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Editorial

Diabetes Mellitus: Test and Tools

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In 2017, there are 425 million patients living with diabetes in the world. This figure is estimated to increase by 45% or the equivalent of 629 million patients by 2045. Cardiovascular and kidney complications are the leading cause of death for diabetic patients in the world. Indonesia ranked 6th out of ten countries with the highest number of diabetes patients, which is 10.3 million patients in 2017, and expected to increase to 16.7 million patients in 2045.[1]

Since 2014, a national social security system has been implemented in Indonesia, thus began the tiered referral era. In this condition, most diabetes mellitus patients will be treated at the first level of health facilities. Only when complications arise or things that require being referred to a specialist will be sent to a referral hospital. This causes most of the diagnosis and monitoring of therapy results to be carried out at the first health facility, which generally has limitations in medical facilities and personnel. For that, we need tools that can bridge the boundaries of medical facilities and personnel with the accuracy of diagnosis and monitoring therapy in diabetes mellitus patients.

What does the guideline say?

According to American Diabetes Association, diabetes may be diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG) value or the 2-h plasma glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or A1C criteria.[2] Guidelines for the management of diabetes mellitus in Indonesian adults issued by the Indonesian Society of Endocrinology (PERKENI) states that the diagnosis of diabetes mellitus includes examining fasting blood glucose ≥ 126 mg/dl, oral glucose tolerance test ≥ 200 mg/dl, or random blood glucose ≥ 200 mg/dl with classical diabetes symptoms, or HbA1C ≥ 6.5 % with standardized examination.

HbA1C examination has been used as a standard for the diagnosis of diabetes mellitus for years. This examination has begun to be used as a definitive diagnosis of diabetes mellitus since the Diabetes Control and Complications Trial (DCCT) study showed an association between HbA1C levels and the risk of complications from diabetes mellitus. Subsequent studies from United Kingdom Prospective Diabetes Study (UKPDS) also

demonstrated an association between Hba1C levels and risk of complications, such as the level of HbA1c 10 % indicating a three times greater risk of retinopathy than the level of HbA1c 7.5%. [3]

What kind of tools are available?

Many methods have been developed to perform HbA1c assays based on differences in the structure between glycosylated and those that are not. The developed methods include ion-exchang echromategraphy, electro-phoresis, boronated affinity chromatography, and immunoassay methods. The existence of these several methods implies challenges in standardizing HbA1C examinations. This standardization is needed to ensure that HbA1C results can be correlated with studies from DCCT and UKPDS that show an association between HbA1C levels and complications that can occur. For this reason, a standard HbA1C examination was created by the American Association for Clinical Chemistry (AACC), which implemented The National Glycohemoglobin Standardization Program (NGSP) to standardize HbA1C tests since 1996.[4]

High-Performance Liquid Chromatography (HPLC) and electron spray mass spectrometry are some of the methodologies used as references for measuring HbA1c. Still, these two examinations require substantial costs, facilities, and infrastructure. This is, of course, a problem related to the financing that must be issued if this examination is a routine examination for diabetes patients in Indonesia.

What are the options?

In the last few months, HbA1c examination by Point of Care Testing (POCT) is developed in Indonesia. POCT is a method of examination performed outside the central laboratory using instruments. In diabetes mellitus patients, POCT is commonly used for blood glucose control such as glucometer examination. Currently, POCT checks are also being used to monitor HbA1C. The challenge that has been raised is the need for standardization with a tool that has been established as a definitive diagnosis. POCT for HbA1C examination has several advantages such as the use of a practical tool in the sense that it can be used immediately without having much special preparation, results that can be seen in a short time, the use of fingerstick instead of venous blood so that the patient will be more comfortable.[5]

Several studies report improved outcomes from the implementation of POCT to use in the outpatient and inpatient setting, both in the form of medical outcomes such as enhanced disease control, operational outcomes such as decreased length of stay, and financial outcomes (cost control).[6] In his review article, *Schnell* concluded that examining HbA1 utilizing POCT would improve compliance with the implementation of HbA1c examination recommendations as a method of monitoring blood glucose control, improving clinical outcomes, and improving patient education and motivation, improve the quality of life of patients, also saving costs.[7]

Most of the data above were obtained from patients who performed blood glucose control at the referral hospital. With the tiered referral system of the *Sistem Jaminan Sosial Nasional* currently in effect in Indonesia, the largest number of patients will be in primary health care. A diagnostic examination system and monitoring of therapeutic results are simple but high accuracy is needed. A study is needed to see how the accuracy and efficiency

of the POCT examination for Hba1c used in primary health services in Indonesia so that it can be used as the basis for the wider use of POCT-HbA1C.

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Original Article

Comparison of Diagnostic Value between Point of Care Testing (POCT) and Standardize HbA1c Testing in Primary Health Care

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ABSTRACT

Diabetes Mellitus is one of the biggest health problems. HbA1c is used to diagnose, to monitor treatment and treatment adjustment. High Performance Liquid Chromatography (HPLC) and electron spray mass spectrometry are recommended to measure HbA1c. But both methods need expensive cost, facility, and potentially increase health cost burden. HbA1c measurement using Point of Care Testing (POCT) is developed in Indonesia. POCT is more comfortable, has lower cost and easily brought to primary health care. **Aim:** to assess the clinical efficacy of POCT compared with a standard examination by HPLC. **Methods:** It is a cross-sectional observational study conducted at a first-level health facility (FKTP) in Malang. The subject population was patients who went to an outpatient unit in FKTP with inclusion criteria aged 20-75 years, were participants in the chronic disease management program (PROLANIS), both diabetes and non-diabetes. **Results:** Three hundred and thirty eight subjects were participated in this study. Five subjects were excluded because the presence of anemia. We used Wilcoxon test to compare HbA1c level between two methods and Rank Spearman correlation test to find correlation between two methods. This study showed measurement HbA1c level using POCT method had good accuracy (>80%). Other than diagnosis value, increased utilization of POCT HbA1c might also be caused by its portability and patient's comfort. **Conclusions:** This study showed a strong correlation (R=0.016) between POCT HbA1c and standardized measurement. POCT HbA1c also showed good accuracy in all HbA1c groups.

Keywords: diabetes mellitus, HbA1c, High Performance Liquid Chromatography (HPLC), Point of Care Testing (POCT)

INTRODUCTION

Diabetes Mellitus (DM) is one of the biggest health problems in the twenty-first century. At present, there are an estimated 415 million people with diabetes worldwide and 318 million people with impaired glucose tolerance that have the potential to become diabetes.[1] Data released from the results of basic health research (RISKESDAS) show that the prevalence of diabetes in Indonesia is 5.7% while the prevalence of people with impaired glucose tolerance or who are often grouped into prediabetes is 10.2%.[2]

The diagnosis of DM can be made by using fasting blood glucose (GDP) or blood glucose testing 2 hours after 75 grams of glucose (OGTT) or by HbA1c. These three

examinations have the same place in establishing the diagnosis of DM. [3,4] In addition to establish the diagnosis, blood glucose, and HbA1c checks are also important to carry out monitoring that could be used as a guide to improve management such as changes in diet / physical activity or adjusting the dose of drugs/insulin. [5,6]

Two large-scale studies, Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) showed that intensive therapy using HbA1C as a target can reduce the risk of developing microvascular complications. The decrease in HbA1C is identical to the reduced risk of diabetes-related complications. Data from UKPDS shows that each 1% decrease in HbA1c, it will decreases the relative risk of myocardial infarction by 14% and the risk of microvascular complications by 37%.[7] Besides, HbA1c examination is an indirect test to measure the average blood glucose. Several other factors can affect HbA1c such as age, race/ethnicity, the presence of anemia /hemoglobinopathy, history of blood transfusion in the last 2-3 months and disorders of kidney function. [8,9]

Guidelines issued by the American Diabetes Association (ADA) as well as from the Indonesian Endocrinologist (PERKENI) states examination of HbA1c for diagnosis must use methods that certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized by studies from Diabetes Control and Complications Trial (DCCT).[3,4] In Indonesia, not all laboratories use NGSP standards, accuracy and caution are needed in interpreting the HbA1C examination.

High-Performance Liquid Chromatography (HPLC) and electron spray mass spectrometry are some of the methodologies that used as a reference for measuring HbA1c, still both of these checks require substantial costs, facilities and infrastructure.[10] This indeed becomes a problem related to financing that must be issued by the country if this examination is a routine examination for diabetic patients in Indonesia.

In recent months, the HbA1c examination with Point of Care Testing (POCT) is developed in Indonesia. POCT is a method of the investigation carried out outside a central laboratory that uses tools. Some of the advantages of this POCT examination include: providing real-time results so that doctors can immediately provide motivation and management plans based on the results obtained; using capillaries so that the patient is more comfortable; and is cheaper and easier to carry to primary health facilities. [5, 11-14]

This POCT examination has not recommended for the diagnosis of T2DM. This examination has several shortcomings, such as the absence of evidence that states that this examination will provide better outcomes for patients, better optimize diabetes services, and improve HbA1C. Besides, standardization of the use of HbA1C is also still not available properly. [15-19] For this reason, a study aimed at assessing the clinical effectiveness of POCT, which includes its accuracy is compare with a standard examination by the HPLC method.

METHODS

Study Design

This study is a cross-sectional observational study conducted at a first-level health facility (FKTP) in Malang. The subject population was patients who went to an outpatient unit in FKTP with inclusion criteria aged 20-75 years, were participants in the

chronic disease management program (PROLANIS), both diabetes and non-diabetes. While the exclusion criteria are hemoglobin levels <10 gr/dl or hemoglobinopathy. This study was approved by Ethics commitee of General Hospital dr. Saiful Anwar Malang.

Sample size

The prevalence of type 2 diabetes and glucose intolerance in Indonesia is currently 5.7% and 10.2%. An expected sensitivity level of 80% and an error rate of 5%, as many as 307 subjects need in this study.

Study procedures

The study carried out after the patient signed the consent form following this study. The patient was anamnesis and anthropometric, physical examination including blood pressure, body mass index. Then the HBA1C POCT examination was carried out in the form of capillary blood followed by venous blood sampling to examine the Hba1C by the HPLC method. POCT examination uses Borronate affinity Tri-stat™ technology. The analysis of the HPLC method uses Premier Hb9210TM. The HbA1c results grouped into three groups, namely groups with HbA1c <5.7%, groups with HbA1c ≥ 6.5%, and groups with HbA1c ≤ 7%. Complete blood tests are also perform to rule out anemia (Hb <10g / dl) and hemoglobinopathy.

Statistical Analysis

We used paired t-tests to compare HbA1c levels from the two methods or alternative statistical analysis which appropriate with types of the data. Analysis of sensitivity, specificity, positive predictive value, and accuracy. All data were analysed by computerization using Statistical Product and Service Solution software, IBM SPSS Statistics 20, with a significance level of 0.05 (α=0.05).

RESULTS

There were 338 subjects who participated in this study. Five subjects were excluded due to anemia, resulting in a total of 333 subjects. Based on **Figure 1**, it can be seen Correlation test results showed a significant correlation between POCT HbA1c levels with standard HbA1c levels. The correlation value is 91.6% and it has positive correlation. It means that the higher the POCT HbA1c levels, the higher HbA1c levels obtained by the standard HPLC method.

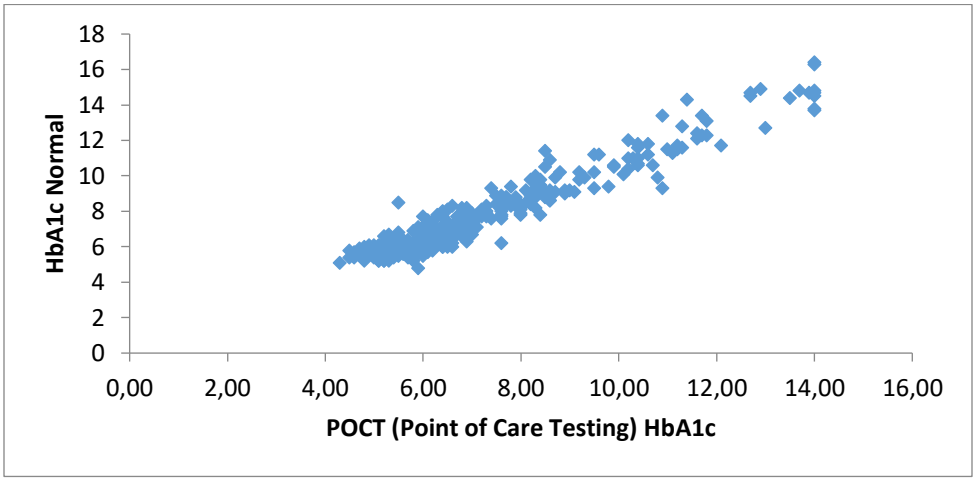

Figure 1. Correlation of POCT HbA1c levels with standard HbA1c

3.1 Group of Diabetic Patient with HbA1c < 5.7 %

The results of cross-tabulation, POCT HbA1c with standard HbA1c for groups with HbA1c levels <5.7% can be seen in **Table 1**.

Table 1. POCT HbA1c levels compared to standard HbA1C levels for HbA1c < 5.7 %.

POCT HbA1c	HbA1c standard		Total
	< 5.7	≥ 5.7	
< 5.7	18	52	70
≥ 5.7	6	257	172
Total	24	309	333

**POCT, Point of Care Testing; HbA1c, Haemoglobin A1c*

In this group, POCT sensitivity was 0.79, specificity was 0.99, positive predictive value was 0.98, and the negative predictive value was 0.82. The method accuracy is outstanding (0.89 / 88.89%)

3.2 Group of Diabetic patient with HbA1c ≥ 6.5 %

The results of cross-tabulation (table 2x2) POCT HbA1c with standard HbA1c for groups with HbA1c levels ≥ 6.5% can be seen in **Table 2.**

Table 2. POCT HbA1c levels compared to standard HbA1C levels for HbA1c ≥ 6.5 %.

POCT HbA1c	HbA1c Standard		Total
	≤ 6.5	>6.5	
≤ 6.5	123	38	161
> 6.5	5	167	172
Total	128	205	333

*****POCT, Point of Care Testing; HbA1c, Haemoglobin A1c*

Table 3. POCT HbA1c levels compared to standard HbA1C levels for HbA1c ≤ 7 %.

POCT HbA1c	HbA1c Standard		Total
	≤ 7	> 7	
≤ 7	163	35	198
>7	2	133	135
Total	168	165	333

*****POCT, Point of Care Testing; HbA1c, Haemoglobin A1c*

In this group, POCT sensitivity was 0.79, specificity was 0.99, positive predictive value was 0.98, and the negative predictive value was 0.82. The method accuracy is excellent (0.89/ 88.89%)

DISCUSSION

Overall, the results of this study indicate that the use of the POCT HbA1c examination has reasonably good accuracy (> 80%) compared to the standard examination for HbA1c. These results are the same as the study conducted by Wiwanitkit, *et al*. from Thailand who saw a correlation between the POCT HbA1c examination and with the standard HbA1c examination tool with a correlation coefficient (R) 0.99.[19] It is not too different from our study where we obtained a correlation coefficient (R) between the two examinations amounting to 0.92. Likewise, a comparative study conducted by Sicard, *et al*. in 23 patients found a reasonably good correlation (r = 0.76) between the two methods used for this HbA1c examination.[20] The same results in terms of accuracy also reported by Knaebell, *et al*. testing 3 POCT HbA1c devices at the same time compared to the standard where the correlation coefficient is 0.98, 0.99 and 0.99.[12] In addition to diagnostic values, POCT HbA1c is increasingly used because it is more economical than standard inspection, is portable, so it is easy to be moved and used, and greater satisfaction from the patient's side.[21,22]

CONCLUSION

The results of this study show a strong correlation (r = 0.916) between POCT HbA1c and standard inspection. They also obtained useful accuracy data from the use of POCT HbA1c in all groups.

This study can be a basis for consideration of the use of POCT HbA1c in FKTP as part of the management of Diabetes Mellitus Type-2. However, it is always necessary to understand the limitations of using all diagnostic tools to avoid the results misinterpretation.

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Original Article

Correlation between Risk Stratification of Complications and Types of Antihyperglycemia Drugs with Incidence of Acute Diabetic Complications in Patients with Diabetes Mellitus during Ramadan Fasting

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ABSTRACT

Ramadan fasting for Diabetes Mellitus (DM) patients can lead to acute complications such as hypoglycemia, hyperglycemia, diabetic ketoacidosis (DKA) and thrombosis. Risk stratification predicts fasting safety of DM patients. Dose and timing of antihyperglycemia drugs adjusted during Ramadan fasting. **Aim:** To know the correlation between the risk stratification of Ramadan fasting and type of antihyperglycemia drugs with the incidence of acute complications in DM patients undergoing Ramadan fasting. **Methods:** DM patients in Endocrinology Clinic, dr. Saiful Anwar, General Hospital Malang who intend to fast during Ramadan classified in IDF-DAR risk stratification, conduct blood glucose monitoring and filled out a daily logbook during fasting. **Results:** Thirty-seven subjects were included in the study, only 1 patient with type-1 DM. Average fasting time is 18 days. Acute complications found higher in very high-risk group (5/6) compared to mild/moderate (2/13) and high-risk group (7/18) (p=0.009). Acute complications found higher in group with OAD and insulin combination regiment (2/4) compared to OAD (9/24) or insulin group (3/9) (p= 0. 731). One subject in very high-risk group suffered from acute coronary syndrome. Relationship between risk stratification and the incidence of hypoglycemia (p=0.040) and hyperglycemia (p=0.031) was significant. Relative risk in the very high-risk group was 2.538 compared to mild/moderate risk RR (95% CI)= 0.77 (0.62-0.96). **Conclusions:** There is a correlations between risk stratification and acute complications in DM patients undergoing Ramadan fasting. No relationship between type of antihyperglycemia drugs with acute complications of Ramadan fasting.

Keywords: diabetes mellitus, Ramadan fasting, risk stratification, acute complication, antihyperglicemic drug

INTRODUCTION

Doing Ramadan fasting is one of the five pillars in Islam which obliges Muslims to refrain between dawn and dusk from food, drink, and all forms of immoral behavior that can make the fasting is not valid. Epidemiology of Diabetes and Ramadan (EPIDIAR) study in 2001 which investigated about the diabetic patient in 13 Islamic states resulted 42.8% type-1 DM patient and 78.7% type-2 DM patient who fast during Ramadan.[1] Indonesia is one of country with the largest Muslims population in the world. In 2015, there are 10 million of DM patients in Indonesia and 87.2% among them are Muslims (WHO-*Diabetes country profile*, 2015). [2]

During fasting, there are some changes which are diet, sleep pattern, physical activities, dosage, also the timing antihyperglycemic drugs consumption.[3] It is potentially causing an uncontrollable blood glucose in diabetes patients who fast during Ramadan. The diabetes patient who fasts can lead to acute complication such as hypoglycemic, hyperglycemic (with or without ketoacidosis), dehydration and thrombosis (IDF-DAR, 2016). [2]

The Result of EPIDIAR study showed Ramadan fasting can increase severe hypoglycemia (SH) risk which needs medical treatment at hospital. Hypoglycemia had increased 4.7 times to type-1 DM patient and 7.5 times as frequent to type-2 DM patient. Those numbers were not included in home treatment.[2] The use of oral antihyperglycemic drug which have function to increase insulin secretion to the pancreas can lead to the hypoglycemia incident.

During fasting, blood glucose level will be decreased, thus it causes secretion degradation by the insulin and improvement of contra-insulin hormone, which are glycogen and glucose. It will cause fatty acid release from the adipocyte and producing ketone which will be used as an energy by the organs.[4] In normal body, insulin and contra-insulin hormones will be at the balance-condition. However, in diabetes patient, the balance will be disturbed. Fasting can cause breakdown of excessive glycogen and increased gluconeogenesis also ketogenesis that cause hyperglycemia and ketoacidosis.[5]

Study of EPIDIAR showed the incidence of higher risk of hyperglycemia in Ramadan that needs hospitalization was increased by 5 times in type-2 DM patients, while the incidence of higher risk of hyperglycemia (with or without ketoacidosis) in type-1 DM patients was increased by 3 times. Hyperglycemia can be caused by increasing food consumption and excessive glucose also excessive doses decreased that had done to prevent hypoglycemia.[6]

A limited fluid intake during fasting, especially in a long term can cause dehydration. It makes the patient heavy in hot weather and high-humidity areas. Also, it might be happened frequently to individual who performs strenuous activities.[7] Besides, hyperglycemia can cause osmotic diuresis which can provoke volume depletion and electrolyte. Patient with autonomic neuropathy may develop orthostatic hypotension which cause syncope.[8] Intravascular volume reduction as a result of hypovolemia will increase thrombosis tendency, especially in patient who tend to have hypercoagulable state. The blood viscosity increase due to dehydration will increase stroke risk and thrombosis.[9] However, one investigation showed the incident of hospitalization due to coronary diseases or stroke did not increase during Ramadan.[10]

Fasting during Ramadan might be can cause serious health issues for diabetes patient. But there were a lot of diabetes patient who still want to do Ramadan fasting even though it can be harmful for themselves medically. Doctor plays a role to give insight and guidance about the impact of fasting to the patient's medical condition.[11] The last decision whether the patient is willing to fast or not is on themselves. If they insist to fast, then they must get the guidance and suggestion about the meal plan, physical activities, periodic blood glucose monitoring, and timing of

antihyperglycemic drug consumption which be applied to patient during fasting.[12]

To help giving a guidance about the safety of Ramadan fasting for diabetes patient, *International Diabetes Federation* collaborated with *The Diabetes and Ramadan International Alliance* (IDF-DAR) make a risk stratification which classify diabetes patients into some groups. Those were mild-moderate risk group, high-risk, and very high-risk to experience an acute complication during Ramadan fasting.[11] These stratifications were applied in pre-fasting screening as an advice for the diabetes patient considering to their safety during fasting.

All this time, the use of risk stratification is for knowing the DM patient's characteristics based on complication risk that may be happened.[12] Majority of Muslims are insisting to fast despite there is an exception in Islam for the one who suffers from serious health problems like Diabetes Mellitus. It leads DM patient who is fasting have various risk stratification, which means they have different susceptibility to acute complications.[13]

This study aims to find out a correlation between risk stratification for complications and correlation between types of antihyperglycemic drug to the incidence of acute complication in DM patient who fasts in Ramadan.

METHODS

It used a survey method by taking the pre and post data. Subject of the research is DM patients who fast in Ramadan, especially those who visit the Endocrine-Metabolic Outpatient Installation regularly in dr. Saiful Anwar General Hospital, Malang. Only those who meets the inclusion criteria and not listed in exclusion criteria that became the subject of the research.

We conducted the research during Ramadan 1438 H, which was May 25, 2017 until June 25, 2017. We conducted the screening of risk categorization and counseling since 4 until 6 weeks ago before Ramadan. This study has received approval from the Health Research Ethics Commission dr. Saiful Anwar by number 400/98/K.3/302/2017.

We gave the patient an *Accu-chek* Performa II Glucose Meter Kit which has been calibrated first, daily note during Ramadan fasting, alcohol swab, and lancet. Subjects and the associate will get education about the way of Self-Monitoring of Blood Glucose (SMBG) with the way demonstrating it in front of the subject. To assure the understanding of the subject about the kit, we asked the subjects to demonstrate it in front of the researchers based on what we explained before.

We explained the way of filling out the daily note that must be filled during Ramadan fasting. It covered description about patients' food consumption, their physical activities, the timing and doses of antihyperglycemic drug during fast in Ramadan based on the researcher's recommendation, blood glucose rate with SMBG, also the complaints during fasting in Ramadan. We explained regarding to the signs and symptoms of hypoglycemia, hyperglycemia, dehydration, and thrombosis. We also told them to do SBGM as soon as they felt the signs of acute complication.

During first week of Ramadan fasting, patients measured blood glucose rate 1 once/every day between 9.00 a.m. till 10.00 a.m. and when they felt the acute complication signs. In the second and third week of Ramadan, patient should check their blood glucose before *iftar* and 2 hours before having heavy meal or having iftar every twice a day and when the problem arose that lead to acute complication. We told them to contact the researchers if they experienced the problems. The research team contact the subject by phone minimum once a week. Patients had to record in daily note regarding to the day when they did not fast or the time when they ruined their fast. We asked them to go back to Endocrinology Clinic, dr. Saiful Anwar, General Hospital, Malang in 3-5 days before Eid Al-Fitr to return the kit and daily note, also taking data of post-fasting; weight, blood pressure, and discussed about their process during Ramadan fasting.

We analyzed the data using univariate and bivariate analysis. In univariate, we served the data in the form of frequency distribution for categorical data and served in the form of average (mean ± SD) for continuous data. In bivariate, on the correlation examination between risk stratification and types of drug toward acute complication was done by *Chi-Square* analysis. If the *Chi-Square* requirements cannot be fulfilled, then we used *Kruskal Wallis.* We used *Mann Whitney* to examine the correlation between the first categorical data and the other categorical data. An Analysis *Pearson* correlation was used in normal data distribution and *Spearman* correlation analysis was used in abnormal data distribution.

We computerized analysis a whole techniques of statistical analysis using *Statistical Product* and *Service Solution* (SPSS) software, IBM SPSS Statistics 20 with the significant level or probability value 0.05 (p=0.05) and confidence level 95% (α = 0.05).

RESULT

There were 37 subjects of the research. The sample size was explained below.

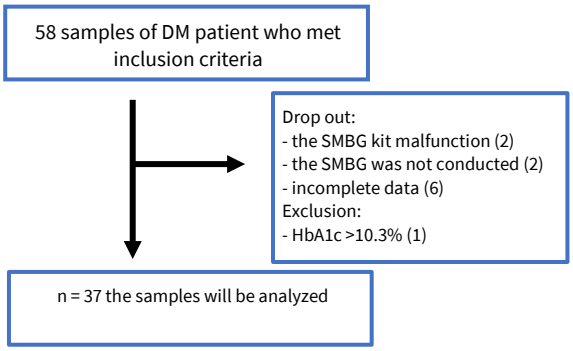


Figure 1. Demography and Description of the Clinical Subject

Characteristics of Subject

Characteristics of research subject are listed on **Table 1**. Subject was categorized into 3 risk stratification groups based on IDF-DAR 2016 which were mild-moderate risk group, high-risk group, and very high-risk group with the characteristic description. It was explained on **Table 2**.

Table 1. Characteristics of Research Subject

Characteristics	Mean/ Proportion
Total (n)	37 (100%)
Gender	
Male	18 (48.35 %)
Female	19 (51.35%)
age (years)	60.08±11.34
DM Types	
Type-1 DM	1 (2.70%)
Type-2 DM	37 (97.30%)
Duration of DM (years)	7.14 ± 5.27
< 10 years	25 (67.57%)
≥ 10 years	12 (32.43%)
Weight(kg)	62.94± 9.86
Medical History	
OAD	24 (64.9%)
SU (Sulfonylurea)	5 (20.8%)
Biguanide + SU	4 (16.7%)
SU + Acarbose	11 (45.8%)
Biguanide + SU + Acarbose	4 (16.7%)
Insulin	9 (24.3%)
OAD + Insulin	4 (10.8%)
HbA1c of pre-Ramadan (%)	8.03 ± 2.03
FBG (Fasting Blood Glucose) (mg/dl)	134.32 ± 50.11
GD2PP (2-hours post-prandial) (mg/dl)	181.59 ± 51.48
Lipid Profiles in pre-Ramadan	
Total of Cholesterol (mg/dl)	185.91 ± 37.64
HDL (High Density Lipoprotein) (mg/dl)	50.31 ± 24.00
LDL (Low Density Lipoprotein) (mg/dl)	118.06 ± 34.15
Triglyceride (mg/dl)	170.85 ± 93.30
Complication History of DM	
Hypoglycemia	0 (0%)
Nephropathy	7 (18.4 %)
Microvascular (Retinopathy, neuropathy)	8 (21 %)
Diabetic Ulcer	1 (2.6 %)
Coronary Heart Disease	0 (0%)
Cerebrovascular Disease	3 (7.9 %)
Average Lengths of Fasting (day)	18 days

**DM, Diabetes Mellitus;OAD, Oral Antidiabetic; HbA1c, Haemoglobin A1c; FBG, Fasting Blood Glucose; GD2PP, 2-hours post-prandial; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein*

Table 2. Patient Risk Stratification

Characteristic	Mild/moderate n=13	High n=18	Very High n=6	*p*-value
Age (years)	62.00±9.65	57.78 ±8.18	63.17 ±21.06	0.475
DM Types				
Type-1 DM	0 (0%)	0 (0%)	1 (7%)	0.065
Type-2 DM	13 (100%)	19 (100%)	5 (83.3%)	
Duration of DM (years)	6.54±3.89	7.50±6.55	7.33 ± 4.03	0.883
Therapy History	11 (78.57%)	8 (44.44%)	5 (83.33%)	0.070
OAD	1 (7.7%)	7 (38.89%)	0 (0%)	
Insulin	1 (7.7%)	3 (16.67%)	1 (16.67%)	
OAD + Insulin				
FBG	130.08±38.05	155.61±74.5	123.0±60.39	0.393
GD2PP (2-hours post-prandial)	182.31±42.95	183.56±67.24	184.5±42.14	0.996

**DM, Diabetes Mellitus;OAD, Oral Antidiabetic; HbA1c, Haemoglobin A1c; FBG, Fasting Blood Glucose; GD2PP, 2-hours post-prandial*

Acute Complications

Acute complications which happened during Ramadan fasting are known through daily record that contains blood glucose through SMBG based on scheduled monitoring in the first, second, third, and when patients get complaint or clinic symptoms that lead to acute complication, also patient's live report to the research team.

Table 3. Incidence of Acute Complications

Risk Stratification	Number of events	Times of Events		
		1st week	2nd week	3rd week
Very high				
Hypoglycemia	5	4	0	1
Hyperglycemia	12	6	2	4
Thrombosis	1	0	0	1
DKA	0	0	0	0
High				
Hypoglycemia	17	7	3	7
Hyperglycemia	19	10	6	3
Thrombosis	0	0	0	0
DKA	0	0	0	0
Moderate				
Hypoglycemia	1	0	1	0
Hyperglycemia	2	2	0	0
Thrombosis	0	0	0	0
DKA	0	0	0	0

*****_DKA, Diabetic Ketoacidosis_

Results of Blood Glucose Monitoring based on Complaints and Clinical Symptoms

In the first week, hypoglycemia occurred 11 times, which were 7 times on 1 subject in high-risk group, and in 2 subjects of very high-risk group who has hypoglycemia occurred once and 3 times. It occurred between 12.00 p.m. till before iftar. The lowest recorded blood glucose level was 53 mg/dL.

In the second week, hypoglycemia occurred 3 times out of the monitoring schedule. It occurred at 14.00-15.00 WIB, which were once in one subject of mild/moderate risk group, twice in one subject in high-risk group. In the third week, it occurred 4 times out of the monitoring schedule. It occurred at 14.00-15.00 WIB, which were on 2 subjects in high-risk group and one subject of very high-risk group.

There is no subject who needs special medical treatment though hospital care related to hypoglycemic complication or hyperglycemia.

Results of Blood Glucose Monitoring of the First, Second, and Third Week

Description of changes of blood glucose mean in each risk group is in **Figure 2**. The most noticeable blood glucose fluctuation in the first week of Ramadan fasting was in very high-risk group, especially in the 2nd and 3rd day of fasting.

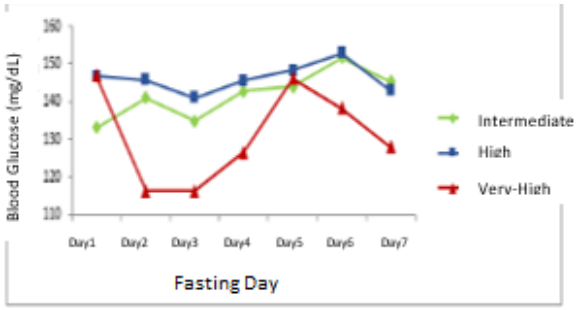
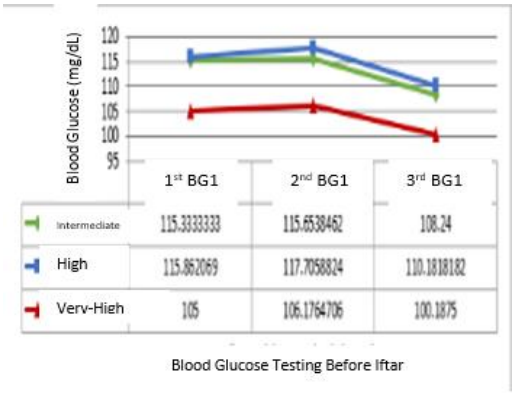


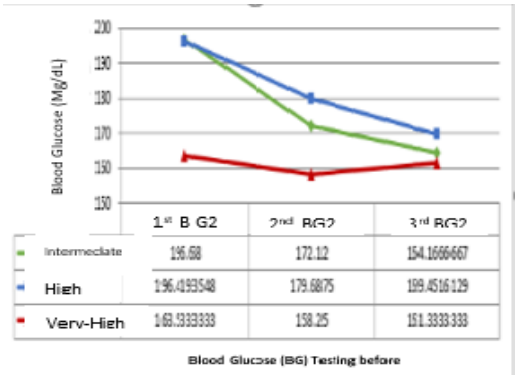
Figure 2. Mean of Blood Glucose in the 1st Week of Ramadan Fasting Based on Risk Category

Blood glucose level before iftar in the 2nd week of 3 risk groups was relatively balanced. It showed in **Figure 3** and **4**. Blood Glucose 2-hours after Iftar in the 2nd week seemed higher than the first testing for each risk group. In second and third testing, the blood glucose level of 2-hours after meal seemed dropping and the lowest seemed at the third testing for mild/moderate and high-risk groups.



*****_BG, Blood Glucose_

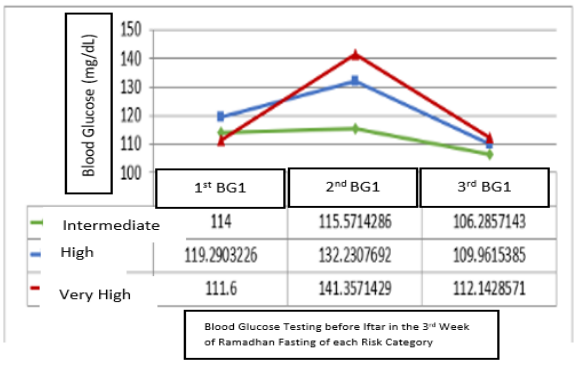
Figure 3. Mean of Blood Glucose Before Iftar in the 2nd Week of Ramadan Fasting of each Risk Category



**BG, Blood Glucose*

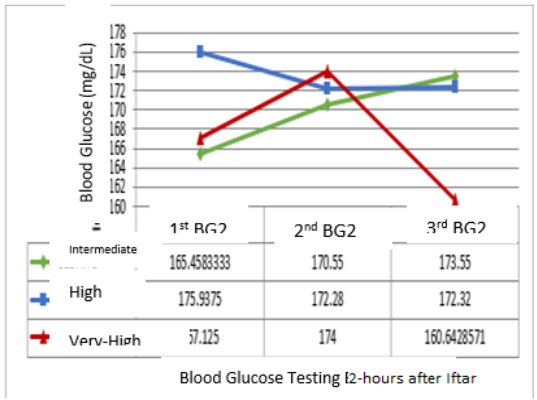
Figure 4. Mean of Blood Glucose 2-hours after meal in the 2nd Week of Ramadan Fasting of each Risk Category

Explanation of mean of blood glucose before iftar in the third week is in **Figure 5** and **6**. It shows glucose blood level in 3 groups are relatively balanced. Mean of blood glucose 2 hours after iftar in the 3rd week of Ramadan fasting is lower than 2nd week.



**BG, Blood Glucose*

Figure 5. Mean of Blood Glucose Before Iftar in the 3rd Week of Ramadan Fasting of each Risk Category



**BG, Blood Glucose*

Figure 6. Mean of Blood Glucose 2-hours after Iftar in the 3rd Week of Ramadan Fasting of each Risk Category

Others Acute Complications

Apart from hypoglycemia and hyperglycemia, there was one subject who has complication, such as acute coronary syndrome. It was a subject of very high-risk group. Subject who got *Unstable Angina Pectoris* in 3rd week of Ramadan fasting and need a hospital care. Diabetic ketoacidosis (DKA) was not found in subject who undergo Ramadan fasting.

Correlation between Risk Stratification and Acute Complication

We used comparative analysis (categorical) which is *Chi-Square* to determine the relationship between the risk stratification of Ramadan fasting on the incidence of acute complications in DM patients who did Ramadan fasting, a categorical comparative analysis is used, namely *Chi-Square*. From the analysis, it is known that the *Chi-Square* assumptions have not been fulfilled so that the test is replaced by using *Kruskal Wallis* with a *p value* = 0.009. The *p value* <0.05 indicates that there is a correlation between the risk stratification of Ramadan fasting and the incidence of acute complications in DM patients who observe Ramadan fasting. This is explained in **Table 3**.

Table 3. The Correlation between Risk Stratification and the Incidence of Acute Complications.

Risk Stratification	Total						*P-Value*
	Hypo glycemia	Hyper glycemia	DKA	Thrombosis	>1 Complication		
Mild/ moderate n=13	1 (7.7%)	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)	13 (100%)	0.009
High n=18	3 (16.7%)	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	18 (100%)	
Very high n=6	1 (16.7%)	1 (16.7%)	0 (0%)	1 (16.7%)	2 (33.3%)	6 (100%)	

a= hyperglycemia and hypoglycemia

Based on these results, analysis was performed with the *Mann Whitney* test to find out which risk stratification has a different complication result, which is described in **Table 3**. From the results of the *Mann Whitney* test, it is known that there is a significant difference in the incidence of acute complications between the

mild/moderate and very high-risk stratification groups (p = 0.003). Comparative tests between high and very high-risk groups also had a significant difference in the incidence of acute complications (*p*= 0.038).

To determine the relationship between the risk stratification of Ramadan fasting on the total incidence of hypoglycemia and hyperglycemia in DM patients during Ramadan fasting, correlation analysis *Spearman's* (categorical vs numeric) was used.

From the results of the *Spearman* correlation analysis obtained *p-value* in DM risk stratification correlation with a total incidence of hyperglycemia complication is 0.040. The *p- value* <0.05 indicates that there is a correlation between the risk stratification of DM with the number of complications of hyperglycemia. In relation to the risk stratification of DM with the number of complications of hypoglycemia, the *p-value* is 0.031. The *p-value* <0.05 indicates that there is a correlation between the risk stratification of DM and the number of complications of hypoglycemia.

To determine the relative risk of acute complications of fasting based on risk stratification, the mild/moderate risk group was used as a comparison. The results are described in **Table 4**. Patients in the very high-risk group had 2.5 times chance of developing complications from experiencing acute complications compared to patients in the moderate risk group.

Table 4. Relative Risks of Complications of Acute Fasting Based on Risk Stratification

		No Complication	Complication occured	P-value	RR (IK 95%)
		n (%)	n (%)		
Risk Stratification	Very high	1 (16.7)	5 (83.3)	0.046	2.53 (0.800–8.058)
	High	11 (61.1)	8 (38.9)	0.237	1.38 (0.896–2.288)
	Mild/moderate	11 (84.6)	2 (15.4)		Standard of comparison

Correlation of Risk Stratification and Types of Antihyperglycemic Drugs

We used *Kruskal Wallis* analysis to know correlation between types of therapy of acute complication in DM patient who fast in Ramadan because *Chi-square* provision was not fulfilled. It was explained in **Table 5**.

Table 5. Correlation between Types of Antihyperglycemic and Acute Complication

Types of OAD	Acute Complication						*p-Value*
	Hypoglycemia	Hyperglycemia	DKA	Thrombosis	>1 Complication	Total	
OAD n=24	4 (16.6%)	3 (12.5%)	0 (0%)	1 (4.2%)	1 (4.2%)	24 (100%)	
Insulin n=9	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)	9 (100%)	0.731
OAD + Insulin n=4	0 (0%)	1 (25%)	0 (0%)	0 (0%)	1 (25%)	4 (100%)	

*OAD, oral antidiabetic

The most acute complication was found in OAD combination with insulin therapy group (2/4) compared to OAD combination and insulin therapy without combination, with p-value >0.05 showed that there is no correlation between types of therapy on acute complication in DM patient who did Ramadan fasting.

DISCUSSION

Mean of fasting day in this study is 18 days. It is in line with EPIDIAR result which is the largest research discussed about Ramadan. The result was a total of 79% of type-2 DM patient and 43% of type-1 DM patient were fasting in at least 15 days during Ramadan.

In this study, acute complication was not happened to 23 subjects during Ramadan fasting. The prevalence of hypoglycemia and hyperglycemia were 13.51% (5/37) and 10.8% (4/37), and there are 10.8% subjects (4/37) who experienced acute complication like hypoglycemia and hyperglycemia alternatively during fasting day in Ramadan. A total of 14 subjects who experienced acute complication, there are 57 incidence of complication report that 22% of it, is hypoglycemia and 57.89% is

hyperglycemia. Ahmedani, *et al*.'s study showed the prevalence of hypoglycemia (21.7%) was a slightly higher than hyperglycemia (19.8%) in 327 DM patients who were fasting in Pakistan and there is no difference between prevalence of hypoglycemia and hyperglycemia in type-1 DM patient and type-2 DM patient during Ramadan.[11]

There is none hypoglycemia found in this study need to be hospitalized. Hypoglycemia was found to occur most frequently between 13.00 until before Iftar. Some subjects reported the hypoglycemia to the research team and cancelled their fasting when hypoglycemia occurred, but there were also subjects who did not report this finding and continued fasting despite knowing that they got hypoglycemia.

Hyperglycemia was occurred at various times but most commonly happened after Iftar, with the highest blood glucose level recorded being 540 mg/dL in patients after Iftar. However, there were no subjects with severe hyperglycemia requiring further hospitalization. Factors that contribute to the increased risk of hyperglycemia during Ramadan include the food consumed during iftar, which is heavy food with high carbohydrate content and cooked by frying, and consumption of drinks and sweet snacks.[14] The reduced physical activity during the fasting month of Ramadan also contributes to the incidence of hyperglycemia.[15] However, in this study, it cannot be known about the measurable changes in the physical activity of the subjects, as well as changes in dietary patterns during Ramadan compared to pre-Ramadan so that the correlation between the two with the incidence of hypoglycemia and hyperglycemia cannot be concluded.

Although there is an acute complication of hyperglycemia, this study did not find subjects who had DKA. DKA itself is often mentioned in expert recommendations as one of the most likely acute complications during Ramadan fasting. The risk of DKA is considered to increase during Ramadan because fasting itself will result in hypoinsulinemia and hyperglucagonemia, as well as the formation of ketone bodies which will then cause DKA.[16] However, it becomes a speculation among experts, that there are no studies showing that the incidence of DKA does increase during Ramadan. One study showed that only 2.5% of DM patients in Libya studied had DKA during Ramadan fasting.[10] Another study showed no increase in incidence and mortality due to DKA during Ramadan which indicates that fasting in Ramadan is a significant risk factor for DKA.[16] A *critical reappraisal* by Beshyah, *et al.* added although DKA can occur during the fasting month of Ramadan, this may be due to precipitation from other factors not related to fasting itself, and there is no sufficiently strong evidence that the risk of DKA will increase during Ramadan fasting. Further study with a larger sample is needed to prove this.[17]

Acute complications were recorded most frequently in the 1st week of fasting in Ramadan, namely 50.88% (29/57), of which 62% (18/29) were hyperglycemia. This may be because the patient is still adapting to changes in meal times, and the increased consumption of high-carbohydrate foods at iftar and dawn, accompanied by changes in physical activity. These findings suggest that monitoring of blood glucose and the patient's general condition should be done more intensively during the first week of fasting in Ramadan.

There is a statistically significant correlation between risk stratification and the incidence of acute complications. Based on these results, it can be concluded that the DM patients' classification on the risk stratification of Ramadan fasting complications is an important thing to do and should be part of the pre-Ramadan education for DM patients who want to fast in Ramadan.

By classifying based on risk complication initially, patients can get medical advice that it would be more safety for them not to fast if there is a high-risk of complications during fasting, and if they still decide to fast, the patient already knows the types of complications risk and how big the chances of complications are that can happen

to them, and able to take steps to prevent complications, and tighter supervision during fasting by themselves, the people around them and the doctors who treat them.[17]

There are many factors that can affect the incidence of acute complications during Ramadan fasting, one of which is type of antihyperglycemia drug. To determine the correlation between the type of therapy and the incidence of acute complications in DM patients undergoing Ramadan fasting, *Kruskal Wallis* categorical comparative analysis was used with a value of p = 0.731. The *p value*> 0.05 indicates there is no correlation between the type of therapy and the incidence of acute complications in DM patients who observe Ramadan fasting.

In this study, the observed pharmacological therapy factor was the type of antihyperglycemic drug, without considering whether the patient had adjusted the therapeutic regimen according to previous pre-Ramadan counseling, and the factors for changing the time to take antihyperglycemia drug during Ramadan fasting. However, a study by Ahmedani, *et al* showed that changes in the timing of taking antihyperglycemia had no significant effect on the incidence of hypoglycemia or hyperglycemia during Ramadan.[11] This finding is not in line with the EPIDIAR study which showed a significant relationship between the incidence of severe hypoglycemia with changes in insulin and OAD during fasting Ramadan and changes in physical activity during fasting Ramadan. [18]

This study is a pilot study which determine glucose control in DM patients who are fasting in Malang. Further research is needed with maximum research handling to determine the factors that influence the incidence of acute complications such as diet, pre-Ramadan education, physical activity and sleep patterns, and to find out whether there are other factors related to therapy that affect the incidence of acute complications, such as changes in therapy regimens and changes in dosage, as well as changes in the timing of drug consumption.

CONCLUSION

There is a correlation between risk stratification and the incidence of acute complications in DM patients who undergo Ramadan fasting. There is no correlation between the type of antihyperglycemic drugs and the incidence of acute complications in DM patients who undergo Ramadan fasting.

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Original Article

The Impact of Subchronic Soybean Milk and Genistein Supplementation on Pancreatic Fatty Infiltrations of Sprague Dawley Male Mice

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ABSTRACT

Isoflavones (genistein, daidzein) on soybean milk have phytoestrogenic properties. In Asian, the blood phytoestrogen levels can reach 160 ng/ml (80 times higher than Western). This may potentially disrupt endocrine functions regarding its binding with estrogen receptors.. Since the function and distribution of adipose tissues are regulated by estrogen receptors, the reduction of estrogen receptor-α(ERα) results in ectopic fats distribution around visceral tissues, such as the pancreas. **Aim:** To investigate the impact of subchronic soybean milk and genistein supplementation on pancreatic fatty infiltrations in mice. **Methods:** The experiment used 35 Sprague dawley male mice under 7 treatment groups within 60 days: negative control with standard rationed food, 3 groups with variable dose of soybean milk: 100 mg, 200 mg, and 400 mg, and 3 groups with variable dose of genistein: 0.4 mg, 0.8 mg, and 1.6 mg. Histological measurements on the level of pancreatic fatty infiltrations were conductedafter. Analyses used Kruskal-Wallis and post-hoc Mann-Whitney. **Results:** Medium to high level of pancreatic fatty infiltrations was found at the control group while there is a decreasing trend on the level of pancreatic fatty infiltrations on groups with soybean milk and subchronic genistein compared with control group, proportional to higher dosage supplementation. Reduction of pancreatic fatty infiltration levels on groups with soybean milk and subchronic genistein supplementation is not statistically significant compared to control. **Conclusion:** Supplementation of soybean milk and subchronic genistein do not significantly reduce the levels of pancreatic fatty infiltrations in Sprague dawley male mice.

Keywords: soybean milk, genistein, pancreatic fatty infiltrations

INTRODUCTION

Soybean and its derivatives are the main source of vegetable protein for the Asian population.[1-3] Isoflavones found in soybeans (genistein, daizein, glysitein) have the benefit of increasing high-density lipoprotein (HDL) levels and reducing low-density lipoprotein (LDL) levels and have antioxidant effects.[4]

Isoflavones in soybeans are phyto-estrogens. In the population of Asia, the level of phytoestrogens in the blood reaches 160ng / ml (80 times higher than the western population).[5] Phytoestrogens can bind to estrogen receptors so that by WHO categorized as *Endocrine Disrupting Chemicals* (EDC) because they have the potential to interfere with endocrine function.[6] Previous studies have shown that in male experimental animals, subchronic administration of genistein can reduce the expression of estrogen and androgen receptors in the prostate, thereby reducing prostate size.[7]

The function and distribution of adipocyte cells are regulated by estrogen receptors.[8] Healthy adipose expansion in the form of adipocyte hyperplasia, subcutaneous fat distribution, and high adinopectin secretion is regulated by normal ERα expression. Meanwhile, pathological adipose expansion is in the form of adipocyte hypertrophy and fat infiltration to the visceral organs. Furthermore, there is a decrease in insulin sensitivity, a tendency to develop metabolic syndrome, and inflammation in pancreatic beta cells which increase the risk of *pancreatic malignancy*.[8-11]

The nature of soy milk as an EDC raises a debate about the benefits and ill effects of soy and its derivatives, especially in the long term.[12, 13] To date, no studies have specifically examined the soy milk and genistein supplementation effect on pancreatic fat infiltration in males. This study aims to determine the effect of supplementation of soy milk and sub chronic genistein on pancreatic fat infiltration of Sprague Dawley male mice.

METHODS

Study Design

This study used a true experimental in vivo test design. Post-test only done to control group.

Experiment and Treated Animals

The study was conducted on *Sprague Dawley* male mice those were 6-8 weeks old and 160-250 grams body weight. The experimental protocol has been approved by the Research Ethics Commission of Faculty of Medicine, Universitas Brawijaya by number 96/EC/KEPK/03/2017 and conducted at the Biomedical Laboratory of the Faculty of Medicine, Universitas Brawijaya. A total of 35 Sprague Dawley male mice, divided into 7 treatment groups. The negative control group was only given standard rationed food. The treatment group was supplemented with soy milk 100 mg, 200 mg, and 400 mg and pure genistein supplementation with 0.4 mg, 0.8 mg, and 1.6 mg. This dosage is a conversion of genistein consumption in humans, which is 20 mg, 40 mg, and 80 mg/day.[1] Duration of treatment for 60 days. Subjects who died were excluded from the study. Body weight, height, and *body mass index* (BMI) were measured from the beginning and the end of the study.

Ingredients and Dosage Calculations

The standard rationed mice's food consists of a mixture of chicken feed (PAR-S produced by JAPFA COMFEED) 66.6% and 33.4% wheat flour which has a calorie content of 2700-2800 kcal /kg given as much as 70-80 grams/day.[14] Soy milk is made with a ratio of 20 grams of soybean powder and 160 ml of distilled water. The concentration of the solution is 0.125g/ml. Soybean powder uses Fressoya with license number Food-Home Industry (P-IRT) 815350701862 which is produced by CV. Fresco Food Industry. Genistein content in soybean powder is 4.4 mg / g. Genistein is a solution with a concentration of 0.5mg / ml. Pure genistein is produced by the Wuhan Economic and Technological Development Zone, Wuhan, Hubei with number CFN98681.

The treatment dosage is based on human genistein consumption in Asian race (standard weight 60 kg) is 20-80 mg/day, with low dose (20 mg/day), medium dose (40 mg/day) and high dose (80 mg/day).[1] The dose is converted into a dose in mice with the formula: mice dose (mg/kg)= human dose /day (mg/kg) x 6.2 (constant).[15] Then, the conversion for low, medium and high doses was obtained of 0.4 mg, 0.8 mg, and 1.6 mg/200 g mice weight /day.

Soy milk supplementation that was given was 0.8ml, 1.6ml, and 2x1.6ml for each treatment group with low (K2), medium (K3) and high (K4) doses. While the genistein supplementation given was 0.8 ml, 1.6 ml and 2x1.6 ml for low (K5), medium (K6) and high (K7) doses.

Pancreatic Histopathology Examination

Mice were sacrificed on the 61st day. The pancreas was fixed in 10% formalin buffer and made paraffin blocks. Histopathological preparations were done by staining with hematoxylin and eosin. Observation of the degree of pancreatic fat infiltration using the modified Papacio and Dembinsky scoring in **Table 1**.[16, 17]

Table 1. Degree of fat infiltration

Score	Fat Infiltration
0	No vacuolization of acinar cells
1	<25% vacuolization of acinar cells
2	25% -50% vacuolization of acinar cells
3	> 50% vacuolization of acinar cells

Statistical Analysis

Box plots of the subject's baseline and final body weight were used to determine the existence of extreme values. Subjects with extreme body weight values were not used for further statistical analysis. Weight gain, body length and BMI in each group were analysed using a paired T-test. The effect of treatment on body weight and BMI of mice was tested using One Way ANOVA. Analysis of the degree of pancreatic fat infiltration, Kruskal-Wallis test, and if a significant effect was found, it was continued with the Mann-Whitney test for each treatment on the control. The software used for the analysis was IBM © SPSS © version 26.

RESULTS

In this study, there are four subjects were excluded due to death, which is one in group 2, two in group 5, and one in group 6. Subjects who had extreme body weight values at the beginning and end of the study were not used in the statistical analysis. The characteristics of the research subjects are described in **Table 2**. In this study, the total number of samples used for statistical analysis was 25 samples. Sample characteristics in **Table 2**. One-Way Annova test with Tukey HSD post-hoc test showed no significant difference in final body weight, body length and BMI between control and treatment groups.

Histopathological observations are presented in **Figure 1** and followed by a frequency graph to see the trend of changes that occur in each treatment. Statistical tests using the Kruskall Wallis method showed no significant difference in the infiltration of pancreatic fat cells in the supplementation of soy milk or sub chronic genistein ($p > 0.05$). However, there was a trend of decreasing fat infiltration in the pancreas in the treatment of subchronic soy milk and genistein compared to controls. One-way ANOVA test results showed the effect of giving soy milk and genistein on body weight ($p = 0.022$). However, the results of the post hoc test with Tukey HSD showed that there was no effect of giving soy milk on body weight and BMI between the control and treatment groups at all dose.

Table 2. Baseline Characteristics of Subjects

Karakteristik	K1	K2	K3	K4	K5	K6	K7
Total (n)	3	4	4	3	3	4	4
Initial Weight	166.9±1.4	201.3±10.9	219.0± 5.3	202.5±10	186.7±7.3	189.8±7.1	206.4±5.4
Final Weight	325.4±8.7	334.2±9.4	344.1±15.9	333.8±1.4	263.6±18.7	329.3±15.4	344.6±13.4
Initial Length	16±0.2	17.9±0.2	17.7±0.13	17.9±0.2	17.8±0.2	17.42±0.5	18.07±0.21
Final length	20.4±0.08	20.3±0.2	20.6±0.2	20.03±0.1	19.7±0.5	20.2±0.1	20.5±0.1
Initial BMI	0.65±0.02	0.62±0.02	0.69±0.02	0.63±0.03	0.59±0.01	0.62±0.02	0.63±0.01
Final BMI	0.78±0.05	0.8±0.02	0.81±0.04	0.83±0.01	0.67±0.02	0.81±0.03	0.81±0.02

**Description: Data is presented in the form of Mean + SEM (Standard Error of Mean); BMI, body mass index.*

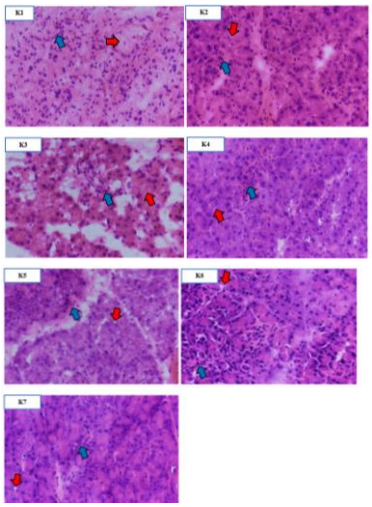

Figure 1. Comparison of the histopathological features of the pancreas in various treatment groups. The red arrow indicates the presence of fat micro-droplets (fat deposits) in the pancreatic acini. The blue arrow indicates the presence of inflammatory cell infiltration (fibrosis cells). H.E. 400x

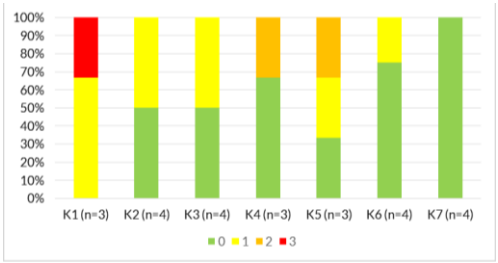

Figure 2. Degree of pancreatic fat infiltration in each treatment group (p = 0.204)

Each group showed significant weight gain in the K2 (p=0.025), K4 (p=0.029), K6 (p=0.017), and K7 (p=0.014) groups, this is consistent with the increase in BMI in the K2 group (p=0.025), K4 (p=0.029), K6 (p=0.017) and K7 (p=0.014). Groups K2 (0.8 mL of soy milk and K4 (3.2 mL), which are groups with small doses of soy milk and large doses equivalent to 2.1 and 8.3 mg/kg/day for mice or equivalent 20 and 80 mg/day for human doses (bb = 60kg). In the K6 and K7 groups the dose was equivalent to the daily consumption of genistein 4.1 and 8.3 mg / kg / day which is equivalent to the dose of 40 and 80 mg / day for the human dose (body weight = 60kg).

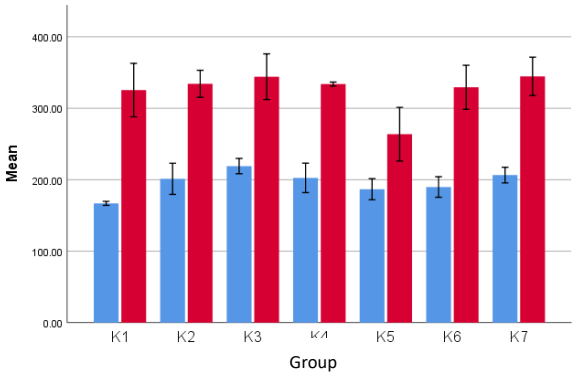

Figure 3. Figure of body weight of each treatment group. Blue: initial weight, Red: final weight.

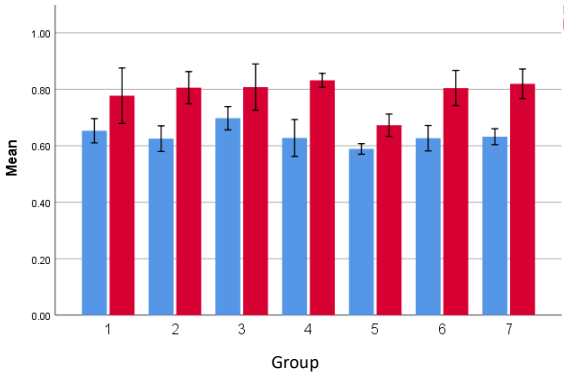

Figure 4. Figure of BMI in each treatment group. Blue: initial BMI, Red: final BMI. BMI, body mass index

DISCUSSION

In this study, 4 subjects were excluded due to death. Each of them is 1 rat in the soy milk group and 3 in the genistein group. The proportion of sample deaths reached 11.3%, exceeding the initial estimate of 10%. In group 5, there were 2 mice that died so that the number of samples was only 3, less than the minimum sample requirement in the calculation of the federer's formula.

Giving soy milk and genistein did not significantly affect differences in body weight and BMI between control and treatment groups at all doses. However, weight gain in the soy milk and genistein groups was not consistent with previous studies. Research by Wang, *et al*. shows that giving large doses of soy milk and genistein supplementation in male mice tends to lose weight. Whereas at low doses it tends to increase body weight. However, there is a gap between invitro and in vivo studies due to the overlapping of transcription factors, metabolism and the stability of active substances.[18] The use of soy milk and genistein also gave different results, because in soy milk there is another isoflavone, daidzein, which affects lipogenesis via the PPAR-α independent pathway in hepatocytes rather than via estrogen receptors in adipocyte tissue. Microarray gene analysis, showed adipogenesis in low-dose genistein was influenced by the phospholipase A2 group 7 gene and phospholipid transfer protein. Meanwhile, the anti-adipogenic activity of genistein and down-regulation of the adipogenic gene require expression of ERβ.[19]

From **Table 2** it can be seen that all groups of mice are obese. Normal BMI in adult male mice ranges from 0.45 to 0.68 g / cm2.[20] This result is not in accordance with the initial study design, because the control used is a negative control, so that obesity is not expected in the control group. This study did not calculate the amount of leftover feed, and the physical activity of the mice. Obesity that occurred in all groups in this study could occur due to excess calorie intake compared to daily calorie requirements compared to physical activity of mice.[21] Daily calorie requirements of Sprague Dawley male mice are 110 kcal ME/$BW_{0.75\ kg}$/day.[22] While the food intake given in this study in all groups was 70-80 g/cage/day which was equivalent to 120-137 kcal/rat/day.

In this study, the control group contained mild to severe pancreatic fat infiltration. This indicates that the control group occurred an ectopic fat infiltration process. Ectopic fat infiltration is preceded by inflammation of the acinar cells, followed by acinar cell death and replaced by adipocyte droplets.[11] This figure is not in accordance with the initial hypothesis of the study, namely that the negative control is expected to have no picture of pancreatic fat infiltration. The presence of pancreatic fat infiltration in the control group, seems to be related to the incidence of obesity in the control group. BMI at the time of the surgery will be performed the control group was 0.78±0:05 g/cm^2. Normal BMI in mice ranged from 0:45 to 0.68 g/cm2.[20] In male sex, decreased expression of ERα due to the absence of estrogen stimulation causes the distribution of adipocyte tissue to go to the visceral organs. [8, 23]

Research by Ahmad (2014), concluded that feeding the standard chicken feed to mice significantly increased body mass and liver compared to the group with standard laboratory feed. In mice that were given chicken feed also found obesity. An increase in liver weight indicates the production of excess protein and fat so that the liver cells hypertrophy. This is due to the high levels of cholesterol, amino acids and fat precursors in the feed. The study also compared feeding with pure soybeans, where pure soybeans significantly reduced rat body weight compared to standard lab feed, as well as chicken feed.[24]

In this study, there was no effect of weight loss from supplementation of soy milk or genistein.

The absence of a trend in weight loss in this study is due to the different gut microbiota of mice from humans. Intestinal absorption of genistein is the main prerequisite for genistein to work. Bacteria contained in the small intestine of mice can change the structure of β-glucosides. However, because genistein is stable in the intestinal lumen, it is difficult to change the structure of β-glucoside from genistein to genistein during hydrolysis.[25] Isoflavones in the form of glycosides cannot be completely absorbed by intestinal cells and their bioavailability requires initial hydrolysis by the β-glucosidase enzyme to then be carried to the peripheral circulation.[26] In rat intestinal tissue, genistein (isoflavone in the form of glycosides) and / or its metabolites are not absorbed.[27] This study did not assess these differences in microbiota.

The results of this study showed that there was no significant difference in the effect of giving soy milk and genistein compared to control on pancreatic fat infiltration (p=0.204). However, the trend showed a decrease in fat infiltration in the soy milk and genistein groups and was directly proportional to the dose of soy milk and genistein. This may be influenced by the function of soy milk isoflavones and genistein which function as selective ligands for ERβ. This is because the affinity of these phytoestrogens to ERβ is 20-30x higher than ERα.[28]

Expression of adipocyte tissue ERβ functions to reprogram preadipocytes and mesenchymal stem cells to turn into *brown fat* (BAT) and increase mitochondrial respiration. This expression also increases energy biogenesis and oxygen consumption from the pathway's *tricarboxylic acid-dependent* and *independent*. The cumulative effect that can occur is a reduction in adipocytes and body weight.[29] However, this pathway was not seen in this study. Because there are no significant differences and trends in weight loss and BMI.

Factors that can influence the trend of decreasing pancreatic fat infiltration are the anti-inflammatory effects of isoflavones in soy milk and genistein. Visceral fat, tends to release adipokines and pro-inflammatory mediators in large quantities, which will trigger insulin resistance, increase triglyceride lipolysis and release free fatty acids into the circulation.[11] Genistein increases the potency of AMPK (AMP-activated protein kinase) / mitofucine 2 activation in preadipocytes and *white / brown adipocytes* thereby protecting them from the effects of hydrogen peroxidase by inhibiting ROS and maintaining mitochondrial function.[30]

Estrogen receptor modulation is only one pathway in the cascade of adipocyte tissue function and metabolism. The phenotype of adipocyte tissue is the resultant of a variety of other metabolic pathways, and is influenced by gender and dose, as well as length and time of exposure, of isoflavones and genistein.

In vitro studies, concluded that genistein can cause adipogenesis through disruption of estrogen receptors (ERs). Exposure to genistein given inhibits differentiation of human adipocytes cells with the *down* regulation of ERα (doses of 25 and 50 m) and ERβ (6:25 and 25 m). The biggest decrease that occurred was in Erβ. The dosage in this study was very high when compared to 6.6 µM for infant soy formula and 2.4 µM for soybean powder in adults.[31] However, there is also a biphasic effect, that is, at low doses it tends to produce antiadipogenic effects. This effect occurs when genistein is given in the early phase of adipocyte differentiation, and has a long-term effect on the reduction of tissue volume of adipocytes.[18]

In animal studies, the effects arising from the administration of soy milk isoflavones (geneistein, daidzein, glycetein), consistently reduce obesity and improve lipid metabolism

profiles in male and female animals. Fat deposits in the visceral organs were also decreased in the high-fat and normal model diets. But the biphasic effect still appears in male experimental animals. Low doses of genistein appear to increase visceral fat, but at high doses (200 mg/kg /day) reduce visceral fat. The difference between in vitro and in vivo studies is due to the *overlapping* roles of transcription factors and metabolic factors, as well as the stability of the active substances used.[18]

Human studies show changes in fat distribution in menopausal women. This shows that the action of soy milk isoflavones is closely related to ERs. Administration of soy milk isoflavones and their derivatives also upregulated anti-inflammatory genes at all doses. However, the biphasic effect is still visible, namely at low doses there is a downregulation of genes that express fat energy consumption. Further studies have shown the role of the gut microbiota in converting isophavones into active metabolites. In patients with an obesity profile, 2.8x were less likely to produce active metabolites of isoflavones in the intestine.[18]

Another effect seen in human studies is a change in fat distribution from visceral to subcutaneous. The pathway of change is through increased lipoprotein lipase activity. Another important pathway is the reduction of inflammation through *downregulation of* IL-6 through its transcription factor, NF-kB, (this substance plays a major role in *downregulation* of estrogen receptors).[18]

In this study, we suspect the trend of decreasing degree of pancreatic fat infiltration is due to the anti-inflammatory effects of soy milk and genistein. The effect will prevent the *down* regulation of the estrogen receptor, so that isoflavones and genistein soy milk can induces estrogen receptors on adipocytes network, presence of hormone stimulation modulation of estrogen receptors on adipocytes male network will improve the function and tissue distribution of adipocytes.

Limitations of the Study

These are limitations of the study1) The number of samples is too small because the number of deaths exceeds the estimated number and the existence of data with extreme values that are not included in the statistical analysis. 2) The content of other isoflavones in soy milk was not checked. The examination is needed because the isoflavone compound other than genistein in soy milk is a phytoestrogen which can become EDC on the function and distribution of adipocytes. 3) The negative control in this study is obesity and there is an infiltration of fat in the pancreas due to factors beyond the knowledge of researchers that were not considered at the time of the initial study design. 4) This study did not use estrogen treatment as a positive control as a comparison of the endocrine disruption effect of the studied phytoestrogens. 5) Confounding factors such as gut microbiota were not assessed.

CONCLUSION

The supplementation of subchronic soy milk and subchronic genistein did not significantly reduce the pancreatic fat infiltration of Sprague Dawley male mice. There was no difference in the degree of pancreatic fat infiltration of male Sprague Dawley mice in the supplementation group with soy milk and subchronic pure genistein.

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Original Article

The Effect of Vitamin E on Oral Mucositis Induced by Chemotherapy in Non-Hodgkin Lymphoma Patients Receiving Chemotherapy

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ABSTRACT

Chemotherapy-induced mucositis is a side effect of chemotherapy that often occurs in patients with solid tumors and lymphoma. Oral mucositis can affect nutritional status and the risk of infection, both local and systemic. Antioxidant Vitamin E is beneficial for the prevention and therapy of both oral and gastrointestinal mucositis. **Aim:** To determine the effect of vitamin E therapy on the incidence of oral mucositis in non-Hodgkin lymphoma (NHL) patients receiving chemotherapy. **Methods:** This is a single blind experimental study in 62 NHL patients undergoing chemotherapy who meet the inclusion criteria. Patients who met the inclusion criteria were randomly divided into 2 groups, namely 31 patients (treatment group) received vitamin E 400 mg / IU per day for 7 days and 31 patients in the placebo group. The incidence and grade of oral mucositis were observed on day 7. Statistical analysis used Chi Square and Mann Whitney test according to the data type. **Results:** a total of 67% NHL patients were male, most of whom were over 46 years of age and as many as 50% of patients used chemotherapy regimens RCHOP and CHOP. There was an incidence of oral mucositis in 35% in the placebo group and 12.9% in the treated group (p = 0.038). In the placebo group there were 4.8% of patients with grade 2 and 3 oral mucositis, which were not found in the therapy group. **Conclusion:** treatment with vitamin E in NHL patients undergoing chemotherapy can prevent chemotherapy-induced oral mucositis and prevent its severity.

Keywords: oral mucositis, chemotherapy, vitamin E, non-Hodgkin's lymphoma

INTRODUCTION

Chemotherapy-induced mucositis is an erythematous and ulcerative lesion of the oral mucosa observed in patients with cancer treated with chemotherapy, and / or with radiation therapy. Oral mucositis lesions are often very painful and can affect nutrition and oral hygiene and can increase the risk of developing both local and systemic infections. Mucositis can also affect other areas of the digestive tract; for example, gastrointestinal (GI) mucositis which can manifest as diarrhea. Thus, it is clear that the effect of mucositis is very significant on the quality of life of patients. Likewise, the presence of mucositis often requires limiting the dose of cancer therapy. [1,2]

One study reported the incidence of oral mucositis in 303 of 599 patients (51%) with solid tumors or lymphoma who received

chemotherapy.[25] Oral mucositis developed in 22% of 1236 chemotherapy cycles, whereas GI mucositis occurred in 7% of chemotherapy cycles.[25] The incidence of mucositis is also influenced by the dose of chemotherapy. In hematopoietic cell transplant patients who receive high doses of chemotherapy, the incidence of mucositis is around 75-80%. [3,4]

Chemotherapy-induced mucositis causes significant pain which can affect nutritional intake, oral hygiene and quality of life. Infections associated with oral mucositis lesions can lead to life-threatening systemic infections. Moderate to severe chemotherapy-induced mucositis correlated with infection-related mortality. In patients with solid tumors or lymphoma who received chemotherapy and developed mucositis, the degree of infection during the cycle was two times higher and was directly proportional to its severity compared with the group without mucositis. Infection-related deaths during chemotherapy cycles are more common in patients with oral or GI mucositis. Likewise, the mean duration of stay during chemotherapy was significantly longer in patients with mucositis. The incidence of chemotherapy dose reduction in subsequent cycles was two times higher in patients with mucositis than without mucositis.[5]

The pain from mucositis causes eating disorders by mouth, so it is often necessary to get nutrition via gastrostomy or intravenous lines. Patients with oral mucositis were significantly more likely to have a more severe condition with a weight loss of more than 5%.[5,8] In terms of medical costs, there is a significant difference between patients with mucositis and without mucositis. Patients without mucositis required an inpatient fee of approximately $3893 per chemotherapy cycle, with oral mucositis $6277 per cycle whereas those with oral and GI mucositis were $9132 per cycle. [3,4]

So based on the above conditions, it seems that prevention of mucositis is important. Various attempts have been made, but until now they have not yielded satisfactory results. Based on the role of oxidants in the mucositis process, Vitamin E which is known to have antioxidant properties in the inflammatory [7,8] process is expected to prevent mucositis in solid tumor patients undergoing chemotherapy.

METHODS

Study Design

This study is a single blind clinical trial with random sampling which aims to determine the incidence of oral mucositis in NHL patients receiving chemotherapy. The research subjects were 62 NHL patients who met the inclusion criteria. Subjects were divided randomly into 2 groups, namely the treatment group and the control group (placebo) each of 31 patients. The inclusion criteria were NHL patients who were undergoing chemotherapy, were over 16 years of age, were not experiencing mucositis, and were willing to sign an informed consent to participate in the study.

Subsequently, subjects who met the inclusion criteria were subjected to an anamnesis regarding their main complaints, medical history and previous therapy as well as a general physical examination, including awareness, vital signs, examination of the head, neck, chest, lungs, heart, stomach, and extremities. Then the patients in the treatment group were given vitamin E and the placebo group was given placebo therapy for 7 days which was physically the same as vitamin E. On the 7th day of chemotherapy, the patients were examined to assess the incidence of oral mucositis and the degree of mucositis. This study has received approval from the Health Research Ethics Commission dr. Saiful Anwar by number 400/282/K.3/302/2019.

Statistical analysis

Statistical analysis using statistical tools, IBM *Statistical Products and Service Solutions Statistics* (SPSS) *version 25.0 for*

Windows. Data were analyzed using Chi Square test and Mann Whitney test according to the type of data.

RESULTS

Characteristics of subjects

The proportion of the incidence of oral mucositis between men and women was not significantly different, namely 26.2% in men and 25.0% in women. Based on age, it was found that the highest incidence of oral mucositis was in the age group over 46 years (61.3%), whereas proportionally the incidence of oral mucositis was higher in the 26-45 years age group than in the 45-45 years age group (46.7% vs 26.7%; p <0.05) **(Table 1)**.

Table 1. Characteristics of Subjects

Variables		Oral mucositis (n)		Amount (n, %)
		(+)	(-)	
Sex	Male	31	11	42 (67.7)
	Female	16	4	20 (32.3)
Age (range)	16-25	2	0	2 (3.2)
	26-45	15	7	22 (35.5)
	>45	30	8	38 961.3)
chemotherapy regimen	CEOP	0	1	1 (1.6)
	CHOP	14	6	7 (35.5)
	COP	1	0	1 (1.6)
	RCEOP	7	0	7 (11.3)
	RCHOP	23	7	30 (48.4)
	RCOP	2	1	3 (4.8)

** R, rituximab; C, cyclophosphamide; H, hydroxydaunorubicin (doxorubicin) hydrochloride; O, oncovin (vincristine); P, prednisone*

Table 2. incidence of Oral Mucositis between Placebo and Therapy Groups

	Placebo group (n=31) n (%)	Vitamin E group (n=31) n (%)	P value
(+)	11 (35.0)	4 (12.9)	0.038*
(-)	20 (65.0)	27 (87.2)	

**Chi-Square test*

Table 3. The severity of oral mucositis between the placebo and therapy groups

Severity of oral mucositis	Placebo group (n=31) n (%)	Vitamin E group (n=31) n (%)	Total (n=62)
0	20 (32.2)	27 (43,5)*	47 (75,8)
1	4 (6,5)	4 (6,5)	8 (12.9)
2	4 (6,5)	0 (0)	4 (6.5)
3	3 (4,8)	0 (0)	3 (4.8)

**Chi-Square test*

Based on **Table 3**, it shows the incidence of oral mucositis was found in 15 (24%) patients of the total patients who underwent chemotherapy. The highest degree of mucositis was grade 0 as much as 47 (75.8%) and the lowest degree of mucositis was grade 3 as many as 8 patients (4.8%). In the vitamin E therapy group, the proportion of grade 0 oral mucositis was significantly different compared to the placebo group (43.5% vs 32.2%; p <0.05) and there was no grade 2 and 3 oral mucositis (**Table 3**).

Table 4. Correlation between Vitamin E T herapy and degree of oral mucositis.

	Mean Ranks		**p value**
	Placebo group (n=31)	Vitamin E group (n=31)	
Severity of oral mucositis	35,45	27,55	0,021*

**Mann-Whitney test*

Based on **Table 4**, there is a significant difference in the degree of mucositis in the placebo group compared to the treatment group (p=0.021).

DISCUSSION

The age range of the subjects in this study was 18-79 years with the largest age being over 46 years. This is consistent with a study that

reported the largest incidence of NHL was at the age of 35-65 years. [11,12] (**Table 1**).

Based on data in the UK in 2012 the incidence of NHL (Non-Hodgkin Lymphoma) increases with older age. The American Cancer Society in 2019 also stated that the risk of developing NHL increases at older people over 65 years compared to younger ages.[13]

Based on available evidence, it has been shown that an increased incidence of chemotherapy-induced mucositis in elderly patients is associated with changes that accumulate with time, affecting both genetic and environmental causes. The presence of prolonged exposure to carcinogens in the elderly and increased risk of epigenetic gene mutations, telomere dysfunction, limited replication potential, altered environment, apoptosis, all contribute to the changing environment leading to chemotherapy-induced mucositis.[14]

Other studies have also shown that the incidence of chemotherapy-induced oral mucositis is higher at older ages (over 45 years) compared to younger ages.[15] In accordance with the results of this study, the highest incidence of chemotherapy-induced oral mucositis was found at the age above 45 years or about 50% of the total sample.

In this study, there were more male than female patients (61.7% vs 32.3%) (**Table 1**). Sex differences in the incidence of NHL were related to differences in epidemiology, clinical features and response to therapy. The incidence of NHL in the last 20 years shows that the incidence rate for the male sex is significantly higher than that for the female gender. Clinical characteristics are also different, in women the predominant location of NHL in extra nodals such as mother, thyroid and respiratory system, while related to therapy response, in women the response to therapy with anti-CD 20 or rituximab was better than men.[18]

Chemotherapy-induced mucositis in this study was found to be more in male patients than female patients. Previous studies have shown that gender is considered to be a factor influencing the incidence of mucositis although some studies have shown conflicting results.

The effect of sex hormones has been investigated in vitro and resulted in a theory about the direct effect of estrogen on the cell proliferation process or the effect on the anti-tumor response on the female immune system is thought to be responsible for explaining the decrease in the incidence of chemotherapy-induced mucositis in women.[20] Another mechanism thought to be related to the effects of estrogen is the immune response. The study found that 17β-estradiol spontaneously decreased the production of IL6 by MN cells resulting in lower levels of IL6. Meanwhile, high IL6 levels are associated with the incidence of chemotherapy-induced mucositis.[20] Estrogen exerts a protective effect by lowering IL6 (Interleukin-6) levels. Immunological effects that are not directly related to sex hormones were also explored. Helper T cells are essential for the body's immune response. T Helper 1 (TH1) cells secrete cytokines that promote cellular immunity to fight intracellular pathogens while T helper 2 cells (TH2) control humoral immunity by regulating antibody production. Unbalanced regulation and expression of cytokines TH1 and TH2 play an important role in the development of chemotherapy-induced mucositis.[23] Another proposed mechanism is through the direct effect of estrogen on all types of lymphocytes that express estrogen receptors. The mechanism of estrogen inhibition on cell proliferation is unclear, but it is thought to play a role in chemotherapy-induced mucositis.[24] Based on the information above, it can be concluded that the gender factor in the incidence of mucositis still causes controversy, so in this study gender cannot be ruled out as a confounding factor.

Doxorubicin-based chemotherapy remains the gold standard of first-line NHL

therapy. In this study the CHOP regimen was administered to 14 (22.5%) patients, whereas the COP regimen was administered to only 1 (1.2%) patient. The LNH management guidelines recommend CHOP as the regimen of first choice in LNH patients. [35]

Doxorubicin is a drug belonging to the anthracycline class that has the potential to cause chemotherapy-induced mucositis. There are two mechanisms of doxorubicin activity against cancer cells, the first is through the intercalation bond into DNA which interferes with the work of 2-topoisomerase which is useful in the DNA replication process and the second is through the formation of free radicals to damage cell membranes, DNA, and proteins.[26] Doxorubicin is oxidized to semiquinone, an unstable metabolite which is converted back to doxorubicin. This conversion process releases ROS (Reactive Oxygen Species) which can result in fat peroxidation and damage to membranes, DNA, oxidative stress, and triggers apoptosis from cells. Gene candidates that regulate this conversion process involve enzymes that can carry out oxidation reactions (NADH dehydrogenase, nitric oxide synthase, xanthine oxidase) and deactivate glutathione peroxidase, catalase, superoxide dismutase.[6] There is another possibility that doxorubicin can enter the nucleus and interfere with 2-topoisomerase which results in DNA damage and cell death.[26]

Doxorubicin is a cytostatic drug which also often causes serious side effects in the form of mucositis. Doxorubicin acts primarily at the DNA level by forming covalent bonds to DNA and is associated with increased apoptosis of actively multiplying cells which ultimately leads to inhibited proliferation of new mucosal cells. This condition supports the occurrence of mucositis due to chemotherapy in patients.[26]

Another agent is Cyclophosphamide (CYC). Cyclophosphamide is a chemotherapy agent with alkylating activity related to nitrogen that binds to DNA and interferes with mitosis and cell replication. CYC targets rapidly dividing cells and is often used in antineoplastic management in the context of solid tumor and hematological malignancies. CYC has been shown to be effective in the treatment of lymphoma, leukemia, multiple myeloma, breast cancer, ovarian adenocarcinoma, retino-blastoma, neuroblastoma, nephritic syndrome in children, and others. Cyclophosphamide also has immunosuppressive effects in addition to anti-mitotic and anti-replication effects. Specifically, CYC induces suppression of cellular and humoral immunity through its action on T and B cells. [32] The activity of cyclophosphamide as an immunosuppressant agent is derived from its ability to kill proliferating lymphocytes, including natural killer cells, T cells, and B cells, all of which are sensitive to cyclophosphamide.[32]

Some other chemotherapy agents that have been shown to have high mucosal toxicity are daunorubicin, ara-C, etoposide, cyclophosphamide, doxorubicin, idarubicin and busulfan / melphalan.[32]

Based on the results of this study, it was found that the regimen using doxorubicin caused the most chemotherapy-induced mucositis (CHOP and RCHOP) so it was suspected that doxorubicin might play an important role in the incidence of oral mucositis.

Effect of Vitamin E on Chemotherapy-Induced Mucositis

Chemotherapy-induced oral mucositis is defined as the appearance of ulcerated lesions in the mouth area after chemotherapy. Oral mucositis appears on day 1-14, influenced by many factors including nutritional status, comorbidities such as metabolic diseases (DM, CKD), viral, bacterial and fungal infections. In this study patients with previous mucositis were excluded from the study. The examination of the incidence of mucositis which was checked on the 7th day of chemotherapy showed that the incidence of chemotherapy-induced mucositis

occurred on the 7th day of chemotherapy drug administration. This condition is in accordance with the theory of the nadir point of chemotherapy agents, namely the 7th day of chemotherapy agents at the lowest point of the immune system. usually characterized by the onset of neutropenia in the patient.[29]

This study showed that the administration of vitamin E significantly reduced the incidence of chemotherapy-induced oral mucositis in NHL patients undergoing chemotherapy **(Table 2).** The ability of vitamin E to protect epithelial cells thereby reducing the degree of mucositis is due to its ability to increase the order of the lipid structure of the cell membrane to become tighter. Free radicals make the membrane phospholipid its main target and in this case vitamin E efficiently prevents the peroxidation of fat in the cell membrane. Therefore, vitamin E improves the quality of cell membrane recovery by preventing the formation of oxidized phospholipids that can interfere with the fusion of the cell membrane.[28]

The effect of vitamin E on the degree of chemotherapy-induced mucositis

The degree of chemotherapy-induced oral mucositis was higher in the placebo group than in the vitamin E group **(Tables 3 and 4).** This is similar to the study of alpha tocopherol administration in the incidence of chemotherapy-induced mucositis in patients where the results of the treatment group were lighter in degree than the placebo group. The study stated that patients in the placebo group had grade 1, 2, 3 and 4 mucositis, while the treatment group only experienced grade 1, 2 and 3 mucositis without anyone getting grade 4 mucositis.[26]

Factors affecting the degree of chemotherapy-induced oral mucositis include complaints of pain in the oral cavity before chemotherapy and lack of attention to oral hygiene before, during and after chemotherapy. Additional risk factors are the type of cancer, the location of the cancer, the antineoplastic substance used, the dose, the administration schedule, the radiation area, and the patient's age.[30]

As previously explained, vitamin E can act as an anti-oxidant or anti-inflammatory in chemotherapy-induced mucositis. One way to further explain whether the antioxidant mechanism underlying the decrease in the incidence of mucositis due to chemotherapy requires examination of the antioxidant marker, namely Malondialdehyde (MDA). MDA is the end product of lipid oxidation. High levels of MDA are influenced by levels of lipid peroxidation, which indirectly also indicates a high number of free radicals. Free radicals are highly reactive, can cause biochemical changes and damage various components of living cells such as proteins, lipids, carbohydrates, and the nucleus of the cell membrane which consists of lipid components.[35]

Limitations of the study

Some of the limitations of this study were the uneven proportion of patients based on age, type of chemotherapy and duration of chemotherapy for each subject so that they could not control for this as a confounding factor. Besides, adherence to taking vitamin E in the study subjects was only assessed based on anamnesis.

CONCLUSION

Patients with NHL who are receiving chemotherapy, giving Vitamin E can prevent oral mucositis. Giving vitamin E can also reduce the degree of chemotherapy-induced oral mucositis.

More specific research is needed regarding chemotherapy regimens and duration of chemotherapy on the incidence of chemotherapy-induced mucositis and further research related to the factors that influence the occurrence of chemotherapy-induced mucositis.

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Review Article

Renal Artery Stenosis: Diagnostic and Management Problems

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ABSTRACT

Renal artery stenosis is one common clinical problem. It has wide spectrum of pathophysiology with 3 most common clinical syndromes, ischemic nephropathy, hypertension, and cardiac destabilization syndrome. **Aim:** To date there was not any specific diagnostic criteria for renal artery stenosis. **Method:** Clinicians only used some clinical syndromes to guide the diagnostic possibility of renal artery stenosis. RADUS as one sensitive and specific diagnostic method, still had some disadvantages. **Results:** it gives false negative results in 10-20% of patients due to confounding factors such as operator capability, obesity, or abdominal gas distribution. CTA and MRA was excellent, but possessed some risks for the patient. Therefore, CTA was mostly recommended in patient with the planning of revascularization. Management of renal artery stenosis was still debated between optimal medical management and revascularization because the complexities of mechanisms underlying the renal artery stenosis. Because of the complicated pathophysiology of renal artery stenosis, revascularization could not entirely improve renovascular hypertension and nephropathy. Revascularization offered best results in fibromuscular dysplasia, although procedure related complication was still high. Revascularization, even though it might have high success rate in atherosclerotic renal artery stenosis, but the incidence of re-stenosis was also fairly high. **Conclusion:** Overall, revascularization was recommended in FMD, but should only be preserved for atherosclerotic renal artery stenosis after the failure of optimal medical management.

Keywords: renal artery stenosis, diagnosis, medical management, revascularization

INTRODUCTION

Renal artery stenosis becomes a substantial clinical problem especially in countries with poor resources. Generally, renal artery stenosis was categorized into atherosclerotic and non-atherosclerotic renal artery stenosis. 90% of renal artery stenosis was caused by atherosclerosis. Non-atherosclerotic renal artery stenosis was mostly caused by fibromuscular dysplasia which composed 10% of all renal artery stenosis.[1,2] Diagnosis of renal artery stenosis is important because it could decrease renal blood flow and renal perfusion. Severe renal artery stenosis could activate the renin angiotensin aldosterone system. It would induce vasoconstriction and increase of peripheral artery resistance.[1,3,4] Literatures

suggest the relationship between renal artery stenosis and reno-vascular hypertension.

Renal artery stenosis includes a wide spectrum of pathophysiology with 3 most common clinical syndromes, ischemic nephropathy, hypertension, and cardiac destabilization syndrome.[2] To date, there are not any diagnostic criteria for renal artery stenosis. Some authors suggested clinical suspicion as a guide for diagnosing renal artery stenosis.[1,5] Renal artery duplex ultrasonography is recognized as a sensitive diagnostic method for renal artery stenosis, although there are still many confounding factors. It gave negative results in 10-20% of cases. Another imaging examination such as CTA (computerized tomography angiography) and MRA (magnetic resonance angiography), although highly sensitive and specific, both are expensive and inflict contrast and radiation exposure.[5,6]

Recently, studies indicated renal artery revascularization did not improve patients' outcomes in the aspects of renal function and cardiovascular outcomes.[7] Angioplasty and stenting procedures offered excellent results for fibromuscular dysplasia, but evidence was not enough to prove benefit for blood pressure control and renal function improvement. Both procedures also possessed high prevalent risks and did not provide any prevention from cardiovascular risk.[8,9] This review article explained about problems in the diagnosis and management of renal artery stenosis, especially in the setting of poor resources.

Renal Vascularization and Glomerular Filtration Regulation

Kidney worked as a unit of excretion. Beside excreting residual substances such as ammonia, urea, creatinine, and uric acid, it also excretes drugs and toxin. Kidney has another function as regulator of water and electrolyte equilibrium. It regulates the balance of acid base, calcium, phosphate, vitamin D metabolism, and red blood cell production. Every nephron in kidney contains glomerulus, which function as ultrafiltration membrane, loop of Henle, distal renal tubules, and collective tubules.[4,8]

Blood flows through afferent arterioles to glomerular capillaries. In the glomerulus, capillaries rolled up densely, surrounded by Bowman capsules. Blood in glomerulus undergo ultrafiltration in glomerular basement membrane (GBM), which consisted of basal membrane of tubular epithelia and vascular endothelial cells. Majority of water-soluble substances will be filtrated through membrane. Filtration of glomerulus forms relatively large volume of plasma ultra-filtrate (about 120 ml/minutes or 170 L/day). The ultra-filtrate will pass through tubules and undergo selective reabsorption. Distal end of glomerular capillaries merge and continue as efferent arterioles. Efferent arterioles will continue as second capillary networks (cortical peritubular capillaries or vasa recta medulla) around tubules. Efferent arterioles regulate hydrostatic pressure in both capillary networks.[4,8,9]

Hydrostatic pressure along capillary walls of glomerulus is the main force of glomerular filtration. Oncotic pressure inside capillary lumens plays the role of filtration barrier. Oncotic pressure was influenced by unfiltered plasma protein concentration. During filtration process, oncotic pressure will increase along glomerular capillaries with the decrease of filtration pressure. It reaches 0 mmHg in proximal of efferent arterioles. About 20% of plasma will be filtrated into Bowman capsules. Ratio of eGFR (estimated glomerular filtration rate) to renal blood flow determines filtration fraction.[4,9]

Although glomerular filtration rate was affected by renal arterial pressure, but the relationship was not linier. It was because of

autoregulation of GFR. Glomerular filtration rate is the rate of blood that flows through nephrons after being filtered. GFR did not reflects general renal function and it could be affected by body surface area. GFR was calculated using some substances which were filtered entirely by glomerulus and were not reabsorbed by tubules, such as creatinine. Glomerular filtration pressure was constantly maintained under autoregulation process. It provided relatively constant glomerular filtration pressure under systemic blood pressure and cardiac output variation.[4,8]

Rate of filtration and reabsorption in nephron was controlled by hormonal signals and hemodynamic factors. Those components were also important in blood pressure regulation. Juxtaglomerular part of kidney secreted renin as response to decrease of afferent arteriolar pressure, sympathetic nerve stimulations, and sodium concentration changes in distal contorted tubules at macula densa. Renin production is the first step of angiotensin II production and release of aldosterone which will continue to induce systemic vasoconstriction and regulate extracellular volume. Renin cleaved angiotensinogen into angiotensin I, in which it would be converted by angiotensin converting enzyme into angiotensin II. Angiotensin II will return the pressure of glomerular perfusion rapidly by vasoconstriction of efferent arterioles inside kidney and through systemic vasoconstriction. Both conditions will induce increase of kidney perfusion. In the long term, angiotensin II will increase plasma volume through stimulation of aldosterone release in which it will induce sodium reabsorption through renal tubules.[8,10]

The increase of arteriolar afferent pressure will be accompanied by increase of urine output through the phenomenon of pressure diuresis. This condition will be reflected by renal urinary output curve or renal function curve. In artery pressure of 50 mmHg, almost there is not any urinary output. In artery pressure about 100 mmHg, urinary output will be normal and in artery pressure of 200 mmHg, the urinary output will be 8 times than normal. Increase of arterial pressure also increases sodium output through the process of pressure natriuresis.[10]

Epidemiology and Etiology of Renal Artery Stenosis

Prevalence of renal artery stenosis in general population is relatively small. About 1-6% of patients with hypertension, was suspected to have element of renal artery stenosis. In population of patients that undergone coronary arteriography, the prevalence increased significantly to >20%. In a study of 1302 patients which undergone coronary arteriography, renal artery stenosis occurred in 15% of participants.[2,11]

Renal artery stenosis is a spectrum of conditions with different pathophysiology, so it needs comprehensive approach of diagnosis and management. Generally, it is categorized as atherosclerotic and non-atherosclerotic renal artery stenosis. About 90% of renal artery stenosis was caused by atherosclerosis. Atherosclerosis often affects 1/3 proximal of renal artery stenosis, includes perirenal aorta and ostium. Non-atherosclerotic renal artery stenosis includes a large number of etiology such as fibromuscular dysplasia, aneurysm, arteriovenous fistulas, vasculitis, neurofibromatosis, trauma, emboli, congenital band, radiation therapy, and vascular dissection. Fibromuscular dysplasia is the second most common cause of renal artery stenosis, which about 10% of all renal artery stenosis. Although it was similar to vasculitis, definitive cause of fibromuscular dysplasia is still not known.[1,2] **Table 1** presents some causes of renal artery stenosis.

Table 1. Causes of Renal Artery Stenosis[1]

Classification	Causes
Atherosclerotic	Atherosclerosis
Non-Atherosclerotic	Fibromuscular dysplasia
	Nephroangiosclerosis (Hypertensive injury)
	Diabetic nephropathy (small vessels)
	Renal thromboembolic disease
	Atheroembolic renal disease
	Aortorenal dissection
	Renal artery vasculitis
	Trauma
	Neurofibromatosis
	Thromboangiitis obliterans
	Scleroderma
	Extrinsic compression

Renal artery stenosis seldom generated major hemodynamic effects. Atherosclerotic renal artery stenosis often related to renovascular hypertension and nephropathy. Atherosclerotic renal artery stenosis was relatively common in population (6.8% in population >65 yo) and the prevalence continues to increase with the increase of age. It was also commonly found in patient with other vascular diseases such as coronary artery disease (18-23%) and/or peripheral artery disease and lower extremity artery disease (>30%). More than 50% atherosclerotic renal artery stenosis will worsen in 5 years if it leaved without any treatment.[2,4]

Fibromuscular dysplasia was reported in 3-5% of potential kidney donor candidate without any hypertensions. But this condition was also reported in young patient with hypertension (with age about 15-50 y.o.), especially women. FMD rarely disrupts kidney function but sometimes FMD causes total occlusion and renal artery aneurysm.[4,12]

Pathophysiology of Renal Artery Stenosis and Clinical Manifestation

Classically, renovascular diseases were classified into two broad groups, hypertension and nephropathy, in which both of them are related to renal artery stenosis.[1] Severe unilateral renal artery stenosis would activate renin angiotensin aldosterone system (RAAS). It actually increased vasoconstriction and peripheral arterial resistance. If the contralateral kidney was normal without any stenosis, the effect of volume expansion would still be restricted by sodium and water excretion through contralateral kidney. But in the condition of bilateral renal artery stenosis, peripheral arterial resistance and blood volume continued to increase which lead to high blood pressure and overload condition.[3,4]

Renin angiotensin aldosterone system has role in maintaining vascular tones, water-sodium balance, and cardiac function through the interaction of sympathetic nervous system and some other hormones. The system will be activated in the condition of hypotension, decrease intravascular volume, hyponatremia, hypokalemia, and chloride changes. The system detects changes of intravascular volume through changes in Na-K-Cl co-transporter at macula densa in kidney. Activated RAAS induces release of renin from juxtaglomerular apparatus inside kidney. Renin cleaves angiotensinogen into angiotensin I. Angiotensin I will be cleaved by angiotensin converting enzyme into angiotensin II. Angiotensin II binds to AT1 receptor in the kidney. The binding induces cascades that will eventually causes vasoconstriction of efferent arterioles, aldosterone stimulation, and increase sodium reabsorption.[1,13]

Renal artery stenosis progresses in a long period of time. It allows both kidneys to slowly adapt to decrease of blood flow through autoregulation.[3] Activated renin angiotensin aldosterone system aims to increase renal

perfusion through systemic blood pressure rise. Beside vasoconstriction, water and sodium retention, aldosterone secretion, and sympathetic nerve activation, activated RAAS also induces vascular remodeling and hypertension. Vasoconstriction in efferent arterioles of glomerulus will maintain glomerular filtration function in short time period and prevent kidneys from hypoxia. But in long period of time, RAAS will induce ischemic nephropathy in stenotic kidney, nephro-sclerotic hypertension in non-stenotic contralateral kidney, and glomerulosclerosis and interstitial fibrosis in both kidneys. Glomerulosclerosis and tubule-interstitial fibrosis was presumably due to increase of angiotensin II which related to increase of pro-inflammatory cytokine, which in turn increase inflammatory and pro-fibrogenic pathway. In the condition of severe bilateral renal artery stenosis or stenosis in one functional kidney, kidney will losses its capacity to maintain balance of sodium and fails to induce pressure natriuresis against blood pressure increase. This will lead to inappropriate peripheral vasoconstriction with significant increase of afterload, which can lead to myocardial ischemia and heart failure.[1,5,9]

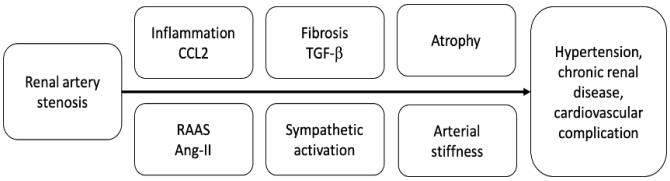

Figure 1. Pathophysiology of renal artery stenosis[7]

In an experimental study, significant hemodynamic changes that causes hypertension occurred in 75-80% of lumen occlusion. But critical point for lumen occlusion in human still could not be determined. Generally, stenosis for >50% or 70% of lumen was defined as significant renal artery stenosis.[3]

Hypertension in renal artery stenosis was also caused by some other mechanisms, such as increase sympathetic tones, endothelial dysfunction, oxidative stress, ischemic nephropathy, and end organ damage.[3] Overall, renal artery stenosis will cause systemic hypertension which indicated by dependencies to angiotensin in initial stage, wide range of blood pressure variation, losses of circadian blood pressure rhythm, and acceleration of end organ target damage, such as left ventricular hypertrophy and renal fibrosis.[4,13]

Some recent studies oppose the idea of RAAS as the main factor played role in renovascular hypertension in renal artery stenosis. Studies using imaging technique showed that kidneys with arterial stenosis were not entirely hypoxic and even the stenotic kidney received higher blood flow than non-stenotic kidney. Studies also observed that kidneys had adaptation capacity to significant decrease of renal artery so they can maintain their oxygen capacity. Systemic RAAS activation tend to be temporarily but progress of kidney disease and cardiovascular dysfunction could happen without any persistent increase of angiotensin II. It indicated that even though systemic RAAS activation only last for a short period of time, but intra-renal RAAS activation was persistent because kidney could produce all elements needed in RAAS activation. Persistent RAAS activation occurred in stenotic kidney, but not in contralateral kidney.[7]

Fibromuscular dysplasia (FMD) is often found in pre-menopausal women with age between 15-50 years old and it closely related to history of hypertension and smoking. FMD patients generally had good clinical condition with low cardiovascular risk. Hypertension in FMD did not related to obesity, oral contraception, and family history.[1] Histologically, FMD includes intimal, medial, and adventitial layer of renal artery, but 90% was found in medial layer. In angiography, FMD was described as beads-on-a-string because

the contrast filled the aneurysm parts alongside renal artery. Most FMD causes stenosis at 2/3 of distal renal artery. But it could also involve carotid and vertebral arteries. FMD had good prognosis and did not progress into total occlusion. But in 2/3 of patient, FMD is bilateral and causes renovascular hypertension, but rarely become nephropathy.[2,9]

Pathologically, FMD had 3 subtypes:[2,4,6]

a. Intimal fibroplasia: It encompasses 5-10% of FMD and is often found in children and young adult. But it is not always found in women. Histologic examination showed collagen fibres deposit circularly at intimal layer which produced smooth tubular stenosis.
b. Medial fibroplasia: It is the most common pathological finding in FMD (75-80%), which commonly found in middle aged women. There was the thinning of intimal and medial layer with losses of elastic lamina, which lead to aneurysm formation. The area of aneurysm was interspersed with local areas of fibrosis at medial layer. In angiography examination, those areas were referred as string of beads. One third of arteries were usually normal and 40% cases were bilateral.
c. Perimedia fibroplasia: It is found in about 10% of FMD. Fibrosis tissue was formed in the outer layer of medial muscle layer. It causes severe stenosis without aneurysm.

Atherosclerotic renal artery stenosis often happened in older individuals, in which 7% was diagnosed in patient >65 years old and 60% was diagnosed in patient with history of hypertension, coronary artery disease, peripheral artery disease, and renal insufficiency. Atherosclerotic renal artery stenosis commonly involved artery ostia, 1/3 proximal of renal artery, and nearby aorta. Atherosclerotic renal artery stenosis related to renovascular hypertension and nephropathy.[1]

Renovascular hypertension in renal artery stenosis typically arise in young age and worsened with increasing age. It could also manifest as accelerated hypertension or resistant hypertension. Resistant hypertension is defined as uncontrolled hypertension despite consumption of 3 or more anti hypertension drugs including diuretics, or malignant hypertension with end organ damage.[8] Clinically, there could be some abdominal systolic or diastolic bruit and also hypokalemia in renovascular hypertension. Most common acute cardiovascular manifestation in renovascular hypertension includes flash pulmonary edema, which is not caused by any other coronary arterial diseases. Other cardiovascular manifestation includes hypertension related to acute coronary syndrome, aortic dissection, transient cerebral ischemia, stroke, intracranial hemorrhage, encephalopathy, and papil edema.[12]

Renal manifestation of renal artery stenosis could include acute renal failure which was characterized by increase of creatinine level after consumption of ACE-inhibitor or ARB. Acute renal failure typically happened in 10-14 days after first drug consumption, but occasionally it happened randomly. Although acute renal failure induced by ACE-inhibitor or ARB was considered as classic signs of renal artery stenosis, but it was not a sensitive nor specific indication for renal artery stenosis. Another renal manifestation is unreasonable chronic renal failure, asymmetrical renal size, and renal atrophy. About 5-15% of dialysis needed chronic ischemic nephropathy was estimated to be caused by renal artery stenosis.[1,12]

Diagnostic Criteria for Renal Artery Stenosis

Renal artery stenosis is a general term for any vascular lesion which induced constriction of renal artery lumen and actually decrease renal

blood flow. Two most common etiologies of renal artery stenosis are fibromuscular dysplasia and atherosclerotic renal artery stenosis. Atherosclerotic renal artery stenosis is typically accompanied by atherosclerosis in other arteries. Renal artery stenosis creates a wide spectrum of pathophysiology, with 3 main clinical syndromes: ischemic nephropathy, hypertension, and cardiac destabilization syndrome.[2]

Diagnostic criteria for renal artery stenosis is not yet established, but there are some clinical conditions that can guide into the possibility of renal artery stenosis:[1,5]

- Onset of hypertension <30 y.o. or severe hypertension in age >55 y.o.
- Accelerated, resistant, or malignant hypertension
- Renal atrophy without any defined causes or renal size discrepancies >1.5 cm
- Flash pulmonary edema without any definitive causes
- Unreasonable renal dysfunction, even need a renal replacement therapy
- New azotemia or worsened of renal function after ACE inhibitor or ARB consumption
- Multi-vessels coronary artery disease or peripheral artery disease
- Congestive heart failure or refractory angina without any possible causes

Patient with clinical suspicion of renal artery stenosis could have sent for diagnostic evaluation. Physical examination sometimes only gave limited clues such as systolic/diastolic abdominal bruit which radiated into flank area.[5] Prevalence of abdominal bruit was only about 78-87% even in patient with established renal artery stenosis. Systolic and diastolic bruit had sensitivity about 39% and specificity about 99%. Epigastric bruit or flank bruit had sensitivity about 63% and specificity about 90%.[6]

Biochemical blood analysis indicated renal dysfunction with increase of creatinine level in serum and increase of plasma renin activity. Sometimes, renal dysfunction also caused hypokalemia due to hyperaldosteronism.[8] Urinary analysis showed increase urine concentration with decrease of sodium concentration, especially in kidney with ischemia. Estimated GFR will also lower in ischemic kidney.[9]

Some diagnostic evaluation for renal artery stenosis includes captopril-stimulated nuclear renal flow, magnetic resonance angiography (MRA), and computerized tomography angiography (CTA). Captopril stimulated nuclear renal flow was used for diagnosing renal artery stenosis in patient with normal renal function and considered as one effective diagnostic method. But its accuracy decreased in patient with bilateral renal artery stenosis.[1]

Table 2. Classification of Renal Artery Stenosis according to Angiography Results[5]

Angiographic stenosis severity[a]	Physiologic testing	Significance
<50%	None	Mild
50-70%	None	Indeterminate
50-70% with	Resting mean pressure gradient[b] >10 mmHg	Significant
50-70% with	Systolic hyperemic pressure gradient >20 mmHg	Significant
50-70% with	Renal Pd/Pa ≤0.8[c]	Significant
≥70%	None	Significant

[a] *Visual estimation*

[b] *Translesional gradient measured with a non-obstructive catheter, i.e. ≤4 Fr or with 0.014-in pressure wire (Pd/Pa)*

[c] *Hyperemia may be induced with intrarenal bolus of papaverine 30 mg or dopamine at 50 μg/kg*

Renal artery duplex ultrasonography (RADUS) is one of the sensitive and specific procedures for renal artery stenosis. It is not expensive and can be repeated without any large

risk. RADUS has sensitivity range about 84-98% and specificity range about 97-99% according to studies from Taylor, *et al.*[14] and Olin, *et al*.[15] RADUS defined renal artery stenosis through the evaluation of ratio between peak velocity of renal artery systolic blood flow to peak velocity of aorta. But ultrasound has some limitation such as it depends on body habitus, bowel gas around kidney sometimes obscure kidney image, and the quality of examination depends on operator. To evaluate nephropathy in RADUS, clinician can use renal resistive index (RI) which is the evaluation of arterial flow resistance in the renal vascular networks and it is calculated using Doppler arterial pulsation. RI value >0.8 is considered significant for severe renal parenchymal disease.[1,5] Some other criteria evaluate renal artery stenosis using peak systolic velocity (PSV), renal aortic ratio (RAR), and acceleration time (AT) in RADUS. PSV value >180 cm/s is the most accurate diagnostic value for renal artery stenosis. Hemodynamic effect due to lumen occlusion such as changes of pressure trans-lesion or changes of flow trans-lesion, will be difficult to detect unless the occlusion is critical (occlusion of 70-80% of lumen).[6,15]

Some axial imaging techniques such as CTA and MRA, also have high sensitivity and specificity for diagnosing renal artery stenosis, but with much higher cost. Although CTA has sensitivity about 94% and specificity about 93%, CTA also possess high risk of contrast and radiation exposure.[5,6] MRA uses non-ionic contrast and does not use radiation, but MRA is not recommended for only estimating the degree of severity. MRA has 90% of sensitivity and 94% of specificity. Patient with renal dysfunction has the risk of contrast induced nephropathy because of ionized contrast in CTA. Gadolinium contrast in MRA can induce nephrogenic systemic fibrosis.[16] Invasive angiography may be indicated in patient with inconclusive results of imaging and clinical manifestation of renal artery stenosis, especially in patient with indication of intervention and revascularization.

In his review, Gottam, *et al*.[1] mentioned, with the sophistication of technology, some studies showed significant increase of sensitivity and specificity of CTA and MRA for renal artery stenosis. Study by Vasbinder, *et al*.[16] showed MRA sensitivity about 62%, CTA sensitivity about 64%, MRA specificity about 84%, and CTA specificity about 94%.[16] In the studies by Postma *et al*[17] and Williams, *et al*.[18] CTA showed sensitivity about 98% with specificity about 94% and MRA could show sensitivity as high as 100% and specificity about 96%.

Renal angiography is the golden standard for invasive diagnostic evaluation for significant renal artery stenosis especially in patient with hemodynamic changes. Degree of stenosis was classified according to **Table 2**, but it did not accurately reflect clinically significant hemodynamic changes. Stenosis >70% was considered severe and significant hemodynamically. Stenosis between 50-70% might not be significant hemodynamically and it indicated repeated evaluation. Consensus and some experimental studies classified severe hemodynamic dysfunction if the average trans-lesion gradient was >10 mmHg, peak hyperemic systolic gradient pressure was >20 mmHg, or renal fraction flow reserve (FFR) $\leq$0.8.[5,18]

Management of Renal Artery Stenosis

The main objective of renal artery stenosis management is to prevent decrease of renal function and reduce water and sodium overload. Most patient with renal artery stenosis had increase of mortality due to increase of cardiovascular dysfunction. Clinician had to ensure modification of changeable cardiovascular risk beside optimal management of renal artery stenosis.[9] Medical management is the main

therapy of renal artery stenosis especially in atherosclerotic condition, in which patient was given oral anti hypertension, statin, and low dose aspilet.[8] Some studies also suggest diet, exercise and physical activity, and controlling other risk factors such as smoking and alcohol.[6]

Conservative or medical management is indicated for patient with RI >80, pulse pressure >100, and severe nephropathy with urine protein excretion >1 g/day, creatinine clearance <40 ml/minute, hyperuricemia, and hypertension in nighttime. Some authors suggest pulse pressure >70.[1] Hypertension could be controlled with ACE inhibitor. But ACE-inhibitor might decrease renal perfusion which could be significant in post stenotic level. ACE inhibitor might also cause acute kidney injury in patient with hemodynamically significant bilateral renal artery stenosis.[9] Beside ACE inhibitor, other anti-hypertension which could be recommended is angiotensin receptor blocker (ARB).[7]

When there is failure of conservative therapy in which the blood pressure did not come down, and accompanied by recurrent flash pulmonary edema, worsened azotemia, with evidence of significant renal artery stenosis, patient could be considered to be sent for revascularization. Revascularization should also consider about patient's age and pulse pressure. Optimal outcome of revascularization decreases with increasing age.[1] Patient with pulse pressure <70 might still gain benefit from revascularization especially for blood pressure control with about 97% of sensitivity and specificity. Improvement of renal function has 80% sensitivity and 88% specificity. Revascularization in patient with pulse pressure >100 would even worsened blood pressure control with sensitivity about 73% and specificity about 99%. It would also decrease renal function with sensitivity about 84% and specificity about 95%.[9,19]

The main objective for revascularization is to improve blood pressure control, prevent progressive ischemic nephropathy, and improve heart failure, chronic angina or flash pulmonary edema. Revascularization is only indicated in hemodynamically significant renal artery stenosis. **Table 3** shows some indications for revascularize-ation in renal artery stenosis.[5,9]

Table 3. Indications of revascularization in renal artery stenosis[5,9]

Cardiac dysfunction	Hemodynamically significant renal artery stenosis • Recurrent congestive heart failure with undefined causes • Flash pulmonary edema without definitive causes Renal artery stenosis with unstable angina
Resistant hypertension	Renal artery stenosis with • Accelerated, resistant, or malignant hypertension • Hypertension with unilateral kidney shrinkage • Hypertension with drug intolerance
Ischemic nephropathy	Renal artery stenosis and chronic renal insufficiency Renal artery stenosis and chronic renal insufficiency with unilateral renal artery stenosis Asymptomatic unilateral or solitary viable kidney Asymptomatic unilateral significant renal artery stenosis but in only viable kidney Chronic renal failure with hemodialysis dependency Progressive renal failure without definitive causes

Main recommendation for FMD revascularization is balloon angioplasty. Some studies revealed successful outcome of balloon angioplasty for FMD with nearly 100% success rate with re-stenosis for only <10% in 10 years. Angioplasty was proven to be more effective if the FMD was localized in major renal artery than

diffused FMD. Some FMD cases were accompanied by aneurysm at renal artery in which if the aneurysm had big enough, it could be managed by graft stenting, occlusion with coil, or surgical reconstruction.[1,12]

Diagnostic and Management Problem in Renal Artery Stenosis

Fibromuscular dysplasia had some challenges in diagnostic process because although many patients showed symptoms and signs of resistant hypertension, but also many patients did not indicate any abnormalities in the physical examination and routine laboratory examination. Even RADUS was considered to be sensitive for diagnosing of renal artery stenosis, RADUS could produce false negative results in 10-20% of patient because of some confounding factors such as operator capability, obesity, or gas distribution at abdomen.[12,14] Other imaging technique such as CTA and MRA were sensitive and specific, but very expensive. CTA also poses risk of exposure to ionized contrast and radiation. MRA contrast, gadolinium, gives the risk of nephrogenic systemic fibrosis.[5,6] Therefore CTA was mostly recommended in patient with planning of revascularization.

Some doubts were proposed regarding medical management for renal artery stenosis. Aggressive blood pressure control was considered to aggravate renal dysfunction, even though generally, blood pressure control could improve renal function with protecting contralateral kidney. Patient with medical management still had some risks to develop progressive renal dysfunction and cardiovascular events. Some studies observed inflammation reducing therapy may offer some benefit for some patients with chronic renal failure with renal artery stenosis.[7]

Best result of revascularization was obtained in non-atherosclerotic FMD in which stenosis correction often improve blood pressure control and renal function. ASTRAL study revealed angioplasty and stenting may give some benefit for atherosclerotic renal artery stenosis but the evidence was still scarce regarding improvement of blood pressure control and renal function. Angioplasty and stenting, on other side, also had procedural risks such as renal artery occlusion, renal infarct, and atheroembolism.[8,23] Even 30% of renal intervention had complication because of atheroembolism. Recent studies mentioned renal artery stenting did not bring any significant benefit for preventing comorbidities such as cardiovascular event, renal disease progression, or the need of renal replacement therapy.[9,19]

According to Tegtmeyer, *et al*.[25] angioplasty in FMD, although had better prognosis than atherosclerotic renal artery stenosis, only offered overall recovery rate about 36% with post-operative recovery rate about 54%. Re-stenosis rate for FMD was 20%. Another recent study in more than 2000 patients, which recovery was defined as blood pressure <140/90 mmHg without any oral hypertension drugs, it was found that blood pressure outcome was significantly related with age.[28] Long term blood pressure control in FMD was about 93%. Improvement or stabilization of renal function was observed in about 92%. But complication of intervention procedure in FMD was as high as 12% after angioplasty and 17% after surgery. Major complication was observed in 6% cases after angioplasty and 17% cases after surgery. Besides, medical management with oral anti hypertension was considered effective in FMD in which clinician seldom attempted intervention management.[9] But some authors found blood pressure control in FMD after revascularization was as high as 79% and 65% patient had successfully maintain blood pressure control until 8 years after revascularization.[1,29]

Angioplasty with stent placement was more recommended for atherosclerotic renal artery

stenosis to prevent elastic recoil, minimalize arterial dissection, and maximize lumen widening. Successful outcome of angioplasty with stent placement in atherosclerotic renal artery stenosis, although could be as high as 95-100%, but re-stenosis rate was also as high as 10-30% in 1-year period.[1] Procedural related complication was also high which included arterial dissection, atheroembolism, and renal dysfunction. Even with newest techniques such as catheter-in-catheter and no-touch technique to minimalize contact between guiding catheter with atherosclerotic plaque, but renal embolism was found in 60% of cases and became the main major factor for worsening renal function.[30,31]

There were three randomized clinical studies which compared angioplasty with medical management for blood pressure control in patient with renal artery stenosis. But none of those studies showed any significant differences in systolic blood pressure between 2 groups. Although DRASTIC study showed significant blood pressure improvement in angioplasty group than medical management group (68% vs 38%) and angioplasty group showed fewer worsening of blood pressure control (9% vs 33%) and fewer renal artery occlusion (0% vs 16%) in 12 months.[29] But to date, there was not any study comparing medical management with angioplasty and stent placement because recently stent placement had become standard therapy. Stent placement in renal artery stenosis improved technical outcome and clinical outcome in long term period, compared to angioplasty alone, especially in ostial stenosis (80% of lumen). In a meta-analysis of 1.322 patients, stent placement had better technical outcome and lower re-stenosis rate when was compared to angioplasty alone (98% vs 77% and 17% vs 26% respectively). It also offered higher recovery rate.[28]

Stent placement in renal artery stenosis generally related to systolic and diastolic blood pressure improvement and decreased the need of oral anti hypertension. Even blood pressure improvement could be observed in 24 hours after successful stent placement and maintained for 24 months. ASPIRE-2 study[26] evaluated safety and efficacy of balloon expandable stent placement after failure of angioplasty. It revealed re-stenosis in 17.4% of participants. Some predictors for re-stenosis in the study was diabetes, pre-procedural small diameter of vessels, and small minimum diameter of vessel after procedure. But this study also found the increase of average creatinine level from 1.36±0.52 mg/dl in baseline to 1.40±0.61 mg/dl in 9 months and became 1.46±0.81 mg/dl in 24 months. In a subgroup of patient with baseline creatinine level 1.46±0.81 mg/dl, there was increase of creatinine to 1.46±0.81 mg/dl in 9 months and 1.46±0.81 mg/dl in 24 months. In 24 months, 7.5% of patient with abnormal renal function at baseline, had worsening of renal function although there were not any patients requiring permanent nor temporary hemodialysis.

CORAL study [28] compared optimal medical management with angioplasty and stent placement in patient with hemodynamically significant atherosclerotic renal artery stenosis and refractory systolic hypertension. Stent placement used distal protection. Optimal medical management included aggressive hypertension management, dyslipidemia management, diabetes, chronic kidney disease, smoking cessation, and anti-platelet drugs. Primary outcome for study was cardiovascular event or major renal dysfunction and death which related to cardiovascular event, renal dysfunction, stroke, myocardial infarction, hospitalization due to congestive heart failure, and the need of permanent renal replacement therapy. Primary outcome did not significantly differ between

medical management group and stent group with hazard ratio 0.94 (35.8 vs 35.1%). Mortality rate was also not significantly different. Systolic blood pressure decreased in both groups with higher decrease in stent group (average -2.3 mmHg).[30]

ASTRAL study[27] compared revascularization therapy and optimal medical management. This study showed higher rate of renal dysfunction in medical therapy group with difference about $0.06x10^3$/L every year. Average creatinine serum concentration was 1.6 µmol/L lower in patient with revascularization compared to medical therapy group. Systolic blood pressure was not significantly different between two groups but diastolic blood pressure had higher decrease in medical therapy group. But in this study, renal event (hazard ratio 0.97), cardiovascular event (hazard ratio 0.94), and mortality rate (hazard ratio 0.90) was equivalent between two groups.

In STAR study[30], authors observed the efficacy and safety of stent placement in patient with renal artery stenosis and decrease of renal function, compared to medical therapy management. Primary outcome in this study was the decrease of creatinine clearance 20% or more from baseline with two measurement. In stent group, if the primary outcome has been achieved, patient was sent for imaging examination to exclude re-stenosis of renal artery. Primary outcome was achieved in 22% of medical therapy patient and 16% of stent patient in 10 months. 5 from 10 patients in stent group had repeated angioplasty before they could achieve primary outcome in which 2 of them had re-stenosis. This study also indicated there was not any significant difference for blood pressure control, cardiovascular morbidity and mortality, and incidence of worsening renal function.[31]

True renovascular hypertension occurred when hypertension caused by renal artery stenosis induced activation of renal angiotensin aldosterone system. This condition happened when the blood pressure did not improve after renal revascularization. It became special problem in diagnostic procedure because if the blood pressure did not improve after revascularization, patient might have renovascular hypertension or might be there were structural changes that prevent blood pressure from falling. Patient might also have essential hypertension accompanied by atherosclerotic renal artery stenosis with or without renovascular component. Renovascular hypertension could only happened in the condition of renal artery stenosis, but renal artery stenosis could occur without any renovascular hypertension.[3] Some studies showed failure of revascularization to improve clinical condition and anatomical diameter could not be used as prognostic factor in revascularization.[19,20,21] Other studies used pressure gradient as predictor of significant stenosis in which fractional flow reserve <0.80 might be a good response predictor to revascularization.[31]

Gottam, *et al.* [1] mentioned that although renal artery stenosis was assumed to improve with revascularization and revascularization would also improve renovascular hypertension and nephropathy according to their pathophysiology, but some clinical studies did not always observe those same facts. Reasons behind those phenomena were complex and many studies is still on going to reveal many more of those reasons. Some process which could be related to hypertension after revascularization were sympathetic activation and endothelial dysfunction.[3] Recent evidences indicated improvement of blood flow alone could not improve outcome of renal function and cardiovascular event in patient with renal artery stenosis.[11]

CONCLUSION

Current problem regarding renal artery stenosis was dilemma in diagnostic process and management. To date there was not any specific diagnostic criteria for renal artery stenosis. Some authors only suggest clinical suspicion which could possibly guide the diagnosis of renal artery stenosis. RADUS as one sensitive and specific procedure for diagnosing renal artery stenosis, sometimes was still confounded by some confounding factors. Imaging techniques such as CTA and MRA were excellent in diagnosing renal artery stenosis, but they were expensive and possessed the risk of ionized contrast and radiation exposure. To define severity of stenosis, clinician might use RADUS with and followed by CTA in some cases. But CTA was more recommended to performed in patient with planning of revascularization.

Clinical outcome for revascularization was best found in fibromuscular dysplasia with the using of balloon angioplasty, with success rate almost 100% with blood pressure control rate about 93%. But complication rate of the procedure was still as high as 12% after angioplasty and 17% after surgery, that included arterial dissection, atheroembolism, and renal infarction. Some studies even showed equivalent effectivity of conservative medical therapy and angioplasty so the angioplasty was not recommended unless medical therapy had failed. In atherosclerosis, even though success rate of stenting achieved 95-100%, but re-stenosis rate was 10-30% in 1 year.[1] Revascularization procedure could not entirely improve renovascular hypertension and nephropathy because they had complex mechanisms and improvement of renal blood flow alone could not ameliorate renal function nor cardiovascular event. From clinical studies, it was still recommended to choose revascularization in FMD, but revascularization should only be performed after the failure of optimal medical management in atherosclerotic renal artery stenosis

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Case Report

Fork Rib: A Rare Musculoskeletal Etiology of Chest Pain

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ABSTRACT

Chest pain is a common clinical presentation in daily practice. Musculoskeletal origin is a rare etiology of chest pain, compare to cardiorespiratory problem and often underrecognized. Fork-rib or bifid-rib is a rare anomalies which uncommonly present with clinical symptoms, since in most cases fork-rib incidentally found during cadaveric dissection. Here we report a 27 years old man presenting with chest pain and radiographic examination showing bifid rib of the fifth left rib, without any abnormalities from physical examination and electrocardiography. Patient treated with intravenous painkiller and anticonculsants. Fork-rib should be a considered as differential diagnosis for chest pain of musculoskeletal origin especially in young adults or chest pain precede with minor trauma.

Keywords: chest pain, musculoskeletal, fork-rib

INTRODUCTION

Chest pain is a common clinical presentation in daily practice. Chest pain can be originated from several organ, cardiovascular, pulmonary, gastrointestinal, musculoskeletal, and psychological cause. In clinical settings, acute coronary syndrome should be excluded at any presentation of chest pain. Chest pain could be an emergency conditions, requiring immediate management. In the other side, non-emergency etiologies of chest pain also common.

The most common musculoskeletal origin of chest pain is costochondritis, counted 13 - 30% approximately of chest pain etiologic.[1] In this report, we would like to report a rare cause of musculoskeletal chest pain in young adult. Fork rib is an anatomical variation where one rib separated or bifurcated, often in anterior part of rib. This anomaly can be symptomatic or asymptomatic, symptoms include anterior bulging and pain. Literature search found only one report of fork rib presenting as chest pain in children, while most reports incidentally found on cadaver. It is

important to identify this anomaly since it might be part of particular syndromes with another serious clinical manifestations.

CASE REPORT

Mr. A, 27 years old visiting internal medicine outpatient clinic with chief complain of chest pain in the lateral side of the left hemithorax. This is the first onset, experienced a day before, the pain was localized with excruciating characteristic, the pain does not radiate and not alleviated by strenuous activity or respiration. The pain was persistence during the night, slightly relieved with paracetamol given at emergency department visit a night before.

Physical findings showing no cardiopulmonary abnormalities, moderate pain with Wong-Baker face pain rating scale 8/10. Electrocardiogram performed with normal results. Chest radiography showing branching of the anterior part of left fifth ribs.

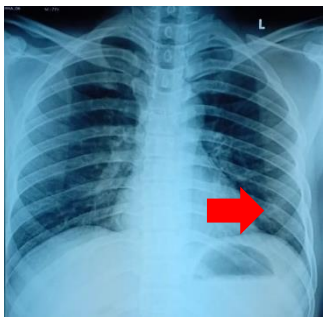

Figure 1. Chest radiograph showing branching of the anterior part of left fifth ribs (costae bifida, bifid costae, fork rib, bifurcated rib)

Patient finally diagnosed as fork rib of left fifth rib and was treated with tramadol infusion combined with gabapentin and discharged on the other day with ibuprofen and gabapentin, regarding possibility of neuropathic pain.

DISCUSSION

Chest pain of musculoskeletal origin can be caused by several aetiologies, i.e. costochondritis, Tietze syndrome, osteitis, fractures, strains, myofascial pain syndrome, or congenital anomalies of bones and adjacent structures. Costochondritis accounts for 13 – 30% cause of chest pain, results in the most prevalence cause of musculoskeletal chest pain.[1]

Fork rib (or bifid costae or bifurcated ribs) is an anatomical abnormality of costae. These findings might be related to a particular syndrome or isolated abnormality. Often this abnormality does not manifest into a clinical problem, several reports found this abnormality in cadaveric dissection during medical students' learning. While onset of clinical manifestation, when occur, ranging from early childhood into elderly. It is estimated that fork rib occurs in 28% of rib anomalies.[2] Fork rib could be identified from plain radiography and yet confirmed by 3D reconstruction CT. [3]

Several particular syndrome or complexes of abnormality reported shown in **Table 1**, while most of reports incidentally found fork ribs in cadaver. [4-7][8-10]

Table 1. Complex of Abnormalities Reported Associated with Fork Rib

Patients	Abnormalities	Bifid rib side	Bifid rib number	Reference
Male, 43 yo	Intrathoracic ribs Supernumerary ribs Vertebral block Hypoplastic left lung	Left	3rd rib	(11)
Female, 85 yo (cadaver)	Suspected Gorlin's syndrome (nevoid basal cell carcinoma): Breast cancer Multiple brain tumours (craniotomised) Renal cysts Hysterectomised Cholecystectomised Hip fracture	Right	4th rib	(6)
Female, 19 yo	Unerupted 3rd molar Congenital cataract Skin lesions	Left Right	3rd, 4th, 8th ribs 4th rib	(12)
Female, 30 yo	Bilateral dental follicular cysts Multiple body papules		6th rib	Reported in (2)
Male, 9 yo	Epithelioma adenoids cysticum Dental cysts on the left mandible and maxilla	Bilateral	6th rib	Reported in (2)
Female, 38 yo	Multiple dentigerous cyst	Left	4th rib	(13)
18 yo	Multiple maxillary and mandibular cysts Multiple basal cell lesions on the skin	Bilateral	6th rib	Reported in (2)

Based on literature search, we found 3 cases reporting fork rib with chest pain as clinical manifestations, one report a 9 years old girl

complaining chest pain after minor trauma, further evaluation showing fork rib in 5th right rib.[14] Second case reporting 9 years old boy complaining chest pain after minor trauma, the chest pain was localized at left posterior axillary line projection, fork rib was found in 3rd left rib. In the third case, the patient was 23 years old soldier, also experience 1-meter fall and complaining chest pain in the left lateral side but from radiograph, fork rib was found in the 2nd right rib. [15]

Of the three cases reported, minor trauma precedes the clinical manifestation of chest pain. Interestingly, in the third case, the pain was contralateral of the anomaly. Whether trauma triggered this manifestation remain unknown. The mechanism of pain suspected due to injury to intercostal nerve, intercostal nerve usually adjacent to the lower part of the branch [2,7], while anatomical variation may still occur such as nerve passing the space between bone branches. Our patient treated with tramadol on admission due to severe pain, and was discharged with ibuprofen and gabapentin, unfortunately he did not come for further evaluation.

CONCLUSION

Fork rib is a rare anomaly, most of cases detected during cadaver dissection, without known history of clinical complaints. Some of cases might detect incidentally during radiologic examination as single clinical manifestation or part of certain syndrome. Chest pain might occur as clinical manifestation with suspected mechanism of intercostal nerve injury.

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