OXIDATIVE STRESS BIOMARKERS AND THEIR RELEVANCE TO FATTY ACID BINDING PROTEINS (FABP) IN STAGE 5 CHRONIC KIDNEY DISEASE PATIENTS (CKD-5)

Rishima Sharma

1. Cardiovascular Institute, Hemostasis and Thrombosis Research unit, Loyola University Chicago, Health Science Division, Maywood, Illinois, USA.

INTRODUCTION

- CKD5 is the final stage of chronic kidney disease in which the patient has end stage renal disease (ESRD) and their kidneys are about to fail
- Experts estimate that around 37 million people in the United States have chronic kidney disease
- Given the increasing number of cases of CKD5, understanding how it works could improve the patient's outcome and increase their chances of survival
- Oxidative stress occurs when there's an imbalance between free radicals and antioxidants in the body. DNA, proteins, and lipids can become damaged as a result of this imbalance
- Stable derivatives of oxidants are used as biomarkers of oxidative stress
- When renal damage occurs, likely due to oxidative stress, the levels of biomarkers will likely be more elevated in ESRD patients compared to control samples
- L-FABP is a cytoplasmic protein involved in facilitating and transporting long-chain polyunsaturated fatty acids and is very significant in many research experiments related to CKD5

INTRODUCTION

- Stable derivatives of oxidants are used as biomarkers of oxidative stress
- When renal damage occurs, likely due to oxidative stress, the levels of biomarkers will likely be more elevated in ESRD patients compared to control samples
- L-FABP is a cytoplasmic protein involved in facilitating and transporting long-chain polyunsaturated fatty acids and is very significant in many research experiments related to CKD5.

PURPOSE

- The purpose of this research experiment is to explore the relationship between oxidative stress biomarkers in ESRD patients and how the presence of ESRD affects the concentrations of oxidative stress biomarkers in patients

MATERIALS

- Equipment in the Pathology and Pharmacology labs
  - Centrifuges
  - Freezers
  - Analytical instruments
  - PRISM Graphpad Software
  - IBM SPSS

METHODS

- Blood samples will be collected from CKD5 patients on hemodialysis in sodium citrate tubes
- Samples will be centrifuged and the resulting plasma will be divided and stored at -80°C
- Control plasmas will be purchased from a commercial vendor
- The CKD5-HD and control plasma samples will be used to profile the biomarkers through commercial and sandwich ELISA kits
- Electronic medical record charts of the CKD5-HD patients will be reviewed for levels of ferratin as well as the lipid profile
  - Other patient information such as their gender, age, ethnicity, BMI, comorbidities, and medications will also be obtained

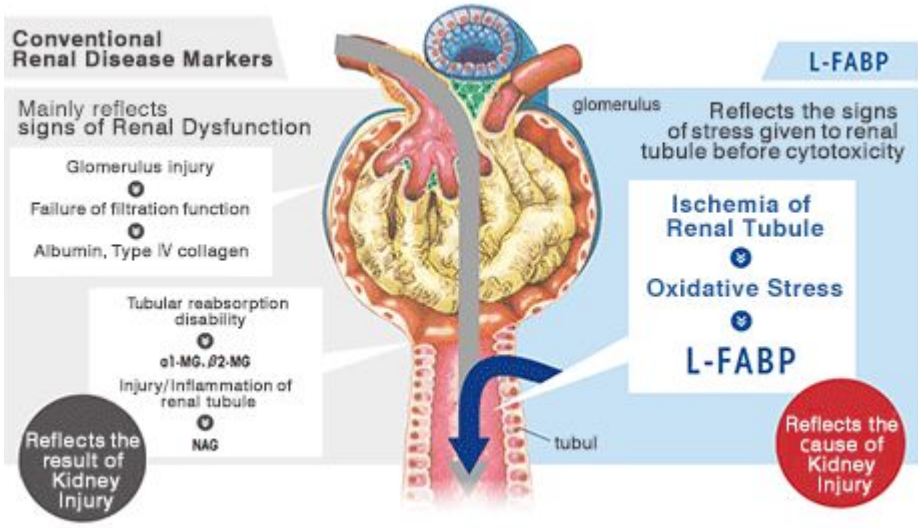
METHODS

- The data will be collected in Excel and analyzed using Prism
  - Plasma levels of biomarkers will be recorded as means +/- standard errors
  - Two-tailed Mann- Whitney U tests will be carried out to compare biomarker levels between patients with CKD5-HD and normal controls
  - In the CKD5-HD cohort, Spearman coefficients will be used to measure correlations between L-FABP and the other oxidative stress biomarkers
  - The R values will be generated to assess the strength of correlation between 2 biomarkers
  - Statistical significance will be defined as $p<0.05$

BIOMARKERS

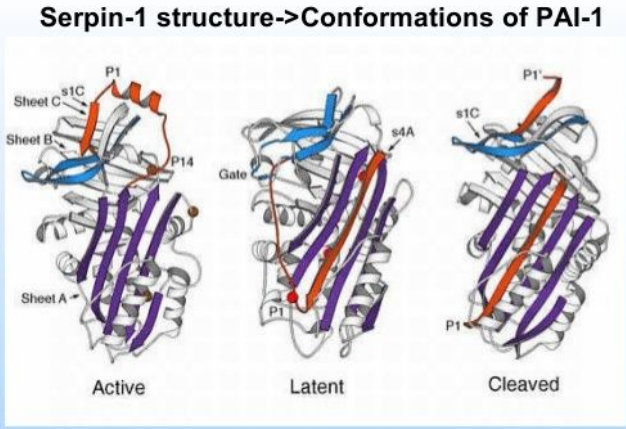
L-FABP

- Cytoplasmic protein involved in cellular fatty acid metabolism
- Facilitates and transports long-chain polyunsaturated fatty acids from the plasma membrane to the intracellular sites for oxidation
- Very significant in many research experiments related to chronic kidney disease and is a potential biomarker for other pathological conditions



PAI-1

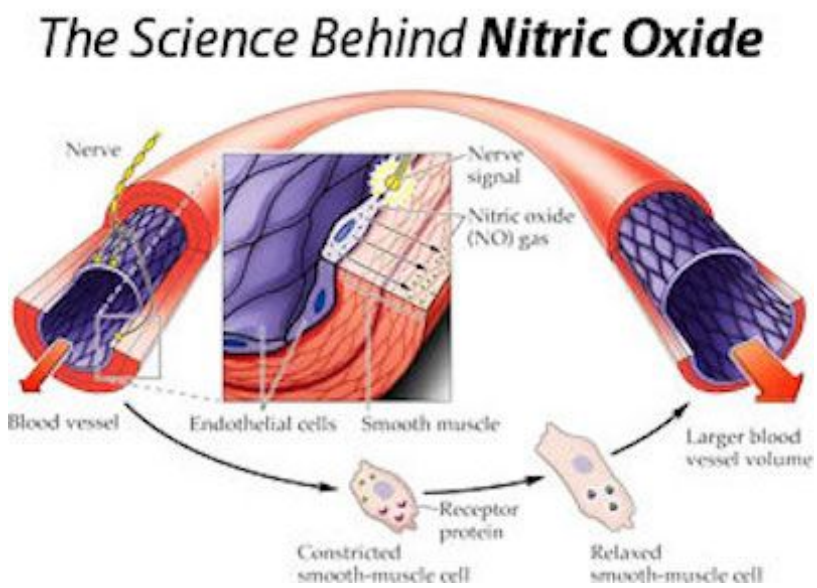
- One of the most important inhibitors of plasma fibrinolytic activity
- Single chain glycoprotein involved in normal blood clotting
- Helps protect the body by sealing off damaged blood vessels and preventing further blood loss
- Inhibits the action of other proteins known as plasminogen activators



The serpin plasminogen activator inhibitor-1 (PAI-1) folds into an active structure then converts slowly to a more stable, but low activity, "latent" conformation.The structure of active PAI-1 has to be different from the binding regions in latent and cleaved PAI-1. ***Crystal structures available in all conformations without any known ligand.***

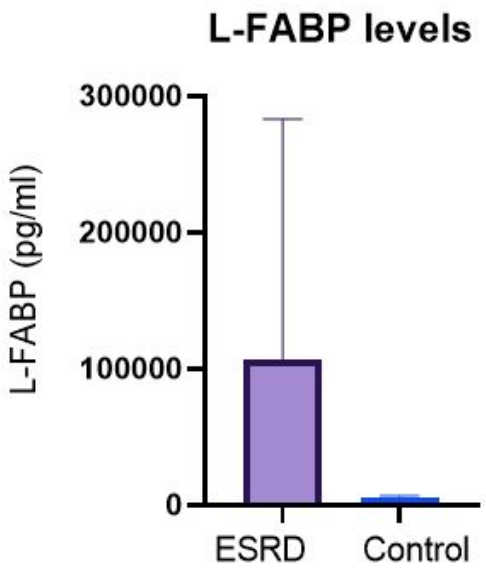
NO

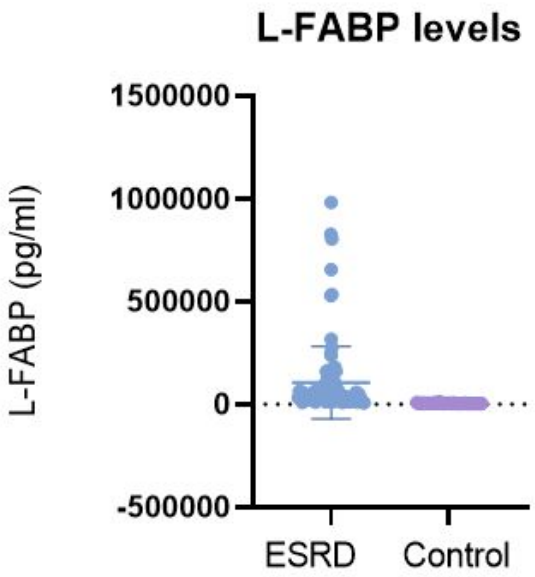
- One of the most important molecules for controlling renal function and long term BP regulation
- Vasodilator
  - Increases blood flow and lowers BP
- As a biomarker, it promotes the excretion of sodium through urine and an increased production of urine



RESULTS

L-FABP Data





	Control Data	ESRD Data
Average	4888.45	106599.43
SD	1767.882	175796.36
SEM	250.02	18132.009
Min	3408.44	8136.72
Max	12670.86	982739.09

% Change (from control values):

- Average: 95.41% increase
- SD: 9843.9% increase
- SEM: 7152.22% increase

PAI-1 Data

Statistics

Control

N	Valid	24
	Missing	53
Mean		10.4667
Std. Error of Mean		2.83321
Median		4.8850
Mode		.82[a]
Std. Deviation		13.87982
Minimum		.82
Maximum		54.02
Percentiles	25	1.5550
	50	4.8850
	60	7.9800
	75	13.3075

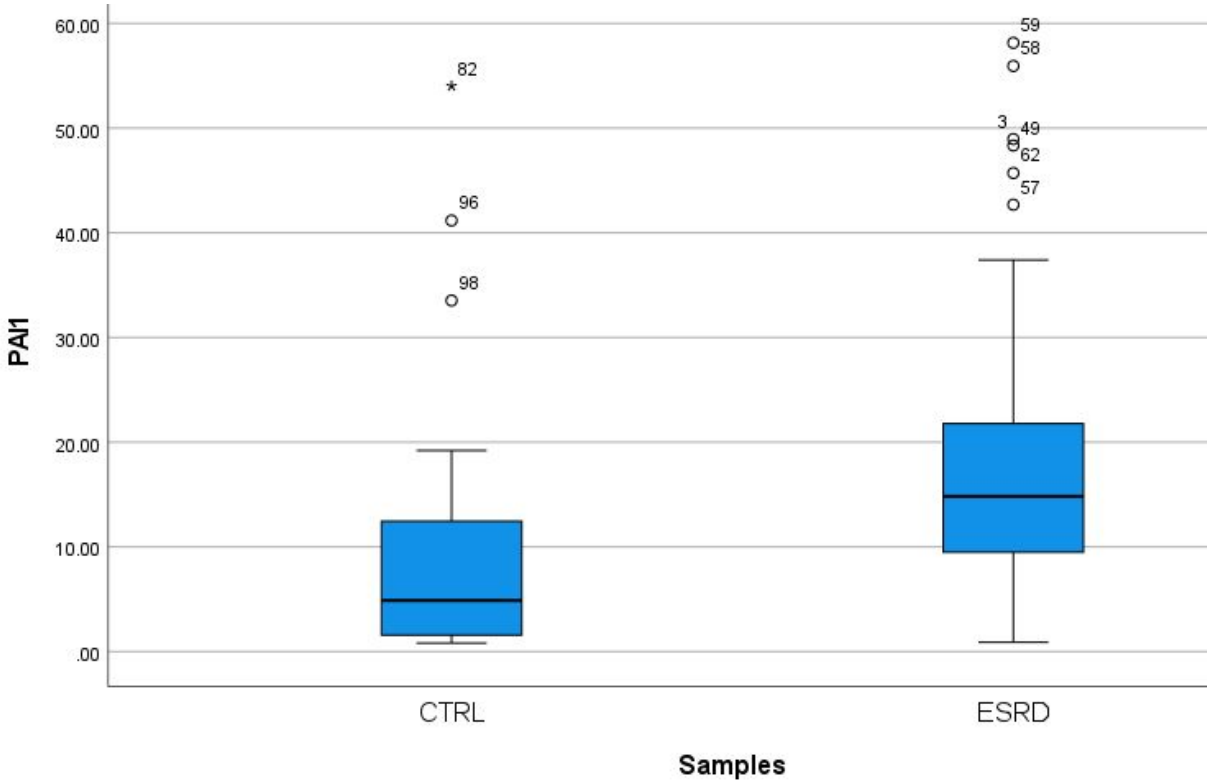
a. Multiple modes exist. The smallest value is shown

Statistics

ESRD

N	Valid	77
	Missing	0
Mean		17.8991
Std. Error of Mean		1.40232
Median		14.8372
Mode		.91[a]
Std. Deviation		12.30532
Minimum		.91
Maximum		58.15
Percentiles	25	9.3139
	50	14.8372
	60	17.6882
	75	21.9674

a. Multiple modes exist. The smallest value is shown



% Change:

- Average: 71.01% increase
- SD: 11.34% decrease
- SEM: 50.47% decrease

LOYOLA UNIVERSITY CHICAGO

Preparing people to lead extraordinary lives

Nitric Oxide Data

Statistics

Control

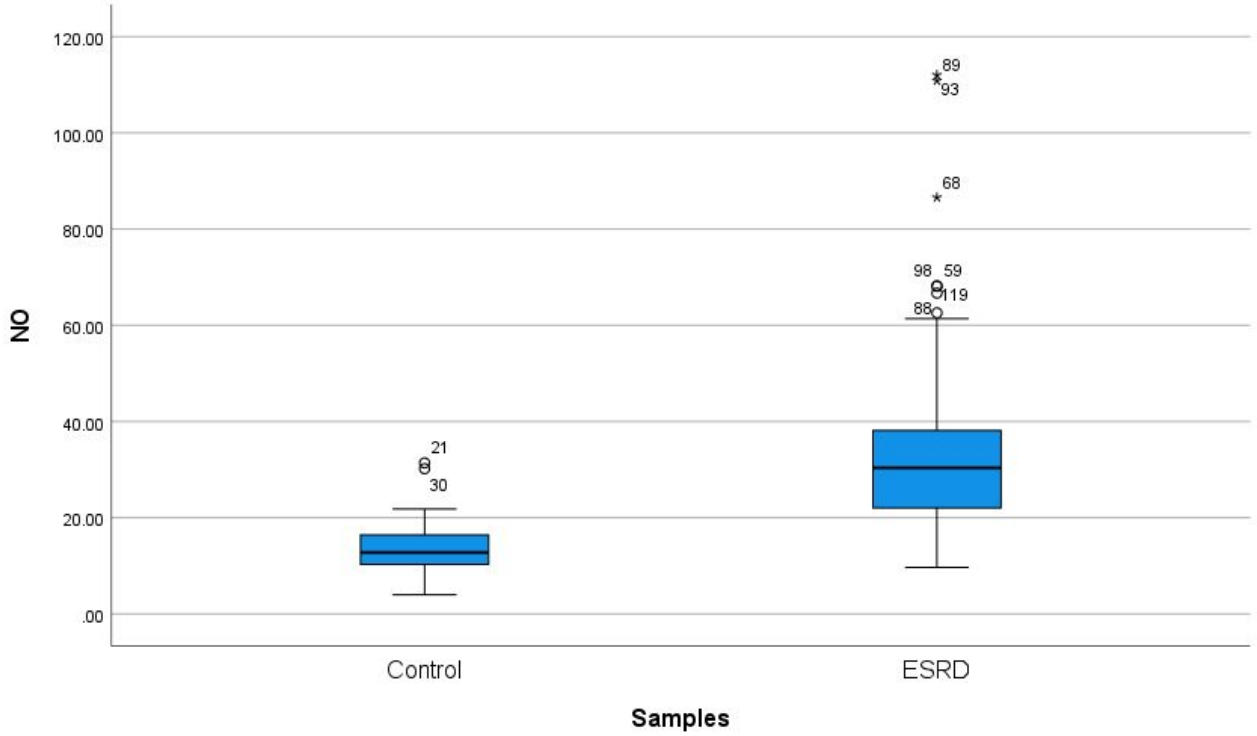
N	Valid	51
	Missing	23
Mean		13.8894
Std. Error of Mean		.78238
Median		12.8000
Mode		15.10
Std. Deviation		5.58730
Minimum		4.00
Maximum		31.40
Percentiles	25	10.2000
	50	12.8000
	75	16.8000

Statistics

ESRD

N	Valid	74
	Missing	0
Mean		34.7430
Std. Error of Mean		2.29502
Median		30.3700
Mode		30.96[a]
Std. Deviation		19.74247
Minimum		9.67
Maximum		112.04
Percentiles	25	21.9750
	50	30.3700
	75	38.4175

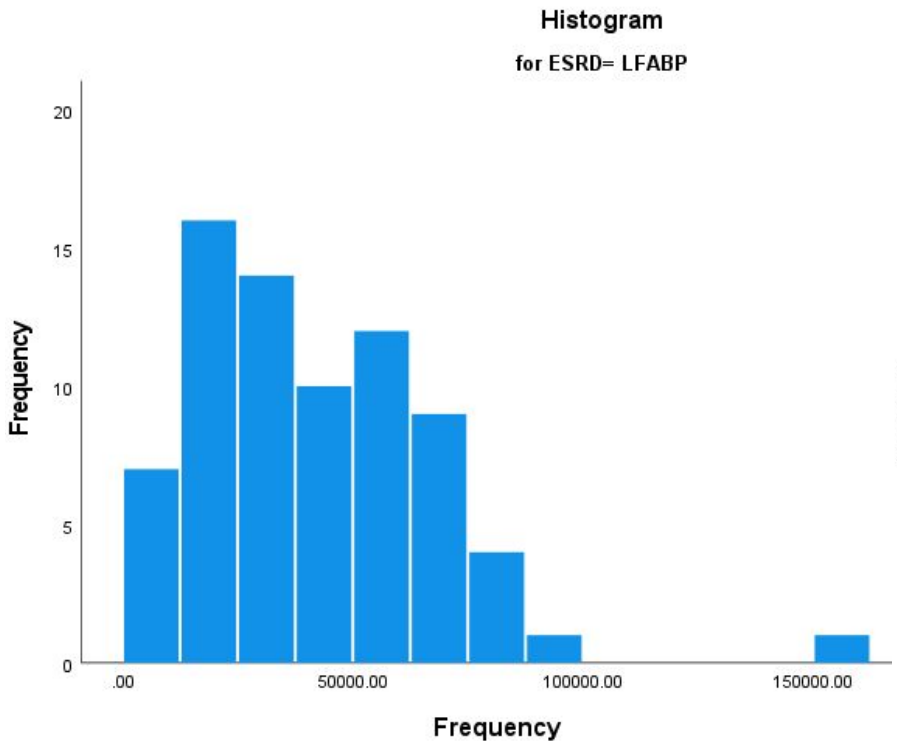
a. Multiple modes exist. The smallest value is shown

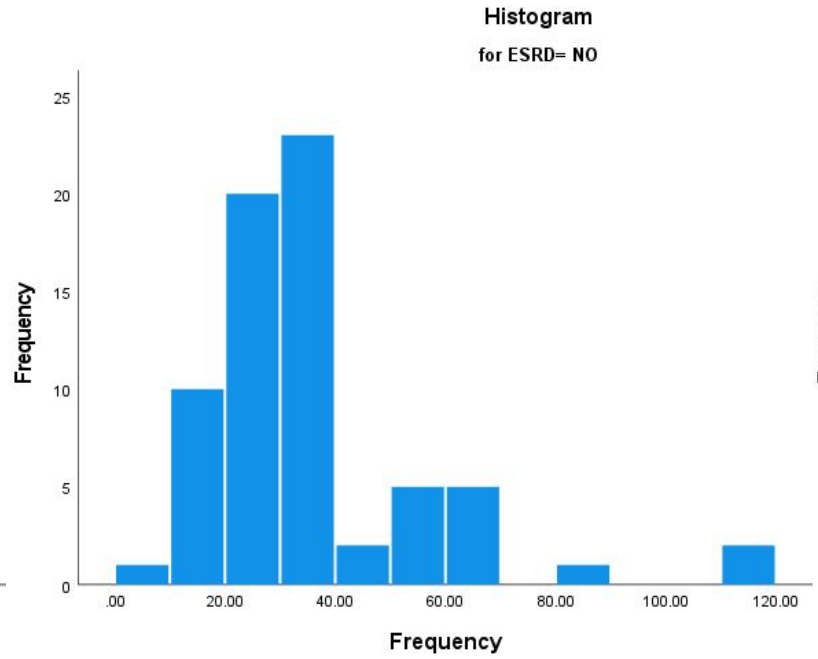


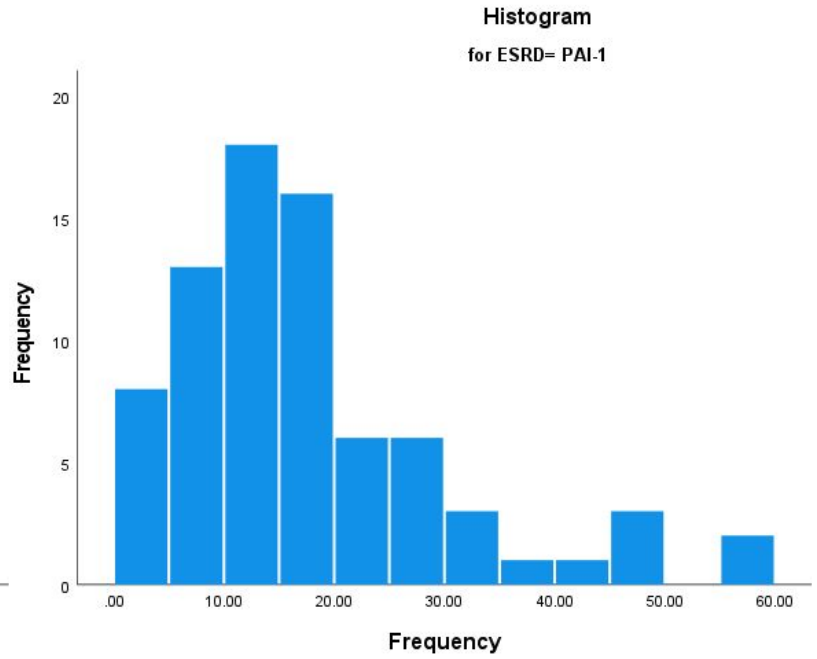
% Change:

- Average: 150.14% increase
- SD: 253.35% increase
- SEM: 193.34% increase

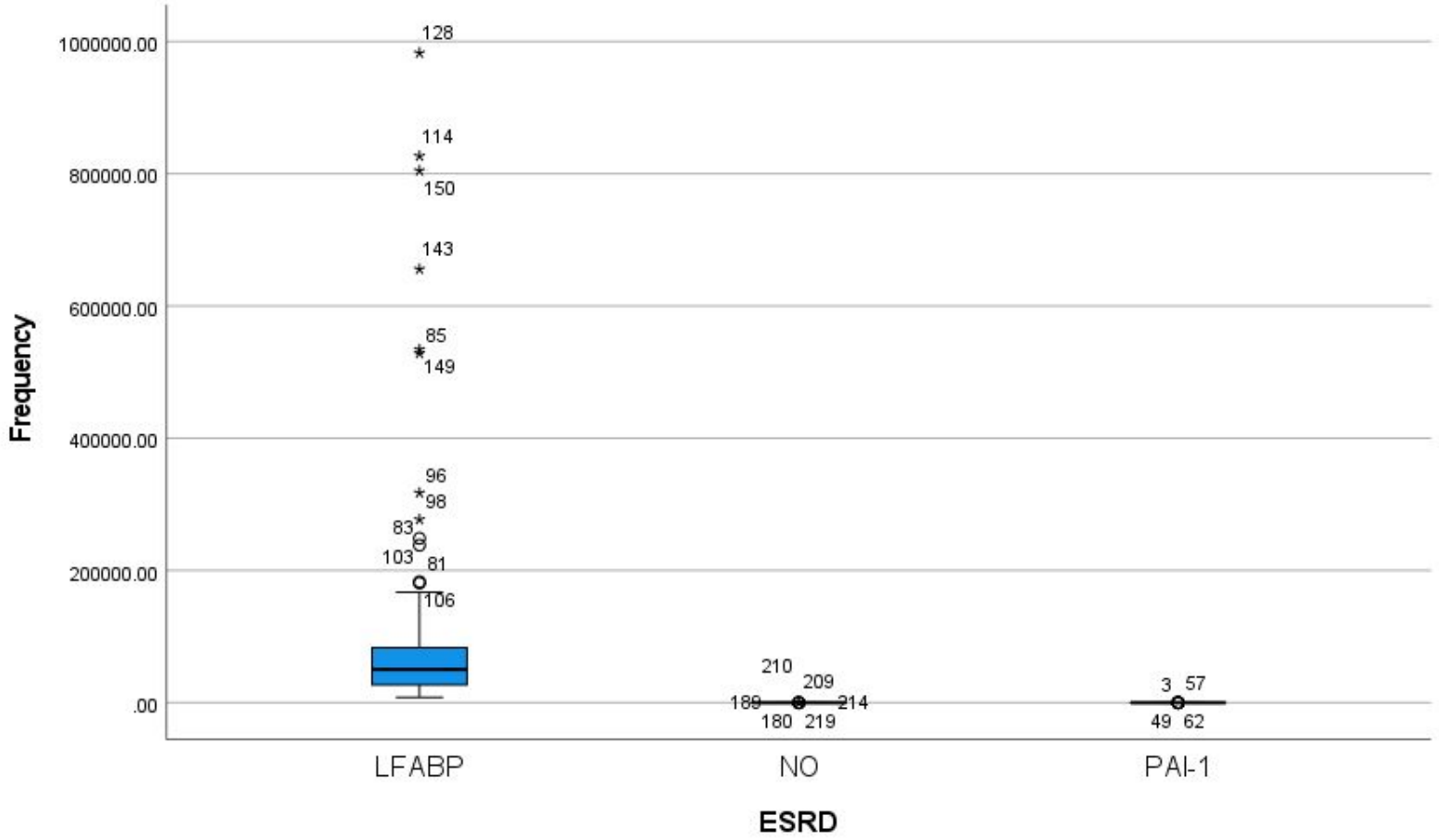
Skewness Values



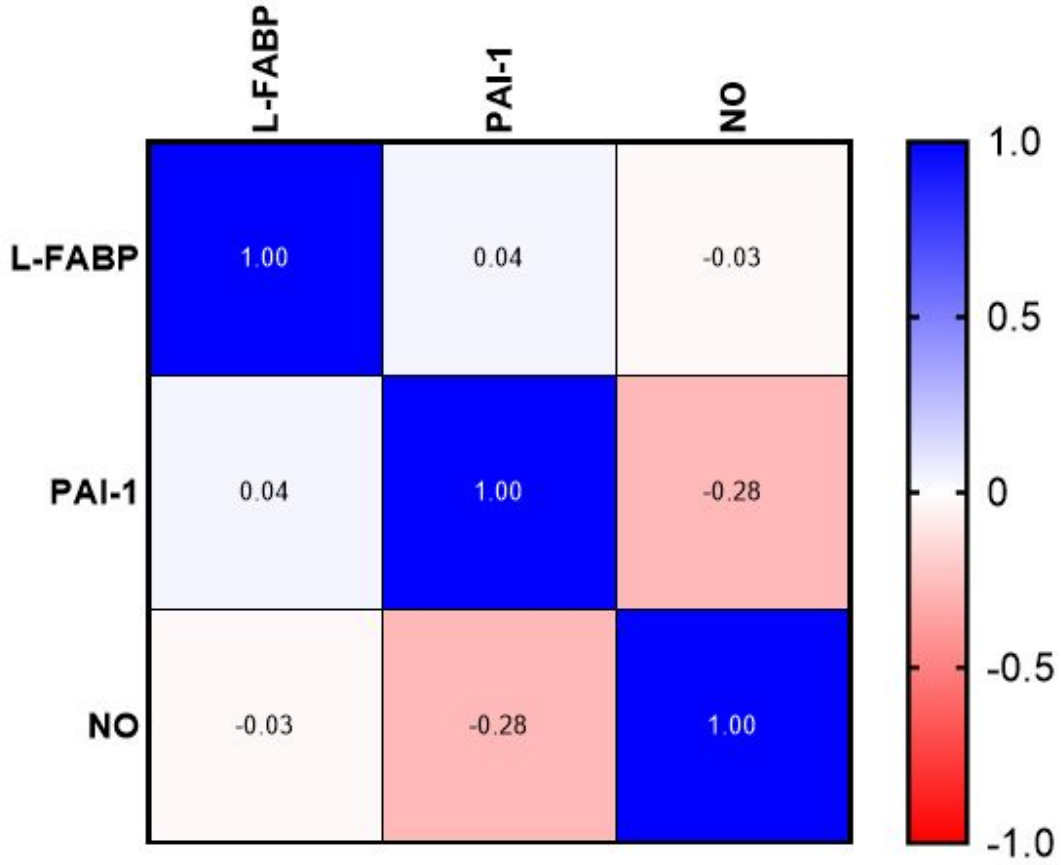




L-FABP, PAI-1, and Nitric Oxide



Correlation Analysis



	LFABP	PAI-1	NO
LFABP	1.000	0.037	-0.029
PAI-1	0.037	1.000	-0.278
NO	-0.029	-0.278	1.000

CONCLUSION

- ESRD L-FABP levels were statistically significantly higher than the levels in the control group
- My biomarkers have a very weak correlation with each other and some are inversely correlated
  - Strongest correlation: PAI-1 and NO at -0.28
- Biomarker data is skewed to the right
  - L-FABP is the most skewed to the right
- The results suggest that impaired renal function and kidney damage contribute to the marked increase of L-FABP in ESRD patients

Acknowledgements

I would like to take a minute to thank the following people for mentoring and supporting me throughout this internship:

- Dr. Fareed
- Dr. Siddiqui
- Dr. Laddu
- Divya Sridharan

Thank You

Preparing people to lead extraordinary lives