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The Effects of Allopurinol on Levels of Cardiac Troponin Following Non-ST Elevation Myocardial Infarction: A Pilot Randomized Clinical Trial

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Abstract

Purpose: Given the potential anti-ischemic effects of allopurinol, we aimed to assess whether allopurinol administration may reduce myocardial injury following non-ST elevation myocardial infarction (NSTEMI).

Methods: A randomized clinical trial (RCT) was conducted on 100 individuals with NSTEMI. The intervention group (n=50) received 600 mg oral allopurinol at the time of diagnosis of NSTEMI, followed by 300 mg every day for two next days and the standard treatment of NSTEMI, while the control group (n=50) received only the standard treatment. Serum concentrations of cardiac troponin I (cTnI) were measured at baseline, and 8, 16, 24, and 32 hours after the treatment.

Results: The baseline demographic and clinical data of the patients were not statistically different between the intervention and control groups (all $P > 0.05$). The comparing estimated marginal mean ± standard error for cardiac troponin I (cTnI) levels revealed no significant difference between the study groups (2.93 ± .27, 2.25 ± .27; P=0.082). The linear mixed model results showed that the interaction of time and group was not statistically different (P=0.751). Moreover, there was a decreasing trend over time for cTnI in both groups (P=0.039).

Conclusion: The present pilot RCT did not support the potential cardio-protective benefits of allopurinol administration on decreasing myocardial injury following NSTEMI.

Keywords: Allopurinol, NSTEMI, Myocardial injury, Inflammation, cTnI

Introduction

Acute coronary syndrome (ACS) refers to an assemblage of clinical manifestations caused by acute myocardial ischemia. It results in significant mortality and morbidity and accounts for approximately 50% of cardiovascular disease-related deaths. More than 30% of ST-elevation myocardial infarction (STEMI) patients die within 24 hours of ischemia onset. Although the mortality and morbidity are lower in patients with non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA); however, it is still considerable, and about 15% of patients die or develop a re-infarction within one month of diagnosis.[1, 2] In the United States, approximately 18.9% and 20% of individuals are readmitted to the hospital for the same condition following one month after an episode of acute MI in summer and winter, respectively.[3]

Cardiac troponin (cTn) level is an indicator of ischemia severity for patients with NSTEMI. Heidenreich et al., in a systematic review and meta-analysis of 7 clinical trials and 19 cohort studies showed that mortality rate was significantly higher in NSTE-ACS individuals with a positive cTn test compared with patients with a negative test (5.2% vs. 1.6%, odds ratio [OR] 3.1).[4] Furthermore, based on the 2020 European Society of Cardiology (ESC) NSTE-ACS Guidelines, initial cTn levels add prognostic data regarding short-term and long-term mortality to electrocardiogram and clinical variables.[5]

ACS is a complex syndrome with various etiologies. Erosion or rupture of atherosclerotic plaque and consequential exposure of the atheroma core to circulating platelets and coagulation proteins leads to the formation of intracoronary thrombus, which is partially obstructive or transiently occlusive in patients with NSTE-ACS. Besides, five principal causes in the pathogenesis of ACS

include plaque rupture with acute thrombosis, secondary unstable angina, progressive mechanical obstruction, dynamic obstruction, and inflammation.[6, 7]

Accumulating evidence suggests that the inflammatory reaction is associated with cardiomyocyte apoptosis, and necrosis, and myocardial hypertrophy. Neutrophils infiltration at the ischemia onset and following monocytes and macrophages invasion lead to a significant increase in inflammatory mediators and reactive oxygen species (ROS). A large amount of ROS in the ischemic myocardium could persuade apoptosis of cardiomyocyte. It has been indicated that up-regulated expression and activity of xanthine oxidase (XO) could mediates myocardial hypertrophy and dilatation through increase in ROS levels.[8-11]

Allopurinol is an inexpensive purine analog with well-known potent anti-inflammatory properties. The drug was firstly used for the treatment of gout in 1966. Currently, allopurinol is also known as an anti-angina and cardio-protective agent. The potential cardiovascular benefits of allopurinol principally were originated from inhibition of xanthine oxidase (XO), improvement of endothelium function, reducing smooth muscle cell proliferation, reducing oxidative tissue stress, and increasing oxygen supply in tissue.[12, 13]

According to Xiao et al. study in rats, allopurinol (50mg/kg -Day 1) decreased apoptosis index as well as expression of fatty acid synthase (FAS) and Caspase-3 in non-infarcted zones. Besides, allopurinol administration decreased O^{2-} and OH-scavenging activity.[14]

Furthermore, numerous clinical trials have shown the benefits of allopurinol in the individuals with ischemic heart disease (IHD). Noman et al., in a randomized, double-blind, crossover trial in 65 patients with coronary disease indicated that allopurinol (600 mg/day) significantly extended

the time to ST-segment depression , the medial overall exercise time, and the time to chest pain. Another clinical trial showed that allopurinol could reduce myocardial oxygen consumption for each cardiac output unit of a particular stroke volume.[15, 16] According to Rekhraj et al. randomized clinical trial (RCT), administration of allopurinol in individuals with left ventricular hypertrophy and IHD is associated with improving endothelial function, decreasing left ventricular end-systolic volume, and regression of left ventricular hypertrophy.[17] Furthermore, a RCT on 100 patients with ACS was done to evaluate effects of allopurinol in patients with ACS. The patients were allocated to the allopurinol and control groups and followed up for two years. Data analysis showed that the serum levels of tumor necrosis factor alpha (TNF-α), high-sensitivity C-reactive protein (hs-CRP), oxidized low-density lipoprotein (OX-LDL), malondialdehyde (MDA), glucose, lipid, creatinine, uric acid, brain natriuretic peptide (BNP) were lower in the intervention group than the control group (all $P < 0.05$). Besides, allopurinol administration led to a significant elevation in the nitric oxide (NO) level compared with the control group ($P < 0.05$).[18]

Recently a systematic review and meta-analysis of nine studies comprising 850 patients revealed that allopurinol administration in patients undergoing coronary artery bypass grafting (CABG) leads to a statistically significant reduction in the periprocedural adverse events, including cardiovascular mortality ($P = 0.01$) and ACS ($P = 0.05$).[19]

Given the underlying mechanisms of ACS, the potential cardiovascular benefits of allopurinol through decreasing inflammation and free radial production, increasing NO level, improving endothelial dysfunction, and its well-known cardio-protective effects in the setting of ischemic heart diseases from the previous clinical trials, this pilot RCT was performed to assess allopurinol effect on the myocardial injury measuring cTnI concentrations in individuals with NSTEMI.

In Huang et al. trial, all the patients, including 42 patients with NSTEMI, 30 with STEMI, and 28 with unstable angina pectoris were analyzed together. Consequently, the cardio-protective effects of allopurinol was not determined in the patients with NSTEMI.[18] To the best of our knowledge, the present study is the first clinical trial that especially investigates allopurinol cardio-protective effects on the myocardial injury following NSTEMI.

Methods

Study design and setting

The study was a prospective pilot RCT carried out in the Shahid Madani Heart Center (SMHC), a major teaching referral hospital for cardiovascular diseases in the northwest of Iran, between June 2019 and March 2020. One hundred eligible patients were randomly allocated 1:1 to the intervention (n=50) and the control groups (n=50). The randomization was performed based on computer-generated random numbers list using online Graphpad randomizer software (https://www.graphpad.com/quickcalcs/randMenu/) and an allocation ratio of 1:1 in two the intervention (group A) and the control (Group B) with sample size n=100. After randomization 50 patients allocated in group A and the other 50 patients allocated in group B.

In the intervention group, patients received a single dose of 600 mg oral allopurinol (Hakim Pharmaceutical Company) at the time of diagnosis of NSTEMI, followed by 300 mg every day for two next days. High dose allopurinol was chosen because it has been shown that it improved oxidative stress and endothelial function much more than did allopurinol 300 mg.[20] Furthermore, patients of the intervention and control groups received the standard regimen of NSTEMI,

including antiplatelet agents (325 mg chewable aspirin and 300 mg of clopidogrel), anticoagulant of heparin, a beta blocker, a nitrate, an ACEi (Angiotensin-converting-enzyme inhibitors)/ARB (angiotensin receptor blocker), and an opioid (morphine or pethidine) as needed, according to the 2014 American Colleague of Cardiology/American Heart Association (AHA/ACC) for the management of patients with non-ST-elevation ACS as well as availability of medications in the hospital.[21] The demographic data of patients such as body mass index, age, sex, drug history, past medical history, positive family history of CVD, and laboratory data and were recorded in data collecting forms.

Study population

All the consented patients with NSTEMI and an age of 18 to 80 years old were included.

The exclusion criteria of the study include cardiogenic shock, renal dysfunction (GFR $\leq$30 ml/min), hepatic disease or dysfunction (Child-Pugh classes B and C), uncontrolled autoimmune and inflammatory diseases, severe infection, cancer, and any contraindications to the used medications.

Blood sampling and end-point outcomes

Blood samples were collected from all patients for measuring cTnI at the baseline, 8, 16, 24, and 32 hours after the treatment. Detection limit for cTnI blood level measurement was 0.1 ng/ml. The primary outcome of the study included a comparison of cTnI blood levels at baseline with 8, 16, 24, and 32 hours following the diagnosis of NSTEMI.

Sample size calculation

Because the present study was the first trial that evaluated the effects of allopurinol among patients with Non-STEMI, we could not calculate the sample size. Therefore, we decided to set a pilot survey, including 100 patients (50 in each group).

Statistical analysis

Data analysis was performed in IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). The normality distribution of data was assessed by the Kolmogorov–Smirnov test. Data were shown as number (%) for categorical variables and mean ± standard deviation (SD) for normal continues variables. Also, for non- normal continues variables such as cTnI concentrations, median and interquartile range (IQR) were reported. Mann Whitney test and/or independent t-test were used to compare means between the groups. Freidman test was used to assess effect of time on cTnI levels according to the two study groups. Chi-square and/or Fisher's exact tests were applied to set of categorical data. Due to the departure of cTnI levels from normality assumption, we used mixed model for data analysis since mixed models are robust against deviation from normality.[22] So, Linear mixed model with considering subject effect as random effect and group, time, and interaction of them as fixed effects was used for analyzing cTnI levels in two groups over time. Due to the existing difference between two group for baseline values of cTnI (p-value=0.06), assessment the effect of intervention was performed by adjusting for baseline values of cTnI in the linear mixed model. The p-values less than 0.05 were assumed as statistically significant.

Results

A total of 107 individuals the diagnosis of NSTEMI were assessed for eligibility. Among them, seven patients were excluded due to renal dysfunction (n=5), hepatic dysfunction (n=1), and cancer (n=1); therefore, 100 patients were allocated 1:1 to the intervention (n=50) and the control (n=50) groups and analyzed. (|Figure 1)

No statistically significant difference was observed between the study groups regarding the baseline demographic and clinical data (all P > 0.05) (Table 1). The median and mean levels of cTnI were reported in Table2. Also, the mean of cTnI levels over time in two groups were reported in Figure 2.

According to the results of linear mixed model included effect of group, time, baseline value of cTnI, and interaction effect of group and time, the interaction of time and group was not statistically different (P=0.751).

The estimated marginal mean ± Standard Error for cTnI levels were not statistically different between the intervention (2.93 ± 0.27) and control (2.25 ± 0.27) groups (P=0.082). The estimated marginal mean for cTnI levels 8, 16, 24, and 32 hours after the treatment were 3.62 ± .48, 2.50 ± .27, 2.36 ± .40, and 1.84 ± .37, respectively, which showed a decreasing pattern over time (P=0.039). (Table 3)

Pairwise comparison by Bonferroni test indicated that the estimated marginal mean of cTnI levels between 8 and 16 hours, 8 and 32 hours, and 16 and 32 hours were not significantly different; however, a significant difference was observed regarding the cTnI concentrations of 8 and 32 hours after the treatment (1.12 ± 0.57 vs. 1.87 ± 0.61; P=0.024). Estimated marginal means for time

according to the group were shown in Table 3. Due to the non-significant effect of interaction between time and group (P=0.751), the trend of cTnI means in both group was similar and there was a decreasing trend over time for cTnI in both group. (Table 3). No adverse effect was observed during the study follow-up period.

Discussion

In the previous trials, the effects of allopurinol on decreasing myocardial injury were evaluated in the other settings such as elective percutaneous coronary intervention (PCI), CABG, and ACS. The present RCT was the first study that especially assessed the cardio-protective role of allopurinol in the decreasing of myocardial injury following NSTEMI. This pilot RCT did not show the potential cardio-protective effects of allopurinol on myocardial injury in patients with NSTEMI.

It has been showed that XO contributes to local oxygen depletion through consuming molecular oxygen as a substrate of oxidation reactions. It also inactivates the mitochondrial electron transport chain and production of adenosine triphosphate (ATP), resulting in ATP depletion in ischemic myocardium.[23, 24] Allopurinol as a low-cost purine analogue XO inhibitor has been widely used in Gout, nephrolithiasis, and tumor lysis syndrome, and also emerged with promising results in cardiovascular disease. A potential role for allopurinol as an anti-ischemic tool was first proposed in animal models with heart failure.[25] The potential cardiovascular benefits of allopurinol mainly is mainly due to inhibition of XO, increasing oxygen supply in tissue, decreasing myocardial oxygen consumption for a determined stroke volume, anti-inflammatory properties, improvement of endothelium function, and reducing oxidative tissue stress.[12-16]

A systematic review and meta-analyses of ten RCTs with 594 patients was carried out to evaluate the allopurinol effects on flow-mediated dilation in individuals with cardiovascular risks. Data analysis showed that allopurinol administration significantly improved flow-mediated dilation in these individual (WMD=1.67%, 95% CI: 0.83% ~ 2.50%, P<0.001; I2=86%). Besides, it has been indicated that that the benefit of allopurinol to flow-mediated dilation is not related to the UA lowering properties. Accordingly, it seems that allopurinol administration is associated with more incredible improvement of flow-mediated dilation in individuals with lower serum UA, compared with those with higher UA (<7 mg/dL: WMD=2.62%, 95% CI: 1.10% ~ 4.14%; ≥7 mg/dL: WMD=0.87%, 95% CI: 0.37% ~ 1.38%; P for subgroup difference=0.03). These results highlighted the potential cardiovascular benefits of allopurinol with mechanisms rather than the lowering UA level properties. In our pilot RCT, serum UA level was not considered in the eligibility criteria.[26]

Allopurinol cardioprotective effects have been indicated in reducing occurrence of perioperative MI in patients undergoing CABG. Based on a systematic review and meta-analysis of six studies, including 229 patients, incidence of MI was higher in control group compared with allopurinol group 12.07% vs. 1.77%). A fixed-effects meta-analysis showed significant reduction in occurrence of MI (RR 0.21, 95% CI: 0.06, 0.70, p = 0.01) in the allopurinol group. It is important to mention that the effects of allopurinol were greatest in Rashid et al. study, wherein patients received highest dose (300 mg) and longest duration (five days) of allopurinol(RR 0.06 95% CI: 0.00–0.99, p = 0.05). Moreover, the allopurinol effect became non-significant with the exclusion of this study.[27, 28] In our RCT, high dose of allopurinol was evaluated; however, the duration of treatment was lower than Rashid et al. study.[28]

It has been indicated that allopurinol 600 mg improved oxidative stress as well as endothelial function much more than did allopurinol 300 mg.[21] Huang et al. showed remarkable effect of 600 mg/day of allopurinol throughout the acute phase of ACS followed by 200 mg/day in oxidative stress and inflammatory reaction indicators as well as ACS treatment.[18] Furthermore, Noman et al. revealed that allopurinol 600 mg per day could increase the time to ST-segment depression ($p = 0.0002$), the medial overall exercise time ($p = 0.0003$), and the time to chest pain ($p = 0.001$) in individuals with coronary artery disease. [15]

In our pilot trial, the patients received 600 mg oral allopurinol at the time of diagnosis of NSTEMI, followed by 300 mg every day for two next days. Our RCT did not support the potential cardio-protective beneficial of high dose allopurinol administration on decreasing myocardial injury following NSTEMI. In line with our results, Alemzadeh-Ansari et al. in a placebo-controlled RCT 254 patients with coronary disease undergoing elective PCI showed that high dose of allopurinol could not decrease CK-MB (OR = 0.1; P-value = 0.67) and cTnT (OR = 0.1; P-value = 0.61) levels.[29] In this double-blind RCT, 133 patients received 600 mg allopurinol in the day before performing PCI, and 600 mg in the day of PCI; while, 121 patients in control group received placebo. Notably, no significant adverse drug reaction was observed in both of trials.[29] In Haung et al. RCT, a total of 100 patients with ACS revived allopurinol (600 mg/day throughout the acute phase of ACS, followed by 200 mg daily up to 4 weeks) and were followed up for 24 months. Data analysis revealed that allopurinol administration improved oxidative stress and inflammatory reaction including indicators including serum levels of creatinine, uric acid, BNP, TNF-α, blood glucose, hs-CRP, blood lipid, OX-LDL, and MDA, and had beneficial effect in the ACS treatment (all $P < 0.05$).[18] Furthermore, Separham et al. in a RCT showed that allopurinol (400 mg before treating with streptokinase, followed by 200 mg/day for 28 days) administration in patients with

STEMI led to lower levels of cTnI ($P < 0.001$), peak creatine kinase (CK) ($P = 0.003$), and CK-MB ($P = 0.005$).[30]

Limitations and future recommendations

Due to some limitations, the result of the present RCT should be cautiously interpreted. First, due to cost limitations, we could not measure the levels of CK-MB and inflammatory biomarkers. Although according to the AHA/ACCF guideline, the definitions of myocardial injury and MI are based on the levels of cTn.[30] Second, to avoid the risk of severe cutaneous adverse reactions, such as drug-hypersensitivity syndrome, toxic epidermal necrolysis, and Stevens–Johnson syndrome, human leukocyte antigen-B*5801 (HLA-B*5801)-positive participations should avoid allopurinol use. However, based on the American College of Rheumatology recommendation, HLA-B*5801 screening is only limited to high risk patients including all individuals with Han Chinese or Thai descent as well as Korean patients with kidney insufficient.[31] In our trial, patients had the same geographical and ethnic origin and did not belong to the mentioned high risk descents. Consequently, HLA-B*5801 screening is not required. Notably, no severe adverse effect was reported in out trial. Third, the present RCT is a pilot study and dose and duration of allopurinol in patients with NSTEMI have not yet been identified. In our trial, high dose allopurinol was chosen because it has been showed that it improved oxidative stress and endothelial function much more than did allopurinol 300 mg. Some of previous trials in similar settings have been shown the promising effects of allopurinol in higher treatment duration than our study; however, due to accessibility problems, the duration of treatment was low in our trial.[18, 21, 30] Numerous trials are needed to show precise dose, time, and duration of allopurinol administration in patients with

NSTEMI. Fourth, the sample size of this RCT does not seem to be enough to reveal the allopurinol exact effect in the lessening of myocardial injury in the patients with NSTEMI. However, the present trial had the nature of a pilot; consequently, this sample size was suitable for a pilot RCT. Fifth, because of the availability problems, we did not use an allopurinol placebo to reduce treatment bias. Finally, the present study was a single-center trial. Hence, double-blinded, placebo-controlled, multi-center studies with higher sample size and longer duration of treatment are warranted to show the precise impact of allopurinol in the setting of NSTEMI.

Conclusion

In the present RCT, we did not observe the potential benefits of 600 mg oral allopurinol, followed by 300 mg every day for two next days on decreasing myocardial injury in the patients with NSTEMI. Further well-designed RCTs with larger sample size and longer treatment duration are required to define the role of allopurinol in the setting of NSTEMI.

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Ethical Issues

The present study protocol was approved by the Research Ethics Committee of the Tabriz University of Medical Sciences (the registration number: TBZMED.REC.1398.424) and then

registered in www.irct.ir under the ID: IRCT20111206008307N36. The present study was performed according to the Declaration of Helsinki and later revisions on ethical principles for medical research.[33] All patients were informed about how the study would be done. Written informed consent for participation was obtained from all patients or their guardians. Patients were free to withdraw from the study at any time. The information of patients will remain confidential to the researchers.

Data Sharing

Applicants can obtain data by contacting the corresponding author.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Table 1.Demographic and Clinical Data of the Study Groups

Demographic/Clinical Data	Intervention (n=50)	Control (n=50)	P-value
Age (years), mean ± SD	62.6 ± 12.2	69.0 ± 10.6	0.375
Sex, male, n (%)	36 (72)	36 (72)	1
BMI (kg/m2), mean ± SD	25.8 ± 2.0	25.9 ± 2.3	0.646
Serum creatinine (mg/dL), mean ± SD	1.1 ± .23	1.1 ± 3.2	0.128
Blood urea nitrogen (mg/dL), mean ± SD	14.8 ± 4.4	16.0 ± 5.4	0.600
Fasting blood sugar (mg/dL), mean ± SD	166.7 ± 99.7	178.5 ± 103.4	0.779
Hemoglobin (g/dL), mean ± SD	14.4 ± 1.6	13.8 ± 1.5	0.264
Ejection fraction (%), mean ± SD	42.2 ± 13.7	42.5 ± 12.4	0.601
Smoking, n (%)	7 (14)	10 (20)	0.424
Diabetes mellitus, n (%)	23 (46)	28 (56)	0.317
Hypertension, n (%)	27 (54)	28 (56)	0.840
Dyslipidemia, n (%)	11 (22)	12 (24)	0.812
History of other disease, n (%)	8 (16)	6 (12)	0.564
Positive family history of cardiovascular diseases, n (%)	13 (26)	18 (36)	0.425
Previous coronary intervention, n (%)	17 (34)	13 (26)	0.764
Previous MI	1 (2)	2 (4)	1
Previous stroke	3 (6)	5 (10)	0.715
Anti-diabetic drug history, n (%)	4 (8)	2 (4)	0.678
Anti-lipid drug history, n (%)	20 (40)	28 (56)	0.109
Beta blocker drug history, n (%)	24 (48)	21 (42)	0.524
Calcium channel blocker history, n (%)	10 (20)	7 (14)	0.424
ACE inhibitors or ARB history, n (%)	19 (38)	17 (34)	0.677
Hydrochlorothiazide history, n (%)	9 (18)	12 (24)	0.461
Nitrate drugs history, n (%)	4 (8)	2 (4)	0.678

Table2. Descriptive statistic for cTnI levels in different time by two study groups

Troponin-I Level (ng/ml)	Intervention		Control	
time	Median, (IQR)	mean, (SD)	Median, (IQR)	mean, (SD)
Baseline	1.30, (2.85)	3.14, (4.82)	0.90, (2.18)	2.65, (4.58)
At 8 hours	1.85, (4.53)	4.16, (6.10)	1.10, (1.80)	3.09, (5.13)
At 16 hours	1.50, (2.48)	2.71, (3.31)	0.80, (2.75)	2.29, (3.54)
At 24 hours	1.20, (2.78)	2.70, (4.47)	0.50, (1.75)	2.08, (4.19)
At 32 hours	0.85, (2.93)	2.53, (4.88)	0.30, (1.48)	1.15, (1.82)
p-value** time	<0.001		<0.001	

IQR, interquartile range; SD, standard deviation

*P-value from Mann Whintey test

** P-value from Freidman test

Table 3. Estimated marginal means± standard error for group, time, and combinations of time and group base on the linear mixed model

Troponin-I Level (ng/ml)	Intervention	Control	Overall
At 8 hours	4.06 ± .68	3.18 ± .68	3.62±.48
At 16 hours	2.62 ± .38	2.39 ± .38	2.50±.27
At 24 hours	2.61 ± .56	2.18 ± .56	2.39±.40
At 32 hours	2.43 ± .52	1.25 ± .52	1.84±.37
Overall	2.93±.27	2.25±.27	
p-value for group=0.082			
p-value for time= 0.039			
p-value for interaction time and group= 0.751			

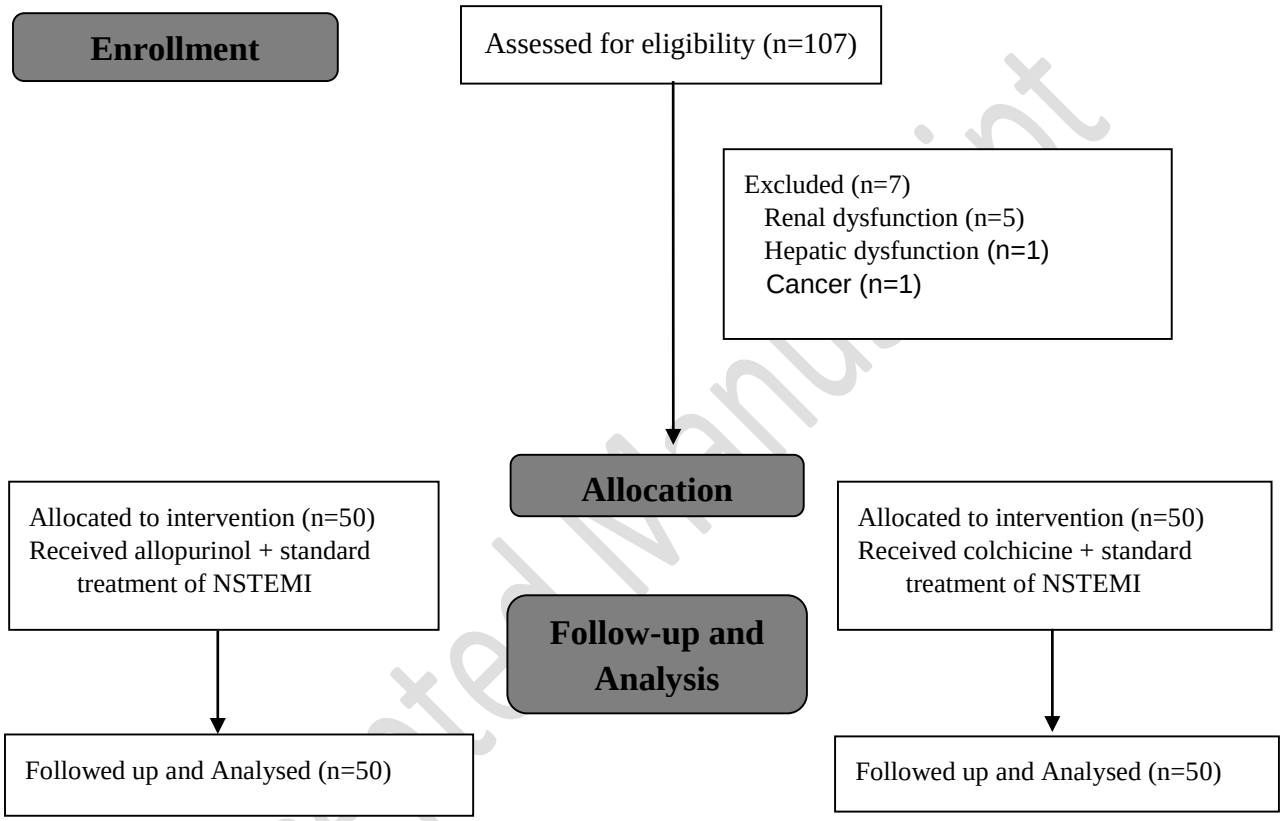

Fig. 1. CONSORT Flow diagram of the study.

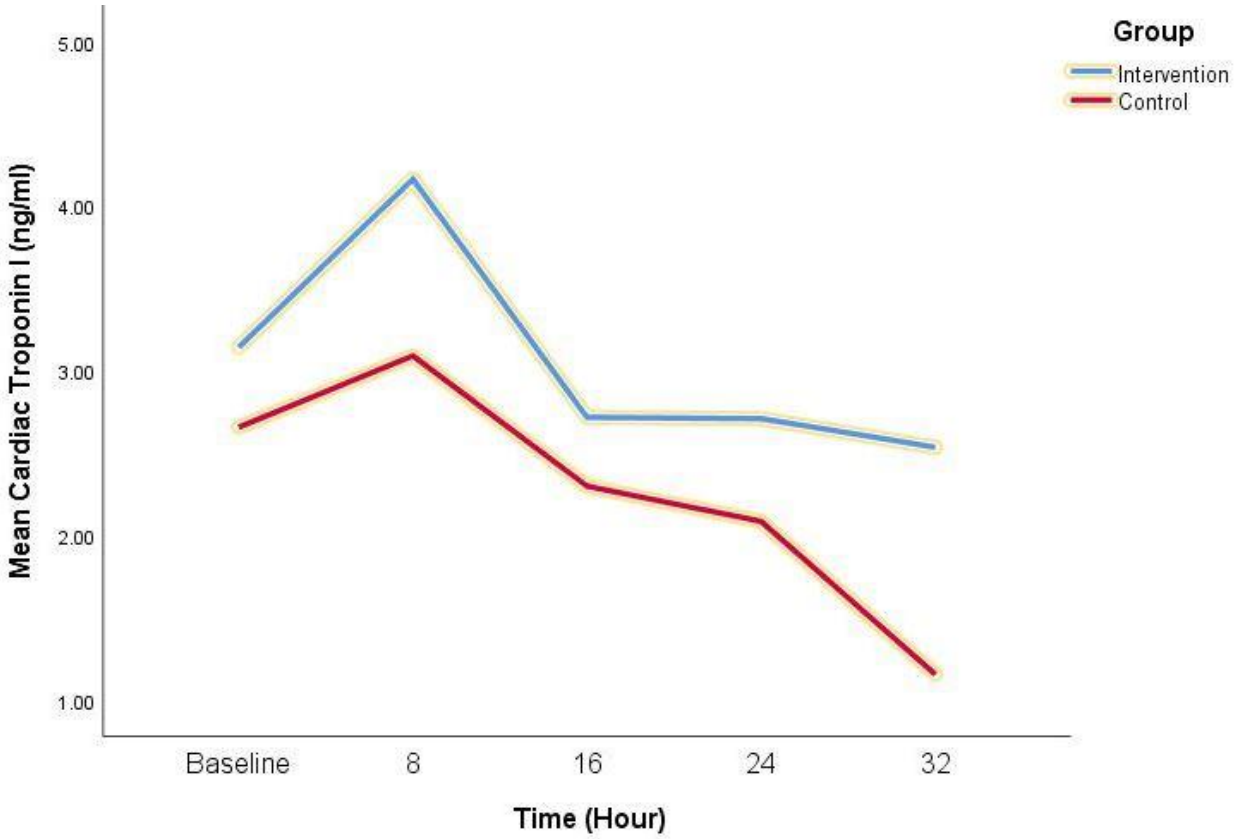

Fig. 2. The mean troponin I level during the study (mean, (SD)).

Baseline (3.14, (4.82); 2.65 (4.58)), 8 hours (4.16 (6.10); 3.09 (5.13)),

16 hours (2.71 (3.31); 2.29 (3.54)), 24 hours (2.70 (4.47); 2.08 (4.19)),

32 hours (2.53 (4.88); 1.15 (1.82))