice with a total of 7 samples in the control group (K1), 5 samples in the high-calorie diet without interval restriction group (K2), and 7 samples in the high-calorie diet with interval restriction group (K3), in the comparison between groups, the proestrus phase was most commonly found in the negative control group (K1), the diestrus and metestrus phases were most common in the high-calorie diet group (K2). The estrus phase was most commonly found in the high-calorie diet with interval restriction group (K3). The fisher's exact test gave the results $p > 0.05$, so that there was no significant difference between the control group (K1) and the high-calorie diet without interval restriction group (K2), and the high-calorie diet with interval restriction group (K3) on the results of the vaginal swab. This situation occurred, because it was influenced by external factors, namely environmental and internal (physical and psychological) in mice that could not be avoided.

Strength and limitation

This study focuses on an important topic of how high-calorie diets can affect reproductive health in female mice. The study uses a randomized controlled trial design and statistical tests to analyze the data, which is a robust method to test the intervention's effect on the outcome and provides reliable results.The study uses probability sampling to select the population, which increases the generalizability of the findings.The study only measures the weight of the uterus as a proxy for reproductive health and does not evaluate other indicators such as hormonal levels, ovulation, or pregnancy outcomes.

CONCLUSION

A high-calorie diet with interval restriction combination did not impact the reproductive cycle, but had an effect on the uterine weight of mice, compared to subjects who did not do interval restriction combination. To determine the underlying mechanism, further research was needed to determine the thickness of the endometrium and the influence of hormones, such as FSH, LH, estrogen, and progesterone.

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Conflict of interest

None.

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None.

Author contribution

ZO, ADK, and RA collected, analyzed, and interpreted the data, drafted, revised, and approved the final manuscript. LLand LH conceptualized the manusript. AGN conceptualized the design of the study, analyzed and interpreted the data, revised, and approved the final manuscript.

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Original Research

SELECTION OF MEDICAL THERAPY IN GLAUCOMA PATIENTS IN THE OUTPATIENT EYE CLINIC SOETOMO GENERAL ACADEMIC HOSPITAL SURABAYA

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ABSTRACT

Glaucoma is the second leading cause of blindness and the third cause of visual impairment. Glaucoma management includes medical therapy, laser, and incisions. This study aims to determine the selection of medical therapy in the outpatient eye clinic Soetomo General Academic Hospital Surabaya. This study was a retrospective descriptive study. The variables were type of glaucoma, visual acuity, intraocular pressure, and drug therapy. Data processing was done by collecting, grouping, and describing data. Glaucoma was mostly common in 50-64 years old (37.66%), male (50.65%), most patients lived in Surabaya (53.68%), the most common type was Primary Open-Angle Glaucoma (32.90%), the most medical therapy given in the first visit in 2019 was single medical therapy (67.97%), the most single drug therapy was latanoprost (49.68%), the most drugs combination was timolol maleat with acetazolamide (35.14%), the most commonly drugs class were prostaglandin analogues (48.48%), most of the visual acuity values were 6/6 - 6/18 (1,00 – 0,33) (37.88%), most intraocular pressure was in the 11-21 mmHg (62.55%). The most common type of glaucoma was primary open angle glaucoma with the most therapy given was prostaglandin analogue.

***Keywords**: Glaucoma; human & medicine; single drug therapy; combination drugs therapy; prostaglandin analogue; latanoprost; health risk*

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Highlights:

1. Medical therapy selection in the outpatient eye clinic were determined.
2. Treatment of prostaglandin analogue therapyfor primary open angle glaucoma was the most common glaucoma type.

INTRODUCTION

According to World Health Organization, glaucoma is the second leading cause of blindness in the world with a prevalence of 8%. In 2019, World Health Organization declared that glaucoma is the third leading cause of visual impairment in the world. Meanwhile, the Ministry of Health in 2007 stated that the prevalence of glaucoma in Indonesia was 0.46%.

Glaucoma is an optic neuropathy characterized by an increase in intraocular pressure. There are three types of glaucoma, namely primary, secondary, and developmental glaucoma (Japan Glaucoma Society 2006). Glaucoma clinical manifestations usually arise in severe damage. Glaucoma management includes medical therapy, laser therapy, and incisions (Japan Glaucoma Society 2006). Classes of drugs for glaucoma

Glaucoma Society 2006). Classes of drugs for glaucoma include prostaglandin analogues, beta-blockers, α-adrenergic agonists, parasympatomimetics, carbonic anhydrase inhibitors, and hyperosmotic agents (Prum et al. 2016).

Data on the selection of medical therapy in glaucoma patients in Surabaya need to be updated. Based on the previous evidence, we were interested to determine the selection of medical therapy in glaucoma patients. This study was expected to provide information (data) about the selection of medical therapy for glaucoma services, so that it could be the basis for any research related to glaucoma.

MATERIALS AND METHODS

This study was a retrospective descriptive study that involved data from the medical records of glaucoma patients in the Outpatient Eye Clinic Dr. Soetomo General Academic Hospital, Surabaya, in January - December 2019. The variables observed were the type of glaucoma diagnosis, visual acuity, intraocular pressure, and drug therapy given to glaucoma patients. Medical record data were collected and grouped based on the criteria to be studied, then described qualitatively and quantitatively.

RESULTS

231 patient medical records were obtained. The data were recorded in the form of the patient's medical record number, name, age, gender, area of residence of the patient, type of glaucoma diagnosis, selection of medical therapy either singly or in combination, class of drug administered, visual acuity, and intraocular pressure of the patient.

Age

Glaucoma was mostly found in 50-64 years old with 87 patients (37.66%), while the lowest distribution of glaucoma patients was in the ≥80 years age group were 5 patients (2.16%).

Table 1. Frequency distribution based on patient age

Age (Years)	Quantity (People)	Percentage
20-34	11	4.76%
35-49	47	20.35%
50-64	87	37.66%
65-79	81	35.06%
≥80	5	2.16%
Total of Patients	231	100%

Gender

The frequency distribution by gender showed that the total of male patients (50.65%) was more than female (49.35%). The ratio of male and female patients was 1.03: 1.

Table 2. Frequency distribution by patient's gender

Gender	Quantity (People)	Percentage
Male	117	50.65%
Female	114	49.35%
Total of Patients	231	100%

Residential area

The frequency distribution based on residential area were mostly in Surabaya (53.68%), followed by Sidoarjo (8.23%), Gresik (6.06%), and Bangkalan (6.06%). Glaucoma patients also came from various regions in Indonesia (Table 3).

Table 3. Frequency distribution based on residential area

Residental area	Quantity (People)	Percentage
Bangkalan	12	5.19%
Bojonegoro	4	1.73%
Bondowoso	1	0.43%
Gresik	14	6.06%
Jember	1	0.43%
Jombang	4	1.73%
Kediri	1	0.43%
Konawe Selatan	1	0.43%
Kupang	2	0.87%
Lamongan	5	2.16%
Lumajang	2	0.87%
Madiun	4	1.73%
Magetan	1	0.43%
Malang	1	0.43%
Manokwari	1	0.43%
Mataram Kota	2	0.87%
Mojokerto	7	3.03%
Nganjuk	4	1.73%
Palu Kota	1	0.43%
Pamekasan	3	1.30%
Pasuruan	5	2.16%
Ponorogo	1	0.43%
Sampang	1	0.43%
Sidoarjo	19	8.23%
Sleman	1	0.43%
Sukoharjo	1	0.43%
Sumenep	1	0.43%
Surabaya	124	53.68%
Trenggalek	1	0.43%
Tuban	4	1.73%
Tulungagung	2	0.87%
Total of Patients	231	100%

Table 4. Frequency distribution by type of glaucoma diagnosis

Type of glaucoma	Quantity (People)	Percentage
Glaucoma Suspect	25	10.82%
Primary Open-Angle Glaucoma	76	32.90%
Primary Angle-Closure Glaucoma	41	17.75%
Glaucoma Secondary To Eye Trauma	2	0.87%
Glaucoma Secondary To Eye Inflammation	6	2.60%
Glaucoma Secondary To Other Eye Disorders	41	17.75%
Glaucoma Secondary To Drugs	1	0.43%
Other Glaucoma	19	8.23%
Glaucoma Unspecified	17	7.36%
Absolute Glaucoma	3	1.30%
Total of Patients	231	100%

Type of glaucoma

The most common types of glaucoma diagnosis were primary open-angle glaucoma as many as 76 patients (32.90%). It followed by primary angle-closure disorders with each percentage was 17.75%. Meanwhile, the fewest type of glaucoma diagnosis was glaucoma secondary to drugs as many as 1 patient (0.43%) (Table 4).

Glaucoma medical therapy

Drug class

The most glaucoma medical therapy given at the first visit in 2019 was single drug therapy (68.83%).

Table 5. Distribution of choice of medical therapy in glaucoma patients

Glaucoma Therapy	Quantity (People)	Percentage
Single drug therapy	159	68.83%
Combination drugs therapy	72	31.17%
Total of Patients	231	100%

Single drug therapy

The most single drug therapy given was latanoprost (49.06%) followed by timolol maleate (37.74%), while the fewest was betaxolol (0.63%).

Table 6. Distribution of single drug therapy

Single Drug therapy	Quantity (People)	Percentage
Brinzolamide	3	1.89%
Betaxolol	1	0,63%
Acetazolamide	17	1.69%
Timolol maleat	60	3.74%
Latanoprost	78	49.06%
Total of Patients	159	100%

Combination of drugs therapy

The most common drug combination given was timolol maleate with acetazolamide (36.11%). The fewest combinations of drugs include Latanoprost, Acetazolamide, Brinzolamide with a percentage of 1.39%.

Table 7. Distribution of combination drugs therapy

Combination Drugs Therapy	Quantity (People)	Percentage
Timolol Maleat, Acetazolamide	26	36.11%
Timolol Maleat, Acetazolamide, Brinzolamide	4	5.56%
Timolol Maleat, Latanoprost	3	4.17%
Timolol Maleat, Latanoprost, Brinzolamide		
Latanoprost, Brinzolamide	6	8.33%
Latanoprost, Pilokarpin	2	2.78%
Latanoprost, Acetazolamide	20	27.78%
Latanoprost, Acetazolamide, Brinzolamide		
Total of Patients	72	100.00%

The most widely administered drug group was prostaglandin analogues (48.48%), followed by beta blockers (45.02%), carbonic anhydrase inhibitors (37.66%) and the least administered was parasympathomimetic (0.87%).

Table 8. Distribution of the drug class

Drug Class	Quantity (People)	Percentage
Beta-blockers	104	45.02%
Prostaglandin analogues	112	48.48%
Carbonic anhydrase inhibitor	87	37.66%
Parasympathomimetic	2	0.87%

Visual acuity

The most visual acuity value of the right eye was in the range of 6/6 - 6/18 (1.00 – 0.33) as many as 34.63%, while the fewest was in the range of 3/60 - 1/60 (0.049 – 0.02) equal to 5.19%. The most visual acuity of the left eye was in the range of 6/6 - 6/18 (1.00 – 0.33) as many as 41.13%, while the fewest was in the range of 3/60 - 1/60 (0.049 – 0.02) equal to 5.19%. The highest visual acuity was 6/6, and the low west visual acuty was to hight pereption.

Table 9. Frequency distribution based on visual acuity

	Quantity (People)	Perentage
Visual Acuity of Oculi Dextra		
6/6 - 6/18 (1,00 – 0,33)	80	34,63%
6/18 - 6/60 (0,32 – 0,10)	44	19,05%
6/60 - 3/60 (0,09 – 0,05)	15	6,49%
3/60 - 1/60 (0,049 – 0,02)	12	5,19%
1/60 - LP+ (0,019 – LP+)	56	24,24%
LP-	24	10,39%
Total of Patients	231	100%
Visual Acuity of Oculi Sinistra		
6/6 - 6/18 (1,00 – 0,33)	95	41.13%
6/18 - 6/60 (0,32 – 0,10)	37	16.02%
6/60 - 3/60 (0,09 – 0,05)	22	9.52%
3/60 - 1/60 (0,049 – 0,02)	12	5.19%
1/60 - LP+ (0,019 – LP+)	47	20.35%
LP-	18	7.79%
Total	231	100.00%

LP: Light Perception

Intraocular pressure

The most intraocular pressure of the right eye was in the range of 11-21 mmHg, as many as 62.77%, while the fewest was in the range of <11 mmHg, as many as 2.16%. The highest value was 81.7 mmHg, and the lowest value was 8.5 mmHg. The average value was 77, and the median value as 81.

Table 10. Frequency distribution based on intraocular pressure on treatment

	Quantity (People)	Percentage
Intraocular Pressure Oculi Dextra		
<11	5	2,16%
11 - 21	145	62,77%
>21	81	35,06%
Total of Patients	231	100%
Intraocular Pressure Oculi Sinistra		
<11	5	2,16%
11 - 21	144	62,34%
>21	82	35,50%
Total of Patients	231	100,00%

The most intraocular pressure of the left eye was in the range of 11-21 mmHg, as many as 62.34%. The fewest was in the range of <11 mmHg, as many as 2.16%. The highest intraocular pressure value was 81.7 mmHg and the lowest intraocular pressure value was 4 mmHg. The average value was 77, and the median value was 82.

DISCUSSION

Age

The distribution of glaucoma patients in the Outpatient Eye Clinic, Dr. Soetomo General Academic Hospital, Surabaya in the period of January - December 2019 was mostly in the 50-64 years age group (37.66%), while the fewest was in the ≥80 years age group (2.16%). Based on the study of Ashica et al. (2011), the higher the age of the patient, the higher the incidence of glaucoma, the peak was at the age of 60-69 years and decreased at the age of 70 years.

Aging is a risk factor for glaucoma (Hashemi et al. 2019). Aging can be associated with tissue aging, duration of exposure to other risk factors, and duration of illness (Ismandari & Helda 2011). The increasing age is correlated with degeneration of retinal ganglion cells and their axons which also causes vision loss. The natural aging associated with changes in the extracellular matrix results in a stiffer extracellular environment throughout the body. The trabecular meshwork and optic nerve head undergo extensive extracellular matrix remodeling characterized by fibrotic changes associated with cellular and molecular events (including myofibroblast activation) that promote fibrosis and further tissue ossification (Liu et al. 2018).

The result of increased deposition of extracellular matrix is hardening of the trabecular meshwork which causes an increase in outflow resistance and contributes to an increase in intraocular pressure. Age and glaucoma are also associated with ossification of the lamina cribrosa. Although tissue hardening is strongly correlated with fibrosis, the initiation of this molecular cascade further inhibits the character of healthy living cells and mediates the differentiation of various precursors into fibrogenic myofibroblasts. Although the normal contractile properties of myofibroblasts are central to wound healing and regulation of tissue architecture, a stiffer matrix enhances myofibroblast differentiation and contractility in a TGF- dependent manner leading to pathological wound healing (Liu et al. 2018). It can be concluded that age is associated with an increased risk of developing glaucoma and is in line with our research.

Gender

In this study, the number of male patients (50.65%) was slightly more than female (49.35%). The results of a study at Dr. M. Djamil Hospital, Padang also showed that the gender of most glaucoma patients was male (Ariesti & Herriadi 2018). Based on a study in Saudi Arabia, glaucoma profile by gender showed that men were higher (52.9%) than women (47.1%) (Helayel 2021). Other studies showed different results. Hashemi et al. (2019) showed that 57.1% of glaucoma patiens were women and 42.9% were men. Meanwhile, Dizayang et al. (2019) showed that the majority of primary glaucoma patients were women (57.8%)

Several studies had shown that the prevalence of women was more than men in angle closure glaucoma, because the anterior chamber angle of women was shallower than men (Stamper et al. 2009). Meanwhile, in open-angle glaucoma, the prevalence of gender varied. However, several studies had shown a higher prevalence of males than females (Stamper et al. 2009).

Residential area

The data obtained from this study indicated that the most glaucoma patients lived in Surabaya (53.68%), followed by Sidoarjo and Gresik. Based on the data on Center for Statistics, East Java in 2021, the population of Surabaya in 2019 is the highest in East Java. Meanwhile, East Java is the province with the second largest population in Indonesia. That is why the incidence of glaucoma from Surabaya to be higher than other areas.

Type of glaucoma

The results of this study indicated that the most common type of glaucoma diagnosis was primary open-angle glaucoma (32.90%). Primary open-angle glaucoma often occurred with a prevalence of 3.05% (Tham et al. 2014). A study by Pratista (2018) in Dr. Soetomo General Academic Hospital in the period of January – December 2017, found that the most common type of glaucoma was open angle glaucoma (29.03%). In addition, a study on a Chinese urban population in Singapore, showed that primary open-angle glaucoma was the most common type of glaucoma with a prevalence of 1.4% (Baskaran et al. 2015), while in Saudi Arabia, primary open-angle glaucoma was the most common (Helayel et al., 2021).

Other studies showed different results. Pusvitasari and Triningrat (2018) resulted that primary open-angle glaucoma was the second most common type of glaucoma (33%) after secondary glaucoma (37%), while in Nigeria, was unclassified glaucoma (56.6%) (Kyari et al. 2015).

Based on the literature, primary open-angle glaucoma is a type of glaucoma that is often found in both black and white races, because primary open-angle glaucoma often causes bilateral visual field narrowing, slow onset, progressive, asymptomatic, and often undetected until extensive visual field narrowing occurs (Riordan-Eva & Whitcher 2007).

Glaucoma medical therapy

Medical therapy is still the first-line therapy in glaucoma patients (Schehlein et al. 2017). In this study, the highest selection of medical therapy given to glaucoma patients at the first visit in 2019 was single drug therapy (68.83%), while the selection of combination drugs therapy was 31.17%. A study by Schwartz et al. (2021) proved that the combination therapy group was less than the single therapy group, while a study in Zimbabwe showed that the administration of single drug therapy was 71.8% of the total antiglaucoma drug prescriptions (Kyei et al. 2020).

Other studies had shown that combination drugs therapy was used more often than single drug therapy (Pratista 2018). Research in India also proved that single drug therapy was only used by 15% of patients (Mittal & Mittal 2020). Approximately, 50-75% of glaucoma and ocular hypertension patients required combination of drugs therapy to achieve the target of lowering intraocular pressure (Mittal & Mittal 2020).

Single drug therapy reduces intraocular pressure by up to 35%. Meanwhile, combination drug therapy can reduce intraocular pressure by up to 50% (Mittal & Mittal 2020). Reduction of intra ocular pressure as a target of glaucoma therapy should be achieved using drugs and minimal side effects (Yu et al. 2020). Combination of drugs therapy with different mechanisms of action can be given if single drug therapy is not able to achieve the target of lowering intra ocular pressure (Yu et al. 2020). Combination therapy should enhance the therapeutic effect and patient comfort (Yu et al. 2020).

Single drug therapy

The most single drug therapy given was latanoprost as much as 49.06% of 159 patients, followed by timolol maleate as much as 37.74%, while the fewest was betaxolol as much as 0.63%. Latanoprost is a prostaglandin analogue. These drugs decrease intraocular pressure by increasing uveoscleral outflow with a 25-33% decrease in intraocular pressure (Li et al. 2014; Prum et al. 2016). The efficacy of lowering intraocular pressure persists for up to 24 hours after a single topical dose, which allows once-daily use (Perry et al. 2003).

A meta-analysis of the comparison of the use of latanoprost and timolol in patients with chronic angle-closure glaucoma in the Asian population showed that once-daily latanoprost reduced intraocular pressure less than twice-daily timolol, so that patient adherence to the drug was higher (Li et al. 2014). The systemic side effects of latanoprost are also lower than the systemic side effects of timolol (Li et al. 2014). In a multicenter study of glaucoma therapy in the United Kingdom, primary open-angle glaucoma patients receiving latanoprost therapy demonstrated longer visual field maintenance (Garway-Heath et al. 2015). In addition, a study on clinical and sociodemographic characteristics in Zimbabwe showed that prostaglandin analogue single therapy was most commonly prescribed in glaucoma patients (Kyei et al. 2020).

Meanwhile, a study in the RS Mata Masyarakat Jawa Timur in the period of January - December 2015 showed that the single drug that was often given was timolol (32.67%) followed by acetazolamide (30.99%), while the percentage of latanoprost therapy was 8.87% (Mustofa et al. 2016). On the other hand, a study conducted in Dr. Soetomo General Academic Hospital in 2017, found that the single drug therapy that was often used was timolol (65.91%), the least used were betaxolol (6.82%) and acetazolamide (6.82%) (Pratista 2018).

Based on the explanation above, latanoprost is an antiglaucoma drug that is often used. In some places, latanoprost has become the first-line antiglaucoma drug because of its advantages, including once-daily use, so that patient compliance is higher, lower intraocular pressure reduction compared to timolol, longer visual field maintenance, and lower systemic side effects.

Combinations drugs therapy

The most commonly given drug combination was timolol maleate with acetazolamide (36.11%). The second most common drug combination was the combination of latanoprost and acetazolamide (27.78%). The results of this study are in accordance with Hazhar (2017) who gave the results that the most widely administered drug combination was beta-blocker + carbonic anhydrase inhibitor with a percentage of 39.50%. In addition, in research in Soetomo General Academic Hospital regarding the Effectiveness of Medical, Surgery, and Combination Therapy on Reducing Intraocular Pressure in Glaucoma Patients, the combination of drugs that is often given was timolol with acetazolamide (30.61%) (Pratista 2018).

In a cohort study in Japan, most patients were prescribed a combination of a prostaglandin analogue and a beta-blocker (Shirai et al. 2021). The study also showed that the fixed combination of latanoprost + timolol was high with a percentage of 54.5% (Shirai et al. 2021). The combination of prostaglandin analogues and beta-blockers is a good combination because of the high effectiveness of each drug class. Both classes of drugs are first-line antiglaucoma therapy.

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However, in this study, the most common drug combination was timolol maleate + acetazolamide, which is a combination of a beta-blocker with a carbonic anhydrase inhibitor. Timolol maleate is an antiglaucoma drug that is available at level 3 health facilities, such as Dr. Soetomo General Academic Hospital, because it is guaranteed by government health insurance and the price is affordable (Ministry of Health, Republic of Indonesia, 2013). Some drugs are given if the patient does not respond to timolol (Ministry of Health, Republic of Indonesia 2013). Acetazolamide is an oral carbonic anhydrase inhibitor available at level 3 health facilities. This drug is able to reduce intraocular pressure by 20-30% (Prum et al. 2016). Based on the Regulation of the Ministry of Health, No. 5 of 2014, the management of acute glaucoma at first-level health care facilities is to simultaneously give several drugs to reduce intraocular pressure, including acetazolamide HCl 500 mg followed by 4x250 mg/day, KCl 0.5 g 3x/day, timolol 0.5%, eye drops a combination of corticosteroids + antibiotics, as well as symptomatic therapy (Ministry of Health, Republic of Indonesia 2014). Based on this explanation, timolol maleate and acetazolamide are drugs that are often used in health facilities in Indonesia because of their availability and regulated in health regulations in Indonesia.

Drug class

The data in this study showed that the highest drug class administered were prostaglandin analogues (48.48%), followed by beta-blockers (45.02%), carbonic anhydrase inhibitors (37.66%), and the fewest was parasympathomimetics (0,87%). Based on a study in China, prostaglandin analogues were prescribed 30.21% of all visits and beta-blockers were prescribed 27.1% of all visits (Yu et al. 2020). Analysis by class of antiglaucoma drugs in 2013 showed

Timur for the January-December 2015 period showed that the non-selective beta-blocker group was the most widely used drug class (34.6%) and was followed by carbonic anhydrase inhibitors (32.03%) (Mustofa et al. 2016).

Prostaglandin analogues decrease the resistance to aqueous humor out of the eye, thereby increasing its flow in the uveoscleral pathway. Prostaglandin analogs have a higher effectiveness in lowering intraocular pressure than beta-blockers and have lower side effects (Conlon et al. 2017). Prostaglandin analogues are given once a day (at bedtime), because their duration of action can last for several days (Bowling & Kanski 2016). Prostaglandin analogs can be used as monotherapy or in combination therapy to control intraocular pressure (DiPiro et al. 2008). This class of drugs can be an effective sole therapy or adjunct therapy for patients who do not respond to other classes of antiglaucoma drugs (DiPiro et al. 2008). Because of these advantages, prostaglandin analogues are the first-line therapy of choice for glaucoma, especially for primary open-angle glaucoma (Bowling & Kanski 2016; DiPiro et al. 2008). The choice of medical therapy in glaucoma patients should pay attention to various things, such as intra ocular pressure target, eye structure, systemic side effects, quality of life, cost, and patient comfort (Kyei et al. 2020).

Prostaglandin analogues have high effectiveness in lowering intraocular pressure, but topical ocular side effects and low cost should be considered when prescribing to patients (Mittal & Mittal 2020). In this case timolol maleate, beta-blockers are less expensive than prostaglandin analogs. In addition, if the target for intraocular pressure reduction is about 20% from baseline, beta-blockers may be the first choice (Mittal & Mittal 2020). If a reduction of 30-35% is desired, a prostaglandin analogue is an appropriate choice. If a person wants a higher reduction in intraocular pressure and consider other aspects, such as cost, a combination of drug classes with different mechanisms can be an option (Mittal & Mittal 2020).

Visual acuity

From the results of this study, the most visual acuity values in the right eye were in the range of 6/6 - 6/18 (1.00 – 0.33), which was 34.63%. The visual acuity value of the left eye was mostly in the range of 6/6 -6/18 (1.00 – 0.33), which was 41.13%. The visual acuity value was in the normal range to mild visual impairment. The highest visual acuity was 6/6 and the lowest visual acuity was LP-.

This study was in line with a study in RS Khusus Mata in Medan Baru, the visual acuity of the right eye 6/6-6/18 was the highest at 48% and the visual acuity of the left eye was 6/6-6/18; the highest with a percentage of 50% (Fadly, 2020). According to Mokhles et al. (2020), the average visual acuity was 0.61 for the right eye and 0.63 for the left eye.

In primary open-angle glaucoma, visual acuity examination results are usually normal except in advanced glaucoma (Bowling & Kanski 2016). In chronic primary angle-closure glaucoma, visual acuity examination results are usually normal except in advanced glaucoma (Bowling & Kanski 2016). In acute primary angle-closure glaucoma, visual acuity was 6/60-hand waving (Bowling & Kanski 2016).

Normal visual acuity to mild visual impairment occurs in glaucoma patients is quite common. Normal visual acuity to mild visual impairment in glaucoma patients may be caused by the slow course of the disease, so that the decrease in visual acuity has not been seen.

Intraocular pressure

The frequency distribution based on intraocular pressure which was measured under anti glaucoma treatment at the first visit in 2019 in the right eye was mostly in the range of 11 - 21 mmHg, which was 62.77%. In the left eye the most were in the range of 11-21 mmHg, which was 62.34%.

The results of this study are in line with Hazhar's research (2017) at RSUD Dr. Soetomo Surabaya, the majority of the intraocular pressure of the right and left eyes is between 10.5-20.5 mmHg. The majority of respondents with glaucoma at the Medan Baru Eye Special Hospital had intraocular pressure in the right eye in the range of 10-21 mmHg/normal with a percentage of 88% and intraocular pressure in the left eye within the normal range as much as 86% of the total respondents (Fadly 2020). The study of Helayel et al. (2021) also indicated that the average intraocular pressure of glaucoma patients was 14.5 mmHg, which meant that the patient's intraocular pressure was in the normal range.

The results of other studies showed that intraocular pressure >21 mmHg was more common than intraocular pressure <21 mmHg (normal) (Dizayang et al. 2019). Glaucoma patients generally had a high intraocular pressure, except in normal-tension glaucoma. A study by Dienda et al. (2013) at Muhammadiyah Hospital Palembang observed intraocular pressure when first coming and after being given therapy. Most of the intraocular pressure when it firstly came was in the

range of 20-50 mmHg. After treatment, the intraocular pressure was checked for the second and the third time. Most patients had decreased intraocular pressure. Some glaucoma patients did not experience a decrease in intraocular pressure, because there was differences in glaucoma severity and drug use factors (Dienda et al. 2013).

The distribution of intraocular pressure in glaucoma patients in this study was slightly different from the above studies. This study evaluated the first-visit patients in 2019 who were given glaucoma therapy in the Outpatient Eye Clinic, Dr. Soetomo General Academic Hospital, Surabaya, for the period of January – December 2019. It was possible that the first visit in 2019 was the patient's visit to the eye clinic for the umpteenth time. Therefore, it as also possible that the patient had received therapy from previous visits, so that the patient's intraocular pressure had been controlled with this therapy.

In addition, Dr. Soetomo General Academic Hospital is a level 3 hospital and most glaucoma patients are referred patients from other hospitals in Surabaya and other areas, so that the patient's intraocular pressure has been controlled from previous glaucoma therapy.

Strength and limitation

This study was retrospective, and it did not record the use of previous anti glaucoma therapy. It also did not assess the success of therapy by comparing intraocular pressure before and after therapy. Drug administration in the hospital follows the procedures and regulations that had been determined, so that latanoprost and timolol maleate could be given simultaneously in one visit. Some drugs, such as latanoprost and travoprost, were sometimes not available, because their availability was highly dependent on the health insurance that coverd the patient. It means that it will affect the choice of antiglaucoma drugs by the doctor.

Future studies were recommended to evaluate the success of glaucoma therapy and discuss the selection of glaucoma therapy based on drugs provided by health insurance.

CONCLUSION

The most common type of glaucoma was primary open angle glaucoma with the most therapy given was single drug therapy. The choice of single drug therapy for glaucoma was prostaglandin analogue, while the choice of combination drugs therapy was timolol maleate with acetazolamide.

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Conflict of interest

None.

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Author contribution

VMP, YP, and MFP- collected and analyzed the data, VMP- conceptualized, wrote and revised the manuscript. RL-validatied the final manuscript.

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Case Report

SPLENECTOMY FOR BACTERIAL CULTURE STERILE SPLENIC ABSCESS MANAGEMENT

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ABSTRACT

We presented a rare case, a 26-year-old woman have sterile aerobic and anaerobic bacterial cultures. Splenic abscess is a common case, the increased immunosuppression and treatment incidence. Clinical examination of the patient showed a dense cystic mass in the left upper abdomen. Ultrasonography examination suspected a dermoid cyst. However, MRI examination of the abdomen showed turbid cystic lesions. The surgery revealed a splenic abscess, while pus and splenic tissue examination revealed no bacterial proliferation. Abdominal CT scan or MRI imaging and splenectomy was very helpful for successfully this surgery. Based on the literature, the patient had a good prognosis.

Keywords: *Splenic abscess; splenectomy; woman; health risk*

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Highlights:

1. A 26-year-old woman have sterile aerobic and anaerobic bacterial cultures.
2. Pus and splenic tissue examination revealed no bacterial proliferation, while the surgery revealed a splenic abscess.

INTRODUCTION

Spleen is a vascular-rich organ in a reticuloendothelial immune system part. If splenectomy is mandatory, it will give consequence on the susceptibility to encapsulated bacterial infections and intraerythrocytic parasites. Splenic abscess may cause bacteremia. It is usually trauma, embolization or hemoglobinopathy consequence (Mustafa et al. 2015). Immunodeficiency is HIV virus risk factor impact (Haider et al. 2019, Kusumaadhi et al. 2021). Splenic abscess is a rare case. Based on discovery autopsy, it has an incidence rate ranging from 0.14%-0.7%. Splenic abscess incidence was higher in the tropics, where it is associated with splenic vascular thrombosis and infarction in sickle-cell anemia patients. Splenic abscess is potentially life-threatening. The mortality rate ranges from 15%-20% in healthy patients with unilocular lesions. It can be 80% in multiple-immunocompromised abscess patients.

The most recent case report reveals that spontaneous rupture due to various infections (malaria, listeria, fungal infections, dengue and Q fever), while neoplasms is the cause of splenic abscesses (Lal & Clark 2015, Townsend et al. 2016). The most common organisms causing splenic abscess are Streptococcus and Staphylococcus species, but Salmonella species, Gram-negative *Escherecia coli*, Enterococcus species, and fungal infections can be also the causative organisms (Yeo 2018).

About two-thirds of adult abscess cases are solitary and one-third are multiple. Children's conditions are the opposite of those in adults. Splenic abscess has a

bimodal age distribution with peak cases in the 3rd and 6th life decades. The mortality rate of splenic abscess is quite high and varies, depending on immune status and abscess type. The mortality rate shows about 80% in immunocompromised patients with multilocular abscesses, and 15% in immunocompetent patients with unilocular abscesses (Petroianu et al. 2009). Pathogens ware often isolated include *Streptococcus spp*., Staphylococcus (endocarditis is the most common cause of splenic abscess), mycobacteria, fungi and parasites (Kaczorek-Łukowska et al. 2021). Based on Sabah Malaysia research reports, *Burkholderia pseudomallei* is the most common cause of splenic abscess in certain individuals (Mustafa & Jayaram 2004).

Splenic abscess classified according to the cause. The most common cause was primary hematogenous spread from the nearest septic source. The common cause was bacterial endocarditis associated with valvular disease, intravenous drug consumption, bacteremia, and postoperative or primary intra-abdominal infections (McKinney 2012). Patients with abnormal functions and spleen forms were highly susceptible to this disease, such as lymphoproliferative, myeloproliferative, trauma and systemic arterial embolism. Immunocompromised patients, such as malignancy, organ transplants, HIV/AIDS and chronic steroid consumptions reach 35% for splenic abscesses (Yeo 2018). However, in this case report, disease exact cause was unknown, because clinical and supporting examinations could not identify the cause of the splenic abscess patient.

An initial supportive therapy and parenteral broad-spectrum antibiotics were prominently important to undertake while waiting for further diagnostics and therapies arrangements were made (Chang et al. 2008). The splenic abscess standard therapy is splenectomy. However, recent studies show different success rates with different abscesses approaches (Cash et al. 2006). Medical technology development, such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) can improve diagnosis and therapy, so that the prognosis also improves (Mustafa et al. 2015).

Splenic abscess can occur in immunocompromised patients, neoplasm processes, immunodeficiency, trauma, infection spread, splenic infarction, and diabetes. These conditions can aggravate the single or multiple abscess progression. Multiple splenic abscess accompanied by lymphoma has not been documented (Abbasi et al. 2016). Variations of microorganisms isolated from splenic abscess were quite diverse. The most common aerobic and facultative bacteria were *Eschericia coli*, *Proteus mirabilis*, *S. bovis*, *Klebsiella pneumoniae*, and *S. aureus*, whereas the anaerobes were Pepto *Streptococcus spp*., *Bacteroides spp*., *Fusobacterium spp*. and *Clostridium spp.*

Successfully cultured organisms often reveal the underlying pathogenesis, for example, *S. aureus* and *S. bovis* are associated with endocarditis, *K. pneumoniae* with respiratory infections or liver abscess, *E. coli* with urinary and abdominal tract infections, *Bacteroides spp.* and *Clostridium spp.* with abdominal infections (Lee et al. 2011). Splenic abscess cases caused by *S. bovis* are rare and usually associated with septic emboli from endocarditis patients with colon cancer (Fitzsimmons et al. 2020). Aseptic splenic abscess was less common than splenic abscess in general. Most cases occur in Europe (Brooks & Ghaffari 2014). A small number of case series assess patients with sterile splenic abscess specifically. One case series found M-TB as the cause of negative culture in splenic abscess. A case series states that about 13% of the patients produced sterile abscess pus culture (Birkenmaier et al. 2006).

Some case reports found that splenic abscess is the first sign of Crohn's Disease. A case report in 2010 explained that aseptic splenic abscess patients showed Crohn's disease initial manifestations. Crohn’s disease was diagnosed after endoscopic biopsy that revealed cryptitis, inflammatory cells in lamina propria and epithelioid granuloma (Calzado et al. 2010). Infective endocarditis can associated with ischemic embolization and 10% is associated with splenic abscess. When clinician discovers a splenic abscess case, other investigations should immediately undertake to find the infection primary focus. In splenic abscess cases with suspected cause of infective endocarditis, a serial transthoracic echocardiogram can examined if it was not found other infection focus (McOwat et al. 2014). Here, we presented a rare case of aseptic splenic abscess in 26-year-old woman.

CASE REPORT

A 26-year-old female patient referred to the Digestive Surgery Clinic, Dr. Soetomo General Academic Hospital, Surabaya. The patient complained of a lump in stomach since 1 month before coming to the hospital. The lump was enlarged (Schuffner 2; Hacket 4) within 2 weeks felt a little sting when pressed. The patient also complained of nausea, subfebris fever and weakness in the last 1 week. The patient had no complaints of respiratory and cardiovascular organs, urinary tract organs, the change of bowel habit nor significant weight loss in the past 1 month. There was no previous trauma history.

The clinical presentation, general condition patient was moderate, compos mentis, cooperative, adequate nutrition, and sepsis (subfebrile temperature 37.5 ºC, leukocytosis 18.450, and qSOFA score 1). The patient's general status showed edema in both legs. Patient's local status inspection showed distended abdomen without *darm contour* and *darm steifung* description. Auscultation showed normal bowel sounds without abnormal sounds, such as metallic sound or borborygmi. Palpation showed a well-differentiated mobile cystic mass measuring 22 x 14 x 16 cm in the left upper abdominal region. The lump was painful when pressed and percussion showed liver dullness and shifting dullness. There were no enlarged lymph nodes in the bilateral inguinal region.

Upper lower abdominal ultrasound showed a mass measuring >20 cm firmly isoechoic with spleen and moderate vascularization suspected of a dermoid cyst. The abdomen MRI without and with contrast showed cystic lesions image with large turbidity inside in the upper left to lower abdomen (between the spleen and the right kidney). The spleen appeared pressed to the left rear and right kidney to down as high as the L5 with a lesion size of 15 x 20 x 24.2 cm.

The diagnosis was an intra-abdominal tumor. Regular blood tests indicated patient was suffering from anemia and hypoalbuminemia (Hb 9.9 g/dL, albumin serum 2.5 gr/dL), PPT/APTT was within normal limits (Ppt 13.4, cPpt 14.6, Aptt 38.4, cAptt 28.5). On July 19, 2018, surgery was carried out in general anaesthesia in exploratory laparotomy form, adhesiolysis, abscess drainage, and splenectomy. Based on clinical microbiology examination, aerobic form, anaerobic, and gram pus cultures operation did not find a germs formation, only polymorphonuclear (PMN) cells formation. Informed consent of fasting 6-8 hours pre-surgery procedure was obtained from the patient's family.

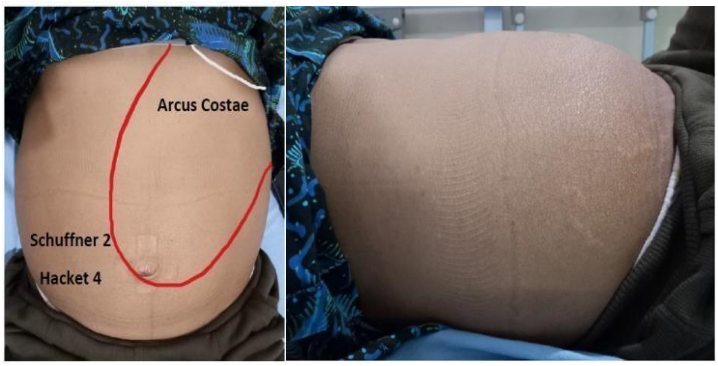

Figure 1. Patient's clinical presentation

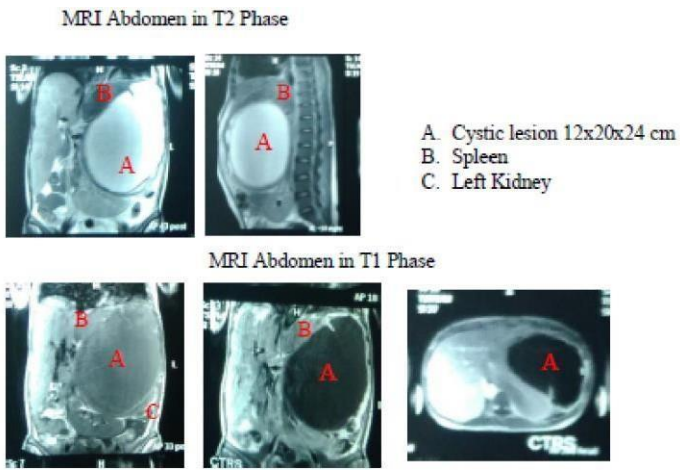

Figure 2. MRI of the patient's abdomen without and with contrast

Figure 3. The surgical procedure

Anatomic pathological examination of the spleen showed stroma with infiltration of neutrophil inflammatory cells, lymphocytes, histiocytes, and plasma cells. The fibrosis and necrosis area did not show malignancy signs. Cytological examination of pus fluid also showed spread of inflammatory PMN cells, signs of malignant cells, mononuclear, and macrophage. The operation began with the patient in supine position on the operating table in general narcosis with abdominal exposure as the operating object. A septic marker procedure was undertaken in the area to be incised, followed by an incision median line, starting from the xyphoideus process to suprapubic (Figure 3).

Then, the incision was deepened layer-by-layer until it opened the peritoneum and reached the peritoneal cavity. The exploration showed 5000 ml of turbid peritoneal fluid mixed with pus and intestine adhesion grade I-III. The omentum was walling off to the left upper and an enlarged spleen was found measuring 20 x 15 cm. Abscess was found. Then, adhesiolysis and splenectomy were undertaken, followed by washing the abdominal cavity with 0.9% NaCl as much as 10 liters. Bleeding was overcome by ligation and cauterization. The surgical wound was closed layer-by-layer and the operation was complete.

On the 19th day of post-surgery, the status patient was outpatient. Complaints of pain in minimal surgical wounds, no fever, and no swelling of both legs. On physical examination, dry surgery scars, hyperemia, and tenderness were not found. Furthermore, wound care was done once a day at home. Upon returning home, the patient was given therapy for 500 mg mefenamic acid, taken 3 times a day and 200 mg cefixime antibiotics taken 2 times a day. The patient was given education to vaccinate *Haemophilus influenzae*, meningococcal, and pneumococcal 4 weeks post-operatively.

DISCUSSION

Although splenic abscess is a rare case, basically this case is very fatal if not recognized and treated. With the increased incidence of immunosuppression and treatment, splenic abscess is a common case. Patients may have fever symptoms, leukocytosis and left upper abdominal pain as an immune response form. Radiological examination of the thorax and abdomen often shows left pleural effusion, increased left hemidiaphragm, mass in the upper left quadrant, and extra luminal air. CT Scan and MRI are imaging modalities that have very high sensitivity. Splenic abscess typically shows a thick irregular edge with hypodense in the middle, but multiple abscesses may be difficult to recognize (Krokos et al. 2011, Zavariz et al. 2017). In this case report, we presented a rare case about splenic abscess according to the literature.

In this splenic abscesses patient, bacterial culture was found to be aseptic. The case report by Brooks and Ghaffari (2014) stated that aseptic spleen abscess unresponsive to antibiotics were precursors of Inflammatory Bowel Disease and require further examination of colonoscopic biopsy (Brooks & Ghaffari 2014). Whereas, in this case report, the patient showed clinical improvement in antibiotics after splenectomy. Lee also reported K. pneumonia as a coinfected bacteria found in splenic abscesses originating from liver abscess or as sequel bacteria that spread from the liver to the spleen (Suk et al. 2011).

Treatment options for splenic abscess include broad spectrum antibiotics, splenectomy, and upper left quadrant drainage. If the patient's condition is critical and surgery is not possible, image-guided drainage should be considered (Yeo 2018). Percutaneous image-guided drainage has an open surgery advantage, including minimally invasive procedures, useful in high-risk patients who avoid general anesthesia with a

short operating time (Hamarayan et al. 2012). In this case, the splenic abscess patient had splenectomy and intravenous antibiotics with 750 mg levofloxacin in 24 hours and 500 mg metronidazole every 8 hours.

Typical signs and symptoms include fever, abdominal pain and tenderness in the left upper quadrant or palpable spleen due to enlargement (in 40% of the patients) along with chest symptoms (Plummer et al. 2004). Clinical examination did not reflect anything other than abdominal pain, which was more dominant in the left upper quadrant (Hamarayan et al. 2012). Diagnosis may be difficult given that only two-thirds of patients showed classic triad symptoms, e.g., fever, splenic enlargement, and left upper quadrant pain (Nagem & Petroianu 2009).

In splenic abscess cases laboratory findings, such as leukocytosis, increased C Reactive Protein (CRP), liver function, and kidney function were normal. Blood culture in 24-50% of the cases was positive (Ng et al. 2008). Chest and abdominal photos often show unspecified changes and can be used as a screening tool because they have high sensitivity but low specificity. Abnormal findings include infiltrates in the left lower lung lobe, left pleural effusion, and increased left hemidiaphragm. Abdominal ultrasound has very useful and added advantage for guideline of percutaneous needle aspirations, and catheter placement for continuous drainage. CT scan and MRI of the abdomen are more superior imaging modalities than others for splenic abscess evaluation (Hamarayan et al. 2012, Krokos et al. 2011).

In this case, the right decision for the patient was splenectomy, both with open and laparoscopic splenectomy. Significant splenomegaly (length >22 cm or weight >700 grams) was considered a contraindication, so that laparoscopic resection was performed, because it was difficult to manipulate and there was a risk of bleeding (Krokos et al. 2011). There are only three therapeutic modalities, namely (a) antibiotics only, (b) antibiotics with percutaneous drainage, and (c) splenectomy. Of the three therapeutic modalities, splenectomy provides the best results (Llenas-Garcia et al. 2009).

The surgery combination and antibiotic therapy were considered the most suitable treatment method. However, broad spectrum antibiotics should be given to patients who cannot be operated (Krokos et al. 2011). Minimally, the invasive method has the advantages of surgical risk minor and hospitalization short duration (Fotiadis et al. 2008).

Based on the results of examination (history, physical examination, laboratory, and pathology) and prognosis on the patient, we decided to perform splenectomy and provide antibiotics to obtain optimal operating results to reduce morbidity and mortality rate. As to the splenic abscess treatment, intravenous antimicrobial therapy, CT guided percutaneous aspiration, and splenectomies were the options.

An initial study had shown that the use of intravenous antimicrobial therapy alone resulted in 100% mortality (Ooi & Leong 1997). Percutaneous drainage was only performed when the abscess is unilocular or bilocular with a discrete wall, no internal septa, and liquid content (Llenas-Garc´ıa et al. 2009). Lee and Lee made a clinical review from 2012-2016 about the comparison of recovered and mortality rates with various treatment options, patients who get antimicrobial therapy alone (81,8% recovered; 18,2% mortality rate), antimicrobial therapy with splenectomy (100% recovered; no mortality rate), and antimicrobial therapy and percutaneous drainage (100% recovered; no mortality rate). Since there are no guidelines regarding its diagnosis and management. The best therapeutic approach for splenic abscess is still a matter of debate (Lee & Lee 2018). Complications of splenic abscess include fistula formation, rupture (43% mortality) and sepsis (Krokos et al. 2011).

In this case report, the patient's microbiological examination did not find bacterial growth. Sterile abscess indicated that the optimal isolation technique for anaerobic bacteria was not done (Krokos et al. 2011). The reported frequency of negative culture results reached 30%. This showed that the patient had been treated with antibiotics, sterile necrosis presence due to infarction, anaerobic cultures failure, and organisms that had not been identified (Brook & Frazier 1998). The bacterial culture sterile potential reason was antimicrobial treatment 3 days in this patient before. However, it seems to have geographical variations and a different population.

Klebsiella pneumoniae was a leading pathogen causing splenic abscess in Taiwan (Chang et al. 2006). *Mycobacterium tuberculosis* had been reported to be the most common liver abscess pathogen in Spain (Llenas et al. 2009). In Thailand, *Burkholderia pseudomallei* had been prescribed to be the most predominant pathogen in 60 cases of retrospective study on splenic abscess (Sangchan et al. 2003). Regarding fungal splenic abscesses, they were found predominantly in immunocompromised patients (Lee & Lee 2018). In this patient, we did not perform fungal and parasitic infection tests, because the HIV Screening test was

negative, and there was no immunocompromised clinical features.

Splenectomy patients have overwhelming post splenectomy infection (OPSI) lifelong risk. A condition associated with high mortality rates (Casciani et al. 2020). Encapsulated organisms are frequently associated with sepsis in splenectomies patients. An encapsulated organism, such as Streptococcus pneumoniae is particularly resistant to phagocytosis, but quickly overcome in the presence of even a small amount of type-specific antibody (Davidson & Wall 2001). Without the spleen, prompt antibody production against a newly encountered antigen is impaired and bacteria proliferate rapidly. Therefore, the invasive pneumococcal disease risk in patients without a spleen is 12-25 times greater than that in the population at large (Hirose et al. 2016).

The invasive disease in splenic patient due to such encapsulated organisms as *Streptococcus pneumoniae* (50%-90%), *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pyogenes* (25%) leads to uninhibited bacterial overgrowth (Okabayashi & Hanazaki 2008). According to the CDC, adult patients are required to have several vaccinations after splenectomy. Haemophilus influenzae B vaccination should be given once, Human Papilloma Virus (HPV) three times, Meningococcal A and B two times at interval of 8 weeks, Pneumococcal (Previary and Pneumovax) 1 to 2 times, and DPT (Diphtheria, Pertussis, Tetanus) and Zoster vaccinations, while vaccination for Hepatitis A, Hepatitis B, MMR (Mumps, Measles, Rubella) and varicella are given if necessary (Center for Disease Control and Prevention 2015). In this patient, vaccinations post splenectomy will be given based on CDC recommendation.

Strength and limitation

The strength of this case is the successful diagnosis and management of a rare condition, splenic abscess, despite the absence of bacterial proliferation on cultures. The use of advanced imaging techniques, such as abdominal MRI, allowed for the accurate identification of the lesion. The surgical intervention, splenectomy, was also effective in treating the abscess. Additionally, the prognosis for the patient was favorable, as reported in the literature. This case was the importance of considering unusual diagnoses and utilizing appropriate diagnostic tools to achieve successful outcomes in patient care.

CONCLUSION

Since splenic abscess has a high mortality rate when too late recognized. It should suspect diagnosis if a mass was found in the left upper abdomen. Careful clinical examination, supported with an initial imaging examination in the form of ultrasound abdomen as screening, was very helpful in establishing the diagnosis. Abdominal CT scan or MRI imaging can be used as a very sensitive and specific imaging tool for splenic abscess cases. For therapeutic modalities, splenectomy is one modality that is still the main choic for reducing mortality and morbidity by giving broad spectrum antibiotics to optimize therapy results.

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Conflict of interest
None

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Author contribution

APK conceived the idea of the study and wrote the manuscript. ED have to lead the surgery, validation the grammar and content. All authors were involved in the revision of the manuscript and have agreed to the final content.

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Case Report

A CASE OF MALIGNANT RIGHT CORONARY ARTERY: FREQUENT ANGINA IN YOUNG PERSON

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ABSTRACT

We presented a case a young adult with activity-triggered atypical chest pain and diagnosed with anomalous origin of right coronary artery (RCA) from the left coronary sinus with an interarterial course between the aorta and the main pulmonary artery that was detected by CT coronary angiography. This anomaly has been called malignant RCA. Coronary artery anomaly is a congenital condition. Most of the cases remain asymptomatic. This condition is also one of the most causes of sudden cardiac death, because the coronary artery examination is not regularly done. Nevertheless, during high intensity activity, it could be symptomatic and might be lethal. Diagnosing coronary artery anomalies might be tricky and cardiologists must be aware of this. The CAAs condition is a rare situation. The CAAs condition is associated with sudden death, especially intense physical activity. There was no rigid guideline for the management of the CAAs condition, so that planning a treatment in the inter-specialist team should be done.

***Keywords**: Malignant RCA; chest pain; health risks; CT coronary angiography*

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Highlights:

1. A young adult has activity-triggered atypical chest pain and diagnosed malignant RCA.
2. Congenital anomalies needed to be aware by cardiologists to help clinical practice.
3. Planning a treatment about management of the CAAs condition should be undertaken by the inter-specialist team.

INTRODUCTION

Coronary artery anomalies (CAAs) are still becoming an important matter to discuss in basic and clinical practice. There is extensively discussed in the literature regarding the definition of CAAs, especially in how it can be different with a normal variant of the coronary artery. Nevertheless, a previous study has proposed a comprehensive classification of CAAs based on several groups of abnormalities; anomalies of origination and course, anomalies of intrinsic coronary arterial anatomy, anomalies of coronary termination, and abnormal anastomotic vessels (Angelini 2007). Moreover, Angelini (2002) had proposed an accessible definition of the normal variant shown in Table 1. The clinical spectrum of CAAs is enormously extensive, from asymptomatic to resting ischemia. The awareness of atypical coronary anatomy could help the cardiologist in giving precise diagnosis and treatment, including catheterization laboratory and surgical procedure (Angelini 2002).

The CAAs are a rare congenital condition, andtheir incidence is around 0.17%-1.2% (Graidis et al. 2015). Regardless of its incidence, around 20% of the CAAs are malignant and could undergo life-threatening manifestation, such as

lethal arrhythmias, syncope, myocardial infarction, or even sudden death (Datta et al. 2005). Specifically, the incidence of the malignant CAAs from the right coronary artery origins is around 0.03-0.17% from patients undertaking angiography (Satija et al. 2012). Here, we presented the right CAAs malignant type with symptomatic atypical chest pain in 34 years old man.

Table 1. Purpose definition of the normal heart variant conditions

Feature	Range
Number of Ostia	2 – 4
Location	Right and left anterior sinuses (upper mid-section)
Proximal orientation	45-90 degrees off the aortic wall
Proximal common stem or trunk	Only left (left anterior descending and left circumflex artery)
Proximal course	Direct, from ostium to destination
Mid-course	Extramural (sub-epicardial)
Branches	Adequate for the dependent myocardium
Essential territories	Right Coronary Artery (Right ventricle free wall)
	Left anterior descendent (anteroseptal)
	Obtuse Marginal Artery (Left ventricle free wall)
Termination	Capillary bed

CASE REPORT

A-34-years-old man has complained of vague chest pain since more than six months ago. There were no risk factors or any significant past medical history. No remarkable result from routine blood examination and rest electrocardiography (ECG). However, during exercise the treadmill test showed ST depression in the lead VI. The patient was referred to the radiology department to undergo CT coronary angiography.

CT coronary angiography was performed using a 64 slice CT scanner (Brilliance-64, Philips, The Netherland). The following parameters were used: 120 KVp, 800 mAs, rotation time: 0.4 s, and collimation: 64 × 0.625mm. Using a dual-head pressure injector, 80 ml of non-ionic iodinated contrast was administered at the rate of 5 ml/sec, followed by 20 ml of saline. The total scan time was 8.6 seconds. Reconstruction was done with 0.6 mm slice thickness 0.5 mm increment.

CT coronary angiography showed the right coronary artery originating from the left coronary sinus, coursing between the aortic root and pulmonary artery (Figure 1a). The rest of the coronary arteries, the left coronary artery and its branches, a normal course (Figure 1b, and Figure 1c). However, we did not yet decide on the treatment for this patient, and still need to follow-up for reassurance (Figure 1).

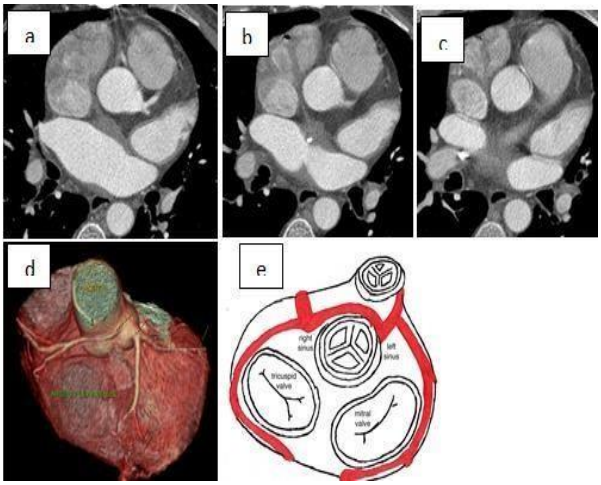

Figure 1. The schematic representation of malignant right coronary artery

Figure 1. CT coronary angiography and schematic figure of malignant RCA. A-c. Maximum intensity projection of the heart showing RCA and LCA originating from LCS. RCA courses between PA and Aorta. d. Volume rendered image showing malignant RCA of this patient with intra-arterial course of RCA. e. Schematic figure of malignant RCA.

DISCUSSION

The CAAs were frequently found as the cause of sudden death (SD) cases in the young. Even though it is a congenital condition, many subjects survive asymptomatic until young adult (Basso 2005). The anomalous coronary artery (CA) origin, either left the main artery from the right sinus or vice versa, had been found 0.17% during autopsy (Basso 2005). Moreover, recent studies found four children from 2,388 children underwent angiogram diagnosed with either anomalous origin of the left main coronary artery (ALMCA) from the right sinus or anomalous origin of the right coronary artery (ARCA) from the left sinus (Davis et al. 2001). Two and half week-old patients with ARCA had normal serial resting ECG after repeated 12 ECG exams one, a half months later and at 35 months. However, the 12 years old patient showed uniform ventricular ectopy with a left bundle configuration consistent with right ventricular origin.

The ectopy diminishes during exercise ECG. In exercise ECG, there was also abnormal repolarization of the lateral chest lead with T-wave inversion and ST elevation. The treadmill exercise perfusion study exhibited inferior wall ischemia of moderate intensity in the RCA perfusion area. The patient persisted

asymptomatic during the test. In our case, the patient never underwent either resting or exercise ECG before and remained asymptomatic. Until the patient reached 34 years old, when he was busy with his job, he suffered atypical chest pain. The resting ECG resulted in no remarkable result. On the other hand, treadmill ECG examination showed notable change with suspicion from right ventricular origin.

Anomaly aortic origins of coronary arteries account for 14-17% of sudden cardiac death for healthy children or young athletes during or immediately after physical exercise (Silva et al. 2018). The lethal mechanisms of the CAAs are considered through malignant arrhythmias and ischemia-myocardial dysfunction (Lluri & Aboulhosn 2014). Those mechanisms are triggered by vigorous physical exercise (Hill & Shepprad 2014). Anomaly aortic origins of coronary arteries further classified into two subgroups; anomalous origin of coronary arteries from the opposite sinus (ALMCA and ARCA), and anomalous left coronary artery from the pulmonary artery (ALCAPA). Despite the classification of the two subgroups, the correlation with sudden cardiac death is quite high (Lluri & Aboulhson 2014). The physiological supply and demand of the heart depend on its exercise type.

During isotonic and isometric exercises, the venous return, and left end-diastolic volume will increase. The adrenergic system stimulation also increases the heart rate, blood pressure, cardiac output, and myocardial contractility. The purpose of the mechanism is to increase oxygen supply to the heart. Unfortunately, the CAAs condition is contrary to that purpose (Wasfy et al. 2015; Cheitlin & MacGregor 2009). In this patient, asymptomatic condition (until he grew up to adult), seemed rational since the patient was not an athlete, and he was probably not doing high-intensity exercise until he became very busy with his work now. Nonetheless, this type of CAAs are associated with a high risk of sudden cardiac death based on previous evidence (Basso 2005). We strongly suggested the patient to reduce his work intensity and scheduled a follow-up meeting to plan the treatment.

In ALMCA or ARCA conditions, the proximal segment of the anomalous CA may course anterior to the pulmonary trunk, posterior to the aorta, or between the pulmonary artery and the aorta (Basso 2005). At some points, especially during exercise, the CA squeezes due to the increased cardiac output with a diastolic expansion of great vessels. As a result, myocardial ischemia might be happening (Basso 2005; Silva et al. 2018; Cheitlin & MacGregor 2009). The ALMCA might have a bigger risk of sudden cardiac death compared to ARCA (Basso 2005). In this case, our patient had an ARCA condition, and this condition might have a lower risk than ALMA's risk of sudden cardiac death. However, this condition could still be life-threatening.

Strength and limitation

The strength of this case report is its focus on a rare and potentially life-threatening condition, anomalous origin of the right coronary artery, and the importance of early diagnosis for preventing sudden cardiac death. The fact report that this condition can be asymptomatic and difficult to detect, and emphasizes the need for increased awareness and regular screening for coronary artery anomalies. Additionally, the report emphasizes the importance of interdisciplinary collaboration for effective management of this condition.

CONCLUSION

In conclusion, the CAAs condition is a rare situation. However, cardiologists need to be aware of congenital anomalies because it might help in clinical practice. Even though the incidence is low, the CAAs condition is associated with sudden death, especially intense physical activity. There was no rigid guideline for the management of the CAAs condition, so that planning a treatment in the inter-specialist team should be done.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

SPS-conceptualized the study, SLP-wrote the manuscript, revised, collected and analyzed the data, SPS-validated and finalized the manuscript.

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Case Report

MEDIASTINAL NON-HODGKIN'S LYMPHOMA METASTATIC TO RIGHT ATRIUM MIMICKING RIGHT ATRIAL MYXOMA

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ABSTRACT

In this case report, the anatomical pathology results in the form of B cell type LNH, but at the age of 36 years and the risk factor in this patient was a former active smoker. In the anatomical pathology results, the results of the B-High Grade Cell Type LNH were also obtained. B-cell type non-hodgkin's lymphoma can be mutated in the MYC gene (v-myc avian myceloctomatosis viral oncogene homolog) and the BCL-2 and BCL-6 (B-cell lymphoma) genes. If this morphology is found, then the patient's prognosis is poor. Most of these patients were males and the incidence was in the mediastinal area. Mediastinal NHL could develop and enlarge to involve the heart and pericardium. The spread could occur directly and lymphogens. These metastatic tumors were often misdiagnosed with atrial myxoma. In this case report, exploration of the right atrium and open mediastinal biopsy was performed. An open biopsy of the mediastinum revealed a mediastinal mass that enlarged to enter the right atrium. Atrial myxoma was not found. Primary lymphoma growth could also occur in the heart. This condition was called primary cardiac lymphoid (PCL). This case was very rare and was often considered an atrial myxoma. The patient died 10 days after discharge from the hospital. While the patient was eating, the patient had a seizure and the patient was immediately taken to the emergency department of Dr. Soetomo General Academic Hospital, Surabaya, and entered the ER (Resuscitation) ER room, but the patient died after being assisted for approximately two hours. Most likely the cause of the patient's death was a thromboembolic tumor in the right atrium that was released, so that it entered the bloodstream of the brain, causing the patient to have seizures. It was suspected that the cause of the patient's death was the presence of a tumor thrombus that separated into an embolism from the right atrium due to the large size of the tumor. Patients suffering from high-rate NHL had a greater percentage of suffering from tumor thromboembolism as many as 10.6% compared to the Low type and Hodgkins lymphoma (LH) (5.8% and 7.25%).

***Keywords**: Mediastinal tumor; non-hodgkin's lymphoma; atrial myxoma; health risk*

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Highlights:

1. A 32-year-old male patient suffered mediastinal non-hodgkin's lymphoma metastatic to the right atrium which mimicked right atrial myxoma.
2. The patient died of suspected mediastinal NHL thromboembolism that spread in the right atrium.

INTRODUCTION

Mediastinum is located in the middle of the thoracic cavity, between the diaphragm, pleural cavity, and thoracic inlet. In general, mediastinum is divided into 3 compartments, namely the anterior, medial, and posterior (Broaddus et al. 2016). Mediastinal tumors that grow in the anterior part are lipoma, liposarcoma, teratoma, lymphoma, and others (Silva et al. 2014). Lymphoma has an incidence of between 10%-20%, both in young adults and the elderly. Based on their histopathology, lymphomas are divided into two kinds, namely Hodgkins lymphoma and non-Hodgkin's lymphoma (NHL) (Broaddus et al. 2016).

In Indonesia, NHL, along with Hodgkin's lymphoma, holds the 6th rank of malignancy (National Cancer Prevention Committee 2016). NHL can occur in all parts of body. Mediastinal NHL can develop and enlarge to involve the heart and pericardium. The spread can occur directly and lymphogens, such as spread/metastases usually occur about 20 months after the initial diagnosis. Symptoms that can be experienced by the patients are usually not specific. New symptoms arise when lymphomas grow large, while pericardium effusion has been formed. The usual symptoms are tightness, chest pain, arrhythmias, cardiac tamponade,

myocardial ischemia, and right heart failure (Bligh et al. 2017). In some cases, it is difficult to distinguish whether it is a primary tumor in the heart or the presence of tumor development that enters the heart.

This case report discussed a patient with non-Hodgkins lymphoma mediastinal right atrial metastases resembling right atrial myxoma from initial treatment to death. Therefore, the discussion was limited to non-Hodgkin's lymphoma. The patient was not exposed to other substances except cigarettes. The patient was an active smoker for 10 years ago with 10-12 cigarettes per day, for 5 years, and quit. There was no data regarding the patient's infection to date regarding the etiology of infection. This case report also did not include a post-mortem autopsy report, because it was not performed.

CASE REPORT

A male patient, aged 32 years, came with complaints of intermittent shortness of breath since three months before hospitalization. Cough was rare, and the phlegm was thick white. He had decreased weight and appetite during the past month. Swelling slowly occurred on the face and neck in the last three months. There was no fever, chest pain, and night sweats.

The results of FNAB (Fine Needle Aspiration Biopsy) and Thorax-Guiding CT scan suspected thymoma. The patient had received anti-tuberculosis drugs (ATD) for 3 months, but it did not heal. The patient had a right and left chest fluid collection as many as 1000 mL yellowish liquid for each. The patient smoked approximately 1 pack per day for 5 years and had stopped since 10 years ago. On thoracic examination, the chest wall movement was symmetrical, intercostal space widened bilaterally, and the trachea remained in the middle. A reduction in palpated fremitus was found in lower 2/3 of both hemithoraces, dull in 2/3 of both hemithoraces. The vesicle decreased in 2/3 of both lower hemithoraces. No additional breath sounds were obtained. Non-pitting edema was found on the right and left arm.

Laboratory tests showed an increase in SGPT, direct bilirubin, LDH, and reactive hepatitis-B. BGA (Blood Gas-Analysis) which showed perfectly compensated respiratory alkalosis with mild hypoxemia. Fluid analysis showed pleural exudate. The initial chest x-ray at the hospital for 3 weeks earlier showed a profile of homogeneous opacity in the lower right and left hemithorax, suggesting bilateral pleural effusion (Figure 1).

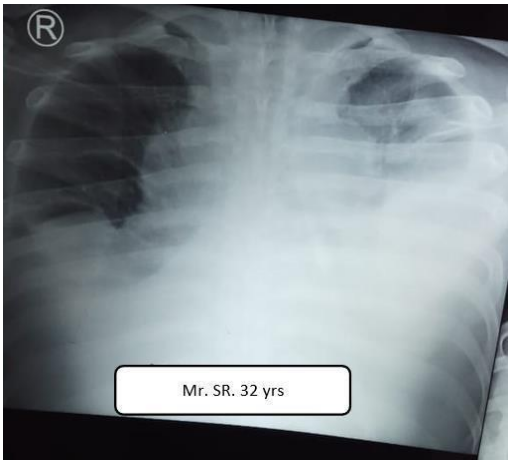

Figure 1. Chest x-ray of homogeneous opacity in lower right and left hemithorax

Contrast thoracic CT examination showed a well-differentiated hypodense lesion measuring about 7.4 x 4.8 cm in the anterior mediastinum attached to and surrounded vascularity entering the left atrium and pleural effusion was found in the right hemithorax (Figure 2).

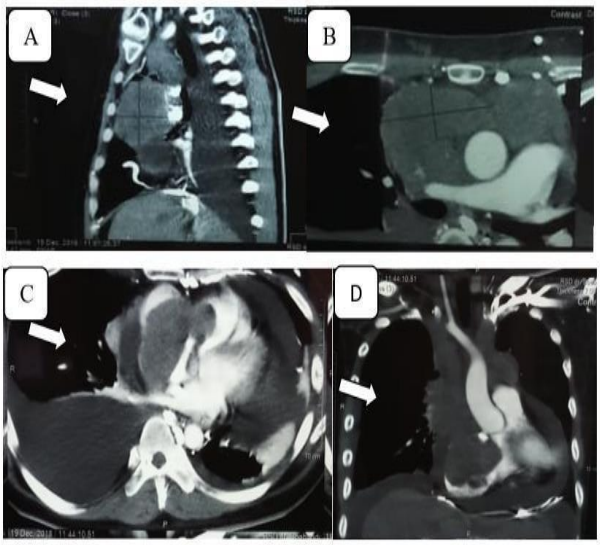

Figure 2. Contrast thoracic CT. A. Sagittal plane. B. Axial plane. C. Pericardial effusion (arrow) D. Suspected thrombus tumor, differential diagnosis of atrial myxoma (arrow)

In radiological evaluation in 3 weeks later, a profile of homogeneous hemithoracic opacity of the left and right hemisphere was obtained, with the one on the left side was more severe. An amount of 900 mL of left pleural fluid was serohemorrhagically evacuated. Tumor marker AFP (beta-fetoprotein) revealed 3.8 ng/mL and CEA (Carcino-embryonic Antigen): 4.63 ng/mL. HCV was non-reactive.

Abdominal USG did not show metastases. Ascites in the pelvic cavity were minimal. During USG-guided core biopsy, we accidentally found a profile of right atrial myxoma. Blood tests showed WBC (White Blood Cell): 21700, neutrophil: 88.8%, SGOT: 51 SGPT: 111, potassium: 2.8 and procalcitonin: 0.09. Echocardiography was performed with the results showing pericardial effusion and mass that filled the atrium, suspected myxoma with a differential diagnosis of atrial thrombus (Figure 3).

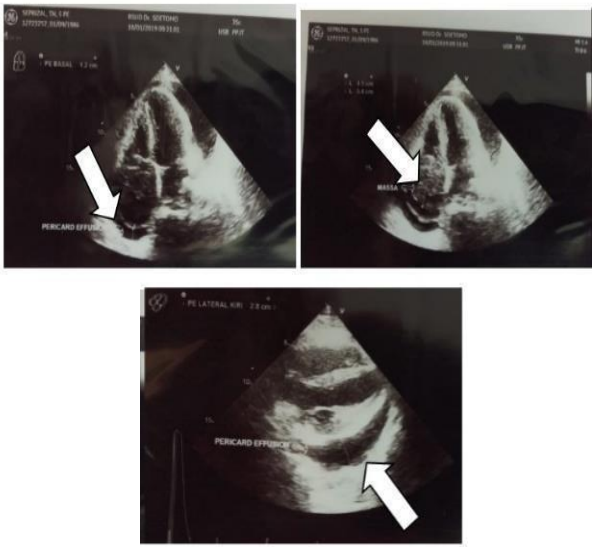

Figure 3. Echocardiography shows pericardial effusion and mass with differential diagnosis of thrombus tumors

Note: Pericardial effusion and mass with differential diagnosis of thrombus tumors filled the right atrium, measuring 4.5 cm x 5.4 cm. Pericardial effusion was massive in the left lateral 2.8 cm, and moderate in the basal 1.2 cm

Thoracic CT examination showed an anterior mediastinal mass (about 10.9 x 5.1 x 7.6 cm) with 55-67 HU contrast enhancement that infiltrated and filled the right atrium and superior vena cava, right and left brachiocephalic vein to jugular vein, and caused thrombus tumors to fill totally the right atrium, superior vena cava, pericardial effusion (density 12 HU, max thickness 2.9 cm) was present, a single nodule in the anterior segment of the superior lobe of the right lung (size 0.6 cm), and there was a sub-centimeter lymph node at left supraclavicular (about 1.4 cm) and right upper paratracheal (about 1.3 cm). No atelectasis fluid in the right and left pleural cavities, and no lytic processes were found (Figure 4).

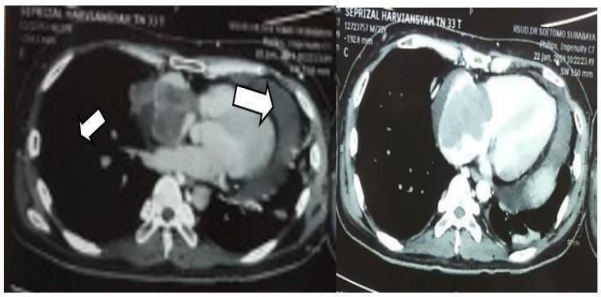

Figure 4. Thoracic CT contrast

FOB (Fiber Optic Bronchoscopy) was performed with the result that no mass was obtained, but narrowing was obtained due to the extraluminal pressure of the main carina and the left main bronchi (Figure 5). The results of BAL (Broncho Alveolar Lavage) examination, brushing cytology, forceps, and biopsy aspiration did not reveal malignant cells.

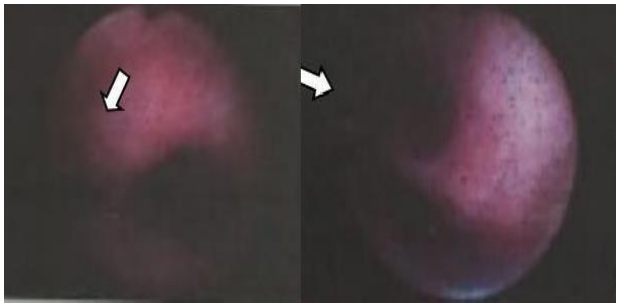

Figure 5. FOB constriction was found due to extraluminal pressure of main carina (left figure) and left main bronchus (right figure)

Open biopsy findings from the mediastinum revealed that the mediastinum mass had enlarged to enter the right atrium. Atrial myxoma was not found. Anatomic pathology (AP) examination showed poorly differentiated carcinoma, non-Hodgkin's lymphoma with IHC (immunohistochemistry) of positive CD45 and negative CK. CD-3 IHC examination revealed negative on the tumor cell membrane, positive on mature T lymphocytes, CD-20: positive on the tumor cell membrane (B-cell), Ki-67: 90% proliferation index. Conclusion: Non-Hodgkin Lymphoma, type-B cells, high-grade. Two months after treatment, the patient reported a seizure and died at home with the probable cause of death of mediastinal NHL thromboembolism that had spread into the right atrium.

DISCUSSION

The etiology of NHL is caused by such infections (Epstein-Barr Virus, Burkitt Lymphoma types related HIV, Human Herpes Virus-8, Helicobacter pylori) or

environmental exposure (herbicides, cigarettes, arsenic, halomethane, asbestos) (Theodore et al. 2019). This patient was a smoker with a Brinkman index of 60. There were no signs of infection.

Primary lymphoma growth can also occur in the heart (Singh et al. 2016). This condition is called primary cardiac lymphoid (PCL). This case is very rare and often considered an atrial myxoma. It was also found that various tumors can metastasize into the heart, ranging from the most common types, such as bronchogenic carcinoma, malignant melanoma, malignant lymphoma, pancreatic carcinoma, and others (Kuriakose et al. 2015, Sweni et al. 2019). These metastatic tumors are often misdiagnosed with atrial myxoma. In this case report, exploration of the right atrium and open mediastinal biopsy was performed. An open biopsy of the mediastinum revealed a mediastinal mass that enlarged to enter the right atrium. Atrial myxoma was not found.

Aging is most likely an important factor in the pathogenesis of B-cells NHL, because this tumor is found mainly in the older age group, and there is an increase in the incidence in each age group over 55 years (Diumenjo et al. 2016). In this case report, anatomical pathology examination showed cell B-type high-grade NHL, but the patient was a 36-year-old with a risk factor of a former active smoker.

In mediastinal NHL, in addition to the manifestations of shortness, systemic complaints were also found, such as body weakness, fever, and weight loss. In addition, there can be superior vena cava syndrome (VCSS), chest pain, hoarseness, and abdominal fluid (ascites) (Mihaljevi et al 2014). In this patient, we found shortness of breath, swelling of the face and neck due to VCSS, and weight loss.

Diagnostic tests included a complete blood examination, clinical chemistry, tumor markers of AFP, LDH, and beta-HCG, continued with examination with core biopsy guided by ultrasound or chest CT scan with contrast. Then, we also examined the anatomy histopathology along with the IHC (immunohistochemistry) (National Cancer Prevention Committee 2016).

Anatomic histopathology examination of the tissue was done by immunohistochemical (IHC) examination of CD45, CD20, CD3, and KI67. According to The National Guideline of Medical Service from the National Cancer Prevention Committee in 2016 states that in lymphoma examination, the proliferation rate was considered high if Ki 67 was more than 30%. In this case, IHC examination was carried out with positive CD45, positive CD20, negative CD3, and Ki67 with a 90% proliferation index (high proliferation rate). Ki67 was a patient's prognostic factor, and it was one of the evaluation parameters after Rituximab chemotherapy. The higher the proliferation index, the worse the patient's prognosis (He et al. 2014).

The NHL staging system was used according to Ann Arbor Staging (Table 1) (National Cancer Prevention Committee, Naeim et al. 2018):

Table 1. Ann-arbor staging

Stages	Description
I	Involvement of a single lymphatic site
II	Involvement of two or more lymph node regions on the same side of the diaphragm: II.2: Involvement of two lymph node regions in one side of the diaphragm II.3: Involvement of three lymph node regions in one side of the diaphragm II.E: Non-diffuse/well-defined involvement of one lymph node region in one side of the diaphragm and one side of a single extralymphatic organ
III	Involvement of lymph nodes in both sides of the diaphragm
IV	Diffuse involvement in one or more extralymphatic organs

Stage information is added behind, A or B or C. A: without constitutional symptoms, B: with constitutional symptoms, such as fever, cold sweats, or weight loss of more than 10%, and C: extranodal involvement (National Cancer Prevention Committee 2016, Naeim et al. 2018).

The patient did not undergo full-body lymph node ultrasound and contrast abdominal CT scan. However, the abdominal ultrasound did not show lymph node enlargement. Thoracic CT scan showed a single nodule (0.6 cm) in the anterior segment of the superior lobe of the right lung, and there was sub-centimeter lymph node in the left supraclavicular (1.4 cm) and right upper paratrachea (1.3 cm). According to the Ann-Arbor staging, it indicated that the patient was at stage III (enlarged lymph nodes on both sides of the diaphragm) with constitutional symptoms: B, so that the patient was at stage IIIB.

The patient died 10 days after being discharged. The patient had a seizure. The most likely cause of the patient's death was a thromboembolic tumor detached within the right atrium, so that it entered the bloodstream of the brain, and caused the patient to spasm.

A patient suffering from high-rate type NHL type had a greater percentage of having tumors thromboembolism as much as 10.6%, compared to those with Low and Hodgkins Lymphoma (HL) types (5.8% and 7.25%) (Mohren et al. 2005). In this case report, the suspected

cause of death of the patient was the presence of a thrombus tumor which was detached and lead to embolism from the right atrium due to the large size of the tumor, because the location of the LNH was in the atrium. It was very likely that parts of the LNH were released following the atrial movement, so that when it released, it became an embolism, and there was a possibility of clogging the pulmonary blood vessels, coronary arteries in the heart and in the central nervous system. Lekovic et al (2010) stated that Compression of Large mediastinal vessels were common in patients with Primary Mediastinal Large B-Cell Lymphoma, predisposing with venous thrombosis. The incidence of this complication is still unknown.

Furthermore, Eltawansy et al (2015) had found that the malignancy had a causal relationship with deep vein thrombosis which can be secondary to the mechanical stress of the tumor on the venous system draining blood from the affected body part or it can be a secondary systemic phenomenon of thrombogenic material released into the circulation from the tumorigenic network. Venous thromboembolism (VTE) is found at autopsy in at least 50% of cancer patients. However, assessment of the true incidence of VTE in cancer patients is difficult, because most patients receive chemotherapy which can trigger VTE. Therefore, this patient was likely to experience thromboembolism too.

Strength and limitation

The strength of this case report lies in the detailed description of the diagnostic process, including the use of radiological imaging, tumor markers, core biopsy, histopathology, and genetic testing to establish the diagnosi of B-cell high-grade non-Hodgkin's lymphoma (NHL). The report also provides valuable information about the incidence and characteristics of mediastinal NHL, as well as the potential for misdiagnosis with atrial myxoma. The case highlights the importance of considering the possibility of tumor thromboembolism in patients with high-grade NHL, which can have significant clinical implications. However, the report also has limitations, such as the lack of information about the patient's treatment regimen and response, as well as the limited follow-up information after the patient's death.

CONCLUSION

A male patient, 32 years old, came with complaints of shortness of breath. A contrast thoracic CT scan showed anteromedial mediastinal tumor and pericardial effusion. Echocardiography showed a suspicion of right atrial myxoma with differential diagnosis of thrombus tumor in the right atrium. Open biopsy showed a suspicion of NHL. IHC revealed CD-3, CD-20 and Ki-67. The patient died of suspected mediastinal NHL thromboembolism that spread in the right atrium, because the location of the LNH was in the atrium. It was very likely that parts of the LNH were released following the atrial movement, so that when it was released, it had become an embolism, and there would be a possibility of clogging the pulmonary blood vessels and coronary arteries in the heart and in the central nervous system.

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Conflict of interest

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Author contribution

GK and LK-conceptualized the study. GK-wrote and revised the manuscript, LK-Lead the surgery, validated, and finalized the manuscript.

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Case Report

A 28-YEAR-OLD MAN WITH MEDIASTINAL SEMINOMA TREATED WITH BEP

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ABSTRACT

Seminoma is a type of germ cell tumor. In this case presentation, a rare primary germ cell tumor was reported in the form of mediastinal seminoma. A 28-year-old man with symptoms of shortness of breath, chest pain, swelling in the right upper extremity, enlarged lymph nodes in the colli region. The diagnosis of malignancy was established through various diagnostic modalities, including chest CT scan with contrast, tumor markers, core biopsy, histopathology, and IHC, which supported the diagnosis of mediastinal seminoma. Thoracic physical examination revealed signs of pleural fluid in the right hemithorax. After obtaining the results of radiological and pathological investigations, a mediastinal mass was obtained, then BEP chemotherapy was given. The underwent clinical and radiological assessment for therapy evaluation. After 3 cycles of chemotherapy, a partial response was obtained. Patients with mediastinal seminoma treated with BEP base chemotherapy gave a partial response. The case demonstrates the successful management of mediastinal seminoma with BEP chemotherapy.

***Keywords**: Germ cell tumor; mediastinal seminoma; health risk*

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Highlights:

1. A 28-year-old male suffered chylothorax and mediastinal seminoma.
2. The patient received bleomycin, etoposide and cisplatin chemotherapy for the management of mediastinal seminomas but he died beforeundergoing 5th cycle chemotherapy.

INTRODUCTION

Seminomas are part of the germ cell tumor. Seminomas of mediastinal masses are rare. Seminomas are a type of malignancy that arise from germ cells, which are cells that give rise to sperm or eggs. They most commonly occur in the testes, but can also arise in other parts of the body, such as the mediastinum. While mediastinal masses are relatively common, mediastinal seminomas are rare. Due to their rarity, diagnosis and management of these tumors require careful evaluation and specialized treatment. The International Germ Cell Consensus Classification classifies mediastinal seminomas as stage III with good, moderate and poor risk categories based on clinical factors including histology, primary tumor location, metastases, and serum tumor marker levels. In this case, we reported on the diagnosis and management of a mediastinal seminoma causing a chylothorax and receiving BEP therapy.

CASE REPORT

Mr. F, a 28-year-old man, came with complaints of shortness of breath, intermittent right chest pain, no complaints of cough, no fever, no decrease in appetite, no weight loss, and no night sweats. The patient complained that his right hand and arm were swollen.

The patient did not reveal the history of trauma, high blood pressure, diabetes mellitus, heart disease, chronic liver disease, asthma and tuberculosis treatment. The patient was a smoker with Brinkman index of 60.

Tachycardia and tachypnea were found in the patient. On head and neck examination, we obtained enlarged lymph nodes in the left and right colli region measuring 2 cm as well as colli edema. Thoracic physical examination revealed pleural fluid in the right hemithorax. Edema was found in the right upper limb.

Laboratory examination showed leukocytosis 10110/µL, granulocytosis 91.7%, hypoalbumin 2.92 gr/dL, and CRP increase of 57.63. Pleural fluid examination obtained dominant polymorphonuclear exudate with cholesterol 10 and triglyceride 858. Blood gas examination obtained metabolic acidosis compensated alkalosis respiratory without hypoxemia.

Chest X-ray showed a blunted right costophrenic angle with homogeneous opacity in 2/3 lower right hemithorax, there was a profile of mass with a blunt edge and obtuse angle on the right perihilar, infiltrates at 1/3 of the right upper hemithorax (Figure 1a).

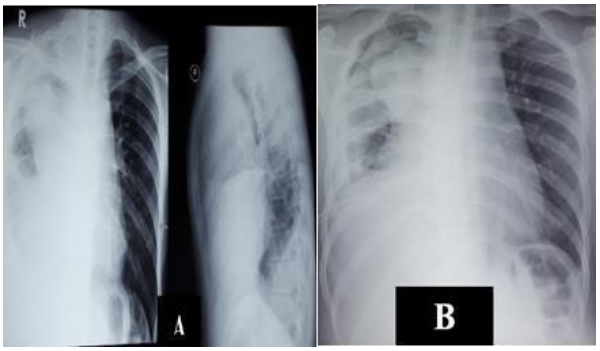

Figure 1. (A) Pre-chest tube thoracic x-ray (B) Post-chest tube thoracic x-ray

The examination of tumor markers showed LDH 551, beta-HCG 12.49 and AFP 3.8. Pleural fluid cell block examination did not show malignant cells. Examination of chest CT scan with contrast (Figure 2) showed solid lesion (34 HU) with calcified component (48 HU) within, well-defined, irregular edges measuring +/- 6.7 x 10.1 x 13.3 cm at anteromedial mediastinum – where by using contrast- showed enhancement (68 HU). The lesion appeared to encase the superior vena cava to the left side. Anatomical pathology examination with Fine Needle Aspiration Biopsy with CT-scan guidance showed malignant round cell carcinoma with suspicion of non-Hodgkin's lymphomaAbdominal ultrasound (USG) examination did not show liver metastases and enlarged lymph nodes in the paraaortic. Abnormalities in the liver, gallbladder, spleen, pancreas, and kidney were not found. The patient underwent a core biopsy with thoracic ultrasound guidance and microscopic examination showed pieces of tissue with tumor growth arranged in lobules, consisting of proliferation of anaplastic cells, rounded nucleus, relatively monotonous, hyperchromatic, narrow cytoplasm, mitosis 6/10 HPF. Fibrous connective tissue was also visible with lymphocyte infiltration. Tumor grew invasively among the fibrous connective tissue stroma, suggesting seminoma. Immunohistochemical examination (IHC) showed positive CD117 on tumor membrane, CD45 negative in tumor cells, positive in mature lymphocytes, positive PLAP in cytoplasm of tumor cells, and negative CK in tumor cells. Immunohistology examination confirmed the diagnosis of mediastinal seminoma.

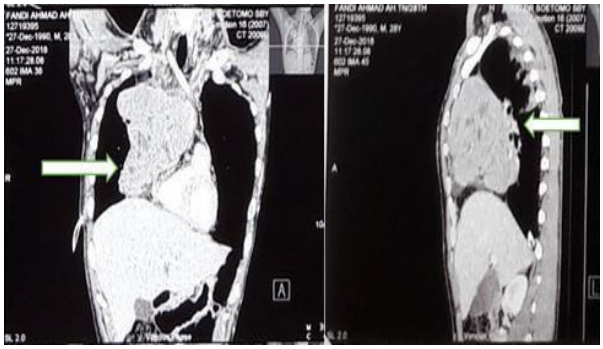

Figure 2. Chest CT-scan with contrast

The patient received 100 mg/m^2 etoposide chemotherapy and 20 mg/m^2 cisplatin on days 1-5. Bleomycin was given 30,000 units on day 2, 9 and 16. During chemotherapy, clinical evaluation showed complaints of nausea, blackened skin, and hair loss. Laboratory tests showed leukopenia as a side effect of degree 2 chemotherapy. The patient received management of chemotherapy side effects.

After the second and third cycles of chemotherapy, side effects of degree 2 chemotherapy appeared, in the form of nausea and blackened skin and accompanied by leukopenia. After 3 cycles of chemotherapy, the general condition of the patient was sufficient, with subjective complaints of intermittent chest pain which improved with the administration of symptomatic therapy.

The patient underwent an evaluation of a thoracic CT scan without and with contrast (Figure 3) with the results, namely solid lesion (30 HU) and necrotic areas (16 HU), with calcification (257 HU) within, well-defined, irregular edges, size +/- 7.4 x 6.6 x 8.4 cm in the anteromedial mediastinum – where by contrast-showed enhancement (65 HU). Lesions appeared attached to the heart, causing pericardial effusion with a maximum thickness of +/- 2.1 cm. The lesion appeared to tighten, constrict and force the superior vena cava to the left side, accompanied by a thrombus in the superior vena cava, right and left brachiocephalic vein, right and

left internal jugular vein, left subclavian vein, encasing ascending aorta, right pulmonary artery and right pulmonary vein, right and left jugular vein, left subclavian vein, encasing ascending aorta, right pulmonary artery and right pulmonary vein, right left jugular vein, left subclavian vein, encasing ascending aorta, right pulmonary artery and right pulmonary vein, abbuting main left bronchus accompanied by collateral veins in anterior right and left hemithorax. There was a bleb (-980 HU) sized +/- 2.7 x 4.4 x 2.6 cm in the apical segment of the superior lobe of the right lung. Lymph node enlargement was found to be +/- 0.6 cm in the right upper paratrachea, +/- 0.8 cm in the left upper paratrachea, +/- 0.7 cm in the lower left paratrachea and +/- 0.8 in the lower right paratrachea. Fluid density (19HU) was found in the right pleural cavity, loculated with thickening of the pleura +/- 0.4 cm, fluid density was visible in the left pleural cavity.

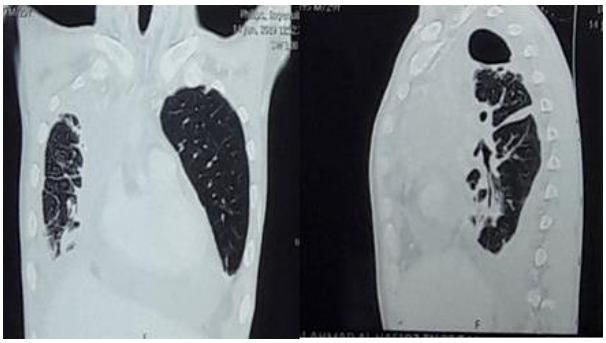

Figure 3. Chest CT scan without and with contrast evaluation after 3 cycles of BEP chemotherapy

The fourth cycle of the chemotherapy was given based on the results of the CT scan evaluation, in which the tumor size shrinked <30% (partial response). Clinical evaluation after the fourth cycle of the chemotherapy showed complaints of nausea and blackened skin, which was the side effect of degree 2 chemotherapy. The patient was unable to continue the 5^{th} cycle chemotherapy, because the patient died.

DISCUSSION

Symptoms, physical and radiological examination of chylothorax are the same as pleural effusion. Because of the presence of fluid in the thoracic cavity, the patient often experienced tightness. Pleuritic pain and fever were rare in the patient, because chyle did not irritate the pleural surface and did not cause inflammation. Long-term loss of chyle causes nutrient loss, hypovolemia, electrolyte disturbances, decreased leukocytes which may cause patient's death (Light & Lee 2016).

Chylothorax is the presence of chyle in pleural cavity and emerges after damage or obstruction of the thoracic duct. In the chyle, there are high concentrations of triglycerides. The diagnosis is made through the analysis of pleural fluid containing high triglycerides and is confirmed by the findings of chylomicrons (Parikh & Rajesh 2018).

In the patient Mr. F, we found symptoms of shortness of breath, no fever, chest pain disappeared. Laboratory tests showed hypokalemia and hypoalbuminemia. Pleural fluid examination showed elevated triglyceride levels and normal total cholesterol levels.

Chylothorax can be classified as traumatic and non-traumatic. The causes of non-traumatic chylothorax include malignancy (50%), sarcoidosis, retrosternal goiter, amyloidosis, superior vena cava thrombosis, benign tumors, congenital duct abnormalities (15%), and lymphatic canal diseases, such as yellow nail syndrome, lymphangioleiomyomatosis (10) %), and hemangiomatosis (17%) (Maldonado et al. 2009, Light & Lee 2016). Chylothorax due to lymphoma malignancy is found in 70% of cases, with higher numbers in non-Hodgkin than Hodgkin. Thoracic duct obstruction can also be caused by a metastatic process (McGrath et al. 2010).

Mr. F underwent thoracic CT-scan without and with contrast, and showed solid lesion (34 HU) with calcified component (48 HU) within, well-defined, irregular edges measuring +/- 6.7 x 10.1 x 13, 3 cm in anteromedial mediastinum which, by contrasting, revealed contrast enhancement (68 HU). The lesion appeared to encase the superior vena cava to the left side which was in the anterior mediastinum. The presence of a mass in the anteromedial mediastinum in Tn. F provided a diagnosis of chylothorax etiology due to malignancy.

After examination of core biopsy and anatomic pathology, microscope observation showed tumors arranged in lobules, consisting of proliferation of anaplastic cells, with rounded nuclei, relatively monotonous, hyperchromatic, narrow cytoplasm, mitosis 6/10 HPF, including visible connective tissue fibrous with lymphocyte infiltration. Tumors grew invasively among the fibrous connective tissue stroma, suggesting mediastinal seminomas.

In mediastinal tumors, serum tumor markers examined are Fetoprotein (AFP), HCG and Lactate Dehydrogenase (LDH). Seminoma never secretes AFP. If there is an increase in AFP levels, the tumor must be classified as a non-seminoma germ cell tumor (NSGCT)

(Friedlander et al 2014). Examination of tumor markers in Mr. F showed AFP 3.8 (normal), LDH 551 (increased), and beta-HCG 12.49 (increased). The results of the tumor marker examination indicated seminoma.

IHC examination is needed to differ mediastinal seminomas from other types of mediastinal tumors. CD45 (leukocyte common antigen) is a receptor-linked protein tyrosine phosphatase expressed in all leukocytes. Positive CD-45 examination is seen in 97% of B cells and in 90% of T cell lymphomas (Alikhan et al. 2016, Al Shaarani et al. 2019, King & Lam. 2020). CD117 (C-KIT) is a glycoprotein transmembrane that functions for the development and defense of germ cells. C-KIT is an IHC tumor marker to differentiate extragonal seminomas from embryonal carcinoma. C-KIT tests were positive in 98% of those examined for seminomas (Nakagawa et al. 2005, Kriegsmann et al. 2015).

Placental Alkaline Phosphatase (PLAP) is a collection of 4 isoenzymes from the liver, bones, placenta and digestive tract. These isozymes are also produced by seminomas. PLAP examination can differ germ cell tumors, including seminoma or nonseminoma germ cell types. On histopathological examination, *yolk sac* tumor and seminoma have almost similar profile (Yao et al. 2012). To differ yolk sac tumor from seminoma, cytokeratin (CK) examination is used. CK examination will be positive for all preparations of yolk sac tumor, while in seminoma, it will be positive in 43% of all examinations (Cheville et al. 2000).

IHC examination results in Mr. F showed positive CD117 on tumor membrane, negative CD45 in tumor cells, positive in mature lymphocytes, positive PLAP in tumor cell cytoplasm, and negative CK in tumor cells. In conclusion, the tumor in the patient was a germ cell tumor of seminoma type.

Determination of the seminoma stage is performed using the American Joint Committee on Cancer (AJCC) (Table 1). The International Germ Cell Consensus Classification (Table 2) classifies stage III seminomas into good, intermediate and poor risk categories based on clinical factors, including histology, location of primary tumors, metastases, and serum tumor marker levels. Patients with rapid metastatic progression and life-threatening symptoms such as coughing up blood and suspected seminoma should be treated promptly using cisplatin-based chemotherapy, although it has not been diagnosed from histological tissue (Boujelbene et al. 2011, Jameson et al. 2018).

Patients with good prognosis are given chemotherapy in 3 cycles of bleomycin, etoposide, cisplatin (BEP) or 4 cycles of etoposide, cisplatin (EP). Patients at intermediate and poor risk of metastases are given 4 cycles of BEP chemotherapy or 4 cycles of etoposide, ifosfamide, cisplatin (VIP). Maintenance doses and schedules are important to note because dose changes and chemotherapy delays are associated with poor prognosis. Serum marker tumors are important to be examined at the time of management and must be normal during or after treatment. Chemotherapy using cisplatin can cause myelosuppression, nausea, vomiting and hair loss. Cisplatin can cause nephrotoxic, ototoxic and peripheral neuropathy. Bleomycin can cause pulmonary toxicity with an increased risk in patients over 40 years, with kidney damage, smokers, and cumulative use of bleomycin (Boujelbene et al. 2011, Jameson et al. 2018).

Based on the AJCC, Mr. F was included in stage 3C seminoma. Based on the International Germ Cell Consensus Classification, the patient was at poor risk. Chemotherapy using BEP regimen had been carried out. After 3 cycles of chemotherapy, an evaluation using a thoracic CT scan without and with contrast was carried out and the results showed that tumor lesions relatively shrinked. Side effects of 2nd degree chemotherapy include nausea, blackened skin, hair loss and leukopenia. In the course of his disease the patient died with complaints of shortness of breath for 5 days before undergoing the 5th cycle chemotherapy.

Table 1. American Joint Committee on Cancer (AJCC)

Clinical Stage	TNM (UICC/AJCC) Category				Blood tumor markers (S)			
	T		N	M	S	LDH	βHCG (mIU/ml)	AFP (ng/ml)
0	pTis	carcinoma in situ	N0	M0	-	-	-	-
IA	pT1	Limited to the testis and/or epididym, without lymphatic or vascular invasion, the tumor can infiltrate the tunica albuginea but not the tunical vaginalis	N0	M0	Any S level	Any LDH level	Any βHCG level	Norm.
IB	pT2	Limited to the testis and/or epididym, without lymphatic or vascular invasion, or spread through the tunica albuginea and invasion of the tunica vaginalis	N0	M0	Any S level	Any LDH level	Any βHCG level	Norm.
	pT3	Infiltration of the spermatic cord						
	pT4	Infiltration of the scrotal wall						
IIA	Any T stage		N1 (≤ 2 cm)	M0	Any S level	Any LDH level	Any βHCG level	Norm.
IIB	Any T stage		N1 (> 2 - 5 cm)	M0	Any S level	Any LDH level	Any βHCG level	Norm.
IIC	Any T stage		N1 (> 5 cm)	M0	Any S level	Any LDH level	Any βHCG level	Norm.
IIIA/B/C	Any T stage		Any N stage	M1a (non-regional nodes or lung metastasis)	Any S level	Any LDH level	Any βHCG level	Norm.
IIIC	Any T stage		Any N stage	M1b (other metastasis sites)	Any S level	Any LDH level	Any βHCG level	Norm.
IIIC	Mediastinal primary tumor		Any N stage	Any M stage	Any S level	Any LDH level	Any βHCG level	Norm.

Source: Boujelbene et al. (2011).

Table 2. International germ cell consensus classification

Risk Groups	Seminoma	Non Seminoma Germ Cell Tumor
Good	Any primary site; Normal AFP, any HCG, any LDH; Absent non-pulmonary visceral metastasis	Gonadal or retroperitoneal primary site; Absent non-pulmonary visceral metastasis; AFP <1000 ng/ml HCG <5000 mIU/mL LDH < 1.5x upper limit or normal
Intermediate	Any primary site; Normal AFP, any HCG, any LDH; Presence of non-pulmonary visceral metastasis	Gonadal or retroperitoneal primary site; Absent non-pulmonary visceral metastasis; At least one of: AFP 1000 – 10000 ng/mL HCG 5000-50.000 mIU/mL LDH 1.5 – 10 x upper limit or normal
Poor	Unapplicable	Mediastinal primary site; Presence of non-pulmonary visceral metastasis; At least one of: AFP >10000 ng/mL HCG >50000 mIU/mL LDH >10 x upper limit or normal

Source: Jameson et al. (2018)

Strength and limitation

This study is a valuable contribution to the medical literature, providing important insights into the diagnosis and manageme of mediastinal seminoma. The study is well-documented, and the findings have important clinical implications, suggesting that BEP chemotherapy may be an effective treatment option for patients with this condition.

The study is well-documented, providing a comprehensive analysis of the clinical and radiological investigations that were used to diagnose the condition, as well as the treatment that was administered. The patient was treated with BEP chemotherapy, and after three cycles of treatment, a partial response was achieved. This response was evaluated using radiological imaging, which is a standard method for assessing the efficacy of chemotherapy in patients with cancer.

CONCLUSION

A 28-year-old man suffered from chylothorax and primary mediastinal seminoma. The main clinical manifestation of the patient's disease was shortness of breath due to pleural effusion. Results of analysis supported the diagnosis of chylothorax. Examination was performed to determine the etiology of chylothorax, comprising laboratory tests, measurement of tumor markers, pleural fluid block cells, chest X-ray, and thoracic CT scan with contrast. The results of the examination supported the diagnosis of chylothorax resulting from malignancy.

The establishment of the diagnosis of malignancy was performed by examining chest CT scan with contrast, tumor markers, core biopsy, histopathology and IHC, the results of which supported the diagnosis of mediastinal seminoma. The patient received bleomycin, etoposide and cisplatin chemotherapy for the management of mediastinal seminomas. Therapy evaluation was carried out by clinical and radiological assessment. Radiological evaluation of CT scan after the third BEP chemotherapy cycle showed tumor size shrinked to <30% (partial response). The patient died before undergoing 5th cycle chemotherapy.

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Conflict of interest

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Author contribution

AR was conceptualized the study and validation the grammar. LW was wrote the manuscript and revisedit, validated, and finalized the manuscript.

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Review Article

THE ROLE OF PHYSICAL EXERCISE INTESITY TO IRISIN LEVELS ON OVERWEIGHT AND OBESE

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ABSTRACT

Physical exercise is a non-pharmacological therapy that can secrete various types of myokines to treat obesity problems. One of the myokines that play a role is irisin. Irisin is a polypeptide hormone with 112 amino acid residues that are synthesized in skeletal muscle after the proteolytic precursor cleavage of fibronectin type III domain-containing protein 5 (FNDC5). The release of irisin in the blood circulation will stimulate the browning process in white fat tissue by inducing the expression of uncoupling protein-1 (UCP-1) through signaling p38 mitogen-activated protein kinase (p38-MAPK) to increase energy expenditure, thermogenesis and reduce fat accumulation. This study described the differences in intensity of physical exercise mechanisms associated with the increased irisin secretion in overweight and obese subjects. This study was designed as a literature review that involved studies from research journals in the last 10 years concerning humans from some databases, such as Science Direct, PubMed, and Google Scholar. This study also discussed the relationship between the intensity of physical exercise and the synthesis, secretion, circulation, and regulation of irisin in preventing obesity.

***Keywords**: Irisin; physical activity; exercise; intensity; obesity*

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Highlights:

1. The differences in intensity physical exercise mechanisms associated with increased irisin secretion in overweight and obese subjects were determined.
2. The secretion of irisin in the right intensity blood on obesity can be reduced because the calories were balanced.

INTRODUCTION

Obesity is a factor in global problems that occur in both developed and developing countries (Norheim et al. 2014, World Health Organization 2019). Based on the data of World Health Organization in 2016, more than 1.9 billion people aged over 18 years were overweight, and 650 million of them were categorized as obesed. In Indonesia, data of the Ministry of Health in 2018 showed that obesity at over 15 years old in 2007 was 18.8%, while in 2018 it was 31.0%. Obesity could increase the risk of metabolic syndrome, such as stroke, type 2 diabetes, and heart disease (Grundy 2004, Gepstein & Weiss 2019). Therefore, we needed an appropriate intervention to reduce the potential rate.

One of the factors that plays a major role in obesity is excessive food intake and inadequacy of physical

activity (Global Burden of Disease 2015, Houben & Jansen 2017). This could increase various risks of metabolic syndrome (Ma et al. 2019). The risk of metabolic syndrome can occur due to the role of sympathetic nervous system activation which contributes to increased vascular and cardiac function which stimulates vasoconstriction of blood vessels, and causes blood pressure to increase. Besides, the metabolic balance is also disturbed, such as increased lipolysis which can drive fatty acid levels, so that it affects blood vessels and heart function (Ciccarelli et al. 2013). Thus, pathological symptoms will appear through an increase in blood pressure, an increase of triglycerides, glucose in blood and insulin resistance that can cause the increased risks of heart disease, stroke, and diabetes (Pescatello 2014, Han & Lean 2016).

As long as the conditions keep happening, it will have an impact on the low quality of public health, so that we needed an appropriate intervention; one of which was by doing physical exercise that could cause secreting various types of myokines to treat obesity problems. One of the myokines that play a role is irisin, but the intensity level of physical exercise can affect synthesis, secretion, circulation, and regulation of irisin in preventing obesity and negative impact.

In 2012, a myokine was identified and induced during physical exercise, known as irisin (Bostrom et al. 2012). This hormone can help to burn fat, so that it can inhibit obesity (Archundia-Herrera et al. 2017, Perakakis et al. 2017). Several studies have shown the many benefits of irisin secretion. A study by Huh et al (2014) showed that high intensity can increase acute response to irisin levels by 30% within 5 minutes after physical exercise in overweight men. However, Soori et al (2016) showed that moderate intensity-physical exercise was effective in increasing irisin secretion in the blood, so that the debate about the effective intensity in increasing irisin secretion was still being debated, as well as the effect of physical exercise intensity on irisin secretion in the overweight and obese categories that was still unclear. Therefore, this study examined differences in the intensity of physical exercise in increasing the secretion of irisin levels in order to find the right dose intensity to reduce the risk of obesity and avoid an increased risk of metabolic syndrome.

This study was designed as a literature review that involved studies from research journals in the last 10 years. We searched for data sources from the Science Direct, PubMed, and Google Scholar database with some keywords, namely irisin, physical exercise, intensity, overweight, and obesity with the inclusion criteria of physical exercise, irisin levels, and one subject with a Body Mass Index (BMI)> 25 kg/m^2. The exclusion criteria included the absence of physical exercise interventions, animal subjects or in vivo studies, meta-analyses, reviews, books, book chapters, editorials, and letters to the editor. According to prior keywords searching, it found 69 articles from science direct, 123 articles from Pubmed, and 42 articles from Google Scholar. However, all articles still needed to be further reviewed to get results according to the specified criteria. After reviewing the results of the review, the final results of the search for the appropriate database resulted in 45 article journals.

OVERVIEW

Irisin

Irisin is a hormone that was discovered in 2012. Initially studied in mice and secreted from mouse muscle expressing Ppargc1a or encoded by transcription cofactor peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) which enters the energy metabolism pathway (Huh et al. 2012). PGC1α stimulates the expression of fibronectin type III domain-containing protein 5 (FNDC5) and synthesizes membrane protein FNDC5 consisting of 212 amino acids to 209 amino acids in mice and mice (Bostrom et al. 2012). This protein sequence is included in the peptide signal located in the cyto serum (Perakakis et al. 2017). After proteolytic cleavage, glycosylation, and possibly a dimerization process from FNDC5, a new protein structure consisting of 112 amino acid chains was called irisin.

The highest irisin in humans was produced in skeletal muscle, so that it was known as a myokine, apart from those several other organs, such as heart, tongue, and rectum also produced it, although not significant (Huh et al. 2012). In contrast, the lowest expression of FNDC5 was in the pancreas and liver. Roca-rivada and Castelao et al (2013) also found FNDC5 / irisin in adipocytes. Yet of course, the expression was 100-200 times lower than the expression in human skeletal muscle (Huh et al. 2012, Perakakis et al. 2017).

When irisin has been secreted from muscle, irisin will communicate with several cells and organs in the body, one of which is fat cells or adipocytes. When irisin enters adipocytes it stimulates the expression of UCP1 via the p38 mitogen-activated protein kinase (MAPK) pathway and extracellular-signal regulated kinase (ERK) which then causes a thermo-genetic reaction in the mitochondria. The increase in thermogenesis causes the white adipose tissue (WAT) to turn brown / Brown Adipose Tissue (BAT). The process of converting WAT-type fat into BAT will cause an

converting WAT-type fat into BAT will cause an increase in the burning of energy reserves stored in the body, so that fat deposits can be burned and used as energy. A more detailed explanation can be seen in Figure 1.

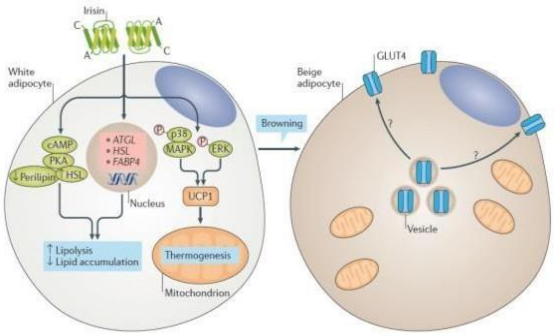

Figure 1. Irisin and communication on adipocyte cells (Perakakis et al. 2017)

In several studies conducted on animals or humans, irisin had also been shown to be strong in secretion during physical exercise. For example, in the study of Löffler et al (2015) on children and adults shown in Figure 2, there was an increase in irisin levels both in Figures A and B of 1.2 fold.

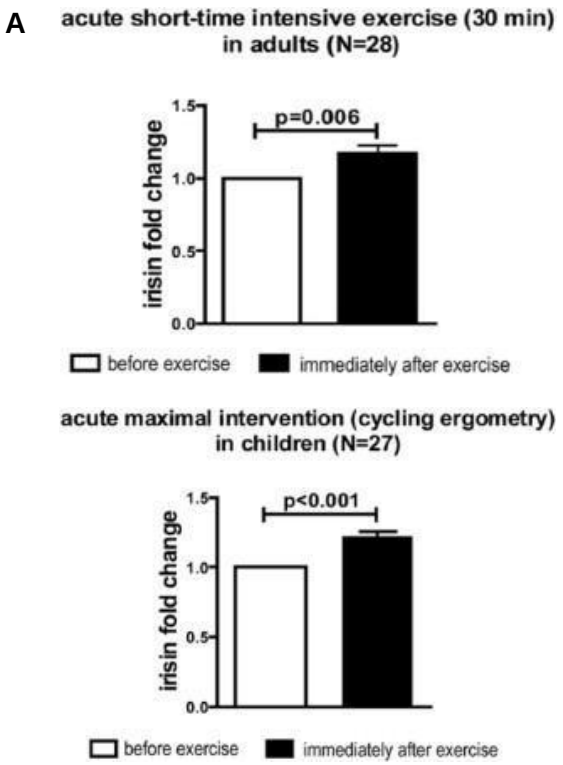

Figure 2. Serum irisin levels induced during physical exercise (Löffler et al. 2015)

In addition, a study by Bostrom et al (2012) showed that plasma irisin levels in mice increased by 65% after 3 weeks of running intervention, while in humans, irisin levels doubled after a 10-week intervention with aerobic-type physical exercise. In addition to increasing lipolysis, the increase in irisin can also create a state of glucose homeostasis in the blood.

Perakakis et al (2017) explains that irisin could activate the AMP-Activated Protein Kinase (AMPK) pathway by reducing intracellular ATP levels, increasing reactive oxygen species (ROS) or intracellular calcium concentrations. Activation of the AMPK pathway would stimulate the expression of Glucose transporter 4 (GLUT4). High GLUT4 expression, combined with the increased translocation of the GLUT4 protein from the cytoplasm to the membrane, induces glucose uptake by cells. In addition, irisin induction in cells will cause an increase in fat metabolism, a decrease in glycogenolysis, and a decrease in gluconeogenesis. In brief, it could be seen in Figure 3 regarding the role of irisin in stimulating the activation of various metabolisms in cells.

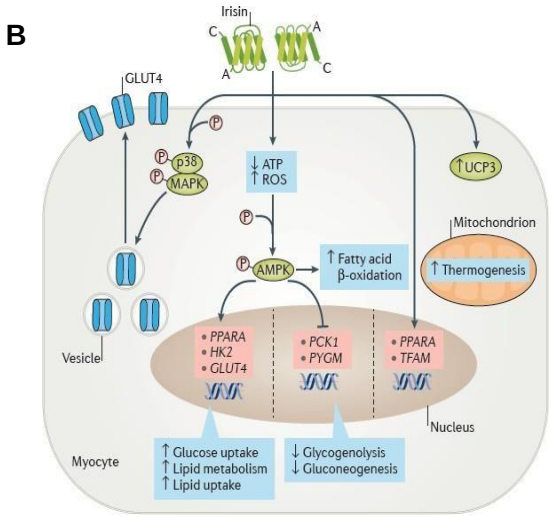

Figure 3. Irisin signal pathways inside the cell (Perakakis et al. 2017)

In the Fukushima et al (2016) study conducted on 22 women and men with the obesity category, physical exercise for 6 months with a diet setting showed a decrease in body weight and BMI. Irisin levels also showed an increase associated with decreases in the percentage of body fat, subcutaneous fat, triglycerides, and fasting blood sugar.

The difference in intensity also affects the secretion of irisin in the blood. This was evidenced from several previous studies that had been conducted. The study of

Cialowicz et al (2020) showed that High Intensity Interval Training (HIIT) caused an increase in irisin levels in the blood by 30% after 8 weeks of intervention.

The increase in irisin can occur, because the body experiences hypoxia and an increase in the glycolytic rate during intense and short physical exercise. This anaerobic process contributes to mitochondrial biogenesis causing increased oxygen and fat burning. Figure 4 shows the differences in irisin secretion in HIIT before and after intervention and their comparison with the control group in a clearer manner.

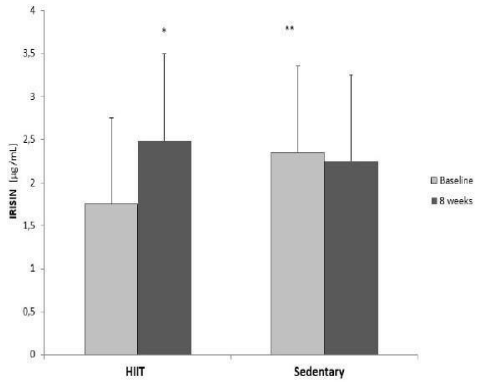

Figure 4. Differences in irisin secretion in the HIIT and control groups (Cialowicz et al. 2020)

This was in line with the research of Huh et al (2014) by involving 78 people divided into two groups, namely 38 adolescents and 40 elderly people. Each group was given physical exercise treatment which was divided into two groups, namely Continuous Moderate Exercise (CME) and High Intensity Interval Exercise (HIIE) which would be observed to increase levels of irisin regularly, namely before intervention, shortly after intervention (10-15 minutes), one hours, and 24 hours. In the results of this study, HIIE had the highest increase in irisin secretion after the intervention compared to CME.

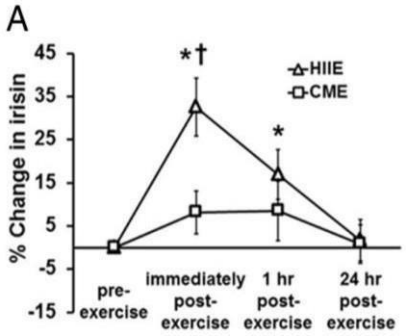

Figure 5. Irisin circulation response between HIIE and CME (Huh et al. 2014)

Obesity is a condition of body weight due to excess fat accumulation (American College of Sports Medicine 2018). This happens, because there is an imbalance of calories in and calories out (Sahoo et al. 2015). In this case, the adipose tissue has a role as a form of body mechanism to store energy reserves in the form of fat. The network was divided into two types, namely White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). WAT stores excess energy in the body, forming triglycerides, and releasing energy in the form of free fatty acids and glycerol. Meanwhile, BAT oxidizes the stored fat to be readily used as energy for physical activity (Lo & Sun 2013).

According to Cui and Chen (2017), WAT has two main storage depots, namely visceral white adipose tissue (vWAT) and subcutan white adipose tissue (scWAT). WAT has a percentage of about 20% of normal adult body weight. Meanwhile BAT is involved in metabolism, especially during heat generation. A study by Saely et al (2012) showed that BAT functioned as an energy homeostasis in the body. Apart from WAT and BAT, there were also beige/ brite/ brownlike adipose tissue (bAT) (Cedikova et al. 2016). BAT has mixed characteristics of WAT and BAT. During the homeostatic state or basal state, BAT will be similar to WAT morphologically, but when stimulated by exercise or physical activity, the morphological appearance will change to BAT, thus transforming stored fat (Cedikova et al. 2016).

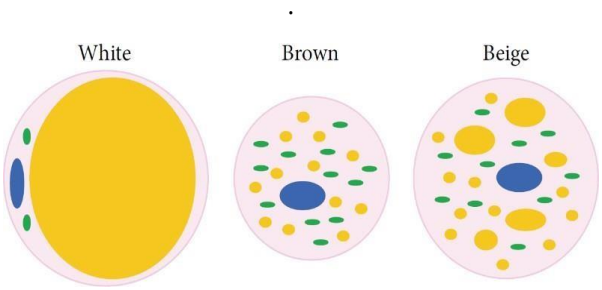

Figure 6. Morphological characteristics from WAT, BAT, and bAT (Cedikova et al. 2016)

There were several measurements that could be done to identify the body was included in the obesity category. These measurements included waist circumference, hip circumference, waist to hip circumference ratio (WHR), and Body Mass Index (BMI) (Ahmad, 2016). The easiest way to do the calculation at BMI. The BMI category of obesity has a value range of 25-29.9 (World Health Organization 2000). However, BMI has disadvantages, such as not being able to differentiate between muscle mass and fat, so that if it is done in a trained person, further measurements are needed.

Table 1. Classification of body weight based on BMI in adults in Asia

Classification	BMI	Risk of Disease
Underweight	<18.5	Low (but may increase the risk of other clinical diseases)
Normal	18.5-22.9	Average
Overweight Risk	≥23	Risk increases
Obesity I	23-24.9	Moderate
Obesity II	25-29.9	
	≥30	Severe

Source: World Health Organization (2000)

Several disease risks can arise due to excess weight gain, especially in the obesity category, such as cardiovascular disease, high blood pressure, cholesterol, glucose tolerance to diabetes mellitus type 2, and other musculoskeletal diseases (Global Burden of Disease 2015, American College of Sports Medicine 2018). This weight regulation is of course influenced by the energy put into the body and the energy released (Pescatello 2014). For someone with obesity, the energy released is lower than that which it enters, and it causes food not to be processed as energy, but stored as energy reserves in the form of fat.

Gender differences will also differentiate fat distribution in each individual (American College of Sports Medicine 2018). In general, there are three types of fat distribution, namely android, gynoid, and intermediate patterns. For females, fat distribution will be spread over the hips and thighs, or commonly known as the gynoid pattern. Meanwhile, in men, the distribution of fat is spread over the neck, shoulders and stomach which is known as android pattern. There is also another form, namely the intermediate pattern; the distribution of fat located in the upper and lower areas of the body, so that it forms a shape like a square appearance (Plowman & Smith 2011).

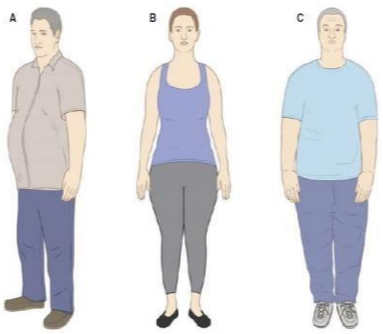

Figure 7. Fat distribution A) Android, B) Gynoid, C) Intermediate pattern (Plowman & Smith 2011)

Effects of physical exercise intensity on irisin levels

Based on the results of several studies, irisin can be improved with acute physical exercise interventions (Tsuchiya et al. 2014, Huh et al. 2015, Löffler et al. 2015, Herrera et al. 2017, Winn et al. 2017). Conversely, several studies had also succeeded in proving that chronic physical exercise interventions could also increase irisin secretion (Bluher & Panagiotou 2014, Kim et al. 2016, Bonfante et al. 2017, Leblanc et al. 2017, Tofighi et al. 2017), so that irisin is a myokin influenced by physical exercise. In overweight and obese subjects, they were able to prove that irisin was secreted higher than the pre-intervention level. One of the changes in the increase in irisin is affected by the intensity of physical exercise. The high intensity of physical exercise can increase irisin secretion more than low intensity (Tsuchiya et al. 2014, Huh et al. 2015, Löffler et al. 2015), but high intensity physical exercise will immediately cause irisin to return to basal value at 15 minutes after physical exercise. Meanwhile, at moderate intensity with continuous type irisin secretion looks more stable, where it can increase up to 2-3 hours after the intervention (Winn et al. 2017).

In addition, the type of weight training with high intensity can also increase irisin secretion than the aerobic type of physical exercise at both moderate and high intensity (Huh et al. 2015, Löffler et al. 2015). It is possible that the higher the intensity, the higher the load on the muscle contraction that occurs so that it can increase the microscopic muscle damage. The microscopic muscle damage will be positively associated with increased production of lactate and creatine kinase as biomarkers of muscle damage (Huh et al. 2015). Other factors (gender and fitness status) had not affected irisin secretion (Löffler et al. 2015, Nygaard et al. 2015). There were also studies that showed the occurrence of irisin levels remained from baseline with aerobic and resistance-type physical exercise interventions (Pekkala et al. 2013). However, these differences may occur due to differences in the methodology.

The difference in results in each study can be determined from the type of sample observed and the sampling time. In most studies, samples to measure irisin levels were observed in serum and blood plasma, and there were those who took samples using saliva (Aydin et al. 2013). The results obtained from the types of serum and saliva samples also showed different results. In saliva, the irisin concentration increased after the intervention of aerobic exercise sessions, while the serum irisin concentration before and after

the intervention did not show any difference (Aydin et al. 2013). However, it is difficult to certainly observe the difference between serum and saliva, because two different methods are used. Saliva is easier to sample without non-invasive action, while serum and plasma use invasive measures using Elisa kits. The most common measurements using these elisa kits are the types EK-067-29 and EK-067-52: Phoenix Pharmaceuticals, Burlingame, CA, USA with a detection range of 0.1-1000 ng/ml and 0.328-204.9 ng./ml (Bluher & Panagiotou 2014, Norheim et al. 2014, Tsuchiya et al. 2014, Huh et al. 2015, Herrera et al. 2017).

The role of optimal physical exercise intensity in increasing irisin secretion occurred at high intensity and resistance exercises. Both were more effective in increasing the concentration of irisin in the blood. However, studies on this effect are still limited and there remains a variety of approaches among studies, so that it is not sufficient to develop a suitable protocol. However, this is sufficient to prove that physical exercise is a non-pharmacological therapy that has a potency to solve the problem of overweight and obesity by increasing irisin secretion, so that it can increase the thermogenesis process.

Strength and limitation

The study is highly relevant and up-to-date. The study provides a detailed explanation of the structure and function of irisin and how it induces the browning of white adipose tissue. The findings could have important implications for the design of exercise interventions for individuals who are overweight or obese, as well as the development of non-pharmacological therapies for the treatment of obesity. This study is a valuable contribution to the field of exercise physiology and obesity research, providing important insights into the mechanisms by which physical exercise can promote the secretion of irisin, and its potential therapeutic benefits for preventing obesity.

CONCLUSION

Obesity is a global problem that occurs today. The increasing prevalence of obesity will lead to various risks of metabolic syndrome which can increase the risk of diseases, such as type 2 diabetes, heart disease, and stroke. In overcoming these problems, physical exercise is a non-pharmacological therapy that can be done to prevent obesity, so that various risk factors for the disease can be reduced. Physical exercise can stimulate irisin that can help the process of converting WAT to BAT, so that it helps to increase lipolysis activation.

The secretion of irisin in the blood with the right intensity is expected to be able to balance calories in and calories out, so that it can reduce the incidence of obesity. The most proven intensity to increase irisin secretion is higher at high intensity. In addition, the type of physical exercise with resistance exercise is also quite high in increasing irisin secretion compared to other types. Therefore, it is important to know the dosage of physical exercise, especially at intensity, which is necessary to prevent overweight and obesity as well as to get health and fitness benefits in a sustainable manner.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

INA-conceptualized the study and wrote the manuscript. S-reviewed, validated, and finalized the manuscript. PSR-Revised, collected, validated and finalized the manuscript.

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Review Article

EFFICACY OF LIVE ATTENUATED DENGUE VACCINES: CYD-TDV, TDV (TAK-003), AND TV003/TV005

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ABSTRACT

Dengue fever is the most common tropical disease, but there still remains no specific therapy that can overcome it. Special attention needs to be paid to this disease, because there were large increases in incidence in the last decade. As an effective preventive strategy, finding a new vaccine for dengue fever with higher potentiation and efficacy is highly necessary to stop dengue transmission especially in the endemic area. Vaccine triggers an immune response, so that it can create a robust immune response when infected. Nowadays, there is only one licensed dengue vaccine that is CYD-TDV (Dengvaxia). However, this vaccine still has many weaknesses, namely its dependency on the serostatus of the recipient. There are also other dengue vaccines that are in ongoing clinical testing and have promising results, TDV (TAK-003) and TV003/TV005. These three vaccines are live attenuated vaccines with various results. This review discussed differences in the efficacy of CYD-TDV against the other TAK-003 and TV003/TV005; considering the known and unknown various factors.

***Keywords**: Live attenuated dengue vaccines; CYD-TDV; TAK-003; TV003/TV005; tropical disease*

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Highlights:

1. Differences in the efficacy of CYD-TDV versus the other TAK-003 and TV003/TV005 were discussed.
2. One licensed dengue vaccine is CYD-TDV (Dengvaxia).

INTRODUCTION

Dengue is an infectious disease caused by dengue virus (DENV) which is transmitted through the bite of Aedes mosquitoes (especially Aedes aegypti), and it is one of the most common tropical diseases in the world. DENV is a single chain positive RNA virus consisting of four serotypes (DENV 1, DENV 2, DENV 3, and DENV 4), belongs to the Flaviviridae family, Flavivirus genus (including Zika, yellow fever, Japanese Encephalitis) (Wang et al. 2020). Someone who has been infected with DENV will have lifetime immunity against the same DENV serotype. It means that a person during his life can be infected four times. Dengue can be found in the tropical and subtropical countries of the world, especially in endemic countries with 10% of fever episodes that are caused by dengue (Wilder-Smith 2020). The incidence of dengue has increased in the last decade. A recent study discovered that the infection incidence is 390 million per year, of which 96 million experienced clinical symptoms (in varying degrees). The incidence rate was 8-fold compared to the last two decades (from 505.430 cases in 2000 to 4.2 million by 2019) (World Health Organization 2014). Another study estimates that 3,900 million people are at risk to have dengue infection. This rapid increase is due to several factors, such as the increasing population, increasing urbanization and migration including international travel, global climate change, and difficulty to overcome vectors effectively and sustainably (Pinheiro-Michelsen et al. 2020, Murray et al. 2013).

Nowadays, there remains no specific treatment to cure dengue. The number of individuals who are at risk of getting dengue makes this disease highly important to be treated. The most effective strategy to overcome dengue is preventive, and is currently done by doing vector control. Yet, the control of this vector is sufficiently complex, because it is multidisciplinary and multi sector that can consume a lot of money and work (Da Silveira et al. 2019), so that the development of effective and safe dengue vaccine is an urgent necessity. The ideal dengue vaccine is a vaccine given in single doses that can protect against all four DENV serotypes, providing long-term protection, and having no side effects. The recognized dengue vaccine has related problems to the immune response and cross-reactive T-cell responses implicated in a dysfunctional immune response that may contribute to more severe cases of secondary DENV infection. However, more recent works point to the value of more robust T-cell immunity in reducing the risk of developing severe manifestations of DENV infection (Collins & Metz 2017).

MATERIALS AND METHODS

This study was a systematic literature review conducted by collecting a number of literatures related to the problem and research objectives. In this study, we searched journal articles about dengue vaccines and its characteristics, efficacy, safety, and clinical trials status by reading it to make a theoretical summary, so that a complete review about dengue vaccines was obtained.

RESULTS

There were several vaccines that had entered the clinical trial phase, namely TV003/TV005, TDV/DENVax (TAK-003), PIV, V180, D1ME100, TVDV, and TLAV Prime. From these vaccines, there was only one vaccine that had been approved and licensed to be consumed in endemic areas, namely CYD-TDV (Dengvaxia) (Deng et al. 2020). In addition, there were two vaccines that had undertaken phase III clinical trials. Many studies had examined the efficacy of dengue vaccines.

In this review, we discussed vaccines that had been approved, namely CYD-TDV and vaccines in phase III clinical trials, TDV (TAK-003), and TV003/TV005 (LATV/Butantan-DV/TetraVax-DV), because many studies in the clinical trials showed promising results, where the live attenuated vaccine provided the advantage to stimulate and neutralize antibodies in humans than other types of vaccines, such as recombinant subunit vaccines (triggering a balanced immune response, problems of endotoxin contamination, and improper protein folding), and DNA vaccines (low immunogenicity), and vaccines with relatively low production prices that generally only required a single dose to provide protection, and had shown satisfactory results for other diseases caused by flaviviruses (Deng et al. 2020, Kallas et al. 2020).

DISCUSSION

CYD-TDV (Dengvaxia)

CYD-TDV (Chimeric Yellow fever 17D Virus-Tetravalent Dengue Vaccine) is a live attenuated, recombinant tetravalent vaccine developed by Sanofi Pasteur and has been licensed in endemic countries in Asia and Latin America under the trade name Dengvaxia. This vaccine has been through phase I-III clinical trials involving more than 45,000 participants from 16 countries before licensed (Pinheiro-Michelsen et al. 2020). This vaccine is a quadrivalent combination of four monovalent chimeric attenuated viruses that comprise the prM (pre-membrane) and E (envelop) sequence of each DENV serotype grafted on to the nonstructural protein backbone of YF17D (yellow fever virus vaccine strain). The live attenuated vaccine, CYD-TDV works as an agent of RNA replication, so that it stimulates the response of the humoral and induces controlled stimulation of dendritic cells and other immune responses. The strains used in CYD-TDV are genetically and phenotypically stable, non-hepatotropic and less neurovirulent than the strains used in YFV 17D (Wang et al. 2020, Collins & Metz 2017). The structure of this vaccine can be seen in Figure 1. This vaccine is given 3 times with a length sequence of 6 months, because in previous studies, seroconversion in vaccine recipients with 3 doses could reach up to 100% compared to 2 doses which only reached 92% (Pinheiro-Michelsen et al. 2020).

There are several factors that can affect the efficacy of this vaccine, including age, virus serotype and vaccine recipient serostatus (Hadinegoro et al. 2015). In its development, in phase IIb clinical trials in Thailand and phase III clinical trials in Asia and Latin America, this vaccine showed protection against DENV3 and also DENV4, moderate protection against DENV1, but it did not provide good protection for DENV2 with the level of efficacy protection against each serotype was 54.7% (DENV1), 43.0% (DENV2), 71.8% (DENV3), and 76.9% (DENV4). Overall, the level of efficacy of protection against all DENV within 1 year after the administration of the third dose vaccine was 59.2% (Collins & Metz 2017).

Table 1. Differences of three vaccines in this review

	CYD-TDV	TAK-003	TV003/TV005
Phase	IV (licensed)	III	III
Age	9 - 45 years old (SAGE)	Phase III Studies: 2-60 years olds	Phase III Studies: 12 -70 years olds
Dosage	3 dosage, 6 months apart	2 dosage, 3 months apart	Single dose
Administration	Subcutaneous	Subcutaneous	Subcutaneous
Duration Protection	Up to 5 years in seropositive	Over 3 years	At least 1 years*
Reaction	Seroconversion with neutralizing antibodies	Seroconversion with neutralizing antibodies and inducing cellular immune responses	Seroconversion with neutralizing antibodies and inducing cellular immune responses
Serostatus	Must be given to seropositive persons	Regardless serostatus	Regardless serostatus
Safety	No serious side effects, must be given to seropositive persons	No serious side effects	No serious side effects, most side effect are Rash
Overall Efficacy	± 59.2%	± 80.6%	Tetravalent neutralizing response 76%
Efficacy to Seropositive	± 76%	± 82.2%	No data*
Efficacy to Seronegative	± 39%	± 74.9%	No data*
Efficacy for DENV1	± 54.7%	± 73.7%	No data*
Efficacy for DENV2	± 43%	± 97.7%	No data*
Efficacy for DENV3	± 71.8%	± 62.6%	No data*
Efficacy for DENV4	± 76.9%	± 63.3%	No data*

*need more data from future studies

The serostatus of vaccine recipients also affects the efficacy of this vaccine, whereby vaccine recipients who have previously been infected with one of the DENV serotypes have better results (more effectively) than those that have never been infected. This indicates that CYD-TDV is better suited to improve and expand the existing immunity rather than provoking or incurring protection against all DENV serotypes in individuals who have never been infected previously (Collins & Metz 2017). This can occur, because the vaccine may play a role in stimulating the existing immune memory.

In a case-cohort study on the effect of serostatus on vaccine safety and efficacy concluded that CYD-TDV protected against severe dengue and hospitalized for dengue in individuals who had been previously infected (seropositive), whereas inversely to those who had never been infected previously (seronegative) (Sridhar et al. 2018). With the use of CYD-TDV, seronegative individuals aged 9-16 years had a hazard ratio of 1.41 (95% confidence interval [CI] 0.74-2.68) for hospital admission due to dengue, while seropositive had a hazard ratio of 0.21 (95% CI 0.14 - 0.31). In the case of severe dengue exposure, the hazard ratio for individuals with seronegative is 2.44 (95% CI 0.47 - 12.56), while that in seropositive is 0.16 (95% CI 0.07 - 0.37). This study also examined individuals aged 2 - 8 years, and those with seronegative had a hazard ratio of 1.95 (95% CI 1.19 - 3.19) for hospital admission due to dengue, while seropositive had a hazard ratio of 0.5 (95% CI 0.33 - 0.77).

In the case of severe dengue exposure, the hazard ratio for individuals with seronegative was 3.31 (95% CI 0.87 - 12.54), while that in seropositive was 0.58 (95% CI 0.26 - 1.30) (Sridhar et al. 2018). From this study, the administration of these vaccines will be more effective in individuals with age above 9 years and also has seropositive status. Other studies also showed that the risk of hospitalization due to dengue and risk of severe dengue increases in the 3 - 4 years after the first dose due to the protection given by the vaccine that had been reduced or due to the disappearance of neutralizing antibodies and leaving only enhancing antibodies (Arredondo-García et al. 2018, Plotkin 2020).

Related to serostatus, overall efficacy of the vaccine to prevent symptomatic dengue in individuals with seronegative was 39% (individuals over 9 years old) and 19% (individuals aged 2-8 years). Efficacy in individuals with seropositive status was 76% (individuals over 9 years) and 60% (aged 2-8 years) (Sridhar et al. 2018, World Health Organization 2018). In regard to age, a study in Asia showed a higher efficacy in children over 9 years of age from children aged 2 - 5 years (Da Silveira 2019). Similarly, another study stated that the efficacy of the vaccine to prevent symptomatic dengue in children over 9 years was 65.6% compared to those under 9 years (44.6%) (Hadinegoro et al. 2015).

The duration of protection that CYD-TDV could provide was at least 5 years for those with seropositivity (Wang et al. 2020, Sridhar et al. 2018). This vaccine is also relatively safe compared to other vaccines given to children (e.g., diphtheria and tetanus vaccines) because it has fewer side effects. However, as explained above, the use of this vaccine in those with seronegative can increase the risk of being hospitalized and suffering from severe dengue. This is probably because the CYD-TDV vaccine is partially mimics primary infection, so that it plays a role in antibody- dependent enhancement (ADE) for a second infection (Sridhar et al. 2018, Swaminathan & Khanna 2019, Harenberg et al. 2016). Giving children under 9 years old also increases the risk of hospital admission and suffering severe dengue. However, the mechanism is still unclear (Deng et al. 2020, Collins et al. 2017).

In the updated recommendation from SAGE (Strategic Advisory Group of Experts) in 2018, World Health Organization (2018) informed that this CYD-TDV vaccine was given only to 9-45 years old with seropositive status (thus pre-vaccine screening is necessary), because it could increase the risk of hospital admission and severe dengue exposure in seronegative individuals. In addition, as of 1st May 2019, the Food and Drug Administration (FDA) indicated this vaccine was only for individuals 9-16 years with a history of previous dengue infection that had been proven by laboratory results in medical records.

TDV (TAK-003)

Similar to the manufacture of CYD-TDV, TAK-003 replaces YF17D with DENV2 PDK-53 (laboratory-derived attenuated virus). DENV2 PDK-53 was chosen, because a study showed that this strain even used in recombine remains replicate uniformly and also relatively safe (Pinheiro-Michelsen et al. 2020). Also, because it used the DENV2 strain, there was a nonstructural (NS) DENV2 protein in this vaccine (Wilder-Smith 2020). To get immunity against all serotypes, prM and E proteins from DENV1, DENV3, and DENV4 have been replaced to DENV2 PDK-53 genetic backbone to get vaccine strains for each serotype (TDV-1, TDV-2, TDV-3, and DENV4 TDV-4). TAK-003 was a combination of TDV 1-4 developed by Takeda Vaccines (Biswal et al. 2019). The structure of this vaccine can be seen in Figure 2.

Similar to CYD-TDV as a live attenuated vaccine, TAK-003 acts as an agent of RNA replication that can stimulate humoral and cellular immune systems. In addition, this TAK-003 vaccine generates a CD8 + pool of NS1, NS3, and NS5 reactive T cells capable of producing IFN-γ, TNF-α, and to a lesser extent IL-2 upon ex vivo restimulation which may arise due to the presence of NS proteins causing cross-reactive T-cell mediated responses to broad protection against dengue (Wang et al. 2020, Wilder-Smith 2020, Waickman et al. 2019, Prompetchara et al. 2020, Sáez-llorens et al. 2017). This vaccine showed seroconversion rates of 84 - 100% for DENV1, 96 - 100% for DENV2, 83 - 100% for DENV3, and 33 - 77% for DENV4 (Pinheiro-Michelsen et al. 2020).

Other studies also proved that the overall seroconversion (all serotypes) for this vaccine was 88% in those with seronegative and 97% in those with seropositive (Macias et al. 2020). TAK-003 vaccine is given twice with an interval of three months, because giving a second dose could increase immunity to DENV3 and DENV4 especially in those with seronegative baseline (Sáez-llorens et al. 2017, Sáez-llorens et al. 2017). This vaccine is currently still in phase III clinical trials in Asia and Latin America.

A study by Shibadas et al in phase III clinical trials with subjects aged 4-16 years, found that the efficacy of this vaccine to prevent virologically confirmed dengue (which was confirmed through RT-PCR testing) was 80.6% (95% CI 73.8 - 85.6) (Biswal et al. 2019). Another study in phase II clinical trials also reported the relative risk (Rr) for the occurrence of virologically confirmed dengue in TAK-003 recipient individuals was 0.35 (CI 0.19 - 0.65) (Tricou et al. 2020). Regarding the type of DENV serotype, in contrast to CYD-TDV, this vaccine showed a high efficacy against DENV2 (efficacy 97.7%) with a different efficacy for DENV1 (73.7%), DENV3 (62.6%), and DENV4 (63.2%) (Biswal et al 2019).

In addition to children aged 4 - 16 years in a phase I clinical trial, Chukiat et al (in Sirivichayakul et al. 2015) showed that this vaccine could induce immunity against all four DENV serotypes in individuals aged 1.5 - 45 years both with seropositive and seronegative.

Regarding the serostatus of vaccine recipient individuals, there were differences in efficacy with seropositive and seronegative people, but this difference was not as great as CYD -TDV. The efficacy of this vaccine against individuals with seronegative was 74.9% and against seropositive was 82.2% (Biswal et al. 2019). In the same study, the efficacy of this vaccine to prevent hospital admission due to dengue was 95.4%

(95% CI 88.4 - 98.2%), where the efficacy in seronegative individuals was 97.2% and in seropositive individuals 94.4%. This is interesting to note, because the efficacy in seronegative sufferers is higher than that of seropositive, so that this vaccine may be suitable for use with seronegative compared to CYD -TDV.

Two studies with phase III clinical trials within 48 months, had shown that this vaccine provided a long-term antibody persistence with evidence of high levels of antibodies above the baseline (Tricou et al. 2020, Biswal et al. 2020). Other studies had also shown that this vaccine could provide protection for at least 3 years after the first dose as evidenced by the presence of antibody titers on peripheral blood tests. Even after 3 years, seropositivity rates for DENV1, 2, 3 were still high (88 - 97%) and moderate for DENV4 (56%) (Sirivichayakul et al. 2020).

Regarding the safety of using TAK- 003, some studies had shown that this vaccine could be well tolerated and classified as safe to use as proven by the incidence of side effects that are relatively small in the vaccine and control group. From the study with a total of 20, 078 subjects (13, 380 vaccine recipients and 6,687 controls) who experienced serious side effects were 1 person from the vaccine group and 4 people from the control group (2 people had hypersensitivity, 2 were diagnosed with dengue, and 1 was diagnosed with dengue hemorrhagic fever) (Biswal et al. 2019).

All previous data may undergo a change later, because the vaccine is still in the clinical trial phase. Further studies were necessary to evaluate the final results of this vaccine. However, this vaccine remains a high efficacy to prevent dengue and also hospitalization due to dengue, including in children under 9 years and regardless of serostatus.

TV003 / TV005

The second vaccine that is currently in phase III clinical trial phase is TV003 / TV005 which is also a life attenuated vaccine. This vaccine was developed by the Laboratory of Infectious Disease (LID) of the National Institute of Allergy and Infectious Diseases (NIAID), which is also licensed by manufacturers from Brazil (Butantan Institute), Vietnam (Vabiotech), India (Panacea Biotech and Serum Institute of India), and global licensed by Merck & Co (Whitehead 2016) This vaccine is a live attenuated tetravalent vaccine (LATV) which is a combination of four attenuated recombinant monovalent DENV: rDENV1Δ30, rDENV2/4Δ30, rDENV3Δ30/31, dan rDENV4Δ30 (Pinheiro-Michelsen et al. 2020, Deng et al. 2020, Swaminathan & Khanna 2019). Figure 1C provides the structure of this vaccine.

This attenuated virus was created using recombinant DNA technology by removing nucleotides from 3' untranslated regions (3'UTR) from each strain of DENV. This region was chosen as a target, because it played an important role in RNA replication (Pinheiro-Michelsen et al. 2020). Of the four components, one component is chimeric strain (rDENV2 / 4Δ30) which is made by replacing prM and E protein from the rDENV4Δ30 backbone with prM, and E protein from DENV2.

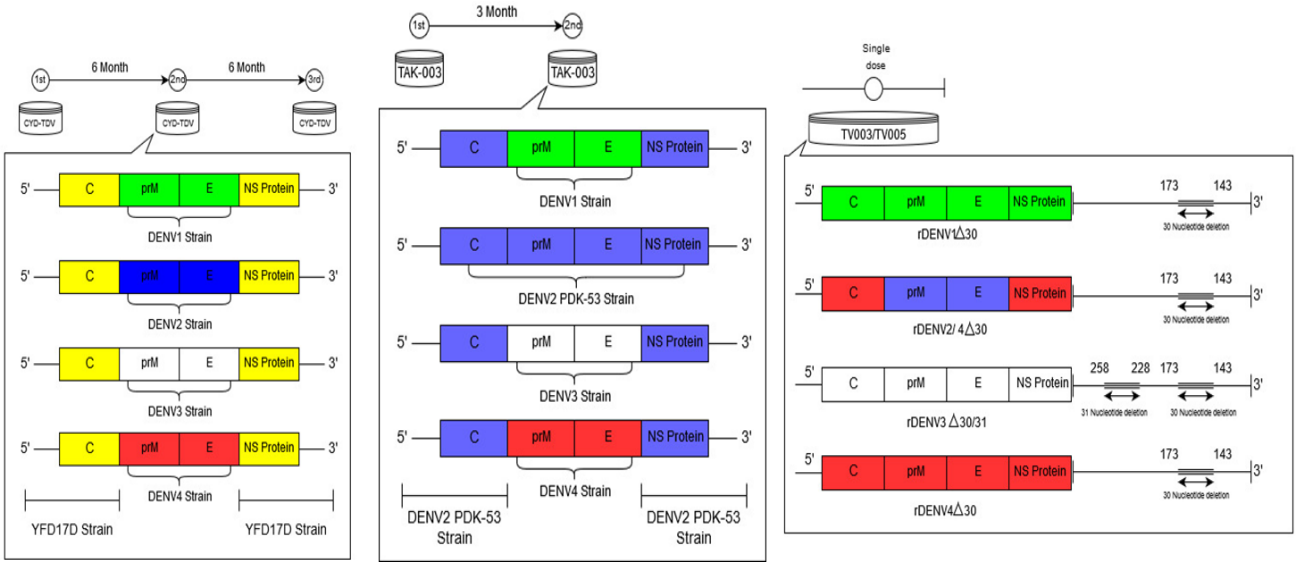

Figure 1. Structure of: (a) CYD -TDV (Dengvaxia); (b) TAK -003; (c) TV003 / TV005
Source: (Pinheiro-Michelsen et al. 2020, Collins & Metz 2017, World Health Organization, 2018)

Because this vaccine is made with three components of the dengue virus mutation, i.e. rDENV1Δ30, rDENV3Δ30/31, and rDENV4Δ30, this vaccine also contains the same non-structural protein as the nonstructural protein of "wild" DENV 1, DENV 3, and DENV 4, so that this vaccine also stimulates cellular immune responses (T cells) besides producing neutralizing antibodies (Pinheiro-Michelsen et al. 2020, Whitehead 2016, Durbin & Gubler 2019).

The difference from TV003 and TV005 is the doses of one component (rDENV2/ 4Δ30), on TV003 each component has a dose of 103 PFU (Plaque Forming Unit), while on TV005 the dose of rDENV2/ 4Δ30 increased to 104 PFU (Pinheiro-Michelsen et al. 2020, Whitehead 2016). This vaccine is a single dose, because in the study, a second dose after 6 or 12 months did not trigger an increase in antibody titers and cellular response to any DENV serotype significantly, which proved that a single dose alone was sufficient to prevent virus replication and protect from disease with sufficient neutralizing antibody responses (Kallas et al 2020, Whitehead et al 2020, Kirkpatrick et al 2015)

Efficacy data of this vaccine is still not available. A study by Whitehead (2016) showed that tetravalent immune responses to TV003 were 87% in those who had been exposed to flavivirus before, and 66% in those who had never been exposed. The frequency of seroconversion of each serotype also varies, namely 89%, 95%, 97%, 100% for DENV 1, 2, 3 and 4 in those who had been exposed to flaviviruses. Those who had never been exposed were 95%, 67%, 98%, 100% for DENV 1, 2, 3, and 4 (Whitehead et al 2017). A recent study showed that the frequency of tetravalent neutralizing antibodies was 76% (Kallas et al 2020). In addition, there were other studies comparing TV003 and TV005 resulting in tetravalent immune responses with TV003 were 74% and increased to 90% with TV005. Besides, the specific immune response for DENV2 also increased from 76% (TV003) to 97% (TV005), whereas for other serotypes, it was not much different (Kirkpatrick et al. 2015).

Regarding safety, the use of the TV003/ TV005 vaccine was classified as safe, because previous studies did not find serious side effects. Most of the side effects that appeared and were also significantly different from the control group were rash (66% - 76% of total vaccine recipients), where these effects could disappear without treatment intervention. The rashes are generally mild, and only a few are classified as moderate due to pruritus. Besides, the side effects that arise were not also influenced by the initial serostatus of the vaccine recipient (Kallas et al 2020, Whitehead et al 2017, Kirkpatrick et al 2016).

Based on previous studies, the use of TV003 / TV005 is classified as safe, tolerable, and can provide protection against dengue in the recipient both seropositive and seronegative. We are now waiting for further research in phase III clinical trials to see the extent of efficacy from this vaccine, so that it can be accepted in the future community.

Strength and limitation

The study is well-researched and up-to-date, considering the latest developments in the field of dengue fever vaccines.The review examines the strengths and weaknesses of each vaccine, including the dependency of CYD-TDV on the serostatus of the recipient and the promising results of ongoing clinical trials for TAK-003 and TV003/TV005.

CONCLUSION

To date, only one vaccine has been approved and licensed (CYD-TDV). However, the vaccine is still less effective and efficient in preventing dengue, so that its use should be reconsidered or restricted. Many other promising vaccines such as TAK-003 and TV003/ TV005 are still in the clinical trial phase, so that they still cannot be fully recognized. Yet from various research reports, this vaccine showed satisfactory results in performance. In general, all of these dengue vaccines show a good outcome to prevent dengue infections. It is expected that the vaccine with one-time administration, independent of serostatus, and can be used at any age range (especially children), and reachable by various levels of society can be developed immediately. More studies on these vaccines can be done in the near future. In addition, further study about dengue vaccine efficacy in younger ages is necessary.

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Conflict of interest

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Author contribution

All authors was contributed in this study. BDN was write and validation the study.

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Cancer-Pain.org (2002). New York: Association of Cancer Online Resources, Inc.; c2000-01. [updated 2002 May 16]. Available from http://www.cancer-pain.org/. Accessed July 9, 2002.