Analysis of the Plasma Concentration of Interleukin-37 in patients suffering from Acute Myocardial Infarction: A case-control study

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A thesis submitted to the Department of Mathematics & Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted, titled "**Analysis of the Plasma Concentration of Interleukin-37 in patients suffering from Acute Myocardial Infarction: A case-control study,**" is our own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

The thesis titled **"Analysis of the Plasma Concentration of Interleukin-37 in patients suffering from Acute Myocardial Infarction: A case-control study,"** submitted by

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Of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 4^{th} September 2025.

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Ethics Statement

The study was conducted in compliance with institutional and international ethical standards. Blood samples were collected from patients at Sher-E-Bangla Medical College Hospital and Dhaka city with full adherence to established ethical guidelines. Ethical approval was obtained and granted by the hospital's review board prior to the initiation of the study. The institutional review board meticulously reviewed and approved the research protocols to ensure compliance with the National Guidelines on Health Research and the International Guidelines for Ethical Considerations in Research.

Furthermore, the study underwent review and received approval from the Ethics and Research Review Committee of the Department of Mathematics and Natural Sciences, MNS, at BRAC University, Dhaka, Bangladesh. The research was conducted under the supervision of Dr.Nadia Sultana Deen, with all aspects of the study aligning with the ethical guidelines set forth by BRAC University.

Informed consent was obtained from the patients before the collection of samples. Patients were thoroughly informed about the study's objectives, the procedures involved, potential risks, and the benefits. Data confidentiality and patients' anonymity were rigorously maintained, with all personal identifiers being removed during data analysis.

This robust ethical framework underpins the study's integrity and validity, ensuring adherence to internationally recognized norms and principles for ethical research.

Acknowledgement

We would like to begin by expressing our deepest gratitude to Almighty Allah for granting us the opportunity to undertake and complete this research. Our heartfelt thanks go to our parents, whose unwavering support has been invaluable from the inception to the completion of this study.

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Abstract

Acute Myocardial Infarction(AMI) is one of the leading causes of morbidity and mortality throughout the world. The regulation of several pro-inflammatory and anti-inflammatory cytokines plays a crucial role during myocardial infarction. Interleukin-37 is one such anti-inflammatory cytokine. This case-control study aimed to investigate the concentration of plasma IL-37 between patients suffering from AMI compared with control groups. This study included 40 AMI patients (STEMI=20 and NSTEMI=20) as the case group and 20 healthy individuals as the control group. Along with demographic and clinical information, Lipid profile tests, Troponin I, and CRP tests were performed from subjects' blood samples. Plasma was isolated to perform an ELISA test for measuring the concentration of IL-37, and clinical experimental data were analyzed statistically. Positive correlation was found between CRP level and IL-37 concentration in the case group (rho=0.3), clearly suggesting anti-inflammatory activity elevated in AMI patients as the disease progressed. However, there was no statistical significance(P=0.87>0.05) in the case of comparing the concentration of IL-37 between the case and control groups. CRP and Troponin I values were very high in the case group compared with the control group, indicating the continuous inflammation in the case subjects and inducing the activity of IL-37. Based on the correlation between IL-37 concentration and CRP value found in the subject group of this study, further large-scale research can pave the way to assess the anti-inflammatory role of IL-37 and its potential use as a biomarker and therapeutic agent that can help the clinician to manage cardiovascular diseases efficiently.

Keywords: AMI; Interleukin-37; Troponin I; STEMI; NSTEMI; ELISA.

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List of Acronyms

AMI	Acute Myocardial Infarction
ACS	Acute Coronary Syndrome
CVD	Cardiovascular Disease
IL	Intereukin
IL-1F7	IL-1 family member 7
IL-18Rα	Interleukin 18 Receptor Alpha
IL-1R8	Interleukin-1 receptor 8
IL-18BP	Interleukin 18 Binding Protein
SMAD3	Mothers against decapentaplegic homolog 3
NF-κB	Nuclear factor kappa B
MAPK	Mitogen-activated protein kinase
ERK	Extracellular Signal-Regulated Kinase
TNF-α	Tumor Necrosis Factor-Alpha
DCs	Dendritic Cells
Th17	T helper 17
SAP	Stable angina pectoris
STEMI	ST-elevation myocardial infarction
NSTEMI	Non-ST-elevation Myocardial Infarction
PCI	Percutaneous Coronary Intervention
ECG	Electrocardiography
TG	Triglyceride
TC	Total cholesterol

LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
ACEI	Angiotensin-Converting Enzyme inhibitors
cTnI	Cardiac Troponin I
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay

Chapter 1

Introduction

1.1. Background

Cardiovascular diseases (CVDs) are responsible for the majority of deaths worldwide, with approximately 18 million fatalities each year (World Health Organization, 2022). Acute myocardial infarction (AMI) with or without ST-segment elevation (STEMI or NSTEMI) is critically manifested as acute coronary syndrome (ACS), a form of cardiovascular disease (CVDs). Despite the development in the reperfusion interventions and medical treatment, e.g., percutaneous coronary intervention (PCI), AMI represents a significant health issue resulting in high morbidity and mortality (Benjamin et al., 2019; Roth et al., 2020). AMI develops following the acute occlusion of a coronary artery through mostly the rupture of an atherosclerotic plaque and the subsequent thrombosis, which causes myocardial ischemia and necrosis (Thygesen et al., 2018).

1.2. The Role of Inflammation in AMI

Inflammation is a critical factor in the pathogenesis and progression of AMI, which induces instability of the plaque, thrombosis, and post-infarction remodeling (Libby et al., 2019). After AMI, a systemic inflammatory response occurs, including the secretion of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor (TNF) that contribute to intensified myocardial injury and favor detrimental ventricular remodeling (Frangogiannis, 2014). Innate immune cells, such as monocytes, macrophages, and dendritic cells (DCs) mediate this inflammatory cascade and infiltrate the infarcted myocardium to enhance the response (Swirski and Nahrendorf, 2013). High levels of inflammatory markers such as C-reactive protein (CRP), troponin I are common predictors of ACS severity and prognosis, with CRP being a marker of systemic inflammation, and cTnI stipulating cardiac muscle deterioration (Danesh et al., 2008; Thygesen et al., 2018). Anti-inflammatory therapies have been suggested to be potentially viable therapeutic mechanisms to alleviate such effects, as

shown by trials of canakinumab, an IL-1b blocker , which decreases recurrent cardiovascular events in post-AMI patients (Ridker et al., 2017).

1.3. Interleukin-37: Structure, Function, and Pathways

Interleukin-37 (IL-37), also referred to as IL-1 family member 7 (IL-1F7), is a new anti-inflammatory cytokine of the IL-1 ligand family, with 11 other members (Dinarello et al., 2016). In contrast to other cytokines of the IL-1 family, IL-37 is particularly unique in that it prevents both innate and adaptive immune responses, and it represents the natural inhibitor of the inflammatory process (Nold et al., 2010). IL-37 has five splice variants (a-e), with IL-37b as the most common isoform in the peripheral blood (Boraschi et al., 2011). It is reported to be expressed at low concentrations in healthy subjects, mainly in peripheral blood mononuclear cells (PBMCs), monocytes, DCs, and macrophages, but it quickly increases during inflammatory situations (Nold-Petry et al., 2015).

The anti-inflammatory effects of IL-37 follow both intracellular and extracellular mechanisms. Via the intracellular pathway, IL-37 binds with SMAD3, translocating to the nucleus and suppressing the expression of pro-inflammatory genes. Extracellularly, IL-37 binds with IL-18Rα and IL-1R8, suppressing NF-κB and MAPK/ERK pathways, thereby reducing the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Nold et al., 2010). Moreover, foam cells of atherosclerotic plaques of coronary and carotid arteries express IL-37, which indicates its role in the stabilization of plaques (Boraschi et al., 2011).

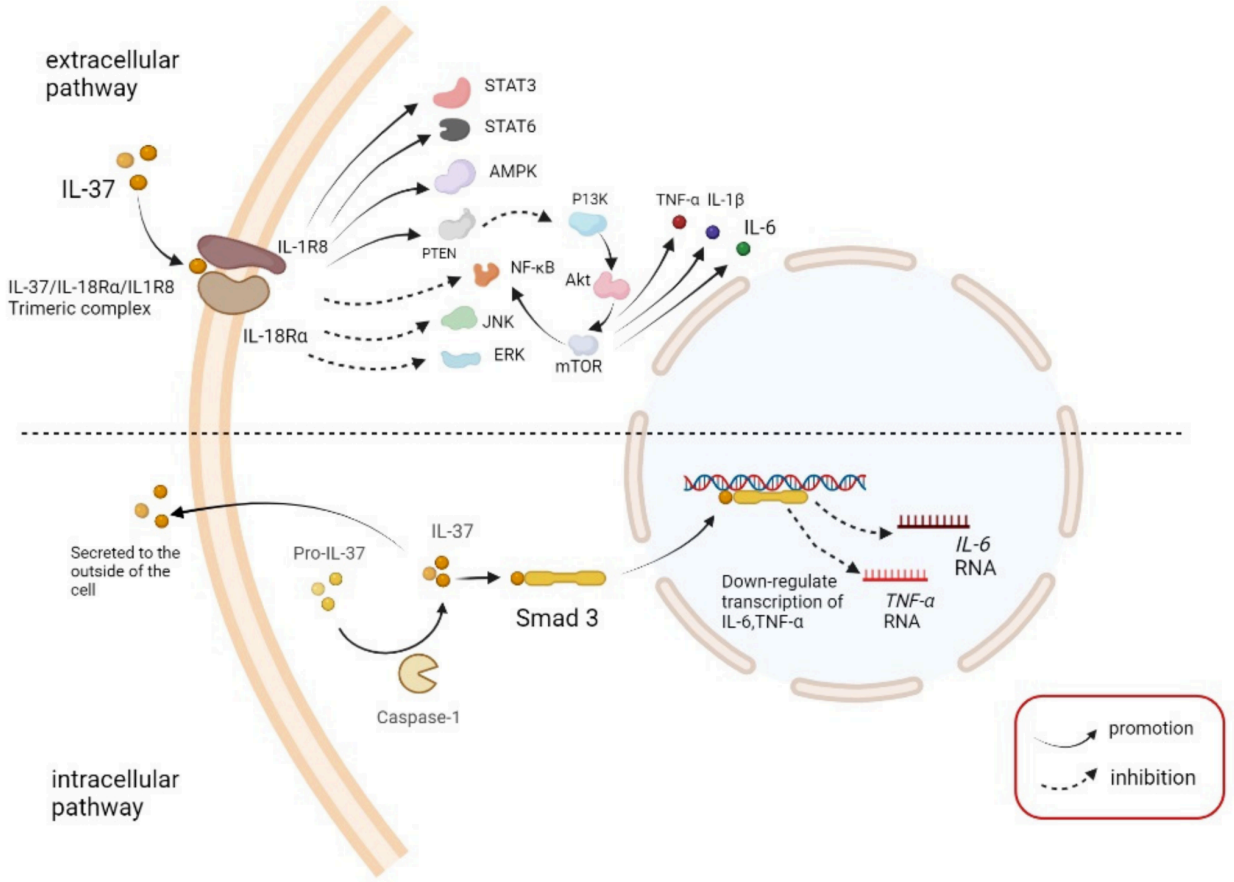

Figure 1.3. Pathway through which Interleukin-37 (IL-37) exerts anti-inflammatory effects(Li et al., 2022).

1.4. IL-37 in AMI

Early research suggests that plasma IL-37 levels are highly elevated in AMI patients as opposed to healthy controls or stable angina pectoris (SAP). Indicatively, in a cohort of ACS patients including patients with ST-elevation myocardial infarction (STEMI), plasma IL-37 levels were markedly elevated in AMI compared with controls (Ji et al., 2014). Some other studies have found the peak IL-37 levels during the early post-AMI phase and reduced thereafter following PCI, indicating that there was a dynamic response to acute inflammation (Yang et al., 2015). In another study, plasma and leukocytic IL-37 were in decline in STEMI patients post-PCI(Wang et al., 2015). These results are supported through experimental models, which reveal that IL-37 treatment reduces the size of the infarct, restricts neutrophil migration, and leads to an increase in cardiac function after ischemia-reperfusion injury (Xu et al., 2015).

Nevertheless, other studies demonstrate the reduced expression of IL-37 in ACS, where less expression is linked with unfavorable outcomes, which implies that incomplete responses to IL-37 can exacerbate the situation (Liu et al., 2021). Such inconsistencies reveal that the IL-37 regulation in the ACS is complicated and can be contingent upon the stage or severity of a disease.

1.5. Correlation Between IL-37 and Clinical Markers in AMI

The IL-37 in AMI patients also exhibits correlations with some cardiac and inflammatory indicators, which confirms its role in disease modulation.. Plasma IL-37 is positively correlated with cTnI and with CRP, suggesting that myocardial damage and systemic inflammation are both linked to it (Ji et al., 2014; Yang et al., 2015). Higher IL-37 levels in ACS cohorts were associated with a higher ratio of IL-18 and IL-18 binding protein (IL-18BP), indicating a remedial anti-inflammatory response (Ji et al., 2014). The IL-37 suppressing Th17 cell differentiation in PBMCs in AMI patients in vitro inhibits the production of pro-inflammatory cytokines (Mao et al., 2019). These associations demonstrate the possibility of using IL-37 in the diagnosis and treatment of AMI severity as a biomarker and treatment with anti-inflammatory agents.

1.6. Rationale and Objectives

Inflammation is central to the pathophysiology of AMI, and recent research indicates that IL-37 is a potent anti-inflammatory cytokine that suggests that the study of the expression of IL-37 in AMI patients is clinically significant. The plasma IL-37 concentration may serve to explain the role of IL-37 as a mediator of early inflammatory response to AMI. Additionally, the comparison of the correlations of IL-37, cTnI, and CRP can be used in the interpretation of whether IL-37 is a regulator of myocardial injury and systemic inflammation.

This study aims to (1) find out the concentration of plasma IL-37 at an early stage of post-AMI, and (2) determine the associations that exist among IL-37, cTnI and CRP. The study would be useful in examining the potential of IL-37 as a biomarker and therapeutic target in order to lessen inflammation and improve outcomes in MI patients.

Chapter 2

Materials and Methods

2.1. Study Design

The study was designed as a case-control study: 40 patients suffering from AMI, and 20 healthy individuals were recruited as the cases and controls, respectively. Both cases and controls were selected based on specific exclusion and inclusion criteria. AMI subjects were divided into two groups: ST-elevation myocardial infarction (STEMI) and non-elevation myocardial infarction (NSTEMI). Samples of cases were collected from a tertiary hospital, and samples of controls were collected from different regions of Dhaka city. Inclusion criteria for cases were: AMI patients above 40 years of age, and admitted to the hospital within 48 hours of onset of symptoms, STEMI and NSTEMI identified by Electrocardiogram (ECG) and a significant increase in Troponin I (TnI) and C-reactive protein (CRP) levels (Mao et al., 2019). Exclusion criteria for cases were: AMI patients admitted to the hospital after 48 hours of onset of symptoms, with a previous history of cardiovascular diseases, strokes, and patients who underwent any revascularization procedures such as PCI, Coronary artery bypass surgery (CABG). Patients with autoimmune diseases, systemic or local infection at the time of hospital admission, or infectious diseases, previous history of pacemaker transplant, diagnosed with cancer, or liver or kidney diseases were excluded from the study (Wang et al., 2015). Subjects above 40 years with no history of cardiovascular diseases or strokes, a normal level of TnI, and a CRP value were included as controls(Yang et al.,2016). Biochemical and biomarker tests were performed using automatic analysers. An ELISA test was performed to quantify the concentration of plasma IL-37. All the demographic and clinical data were collected using a questionnaire from all of the participants of this study. The questionnaire is presented in **Table 1.**

Table 1. Questionnaire for the study participants

Title: Evaluation of the role of biochemical, immunological, and inflammatory molecules in patients with cardiovascular diseases and metabolic disorders.

ID:

Demographic Information

01.	Age				
02.	Gender	Male	Female		
03.	Residence				
04.	Educational Qualification	Less than SSC / SSC or equivalent / HSC or equivalent / Bachelor's or equivalent / Master's or equivalent / More than Masters			
05.	Occupation				
06.	Marital Status	Single	Married	Prefer not to say	

Clinical Information

01.	Weight						
02.	Height						
03.	Body Mass Index (BMI)						
04.	Waist-to-hip ratio						
05.	Do you smoke?	Yes	No	Remarks:			
06.	Do you consume alcohol?	Yes	No	Remarks:			
07.	Do you consume coffee/tea regularly?	Yes	No	Remarks:			
09.	Diet	Vegetarian		Non-vegetarian		Remarks:	
10.	How often do you do physical exercises?	Daily		Several times a week	Once a week	Rarely	Never
11.	Systolic/ Diastolic blood pressure (mmHg)						
12.	Heart rate (beats/min)						
13.	Do you suffer from hypertension?	Yes	No	Remarks:			
14.	Are you diabetic?	Yes	No	Remarks:			
15.	Do you have dyslipidaemia/hyperlipidaemia?	Yes	No	Remarks:			
16.	Do you have a family history of any cardiovascular diseases?	Yes	No	Remarks:			
17.	Do you have any family members with or who has ever had a stroke?	Yes	No	Remarks:			
18.	Did you previously suffer from any cardiovascular diseases?	Yes	No	Remarks:			
19.	Do you suffer from any autoimmune disease?	Yes	No	Remarks:			
20.	Are you suffering from any infectious disease?	Yes	No	Remarks:			

21.	Any local/systemic inflammation for a period of one week	Yes	No	Remarks:
22.	Are you suffering/recovered from any malignant diseases? *	Yes	No	Remarks:
23.	Did you go through any revascularization procedure? (CABG,PCI)	Yes	No	Remarks:
24.	Do you have a pacemaker transplant? *	Yes	No	Remarks:
25.	Did you previously suffer from a stroke? *	Yes	No	Remarks:

Pre-admission and Post-admission Clinical Information

What symptoms did you have before admission?	
What was the duration between the first onset of symptoms and admission?	
What initial tests were prescribed for diagnosis after admission?	
Which drug(s) was(were) suggested right after admission?	
Have you made any lifestyle changes since your stroke to reduce your risk of future strokes? If yes, please specify the changes you've made (diet, exercise, medication adherence, etc.).	

Laboratory findings

Parameter (unit)	Concentration	Parameters (unit)	Concentration
Total Cholesterol (mmol/L)		CRP/Hs-CRP (mg/l)	
Triglyceride (mmol/L)		CK-MB (μg/L)	
LDL-C (mmol/L)		cTnI/Hs-cTnI (ng/L)	
HDL-C (mmol/L)		NT-proBNP (pg/mL)	
Fasting blood sugar (mg/dl)		BUN (mg/dL)	
HbA1c (%)		Sodium (mEq/L)	
Creatinine (μmol/L)		Potassium (mEq/L)	

Uric Acid (mmol/L)		Phosphorous (mmol/L)	
ALP (mmol/L)		Total Homocysteine (µmol/L)	

Which of the following drugs was/were suggested right after admission?

ACEI/ARB	Prescribed	Not Prescribed	Remarks:
Aspirin	Prescribed	Not Prescribed	Remarks:
β-blocker	Prescribed	Not Prescribed	Remarks:
Nitrate	Prescribed	Not Prescribed	Remarks:
CCB	Prescribed	Not Prescribed	Remarks:
Statin	Prescribed	Not Prescribed	Remarks:
Diuretics	Prescribed	Not Prescribed	Remarks:
Anxiolytics	Prescribed	Not Prescribed	Remarks:

Any other information that you would like to mention:

2.2. Sample Collection

Venous blood was taken from both the cases and controls for biochemical tests and an Enzyme-linked immunosorbent assay (ELISA)experiment. Written consents were taken from the participants. Venipuncture was used to obtain blood samples using sterilized, disposable syringes and needles by professional nurses. A plain red-topped tube and a sodium heparin green-topped tube were used to collect blood for biochemical and ELISA experiments, respectively. Samples were transported to the biochemistry laboratory using a sealed iced box (4°C). Sodium heparin tubes were centrifuged for 5 minutes at 3,000 rpm to separate plasma. Using a pipette, plasma samples were collected in a centrifuge tube and stored at -20°C.

2.3. Biochemical Tests

A lipid profile test was performed to measure the levels of [total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol] in the blood. For confirmation of AMI, biomarkers including TnI and CRP were examined along with an ECG. The increased level of TnI and CRP values indicated AMI.

2.4. ELISA

The concentrations of plasma IL-37 were quantified by using a commercial ELISA kit (DY1975-05, R&D Systems). Absorbance of standards and samples was determined at 450 nm, and wavelength correction was set at 540 nm using a microplate reader (BK-EL10C, Biobase). Using the kit, the minimal detectable concentrations of IL-37 were 15.6 pg/ml. Results were plotted against the standard curve. The assays were carried out according to the protocols provided by the manufacturer. All of the samples were measured in duplicates.

2.5. Statistical analyses

Four-parameter logistic regression (4PL model) was used to measure the concentration of IL-37 in both case and control samples. Firstly, a standard curve was created using the concentration of standards and absorbance. Using the standard curve and the equation, other samples were calculated. A student’s t-test was performed to compare the concentration of IL-37 between cases and controls. Additionally, the biomarker and IL-37 concentration data were compared using Spearman's rank correlation coefficient. The arithmetic mean and standard deviation were calculated for some parameters, such as age, medication, etc.

Chapter 3

Results

3.1. Demographic and clinical characteristics of Subjects

This study included 60 subjects: 40 cases (STEMI 20 and NSTEMI 20) and 20 controls. The age of the majority of study participants was between 40 to 60 years. Demographic characteristics included age, sex, physical activity, and food habits. To assess the risk factors of AMI, clinical and demographic data, e.g, family history, smoking, hypertension, hyperlipidemia, and lipid profile were collected. A summary of demographic and clinical characteristics of study subjects is presented in **Table 2.**

Table 2. Demographic and Clinical Characteristics.

Characteristics	**Case**	**Control**
Age (year), mean ± SD	56.5 ± 9.85	46.3 ± 4.57
Male, n (%)	31 (77.5)	16 (80)
Female, n (%)	9 (22.5)	4 (20)
Smoking, n (%)	14 (35)	1 (5)
Hypertension, n (%)	10 (25)	10 (50)
Diabetes, n (%)	10 (25)	6 (30)
Hyperlipidemia, n (%)	12 (30)	7 (35)
Family history of CVD, n (%)	8 (20)	4 (20)
Triglyceride (mgl/dl), mean ± SD	154.90 ± 151.53	147.52 ± 117.84

Cholesterol (mg/dl), mean±SD	209.35 ± 48.61	199.07 ± 44.97
LDL-C (mmol/l), mean±SD	138.91 ± 37	138.68 ± 42.23
HDL-C (mmol/l), mean±SD	40.93 ± 9.06	40.75 ± 9.89
ACEI /ARB takers, n (%)	12 (30)	-
Beta blocker takers, n (%)	18 (45)	4 (20)
Aspirin takers, n (%)	36 (90)	-
Statin takers, n (%)	37 (92.5)	6 (30)
Nitrate takers, n (%)	40 (100)	-
Anxiolytics takers, n (%)	35 (87.5)	-

Among 40 cases, 31 were male and 9 were female, while out of 20 controls, 16 were male and 4 were female. Thirty-five percent (35%) of the cases were smokers, and 25% of them were suffering from hypertension. In the control group, 50% of the subjects were diagnosed with hypertension. Moreover, 25% of the cases were diagnosed with diabetes, and in the case of control subjects, 30% of them are suffering from this disease. Furthermore, 20% of both the case and control groups had a previous family history of cardiovascular disease. Based on the lipid profile test results of both case and control subjects, it was observed that 30% of the subjects were suffering from hyperlipidemia, while the percentage of control subjects was a bit higher, which was 35%. The clinical treatment procedure data of case subjects suggested that all the patients were treated with the nitrate drug class, while Aspirin, Statin, and Antidepressants were broadly used for almost all the patients. Beta blockers and ACEI/ARB drug classes were used in 45% and 30% of the case subjects, respectively. 20% of the control subjects were prescribed beta-blockers, and 30% of them used Statins due to hypertension problems.

3.2. Biomarker level and IL-37 concentrations

In this study, CRP and TnI levels were measured to identify cases suffering from AMI. These tests were also performed on the healthy subjects. The average CRP value was significantly higher in the case group (24.07 ± 28.06) compared with the control group (2.59 ± 3.15). Similar findings were observed in the case of TnI.

This study aimed to measure the concentration of plasma IL-37 in patients suffering from AMI. As a result, the concentration of IL-37 was measured in both the case and control groups, following an ELISA. For ELISA data, 4-parameter logistic regression (4PL model) was used. The average concentration of IL-37 in these cases was 333.87 ± 426.52 pg/ml, whereas 453.18 ± 516.33 pg/ml in the control group. The results of biomarkers and IL-37 concentrations are presented in **Table 3.**

Table 3: Biomarker level and IL-37 concentration

Variables	**Case**	**Control**
CRP (mg/l), mean ± SD	24.07 ± 28.06	2.59 ± 3.15
Troponin I (mg/l), mean ± SD	45.21 ± 29.71	NS
Interleukin-37 (pg/ml), mean ± SD	333.87 ± 426.52	453.18 ± 516.33

3.3. Analysis of biomarker level and Interleukin-37 concentration

Between biomarkers and IL-37 in cases and controls, A student's t-test was performed to compare the concentration of IL-37 between cases and controls. Moreover, Spearman's rank correlation coefficient was used to compare the data of biomarkers and IL-37 concentration. The concentration of IL-37 in STEMI and NSTEMI patients was compared with that of controls, followed by a t-test. No significant differences were observed in IL-37 concentration between controls and STEMI group (p=0.86), and controls and NSTEMI group 0.33 (p=0.33).

Additionally, there was no statistically significant difference in IL-37 concentration between STEMI and NSTEMI groups(*P*= 0.86).

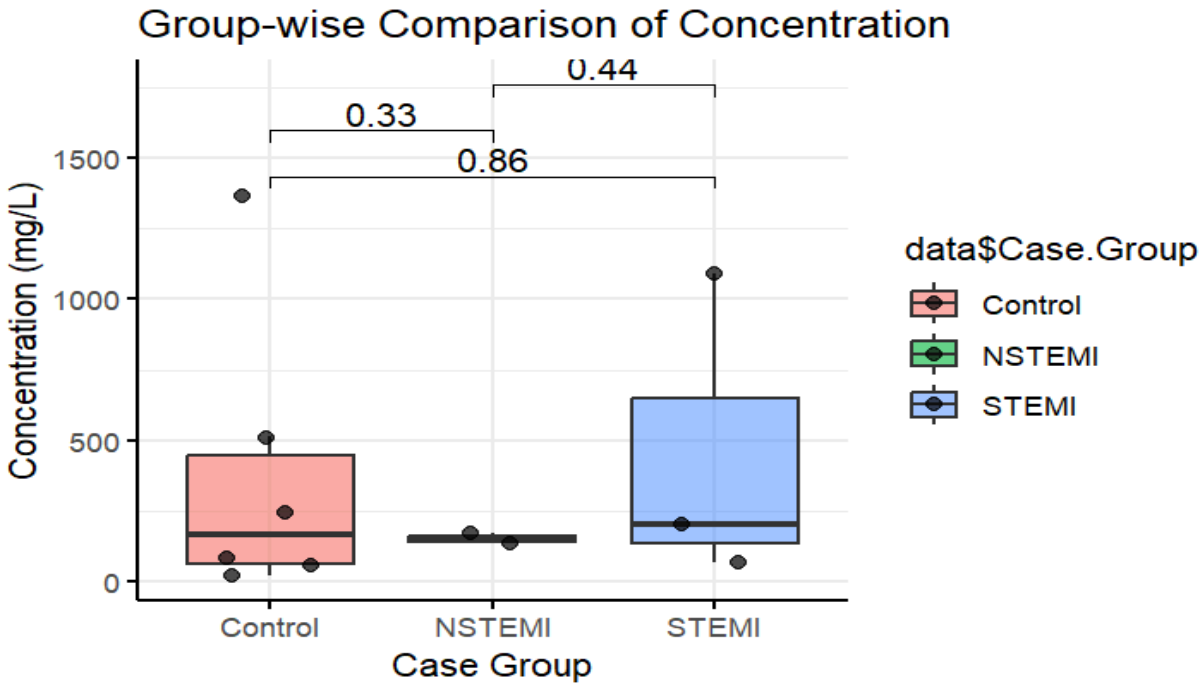

Figure 3.3.1: Relationship between the concentration of IL-37 in the case and control groups.

Spearman's rank correlation coefficient was calculated between the CRP value and the concentration of IL-37 in the case group. Following the calculation, it was found that the "r" value was 0.3 (p=0.68). These results suggested that there was a weaker correlation between the concentration of IL-37 and CRP values.

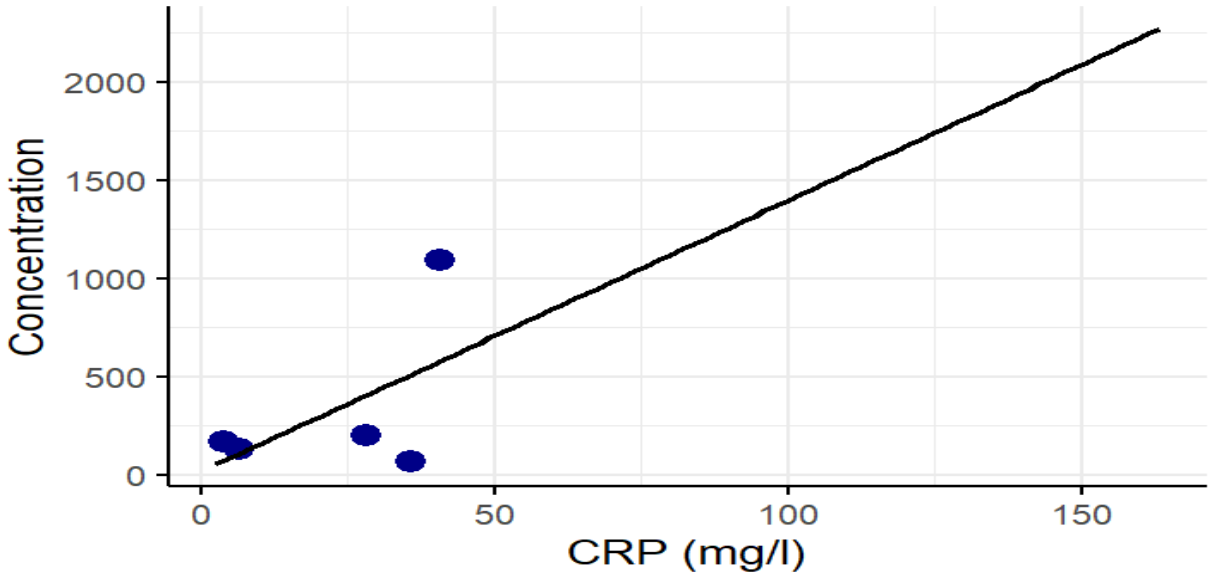

Figure 3.3.2: Spearman's rank correlation between IL-37 concentration and CRP in the case group.

Chapter 4

Discussion

The purpose of this case-control study was to compare the concentration of IL-37 between patients suffering from STEMI and NSTEMI and healthy individuals. This study focused on several demographic and clinical characteristics of cases and controls to assess their risk factors that may lead them to AMI. Thirty-five percent (35%) of the cases were found to be smokers, while 25% of them suffered from hypertension that might lead to the event of AMI. Meanwhile, 50% of the participants in the controls were suffering from hypertension, which indicates a high risk of future AMI or stroke events. Moreover, case and control groups had almost similar rates of hyperlipidemia, a highly considered risk factor, with proportions of 30% and 35% respectively. However, clinical treatment data revealed that all AMI patients were treated with Nitrate drug classes, while aspirin, statin, and anxiolytic drug classes were prescribed significantly. It was observed that the controls suffering from hypertension tend to take statin and beta-blocker drug classes. Biomarker tests that include CRP and TnI were conducted for both case and control groups. The test results of the biomarker showed the cases had higher levels of Troponin (45.21 ± 29.71 mg/l), suggesting significant inflammation while suffering from AMI. Therefore, the level of TnI in the control group had no significant result, which indicates a normal heart condition. Similarly, the study found that the CRP levels were significantly higher in the case group (24.07 ± 28.06 mg/l) compared to the control group (2.59 ± 3.15 mg/l), verifying the inflammatory activity in AMI. However, the concentration of IL-37 in the case group was 333.87 ± 426.52 pg/ml and 453.18 ± 516.33 pg/ml in the control group. The results of IL-37 concentration contradict with similar previously conducted by K. Liu et al. (2017) and Ji et al. (2014). These previous studies suggested that IL-37 concentrations were significantly higher in AMI patients compared to healthy individuals. Sample size could be a key factor for the contradiction between previous work and our study findings. While IL-37 concentrations in some of the subjects were similar to what observed in previous studies, in some other samples (both cases and controls), the concentrations were much higher, which might interfere with the overall mean concentration of IL–37. The possible reasons for this finding could be a lack of rigorous pre-clinical information given by the study subjects, possible contamination during

sample collection, improper handling of the ELISA kit during the importing period from the producers, which affected the reagent. However, the Spearman's correlation coefficient of this study suggested a weaker correlation (rho=0.3) between CRP and the concentration of IL-37 in the case group. This finding may indicate that the concentration of IL-37 is linked to inflammation in AMI patients. Moreover, it was observed that TnI and CRP values were elevated in all the cases. However, there was no statistical significance in IL-37 concentration between the control group and case group. The *p* value between the control group and STEMI was 0.86, whereas the control group vs NSTEMI was 0.33 (*P*> 0.05). Considering STEMI and NSTEMI as the case group, the *P* value was 0.87(P> 0.05).

Our study has some limitations. First of all, the sample size was small considering the prevalence rate of AMI in Bangladesh, which limited the analysis of statistical significance. Selection bias could be a reason, as the case and control groups were selected from different locations. Diagnosis and prognosis procedures in the Bangladeshi Government hospital setup are not quite convincing, which could affect the subject's selection process due to contradictory information given by the patients in the hospital. Moreover, Other inflammatory cytokines (e.g., IL-6, TNF) could signify the result as these cytokines were included in the experiment of previous relevant studies. However, our study was the first of its kind in the South Asian region. This kind of study is required to be conducted for observing the role of inflammatory and anti-inflammatory cytokines, like IL-37, so that their potential therapeutic and biomarker properties can be assessed. Future research based on this study should focus on a larger sample size while considering the prevalence rate of AMI. Professional collaboration is needed with a specialized hospital relevant to this disease and a proper laboratory set-up for storing the samples and testing biochemical and biomarkers perfectly.

Chapter 5

Conclusion

This study aimed to investigate the concentration of IL-37 in patients with Acute Myocardial Infarction(STEMI and NSTEMI) and compared it with the healthy control group. In this study, a correlation between CRP and IL-37 was found in the case group, which suggested that the anti-inflammatory activity of IL-37 increased with the elevation of inflammatory activities in AMI. Although this case-control study is the first in Bangladesh, the small sample size limited the statistical interpretation of the association between IL-37 concentration in the case and control groups. Despite this limitation, this case-control study can be a potential guideline for similar studies for a larger population. Moreover, this study may pave the way for future research on anti-inflammatory cytokines and their potential acceptance as a biomarker or therapeutic agent, along with the prognostic assistance to clinicians. A larger sample size based on this study design, while considering the prevalence rate of AMI in Bangladesh, could provide some important insights regarding the association of risk factors and the anti-inflammatory activities of different cytokines. In conclusion, by demonstrating the association of the anti-inflammatory activity of IL-37 with AMI patients and the control group, this study provides a strong foundation for further research on highlighting the role of IL-37 as a potential biomarker and therapeutic agent that may pave the way to early detection and management of cardiovascular diseases.

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