Examining the Relationship between Ischemic Preconditioning and Apoptosis and Autophagy in ST-Elevation vs. Non-ST Elevation Acute Myocardial Infarction

By

Lacey Maclay

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This thesis was presented

by

Lacey Maclay

It was defended on

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and approved by

Ziad Faramand, MD, MSc

Yvette Conley, PhD, FAAN

Imad Al-Ghouleh, PhD

Mary G. Carey, PhD, RN, FAHA, FAAN

Thesis Advisor: Salah Al-Zaiti, PhD, Rn, ANP-PC, FAHA

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Lacey J. Maclay, BSN Honors

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Introduction: Growing evidence suggests that the differences observed in symptomatology, morbidity, and mortality between ST-Elevation Acute Coronary Syndrome (STE-ACS) and Non-ST-elevation Acute Coronary Syndrome (NSTE-ACS) cannot be simply explained by the severity of coronary occlusion. Apoptosis and autophagy are two known pathways that can induce myocardial cell death or cell survival, respectively. Ischemic preconditioning, on the other hand, is a recognized endogenous protective mechanism that can limit the extent of scaring. It remains unknown if the degree of ischemic preconditioning, and subsequently the extent of apoptosis and autophagy, can partially explain the differences observed between STE-ACS and NSTE-ACS.

Methods: In this exploratory pilot study, we prospectively enrolled consecutive myocardial infarction (MI) patients from a single UPMC-affiliated primary PCI-receiving center. The interventional cardiologist obtained blood samples from each patient using PAX-gene tubes femoral arterial access line prior to the catheterization procedure. qRT-PCR were conducted using TaqMan assays, GAPDH as an endogenous control, and quantified using comparative Ct method. The following candidate genes were analyzed: BAX (apoptosis activator), BCL2 (apoptosis regulator), EGR1 (ischemic preconditioning suppressor), PINK1 (autophagy initiator), ATG5 (autophagy initiator), and DRAM2 (autophagy initiator).

Results: Our sample included 29 patients treated for acute MI (Age 67±12; 83% males). EGR1 was significantly correlated with DRAM2 ($r=0.697$, $p<0.001$), BCL-2 ($r=0.657$, $p<0.001$), and BAX ($r=0.448$, $p<0.05$). EGR1 was negatively associated with age and known history of CAD. Compared to patients with STE-ACS, those with NSTE-ACS had attenuated expression of EGR1 (4.7±2.2 vs. 6.9±0.7, $p=0.001$), BCL-2 (2.7±1.2 vs. 4.8±1.0, $p=0.001$), and BAX (–1.8±3.5 vs. 1.3±0.9, $p=0.007$). EGR1 was also more attenuated in patients with occlusion in left anterior descending or left circumflex coronary arteries.

Conclusions: Overall, these results indicate that ischemic preconditioning promotes more programmed cell death, which is more likely to happen in older patients and in those with existing CAD with progressive coronary occlusion primarily due to LAD or LCX occlusion. These are important and novel pilot results that may help explain the different clinical phenomena associated with STE-ACS vs. NSTE-ACS, which can inform future targeted therapies.

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Preface

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1.0 INTRODUCTION

Acute myocardial infarctions (MI) are one of the leading causes of morbidity and mortality worldwide. Every day, more than 2,500 Americans suffer from an ischemic coronary event and more than 1,400 of them will die of one (Murray et al., 2013). The key in addressing this issue is to reduce the ischemic damage by early identification of disease and by identifying those who would benefit from immediate catheterization. Coronary artery occlusion resulting in acute myocardial injury constitutes the pathophysiological basis of acute coronary syndrome (ACS) (Thygesen et al., 2019). ACS is broadly classified according to the presence or absence of ST-elevation (STE) on the 12-lead electrocardiogram (ECG) with STE-ACS generally linked to complete coronary artery occlusions and NSTE-ACS linked to partial coronary occlusions (Thygesen et al., 2019). However, each of the two presentations has its own clinical phenomena that cannot solely be explained by the degree of coronary artery occlusion.

Current diagnosis and treatment methods fall short as they rely on STE on ECG as the sole marker for risk stratification given that STE events are dynamic and transient. This results in a delay in vital treatment which is also the case with NSTE-ACS patients whose treatment is delayed by hours until definitive diagnosis is confirmed with serial cardiac troponins. NSTE-ACS is often managed with a conservative approach using pharmacological agents (anti-ischemic, anti-platelets, anti-coagulants, and statins) in which early administration has been shown to improve outcomes (Melloni et al., 2013; Sherwood et al, 2014; Navarese et al., 2014). However, ischemia-guided therapy with urgent (<2 hours) or early (2-24 hours) percutaneous coronary interventions (PCI) depending on severity of ischemic burden has been shown to be

superior to initial conservative therapy (Mehta et al, 2005; Hirsch et al., 2007; Hoenig et al., 2010). Early PCI in high-risk patients would improve outcomes by reducing infarct size, limiting ejection fraction reduction, and shortening the length of hospital stay (Wright et al., 2011). However, we lack the ability to quantify the ischemic burden of NSE-ACS in a time period that would allow early and immediate intervention. A better understanding of the pathological mechanisms associated with the presence or absence of STE on the ECG would allow us to identify those most in need for urgent PCI and thus, improve their clinical outcomes.

STE-ACS is not always associated with complete and permanent occlusion of a coronary artery causing transmural ischemia (requiring immediate PCI), and conversely, NSTE-ACS is not always associated with partial occlusion of a coronary artery causing non-transmural ischemia (Kumar et al., 2009). More importantly, current evidence suggests that urgent coronary revascularization (i.e., PCI) might lead to adverse cardiac outcomes in those with NSTE-ACS (Anderson et al., 1995). These observations suggest that STE vs. NSTE presentations represent complex and distinct pathways of acute myocardial injury.

The different ECG presentations observed in ACS patients are thought to be related to ischemic preconditioning (IPC). IPC is one of the most powerful cardioprotective mechanisms (Ravingerova, 2007). IPC is when a brief exposure to ischemia before sustained ischemia markedly enhances the ability of the heart to withstand a subsequent ischemic response (Ferdinandy et al., 2007). Previous studies have demonstrated that patients presenting with NSTE-ACS are more likely to be older, to have existing coronary artery disease (CAD), to have prior history of coronary revascularization, and to have multi-vessel disease (Ferrara et al., 2013; Boyer et al, 2012). The aggregation of cardiovascular risk factors in NSTE-ACS suggests that

these patients are exposed to chronic myocardial insult, which in turn could lead to IPC. In fact, the absence of pre-infarction angina (a strong clinical correlate of IPC) has been found to be the strongest predictor of adverse events in this population (relative risk 9.1, p=0.003) (Papadopoulos et al., 2003).

IPC is a known endogenous protective mechanism that reduces apoptosis (cell death) by inhibiting inflammatory cell activation and altering the expression of anti-and pro-apoptotic proteins (Zhao & Vinten-Johansen, 2002; Maulik et al., 1998). Autophagy which helps to maintain cellular homeostasis under stress conditions has also been linked to IPC. It has been shown to be an important mechanism mediating cardio protection following repetitive episodes of coronary stenosis or coronary occlusion (Huang et al., 2010; Yan et al., 2009). Given that IPC is thought to be more prevalent in ACS patients with NSTE compared to those with STE presentations, it remains unknown if apoptosis and autophagy are mediated differently in these two subtype populations.

In this BSN thesis, we aimed to (1) explore the associations between the gene expression of ischemic preconditioning and various autophagy and apoptotic proteins pathways during the acute phase of ACS; (2) explore the association between these genetic pathways and patients' demographic and clinical characteristics; and (3) compare the expression of these genetic pathways between patients according to ACS subtype (STE-ACS vs. NSTE-ACS) and location of coronary artery culprit lesion (LAD vs. LCX vs. RCA).

2.0 METHODS

2.1 Sample and Setting

This was a prospective observational cohort study. We recruited a convenient sample of 50 patients undergoing invasive angiography for primary PCI at the catheterization lab of UPMC Presbyterian Hospital, a primary-PCI receiving center, between November 2016 and February 2017. Inclusion criteria included: (1) age > 40 year-old; and (2) confirmed ACS diagnosis documented by raise of cardiac troponin in patients presenting with ischemic symptoms. Exclusion criteria include patients who have already received (or started on) reperfusion therapy (i.e., thrombolysis therapy) or those in whom cardiac catheterization revealed non-occlusive CAD (i.e., troponin elevation is attributable to non-ACS causes). A delayed informed consent was obtained within 24-hours of admission to the catheterization lab. Patients who had a diagnostic angiogram without PCI (n=14), refused to give informed consent (n=5); were unconscious or non-competent to consent (n=2) were removed from the study; yielding a final sample size of n=29. These eligible patients were then followed up through their hospitalization. Clinical data about demographics, comorbidities, medications, course of hospitalization and outcomes were obtained from charts by independent reviewers. ACS subtype was confirmed as per interventional cardiologist documentation on the final catheterization report. The study was approved by the University of Pittsburgh Institutional Review Board (IRB).

2.2 Data Collection

The interventional cardiologist obtained a blood samples from each patient using PAX-gene tubes via the femoral arterial access line prior to the catheterization procedure. Drawn blood samples were labeled by patient's MRN and then placed at the nursing station in a predesignated biohazard box provided by study personnel. The study nurse collected these blood samples the following morning, visited the patient to obtain an informed consent, then assigned enrolled patients a study ID number and relabeled the blood sample with only the study ID. The linkage list was kept separate from patient identifiers at all times. Blood samples of patients who refused to participate were discarded of as per IRB regulations (e.g., two witnesses confirmed the disposal). Blood samples of recruited and consented patients were dropped off at the University of Pittsburgh's Molecular Genetics Lab that is located in interconnected building one block away from the clinical site. Samples were stored at -70 degrees Celsius for subsequent analysis.

2.3 Gene Expression Analysis

A lab technician extracted RNA from PAX-gene samples then conducted qRT-PCR using TaqMan assays. GAPDH was used as an endogenous control and gene expression was quantified using comparative Ct method. Following a candidate gene approach based on extensive literature review, the following candidate genes were analyzed: BAX (apoptosis activator), BCL2 (apoptosis regulator), EGR1 (ischemic preconditioning suppressor), PINK1 (autophagy initiator), ATG5 (autophagy initiator), and DRAM2 (autophagy initiator).

2.4 Statistical Analysis

All analyses were preceded by detailed descriptive analysis of the data, yielding standard descriptive summaries (e.g., means, standard deviation, percentiles, ranges) and displayed using graphical techniques (e.g., histograms, scatter plots) to identify data anomalies (e.g., outliers). In particular, the distribution of all key variables was examined to ensure that proposed modeling techniques were suitable. Categorical data were compared between groups using chi-square and continuous data were compared using independent samples t-test or Mann-Whitney test (depending on normality of data). Associations between gene expressions data was evaluated using Pearson's r correlation coefficient. All statistical analyses will be performed using SSPS. The level of statistical significance will be set at .05 for two-sided hypothesis testing.

3.0 RESULTS

3.1 Demographics

Our study included 29 patients, 6 (23%) had STE-ACS and 23 (79%) had NSTE-ACS. The mean age of the total study sample was about 66 years and 83% of the subjects were male. Table 1 describes the demographics of all patients included in the study, as well as a breakdown between the study subgroups. Overall, the majority of patients had a history of hypertension and dyslipidemia. Over half of the patients had a known history of coronary artery disease. The mean BMI for the patients was obese (mean BMI 30.6). In terms of significant occlusions (greater than 70% occluded), the left coronary artery was the most common occlusion found followed by the right coronary artery.

Table 1: Demographic and Clinical Characteristics of Study Sample

	All Patients (n=29)	*STE-ACS (n=6, 21%)*	*NSTE-ACS (n=23, 79%)*
Demographics			
Age (years)	66.6 ± 12.2	61.5 ± 12	68 ± 12.1
Male sex	24 (83%)	5 (83%)	19 (83%)
BMI (kg/m^2)	30.6 ± 7	31.6 ± 5.2	30.4 ± 7.5
Comorbidities			
Hypertension	20 (69%)	4 (67%)	16 (70%)
Known CAD	18 (62%)	1 (17%)	17 (74%)
Diabetes Mellitus	7 (24%)	1 (17%)	6 (26%)
Dyslipidemia	20 (69%)	4 (67%)	16 (70%)
Clinical Data			
Length of Stay	3.8 ± 3.7	5.7 ± 5	3.3 ± 3.2
Peak Troponin	15.8 ± 33.7	71 ± 49.7	2.8 ± 3.8
LAD Occlusion	18 (62%)	3 (50%)	15 (65%)
LCX Occlusion	11 (38%)	2 (33%)	9 (39%)
RCA Occlusion	14 (48%)	3 (50%)	11 (48%)

3.2 Gene Expression Data

Table 2 describes the correlation between the genes that were examined. Most notably, DRAM2, an autophagy inducer, was strongly positively correlated with EGR1, an ischemic preconditioning suppressor gene showing that an increase in autophagy is associated with less ischemic preconditioning in ACS patients. BCL2, an apoptosis regulator, was also strongly positively correlated with EGR1, ischemic preconditioning suppressor gene, showing the relationship between an increase in apoptosis (programmed cell death) and less ischemic preconditioning. Further, EGR1 was moderately positively correlated with BAX2, an apoptosis inducer further showing the correlation between more apoptosis and less ischemic preconditioning. BCL2, apoptosis inducer, and DRAM2, autophagy inducer, were strongly

positively correlated and thus, show the association between apoptosis and autophagy. ATG, autophagy inducer, and DRAM2, autophagy initiator, were moderately positively correlated. BAX, apoptosis initiator, and BCL2, apoptosis regulator, had a small positive correlation. Figure 1 shows the associations between all genes that were significantly correlated.

Table 2. Correlation matrix between Examined Genes

	BAX	BCL2	DRAM2	EGR1	ATG5	PINK1
BAX (apoptosis activator)	1	0.378*	0.318	0.448*	-0.164	0.158
BCL2 (apoptosis regulator)	-	1	0.737**	0.657**	0.246	0.239
DRAM2 (autophagy initiator)	-	-	1	0.697**	0.493**	0.284
EGR1 (IPC suppressor)	-	-	-	1	-0.014	0.183
ATG5 (autophagy initiator)	-	-	-	-	1	0.08
PINK 1 (autophagy initiator)	-	-	-	-	-	1

*correlation is significant at the .05 level

**correlation is significant at the .01 level

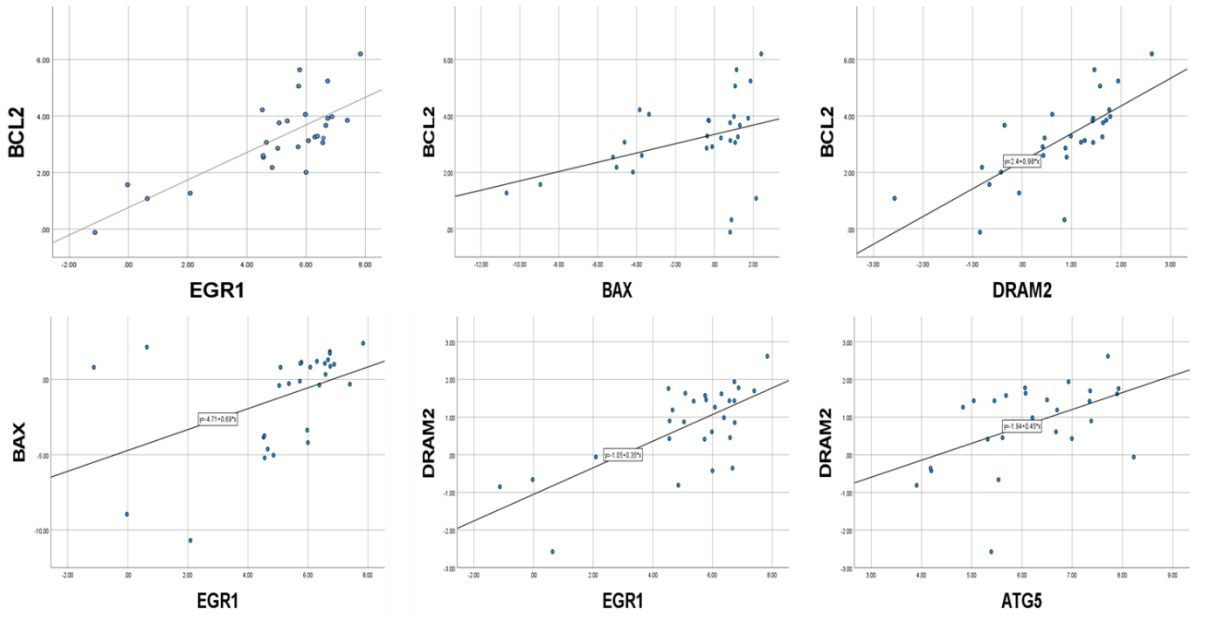

Figure 1: Scatterplots of significant correlations between gene expressions

3.3 Association between Gene Expressions and Clinical Data

The expression candidate genes were examined against the demographic and clinical characteristics of patients in the study. The only gene significantly correlated with clinical patient characteristics was EGR1. Age and EGR1, ischemic preconditioning suppressor gene, were moderately negatively correlated ($r = -0.49$, $p < 0.05$, Figure 2). This association suggests that increased age is associated with an increase in ischemic preconditioning.

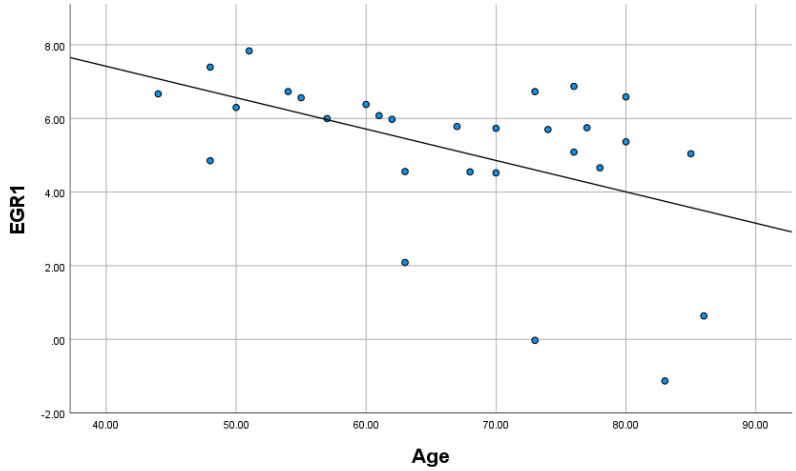

Figure 2: Scatterplot of the association between Age and Ischemic Preconditioning

In addition, there was a relationship between known history of CAD and EGR1 (Chi-square = xx, p = 0.006, Figure 3). Those with a history of CAD appear to have more ischemic preconditioning (less of the ischemic preconditioning suppressor gene EGR1).

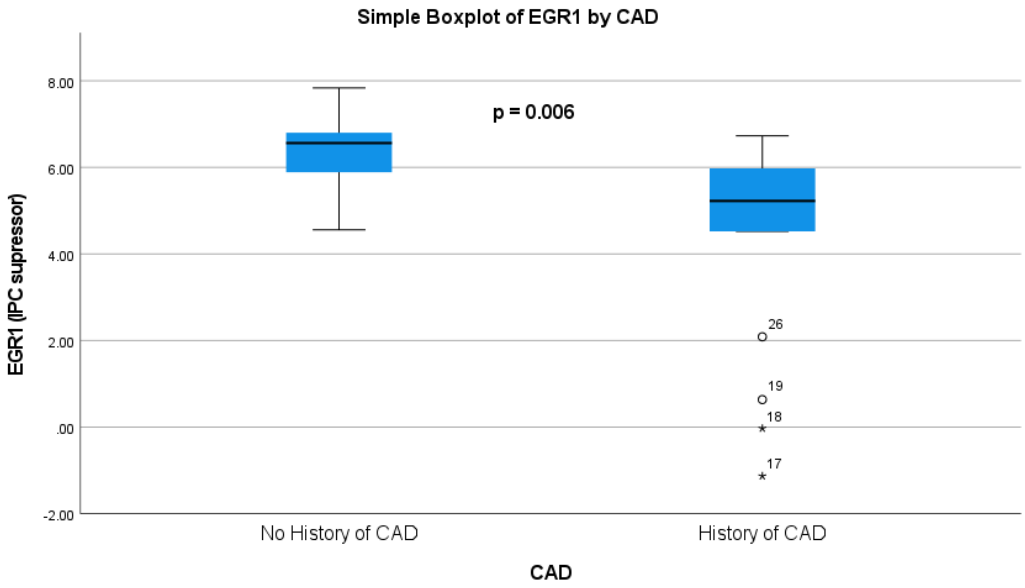

Figure 3: Boxplot of the association between known CAD and Ischemic Preconditioning

3.4 Association between Gene Expression and ACS Subtype

In examining how gene expression differs between STE-ACS and NSTE-ACS, there were significant differences in the BAX, BCL2, DRAM2, and EGR1 genes between ACS subtypes (Figure 4). STE-ACS patients had higher amounts of BAX indicating more apoptosis in STE-ACS patients compared to NSTE-ACS patients. STE-ACS patients also had higher amounts of BCL2, an apoptosis initiator. DRAM2, an autophagy initiator, was found to be significantly higher in STE-ACS patients as well indicating more autophagy occurs in STE-ACS patients compared to NSTE-ACS patients. Lastly, EGR1 (ischemic preconditioning suppressor) was significantly lower in NSTE-ACS patients compared to STE-ACS showing that NSTE-ACS patients had more ischemic preconditioning.

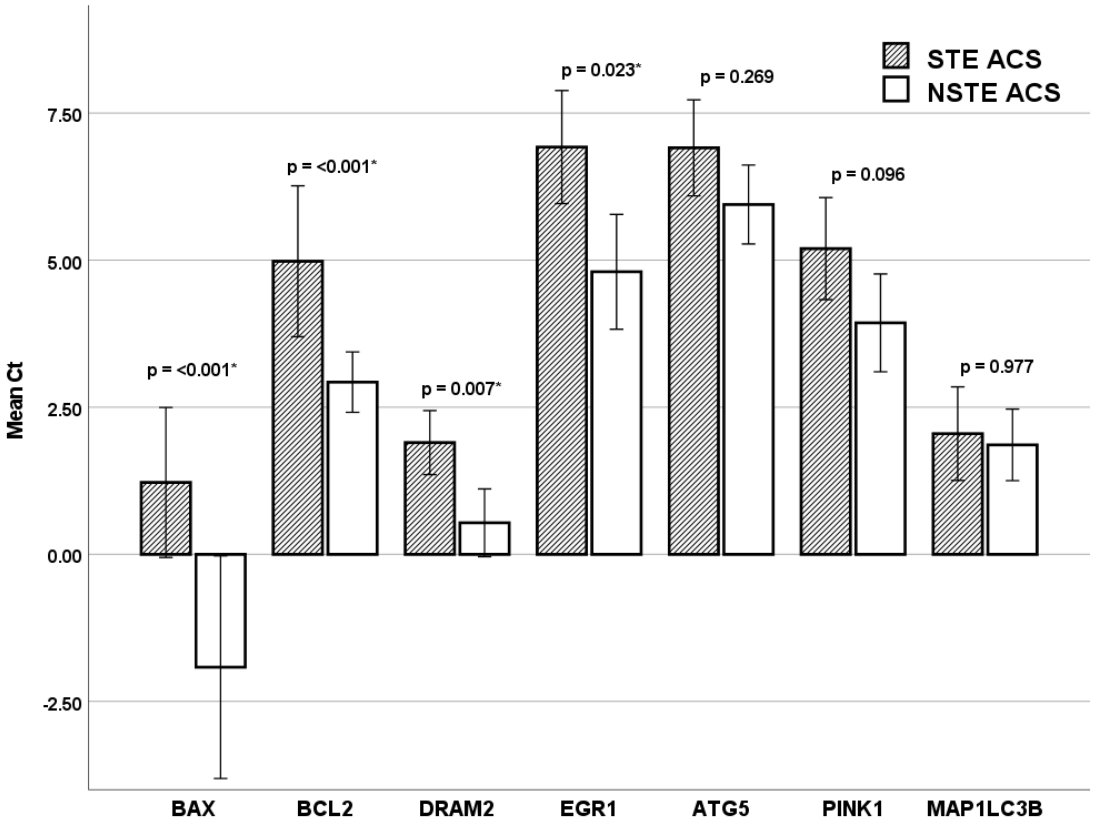

Figure 4: Differences in Gene Expression according to ACS Subtype

3.5 Association between Gene Expression and Location of Culprit Lesion

We examined the correlations between left coronary artery (LAD), right coronary artery (RCA), and the left circumflex (RCx) coronary arteries significant occlusions (greater than 70%) against the gene expression of each candidate gene. For LAD and LCX occlusions, EGR1, ischemic preconditioning suppressor, was the only gene that correlated with occlusion in these

locations. Figure 5 indicates that more ischemic preconditioning was present in individuals who had significant LAD and LCX occlusions, but not RCA occlusion.

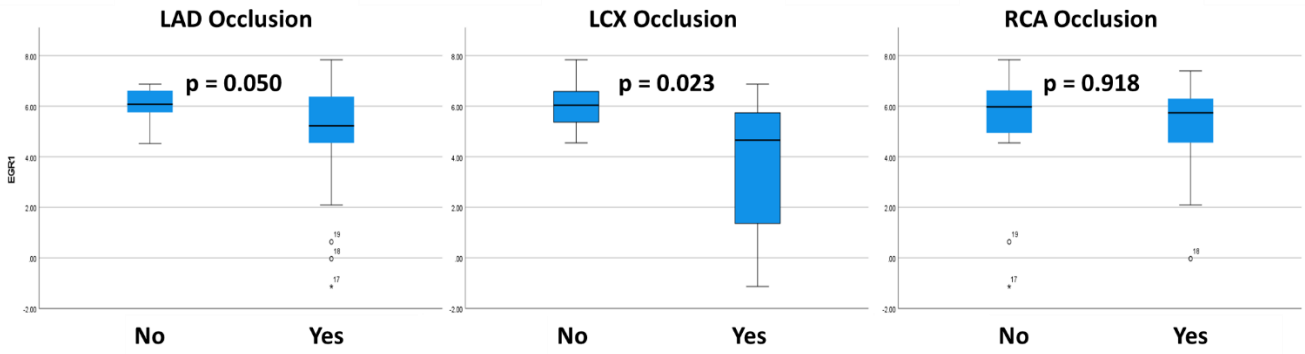

Figure 5: Differences in EGR1 Gene Expression according to Culprit Lesion

For RCA occlusion, significant differences were only found in ATG5 (autophagy initiator) gene expression indicating more autophagy in those with significant RCA occlusions (Figure 6).

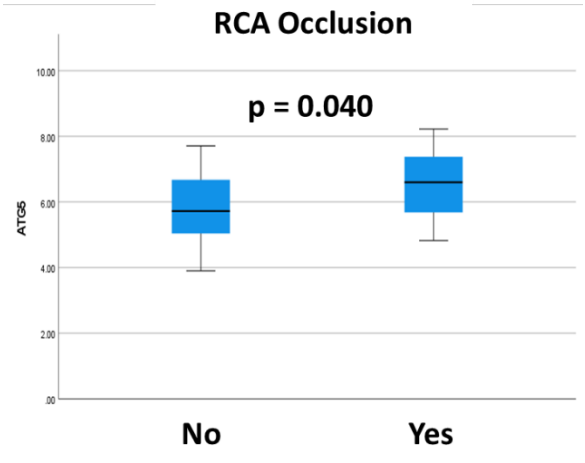

Figure 6: ATG5 Gene Expression according to RCA Occlusion

4.0 DISCUSSION

In this study, we sought to assess the association between the gene expression of ischemic preconditioning, autophagy, and apoptotic proteins and compare their expression across demographic and clinical characteristics, ACS subtype, and location of culprit lesion. IPC was associated with less autophagy and apoptosis. IPC was also associated with older age and with known history of CAD. Compared to NSTE-ACS, STE-ACS patients had less IPC and more apoptosis and autophagy. Patients with LAD and LCX occlusion had more IPC, whereas patients with RCA occlusion had more autophagy. Overall, these results indicate that IPC promotes less programmed cell death, which is more likely to happen in older patients and in those with existing CAD with progressive coronary occlusion primarily due to LAD or LCX occlusion. These are important and novel pilot results that may help explain the different clinical phenomena associated with STE-ACS vs. NSTE-ACS.

4.1 Relationship between IPC and Autophagy

Autophagy is a form of programmed cell death and it plays a central role in maintaining cellular homeostasis through lysosomal-mediated degradation of proteins and organelles (Mialet-Perez & Vindis, 2017). It is activated under stress conditions like hypoxia and starvation, leading to the release of energy. However, excessive autophagy may lead to the depletion of essential molecules leading to cell death. IPC is a known cardioprotective mechanism that enhances the heart's ability to respond to subsequent ischemic events (Ravingerova, 2007). Our study showed

that less IPC is associated with an increase in expression of an autophagy inducing gene, DRAM2. This gene was also found to be greater in STE-ACS patients compared to NSTE-ACS patients. However, the other two autophagy inducing genes that were examined (PINK1 and ATG5) showed no significant difference between STE-ACS and NSTE-ACS or IPC. Overall, more autophagy appears to be associated with less IPC and STE-ACS patients, but further research is needed on this topic.

4.2 Relationship between Ischemic Preconditioning and Apoptosis

Apoptosis is programmed cell death. IPC is known to reduce apoptosis by altering the expression of anti-and pro-apoptotic proteins and by inhibiting inflammatory cell activation (Zhao & Vinten-Johansen, 2002; Maulik et al., 1998). This was shown in this study as the increased gene expression of apoptotic inducing genes was associated with less IPC. Looking at the difference between NSTE-ACS and STE-ACS patients, STE-ACS patients had more apoptosis and less ischemic preconditioning compared to NSTE-ACS patients. More cell death is occurring in STE-ACS patients resulting in worse outcomes. IPC helps to reduce the amount of cell death that is occurring and patients suffering from NSTE-ACS have more IPC which helps to better explain the difference between NSTE-ACS and STE-ACS patients.

4.3 Clinical Implications

The explained differences in gene expression between STE-ACS and NSTE-ACS could help to explain the differences in morbidity and mortality of each condition. The correlation between ischemic preconditioning, apoptosis, and autophagy helps provide insight into the distinct mechanisms of myocardial scarring between STE-ACS and NSTE-ACS patients. IPC differs between the two groups (more IPC with NSTE-ACS patients) and the mechanisms differ as well (less apoptosis and autophagy with NSTE-ACS patients). These differences help to explain the different clinical outcomes observed and will help to inform future targeted therapies. Further research is needed on these mechanisms, as this study was limited by the small sample size.

5.0 CONCLUSION

This exploratory study sought to examine differences in gene expression between STE-ACS and NSTE-ACS patients regarding apoptosis, autophagy, and ischemic preconditioning. STE-ACS patients were found to have less ischemic preconditioning and more apoptosis (cell death) and autophagy compared to their NSTE-ACS counterparts. This helps to explain the differences in clinical presentation between the STE-ACS and NSTE-ACS patients and the myocardial scarring that occurs which can help in developing further targeted therapies.

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