ORIGINAL ARTICLE

Role of colchicine to reduce NLRP3 marker in STEMI patients undergo primary PCI: A randomised controlled clinical trial

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ABSTRACT

Introduction: ST-segment elevation myocardial infarction (STEMI) is a fatal disease with significant burden worldwide. Despite advanced medical treatment performed, STEMI-related morbidity and mortality remains high due to ischemia reperfusion injury after primary angioplasty mediated by NLRP3 inflammasome. Adding colchicine expected to reduce inflammation both in vitro and in vivo. We want to evaluate the effect of colchicine administration on the NLRP3 level of STEMI patient who undergo primary cutaneous intervention (PCI).

Materials and Methods: Randomised controlled trial was conducted on STEMI patients who undergo PCI in two hospitals in Jakarta, 104 patients enrolled to this study, and 77 patients completed the trial. 37 patients were randomly assigned to receive colchicines (2 mg loading dose; 0.5 mg thereafter every 12 hour for 48 hours) while 40 patients received placebo. NLRP3 level was measured from venous blood at baseline (BL), after procedure (AP), dan 24-hour post procedure (24H).

Results: No NLRP3 difference was observed initially between colchicine arm and placebo arm 38,69 and 39,0138, respectively (p >0.05). Measurement conducted at 24H, patients received colchicine demonstrate reduction in NLRP3 level (37.67), while placebo arm results increase in NLRP3 level (42.89) despite not statistically significant (p >0,05).

Conclusion: Colchicine addition to standard treatment of STEMI patients undergo PCI reduce NLRP3 level despite statistically insignificant.

KEYWORDS:
STEMI, inflammasomes, colchicine, acute coronary syndrome, reperfusion injury

INTRODUCTION

STEMI, ST-segment elevation myocardial infarction, is an urgent and potentially life-threatening medical emergency. STEMI occurs when there is a complete blockage of the coronary artery supplying blood to the affected area of the heart. STEMI patients manifested as intense chest pain and have a substantial portion of their heart muscle at risk. Swift access to procedures aimed at restoring blood flow to the coronary arteries is the gold-standard treatment, with a continued focus on minimising the time from admission to balloon angioplasty.[1] Myocardial infarct is still a major global health issue. In 2015, there was an estimated 7.4 million deaths due to coronary heart disease. Prevalence of myocardial infarct varies from 0.06% in men <45 years to 2.46% in men ≥75 years old.[2] However, despite adequate management in the form of percutaneous coronary intervention 3.4% of patients experience mortality within 7 days and up to 12.6% within 1 year after primary PCI conducted.[3,4] Restoring blood flow to previously blocked artery can induce ischemia reperfusion injury, which is attributable to an increased rate of major adverse cardiac events.[5] NLRP3, a crucial component of inflammasome, plays an important role in mediating ischemia reperfusion injury and serve as a potential therapeutic target.[6] Colchicine exhibits inhibition of inflammation in the NLRP3 associated pathway in COVID-19 and has been shown to reverse atherosclerosis mediated by NLRP3.[7,8] However, study assessing the effect of colchicine on the NLRP3 in STEMI patients who undergo primary PCI has not been studied yet. This study aims to address that matter.

MATERIALS AND METHODS

Study Setting

Study was conducted from December 2022 and April 2023 in Cipto Mangunkusumo National Referral Hospital and Jakarta Heart Centre. Study performed according to the Declaration of Helsinki and its 64th World Medical Assembly, Fortaleza, Brazil, 2013 amendments. Study was implemented corresponding to Good Clinical Practice guideline from ICH Tripartite Guideline. Ethical approval was issued by Ethical Committee of Faculty of Medicine University of Indonesia/Cipto Mangunkusumo National Referral Hospital (Ethical Approval Number, KET-1057/UN2.F1/ETIK/PPM.00.02/2022). This study also registered in www.clinicaltrials.gov with identification

This article was accepted: 20 February 2024
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number NCT05734612. Written informed consent was obtained from every study participant.

Population

Eligible patients are defined as those aged 18 to 80 years who have been diagnosed with STEMI and meet diagnostic criteria including typical chest pain, recorded ST-segment elevation and increased cardiac biomarker. Patients were enrolled to either colchicines arm or placebo arm which undergoes primary percutaneous coronary intervention and agree to participate in this study. Exclusion criteria encompass history of malignancy, allergy to colchichine, intolerance to contrast material, type 2 myocardial infarct, severe systolic dysfunction, stroke within 3 months, CABG within 3 years, inflammatory bowel disease, eGFR <30 mL/minute, chronic liver disease, autoimmune and long usage of corticosteroid, or abdominal pain with VAS score >5.

Protocol

This study is double blinded. Randomisation and allocation were made by third parties that didn't participate in data collection. Participants were divided into two groups, colchicine arm and placebo arm. Colchichine arm received loading dose of 2 mg before primary PCI procedure and 0.5 mg thereafter every 12 hour for 1 days. Placebo arm received same amount of glucose as a placebo. Both arm received standard medication for STEMI management. Venous blood sample was obtained before primary PCI and 24 hours after primary PCI procedure.

The samples used for NLRP3 examination is whole blood EDTA or PBMC. The selection of the best sample type is carried out by optimising examination of two samples using whole blood or PBMC. The sample used will be selected based on the most optimal results. The examination method used was flow cytometry with the Facs Canto BD® tool. CD14+-tagged monocytes have intracellular NRLP3. PBMC/whole blood samples will be given a marker for monocytes, namely CD14+. Cells that have CD14+ will be given a permeabilising agent to create pores on the cell surface, in order to the markers NRLP3 is able enter the cells. Cells that have CD14+ and ASC+, NRLP3+, Caspase 1+ markers will be read on a flow cytometry tool.

Data Analysis

Demographic data retrieved include age, sex, onset duration of chest pain, STEMI type and history of risk factors (smoking, hypertension, diabetes mellitus type 2, obesity, coronary artery disease, chronic kidney disease). Results from coronary angiography and primary PCI were collected regarding the number of vessels affected and the artery related to infarct. Ejection fraction was obtained after procedure. Data analysis was carried out using the SPSS version 20 (IBM).

Descriptive data will be arranged in table form. If the numerical data is normally distributed, it will be presented in the form of a mean with a standard deviation, and if it is not normally distributed, it will be presented in the form of a median with a interquartile range. Categorical data is presented in percentage form. The Kolmogorov-Smirnov test was carried out to determine the normality of the data. Analysing differences observed NLRP3 levels between the two intervention groups, independent T test used if the data was normally distributed or the Mann-Whitney test if the data was not normally distributed. Repeated measurement of NLRP3 concentration was done using generalised linear model. P value <0.05 was considered statistically significant.

RESULTS

A total of 104 STEMI patients were screened to participate in this study. 20 patients met exclusion criteria, three patients refused to participate and attending physician of one patient refused to participate. 80 patients were randomised evenly to each group (intervention arm n = 40; placebo arm n = 40). However, three of the intervention group received wrong drugs. 37 patients in intervention arm and 40 patients in placebo arm completed the study with zero dropout. This study recruitment, allocation, follow-up and analysis flow chart was summarised in Figure1.

The mean age of this study participants was 55.22 ± 9.9 years and majority of sex was male (76.6%) (Table I). There was no statistically significant difference of subject characteristics between intervention and control arm. Every study participant has comorbidities, including diabetes, hypertension, dyslipidaemia, history of smoking, obesity, chronic kidney disease and coronary artery disease. The most prevalent comorbidity is history of smoking (71.42%). Larger part of the study participants experienced three vessel disease (3VD; n = 44.15%) with the most frequent infarct-related artery was left anterior descending (LAD; n = 53.24%).

Measurement of NLRP3 concentration from baseline (BL), after procedure (AP) and 24-hour post procedure (24H) shows no statistically significant difference (p >0.05), was observed between colchicine and placebo arm (Table II). However, NLRP3 concentration measured in colchicine arm shows a trend of decrease from BL (38.69) to 24H (37.67) compared in placebo arm (39.01 vs 42.89), despite statistically insignificant.

Neither comparison of NLRP3 median delta (Δ) between colchicine arm and placebo arm yielded statistically insignificant results, whether comparison measured on AP-BL, 24H-BL or 24H-AP (Table III). Observation of median concentration of NLRP3 shows declining concentration in colchicine arm from BL to 24H, meanwhile placebo arm NLRP3 concentration shows increase (Figure 2).

DISCUSSION

Initial assessment of NLRP3 levels in the two intervention groups found no statistically significant difference (p = 0,950). Subsequent comparison of absolute concentrations reveals no significant difference. However, NLRP3 concentration in the colchicine arm decreased from baseline to 24H post primary PCI while the NLRP3 concentration in the placebo arm steadily increased. Comparisons of delta concentrations between AP and BL, 24H and BL, 24H and AP, shows no significant differences. On the other hand, comparison of absolute median between colchicine arm and placebo arm shows that Δ is always smaller or negative in the colchicine arm. Observations at 24H after primary PCI

Table I: Baseline subject characteristics

Subject characteristics	Colchicine N=37	Placebo N=40	*p*-value
Age (Mean ± SD)	55.3 ± 10.01	55.15 ± 9.88	0.930*
Sex, n (%)			
Male	27 (72.9)	32 (80)	0.467†
Female	10 (22.1)	8 (20)	
STEMI nset, (Mean ± SD)	6.0 ± 2.78	7.19 ± 3.34	0.10*
Comorbidities n (%)			
DM	15 (40.5)	13 (32.5)	0.464†
Dyslipidaemia	22 (59.4)	29 (72.5)	0.227†
Hypertension	23 (62.1)	25 (62.5)	0.976†
Smoking	25 (67.6)	30 (75.0)	0.471†
Obesity	17 (45.9)	23 (57.5)	0.311†
Chronic kidney disease	1 (2.7)	1 (2.5)	0.733†
Coronary artery disease	2 (5.4)	1 (2.5)	0.470†
Ejection fraction (Median ± IQR)	54.5% ± 10.70	55.0% ± 10.08	0.756†
Coronary angiography, n (%)			
CAD 1VD	7 (18.91)	14 (35.00)	0.260*
CAD 2VD	11 (29.72)	11 (27.5)	
CAD 3VD	19 (51.35)	15 (37.50)	
Infarct location, n(%)			
LAD	18 (48.64)	23 (57.5)	0.388*
LCx	6 (16.21)	2 (5.00)	
RCA	13 (35.13)	15 (37.5)	

*= Chi quare test, †= Independent t-test

Table II: Comparison of absolute NLRP3 concentration

Measurement	NLRP3 concentration		*p*-value*
	Colchicine arm (n = 37)	placebo arm (n = 40)	
Baseline (BL), mean (SD)	38,69 (16,59)	39,0138 (29,77)	0,950
After procedure (AP), mean (SD)	38,30 (19,91)	38,04 (28,05)	0,938
24 Hour post procedure, mean (SD)	37,67 (17,48)	42,89 (28,39)	0,276

*=Independent T-test

Table III: Comparison of NLRP3 concentration delta

Comparison	Difference in NLRP3 concentration		*p*-value*
	Colchicine arm (n = 37)	placebo Arm (n = 40)	
AP-BL Δ, median (IQR)	-2,86 (16,82)	3,88 (16,71)	0,378
24H-BL Δ, median (IQR)	-0,125 (26,85)	0,415 (38,45)	0,370
24H-AP Δ, median (IQR)	1,415 (26,19)	3,045 (38,3)	0,396

showed that the NLRP3 concentration decreased in the colchicine group while it increased in the placebo group.

Based on the literature exploration by researchers, there has been no previous research examining the effect of administering colchicine on reducing NLRP3 levels in STEMI cases in humans. Similar research conducted by Bakhta et al, conducted using mice subjects who were induced to undergo IMA-EST, found that administration of colchicine could reduce NLRP3 mRNA expression compared to without administration of colchicine.[9] Research on COVID-19 patients showed that administration of colchicine could significantly reduce NLRP3 activity, Measured by a decrease in the final synthesis of the cytokine IL-18.[7] A prospective cohort study assessing the effect of colchicine administration in chronic coronary syndrome patients showed a decrease in the synthesis of NLRP3 end products, namely IL-18, IL-6 and the IL-1 receptor agonist.[10]

Several mechanisms hypotheses to explain colchicine role in reducing NLRP3. Colchicine demonstrated inhibition of pyrin gene expression, thus preventing NLRP3 assembly.[11] Misawa, et al shows that colchicine inhibits transport of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), component of NLRP3, resulted in blocked colocalisation of NLRP3.[12] Colchicine also act as inhibitor of P2X7 induced pore formation, crucial steps in NLRP3 inflammasome response to ATP, which resulted in lower levels of ROS and IL-1β.[13]

The main possible reason that this study yet to achieve statistically significant result is the level of inflammation occurs in STEMI higher than in NSTEMI.[14] Thus, colchicine administered in this study unable to achieve significant reduction in NLRP3 concentration. These data show that the cardioprotective mechanism of colchicine in inhibiting acute or chronic processes when STEMI occurs due to NLRP3 also occurs *in vivo*.

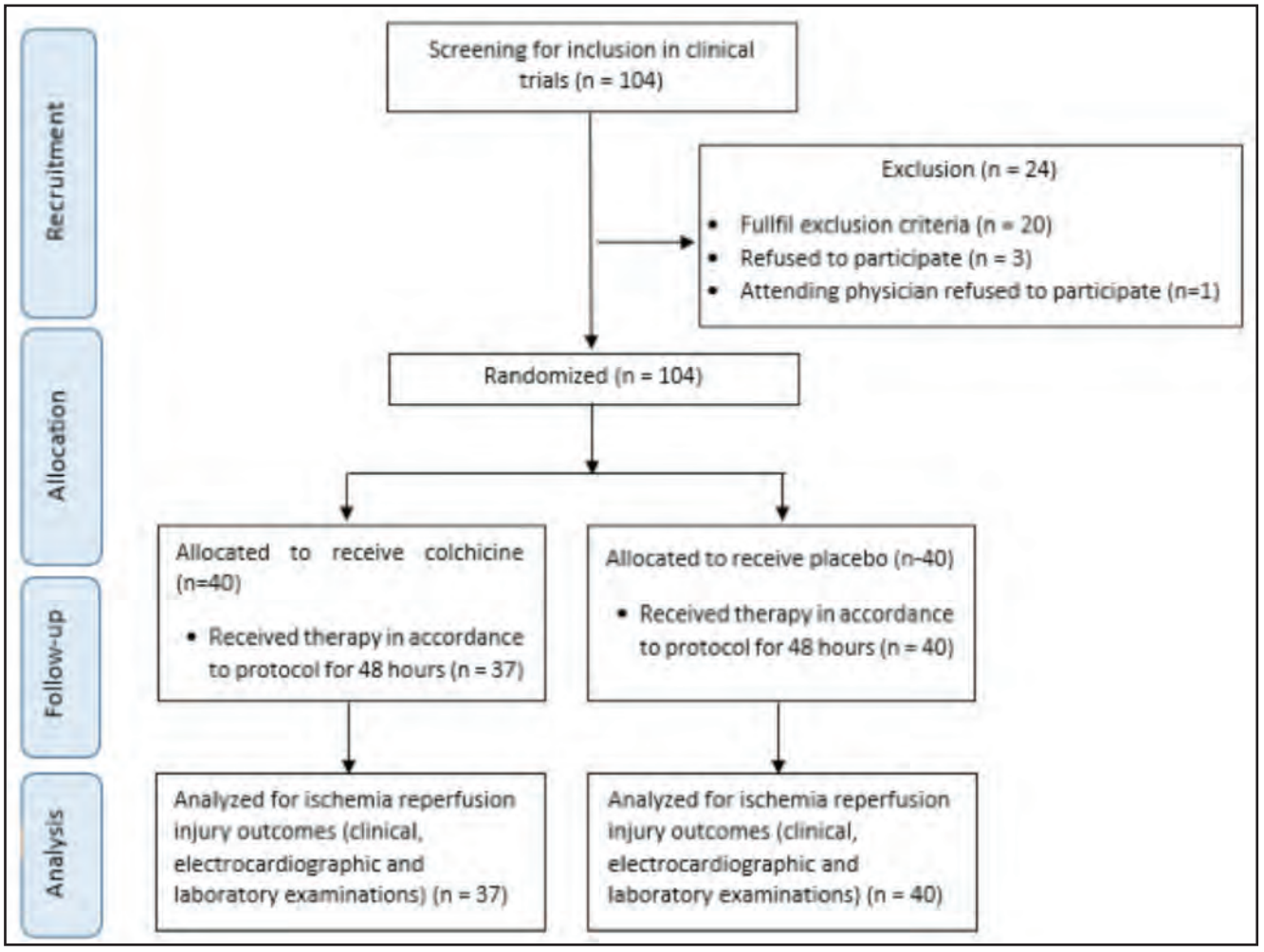

Fig. 1: Study flow chart

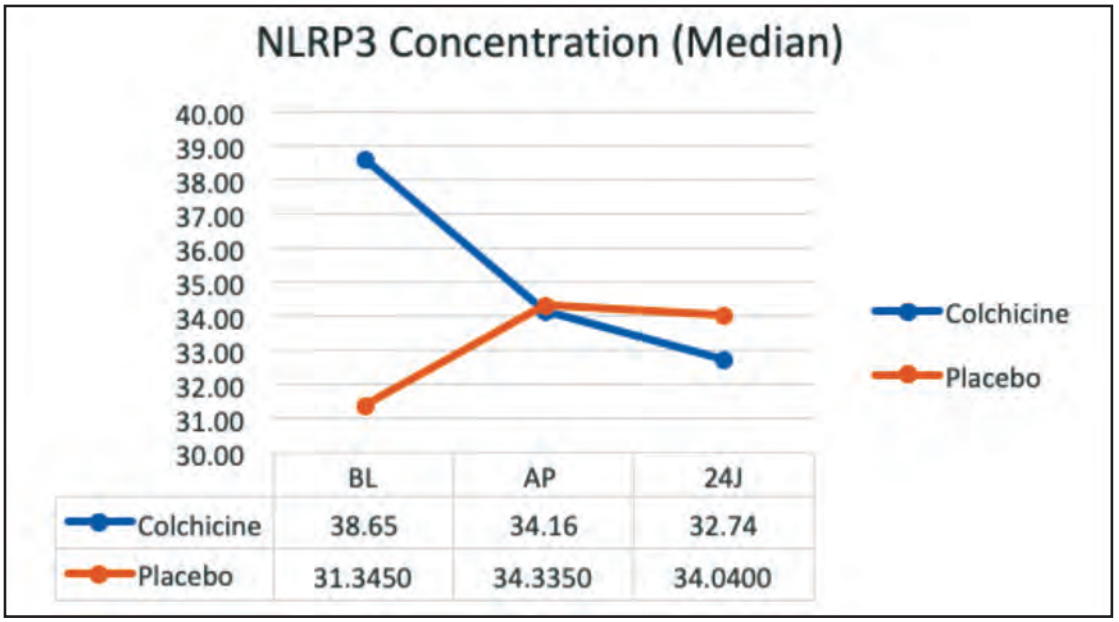

Fig. 2: Comparison of NLRP3 median

ACKNOWLEDGMENTS
All related parties who contributed to this study has been included in the authors list.

FUNDING
No external funding or research grant was awarded to this study.

CONFLICT OF INTEREST
No conflict of interest arises during this study.

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