Tropical Journal of Pharmaceutical Research April 2024; 23 (4): 723-730
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i4.7

Original Research Article

Effect of aspirin combination/clopidogrel combination on high-sensitivity C-reactive protein and P-selectin levels in patients with acute coronary syndrome

Renfen Xie[1], Shengwang Xu[1], Daojiao Ao[1], Yalan Wang[1], Junmin Huang[2]*
[1]Laboratory Department, [2]Internal Medicine Department, Zhaotong Traditional Chinese Medicine Hospital, Zhaotong 657000, China

For correspondence:* ***Email:* *15974890891@163.com*

Sent for review: 25 May 2023 *Revised accepted: 23 March 2024*

Abstract

Purpose: *To investigate the effect of combining aspirin with clopidogrel on high-sensitivity C-reactive protein (hs-CRP) and P-selectin in patients with acute coronary syndrome (ACS).*
Methods: *The medical records of 198 ACS patients treated at Zhaotong Traditional Chinese Medicine Hospital, Zhaotong, China between January 2022 and January 2023 were analyzed retrospectively. Control group (88 patients) received aspirin (300 mg once, and subsequently a maintenance dose of 100 mg daily for 6 months), while the study group (118 patients) received the same dose of aspirin and clopidogrel (150 mg once, and a maintenance dose of 75 mg daily for 6 months). Levels of P-selectin, interleukin-6 (IL-6), hs-CRP, IL-8, tumor necrosis factor-α (TNF-α), efficacy, and incidence of adverse reactions in both groups were determined.*
Results: *There was no significant difference in P-selectin and hs-CRP levels between the two groups before treatment ($p > 0.05$). However, P-selectin and hs-CRP levels in the study group decreased significantly compared to control group ($p < 0.0001$). Similarly, there was no significant difference in IL-8, IL-6 and TNF-α levels between the two groups before treatment ($p > 0.05$). However, IL-8, IL-6, and TNF-α reduced significantly after treatment ($p < 0.0001$), with more significantly lower levels observed in the study group ($p < 0.001$). There was no significant difference in the incidence of adverse reactions between the two groups.*
Conclusion: *Aspirin combined with clopidogrel significantly lowers P- selectin, and hs-CRP, IL-8, IL-6, and TNF-α levels without worsening adverse reactions. A more comprehensive analysis of the combined use of aspirin with clopidogrel including all the associated mechanisms in the treatment of ACS is necessary.*

Keywords: *Clopidogrel, Acute coronary syndrome, Aspirin, hs-CRP, P-selectin*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The incidence of acute coronary syndrome (ACS) is rapidly growing due to improved living standards and social aging [1]. Acute coronary syndrome (ACS) is a large group of clinical symptoms with different clinical features, clinical risk and prognosis. Common symptoms include severe chest pain or discomfort, which may radiate to the shoulders, back, neck, and lower jaw. The pathology is mainly associated with coronary artery disease and related myocardial

injury [2].

As a frequently encountered cardiovascular disease in clinical practice, ACS is mainly triggered by myocardial ischemia, which leads to the formation of atherosclerotic plaques in coronary arteries, and consequently occlusive thrombosis [2]. It is a critical and severe disease, with high global incidence, disability, and mortality. Without timely intervention, it may endanger the lives of patients [3]. Platelets play a crucial part in the development and progression of ACS, so anti-platelet aggregation therapy is a crucial and indispensable part of therapy [4]. Aspirin suppresses platelet aggregation, prevents thrombosis, and exerts an anti-inflammatory effect [5]. It is frequently adopted for the treatment of ACS in clinical practice, but it has the risk of causing bleeding, and some patients do not receive significant therapeutic relief after treatment with aspirin [6].

Clopidogrel, an antiplatelet thiophene pyridine drug, also strongly suppresses platelet aggregation like aspirin and prevents thrombosis [6,7]. Clopidogrel exerts rapid and long-term inhibition on platelet aggregation in coronary thrombosis, quick onset, longer duration and fewer side effects [8]. Therapeutic effect of aspirin and clopidogrel on ACS is one of the major research areas in ACS management. For example, Zhang et al [9] compared the clinical effects of aspirin and clopidogrel monotherapy on ACS. However, there are few studies on the clinical effect of the combined regimen on ACS treatment. Therefore, this study investigated the effects of aspirin combined with clopidogrel on high-sensitivity C-reactive protein (hs-CRP) and P-selectin in ACS patients. This study analyzed the efficacy, and adverse reactions and investigated independent risk factors of unfavorable prognosis by multivariate logistic regression.

METHODS

Patient information

The data of 198 ACS patients treated in Zhaotong Traditional Chinese Medicine Hospital, China between January 2022 and January 2023 were analyzed retrospectively and enrolled into control and study groups. The control group (88 patients) received aspirin while study group (118 patients) received aspirin in combination with clopidogrel. The study was conducted with permission from the Ethics Committee of Zhaotong Traditional Chinese Medicine Hospital (approval no. ztszllsc2021015). The study met the criteria in the guidelines of Declaration of Helsinki [10]. Written informed consent was obtained from the participants and/or guardians prior to enrolment.

Inclusion criteria

Patients who met the diagnostic criteria of ACS developed by the American College of Cardiology (ACC) [11] and were confirmed with ACS based on routine coronary angiography and electrocardiogram, patients who had not received other treatments before enrolment, and patients who complied with the completion of the established treatment plan and follow-up and with complete clinical data.

Exclusion criteria

Patients allergic to the drugs adopted, presence of tumor, peptic ulcer or severe liver or kidney dysfunction. Patients with platelet count > 300 × 10^9/L or < 100 × 10^9/L, sustaining diastolic blood pressure (BP) > 110 mmHg and/or sustaining systolic BP > 180 mmHg (1 mmHg = 0.133 kPa) and pregnant or lactating patients.

Therapeutic regimen

Both groups were given routine comprehensive treatment, which involved absolute bed rest, continuous oxygen inhalation, statins such as atorvastatin and fluvastatin, low molecular weight heparin, β receptor blockers such as carvedilol and bisoprolol nitrates [6]. Control group was also orally given aspirin enteric-coated tablets (Guangdong Jiuming Pharmaceutical Co., Ltd.; SFDA approval no. H44021139; Specification: 100 mg × 30 tablets), with a first dose of 300 mg/time in a day and subsequently a maintenance dose of 100 mg once daily continuously for 6 months. Study group was also orally given enteric-coated aspirin tablets as previously described for control group and clopidogrel tablets (Sanofi Winthrop Industrie; SFDA approval no. 3200J8310; specification: 75 mg × 7 tablets/box) with a first dose of 150 mg once, and a maintenance dose of 75 mg once daily for 6 months [8].

Evaluation of parameters/indices

P-selectin and hs-CRP

Fasting early-morning elbow venous blood (5 mL) was withdrawn from every participant before therapy and 6 months after therapy. The samples were centrifuged and serum P-selectin and hs-CRP levels were quantified using double-antibody sandwich enzyme-linked immuno-sorbent assay (ELISA).

Response rate

Response rate was classified as markedly effective (ME) when clinical symptoms disappeared, and electrocardiographic parameters became normal, or effective (E) when clinical symptoms and electrocardiographic parameters improved and ineffective (I) when clinical symptoms and electrocardiographic parameters were not improved. The overall response rate (R) was calculated using Eq 1.

$$R (\%) = ((ME+E)/N)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where N is the total number of cases.

Levels of inflammatory responses

Levels of IL-6, IL-8 as well as TNF-α were also investigated. Fasting early-morning venous blood (5 mL) was withdrawn from each participant before therapy and after 6 months of treatment. The samples were centrifuged to obtain the supernatant and levels of IL-6, IL-8 as well as TNF-α were quantified using ELISA kits (Beijing Jingmei Company) following kit instructions.

Adverse reactions

Adverse reactions in the two groups were recorded and analyzed.

Prognosis

The one-year prognosis of patients with ACS was analyzed, and the independent risk factors of unfavorable prognosis were identified by multivariate logistic regression.

Statistical analysis

Data was processed and analyzed using Statistical Packages for Social Sciences (SPSS) version 22.0 (IBM, Armonk, NY, USA). GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was utilized for graphic presentations. Categorical data were presented as frequencies and percentages, and compared using chi-square test. Continuous data were expressed as mean ± standard deviation (SD) and compared using independent t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline data

There was no significant difference in gender, age, body mass index (BMI), type of ACS, smoking history as well as drinking history between study and control groups ($p > 0.05$) (Table 1).

Levels of P-selectin and hs-CRP

There was no significant difference in P-selectin and hs-CRP levels before treatment ($p > 0.05$) in both groups. However, P-selectin and hs-CRP levels reduced significantly ($p < 0.0001$) in both groups after treatment with a more significant decrease observed in the study group ($p < 0.0001$) (Figure 1).

Inflammatory factors

There was no significant difference in IL-6, IL-8 as well as TNF-α levels before treatment ($p > 0.05$). However, IL-6, IL-8 and TNF-α levels

Table 1: Baseline data of the two groups

Variable	Sub-factor	Study (n=118)	Control (n=80)	χ^2 value	*P*-value
Age	< 55 years old	50	28	0.950	0.330
	≥55 years old	68	51		
Gender	Male	61	45		
	Female	57	35	0.398	0.528
BMI	≥23kg/m^2	60	46	0.848	0.357
	<23kg/m^2	58	34		
Types of ACS	ST-segment elevation	49	38	0.691	0.406
	Non-ST segment elevation	69	42		
Smoking history	Yes	58	42		
	No	60	38	0.214	0.644
Drinking history	Yes	68	50	0.470	0.493
	No	50	30		
Killip classification of cardiac function	Class I-II	73	48	0.070	0.792
	Class III	45	32		

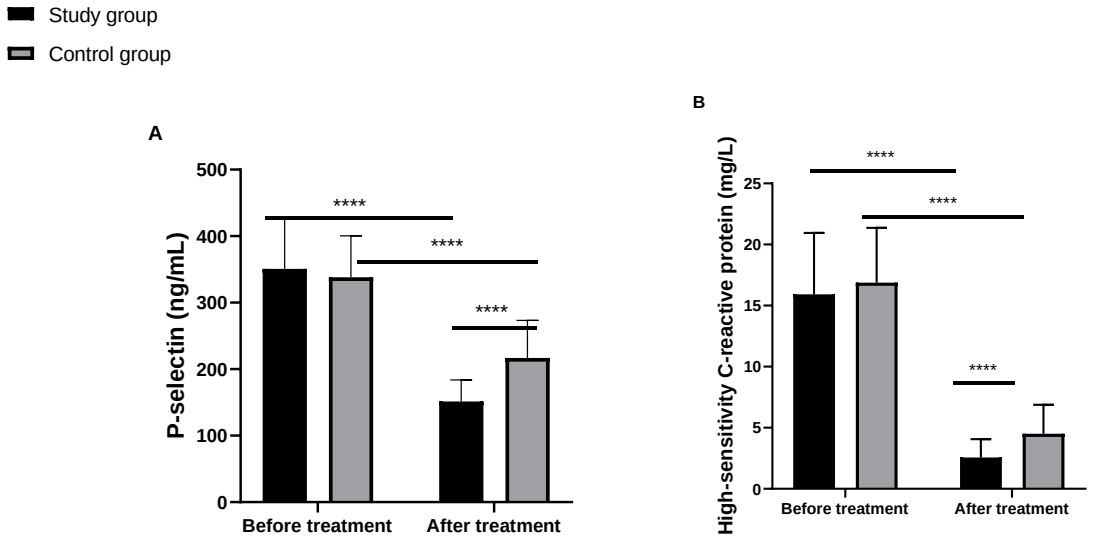

Figure 1: Levels of P-selectin and hs-CRP between the two groups. A: Comparison of P-selectin between the two groups before and after treatment. B: Comparison of hs-CRP between the two groups before and after treatment. ****P < 0.0001

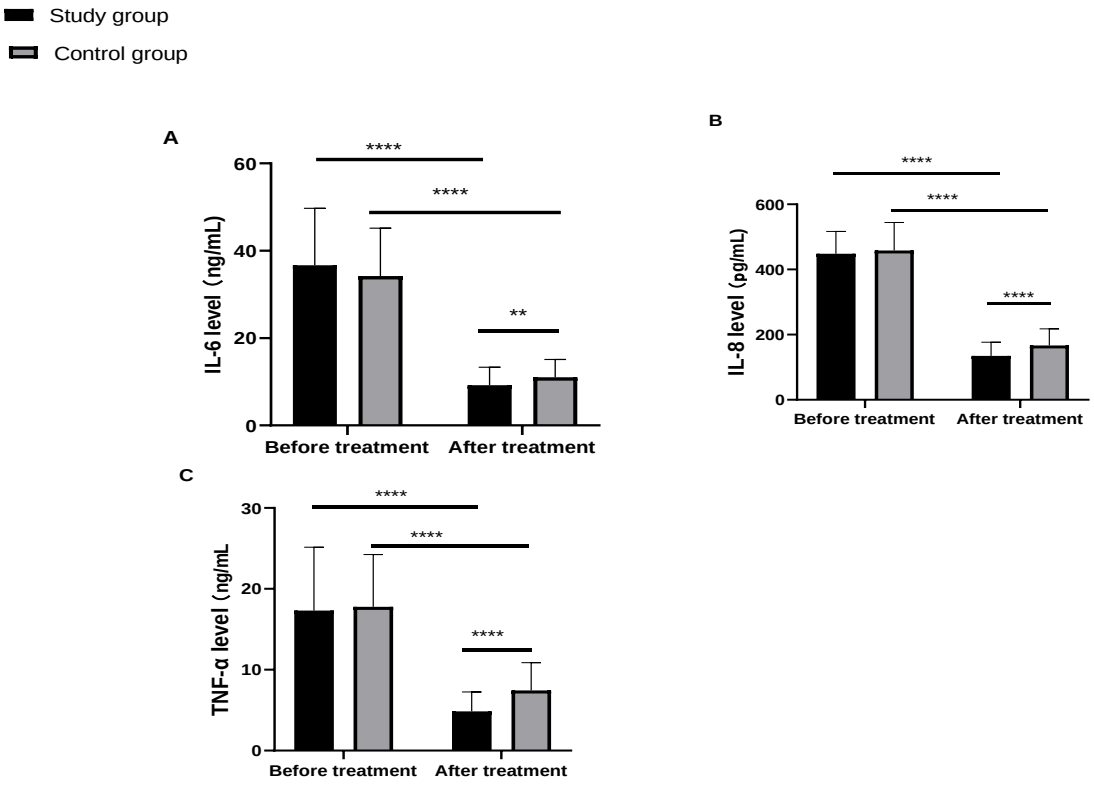

Figure 2: Inflammatory factors between the two groups. A: Comparison of IL-6 between the two groups before and after treatment. B: Comparison of IL-8 between the two groups before and after treatment. C: Comparison of TNF-α between the two groups before and after treatment. **P < 0.001, ****p < 0.0001

significantly reduced (p < 0.0001) after treatment with more significant decrease observed in study group (p < 0.001) (Figure 2).

Efficacy

The study group had significantly higher response rate compared to control group (p < 0.05; Table 2).

Table 2: Efficacy in the two groups (n, %)

Group	Markedly effective	Effective	Ineffective	Overall response rate (%)
Study (n=118)	78(66.10)	33(27.97)	7(5.93)	111(94.07)
Control (n=80)	33(41.25)	27(33.75)	20(25.00)	60(75.00)
χ^2 value				14.721
P-value				0.0001

Table 3: Incidence of adverse reactions (n, %)

Group	Dry mouth	Nausea and vomiting	Rash	Total adverse reactions
Study (n=118)	8(6.78)	4(3.39)	3(2.54)	15(12.71)
Control (n=80)	4(5.00)	4(5.00)	0(0.00)	8(10.00)
χ^2 value				0.341
P-value				0.559

Incidence of adverse reactions

There was no significant difference in the incidence of adverse reactions between study and control groups ($p > 0.05$) (Table 3).

Related factors affecting prognosis

Myocardial infarction and death within 1 year after treatment was taken as criteria for unfavourable prognosis. Patients with either of them after treatment were assigned to the unfavourable prognosis group (n = 55), and the others to the favourable prognosis group (n = 143). Clinical data such as age, smoking history, drinking history and Killip classification of cardiac function were compared as risk factors affecting prognosis (Table 4). Indicators with significant differences were subjected to multivariate analysis (Table 5) and the results revealed that smoking history and Killip classification of cardiac function were independent risk factors affecting prognosis (Table 6).

Table 4: Univariate analysis data

Variable		Good prognosis group (n= 43)	Poor prognosis group (n=55)	χ^2 value	*P*-value
Age	< 55 years old	69	9	16.921	< 0.001
	≥ 55 years old	74	46		
Gender	Male	81	25	1.999	0.157
	Female	62	30		
BMI	≥ 23 kg/m^2	72	34	2.100	0.147
	< 23 kg/m^2	71	21		
Types of ACS	ST-segment elevation	60	27	0.821	0.365
	Non-ST segment elevation	83	28		
Smoking history	Yes	65	36		
	No	78	19	6.358	0.012
Drinking history	Yes	73	45	15.621	< 0.001
	No	70	10		
Killip classification of cardiac function	Class I-II	101	20	19.621	< 0.001
	Class III	42	35		

Table 5: Assignment of variables

Variable	Assignment
Age	< 55 years old = 0, ≥ 55 years old = 1,
Smoking history	No = 0, Yes = 1.
Drinking history	No = 0, Yes = 1.
Killip classification of cardiac function	Class I-II = 0, Class III = 1.
Prognosis	Favourable prognosis = 0, unfavourable prognosis = 1.

Table 6: Multivariate analysis results

Item	B	SE	Wals	df	Sig	Exp (B)	95% C.I. of EXP(B)	
							Lower limit	*Upper limit*
Age	2.037	1.458	1.952	1	0.162	7.667	0.440	133.540
Smoking history	-3.253	1.131	8.271	1	0.004*	0.039	0.004	0.355
Drinking history	0.118	1.495	0.006	1	0.937	1.125	0.060	21.087
Killip classification	2.953	1.047	7.958	1	0.005*	19.167	2.463	149.146

Note: B: regression coefficient; SE: standard error; Wals: Wald statistic; df: degree of freedom; Sig: significance level; Exp (B): exponentiated regression coefficient. $^{*}P < 0.05$

DISCUSSION

Acute coronary syndrome (ACS) is one cause of death among coronary heart disease patients [13]. It is triggered by acute myocardial ischemia and thrombosis due to loosening or rupture of coronary atherosclerotic plaques, which results in several symptoms such as sternal pain, tightness, squeezing, and dyspnea [14]. Currently, therapy is mainly achieved through anti-platelet aggregation and plaque stabilization to reduce thrombosis and cardiovascular effects [15]. Currently, several types of antiplatelet drugs are available in clinical practice which are useful in ACS management. In order to effectively improve prognosis, the selection of the appropriate antiplatelet drug has to be done carefully and systematically [16].

Aspirin, a salicylic acid derivative, substantially inhibits platelet aggregation and controls thrombosis from the source [17,18]. Additionally, a large body of evidence clearly shows that clopidogrel, an antiplatelet drug, helps reduce mortality, and recurrence of cardiovascular events, and increases coronary artery patency [19,20]. Thus, this study investigated the efficacy of aspirin combined with clopidogrel in patients with ACS by evaluating hs-CRP and P-selectin levels. As a member of the family of selectin adhesion molecules, P-selectin is expressed by platelets and vascular endothelial cells after stimulation, and it is a frequently adopted therapeutic target for inflammation, cardiovascular diseases as well as tumor metastasis [21,22]. The Hs-CRP (synthesized in the liver) is a nonspecific marker of the acute phase of systemic inflammatory reaction, and a strong predictor of cardiovascular risk factors [23,24].

In this study, after treatment, P-selectin and hs-CRP levels in study and control groups were significantly reduced. However, study group showed more significant reduction compared to control group. The result shows that combination of aspirin and clopidogrel lowers P-selectin and hs-CRP levels in platelets more effectively than aspirin alone to achieve antithrombosis effect. In addition, there was no significant decrease in IL-6, IL-8 as well as TNF-α levels before treatment. However, IL-6, IL-8 as well and TNF-α significantly reduced after treatment in both groups with the study group showing significantly lower levels compared to control group. The findings imply that aspirin in combination with clopidogrel suppresses the release of these inflammatory factors, activation and proliferation of cells, and weakens the inflammatory response. In this study, there was no significant difference in the incidence of adverse reactions in both groups, implying that the combined application of aspirin and clopidogrel does not greatly worsen adverse reactions in patients. In a previous study, clopidogrel combined with aspirin was significantly more effective than placebo combined with aspirin in patients with extensive ACS [25]. Furthermore, study group had significantly higher overall response rate compared to control group, implying a higher efficacy of the combined medication in the treatment of ACS. Finally, this study also analyzed the prognostic factors of patients with ACS. The results showed that age, smoking history, drinking history and Killip classification of cardiac function were the risk factors affecting prognosis. More specifically, smoking history and Killip classification of cardiac function were the independent risk factors that affected patients' prognosis.

Limitations of this study

The limited sample size of this study may have affect the reliability of the results. In addition, the study did not investigate the effect of different doses of the combined drugs on ACS.

CONCLUSION

Aspirin combined with clopidogrel significantly lowers P-selectin and hs-CRP levels, without worsening adverse reactions. In addition, smoking history and Killip classification of cardiac function are independent risk factors affecting the prognosis of ACS patients. A more complete and comprehensive analysis of the combined use of aspirin with clopidogrel in the treatment of ACS is recommended.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

The study was approved by the Ethics Committee of Zhaotong Traditional Chinese Medicine Hospital (approval no. ztszllsc2021015).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Renfen Xie and Shengwang Xu conceived and designed the study, and drafted the manuscript. Daojiao Ao, Yalan Wang and Junmin Huang collected, analyzed and interpreted the experimental data. Renfen Xie, Shengwang Xu and Junmin Huang revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

REFERENCES

1. *Karimi Galougahi K, Shlofmitz E, Jeremias A, Petrossian G, Mintz GS, Maehara A, Shlofmitz R, Ali ZA. Optical coherence tomography in acute coronary syndromes. Interv Cardiol Clin 2021; 10: 323-332.*
2. *Bergmark BA, Mathenge N, Merlini PA, Lawrence-Wright MB, Giugliano RP. Acute coronary syndromes. Lancet 2022; 399: 1347-1358.*
3. *Babes EE, Bustea C, Behl T, Abdel-Daim MM, Nechifor AC, Stoicescu M, Brisc CM, Moisi M, Gitea D, Iovanovici DC, et al. Acute coronary syndromes in diabetic patients, outcome, revascularization, and antithrombotic therapy. Biomed Pharmacother 2022; 148: 112772.*
4. *Crea F, Libby P. Acute coronary syndromes: The way forward from mechanisms to precision treatment. Circulation 2017; 136: 1155-1166.*
5. *Brener SJ, Mehran R, Lansky AJ, Ayele GM, Stone GW. Pretreatment with aspirin in acute coronary syndromes: Lessons from the ACUITY and HORIZONS-AMI trials. Eur Heart J Acute Cardiovasc Care 2016; 5: 449-454.*
6. *Jacobsen AP, Raber I, McCarthy CP, Blumenthal RS, Bhatt DL, Cusack RW, Serruys P, Wijns W, McEvoy JW. Lifelong aspirin for all in the secondary prevention of chronic coronary syndrome: Still sacrosanct or is reappraisal warranted? Circulation 2020; 142: 1579-1590.*
7. *Zhang Y, Zhao Y, Wang M, Wang J, Yang Z, Lu M. Effect of clopidogrel on post-extraction clotting in patients on dual antiplatelet therapy. Trop J Pharm Res 2023; 22(1):189-197.*
8. *Shao J, Chen W. Mitigating effect of clopidogrel and systematic management on adverse events after interventional therapy for coronary heart disease. Trop J Pharm Res 2022; 21(2): 387-392.*
9. *Zhang L, Lin Z, Yin H, Liu J, Xuan J. Clopidogrel versus aspirin for the treatment of acute coronary syndrome after a 12-month dual antiplatelet therapy: A cost-effectiveness analysis from China payer's perspective. Clin Ther 2018; 40: 2125-2137.*
10. *World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310: 2191-2194.*
11. *Smith JN, Negrelli JM, Manek MB, Hawes EM, Viera AJ. Diagnosis and management of acute coronary syndrome: an evidence-based update. J Am Board Fam Med 2015; 28: 283-293.*
12. *Razavi E, Ramezani A, Kazemi A, Attar A. Effect of treatment with colchicine after acute coronary syndrome on major cardiovascular events: A systematic review and meta-analysis of clinical trials. Cardiovasc Ther 2022; 2022: 8317011.*
13. *Wang L, Jin Y. Noncoding RNAs as biomarkers for acute coronary syndrome. Biomed Res Int 2020; 2020: 3298696.*
14. *Damluji AA, Forman DE, Wang TY, Chikwe J, Kunadian V, Rich MW, Young BA, Page RL 2nd, DeVon HA,*

Alexander KP, et al. Management of acute coronary syndrome in the older adult population: A scientific statement from the American Heart Association. Circulation 2023; 147: e32-e62.

15. *Atwood J. Management of acute coronary syndrome. Emerg Med Clin North Am 2022; 40: 693-706.*
16. *Cole KL, Findlay MC, Kundu M, Johansen C, Rawanduzy C, Lucke-Wold B. The role of advanced imaging in neurosurgical diagnosis. J Mod Med Imag 2023; 1: 2.*
17. *Soodi D, VanWormer JJ, Rezkalla SH. Aspirin in primary: Prevention of Cardiovascular Events. Clin Med Res 2020; 18: 89-94.*
18. *Murphy E, Curneen JMG, McEvoy JW. Aspirin in the modern era of cardiovascular disease prevention. Methodist Debakey Cardiovasc J 2021; 17: 36-47.*
19. *Amsterdam EA. Clopidogrel in the management of acute coronary syndromes: indications, results, obstacles. Crit Pathw Cardiol 2009; 8: 49-54.*
20. *Pereira NL, Rihal CS, So DYF, Rosenberg Y, Lennon RJ, Mathew V, Goodman SG, Weinshilboum RM, Wang L, Baudhuin LM, et al. Clopidogrel pharmacogenetics. Circ Cardiovasc Interv 2019; 12: e007811.*
21. *Ludwig RJ, Schon MP, Boehncke WH. P-selectin: a common therapeutic target for cardiovascular disorders, inflammation and tumour metastasis. Expert Opin Ther Targets 2007; 11: 1103-1117.*
22. *Amrutkar RD, Bhamare VG, Kapse SN. Chemogenomics: Is a promising area for drug target and discovery. J Mod Biol Drug Discov 2023; 2: 2.*
23. *Coner A, Aydinalp A, Muderrisoglu H. Evaluation of hs-CRP and sLOX-1 levels in moderate-to-high risk acute coronary syndromes. Endocr Metab Immune Disord Drug Targets 2020; 20: 96-103.*
24. *Denegri A, Boriani G. High sensitivity C-reactive Protein (hsCRP) and its Implications in cardiovascular outcomes. Curr Pharm Des 2021; 27: 263-275.*
25. *Main C, Palmer S, Griffin S, Jones L, Orton V, Sculpher M, Henderson R, Sudlow C, Hawkins N, Riemsma R. Clopidogrel used in combination with aspirin compared with aspirin alone in the treatment of non-ST-segment-elevation acute coronary syndromes: A systematic review and economic evaluation. Health Technol Assess 2004; 8: iii-iv, xv-xvi, 1-141.*