1-1-2019

Role Of Dyslipidemia On Lipid Metabolism In Maintenance Hemodialysis Patients

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ROLE OF DYSLIPIDEMIA ON LIPID METABOLISM IN MAINTENANCE HEMODIALYSIS PATIENTS

by

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DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2019

MAJOR: NUTRITION AND FOOD SCIENCE

Approved By:

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Advisor Date

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DEDICATION

This thesis is dedicated to all the significant people in my life, who have supported me through this journey:

My parents, Benereta & Sulejman Latifi

First and foremost, I would like to thank both my beloved parents, who never failed to support me through all my ups and downs in life. I would like to thank them for all their sacrifices and teaching me many life lessons that have carried to this point. Moreover, I am grateful for instilling in me the hard work, loyalty, and compassion for others.

My sibling, Ivi Latifi

I would like to thank my beloved brother, who has always been there and support me throughout the years. I thank him for believing in me, my goals, and my dreams in life. Lastly, I am extremely proud of the great person and physician you have become.

Others

I would like to extend my appreciation to my extended family here in the United States and back home to my birthplace Albania, my aunts and uncles, and my beloved grandparents.

ACKNOWLEDGEMENTS

It is a pleasure to thank those who have made this dissertation possible, beginning with my four committee mentors, my colleagues in the lab, and colleagues at the dialysis clinics that were involved in the study. First and foremost, I owe my deepest gratitude to my supervisor, Dr. Pramod Khosla, who has guided and supported me from the very beginning of this journey, from allowing me to join his lab as an undergraduate student, mentoring me as I pursued my master's degree and continue into the doctorate program. Furthermore, I am grateful to him for allowing me to see possibilities of what I can achieve. I would like to extend my appreciation to Dr. Smiti Gupta for the advice and her tutelage throughout the years. I would also like to extend my gratitude to Dr. Ahmad Heydari for investing in me as a graduate teaching assistant, and for his guidance throughout the years. Next, I would like to thank Dr. James Sondheimer for his clinical expertise and guidance for this study. I would also like to extend my gratitude for help and advice throughout the years to Dr. Paul Burghardt and Dr. Diane Cress.

A significant appreciation goes to all the dialysis clinics in the US (Great Lakes dialysis, DaVita, and Henry Ford) and Malaysia with our colleagues Dr. Tilakavati Karupaih, Dr. Karuthan Chinna, Dr. Zulfitri Mat Azuan Daud, Dr. Ban Hock, and Dr. Sharmela Sahatheva. I am also indebted to my lab colleagues starting with Dr. Deepinder Kaur, who has been a mentor and a friend, ever since the day she recruited me to join Khosla's Lab. Lastly, I would like to extend my gratitude to my other colleagues Dina Tallman, Bayan Tashkandi, Tanjina Rahman, Ushaasri Gullapalli, and Jayamini Shanika Arachchige.

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LIST OF ABBREVIATIONS AND ACRONYMS

[D]	:	Dyslipidemic patients
[MD]	:	Mixed dyslipidemic patients
[NL]	:	Normo-lipidemic patients
AA	:	African Americans
ACAT	:	Acyl CoA: cholesterol acyltransferase
AGEs	:	Advance glycation end-products
AHA	:	American Heart Association
Apo	:	Apolipoprotein [A-I; A-II; A-IV; B-48; B-100; C-I; C-II; C-III; E; D]
ATP	:	Adenosine triphosphate
BCAA	:	Branched chain amino acids
BMI	:	Body Mass Index
CE	:	Cholesterol Ester
CETP	:	Cholesteryl ester transfer protein
CKD	:	Chronic kidney diseases
CM	:	Chylomicron
CML	:	Nε-carboxymethyl-lysine
CRF	:	Chronic renal failure
CRP	:	C-reactive protein
CVD	:	Cardiovascular diseases
DL	:	Dyslipidemia
DM	:	Diabetes mellitus
EDTA-K2	:	Ethylenediaminetetraacetic acid K2 anticoagulant

eGFR	:	Estimated glomerular filtration rate
ELISA	:	Enzyme-linked immunosorbent assays
ER	:	Endoplasmic reticulum
ESRD	:	End-stage renal disease
FA	:	Fatty acids
FFA	:	Free fatty acids
GFR	:	Glomerular filtration rate
HD	:	Hemodialysis
HDL-C	:	High-density lipoprotein cholesterol
HMGCR	:	3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A Reductase (HMG-CoA reductase)
hsCRP	:	High-sensitive c-reactive protein
HTN	:	Hypertension
IDL	:	Intermediate-low density lipoprotein
I-HDL	:	Intermediate high-density lipoprotein subfraction particle
IL	:	Interleukin
I-LDL	:	Intermediate low-density lipoprotein subfraction particle
K/DOQI	:	Kidney Disease Outcomes Quality Initiative
Kt/V	:	Dialyzer clearance of urea multiplied by the dialysis time divided by the volume of distribution of urea, approximately equal to the patient's total body water
LCAT	:	Lecithin-cholesterol acyltransferase
LDL-C	:	Low-density lipoprotein cholesterol

LDLR	:	Low-density lipoprotein receptor
LH	:	Lithium heparin
L-HDL	:	Large high-density lipoprotein subfraction particle
L-LDL	:	Large low-density lipoprotein subfraction particle
Lp (a)	:	Lipoprotein (a)
LPL	:	Lipoprotein lipase
LRP1	:	LDL receptor-related protein 1
MC	:	Malaysian-Chinese
MHD	:	Maintenance hemodialysis
MI	:	Malaysian-Indians
MICS	:	Malnutrition-inflammation complex syndrome.
MM	:	Malaysian-Malays
MTS	:	Microsomal triglyceride transfer protein
MUFA	:	Monounsaturated fatty acids
MYI	:	Myocardial infarction
N.S.	:	Not significantly different (no changes statistically)
OS	:	Oxidative stress
PD	:	Peritoneal dialysis
PEW	:	Protein-energy wasting
RCT	:	Reverse cholesterol transport
ROS	:	Reactive oxygen species
RRT	:	Renal replacement therapy
SD	:	Standard deviation

S-HDL	:	Small high-density lipoprotein subfraction particle
S-LDL	:	Small low-density lipoprotein subfraction particle
SR-B1	:	Scavenger receptor class B type I
SREBPs	:	Sterol regulatory element binding protein
TAG	:	Triglyceride or triacylglycerol
TC	:	Total cholesterol
TCA	:	Tricarboxylic acid cycle
TP	:	Tocotrienols
TRF	:	Tocotrienols rich fractions
TT	:	Tocotrinols
URR	:	Urea reduction ratio
USRDS	:	US Renal Data System
VLDL	:	Very low-density lipoprotein

CHAPTER 1: INTRODUCTION

Chronic Kidney Disease (CKD)

Chronic Kidney Disease (CKD) is defined as an irreversible and gradual loss of kidney function over time. Characterized by damage to the kidneys, with structural or functional abnormalities of the organs, with or without decreases in glomerular filtration rate (GFR) manifested by either pathological irregularities such as the composition of the blood or urine or in imaging testing [1]. Moreover, the GFR could be < 60 ml/min/1.73 m^2 for ≥ 3 months or > 60 ml/min/1.73 m^2 with kidney impairment (marked by high levels of albumin in the urine) indicates CKD [1]. Numerous studies have linked CKD as an independent risk factor for cardiovascular disease (CVD) related event [2-4]. Moreover, CKD patients are at an increased risk at all stages of the disease for a CVD event, which is the primary contributor to morbidity and mortality in this population [5]. Adults with CKD are at a higher risk of early death compared to individuals without CKD of the same age. It is estimated that 5-10 times, CKD patients are more likely to die before even reaching ESRD [6, 7]. Also, the decline in the quality of life within this population further exacerbates the disease [8]. The added decline in GFR is also associated with increased risk for a CVD event, irrespective of gender or age [8].

According to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, CKD, and the progressive decline of kidney function can be classified into five stages. Stage-1 of CKD is characterized by a GFR of (> 90 ml/min/1.73 m^2), which is estimated to have a 10% decrease of the filtration rate, leaving the kidneys at a 90% functional capacity, but presented with some urinary abnormality reflective of proteinuria or hematuria. However, as the disease progresses, and the GFR declines furthermore from stage-2 to stage-5, kidney functions reduce below 15% or less, and is unable to filter and remove toxins (**Figure 1-1**) [9]. Moreover, according to the National

Institute for Health and Care Excellence (NICE), there is a rise in mortality at stage-3, therefore, this phase is monitored and is segmented into two additional groupings. Stage-*3a* – where the kidney's filtration rate declines from 59% to 45 % and then as the disease progresses there is a further decline in kidney function from 44% to 30 % defining stage-3b, which is reflective and is associated with an increase in CVD risk. Moreover, stage-3 seems to signify a standpoint of no return in kidney function, which is further validated by the sheer number of CKD patients which happen to be affected (**Figure 1-2**). In the US alone, 14.8% of the general adult population has CKD of which 6.4% are at stage-3 [10-12].

Numerous issues can lead to kidney disease; however, the leading causes of CKD in almost all income groups globally and responsible for two-thirds of the cases are diabetes mellitus (DM), followed by hypertension (HTN) [7]. CKD is further exacerbated by the continuing increase in DM, wherein 1 in 3 adults have been diagnosed. HTN is also an influencing factor, which currently affects 1 in 5 adults and is also on the rise [13]. Further complications include obstructions to the kidneys (caused by kidney stones), tumors, repeated urinary infections, as well as drugs that are toxic to the kidney [14].

Patients with CKD are additionally affected by systemic syndromes such as dyslipidemia (D), oxidative stress (OS), and inflammation [4]. Proteinuria in the urine is an indicator of kidney damage and further increases the risk of CKD progression into end-stage renal disease (ESRD) [7, 8]. In the US, approximately 37 million Americans have CKD [13]. Disease prevalence distribution based on the ethnicities in the US has African Americans (**AA**) at (18%) and Mexican Americans-Hispanics (15%), which are the highest prevalence rate when compared to Caucasian Americans at (13%) (**Figure 1-3**). Moreover, the disease amongst genders affects women (16%) more often than men (13%) (**Figure 1-3**) [6]. Around the world, CKD has increased significantly

in South Asian countries such as Malaysia where multiple-ethnicities are affected by CKD, the percent composition is highest on the local Malays (**MM**) (53.7%), followed by Chinese (**MC**) (21.1%), Indians (**MI**) (20.2%) and other ethnicities at (5%) (**Figure 1-4**). Gender wise, Malaysia is similar to the US, were more women (52.1%) than men (47.9%) are affected (**Figure 1-4**) [15].

End-stage renal disease (ESRD) and Maintenance hemodialysis (MHD)

As CKD progresses, estimated GFR (eGFR) declines from stage 1 all the way to stage 5, leading to kidney failure or ESRD. ESRD observed a steady rise in the US from 1980 to 2001, which then flattened in the early 2000s. While the US has seen a steady and gradual average of a 2% rise in the incidents of ESRD, countries, such as Taiwan. Thailand, Malaysia, the Republic of Korea, Jalisco of Mexico, Singapore, and the Philippines have experienced a 6% to 9% rise in the incidence rate of treated ESRD (**Figure 1-5**) [16]. Due to obesity and other comorbidities, the latest CDC simulation models have projected a rise between 11% % - 18% in crude incidence rate from 2015 – 2030. Moreover, the number of ESRD patients could jump 29% – 68% during the same period (971,000 – 1,259,000 patients) by 2030 (**Figure 1-6**) [17] .

According to K/DOQI guidelines, once CKD patients gradually lose kidney functions, filtration declines and reach its final stage-5, where GFR falls to or below (<15ml/min/1.73 m^2) the least optimal working condition for the kidneys. At this stage, patients are classified to be in kidney failure, wherein the patients need to undergo renal replacement therapy (RRT) [18, 19]. However, not all undertake dialysis treatment; since subjects may or may not have a residual renal function with urine outputs and some clearance. Thus, patients are monitored by health professionals for further loss of renal functions before being placed under dialysis treatment. The most common RRT in the United States (US) is hemodialysis (HD), also termed as maintenance hemodialysis (MHD). MHD treatment accounts for 98% of the total ESRD patients on dialysis

today [20]. Hemodialysis is carried out at regular intervals (generally 3-days per week), serving as a lifesaving procedure that removes toxin build-up and excess fluids in the patients' bodies. This is achieved via the utilization of the principles of hydrostatic pressure and diffusion across the concentration gradient, which is the equivalent of what kidneys are responsible for (**Figure 1-7 A**).

Other treatments are available such as peritoneal dialysis (PD), a similar principle to HD treatment at the dialysis centers, although PD could be conducted at home. In PD treatments, patients' peritoneum membrane linings of the abdominal cavity serves as the filter, which helps facilitate the removal of waste solution and fluid. However, in this case, a sterile dialysate solution composed of ions, bicarbonates, and glucose is introduced in the abdominal cavity via a catheter. For several hours (4 – 6 hours), the dialysate solution helps and allows to facilitate waste exchange across the concentration gradient before being drained and replaced by the new dialysate (**Figure 1-7 B**) [21]. Renal transplant is the treatment of choice for ESRD, which involves the donation of the healthy kidney and surgically placement of the donor's kidney in the iliac fossa (**Figure 1-7 C**).

For years, hemodialysis has been a lifesaving procedure. However, the prevalence of coronary heart disease (CHD) has seen an increase in these patients, and so has the mortality from CVD events at rates 10-30 times higher when compared to the general population [5]. CVDs account for more than 50% of the deaths in ESRD patients, due to acute myocardial infarction (MYI), atherosclerotic vascular disease, which could be characterized by chronic coronary artery disease, strokes, ischemic attacks, and or peripheral arterial diseases [22]. Irrespective of CVD, other factors that lead to the development of ESRD, can be explained by the rise in DM, which is ranked first, followed secondly by HTN and then glomerular disease (**Figure 1-8**). However, the

increased mortality in this population cannot only be explained by traditional cardiovascular risk causes alone, such as DM, HTN, advanced aging, but a combination of abnormal disorders of the non-traditional nature such as, malnutrition, inflammation, oxidative stress (OS), dyslipidemia (D), endothelial dysfunction, and vascular calcification have also been implicated [23, 24].

Lipid and Lipoproteins

Lipids

Lipids are defined as non-polar molecules that can dissolve other hydrocarbons; however, are unable to dissolve in aqueous solution (water). Lipid complexes can be in fatty acids (FA) form, waxes, phospholipids, or sterols (steroids and or cholesterol). When three FAs link covalently to a glycerol backbone molecule via an ester bond, it leads to the formation of triglycerides (TAG) [25]. Fats such as TAGs play numerous roles in the human body biochemistry, since they can be stored, when in excess, in the adipose fatty tissues and can be used for energy production (adenosine triphosphate – ATP) when required. Another function of lipids, in mammals, is to provide protection and insulation, by preventing heat loss, as well as being good at shock-absorbing, which serves as cushions to significant organs (kidneys, gonads, heart, liver, and others.). Accumulated lipids such as phospholipids, glycolipids, and sterols (cholesterol) play a significant role in cellular membrane biogenesis (making new biological membranes). Cholesterol helps the cell membrane to maintain its fluidity. A feature and the interaction with phospholipids of the lipid-bilayer, allows cholesterol to increases membrane packing, thus altering the membrane fluidity and integrity of the cell membrane [26]. Moreover, cholesterol facilitates the synthesis of steroid hormones, which assists in the absorption of the fat-soluble vitamins (A, D, E, and K).

Lipoprotein functions

Lipids are insoluble in an aqueous environment; therefore, they need to be packaged in a form that will allow for transportation throughout the bloodstream. Hence for such transport to ensue, lipids are organized into lipid-protein complexes known as lipoproteins [27]. The most prominent lipoproteins are the chylomicrons (CM), very-low-density lipoproteins (VLDL), intermediate-low density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoprotein (HDL). While the lipoproteins differ in their functionality, they also share similarities, specifically in the inner structure, the amphipathic phospholipid particles, which are composed of hydrophilic polar heads and the hydrophobic nonpolar FA tails. In addition to lipids such as TAG and cholesterol esters (CE) residing in the core of the formed sphere.

The differences in their functionality are due to the surface proteins, known as apolipoproteins or apoproteins. These apoproteins can be peripheral or integral proteins, and many times serve as enzymes or as ligands for cell receptors. Hence lipoproteins have a different proportion of triglycerides, phospholipids, cholesterol, and proteins, which further differentiates their functional roles in metabolism. CMs are primarily involved in exogenous lipid transport. Dietary lipids are packaged into chylomicrons within the enterocyte of the small intestine and distributed to peripheral tissues, mainly the muscle and or adipose tissues [27].

However, before CMs reach the bloodstream, they must follow the path from gut to the blood circulation, passing through the lymphatic system by entering specialized lymphatic vessels, knowns as lacteals, in the villi of the intestine. Once in the lymphatic system, CMs travel through these lymph vessels and will exit at formed ducts (left or right thoracic ducts), which ultimately empty into veins, allowing for CM to reach the circulatory system [28]. Uniquely, CMs are known to have the largest particle size (75-1200 nm) of the lipoproteins, with the lowest density at (<

0.930 g/mL) and are a major transporter of (90%) of exogenous TAG. Additionally, CM particles are composed of roughly (2%) proteins, (3%) phospholipids, and (5%) cholesterol, which is embedded in particle phospholipid and or inside its core. Moreover, due to its versatility, CMs have many apoproteins on the periphery or integrated into the monolayer of the lipoproteins, typified by ApoB-48 which the hallmark of CM particles, in addition to other Apolipoprotein A-I, ApoA-II, Apo-IV, ApoC-I, C-II, C-III, and ApoE (**Table 1-2**).

While CMs play a specific role, mainly being involved in the exogenous lipid transportation, other lipoproteins such as VLDL, IDL, and LDL function as the endogenous transporters in delivering lipids to tissues. An additional key difference between these lipoproteins are noted in the type of ApoB which they carry. Although both ApoB's come from the same gene, due genetic differentiation, ApoB-48 is made explicitly in the intestine and resides in the CMs, while ApoB-100s are made in the liver and found in VLDL, IDL and LDL particles. When comparing the VLDL in relation to CM particles, the VLDLs lipoprotein transports less TAG content (60% vs. 90%), more phospholipid (14%), proteins (6%) and (20%) CE. Furthermore, the VLDL lipoproteins have a greater particle density (0.930 – 1.006 g/mL) with a smaller diameter (30 – 80 nm). Other important apoproteins associated with VLDL are ApoC-I, C-II, C-III, and ApoE (**Table 1-2**). As VLDL goes through changes, depicted by the losses of TAGs due to hydrolysis by lipoprotein lipase (LPL), it gains more CE, the particle converts into IDL. These lipoproteins carry less TAG (20%) compared to CMs, and they attain an increase in phospholipids (22%), CE (40%), and proteins (18%). Moreover, the IDL particles have a higher density of (1.006 – 1.019 g/mL), smaller particle diameter (25 – 35 nm), and are associated having ApoC and ApoE (**Table 1-2**) [27, 29].

Similar to VLDL, there are changes to IDL which are altered into LDL lipoprotein. In the endogenous lipid metabolism pathway, LDL lipoproteins carry significantly less TAG (7%), more phospholipid (22%), proteins (21%), and significantly more CE (50%) content, which is delivered to tissues. The LDL has an increased particle density (1.019 – 1.063 g/mL), and smaller particle diameter (18 – 25 nm) with ApoB-100 as the only apoprotein for the lipoprotein (**Table 1-2**). An increase in plasma LDL is considered to be the "bad cholesterol;" due to having the ability to transports cholesterol to the peripheral tissues and or macrophages where the potential for oxidation is high, in which case can lead to the development of atherosclerosis. Although VLDL, IDL, and LDL are commonly known to transfer lipids such as cholesterol to tissues, these lipoproteins can also ensure the opposite; where cholesterol is transported back to the liver for excretion. However, it is mainly the HDL lipoproteins which are commonly known as the "good cholesterol" since it has the ability to retrieve free cholesterol and brings it back to the liver for multiple fates for excretion, making bile acid and/or hormones. The HDL lipoproteins also are characterized to have the highest particle density when compared to the other lipoproteins at (1.063 – 1.210 g/mL), and the smallest diameter of (5 – 12 nm). Embedded in the bilayer of HDL are numerous apoproteins, with most prominent apolipoprotein being ApoA-I, followed by ApoA-II, and others such as A-IV, ApoC-I, C-II, C-III, and ApoE (detail remarks on apoproteins: **Table 1-3**). Overall, HDL lipoproteins are composed of less TAG content at (5%), in comparison to other lipoproteins, CE (25%), phospholipids at (26%), and the highest content are proteins (44%).

While the main function of the different class of lipoproteins is transportation of lipids in the blood, they also specialize in the type of lipid transportation which are involved in, thus, lipid metabolism can be summarized by the three key pathways: exogenous, endogenous, and reverse cholesterol transport (RCT) [30, 31]. The exogenous transport system revolves around the use of

CM as the lipoprotein, which helps facilitates the transport of dietary fats such as TAG, from the intestine to peripheral tissues for either storage or energy utilization. The system is mainly active during the time when fat-containing meals are consumed. The endogenous system involves VLDL, IDL, and LDL lipoprotein particles, in which case the transportation of triacylglycerol molecules from the liver during normal metabolic conditions to the peripheral tissues occurs again for storage or energy use. Different from these two systems is the RCT pathway, which uses HDL lipoproteins to pick up the excess cholesterol from the peripheral tissues in order to transport and deliver it back to the liver [27].

Exogenous Lipid Metabolism Pathway

The exogenous pathway begins when food, which contains dietary fat, is consumed and digested. Post digestion, the absorption process follows, where fat in the form TAGs are broken down by brush borders lipases and pancreatic lipases located in the intestinal lumen [32]. The breakdown of lipids is further assisted by the release of bile salts, which is created by the liver and stored in the gallbladder. Bile salts are cholesterol derivatives which act both as a detergent and an emulsification agent due to its amphipathic capabilities. This allows for easier access by the lipases. TAGs molecules are hydrolyzed by lipases when they break the ester linkages with the addition of a water molecule, and still within the micelles environment, leads to the production of smaller products which are mono-, di-acylglycerols, free fatty acids (FFA), monoacylglycerol (glycerol) and free cholesterol.

Lipid micelles particles are taken by the enterocytes, lead through various transporters, in order to be further processed by endoplasmic reticulum (ER). In the ER, the broken lipids are then used to resynthesize TAG, phospholipids, and CE. Following the re-synthesis of lipids in the ER, the compounds require to be packaged before they are transported, and the process occurs by a

carrier molecule known as lipoproteins. Specifically, the CMs are the main lipoprotein in the exogenous system. CMs also recruit the aid of ApoB-48 proteins, which are made by the intestine.

Therefore, lipids are packaged into large, spherical TAG-rich lipoproteins along with any other hydrophobic such as cholesterol are sequestered into the core of a protein molecule, which has polar heads, allowing for interaction with the aqueous environment and the hydrophobic molecules which reside in the core. The packaging occurs in the ER, with further help from ApoB-48, a scaffolding protein, and by microsomal triglyceride transfer protein (MTS) another necessary lipoprotein assembler [33, 34]. In addition to ApoB-48, several exchangeable apolipoproteins can be found on the surface of the CM. Once CMs enter the blood via the lymphatic system, they acquire additional apoproteins (mainly ApoE and ApoC-2), which are mostly transferred from HDL lipoproteins through interactions in the circulation.

The entrance of the CMs in the blood circulation is a slow process, which could take up to 14 hours after consumption depending on the TAG-rich dietary food consumed. On the other hand, plasma TAG concentration, unlike chylomicrons, usually, peak 30 minutes to 3 hours after meal consumption and return to nearly normal within 5-6 hours [27]. The final act for the CM is the deliverance of TAG to tissues other than the liver, but mainly muscles and adipose tissues. Once at the surface of the targeted cell, CMs are engaged by LPL enzymes. The enzyme facilitates the hydrolyzes of the TAG, from CM particles into FFA and 2-monoacylglycerols, which then are taken by the cells [35, 36]. Tissues such as adipose and muscle are unable to phosphorylate glycerol which, leads for these molecules being transferred to the serum and picked up by the liver or kidney. As the CMs are depleted from TAG-rich lipoproteins, the molecule becomes a chylomicron remnant, which before it is processed by the liver, it transfers the apoproteins such as (ApoAs and ApoCs) back to the HDL particles. The CM remnants travel to the liver were a specific

receptor; LDL receptor-related protein 1 (LRP1) is able to recognize ApoE component, which allows for the particle to be taken entirely into hepatocytes, where hepatic lipase further hydrolyzes the remnants CM for any leftover TAG or phospholipids.

Endogenous Lipid Metabolism Pathway

The liver is one of the essential organs, which plays a fundamental role in the management of various metabolic mechanisms, and the endogenous lipid pathway is one of these critical processes. When TAGs, come together with CEs, and ApoB-100, they are assembled and packaged by the Golgi apparatus in the hepatocytes, resulting in the formation of nascent VLDL particles. [29]. In its infant stage, the VLDL particles are composed of three important apoproteins, ApoB-100, ApoC-I, and few ApoE. Once the lipoproteins are released in the circulation, the nascent VLDLs interact with HDL particles, where apoproteins such as ApoC-II and additional ApoE are picked up, resulting in the maturation of particle. The mature VLDL particles during circulation are exposed to inactive LPL enzymes, which are expressed on endothelial tissues; however, it is the ApoC-II on the lipoprotein surfaces which leads into activating the enzyme. Ultimately the activated LPL initiates the hydrolyzes of TAG by breaking the ester bonds, which leads to the release of generated glycerol and FA byproducts. The molecules of glycerol and FA are absorbed in the blood by peripheral tissues depictive of adipose and muscle tissues. The compounds are resynthesized back to TAG molecules, inside the cells, in order to be used for energy production or storage. As VLDLs are metabolized, they are depleted of TAG, making the particle denser, in addition to exchanging ApoC-II for more ApoE, which eventually changes VLDL into a transient IDL particle.

The IDL lipoprotein is hydrolyzed by LPL, which leads to the loss of more TAG. It is estimated that 50% of the IDL particles can either be taken up into the liver or the other 50%, also

lose the ApoE [37]. This is followed by an increase in cholesterol concentration, leaving the ApoB-100 as the primary apolipoprotein, thus producing the LDL lipoprotein [38]. It is worth noting that in a transient environment, an added path can be taken by both VLDL, IDL, or any other ApoE containing lipoproteins, which is to utilize the LDL receptor (LDLR) at the hepatocyte surface, where via the process of endocytosis they are removed from circulation. Once inside the liver, the VLDL and IDL are degraded by hepatic lipases were TAG and cholesterol are recycled for other metabolic processes.

The VLDL is characterized as a TAG-rich lipoprotein, whereas the LDL lipoprotein is the composite of the remaining lipids that were secreted by the liver, mainly phospholipids, free cholesterol, and CE making, the LDL particle primarily a transporter for cholesterol [27, 39]. While LDL particles shrink in size as they are depleted of TAG, it only retains apolipoprotein ApoB-100, which constitutes one per molecule. The particle size is additionally affected by the interactions with LPL, as well as other lipoproteins where lipid exchange can occur. Therefore, because of these multitudes of interactions, it leads to various sizes of the LDL particle, which can range from small, intermediate or large. Many clinical studies have associated the small dense LDL (S-LDL) as more atherogenic than larger LDL, emphasizing the importance of LDL cholesterol removal from the blood in order to prevent the accumulation in arterial wall or tissues [40]. Thus, clearance of the LDL from the blood is accomplished via cell surface receptors. Specifically, the LDLR found in the liver and non-hepatic tissues which recognizes the ApoB-100. Upon binding to the LDL lipoproteins, the particles and LDLR are internalized by endocytosis.

In the cell, the LDL particle is carried to the lysosomes, while LDLR is released for either returning on the cell surface or recycled. At the lysosome, the LDL lipoprotein is hydrolyzed via lysosomal enzymes into amino acids, FFA, and free cholesterol. The new influx of cholesterol has

various metabolic fates such as bile acids, and/or hormone production. The entry into the cell also exerts a more profound function on the cellular internal cholesterol products; it acts as a feedback inhibitor. Largely the high amount of cholesterol acts on the rate-limiting enzyme for cholesterol biosynthesis: HMG-CoA reductase as it suppresses the enzyme by decreasing the transcription of the reductase gene and concurrently increases the degradation of the enzyme [41]. Moreover, part of the cholesterol which is not used for metabolic processes is stored away in the form of CE, where is modified by cholesterol esterification by acyl CoA: cholesterol acyltransferase (ACAT).

Lipids that have successfully been targeted by the medical and pharmaceutical community in the past decade, have been the TC and LDL-C. The mechanism by which reduction in TC and LDL-C has been accomplished is through targeting HMG-CoA reductase pathway and the LDLR. Medications such as lovastatin, pravastatin, rosuvastatin, or atorvastatin are HMG-CoA reductase inhibitors drugs which block the rate-limiting enzyme HMG-CoA reductase in the cell which is involved in the synthesis of cholesterol via the mevalonate pathway. The low yield of internal cholesterol forces an upregulating and synthesis of new LDLR, resulting in an increased clearance of LDL particles from the circulation, thus lowering blood cholesterol from the body [42]. The efficacy of statins as lipid-lowering drugs has been considered to be one of the most significant discoveries of the decade. Notably, since it lowers LDL-C, and its use as primary prevention in people with high risk for CVD as well as secondary prevention treatment for those who may develop CVD [43, 44].

There are other medications such as Ezetimibe, which target cholesterol-lowering by reducing the intestinal absorption of cholesterol; however, it is frequently combined with statins [45]. Niacin (B_3), a water-soluble vitamin is another product that lowers LDL by selectively inhibiting hepatic diacylglycerol acyltransferase, thus reducing TAG synthesis and VLDL

secretion [46, 47]. The use of Tocotrienols (TT), a fat-soluble compound derived from the vitamin E family, part of the isomer, especially a delta- and gamma-TT in an in vitro setting have shown the ability to treat high cholesterol. The gamma-TT has also shown to work as another HMG-CoA reductase inhibitor, which reduces cholesterol production. Though there have been various investigations in TT in examining their effects, only to conclude with mixed results [23, 48].

Reverse cholesterol transport pathway

Reverse cholesterol transport (RCT) (**Figure 1-9**) is the process where excess cholesterol from peripheral tissues is picked up by the HDL particles and delivered to the liver for excretion from the body as either bile acids or free cholesterol [27]. The RCT is a multiple-step mechanism involving many cell surface receptors, intravascular enzymes, and transfer of lipids among circulating lipoproteins; moreover, the cholesterol from the peripheral tissues comes back to the liver for removal from circulation [49]. The HDL particle is raised entirely within the intravascular space, starting with ApoA-I. The lipid-free ApoA-I is created and secreted by both liver and the small intestine; where ApoA-I protein can also be picked up from CM and VLDL during their TAG hydrolysis which can allow onto giving rise to new HDL particle.

ApoA-I is the major protein of HDL lipoprotein particles and plays a vital role in its development. It serves as an acceptor for phospholipids and facilitates the cholesterol transfers from the liver via ATP-binding cassette transports (ABCA1) which results in the formation of the nascent HDL particle [50]. Like ApoA-I, the ABCA1 protein, not only aids in the formation of HDL, but it is also known as the cholesterol efflux regulatory protein (CERP) which is a protein coded by ABCA1 gene [51]. Moreover, it is known as a major regulator of cellular cholesterol and phospholipid homeostasis. Additionally, ABCA1 mediates the cholesterol efflux between the lipids and apoproteins such as ApoA-I and ApoE [52]. Nascent HDL acquires additional

phospholipids and cholesterol when it interacts with other ABCA1 protein receptors in the peripheral tissues. An undeveloped HDL particle has a discoidal shape, which retains the amphipathic property to form a bilayer, thus more cholesterol is picked from peripheral tissues through another receptor aside from ABCA1, which happens to be the scavenger receptor class B type 1 (SR-B1); a receptor that is located in the peripheral tissues of muscles, adipose, and macrophages within coronary arteries [27, 53].

The abilities for nascent HDL lipoproteins to accept cholesterol from macrophages not only aids in the HDL particle maturation but also the removal of cholesterol from the circulation is beneficial to the cardiovascular system. The removal of excess cholesterol leads to decreased deposition of CE in the vascular endothelium, thus decreasing CVD or atherosclerotic events [54]. Moreover, the nascent HDL throughout this process acquires an intravascular enzyme known as lecithin-cholesterol acyltransferase (LCAT). This is a key enzyme that helps form CE by catalyzing the transfer of fatty acids from the *sn*-2 position of phosphatidylcholine to free cholesterol within the HDL particle. Furthermore, since the CE particles are nonpolar, they migrate to the core of the lipoprotein, thus forming a mature HDL particle.

Small spherical HDL can further interact with peripheral tissues as the ApoA-I binds to SR-B1 and another receptor ATP-binding cassette sub-family G member 1 (ABCG1) in the peripheral tissues where more cholesterol is acquired during the efflux transportation mechanism. The ABCG1 belongs and is encoded by a gene which is part of a superfamily of ATP-binding cassette (ABC) transporters; which serves as transporters that are involved in the macrophage, cholesterol and phospholipids transportation, which further enables the regulation of cellular lipid homeostasis [55]. On the other hand, mature HDL lipoproteins can bind to the receptor ABCG1

(on the macrophage), but not ABCA1. Whereas both nascent and mature HDL can bind to SR-B1, at the liver or peripheral tissue (macrophase, muscles, or adipose) for the cholesterol exchanged.

The binding to the cell receptors and continuous action of LCAT facilitates esterification of cholesterol into CE, which allows for the new formed CE to be sequestered in the HDL lipoprotein, thus, assisting the particle in growing in size, into a large HDL. However, due to metabolism being very transient, there can be transfers of CE from HDL into other lipoproteins (such as VLDL, IDL, LDL). Enabling such action between HDL and lipoproteins such as VLDL as well as LDL, is the cholesterol ester transfer protein (CETP) enzyme [56]. CETP is in the plasma, is a lipid transfer protein that facilitates the transport of CE and TAG between the lipoproteins in a heteroexchange trade mechanism. It exchanges TAG from VLDL and LDL in return for the CE from the HDL particle, in which case retains the TAG from the two lipoproteins during the exchange, thus depleting an HDL particle of CE, which was on its way back to the liver for excretion. Furthermore, the exchange of CE through CETP in the plasma has been associated in reducing the size of HDL particles, which benefits the lipoprotein into making it more conducive to the interactions with cell surface receptors, thus increasing the HDL's ability to accept more cholesterol for removal.

While but not always, the exchange can lead to health complications in various individuals who are presented with metabolic syndromes featured by low HDL, and high TAG, TC, and LDL. Such individuals are at a higher risk for developing atherosclerosis since the cholesterol, which was meant for excretion was diverted into other pathways. When CE are relocated from HDL into VLDL and or LDL lipoproteins, these molecules take a new path away from being unloaded to the liver, thus, this new fate usually consists with CE being deposited into tissues, especially arteries, where it accumulates, becoming prone to oxidation or leading to an artery blockage which further

exacerbates the risk for cardiovascular disease [57]. Various pharmacological clinical trials have been and are being conducted in improving HDL, by using drugs to inhibit CETP (CETP inhibiting such as a Torcetrapib agent) [58]. However, studies with mortality and decline in cardiovascular events have primarily been inconclusive with disappointing results [59].

One more critical regulatory protein in RCT metabolism is protein phospholipid transfer protein (PLTP), found in the human plasma, which helps to facilitate the transfer of phospholipids or excess of surface lipids from TAG-rich lipoprotein to HDL. PLTP, including CETP, belongs to a family of lipid transferring lipopolysaccharide-binding proteins (LBP) and a bactericidal permeability-increasing protein (BPI). It is worth noting that besides cholesterol and phospholipids, PLTP also has the ability to transfer diacylglycerols and alpha-tocopherol, cerebroside, and lipopolysaccharides thus, the reason why is portrayed as a nonspecific lipid transfer protein. In the plasma, there are two forms of PLTP, that play important roles in lipid metabolism; the *active* form which has been associated with ApoA-I, whereas the *inactive* form has been linked to the ApoE [60]. Hence, the activity of PLTP has lead to the formation of smaller lipoprotein remnants, which form into LDL. On similar roles, like CETP, PLTP helps to facilitate the maturation of HDL particles, as well as regulate the size and composition of the HDL lipoprotein which is crucial to the plasma HDL-C levels in the blood [61]. Further actions by PLTP in lipid metabolism are to assist into the uptake of cholesterol for periphery tissues in the which are transported for degradation and excretion by the liver. Other linked interactions with ApoA-I and ApoA-II have shown that PLTP seems to exert effects onto the apoprotein lipidation as well as nascent HDL biogenesis in the hepatocytes, by stimulating the ABCA1 efflux and remodeling of nascent HDL particles [62-64].

The final step in RCT is the binding of HDL to SR-B1 receptor on the surface of the hepatocytes. Upon binding and removal of CE from the particle core, the HDL lipoprotein enters a two fate pathway. One possible fate, for the HDL lipoproteins, once the cholesterol is removed, is on being released in the circulation for reuse in the collection of more free cholesterol, or the entire HDL lipoprotein is internalized and degraded in the lysosomes similar to the degradation of LDLR. Once internalized for degradation, CE molecules are hydrolyzed by cholesterol ester hydrolase enzymes, thus freeing the cholesterol and directed by the liver for bile acid production or the formation of steroidal hormones.

RCT mechanism and disease complications

In general, HDL is perceived to function as atheroprotective particles, which promotes RCT, induces cholesterol efflux from peripheral tissues such as macrophages foam cells that are taken into the hepatocytes in a known process that is considered to be beneficial to cardiac health. However, this process appears to be compromised in MHD patients. Various studies in MHD subjects have linked HDL particles being defective and compromised, a link which is further exacerbated by dysregulation of lipid metabolism, which has been associated with the dysfunctionality activity in RCT [65]. Other key modulators in the RCT pathway believed to be affected are the LCAT enzymes. Under normal conditions LCAT is involved in HDL maturation to a spherical-shaped particle; however, in ESRD patients, the enzyme has shown low activity. A decreased LCAT activity is problematic since it has been associated with the formation of non-mature or smaller HDL particles. The non-mature HDL lipoproteins have been linked in being ineffective in performing regular RCT functions. Therefore, this would impede the HDL-mediated RCT pathway of disposing of excessive cholesterol from extrahepatic tissues and walls of the blood vessel [66].

While not conclusively, but it is hypothesized that MHD patients have HDL particle impairment, which could lead to lack of particle maturation, thus lipoprotein dysfunction, and this could be due to the downregulation of LCAT as well as increased CETP activity [67]. Additional plasma CETP has shown to play a vital role in RCT, evidenced by the upregulated levels of this enzyme that increases the transportation and exchange of TAG in VLDL or LDL in return for CE from HDL. Other compromised key modulators in RCT metabolism are the efflux genes such as ABCA1, ABCG1, LXR-α, SR-B1 (**Table 1-4**), and the influx of downstream related genes CD36, LOX01 and or SRA1 (**Table 1-5**) [68-70]. Multiple pathways are affected; thus, additional studies are needed to determine what else may increase the risk for CVD events. Therefore, it is imperative to understanding HDL metabolism, specifically how RCT may be impacted by D in ESRD patients undergoing hemodialysis. As is currently understood, dysfunctional RCT in MHD patients is characterized by increased levels of TAG, elevated VLDL, IDL, as well as CETP activity and ApoB-100 protein; whereas TC and LDL-C lipids can be either normal to marginally decreased. Moreover, the HDL-C levels are noted to be decreased, so are the ApoA-I levels, and similar LCAT activities are downregulated as well (**Figure 1-10**).

Furthermore, TAG enrichment of HDL particle could be multifactorial; the transfer of CE occurs between other lipoproteins, apart for CETP being a culprit which facilitates this exchange. It is believed that another influencer has been linked and identified and that being the hepatic lipase (HL), which serves as a catalyst in the hydrolysis of TAG. However, mutations in the HL gene leads to an enzyme deficiency, an event that ultimately results in an increase in TAG level and increased risks for CVD. Moreover, HL facilitates the clearance of TAG from VLDL. Additionally, HL function is further regulated and controlled by the quality and composition of HDL lipoprotein, which in MHD patients is presented by declined HDL levels. Additionally, HDL

regulates the release of HL enzyme from the liver, and the enzyme activation occurs in the circulation for which case is transported by the HDL particles [71].

Patients affected by CKD and ESRD present with hypertriglyceridemia, impaired clearance of TAG-rich lipoproteins and their remnants, abnormal composition are all chiefly due to the downregulation of LPL, HL, VLDL-receptor, LDL-receptor in conjugation with a concurrent upregulated hepatic ACAT [67, 72]. Conversely, an impaired HDL metabolism contributes to the disturbances of TAG-rich lipoprotein metabolism. Intensifying the dysfunction in HDL metabolism for the MHD patients is the downregulation of the apolipoproteins (most abounded protein in HDL particles) ApoA-I, ApoA-II, and ApoC-II [67]. When evaluated together with these abnormalities of the malnutrition-inflammation complex syndrome (MICS), coupled with OS, inflammation, and D, it makes it highly conducive to exacerbate atherosclerosis in HD population.

Dyslipidemia in Lipid Metabolism

MHD patients on dialysis are also affected by the systemic syndromes known as D [4]. It is an intrusive factor, similar to malnutrition and inflammation with a high association with CVD and increased mortality [73]. The term D is defined as dysregulation of lipid metabolism, which is marked by abnormalities in plasma lipids, lipoproteins as well as other lipid-related parameters, including associated enzymes. While the subject of D is not fully understood, more work is required in order to comprehend the overall extent of this dysfunction. Exacerbating the issue is the fact that there is limited knowledge, which is due to the low volume of clinical studies. However, a general hypothesis, by understanding lipid metabolism have been advanced on how D may affect lipids, lipoproteins, and enzyme at different stages of the disease **Table 1-1** [72, 74].

In ***CKD***, the primary complication due to D is associated with the metabolism of HDL and TAG-rich lipoproteins resulting in low HDL-C and elevated TAG [75, 76]. Moreover, the plasma concentration of apolipoproteins A-I and II are reduced, whereas ApoA-IV is increased. The HDL/TAG ratio and pre-β1 HDL (a discoid-shaped HDL particle), which contains ApoA-I, phospholipids, unesterified cholesterol, are elevated, and this due to the impaired maturation of the HDL particle. The failure for the improperly developed HDL particle leads to the impairment of RCT, which is analogous to the decline in HDL-C levels [75, 77]. Furthermore, linked to the decline in HDL-C concentration are two RCT enzymes: CETP, which its activity is seen to be elevated, while LCAT enzyme activity is decreased [72, 74]. Additional lipid parameters affect are shown in (**Table 1-1)**.

As CKD advances to ESRD, the lipid parameters also change. In ESRD patients undergoing hemodialysis, lipid parameters for LDL-C and TC are normal or decreased, whereas, in CKD, they are elevated. In both CKD and ESRD, IDL, VLDL, Lp(a), small-dense LDL, and TAG levels are increased. Irrespective of the disease state, HDL-C levels are decreased in the MHD patients analogous with CKD patients. Non-HDL-C and ApoB are either normal or decreased in ESRD when compared to CKD, which are elevated. As for the reverse cholesterol transport enzymes, CETP is increased, and LCAT is decreased in ESRD, as well as in CKD patients (**Table 1-1**). ESRD patients have an accumulation of oxidized lipids, lipoproteins followed by low plasma HDL-C along with impaired HDL maturation and function [78]. Impaired synthesis and activity of HDL and the delayed catabolism of TAG-rich Apo-B containing lipoprotein subsequently leads to elevated TAG and low HDL-C levels [67]. The rise in TAG levels can be additionally explained by the impaired clearance of VLDL, IDL, and the accumulating CM remnant which, too, are accompanied by a significant increase in plasma ApoC-

III (a potent inhibitor of LPL) and reduced ApoC-II the activator of LPL [67, 79]. The Framingham Study recommended desired lipid parameter ranges reflective of moderate (too low) TAG levels; high HDL-C followed by lower LDL-C because it was shown to be protective against CVD. However, the recommendations were indicative of the general population [80].

ESRD patients are challenged by a phenomenon known as "reverse epidemiology," in which parameters such as high HDL-C and low LDL-C plasma concentration may not be as protective when compared to their healthy counterpart [81]. Various studies have examined how to control DL by raising the HDL and stabilizing the remainder of the lipid profiles. Kilpatrick et al. (2007) found that serum HDL-C showed no clear association to improve or worsen survival however, patients presented with elevated TAG showed improvements in survival between concentrations levels of 200-250 mg/dL and above 250 mg/dL mortality rates increased, likewise LDL-C levels of < 70 mg/dL was associated with low survival. This phenomenon of high TAG and low LDL-C affects AA patients as well; however, elevated serum LDL-C (>100 mg/dL) showed a two-fold risk for CVD, unlike others [82]. On the other hand, Moradi et al. (2014) found that increases in HDL-C concentration up to 50 mg/dL was favorably associated with a reduction of CVD and mortality.

Nonetheless, incremental increases in HDL-C of greater than 50 mg/dL were paradoxically associated with increased deaths [83]. These findings were further validated by Chang et al. (2018) in a large MHD cohort, which found that incremental increases in serum HDL-C over 5-years did not associate with the improved outcome on cardiac incidents [84]. There has been evidence that serum parameters such as non-HDL-C are more accurate predictors for CVD outcomes compared to LDL-C. Chang et al. (2018) looked into the inverse association between serum non-HDL-C levels and mortality in patients undergoing dialysis, and reported the decline in non-HDL levels

(< 100 mg/dL) in addition to the reduced non-HDL/HDL-C ratio (< 2.5) showed paradoxical association with increased all-cause of mortality [85]. High TAG/HDL ratio in the general population has been associated with risk, however, in HD patients, Chang et al. (2017), found that elevated TAG/HDL (ratio ≥ 3.64) when compared with a (ratio < 3.64), the latter had a higher all-cause and CV mortality rates. The findings heighten the importance of qualitative in addition to quantitative evaluation of lipids and lipoproteins in the ESRD population [86]. The 4D study (Die Deutsche Diabetes Dialysis) revealed that the quality of HDL and LDL composition and function might be more critical than their serum levels. Studies suggest that lipoproteins may serve as a biomarker to assess disease; thus, these metabolic proteins can be used in order to discover new therapeutic and diagnostic tools [87].

HDL controversy

HDL-C is recognized as "good cholesterol," which is protective against CHD. In the Framingham study between 1969 and 1971, it was established that HDL in both genders had an inverse association with the incidence of CHD, thus, considered to be protective [80]. Epidemiological studies have further associated the low HDL-C levels as an independent risk factor, and irrespective of LDL-C levels, this is considered to be adverse in attaining CVD outcomes [88]. Moreover, lower concentrations of HDL-C has been recognized to be problematic in both genders and a potent predictor of CHD death, in men with HDL-C less than 40 mg/dL and women less than 50 mg/dL [88, 89]. Such findings were further reinforced by various observational studies throughout the world which have demonstrated that an increase in serum HDL-C levels can be beneficial, by reducing the risk for CHD, even so, many investigations have cautioned this belief by stating that further studies were required for verification [90, 91].

Furthermore, data generated through various meta-analysis studies, in association with understanding HDL metabolism, and its role in RCT pathway, combined with various computer models, have confirmed a positive correlation in raising HDL being correlated in the removal of cholesterol thus, decreasing CVD rates [92]. With no clinical trial intervention to verify the result of HDL protective effects, the pharmaceutical companies entered into the picture, where studies were initiated with the sole purpose of increasing HDL via pharmacological treatments. While the general thought has been that HDL is inversely related with CVD, several failed clinical trials have created doubts on whether HDL has the capacity in being protective and whether if it still attains the capacity to conduct RCT in patients with hypercholesterolemia in order to facilitate the removal of cholesterol and decreasing CHD for those at risk [91]. Clinical trials that used fibrates, niacin, or CETP inhibiting drugs, have shown non-conclusive or at best-scattered results in some patients [93].

Interventions that looked at genetic outcomes of raising HDL observed no association in reducing the risk of an MYI [94]. Various clinical studies have investigated statin drug use as a way to raise HDL-C levels. In rare cases an increase in HDL-C levels was recorded; however, these effects have been inconclusive in a clinical setting, although beneficiary in decreasing LDL-C [95]. In the ENHANCE clinical trial, ezetimibe was combined with simvastatin, resulting in lower LDL-C and raising HDL-C levels; however, the safety and efficacy of the drugs were not determined, thus raising concerns in endorsing prolonged use [96]. Most noted failure in raising HDL-C came through a pharmaceutical intervention when Pfizer, shut down its phase III clinical trial of torcetrapid, a CETP inhibitor since the drug reported to have compromised patients' safety negatively [97].

While the focus for many years has been to raise the HDL-C, doubts due to non-conclusive results raise scrutiny in whether high HDL-C is good and/or protective. This is a further issue in a population of ESRD patients where D has been linked to being a factor that contributes to the formation of dysfunctional HDL particles. Moreover, impaired HDL particles have been associated with lower antioxidant activity, as well as a decrease in anti-inflammatory protective effect; therefore, serum HDL-C has been relegated in being not a dependable indicator for a CVD [66]. In CKD subjects, a U-shaped association between serum HDL-C levels and mortality has been observed; similarly, in ESRD subjects, a J-shape association between HDL-C and mortality, has been noted. Nonetheless, in both populations, CKD and ESRD increased serum HDL-C overtime is linked paradoxically with outcomes worsening in the patients' mortality [84]. This, suggests a window where lower than<30 mg/dL and higher than ≥60 mg/dL of HDL-C levels may be detrimental, and additionally, associated with changes in the lipoprotein functionality [98].

The failed clinical trials in rising HDL-C safely, combined with data results which demonstrated that higher serum HDL-C (≥60 mg/dL) could negatively impact patients' health. Therefore the focus has shifted to where HDL lipoprotein functionality could be more important than the circulating concentration levels itself. It has been postulated that HDL functionality plays a critical role in protection against atherosclerosis than the general HDL-C concentration levels. Furthermore, what makes such particle atheroprotective, are the antioxidant properties, anti-inflammatory, antithrombotic, and cytoprotective function [99].

HDL particles are noted in being protective against CHD; additionally, this protection extends to the LDL particles from inhibiting the generation of proinflammatory oxidized lipid, thus helping in preventing its oxidation. Serving as a protective mechanism to the HDL particle from becoming oxidized or dysfunctional from exposure to a disease state and or inflammation, is

paraoxonase 1 (PON1) hydrolytic enzyme. PON1 is known as an esterase, homocysteine thiolactonase enzyme which is encoded by the PON1 gene [100].

Itself the enzyme is considered to be an antiatherogenic component of HDL, which is highly associated with the lipoprotein and their functional outcome, and its antioxidant properties are protective agents against CHD [101, 102]. It is activated by peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor, which has been implicated various metabolic pathways, in and has been associated with numerous disease; from obesity to DM; nonetheless, it has also been used in the treatment of hyperlipidemia and hyperglycemia [103]. An added property of PPAR-γ is its ability to decrease inflammatory response in many cardiovascular cells, such as endothelial cells; as well as to increase the synthesis of paraoxonase-1 from the liver, and like PON1, the PPAR-γ has been linked to reducing atherosclerosis [104]. PON1 has been investigated in various clinical settings, and its relevance was depicted by Azarsiz et al. [105] and Sharma et al. [106], which demonstrated that serum PON1 activity was significantly low in CHD patients, thus congruently affecting the HDL and its functional behavior.

For LDL particle fractions, based on several investigations, there is strong body of evidence that suggests that small dense LDL (S-LDL) particles are more atherogenic than larger buoyant LDL particles (L-LDL). This is because S-LDL fractions have an enhanced ability to penetrate the arterial wall, which leads to the development of atherosclerotic plaques [107]. On the other side, there are the HDL particles. Wherein could be classified into two subfractions, large buoyant HDL (L-HDL) and small dense HDL (S-HDL).

While the LDL subfractions have a strong body of evidence in the relevance to a clinical setting pertaining to CVD; evidence on the large, and small-HDL is unclear and controversial. Results from two studies the ***IDEAL*** (Incremental Decrease in End Points through Aggressive

Lipid Lowering) trial, and the ***EPIC*** (European Prospective Investigation) a case-control study, which showed that elevated plasma HDL-C levels (≥70 mg/dL) and the L-HDL particles were associated with a higher risk for CVD. In contrast, the S-HDL particle has shown that it may be more functional thus, more efficient in promoting cholesterol efflux from macrophage to remove excess cholesterol [108].

The S-HDL also exhibiting more antioxidant capacity, anti-inflammatory properties, antithrombotic, and anti-infectious activity when compared to the L-HDL particles. Moreover, irrespective of the two lipoproteins, higher levels of ApoA-I in the plasma uniformly show a lower risk for a CVD event [109]. Studies that used CETP inhibitors and niacin have displayed evidence to increase the levels of L-HDL [108, 110]. In this case, data from experimental studies also showed a cholesterol overload HDL, which happens to be abnormal, thus have an impaired antiatherogenic potential and presents with a negative effect on the efflux potential [111].

A retrospective meta-analysis study by Pirillo et al. (2013) agreed upon the fact that the functionality of HDL may be more significant than HDL-C levels. However, these findings revealed that due to dyslipidemia, L-HDL levels are decreased whereas S-HDL particles were increased in CHD patients [112]. A study by Pascot et al., (2001) looked into reducing HDL particle in relation to D due to abdominal obesity; to discover that men presented with more L-HDL, had a more favorable plasma lipoprotein-lipid profile when compared with the S-HDL lipoprotein particles. Additionally, these men with higher levels of L-HDL were also observed to have reduced adiposity, lower visceral fat, and reduced insulin-glycemic response [113]. In patients who have CKD and ESRD, data is scarce and so are the clinical trial, in attempting to understand the functional role of HDL particle size in preventing CVD.

Non-traditional disorders

Malnutrition

Malnutrition is characterized as irregularities in forms of fatigue, loss of body weight accompanied by losses of lean muscle mass that are replaced by fatty tissues, a decline in body mass index (BMI), and a decrease in serum proteins [114, 115]. Approximately 18-75% of CKD patients undergoing MHD are presented with evidence of wasting syndrome, often referred to as uremic cachexia, or MICS or protein-energy wasting (PEW), all of which are a strong predictor for adverse outcomes [116-119]. Kidney disease is also accompanied by metabolic acidosis, which irreversibly destroys the essential branched-chain amino acids (BCAA), proteins, and muscle protein, which leads to low levels of serum albumin (hypoalbuminemia) [115]. Serum albumin serves many functions that help maintain intravascular colloidal osmotic pressure and hypoalbuminemia, where is a key nutritional marker used to identify malnutrition [120].

Inflammation

The elevated inflammation levels are additional critical issues that confront both individuals who are affected by CKD as well as MHD patients. Inflammation is a biological response instigated by stimuli such as injury, pathogen invasion, cell damage, or irritants, and in kidney disease, this is caused due to many factors, associated explicitly with nephron damage [121]. Within physiological limits, an inflammatory response facilitates the removal of the inciting agent and initiates the healing process [122]. Many studies have been conducted to understand the role of inflammation in the CKD population Armdur et al. 2016 [123] found that elevated plasma levels of fibrinogen, interleukins (IL-1, IL-6) and cytokines (TNF-alpha) are involved in the systemic inflammatory mechanism, and these markers have been linked to a faster decline in eGFR which corresponds to the progression CKD into ESRD [123].

Moreover, systemic inflammation is highly prevalent among CKD and HD patients and has been associated with a high mortality rate due to the development and progression of CVD in subjects undergoing hemodialysis [124-126]. In the ESRD population, inflammation is characterized as a multifactorial and an underlying reason for the development of CVD. An acute-phase protein (reaction) has been associated with causing vascular injury by several pathogenetic mechanisms directly. Available data suggests that proinflammatory cytokines also play a central role in the genesis of both malnutrition and CVD in ESRD [127].

Furthermore, the inflammation response in ESRD patients is a multifactorial stimuli event. Some of the influencers are typified by: hypoalbuminemia, malnutrition, advanced oxidation of protein products, atherosclerosis, uremia, anemia, dialysis vascular access infection, obesity, and the elevated activity of the thiobarbituric acid reactive system (TBARS) which leads to the upregulation of multiple pathways, giving rise to inflammation [128]. Elevated inflammation contributes to the progression of CKD by inducing the release of cytokines, which in turn leads to increased production and activity of adhesion molecules. This leads to T-cell adhesion, which migrates into the interstitium, and subsequently attracts pro-fibrotic factors [128]. The prolonged exposure to pro-inflammatory mediators with the concurrent build-up of pro-fibrotic factors, in turn, secretes high levels of matrix metalloproteinases, and other extracellular matrices proteins, followed by degrading enzyme, that converts fibroblast into fibrosis tissue. These fibrotic and connective tissues matrixes accumulate over time and breach the smooth muscles of the heart [129]. Over time a constant pressure of the ventricular contraction causes the weakening of the structural integrity of the heart muscle, followed by the loss of strength in the fibrotic matrix, that leads to compromised ventricles chamber matrix. This ultimately caves and results in MYI or rupture [129].

A systemic review by Agrawal et al. (2015) looked at the importance of both pharmacological and non-pharmacological strategies in the management of coronary artery disease in CKD and in dialysis subjects for what could improve the outcomes in the populations' mortality [130]. However, evidence showed that therapeutic options presented may be helpful only in a set of patients with CHD in the general population, but not beneficial in subjects with advanced CKD. Other studies have examined the effects of antioxidant therapy on markers of inflammation and OS with mixed results. A study by Ikizler et al. (2013), in a placebo-controlled, double-blinded (PATH) clinical trial intervened for 6-month using a combination of mixed tocopherols (666 IU/d) plus a-lipoic acid (ALA; 600 mg/d) looking at the inflammatory markers and OS in MHD subjects. Results showed a non-significant decline in high-sensitivity C-reactive protein (hsCRP), IL-6 concentration, F2- isoprostanes, and isofurans, biomarkers for OS [131].

Since inflammation plays an essential role in the etiology and outcome of atherosclerosis, one prominent maker is C-reactive protein (CRP). Made in the liver, the ringed-shaped pentameric acute-phase protein circulates the blood. Furthermore, the protein is activated due to response to inflammation, which is followed by the increases of interleukin-6 (IL-6), who is further secreted by the macrophages and T-cell. Also, the CRP as a marker is well recognized not only as a predictor of inflammation, but CRP categories were significantly associated with all-cause mortality [132, 133]. Various studies correlate that elevated CRP concentrations (> 3 mg/L) with cardiovascular deaths in the general population and dialysis patients.

Moreover, HD patients are 5- to 10- fold higher at risk of such events than their healthy counterparts [134, 135]. A study by Panichi et al. (2008) followed 757 HD patients for 30 months to understand the relationship between hsCRP, IL-6, IL-8, and serum albumin in relation to mortality and morbidity. It was concluded that CRP was the most powerful predictive factor for a

cardiac event even after adjustments for age, DM comorbidity and BMI, that individuals who present with CRP plasma levels > 5 mg/L were at higher risk for all-cause [136].

Oxidative stress

Another issue that both CKD and ESRD patients face is OS, which has been linked to the development of atherosclerosis within the population [137]. OS is defined as an imbalance between pro- and anti-oxidant systems and is elevated in these patients compared to the general healthy population [138]. In HD patients, OS is attributed to increased reactive oxygen species (ROS) production, reduced clearance, a poor antioxidant defense system, which is associated with CVD by propagating the oxidation and modification of lipids and lipoproteins such as LDL and proteins [139, 140]. Oxidized LDL (oxLDL) can promote and initiate a cascade of reactions, including adhesion of circulating monocytes on the endothelial cell. This leads to migration of the monocytes into the arterial intima, followed by platelet activation, and concomitantly the expression of tissue factor and(plasminogen activator inhibitor 1) PAI-1 by endothelial cells [139, 141]. Foam cell formation in the macrophages at the arterial walls has also been linked to the pathogenesis of atherosclerosis. This is due to increased uptake of oxidized lipids such as oxLDL, which have also been associated to decrease SR-B1 expression and lipid flux while at the same time facilitate the increase of class B scavenger receptor CD36 [142, 143]. Boaz et al. (2000) in the SPACE clinical trial were able to show that 800 IU/day of vitamin E (an antioxidant), in a cohort of MHD patients, could reduce composite CVD endpoints and MYI [144].

OS additionally is implicated in the etiology of other confounders, such as advanced glycation end-products (AGEs) [145], which are pro-oxidative and pro-inflammatory compounds. [145]. In general, AGEs are formed by the Maillard-reaction after initial binding of aldehydes with amines or amides in heated food or in living organisms [146]. Furthermore, AGEs can be derived

from glucose-protein or glucose-lipid interactions, implicated in diabetes, and aging [147]. They are created through a nonenzymatic reaction between reducing sugars and free amino groups of proteins, lipids, or nucleic acids. AGEs are not only produced endogenously, but they can also be obtained through exogenous sources such as diet (dAGEs). The dAGE are produced due to high temperature, boiling, broiling, frying, and roasting of the foods.

Moreover, AGEs in the plasma have been associated with increased risks for higher OS and inflammation that are further linked with DM and higher risk for CVD events [148]. The pathologic effects of AGEs are mainly related to their ability to promote OS and inflammation by binding with cell surface receptors or cross-linking with proteins, which leads to the alteration of structures and functions [149]. Nε-carboxymethyl-lysine (CML) are among the better-studied AGEs due to its compound stability, which can be measured in the plasma and analyzed via dietary records [150]. Moreover, it has also been suggested that in hemodialysis subjects, AGEs could accumulate due to uremic status as a consequence of diminished clearance. This has drastic consequences for the patients because it may accelerate vascular disease, renal failure progression, and dialysis-related amyloidosis [151, 152]. Lopez-Moreno et al. (2016) found that the Mediterranean diet, supplemented with coenzyme-Q10 in elderly men and women showed a decline for CML protein adducts in the plasma [153]. Nevertheless, further investigations specific in the ESRD-MHD population is needed to validate such findings.

Antioxidant studies

Tocotrienol rich-fraction (TRF) is part of the vitamin E family, comprising of eight isomers. Tocopherols (TP) and tocotrienols (TT) have four isomers α-, β-, γ-, and δ- characterized by different numbers and positions of the methyl group attached on the chromanol ring [23]. Unlike TP's which have a saturated aliphatic tail, TT's have an unsaturated chain tail [154]. Additionally,

TT's have a superior antioxidant activity than TP due to their trans-double bound (at 3', 7', and 11' positions) [155]. TTs have gained significant attention due to their diverse capabilities in being cardioprotective, anti-atherosclerotic, anti-hypercholesterolemic, anti-cancer, anti-diabetic, neuroprotective, linked in the regulation of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), as well as being lipid-lowering, and having anti-oxidative properties [154, 156]. Currently, in the United States, TT's have a GRAS chemical recognized status [157, 158].

TRF supplementation on ESRD patients for 16 weeks, showed that TT help to normalize triglyceride (TAG) levels, increase HDL-C concentration, and ApoA-I levels, and decreased in CETP in the TRF group. As for nutritional, inflammatory, and oxidative status, the answer to these parameters is unresolved [23]. Other reviews have emphasized the effects of TT in being able to reduce CRP and AGEs levels [155]. In a six month intervention by Heng et al. (2013) supplementing with TT in healthy young (32±2 yrs.) and older (52±2 yrs.) adult volunteers, resulted in lower CRP levels, a prognostic indicator for CVD [156]. A six-month study by Chin et al. (2011) in healthy elderly adults supplemented with palm oil derived TRF found improvement in plasma cholesterol, increased HDL-C, and reduction of AGEs as well as reduced protein damage [159]. It has been postulated that TRF has the ability to suppress and stimulate ubiquitination and degradation of HMG-CoA reductase, in addition to blocking the processing of sterol regulatory element-binding proteins (SREBPs) which has been demonstrated in vitro studies' [160, 161]. SREBPs are membrane-bound transcriptional factors which bind to sterol regulating elements in the DNA sequence, which plays a critical role in the synthesis of enzymes involved in the sterol syntheses, therefore a key regulator in cholesterol metabolism [162, 163]. Noted regulation by SREBPs via the feedback system is the transcription of HMG-CoA reductase, as well as other enzymes in the cholesterol biosynthetic pathway [163].

Many interventions have tackle ESRD abnormalities by targeting inflammatory and oxidative status parameters, and some studies have been successful (SPACE) and others (PATH) with mix results. In a previews pilot study by our group, we used tocotrienols rich fractions (TRF) supplementation in a small cohort, which showed positive results on D, OS, and inflammation [23].

The rationale for the study

Both CKD and ESRD patients experience accelerated atherosclerosis driven by multifactorial abnormalities such as malnutrition-inflammation complex syndrome, oxidative stress, protein energy-wasting, impaired antioxidant system, and D. Several other factors including the presence of proteinuria, dietary restrictions, pharmacological therapy, genetics, disorders of lipid metabolism and abnormal lipids further exacerbates the progression of CKD into ESRD which has been associated with mortality 10-times higher than in the general population. In the last decade, much research has been conducted to understand the pathophysiology of these disorders; however, the various strategies to correct these problems have been inconclusive. In MHD patients, D is primarily the result of decreased HDL and increased TAG with normal and/or increased LDL. However, the contribution of each of these parameters to D is poorly understood; moreover, the contribution of the following parameters to D in different ethnicities is unknown. Hence, the objective of this study is to characterize D in a multi-ethnic cohort of ESRD patients undergoing MHD.

The current investigation is a snapshot in time, (baseline data analysis only), in a subset of MHD patients, partaking in PATCH USA and Malaysia clinical trial. The primary study is a 1-year trial, double-blinded, placebo-control intervention using Vitamin E Tocotrienol Rich Fraction (TRF) an antioxidant supplement for which subjects receive for consumption 2 capsules, each 150

mg of daily (tocotrienols or placebo) for a total 300 mg. Tocotrienols (TT), are isomers of vitamin E, having anti-inflammatory and antioxidant properties which in some cases are superior to those of tocopherols. Previously, TT was shown to improve D over 16-wks in a pilot study, but the effects on lipoprotein subfractions, inflammation, and OS were unresolved [23].

The central hypothesis of this study is that D amongst MHD patients of different ethnicities will reflect underlying alterations in lipoproteins and enzymes of lipid metabolism. The rationale for the proposed study was that once a dyslipidemic MHD profile is determined, targeted interventions could be used to manage D.

Specific Aims

Specific Aim 1): To document the degree of dyslipidemia in a cohort of MHD patients and characterize lipids, and lipoprotein particle compositions and associated enzymes of lipid metabolism.

- The analysis is depictive of baseline plasma samples (n = 123) from African American (AA) MHD patients participating in the PATCH USA clinical trial (NCT02358967).
- In this study, we measured lipids, characterized lipoprotein subfractions, and examined lipid metabolism enzymes: CETP and LCAT.
- D was assessed based on criteria from the Adult Treatment Panel (ATP III) guidelines, using the category TAG/HDL-C ratios ($\geq$ or < 3.8).

Specific Aim 2): To characterize dyslipidemia across a multi-ethnic cohort of MHD patients.

- Examined baseline plasma samples of (n = 225) from the PATCH Malaysia study (NCT02913690). A cohort composed of three ethnicities: Malaysian-Malays (MM), Malaysian-Chinese (MC), and Malaysian-Indians (MI).

- D was assessed based on criteria from the Adult Treatment Panel (ATP III) guidelines: TAG/HDL-C ratio (≥ or <) 3.8. Ratios are based on ATP cut-offs of < or ≥ 150 mg/dL for TAG and < or ≥ 40 mg/dL for HDL-C.
- Data from the Malaysian cohort (comprising MC, MI, and MM) will be compared with the US cohort of AA patients.

Specific Aim 3): To document the extent to which HDL enzymes contribute to dyslipidemia in MHD patients.

- Plasma samples from the US cohort and Malaysian cohort were analyzed to determine enzymatic activity in:
  - Cholesteryl ester transfer protein (CETP)
  - Lecithin-cholesterol acyltransferase (LCAT)
- The key enzymes which play a role in the HDL RCT pathway were analyzed to understand the role played in association with lipids and lipoprotein subfractions.
- C-reactive protein marker was analyzed in all ethnicities to determine the degree of inflammation between the cohorts and the multi-ethnicities.
- Exclusively for PATCH USA, a subset of the samples were measured for Nε-carboxymethyl-lysine (CML), to discern levels of advanced glycation end-products (AGEs) in the plasma since is recognized as an oxidative stress marker.

Tables Chapter 1

Table 1-1: Dyslipidemic characteristics in lipid parameters for different CRF conditions.

Parameters	CKD (1 – 5)	ESRD-MHD Hemodialysis	Nephrotic Syndrome	PD	RT
TC	↗	↔ **or** ↓	↑↑	↑	↑
LDL-C	↗	↔ **or** ↓	↑↑	↑	↑
HDL-C	↓	↓	↓	↓	↓
Non-HDL-C	↗	↔ **or** ↓	↑↑	↑	↑
TAG	↗	↑	↑↑	↑	↑
$Lp_{(a)}$	↗	↑	↑↑	↑↑	-
IDL	↗	↑	↑	↑	-
VLDL	↗	↑	↑	↑	↑
Sd-LDL	↗	↑	↑	↑	-
ApoA-I	↘	↓	↗	↓	-
ApoA-IV	↗	↑	↑ **or** ↘	↑	-
ApoB	↗	↔ **or** ↓	↑↑	↑	-
CETP	↑	↑	↑	-	-
LCAT	↓	↓	↑	-	-

Symbols: Increase (↗) and decrease (↘) in plasma levels with decreasing GFR; Increase (↑), markedly increased (↑↑) and decrease (↓) plasma levels compared with non-uremic individuals, (↔) normal, (-) data inconclusive.

TAG: Triacylglyceride; HDL-C: high-density lipoprotein cholesterol; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; NonHDL: Non-high-density lipoprotein cholesterol: ApoA-1: apolipoprotein A-1; ApoA-IV: apolipoprotein A-IV; ApoB: apolipoprotein B; S-LDL: small dense LDL CETP: cholesterol ester transfer protein; LCAT: lecithin-cholesterol acyltransferase.

Adopted source: Society of Nephrology. Kwan BCH. [5, 164]

Table 1-2: Lipoprotein classes and their characteristics.

Lipoproteins	Density (g/mL)	Size (nm)	Relative Content %				Apoproteins
			TAG	Ch	Pl	Pr	
Chylomicrons	< 0.930	75-1200	90	5	3	2	ApoB-48; ApoC-I, ApoC-II, ApoC-III; ApoA-I, ApoA-II, ApoA-IV; ApoE
VLDL	0.930 – 1.006	30 – 80	60	20	14	6	ApoB-100, ApoC-II, ApoC-III; ApoE
IDL	1.006 – 1.019	25 – 35	20	40	22	18	Apo B-100, Apo C, Apo E
LDL	1.019 – 1.063	18 – 25	7	50	22	21	ApoB-100
HDL	1.063 – 1.210	12 – 5	5	25	26	44	ApoA-I, ApoA-II, ApoA-IV ApoC, ApoE

Abbreviations: TAG, Triaglycerides; Ch, Cholesterol; Pl, Phospholipids; Pr, Protein

Adopted source: Feingold. K et al. and Kwan. B CH et. al [29, 164]

Table 1-3: Apolipoproteins of human plasma lipoproteins.

Apoprotein	Lipoprotein	Additional remarks
ApoA-I	HDL, CM	• A major component comprises about 70% of the HDL protein mass [165]. • The HDL helps to sequesters phospholipids and cholesterol and interact with plasma enzyme and cellular receptors [166]. • Activates LCAT which helps in the formation of cholesterol ester which leads to the HDL maturation. • Ligand for HDL receptor [167]. • Enables efflux by accepting fats from within cells (interacts with ABCA1, GPLD1, and PLTP) [63, 168]. • Often used as a biomarker for predicting CVD [169].
ApoA-II	HLD, CM	• Second most abundant protein in HDL [165]. • The structure is identical monomers joined by a disulfide bridge [29]. • Gene defects may result in ApoA-II deficiency or hypercholesterolemia [170]. • Has shown to interact with PLTP [63].
ApoA-IV	Secreted with CM but transferred to HDL	• No primary function in humans has been established; however, it has been linked in many pathways. • Synthesis in humans limited to the intestine. • It is associated with the formation of triacylglycerol-rich lipoprotein [171]. • Invitro aids in the activation of LCAT and CETP [172].
ApoB-48	CM only and CM remnant	• Synthesized exclusively in by the small intestine. • Primary protein component of the CM particles. • It contains the same amino acid sequence and similar N-terminal sequence as ApoB-100, however, it lacks C-terminal LDLR-binding region [173]. • After lipids have been absorbed, it returns to the liver with CM remnant to be endocytosed and degraded.
ApoB-100	VLDL, IDL, LDL	• Synthesized in the liver [174]. • Serves as a ligand for the LDLR [173]. • Has been associated as a marker for coronary heart disease [175].

ApoC-I	VLDL, HDL, CM	• Activator for LCAT in order to help with cholesterol esterification [176]. • The main function to inhibit CETP [177].
ApoC-II	VLDL, HDL, CM	• Protein acts as an activator for (LPL), which hydrolyzes TAG, thus freeing FA from cells [178]. • Mutation in the gene causes hyperlipoproteinemia that is characterized by xanthomas, pancreatitis, and hepatosplenomegaly, but not the increased for atherosclerosis [179].
ApoC-III	VLDL, HDL, CM	• Secreted by the liver and small intestine and found on TAG-rich lipoproteins [180]. • Inhibited LPL and hepatic lipase and believed to inhibit hepatic uptake of TAG particles [181]. • It also delays the catabolism of TAG-rich particles [182]. • Overexpression can contribute to atherosclerosis [180]. • It may explain the presence of hypertriglyceridemia associated with ABCA1 deficiency in patients with Tangier's disease [183].
ApoE	VLD, HDL, CM, and CM remnant	• Produced in liver and macrophages. • Ligand for chylomicron remnant receptor [184]. • Transport fat-soluble vitamins and cholesterol into the lymph system and then blood. • Interacts with LDLR [185]. • Mediates cholesterol metabolism; • Principal cholesterol carrier in the brain; • Risk factors for Alzheimer's disease, atherosclerosis, and cardiovascular disease [184].

Table 1-4: Efflux genes linked to RCT metabolism.

Efflux genes	Additional remarks
ABCA1	• The protein *ATP-binding cassette transporter ABCA1*: is encoded by a member of the superfamily of ATP-binding cassette (ABC) transporter gene. Moreover, ABC is divided into seven distinct subfamilies (ABCA, MDR/TAP, MRP, ALD, OABP, GCN20, White) [186, 187]. • Specific (ABCA1) is a protein encoded by the member-1 of the human transporter sub-family of ABCA gene, found exclusively in eukaryotes, which functions as a cholesterol efflux regulatory protein (CERP) [51]. • ABCA1 is an important regulator in cellular cholesterol and phospholipid homeostasis [188]. • Cholesterol is the substrate to ABCA1, whose primary function is as a cholesterol efflux pump in the cellular lipid removal pathway, moreover, it is known as a gatekeeper for eliminating excess tissue cholesterol [189]. • ABCA1 protein is also believed to play a protective role against CVD by mediating efflux of cholesterol to lipid poor ApoA-I and ApoE which leads to the formation of nascent HDL, which ultimately grows into a mature HDL. [189]. • Downregulation of ABCA1 in aging or compromised macrophages disrupts cholesterol from being removed from the cytoplasm. This leads to the promotion of pathologic atherogenesis which can develop into diseases such as atherosclerosis, CVD, cancer, macular degeneration, and dyslipidemia [190, 191]. • In case of a mutation of the ABCA1 gene, it can lead to Tangiers disease, presented with HDL-C deficiency and the increased risk for developing CVD [192].
ABCG1	• The protein *ATP-binding cassette sub-family G member 1*: is encoded by the ABCG1 gene, a member of the superfamily of ABC transporters. • Similar to ABCA1 protein, ABCG1 may be involved in the macrophage, cholesterol, and phospholipid transport and the regulation of cellular lipid homeostasis [193]. • Various studies have suggested that ABCG1 protein is primarily an intracellular sterol transporter, localizes endocytic vesicles, which may facilitate the efflux of cellular sterols to exogenous HDLs lipoprotein [194]. • Aside from performing cholesterol efflux to the HDL lipoprotein, ABCG1 also effluxes cholesterol to the LDL lipoproteins, liposomes and cyclodextrin. Additional it can export sphingomyelin, phosphatidylcholine, and oxysterols to HDL and albumin [195]. • Downregulation of ABCG1 has been associated with a 30% decrease in intracellular cholesterol efflux, followed by increases 3-4 times higher levels of IL-6, and TNFα, as well decreased eNOs protein by 50% [196].

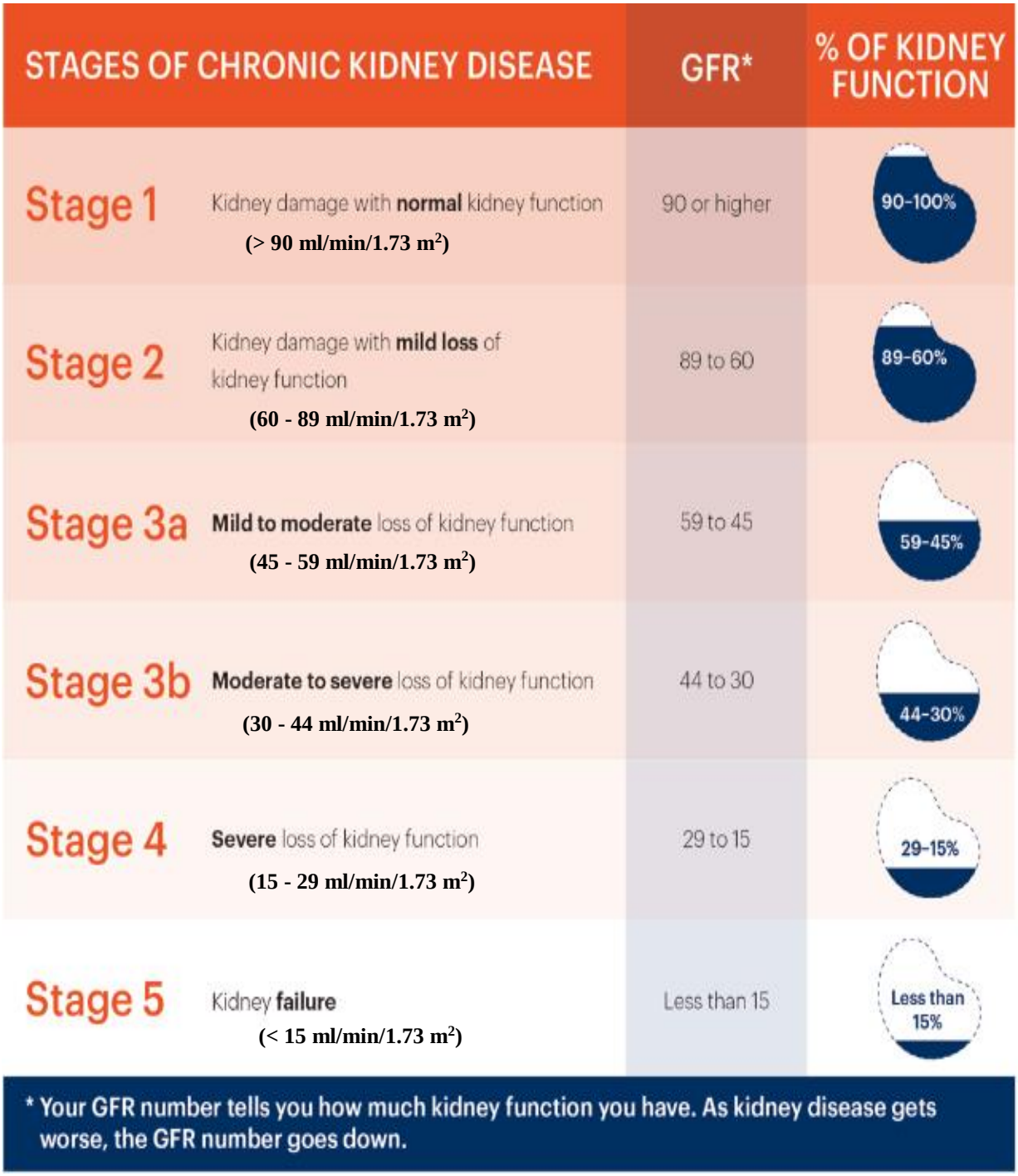
LXR-α	• Liver X receptor alpha, is a nuclear receptor protein in humans encoded by NR1H3 family gene [197]. • The LXR-α is highly expressed in organs such as the liver, adrenal gland, intestine, kidneys, and lungs. It is expressed in tissues such as adipose and macrophages [198]. • Both LXR-α and LXR-β proteins are critical regulators of macrophage functions [198, 199]. • Moreover, both proteins are involved in transcriptional programming which deals with lipid homeostasis and inflammation [199]. • LXR is hypothesized to act as a cholesterol sensor, which inhibits intestinal cholesterol absorption in order to protect from overload. It does so by stimulating cholesterol efflux from cells to HDL lipoproteins, which in the end is transported to the liver to be processed for various fates; either converted into bile acid, biliary excretion or hormone syntheses [200]. • In macrophages, LXR signal leads to the initiation of homeostatic responses to cellular lipid loading, which facilitates cholesterol efflux as ABCA1, PLTP, and ApoE finally transfer the free excess cholesterol to ApoA-I and ApoE-binding lipoproteins. Moreover, synthetic LXRs have shown to reduce the rate of atherosclerosis via the removal of excess cholesterol in animal models [201]. • LXR also works as an agonist which leads to activation of FA synthesis by initiating a cascade that expresses the initiation of lipogenic transcription factor SREBP-1c, which results in TAG elevation in the plasma and liver steatosis [200].
SR-B1 (dual roles)	• Scavenger receptor class B type 1 is a protein encoded by the SCARB1 gene. SR-B1 functions as a receptor for HDL lipoprotein [202]. • This integral membrane protein is found in various types of tissues, including liver, macrophages and adrenal, where the job is to facilitate the uptake of CE from HDL in the liver known as RCT [53]. • Additionally, the function of SR-B1 is crucial in lipid-soluble vitamin update and to viral entry into host cells [203]. • Dual function, primary role to mediate the selective influx of HDL derived CE into cells and tissues; or to facilitate the cholesterol efflux from peripheral tissues, including macrophages back to the liver [203].

Table 1-5: Influx genes linked to RCT metabolism.

Influx genes	Additional remarks
CD36	• Clusters of differentiation 36; are glycoproteins in human coded by the CD36 gene; moreover, they also are known as fatty acid translocases (FAT), platelet glycoprotein 4, scavenger receptor class B member 4 (SCARB3) and glycoprotein 88 (GP88). • Additionally, CD36 is an integral membrane protein found on the surface of several cells (muscle and adipose), which play an essential role in lipid homeostasis, specifically in long-chain fatty acids transportation [204]. • It is part of class B scavenger receptor family; however, CD36 contributes to the atherosclerosis progression due to modification of LDL phagocytosis, disturbed macrophage migration, which leads to foam cell formation [204].
LOX-1	• Lectin-type oxidizes LDL receptor-1, also known as oxidized low-density lipoprotein receptor (Ox-LDL receptor 1) is a protein in humans encoded by the OLR1 gene [205]. • It is known as the main receptor for oxidized LDL on endothelial cells, muscles, and macrophages, but it is worth noting that the TLR4 and CD36 receptor also recognizes oxidize LDL [206, 207]. • Upon binding to Oxi-LDL, the LOX-1 initiates a cascade in which leads to the activation of NF-κB, which leads to monocyte adhesion to endothelial cells, which ultimately leads to the formation of foam cells and atherosclerosis [208].
SRA1	• Steroid receptor RNA activator protein, a human protein coded by the SRA1 gene [209]. • Similar to CD36, SRA1 is a scavenger receptor that has been associated with the modification of LDL upon macrophage loading. In particular, OxLDL which leads to the development of atherosclerosis [210]. • Binding of oxidized LOX-1 and SRA-1 with oxidized phospholipid receptors on monocytes and macrophages initiates a cascade in which pro-inflammatory cytokines and chemokines are released [211].

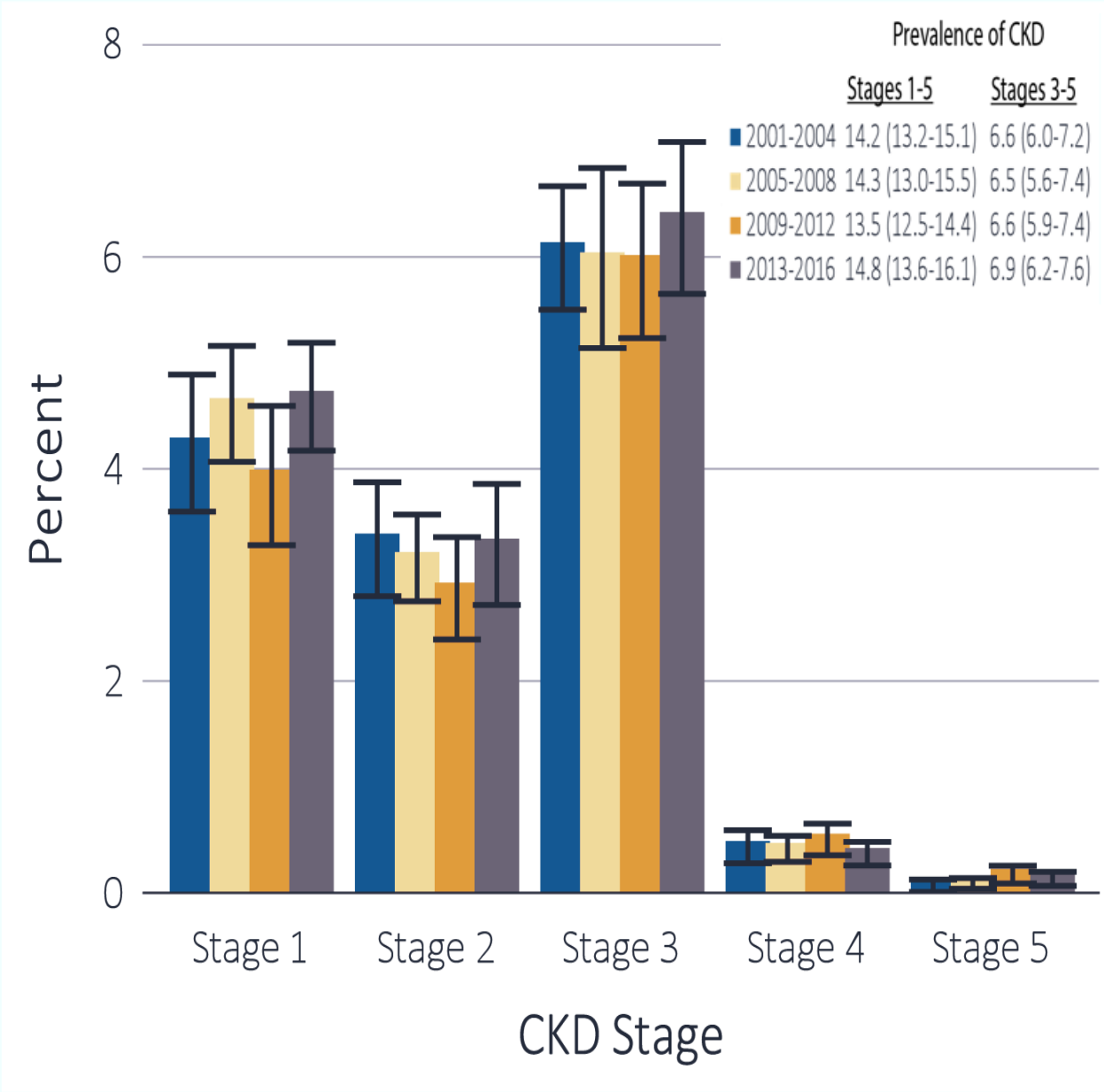
Figures Chapter 1

Figure 1-1: Stages of chronic kidney disease (CKD).



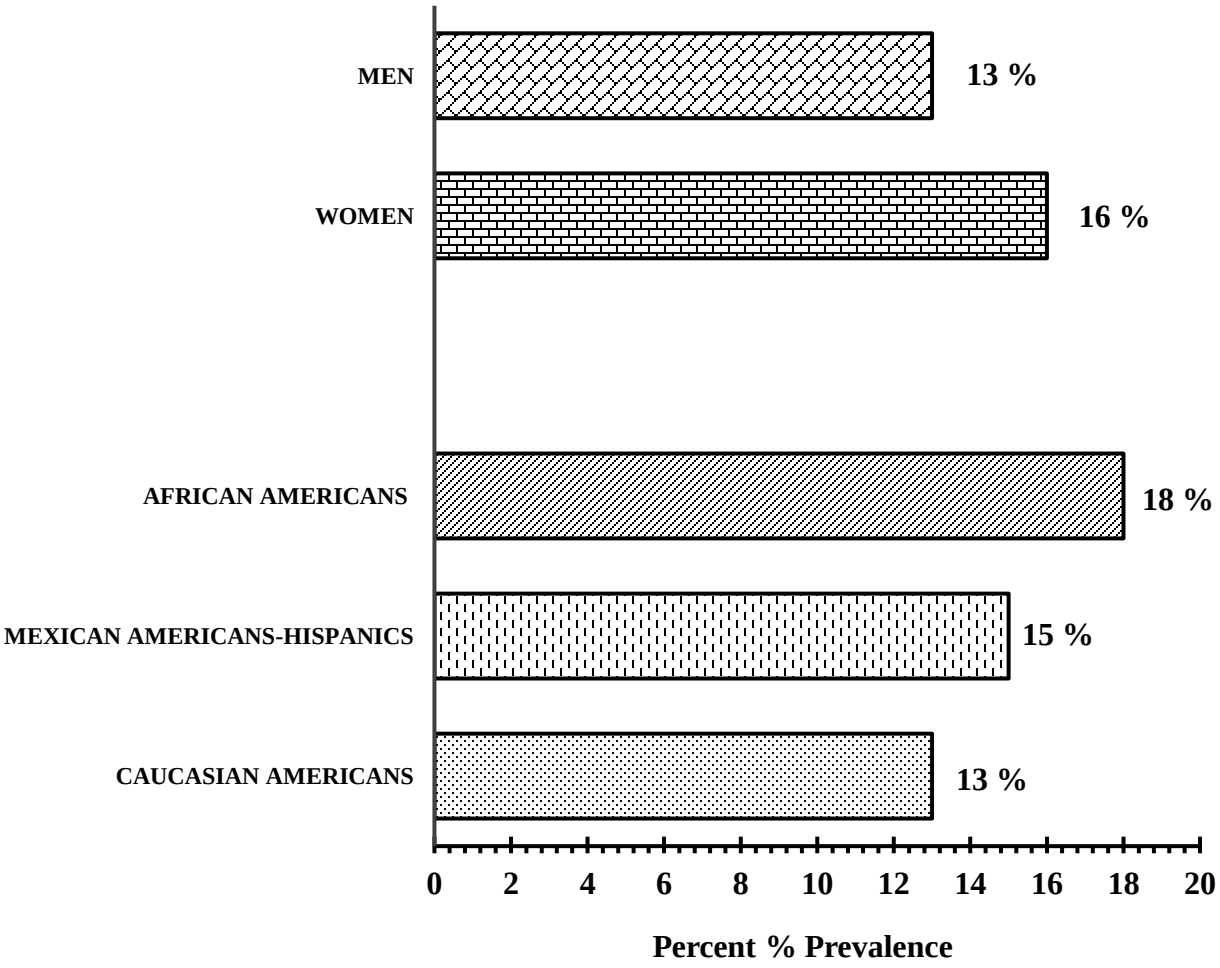
Source by: National Kidney Foundation [1]

Figure 1-2: Prevalence of CKD in the US by stages of NHANES participants.



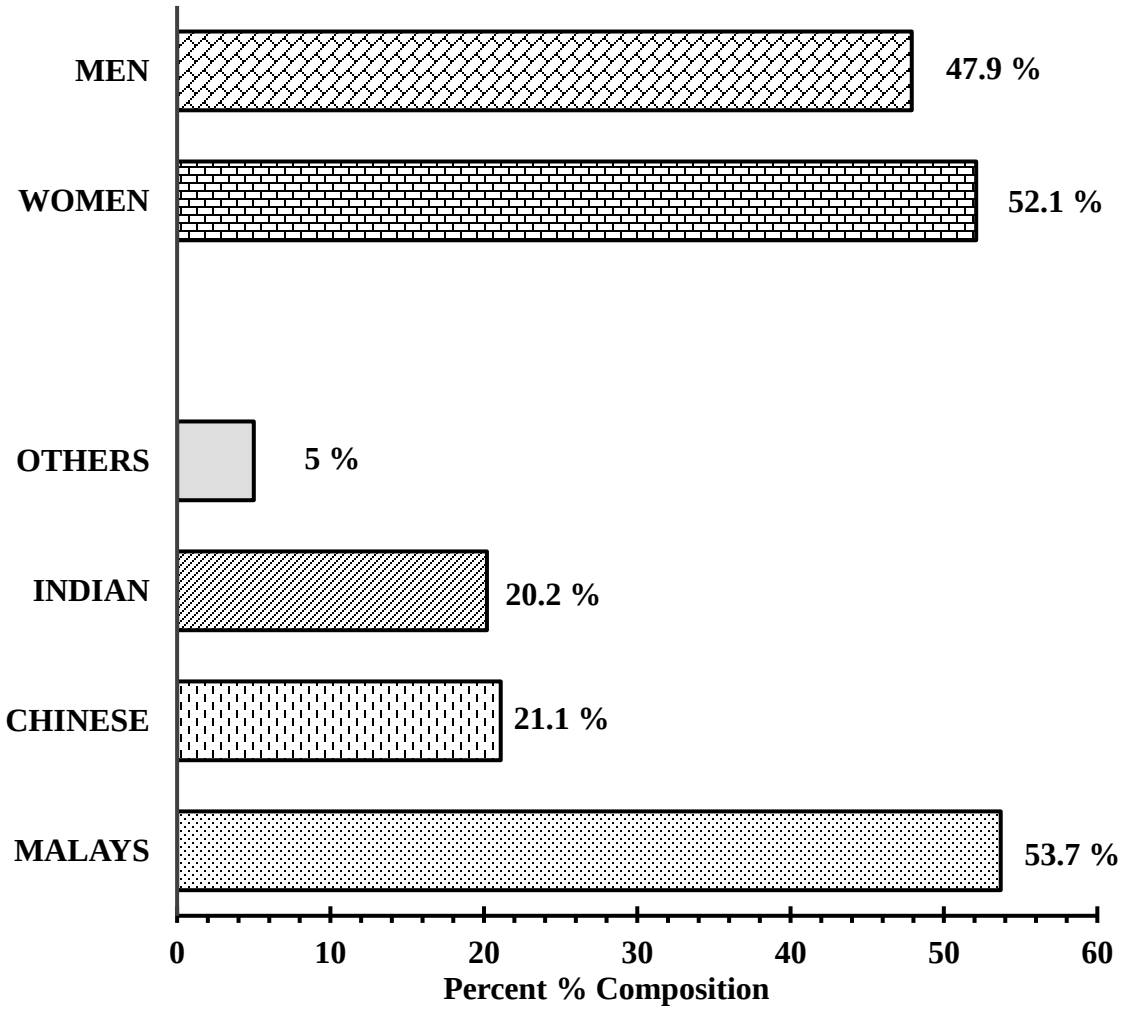
(NHANES), 2001-2004, 2005-2008, 2009-2012 & 2013–2016 participants aged 20 & older. Whisker lines indicate 95% confidence intervals. Abbreviation: CKD, chronic kidney disease, [12].

Figure 1-3: Prevalence of CKD among US adults' gender and ethnic distribution.



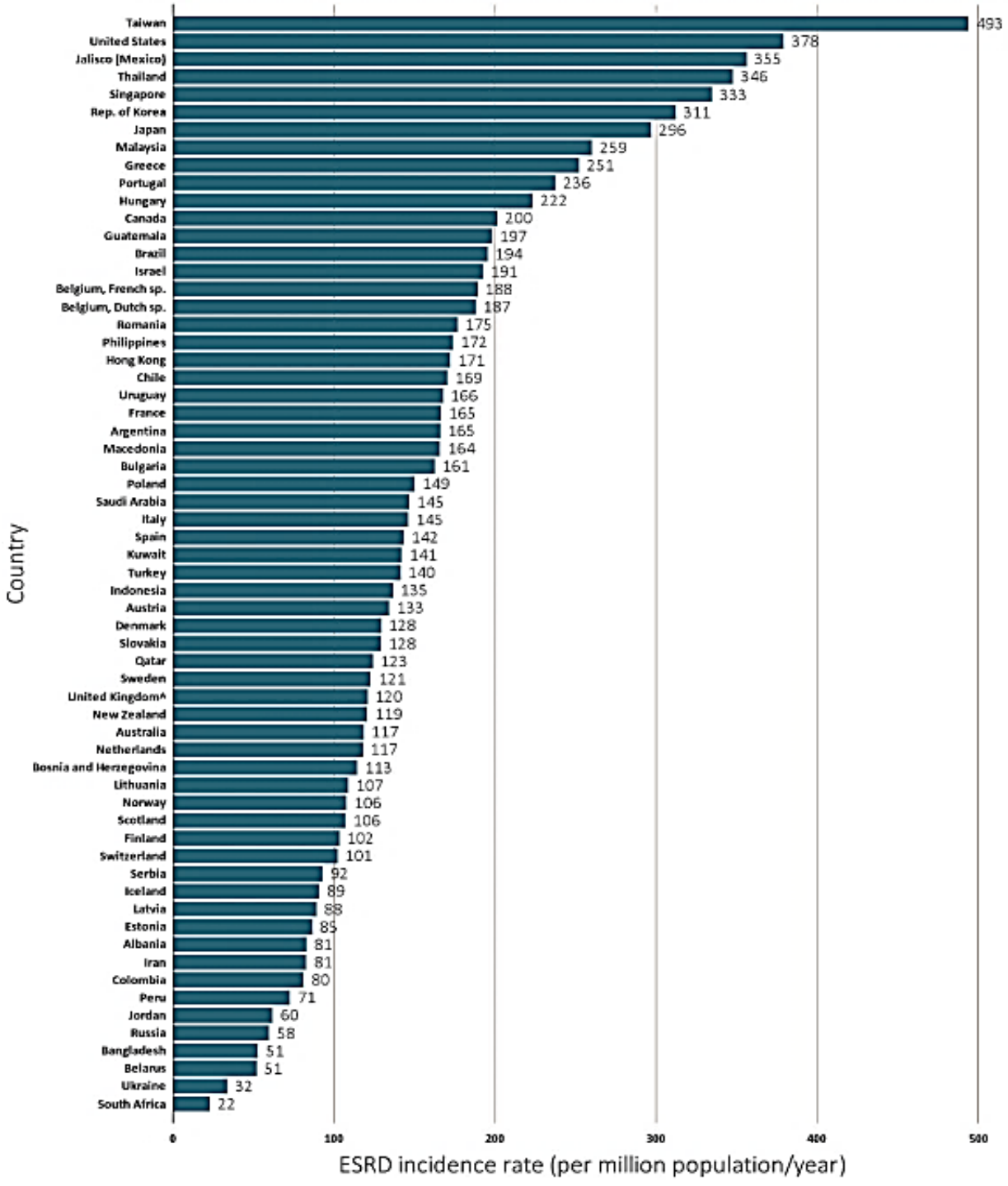
Source: CDC. Kidney Disease Fact Sheet, 2019 [6]

Figure 1-4: CKD among Malaysian adults' gender and ethnic composition.



Source: International Society of Nephrology, Hooi LS, et al., (2013) [15]

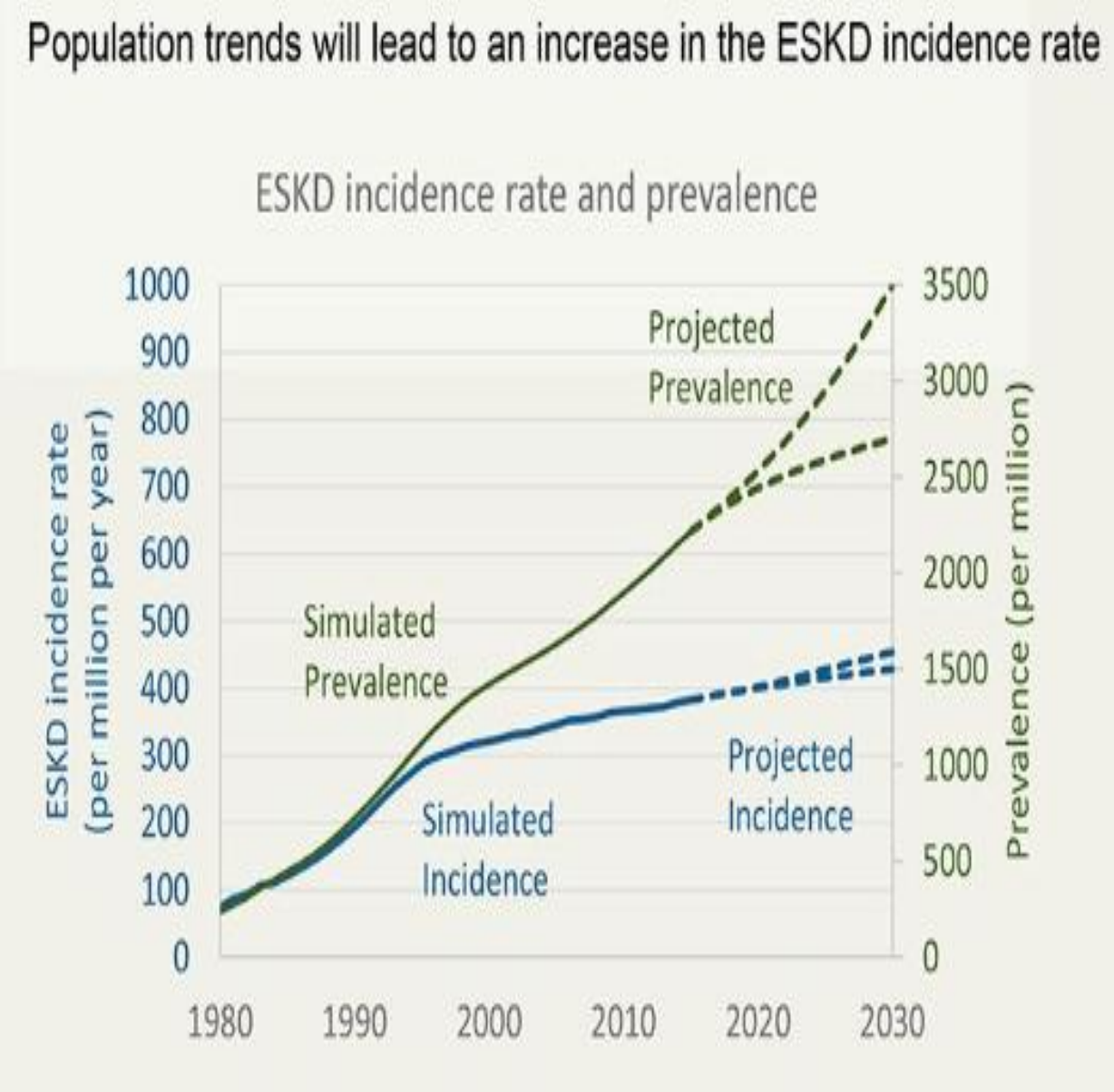
Figure 1-5: Incidence of ESRD internationally.



Source: USRDS, 2016 [16]

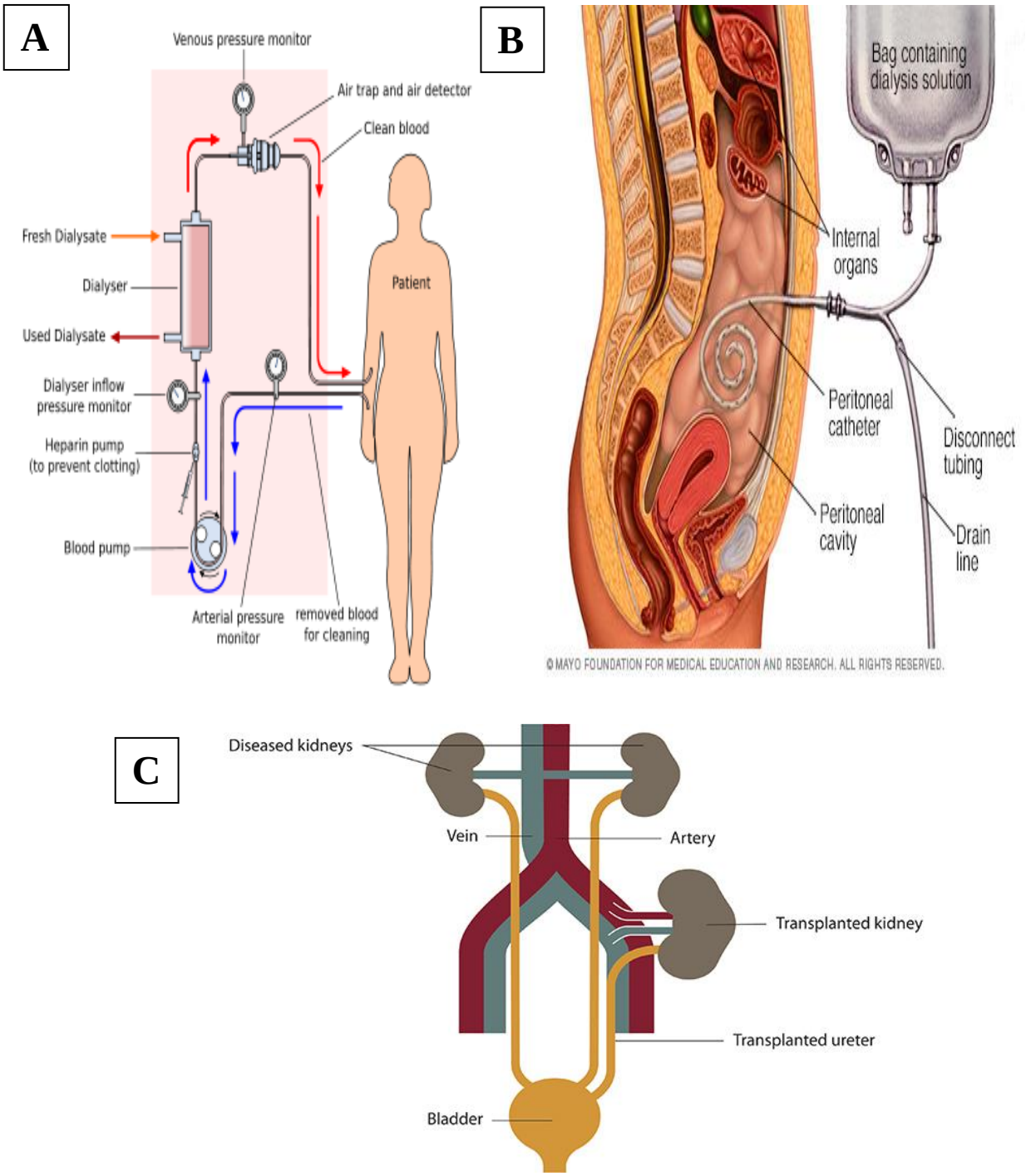
The incidence rate of treated ESRD (pre-million population) by country, 2016.

Figure 1-6: Projected ESRD incidence and prevalence in the US through 2030.



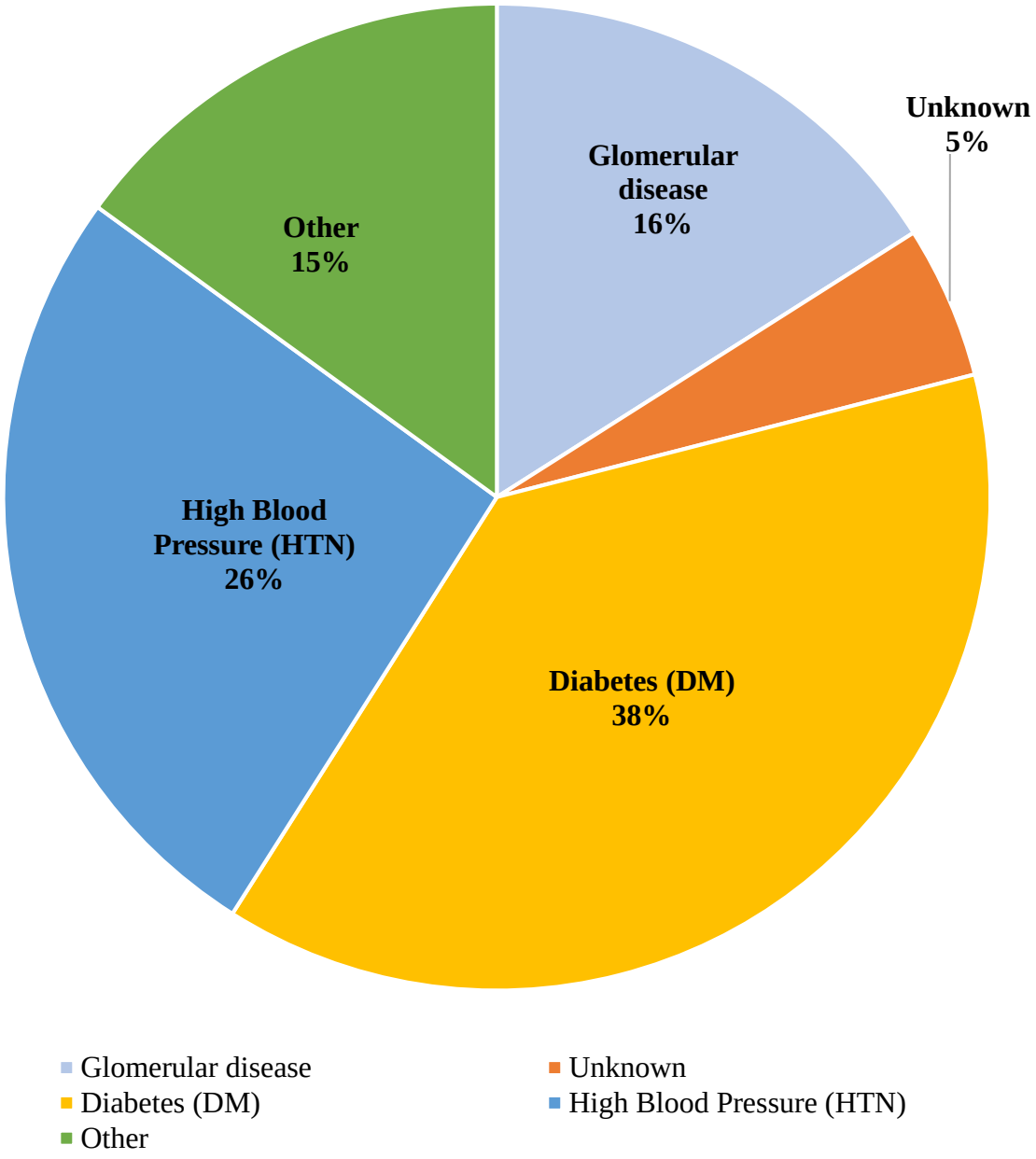
Source: American Society of Nephrology, McCullough, et al., 2019 [17]

Figure 1-7: Treatment options for patients with end-stage kidney disease.



Legend: **A**- Hemodialysis (Source: RenalMed UK); **B**- Peritoneal dialysis (Source: Mayo Clinic); **C**- Renal transplant (Source: NIDDK-NIH)

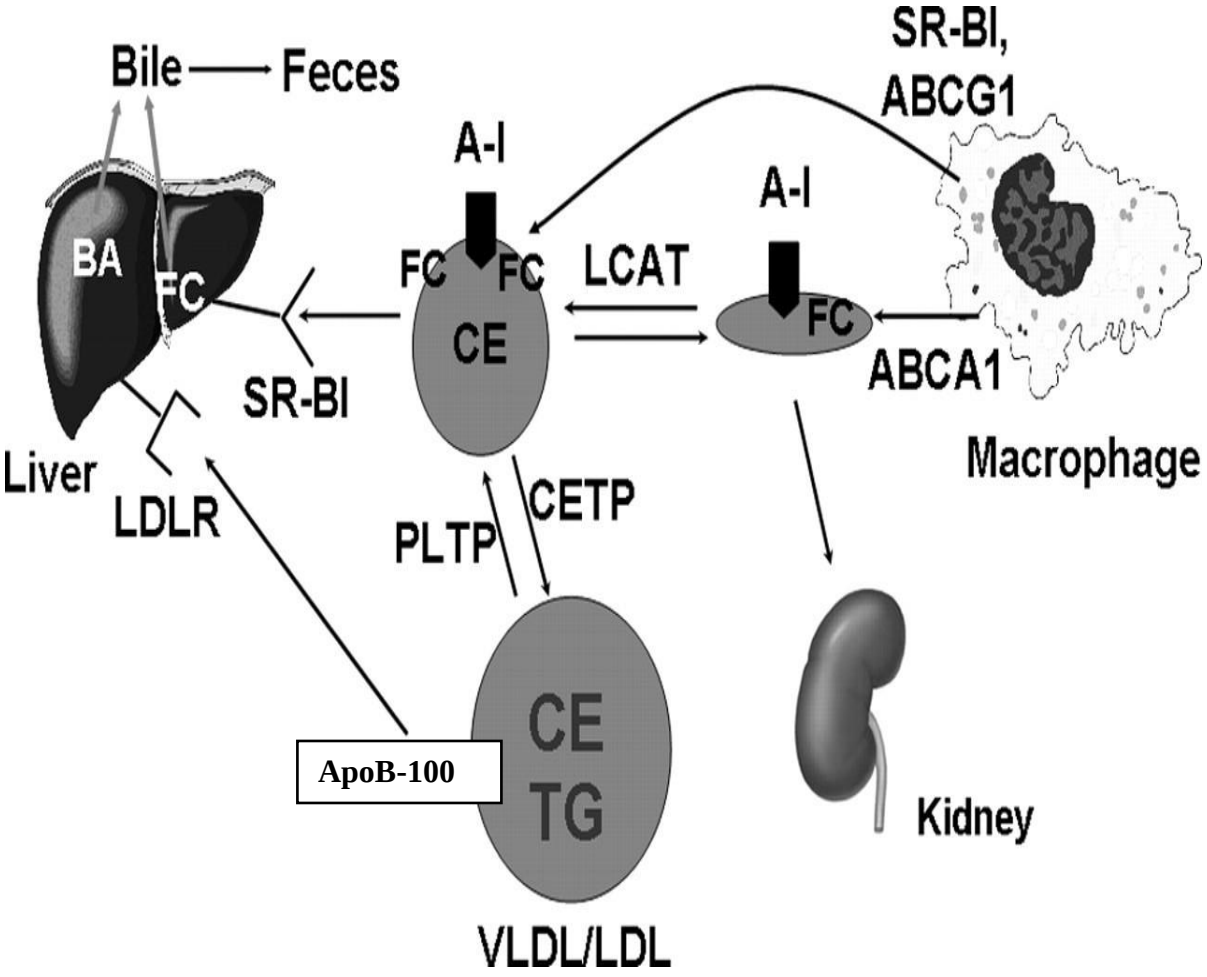
Figure 1-8: Causes of ESRD in the United States.



Source: US Renal Data System, 2019, CDC.gov

N = 726,331 (all ages, 2016); includes polycystic kidney disease (PKD), among other causes. [6].

Figure 1-9: Reverse cholesterol transport.

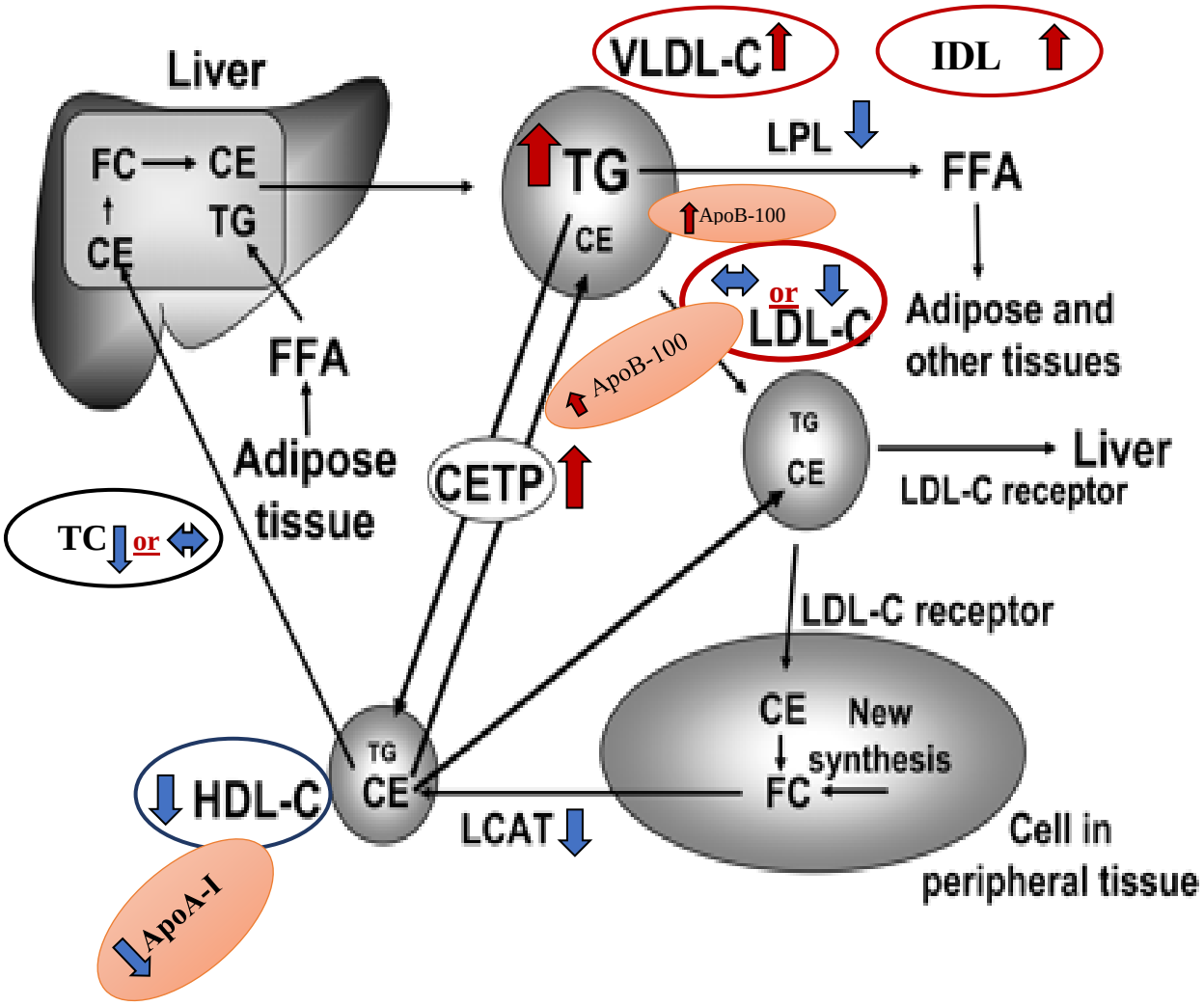


Source: Circulation, Duff D., et al., [212]

HDL lipoprotein promotes and facilitates the process of RCT, whereby excess cholesterol from the macrophages are effluxed into HDL. During the transfer, cholesterol is esterified by LCAT into CE. Through this time, the nascent HDL undergoes through changes as it fills up with CE where it becomes a mature HDL particle. After this point, the mature HDL returns to the liver for excretion of CE via the liver SR-BI receptor. However, this RCT process has a second pathway, wherein CETP working in cohort with ApoB-100 facilitates exchange of CE for TAG between HDL and VLDL/LDL lipoproteins.

(A-1) ApoA-1; (FC) indicates free cholesterol; (BA) bile acids; (LDLR) LDL receptor; (TG) triglycerides; (CE) Cholesterol ester; (PLTP) Phospholipid transfer protein; (CETP) Cholesteryl ester transfer protein.

Figure 1-10: Dysfunctionality of RCT metabolism in MHD patients.



Source: European heart journal Supplements, Barter 2005, [213]

The figure shows an overview of believed lipoprotein metabolism in dyslipidemic MHD patients.

In MHD patients, DL is highly prevalent and is characterized by delayed TAG-rich lipoproteins catabolism which in turn leads to elevated TAG levels, VLDL, IDL, and low HDLC due to suppressed/decreased ApoA-1 production. Moreover, the HDL level may decline because CETP activity may be elevated, and LCAT activity is reduced. Other lipids such as TC, LDL-C may be presented as decreased or normal.

Symbols: Increased (↑) and decrease (↓) plasma levels, (↔) normal.

CHAPTER 2: METHODOLOGY

General study design

The general purpose of this study is to characterize DL in lipids, and lipoprotein subfractions in a multi-ethnic cohort of ESRD patients undergoing MHD. This dissertation integrates MHD subjects from both PATCH USA clinical trial (NCT02358967) comprised of African Americans, and PATCH MALAYSIA clinical trial (NCT02913690) a cohort composed of three ethnicities; Malays, Malaysian-Chinese and Malaysian-Indians. Moreover, this investigation focusses on key HDL enzymes, which partake in the RCT mechanism linked in DL in MHD subjects.

Specific Aim 1 is representative of baseline data, in a cohort of African American (**AA**) patients undergoing hemodialysis treatment thrice weekly, for which we documented individuals' degree of dyslipidemia by characterizing lipids and lipoprotein particle compositions

Specific Aim 2 characterizes dyslipidemia across a multi-ethnic cohort of MHD patients Malays (**MM)**, Malaysian-Chinese (**MC**), Malaysian-Indians (**MI**), and the US- **AA** patients). Moreover, examine differences in lipid parameters amongst the two cohorts of US and Malaysian patients.

Lastly, **specific Aim 3** investigates the extent to which HDL enzyme, in the RCT pathway, contributes to dyslipidemia in MHD patients.

This chapter will describe the specific methodology and procedures of all the experiments and assays that were conducted for the studies as described in the specific chapters (**Chapters III, IV, and V**).

Ethics and Human Subjects'

The Palm Tocotrienols in Chronic Hemodialysis (PATCH) is an ongoing, multi-center, longitudinal, randomized, double-blinded, placebo-controlled study for 15-months involving MHD patients in Michigan, Malaysia, Bangladesh, and India. All study participants were dialyzed thrice weekly.

Subjects were assigned randomly to receive 300 mg of TRF or placebo daily for 12-months, followed by a 3-month washout period. Patients in the US cohort for Great Lakes Dialysis, DaVita: Kresge, Highland Park, and Redford dialysis units were given TRF or placebo capsules during their dialysis sessions under the supervision of a staff member, then the remainder of capsules were consumed at home by the patients. MHD subjects from Henry Ford Medical Centers of Fairlane and West Pavilion dialysis, in addition to all Malaysia, enrolled patients, consumed all capsules at home.

Ethical approvals for the US and Malaysian cohort were obtained from the Human Ethics Committee at Wayne State University (WSU-IRB 123314MP4F), and the Malaysian Ministry of Health. Additional approvals were obtained from participating dialysis units. Written informed consents were also obtained from all the patients. Demographics data and routine biochemical parameters were obtained from patients' medical records provided by the respective units.

Inclusion and Exclusion Criteria's

The two studies for both PATCH USA and Malaysia share mostly but not all requirements, with minor differences. These criteria are shown in **Table 2-1** (for inclusion) and **Table 2-2** (exclusion).

General blood collection, handling, and processing of samples

The US cohort followed a group of African American MHD patients for a duration of 15 months, where blood was obtained every 3 months. Approximately 10 mL of whole blood samples were collected from study subjects using commercially available pre-coated EDTA-K2 or LH vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA), in which case subjects were not required to fast. While past guidelines have suggested measuring fasting blood lipids, new guidelines from the European Federation of Clinical Chemistry and Laboratory Medicine recommend that the use of both fasting and non-fasting measurements are similar for evaluation and thus appropriate to use [214].

The samples were transported to the WSU laboratory within 2-3 hours post blood draw. Plasma was isolated from whole blood via centrifugation (Beckman Coulter Centrifuge, Lakeview Parkway, IN, USA) at 2,500 rpm for 15 minutes at 4°C. Plasma was then dispersed in Eppendorf microcentrifuge tubes and immediately transferred into -80°C freezer until further analysis

The Malaysian blood samples were collected and processed following a similar protocol but with minor differences. Roughly, 10 mL of blood was collected into EDTA-K2 or LH vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were immediately centrifuged at 3000g for 10 minutes to separate plasma [215]. Afterward, plasma was dispersed into Eppendorf tubes and snap-frozen with liquid nitrogen. The frozen aliquots were then placed in dry ice containers and transported to the laboratory for storage at -80°C (Isotemp™, Fisherbrand™, Hampton, NH, USA). A subset of the baseline plasma samples of both LH and EDTA-K2 collected samples from the Malaysian cohort were shipped to Wayne State University, for additional analysis.

Anthropometry and other measurements

The anthropometric measurements for height, weight, estimated dry weight were conducted by PATCH lab investigators. Height was measured to the nearest centimeter (0.1 cm), and body weight was measured to the nearest kilogram (0.1). Body mass index (BMI), was calculated using Quetelet's Index : [BMI (kg/m^2) = weight (kg) / height (m^2)] [216]. Patient medical history, such as medications, were self-reported: blood pressure, diabetic, statin use, were collected.

Biochemical Analysis

Lipid Profile

Lipids in the Malaysian study were measured by an external laboratory in Kuala Lumpur, Malaysia. The lipid parameters for PATCH USA study along with a subset of (n = 57) samples from the Malaysian cohort were measured using EDTA-K2 collected plasma. The TAG, HDL-C, and TC were analyzed using enzymatic kit (Pointe Scientific Inc., Canton, MI, USA), performed according to manufactures protocol, whereas LDL-C was calculated using the Friedewald formula [217]. From the 57 samples measured in both the US and Malaysia; TAG showed a correlation with an $r = 0.934$ ($p < 0.001$), and HDL-C measurements had an $r = 0.854$ ($p < 0.001$). TC had an $r = 0.974$ ($p < 0.001$), whereas LDL-C had an $r = 0.961$ ($p < 0.001$).

HDL and LDL lipoprotein subfractions analysis (Lipoprint)

The assessment of lipoproteins was carried out using **Quantimetrix Lipoprint™** System (Quantimetrix Corp., Redondo Beach, CA, USA). EDTA-K2 plasma samples were used in the measurements for both HDL and LDL lipoprotein subfraction in accordance with the manufacturers' protocol. Plasma was added to a precasted linear polyacrylamide gel tube and loaded with a lipophilic (blue) dye. The dye binds proportionally to the cholesterol in each

lipoprotein, when the prestained lipoproteins undergo electrophoresis. During this phase, particles migrate through the separated gel matrix into appropriate lipoproteins bands according to the particle, charge, and size from largest to smallest due to the sieving action of the gel.

HDL lipoprotein analysis has many subfraction bands. Subfraction bands [HDL1-3] makes up the **large buoyant HDL** (L-HDL) lipoprotein, bands [HDL 4-7] make up the **intermediate-HDL** (I-HDL), and [HDL 8-10] bands constitute the **small HDL** (S-HDL) subfraction. Albumin is the last band in the gel tube with LDL/VLD remaining at the stacking and separating gels interface (**Figure 2-1**).

LDL lipoprotein analysis can also generate multiple bands. There are a total of 12 bands, with the top band being VLDL and the last migrating lipoprotein being HDL. The MID-bands are composed of three sub-bands (IDL-C, IDL-B, and IDL-A). LDL lipoprotein subfractions, are further separated into three categories: [LDL 1] makes up **large buoyant LDL** (L-LDL) subfraction; band [LDL 2] composes the **intermediate LDL** (I-LDL) subfraction, and combined bands [LDL 3-7] includes the **small dense LDL** (S-LDL) subfractions (**Figure 2-2**). LDL-lipoprotein testing can detect and differentiate the mean particle-size in angstroms (Å) units, for which a set range of particle sizes is depictive of a phenotype profile. Phenotype: ***Type-A*** is associated with more large buoyant LDL particles. The atherogenic lipoprotein profile is ***Type-B*** (denser LDL subspecies) and ***Type-intermediate*** or ***[AB]*** phenotype, which is the lesser atherogenic and prone to change [218]. LDL lipoprint program algorithm utilizes the guidelines of the National Cholesterol Education Program and Adult Treatment Panel III (ATP III) as normal reference range.

HDL metabolism enzymes analysis

Cholesteryl ester transfer protein (CETP) activity analysis

CETP was measured in LH plasma samples, using CETP activity assay kit (RB-CETP, Roar Biomedical, Broadway, NY, USA). The assay uses a proprietary substrate donor molecule that enables the detection of CETP mediated transfer of neutral lipid from the substrate to a physiological acceptor. The assay is a fluorometric method, at wavelengths of (465_{Ex} nm / 535_{Em} nm). The analysis was conducted as described in the manufacturer protocol with the results, of CETP activity being expressed in nmol/mL/hrs.

Lecithin-cholesterol acyltransferase (LCAT) activity analysis

LCAT was measured using in LH plasma samples, using the LCAT activity assay kit (RB-LCAT, Roar Biomedical, Broadway, NY, USA). The assay is a fluorometric assay which measures the phospholipase activity of the LCAT. The samples were read using a fluorimeter plate reader at two different wavelengths (340_{Ex} nm/470_{Em} nm) and at (340_{Ex} nm/390_{Em} nm). The two distinct intensity peaks depend upon the concentration of the hydrolized and the intact substrate present in the assay. In the case of the substrate being intact, the fluorophores that are nearby are excited and result in the dissipation of radiationless transitions. The emission is predominately at the less energetic state of 470 nm peak, representative of hydrolysis of the substrate by LCAT, where the fluorophores are not able to energetically interact, leads to a shift that causes intensity in the form of emission at 390 nm. The final results of the LCAT activity were expressed as a ratio between the two readings (470/390 nm), which is the rate of change per min/uL/plasma in accordance with the protocol.

Inflammatory marker: C-reactive protein (CRP)

C-reactive protein (CRP) levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, USA). A total of 10 µL of LH or EDTA-K2 plasma was diluted in the assay buffer into 1:18,200. After sample preparations, the CRP standards were made following a sequence of serial dilutions per manufacturer's protocol. Subsequently, a total of 100 µL of each prepared solution was dispersed in duplicates into pre-coated 96-well plates with a monoclonal antibody for human CRP. Following appropriate incubations and washes, the plate was measured at 450 nm wavelength (Multiskan^{TM} FC, Thermo Scientifc^{TM}, MA USA), where the results were analyzed using Prism (GraphPad, Software v7.0, San Diego, CA, USA), with final concentrations expressed in mg/L.

The Malaysian study measured this inflammatory marker using a high-sensitive CRP (hs-CRP) method through an external laboratory in Kuala Lumpur, Malaysia. During the analyses of the PATCH, USA study, a subset of Malaysian samples (n = 36) were measured using the Cayman Chemicals ELISA kit. Results showed a high correlation between the two techniques with an $r = 0.965$ ($p < 0.001$), which was used to adjust the hs-CRP results (**Appendix Figure 1**).

Oxidative stress marker: Nε-(carboxymethyl) lysine (CML)

CML is a form of advanced glycation end-product (AGE) adduct in human plasma. OxiSelect™ CML competitive ELISA kit (STA-816, Cell Biolabs Inc., San Diego, CA, USA) was used to measure Nε-(carboxymethyl) lysine adducts in LH or EDTA-K2 plasma. Quantity of CML adducts in the protein plasma samples were determined by comparing its absorbances with a CML-BSA standard curve, as specified by the manufacturer's protocol, at a wavelength of 450 nm using a microplate reader (Multiskan^{TM} FC, Thermo Scientific^{TM}, MA USA). Data analysis required a 4PL standard curve, which allowed for extrapolation of the sample's concentrations by using the

graphical analyzer Prism (GraphPad, Software v7.0, San Diego, CA, USA). Concentrations were expressed in ng/mL.

Statistical Analysis

Results were expressed as mean and standard deviation (mean±SD) for continues variables with a normal distribution. Categorical variables of ordinal or nominal nature were expressed as percentages (%) when appropriate. To verify the normal distribution of variables, we used the Shapiro-Wilk test, as for analysis for homogeneity of variances, we used Levene's test. Categorical data and data that were not normally distributed were tested using Chi-square test and nonparametric test, respectively. Differences in the mean between groups were tested using One-Way ANOVA and General linear model (GLM) (Univariate) necessary. One-Way ANOVA analysis (between and or within groups), utilized Post-hoc Bonferroni test was employed for multiple detailed comparisons (two more groups), whereas the Dunnett's T3 test was used in the analysis when equal variance was not assumed when appropriate. Data were corrected for any confounding variables. Covariate factors were adjusted (Age, BMI, Albumin, Systolic BP, Diastolic BP, Risk factor, Kt/V) using ANCOVA, Post-hoc Bonferroni test, in regards to fixed factors (Ethnicities, gender, smoking, DM, HTN, Statin).

An independent sample t-test was applied when was appropriate for non-normally distributed parameters. Additionally, Chi-square χ^2 was used to test for independence of continues categorical or nominal variables when relevant.

Percent risk factor (**PRF**) was used to evaluate the percent increased risk, using an online published calculator for atherosclerotic cardiovascular disease (ASCVD). It covers a 10-years risks of heart disease and stroke factor, with algorithms by ACC/AHA Guidelines based on Framingham risk score (FRS) parameters [219]. The calculator incorporates numerous parameters such as age,

gender, race, blood pressure, lipids, history of DM, smoking, statin use, HTN, and aspirin therapy. The atherogenic risk is an estimated value of the Atherogenic Index of Plasma. It is calculated using the triglycerides and HDL-C in AIP, reflecting the balance between the atherogenic and protective lipoproteins. (AIP = =LOG10 ((TAG*0.0113)/ (HDL-C*0.0259)) [220].

The (α)-value was set to be at ($p<0.05$) for the two-sided tail test, representing statistical significance. GraphPad Prism 7.0 (GraphPad, Software v7.0, San Diego, CA, USA) was used for graphing, and curve fitting in ELISA assays whereas analysis and calculations were completed with the use of (**SPSS)** v25.0 software by (IBM, Chicago, IL, USA)

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Tables Chapter 2

Table 2-1: Study inclusion criteria.

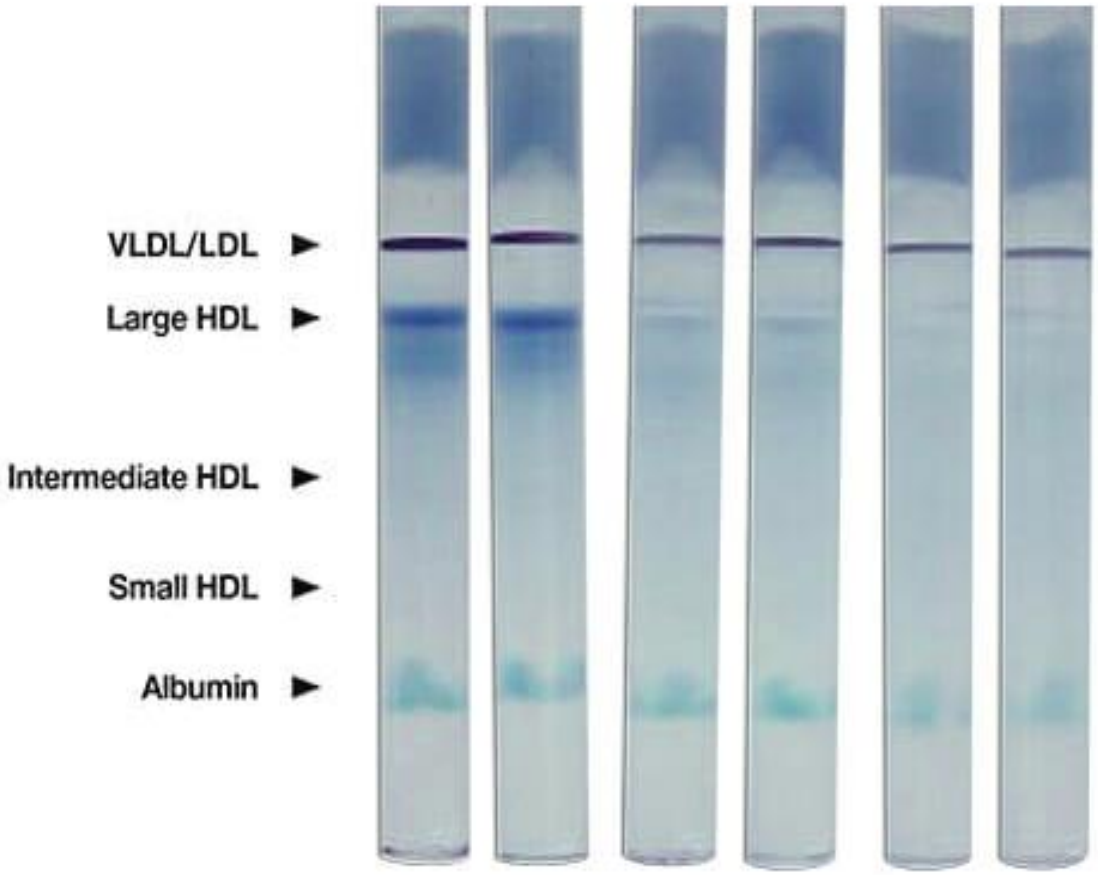
PATCH USA and Malaysia
Patients had to be of age 18 years or older.
They had to be undergoing chronic hemodialysis treatment for more than three months.
To be able and willing to comply with all trial requirements.
Willing to notify his or her Physician/Nephrologist/General Practitioner for participating in the trial.
PATCH Malaysia Only
They must adequately be dialyzed (Kt/V > 1.2 or have a urea reduction ratio (URR) of 65%.
Patients also must have hs-CRP levels of less than 20 mg/dL.

Table 2-2: Study exclusion criteria.

PATCH USA and Malaysia
Subjects who had been involved with another investigatory trial within the past 12 weeks.
Patients with a history of functional kidney transplant 6 months before the study and or waiting for donor kidney transplant over the study duration.
Any participants who had taken vitamin E- containing supplement > 60 IU/day during the past 30 before study enrollment.
Any patients with a history of poor adherence to HD or medical regimen.
Any patients that are currently on active treatment for cancer, excluding basal cell carcinoma of the skins.
Participating subjects diagnosed with HIV/AIDS and/or are on any anti-HIV therapy.
Any patients taking anti-inflammatory medication, except aspirin < 325 mg/d, over the past 30 days before study enrollment.
Any pregnant female patients and or lactating or planning pregnancy during the course of the trial.
Hospitalizations within the last 90 days and more than two times, or one hospitalization within the 30 days preceding enrollment, were excluded.
Subjects receiving nutritional support via enteral and intra-venous routine.
Using a temporary catheter or receiving a graft/fistula within the 6-month study period.
PATCH Malaysia Only
Patients with Hepatitis B or C.
Additional diseases or disorders where the opinion of nephrologists may affect the end result of the study.
Patients with known allergies towards fish-based products.

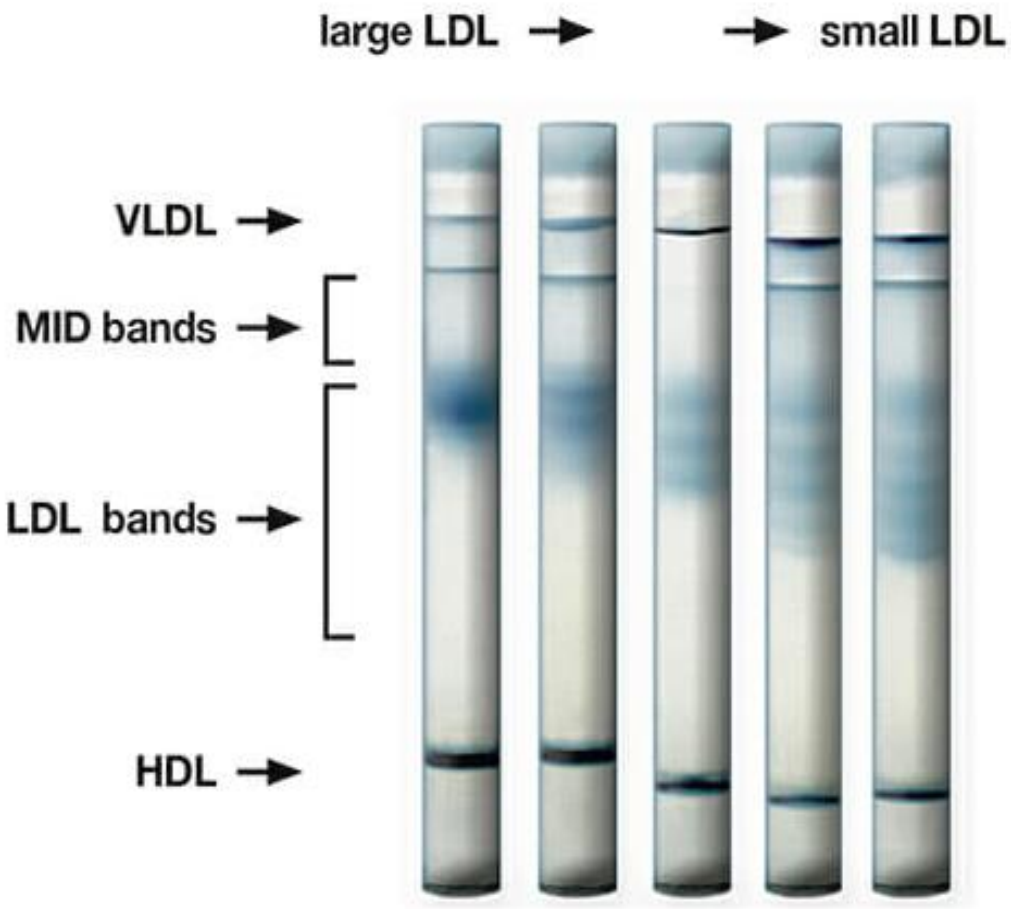
Figures Chapter 2

Figure 2-1: HDL lipoprint subfraction band separation on polyacrylamide gel tube.



Source: Quantimetrix, Laboratory Lipoprint, 2019

Figure 2-2: LDL lipoprint subfraction band separation on polyacrylamide gel tube.



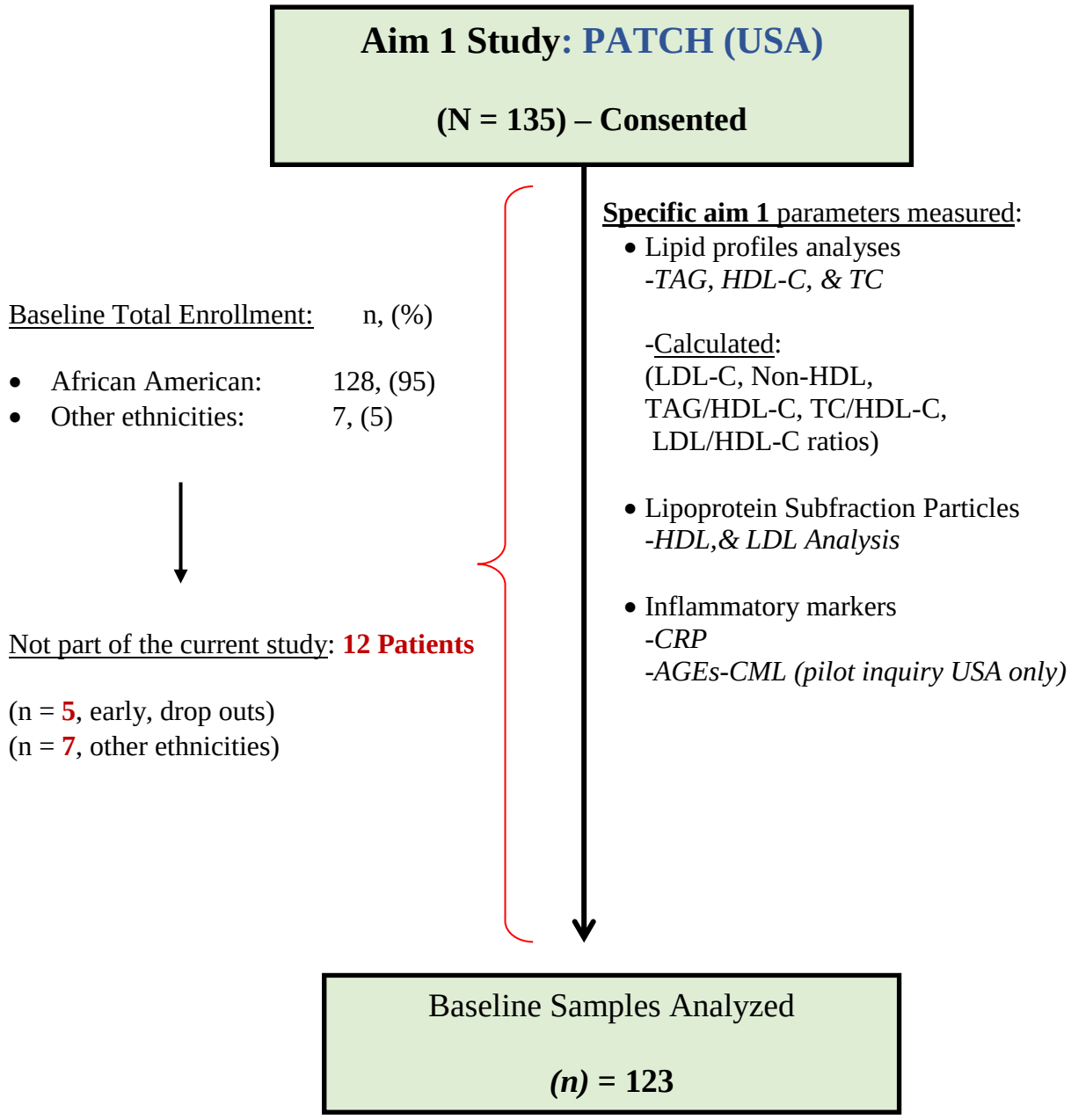
Source: Quantimetrix, Laboratory Lipoprint, 2019

CHAPTER 3: RESULTS – SPECIFIC AIM 1: TO DOCUMENT THE DEGREE OF DYSLIPIDEMIA IN A COHORT OF MHD PATIENTS AND CHARACTERIZE LIPIDS, AND LIPOPROTEIN PARTICLE COMPOSITIONS.

The study flow is shown in **Figure 3-1.** From 135 patients, 12 subjects were excluded from the analyses (not African–American or incomplete data). Thus, 123 subjects (all AA) were used. The plasma analyzed from these subjects represented the baseline samples collected (i.e., prior to study randomization) in the PATCH study (NCT02358967). Patient demographics are shown in **Table 3-1.** The mean age of the cohort was 59±13 years, with 63%% males and 37% females. The majority of the participants (71%) were non-smokers. Almost 55% of the cohort had DM, while 88% were hypertensive. Additionally, 55% were prescribed statin drugs. It should be noted that as part of the patients' routine care, medications would have been prescribed to manage blood pressure and LDL-C to target desirable levels. The overall cohort was classified as obese (BMI 30.0 ± 7.5 kg/m^2). Both systolic and diastolic B/P were within the normal range, creatinine levels were elevated, while albumin was marginally below the recommended values. The Dialysis vintage of the cohort was 66 ± 65 months, and Kt/V was within optimal range

Table 3-2 shows the plasma lipids in this cohort. Collectively, the cohort had lipids in the normal range. To examine the level of dyslipidemia, lipids were separated into two groups; ≥ 3.8 TAG/HDL-C ratio or < 3.8 TAG/HDL-C ratio. (This is based on the recommended target values of TAG <150 mg/dL and HDL-C > 40 mg/dL). This analyses showed that 17 subjects (14% of the cohort) had a TAG/HDL-C ration ratio ≥ 3.8 resulting in significantly higher TAG (179±38 vs. 79±31 mg/dL) and LDL-C (110±36 vs 75±38 mg/dL), while HDL-C was significantly lower (34±6 vs 53±189 mg/dL). As a consequence, ratios of TAG/HDL-C, TC/HDL-C and LDL-C/HDL-C were all significantly higher in subjects with TAG/HDL-C ratios ≥ 3.8

Figure 3-1: Specific Aim 1 study flow.



AA, African Americans; TC, total cholesterol; TAG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; AGEs-CML, Advanced glycation end-products - Nε-carboxymethyl-lysine.

Table 3-1: Characteristics of the African American (AA) cohort.

Demographics	
Age (yrs.)	59 ± 13
Gender (n, %)	
Males	77 (62.6)
Females	46 (37.4)
Ethnicity (n, %)	
African American	123 (100)
Tobacco Smokers (n, %)	
Smokers	36 (29.3)
Non-Smokers	87 (70.7)
Diabetes Mellitus (DM) (n, %)	
DM	68 (55.3)
Non-DM	55 (44.7)
Hypertension (HTN) (n, %)	
HTN	108 (87.8)
Non-HTN	15 (12.2)
Statin (n, %)	
User	55 (44.7)
Non-User	68 (55.3)
BMI (kg/m^2)	30.0 ± 7.5
Creatinine (mg/dL)	9.5 ± 2.8
Albumin (g/dL)	3.83 ± 0.32
Systolic B/P (mmHg)	140 ± 21
Diastolic B/P (mmHg)	79 ± 15
Time on Dialysis/Vintage (months)	66 ± 65
Kt/V	1.52 ± 0.22

Values are mean ± SD, n=123

Table 3-2: Plasma lipids in the AA cohort.

Lipid Parameters	Total	TAG/HDL-C	
	(n = 123)[1]	**ratio ≥ 3.8** (n = 17)	**ratio < 3.8** (n = 106)
TAG (mg/dL)	93 ± 47	179 ± 38 [a]	79 ± 31 [b]
HDL-C (mg/dL)	50 ± 19	34 ± 6 [a]	53 ± 19 [b]
TC (mg/dL)	148 ± 42	179 ± 36 [a]	143 ± 41 [b]
LDL-C (mg/dL)	80 ± 39	110 ± 36 [a]	75 ± 38 [b]
NonHDL (mg/dL)	98 ± 45	145 ± 36 [a]	91 ± 41 [b]
TAG/HDL-C Ratio	2.24 ± 1.59	5.40 ± 1.18 [a]	1.74 ± 0.93 [b]
TC/HDL-C Ratio	3.33 ± 1.51	5.47 ± 1.54 [a]	2.99 ± 1.19 [b]
LDL/HDL-C Ratio	1.89 ± 1.26	3.39 ± 1.46 [a]	1.65 ± 1.05 [b]

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Table 3-3 shows the lipoprotein subfraction composition in the AA cohort. Collectively, the L-HDL (22.0 ± 15.9 mg/dL) and I-HDL (22.1 ± 5.74 mg/dL) are the most prominent HDL particles, whereas the S-HDL (5.94 ± 3.19 mg/dL) is the least. LDL subfraction analysis is made up of multiple subfractions (VLDL, IDL, L-LDL, I-LDL, and S-LDL), which were IDL (46.8 ± 15.4 mg/dL) and VLDL (25.4 ± 10.2 mg/dL) are the most predominant subfractions. This is followed by L-LDL particle (22.3 ± 9.53 mg/dL), as the most prominent subfraction with respect to I-LDL (12.5 ± 8.00 mg/dL) and S-LDL (3.85 ± 6.11 mg/dL). Phenotype analyses resulted in type "A" as the dominant profile 65%, followed by type "B" 19.5%, and "AB," known as the intermediate phenotype, present 15.4% in the AA cohort. D was assessed by separating subjects into two groups, ≥ or < 3.8 TAG/HDL-C ratio. The 17 patients in the ≥ 3.8 TAG/HDL-C ratio group, had significantly lower L-HDL (8.59±4.21 vs. 24.2±16.0 mg/dL), I-HDL (17.5±5.34 vs. 22.9±5.47 mg/dL), and higher S-HDL (7.65±2.96 vs. 5.67±3.15 mg/dL). Furthermore, the subsets had significantly higher IDL (61.4±14.5 vs. 44.4±14.3 mg/dL), and S-LDL (9.47±10.7 vs. 2.94±4.49 mg/dL), with LDL phenotype "B" becoming the predominant profile in 47% of AA subjects.

Table 3-4 shows CETP and LCAT enzymatic activities in the AA cohort. Average CETP activity was noted to be (41.6 ± 18.7 nmol/mL/hr.), whereas LCAT (0.89 ± 0.17 ratio 470 / 390 nm). To discern the effects of D on the two enzyme activity, samples were allocated into two groups. ≥ or < 3.8 TAG/HDL-C ratios. The 17 AA subjects within ≥ 3.8 TAG/HDL-C ratio group displayed a significantly lower CETP activity (32.1±14.0 vs. 43.1 ± 19 nmol/mL/hr.), whereas the LCAT differences were marginal (0.87±0.17 vs 0.89±0.16 ratio 470 / 390 nm).

Table 3-3: Lipoprotein subfraction composition in the AA cohort.

Lipoproteins Subfractions	Total (n = 123)[1]	TAG/HDL-C ratio ≥ 3.8 (n = 17)	TAG/HDL-C ratio < 3.8 (n = 106)
L-HDL (mg/dL)	22.0 ± 15.9	8.59 ± 4.21 [a]	24.2 ± 16.0 [b]
I-HDL (mg/dL)	22.1 ± 5.74	17.5 ± 5.34 [a]	22.9 ± 5.47 [b]
S-HDL (mg/dL)	5.94 ± 3.19	7.65 ± 2.96 [a]	5.67 ± 3.15 [b]
VLDL (mg/dL)	25.4 ± 10.2	37.6 ± 9.86 [a]	23.5 ± 8.82 [b]
IDL (mg/dL)	46.8 ± 15.4	61.4 ± 14.5 [a]	44.4 ± 14.3 [b]
L-LDL (mg/dL)	22.3 ± 9.53	22.2 ± 9.89	22.3 ± 9.52
I-LDL (mg/dL)	12.5 ± 8.00	17.2 ± 8.0 [a]	11.7 ± 7.78 [b]
S-LDL (mg/dL)	3.85 ± 6.11	9.47 ± 10.7 [a]	2.94 ± 4.49 [b]
Mean LDL size (Å)	269.5 ± 4.64	265.1 ± 5.60 [a]	270.2 ± 4.07 [b]
Phenotype "**A**" (n, %)	80 (65%)	6 (35%)	74 (70%)
Phenotype "**B**" (n, %)	24 (19.5%)	8 (47%)	11 (10%)
Phenotype "**AB**" (n, %)	19 (15.4)	3 (18%)	21 (20%)

Values are mean ± SD for the numbers in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

Abbreviations: L-HDL, large-high density lipoprotein; I-HDL, intermediate high-density lipoprotein; S-HDL, small high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate low-density lipoprotein; L-LDL, large low-density lipoprotein; I-LDL, intermediate low-density lipoprotein; S-LDL, small low-density lipoprotein, Phenotype "AB," is a phenotype intermediate between A and B.

Table 3-4: Cholesterol ester transfer protein (CETP) and Lecithin cholesterol acyl transferase (LCAT) activity in the AA cohort.

Enzymes	Total	TAG/HDL-C	
	(n = 123)[1]	**ratio ≥ 3.8** (n = 17)	**ratio < 3.8** (n = 106)
CETP nmol/mL/hr.	41.6 ± 18.7	32.1 ± 14.0 [a]	43.1 ± 19 [b]
LCAT ratio of 470/390 nm	0.89 ± 0.17	0.87 ± 0.21	0.89 ± 0.16

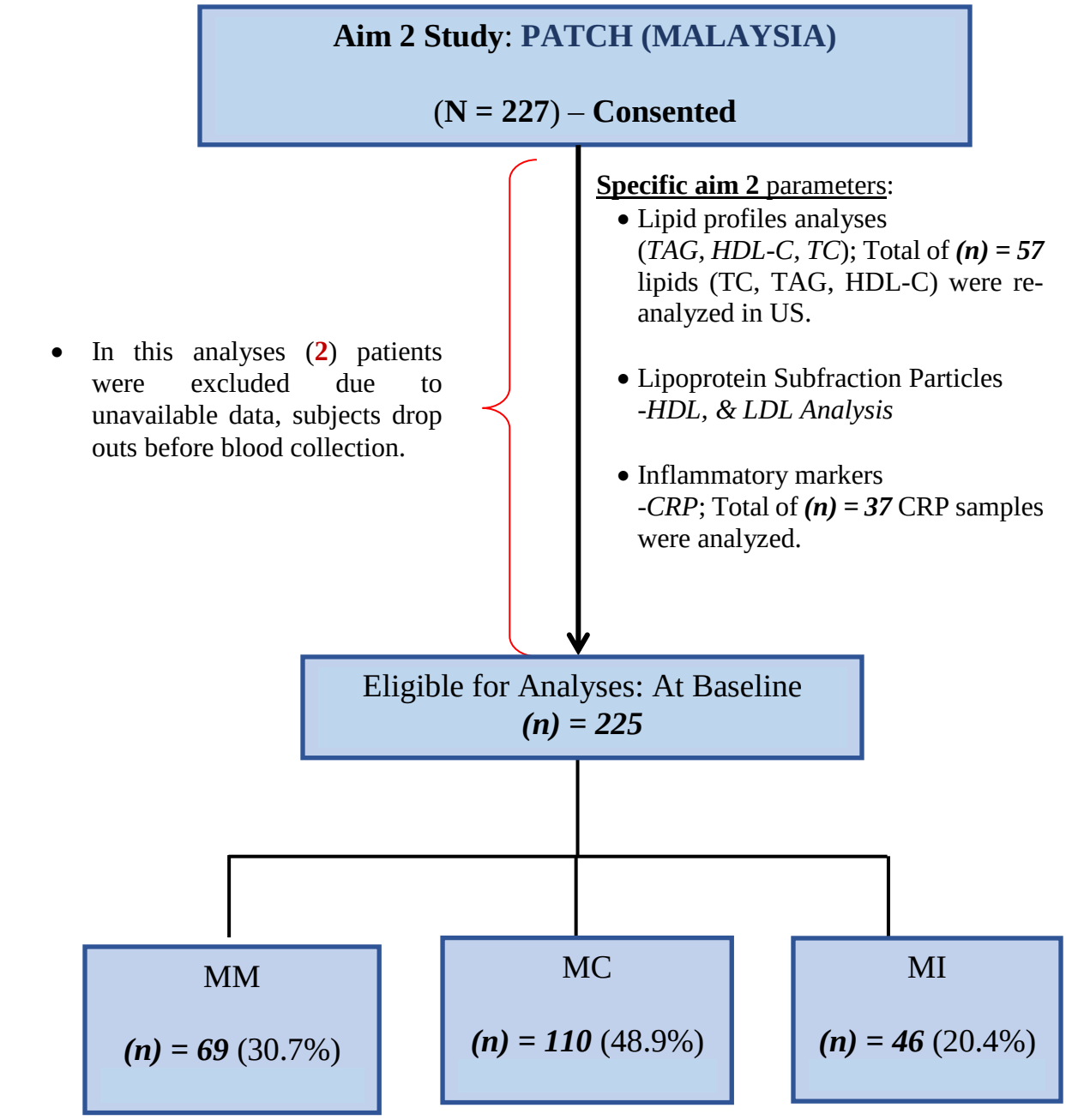
Values are mean ± SD for the numbers in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

CHAPTER 4: RESULTS – SPECIFIC AIM 2: TO CHARACTERIZE DYSLIPIDEMIA ACROSS A MULTI-ETHNIC COHORT OF MHD PATIENTS.

The study flow is shown in **Figure 4-1.** From 227 enrolled patients, two were excluded from the analyses due to unavailable data (patients had drop prior blood collection). Thus, of the 225 patients, the Malaysian cohort was divided into three different ethnicities of which; 69 patients are Malaysian-Malays (MM), 110 are of Malaysian-Chinese (MC), and 46 are of Malaysian-Indians (MI) ethnicity. Similar, to the AA cohort, patients' blood collected was representative of baseline samples gathered before randomization in the PATCH clinical trial (NCT02913690). Patients demographics are shown in **Table 4-1**. The mean age of the cohort is 54 ± 13 years of which 60% are males, and 40% are females. Analyses with respect to age showed that MM (48 ± 14 years) were significantly younger when compared to MC (57 ± 11 years) and MI (55 ± 12 years).

The majority of the participants, 90% were non-smokers. Almost 44% of the cohort had DM, while 84% were hypertensive. Furthermore, about 60% of patients were prescribed statin drugs. Overall, cohort patients were classified as overweight with a BMI of (25 ± 4.1 kg/m^2), specifically MM and MI who had significantly higher BMI than MC. Albumin levels were significantly lower in MM patients (3.8 ± 0.4 g/dL) when compared with MC, and MI. Systolic B/P was marginally elevated, whereas diastolic B/P was within normal range. Cohort vintage was 77 ± 59 months, with MM dialyzed the longest 84 ± 66 months, wherein Kt/V was within an optimal range.

Figure 4-1: Specific Aim 2 study flow.



Abbreviations: MM, Malaysian-Malay's; MC, Malaysian-Chinese; MI, Malaysian-Indians

Table 4-1: Characteristics of the Malaysian cohort.

Demographics	**Total** (n = 225)[1]	**MM** (n = 69)	**MC** (n = 110)	**MI** (n = 46)	*P-value*
Age (yrs.)	54 ± 13	48 ± 14[a]	57 ± 11[b]	55 ± 12[b]	**<0.001**
Gender (n, %)					
Males	134 (59.6)	37 (53.6)	65 (59.1)	32 (69.6)	NS
Females	91 (40.4)	32 (46.4)	45 (40.9)	14 (30.4)	
Tobacco Smokers (n, %)					
Smokers	21 (9.3)	9 (13)	10 (9.1)	2 (4.3)	NS
Non-Smokers	202 (89.8)	59 (85.5)	99 (90)	44 (95.7)	
Other-unknown	2 (0.9)	1 (1.4)	1 (0.90)	-	-
Diabetes (DM) (n, %)					
DM	99 (44)	27 (39.1)	48 (43.6)	24 (52.2)	NS
Non-DM	126 (56)	42 (60.9)	62 (56.4)	22 (47.8)	
Hypertension (n, %)					
HTN	188 (83.6)	57 (82.6)	96 (87.3)	35 (76.1)	NS
Non-HTN	37 (16.4)	12 (17.4)	14 (12.7)	11 (23.9)	
Statin (n, %)					
User	135 (60)	37 (53.6)	73 (66.4)	25 (54.3)	NS
Non-User	90 (40)	32 (46.4)	37 (33.6)	21 (45.7)	
BMI (kg/m^2)	25 ± 4.1	26 ± 4.6[a]	25 ± 4.2[b]	26 ± 3.2[ab]	**0.025**
Albumin (g/dL)	4.0 ± 0.5	3.8 ± 0.4[a]	4.1 ± 0.4[b]	4.0 ± 0.4[b]	**<0.001**
Systolic B/P (mmHg)	156 ± 22	151 ± 21	158 ± 21	159 ± 24	NS
Diastolic B/P (mmHg)	78 ± 15	78 ± 16	79 ± 14	77 ± 16	NS
Dialysis/Vintage (months)	77 ± 59	84 ± 66	76 ± 55	71 ± 56	NS
Kt/V	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	NS

Values are mean ± SD for the numbers in paremtheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA. Data were adjusted using ANCOVA, in relation to covariate factors (age, gender, tobacco use, statin usage, BMI, Albumin, blood pressure, as well as Kt/V. Abbreviations: MM, Malaysian-Malay; MC, Malaysian-Chinese; MI, Malaysian-Indians; NS, Not significant.

Table 4-2 shows plasma lipids for the Malaysian cohort. Collectively, lipids were marginally higher than the AA cohort; however, largely, the Malaysian lipids profile were within normal range. HDL-C results showed to be significantly lower in MM patients (43 ± 10 mg/dL) when compared with MC (48 ± 14 mg/dL). The TAG levels were higher in MM subjects (142 ± 74 mg/dL) when compared with MC (127 ± 62 mg/dL) and MI (124 ± 77 mg/dL) which did not meet statistical significance. Moreover, lipid LDL/HDL-C ratios were significantly higher in MI (2.39 ± 0.91) patients in relation to MC (2.01 ± 0.78).

Table 4-2: Plasma lipids in the Malaysian cohort

Lipid Parameters	Total (n = 225) [1]	MM (n = 69)	MC (n = 110)	MI (n = 46)
TAG (mg/dL)	131 ± 69	142 ± 74	127 ± 62	124 ± 77
HDL-C (mg/dL)	46 ± 13	43 ± 10 [a]	48 ± 14 [b]	43 ±11 [ab]
TC (mg/dL)	163 ± 33	164 ± 34	164 ± 35	163 ± 31
LDL-C (mg/dL)	92 ± 28	93 ± 30	90 ± 28	96 ± 25
NonHDL (mg/dL)	118 ± 34	121 ± 36	115 ± 33	120 ± 33
TAG/HDL-C Ratio	3.29 ± 2.42	3.73 ± 2.58	3.00 ± 2.04	3.32 ± 2.93
TC/HDL-C Ratio	3.81 ± 1.21	4.01 ± 1.29	3.60 ± 1.05	4.03 ± 1.38
LDL/HDL-C Ratio	2.17 ± 0.87	2.29 ± 0.94 [ab]	2.01 ± 0.78 [a]	2.39 ± 0.91 [b]

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Table 4-3 examines plasma lipid, D within the Malaysian cohort. Lipids were separated into two groups; ≥ 3.8 TAG/HDL-C ratio or < 3.8 TAG/HDL-C ratio. Analyses showed 65 subjects (29% of the cohort) had a TAG/HDL-C ratio ≥ 3.8 resulted with significantly higher TAG (216±67 vs. 97±28 mg/dL), and LDL-C (99±32 vs. 90±26 mg/dL) while HDL-C was significantly lower (35±5 vs. 50±12 mg/dL). In the < 3.8 ratio group, MC patients had lower values in TC/HDL-C and LDL/HDL-C ratios, compared to MI subjects.

Table 4-3: Plasma lipid analyses based on TAG/HDL ration in the Malaysian cohort.

Lipids	**Total**	**MM**	**MC**	**MI**
***TAG/HDL ratio ≥ 3.8**	(n =65) [1]	(n=25)	(n = 28)	(n = 12)
TAG (mg/dL)	216 ± 67	219 ± 64	214 ± 52	215 ± 102
HDL-C (mg/dL)	35 ± 5	35 ± 5	36 ± 5	33 ± 5
TC (mg/dL)	176 ± 36	177 ± 38	175 ± 35	176 ± 36
LDL-C (mg/dL)	99 ± 32	100 ± 36	97 ± 32	100 ± 22
NonHDL (mg/dL)	141 ± 35	143 ± 37	139 ± 33	143 ± 37
TAG/HDL-C Ratio	6.34 ± 2.43	6.51 ± 2.28	6.00 ±1.64	6.77 ± 3.98
TC/HDL-C Ratio	5.11 ± 1.18	5.19 ± 1.20	4.88 ± 0.94	5.44 ± 1.60
LDL/HDL-C Ratio	2.85 ± 0.97	2.92 ± 1.08	2.69 ± 0.88	3.10 ± 0.94
*TAG/HDL ratio < 3.8	(n =160) [1]	(n= 44)	(n = 82)	(n = 34)
TAG (mg/dL)	97 ± 28	98 ± 29	97 ± 29	92 ± 23
HDL-C (mg/dL)	50 ± 12	48 ± 9	52 ± 14	47 ± 11
TC (mg/dL)	158 ± 31	156 ± 29	159 ± 33	159 ± 29
LDL-C (mg/dL)	90 ±26	89 ± 25	88 ± 27	94 ± 26
NonHDL (mg/dL)	108 ± 29	108 ± 29	107 ± 29	112 ± 28
TAG/HDL-C Ratio	2.05 ± 0.75	2.15 ± 0.78	1.98 ± 0.73	2.10 ± 0.75
TC/HDL-C Ratio	3.29 ± 0.74	3.35 ± 0.74 [ab]	3.15 ± 0.65 [a]	3.53 ± 0.87 [b]
LDL/HDL-C Ratio	1.90 ± 0.66	1.94 ± 0.63 [ab]	1.77 ± 0.59 [a]	2.14 ± 0.77 [b]

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

***All lipid values, in the TAG/HDL-C ratio ≥ 3.8 group were significantly different from the corresponding values in < 3.8 TAG/HDL ratio group.**

Table 4-4 shows the collective composition of the lipoprotein in the Malaysian cohort, and particle distribution amongst the three ethnicities (MM, MC, and MI). The prominent HDL subfraction in the Malaysian cohort is shown to be I-HDL (22.9 ± 5.0 mg/dL), followed by L-HDL (15.8 ± 10.5 mg/dL), and S-HDL (6.88 ± 3.47 mg/dL). While the I-HDL, predominant subfraction, there are no changes in its content amongst the ethnicities; nevertheless, fluctuations between subfractions are observed in L-HDL and S-HDL subfractions. The 49% of MC patients had significantly higher L-HDL (17.5 ± 11.8 mg/dL) in contrast to the 31% of MM (13.6 ± 8.50 mg/dL) patients. Additionally, MC had a higher content in S-HDL (7.31 ± 3.54 mg/dL) subfraction in relation to MI (5.50 ± 2.63 mg/dL) subjects. The VLDL was also higher in MC patients, (34.8 ± 10.1 mg/dL) and lower MI (30.2 ± 8.60 mg/dL). Congruently a marginal decrease in IDL subfraction in MC (46.0 ± 13.4 mg/dL) is seen while MI patients content rises IDL (50.3 ± 14.7 mg/dL). Overall there no significant changes in LDL content were seen amongst ethnicities. A pattern is perceived for which, a rise in VLDL is concurrently meet with decreases in IDL, L-LDL, and S-LDL are seen. Cohort mean LDL particle size had an average (269.0 ± 5.78 Å) and other changes of significance were recorded amongst the ethnicities. Phenotype analyses showed type "A" LDL pattern as the most prominent, 64% collectively in Malaysian subjects, type "B" is 23%, and type "AB" is 13%. The distribution of the phenotype amongst ethnicities also showed type "A" as the dominant pattern in 61% of MM subjects, 64% of MC, and 70% for MI patients.

Table 4-4: Lipoprotein subfraction composition in the Malaysian cohort.

Lipoprotein Subfractions	**Total** (n = 225) [1]	**MM** (n = 69)	**MC** (n = 110)	**MI** (n = 46)
L-HDL (mg/dL)	15.8 ± 10.5	13.6 ± 8.50 [a]	17.5 ± 11.8 [b]	14.9 ± 9.5 [ab]
I-HDL (mg/dL)	22.9 ± 5.0	22.6 ± 4.27	23.3 ±5.55	22.7 ± 4.67
S-HDL (mg/dL)	6.88 ± 3.47	7.12 ± 3.65 [ab]	7.31 ± 3.54 [a]	5.50 ± 2.63 [b]
VLDL (mg/dL)	33.5 ± 9.81	33.8 ± 9.71 [ab]	34.8 ± 10.1 [a]	30.2 ± 8.60 [b]
IDL (mg/dL)	47.2 ± 13.6	47.1 ± 12.8	46.0 ± 13.4	50.3 ± 14.7
L-LDL (mg/dL)	23.7 ± 8.61	23.0 ± 8.11	23.3 ± 9.01	25.6 ± 8.24
I-LDL (mg/dL)	13.2 ± 7.69	14.1 ± 8.93	12.5 ± 6.92	13.8 ± 7.38
S-LDL (mg/dL)	4.78 ± 7.58	5.41 ± 7.47	4.42 ± 6.87	4.70 ± 9.30
Mean LDL size (Å)	269.0 ± 5.78	268.5 ± 5.97	269.1 ± 5.58	269.4 ± 6.04
Phenotype "**A**" (n, %)	144 (64)	42 (61)	70 (64)	32 (70)
Phenotype "**B**" (n, %)	52 (23)	22 (32)	22 (20)	8 (17)
Phenotype "**AB**" (n, %)	29 (13)	5 (7)	18 (16)	6 (13)

Values are mean ± SD for the numbers in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA. Abbreviations: L-HDL, large-high density lipoprotein; I-HDL, intermediate high-density lipoprotein; S-HDL, small high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate low-density lipoprotein; L-LDL, large low-density lipoprotein; I-LDL, intermediate low-density lipoprotein; S-LDL, small low-density lipoprotein, Phenotype "AB," is a phenotype intermediate between A and B.

Table 4-5 shows the lipoprotein composition when subfractions are separated into two groups; ≥ 3.8 TAG/HDL-C ratio or < 3.8 TAG/HDL-C ratio in order to assess dyslipidemia. Analyses showed that 65 patients (29% of the cohort) allocated in the ≥ 3.8 ration group had significantly lower L-HDL (7.71 ± 3.76 mg/dL vs. 19.0 ± 10.6 mg/dL) lower I-HDL (19.0 ± 3.46 vs. 24.5 ± 4.65 mg/dL) while S-HDL had increased (8.54 ± 3.60 vs. 6.21 ± 3.19 mg/dL). Other noted difference; the VLDL lipoprotein, which was significantly higher in the ≥ 3.8 ratio group (40.8 ± 9.81 vs. 30.6 ± 8.13), also S-LDL was greater (11.6 ± 10.1 vs. 1.99 ± 3.55) whereas the mean LDL size had decreased (263.6 ± 5.88 vs. 271 ± 2 ± 4.09) when compared to the < 3.8 ratio group.

In summary: ≥ 3.8 TAG/HLD-C ratio in HDL subfraction analyses, was distinguished by a decreased in L-HDL, and I-HDL followed by the rise in S-HDL; wherein <3.8 ratio represents the opposite, depictive of increases in large and I-HDL while S-HDL decreases. For LDL lipoprotein analyses ≥ 3.8 TAG/HLD-C ratio showed no changes in L-LDL, but changes were revealed in the form of greater VLDL, I-LDL, S-LDL while mean LDL particle size had significantly decreased in size. A < 3.8 ratio showed decreases in I-LDL, S-LDL (the dense atherogenic particle), decreases for both IDL and VLDL, wherein mean LDL size is depicted by an increase in diameter.

Table 4-5: Lipoprotein composition according to, TAG/HDL-C ration (≥ or < 3.8) in the Malaysian cohort

Lipoprotein Subfractions	Total	MM	MC	MI
*TAG/HDL-C ratio ≥ 3.8	(n =65) [1]	(n=25)	(n = 28)	(n = 12)
L-HDL (mg/dL)	7.71 ± 3.76	7.24 ± 3.50	7.93 ± 3.90	8.17 ± 4.15
I-HDL (mg/dL)	19.0 ± 3.46	19.8 ± 3.49	18.6 ± 3.27	18.3 ± 3.82
S-HDL (mg/dL)	8.54 ± 3.60	8.16 ± 3.54[ab]	9.68 ± 3.52[a]	6.67 ± 3.17[b]
VLDL (mg/dL)	40.8 ± 9.81	39.8 ± 10.6	43.4 ± 9.02	37.0 ± 9.40
IDL (mg/dL)	48.3 ± 13.2	48.4 ± 14.3	47.7 ± 12.8	49.4 ± 12.5
L-LDL (mg/dL)	20.7 ± 7.70	21.0 ± 8.41	19.9 ± 7.92	22.1 ± 5.71
I-LDL (mg/dL)	18.3 ± 7.33	19.5 ± 9.06	16.9 ± 5.81	19.2 ± 6.45
S-LDL (mg/dL)	11.6 ± 10.1	11.8 ± 8.53	11.2 ± 9.23	12.3 ± 15.2
Mean LDL size (Å)	263.6 ± 5.88	263.5 ± 5.64	263.7 ± 5.24	263.9 ±8.04
*TAG/HDL-C ratio < 3.8	(n =160) [1]	(n= 44)	(n = 82)	(n = 34)
L-HDL (mg/dL)	19.0 ± 10.6	17.2 ± 8.41	20.8 ± 11.8	17.3 ± 9.75
I-HDL (mg/dL)	24.5 ± 4.65	24.1 ± 3.89	24.8 ± 5.27	24.2 ± 3.95
S-HDL (mg/dL)	6.21 ± 3.19	6.52 ± 3.61	6.50 ± 3.18	5.09 ± 2.33
VLDL (mg/dL)	30.6 ± 8.13	30.3 ± 7.32[ab]	31.9 ± 8.74 [a]	27.8 ± 6.70 [b]
IDL (mg/dL)	46.7 ± 13.7	46.3 ± 11.9	45.3 ± 13.7	50.6 ± 15.6
L-LDL (mg/dL)	24.9 ± 8.68	24.2 ± 7.78	24.5 ± 9.10	26.8 ± 8.69
I-LDL (mg/dL)	11.2 ± 6.85	11.1 ± 7.37	10.9 ± 6.64	11.9 ± 6.80
S-LDL (mg/dL)	1.99 ± 3.55	1.77 ± 3.23	2.11 ± 3.75	2.00 ± 3.54
Mean LDL size (Å)	271 ± 2 ± 4.09	271.4 ± 3.96	271.0 ± 4.36	270.8 ± 4.10

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

***All lipoprotein values, in the TAG/HDL-C ratio ≥ 3.8 group were significantly different from the corresponding values in < 3.8 TAG/HDL-C ratio group, except for IDL and L-LDL subfraction.**

Table 4-6 it examines D in plasma lipids amongst all ethnicities in the study cohort. Dyslipidemia is defined on the basis of three categories dyslipidemic [D] (8% of the overall study cohort), mixed dyslipidemic [MD] (52%) and normo-lipidemic [NL] (40%). All lipid values, in the [D] group, were significantly different from the corresponding values in [MD and NL] groups. There are also significant differences when comparing [MD] and [NL] lipid groups. The [D] AA patients had significantly lower TAG (189 ± 42 mg/dL) levels, when compared with MI (314 ± 123 mg/dL) patients; congruently, TAG/HDL-C ratios in AA was lower compared to MI (5.73 ± 1.29 vs. 10.5 ± 6.11) subjects. Within the [MD] group, TAG levels (109 ± 35 mg/dL) and TAG/HDL-C ratio (2.87 ± 1.16) of AA patients, which was lower when compared with the MM patients, but not MC or MI. The [NL] group showed many differences between the AA patients and the Malaysian.

In summary, the [D] group is typified by very TAG levels followed by low HDL-C levels. As the degree of dyslipidemic changes and it shifts into mixed dyslipidemia, the very high level of TAG declines, followed by an increase in HDL-C. A further shift from [MD] to [NL] is shown to change the TAG level into low to normal TAG levels, whereas HDL-C increases.

Table 4-6: Dyslipidemia in plasma lipids amongst ethnicities.

***Dyslipidemia [D]** Total (n = 127)	**AA** (n = 9)	**MM** (n =8)	**MC** (n = 7)	**MI** (n= 3)
TAG (mg/dL)	189 ± 42 [a]	231 ± 47 [ab]	212 ± 36 [ab]	314 ± 123 [b]
HDL-C (mg/dL)	33 ± 5	33 ± 4	35 ± 4	32 ± 6
TC (mg/dL)	202 ± 12	215 ± 28	209 ± 18	224 ± 26
LDL-C (mg/dL)	131 ± 17	136 ± 32	132 ± 20	128 ± 7
NonHDL (mg/dL)	169 ± 47	181 ± 27	173 ± 15	192 ± 29
TAG/HDL-C Ratio	5.73 ± 1.29 [a]	7.09 ± 2.05 [ab]	6.16 ± 1.78 [ab]	10.5 ± 6.11 [b]
TC/HDL-C Ratio	6.21 ± 1.33	6.51 ± 0.98	5.95 ± 0.43	7.20 ± 2.11
LDL/HDL-C Ratio	4.06 ± 1.24	4.10 ± 0.90	3.74 ± 0.33	4.07 ± 0.84
***Mixed Dyslipidemia [MD]** Total (n = 181)	(n = 55)	(n = 39)	(n= 56)	(n = 31)
TAG (mg/dL)	109 ± 35 [a]	154 ± 74 [b]	145 ± 68 [b]	124 ± 57 [ab]
HDL-C (mg/dL)	40 ± 12	40 ± 8	43 ± 10	40 ± 8
TC (mg/dL)	161 ± 44	164 ± 31	168 ± 37	164 ± 28
LDL-C (mg/dL)	99 ± 36	94 ± 25	97 ± 30	101 ± 22
NonHDL (mg/dL)	121 ± 38	124 ± 27	125 ± 31	125 ± 24
TAG/HDL-C Ratio	2.87 ± 1.16 [a]	4.13 ± 2.49 [b]	3.63 ± 2.12 [ab]	3.35 ± 1.96 [ab]
TC/HDL-C Ratio	4.11 ± 1.04	4.15 ± 0.72	3.95 ± 0.77	4.26 ± 0.81
LDL/HDL-C Ratio	2.55 ± 0.96	2.36 ± 0.52	2.25 ± 0.58	2.62 ± 0.62
***Normo-lipidemic [NL]** Total (n = 140)	(n = 59)	(n = 22)	(n = 47)	(n = 12)
TAG (mg/dL)	64 ± 26 [a]	89 ± 26 [b]	93 ± 30 [b]	79 ± 21 [ab]
HDL-C (mg/dL)	62 ± 18	52 ± 9	56 ± 16	55 ±9
TC (mg/dL)	128 ± 29 [a]	145 ± 18 [ab]	151 ± 24 [b]	144 ± 19 [ab]
LDL-C (mg/dL)	54 ± 22 [a]	76 ± 18 [b]	76 ± 17 [b]	74 ± 16 [b]
NonHDL (mg/dL)	66 ± 24 [a]	93 ± 20 [b]	95 ± 19 [b]	89 ± 18 [b]
TAG/HDL-C Ratio	1.13 ± 0.56 [a]	1.81 ± 0.75 [b]	1.78 ± 0.74 [b]	1.45 ± 0.37 [ab]
TC/HDL-C Ratio	2.16 ± 0.53 [a]	2.87 ± 0.57 [b]	2.82 ± 0.53 [b]	2.66 ± 0.48 [b]
LDL/HDL-C Ratio	0.94 ± 0.46 [a]	1.52 ± 0.48 [b]	1.46 ± 0.46 [b]	1.38 ± 0.46 [b]

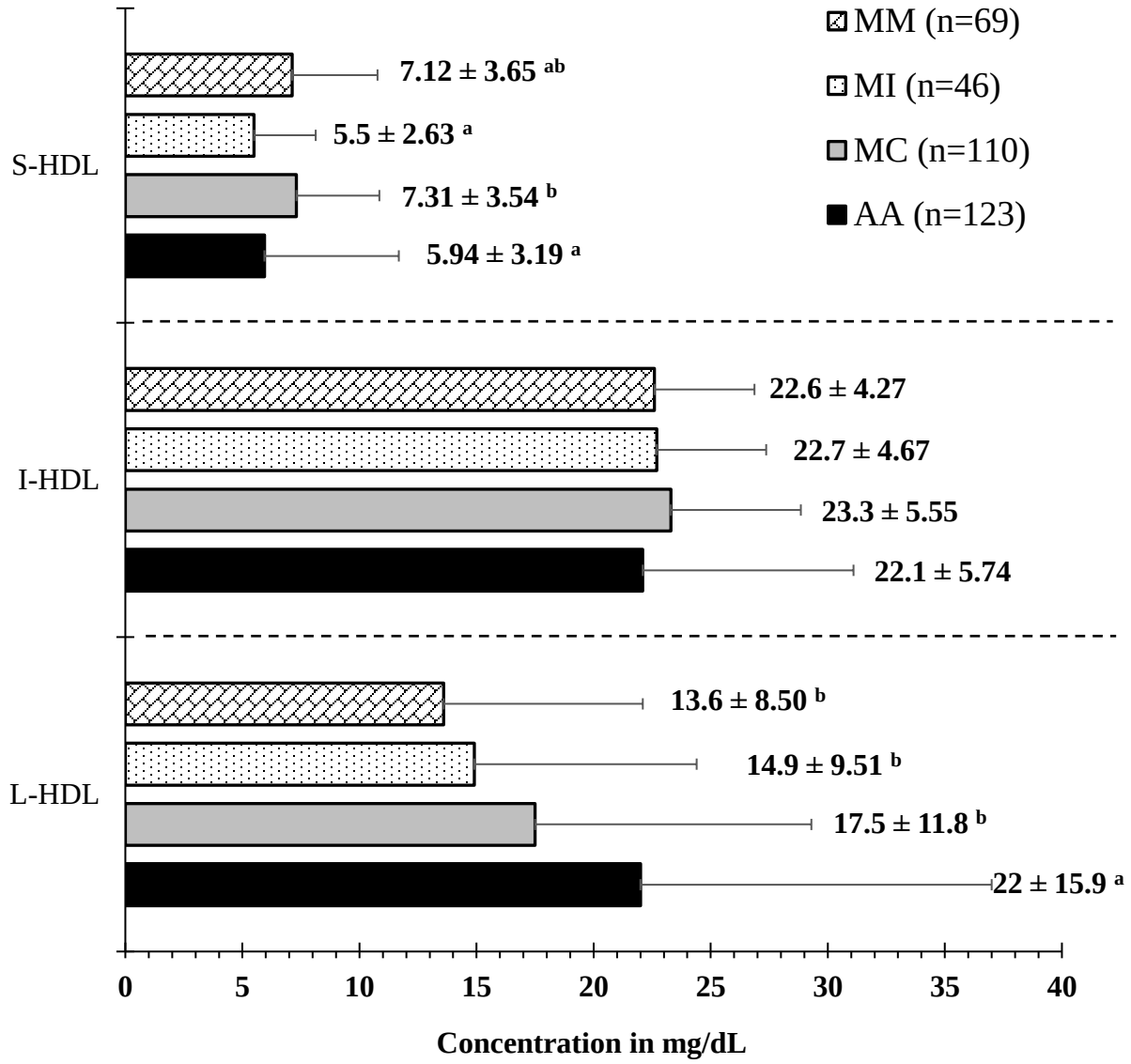
Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA. *****All lipid values, in the D group were significantly different from the corresponding values in MD and NL groups; similarly, there were differences between MD and NL groups. Lipid classification were NL: TAG < 150 mg/dL, HDL-C > 40 mg/dL and LDL-C < 100 m/dL; D: TAG > 150 gm/dL, HDL-C < 40 mg/dL and LDL-C >100 mg/dL; MD: at least one of the D and/or NL group.

Figure 4-2 shows the distribution of HDL lipoproteins measured in both US and Malaysian cohorts. AA patients had significantly higher L-HDL (22 ± 15.9 mg/dL) when compared to MC (17.5 ± 11.8 mg/dL), MI (14.9 ± 9.51 mg/dL), and MM (13.6 ± 8.50 mg/dL). No changes were seen in I-HDL subfraction, in all four ethnicities values were equally distributed. The S-HDL lipoprotein was significantly higher in MC patients (7.31 ± 3.54 mg/dL) when compared with AA (5.94 ± 3.19 mg/dL) and MI (5.5 ± 2.63 mg/dL) subjects. The analyses showed that most of shifting and remodeling of the HDL subfractions seems to reside between the large and small-HDL.

Figure 4-3 shows VLDL and IDL subfraction composition for both PATCH trials (total patients 348). Analyses showed that VLDL lipoproteins were significantly lower (25.4 ± 10.2 mg/dL) with respect to MI, (30.2 ± 8.60 mg/dL), as well as MM (33.8 ± 9.71 mg/dL) and MC (34.8 ± 10.1 mg/dL). IDL lipoproteins did not show any notable changes in the subfraction distribution and amongst the four ethnicities. It is worth noting marginal differences between IDL composition, which was higher in MI patients when comparing differences with MC subjects (50.3 ± 14.7 vs. 46.0 ± 13.4 mg/dL).

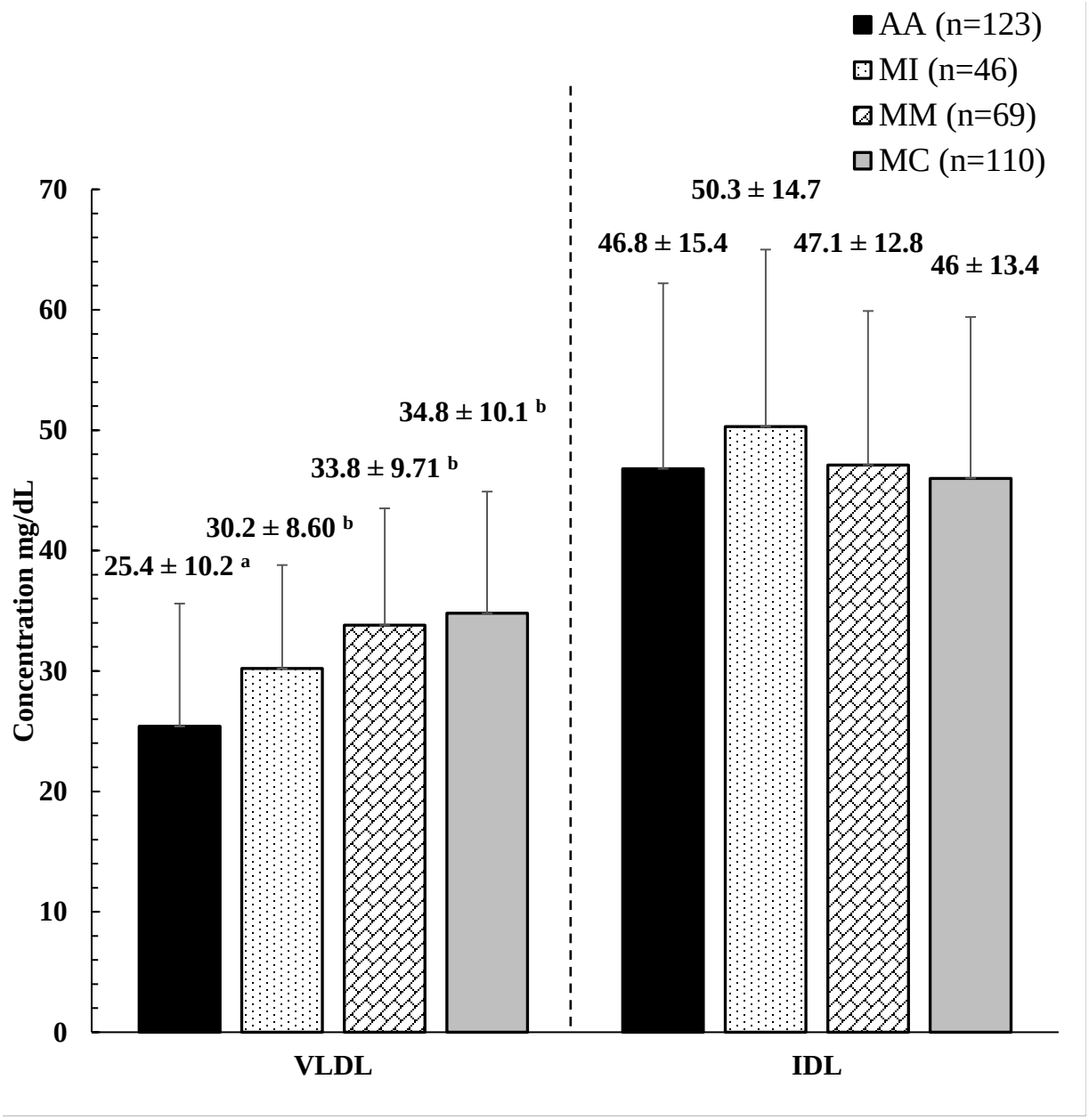
Figure 4-4 shows the overall LDL lipoprotein subfractions measurements amongst the ethnicities. The L-LDL subfraction is the predominant lipoprotein in the LDL lipoprotein analyses. The large buoyant LDL is considered protective with respect to the smaller dense LDL. Current data between the four ethnicities show no changes, and the L-LDL is equally represented. Ethnic MI had significant higher L-LDL content (25.6 ± 8.24 mg/dL), whereas the lowest resides with in AA patients (22.0 ± 15.9 mg/dL). No changes were recorded for the I-LDL, nor in the S-LDL. However, is worth noting, that MM had the highest content of S-LDL (5.41 ± 6.9 mg/dL) and lowest in AA, thus shows minor changes within the subfraction.

Figure 4-2: HDL subfractions composition amongst ethnicities.



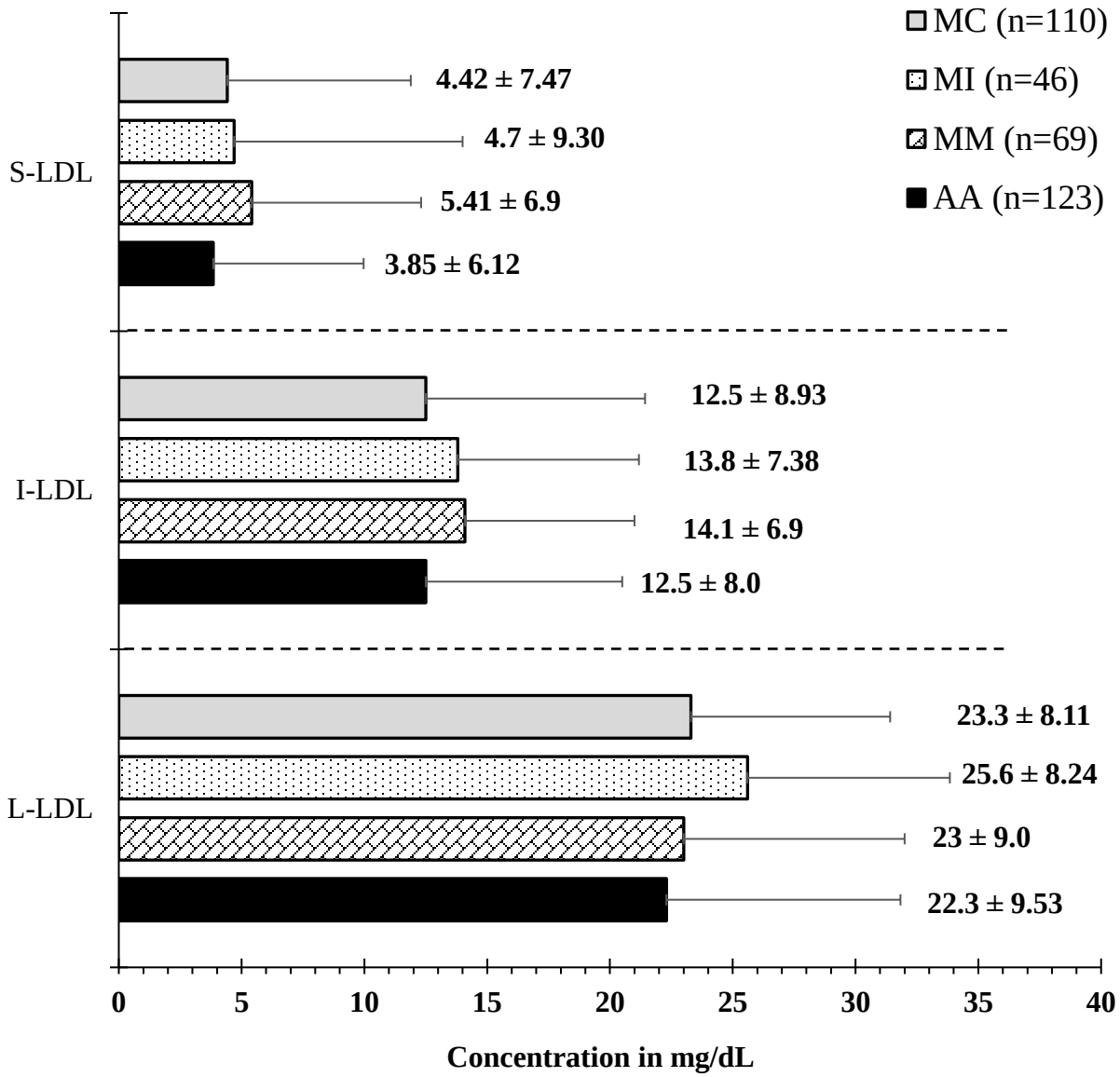
Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Figure 4-3: VLDL and IDL subfraction composition between ethnicities.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Figure 4-4: LDL lipoprotein subfraction composition between ethnicities.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA. **N.S.,** differences were observed between the LDL lipoproteins in the ethnic patients.

Figure 4-5 shows dyslipidemia of lipoprotein subfractions, for the collective cohort of 348 patients analyzed. The investigation found 8% which are [D] patients had significantly lower L-HDL (7.56 ± 3.6 mg/dL) and I-HDL (12.7 ± 7.44 mg/dL) while the S-HDL (8.11 ± 3.18 mg/dL) was higher content.. Additionally, [D] subjects had higher VLDL values (46.6 ± 10.1 mg/dL), as well as IDL (62.9 ± 11.7 mg/dL). The L-LDL, (25.6 ± 8.80 mg/dL) I-LDL (23.8 ± 7.85 mg/dL), and S-LDL (16.4 ± 11.3 mg/dL) were also higher in relation to [MD] and [NL] patients.

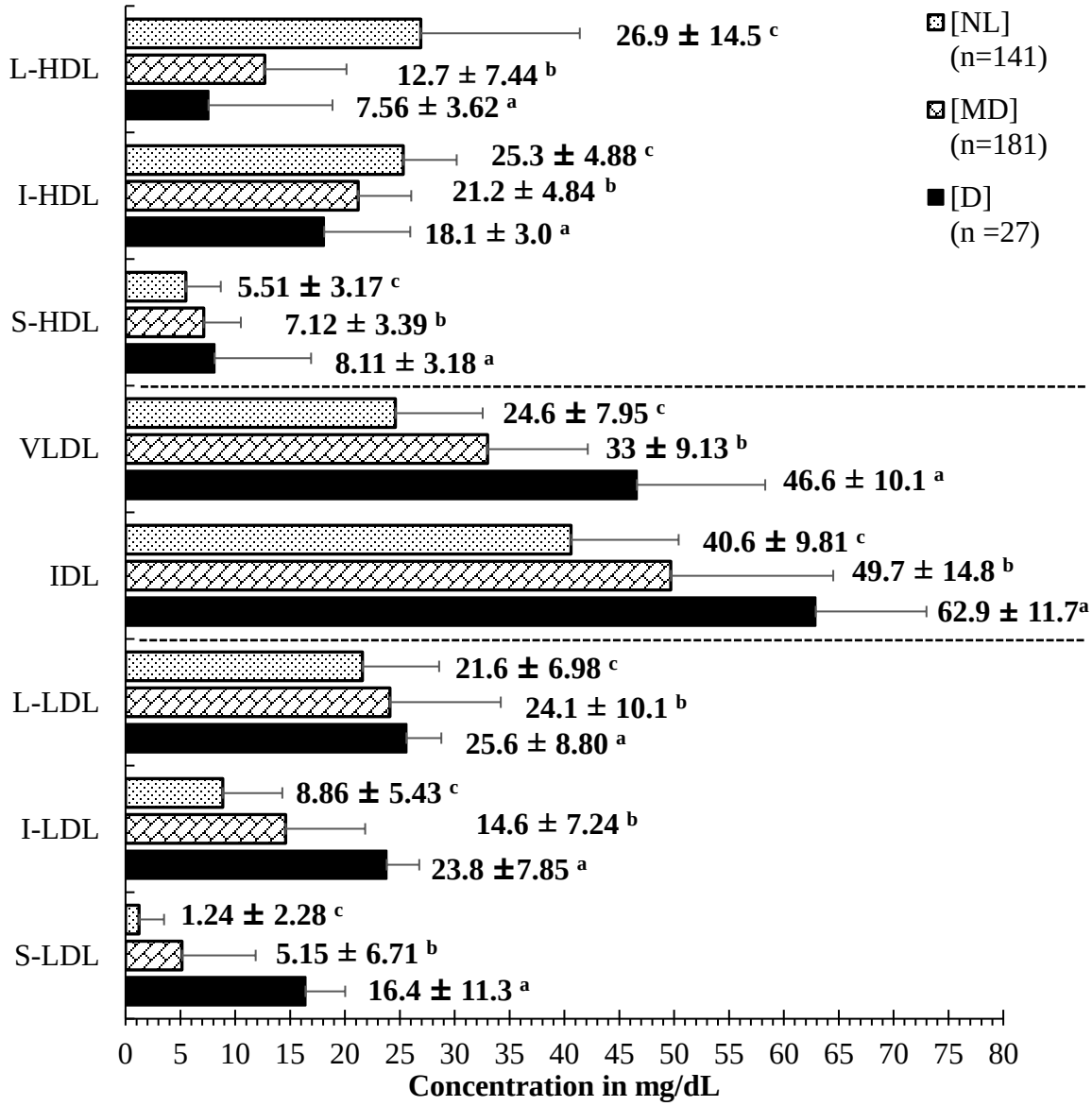
Table 4-7 shows the analyses of the dyslipidemic lipoproteins amongst ethnicity. Samples were separated into three groups [D-8%], [MD-52%], and [NL-40%], which allowed us to compare the subfractions for differences between the four ethnicities. All lipoprotein values, in the [D] group, were significantly different from the corresponding values in [MD and NL] groups; similarly, there were differences between [MD] and [NL] groups.

The key feature with the [D] group; as shown in all patients, irrespective of ethnicity, for which cause subject present a cohort where lower L-HDL, lower I-HDL, followed by the rise in the S-HDL is common. At the same time, [D] subjects are presented by higher VLDL and IDL, marginal difference in L-LDL (concerning MD and NL groups), higher I-LDL, and significantly higher S-LDL followed by a reduced LDL particle size.

Patients in the [D] group, had a greater IDL content, which was found to be higher in AA (71.0 ± 6.04 mg/dL) patients when compared to MM (56.4 ± 16.9 mg/dL), but not significant with MC and MI patients. In the [MD] group, I-HDL was higher in MI patients (22.5 ± 4.6 mg/dL) when compared to AA (19.7 ± 5.09 mg/dL); also, within [MD] group, MC patients (8.04 ± 3.62 mg/dL) had higher values of S-HDL when compared to MI patients (5.81 ± 2.06 mg/dL). VLDL was lower in the AA (28.8 ± 9.40 mg/dL) patients compared to MC (37.1 ± 9.03) as well MI (31.8 ± 6.35 mg/dL). Within the [NL] group, there are differences between AA (31.7 ± 16.3 mg/dL) who

present with a higher content of L-HDL concerning MM (20.0 ± 9.42 mg/dL) and MC (24.1 ± 13.3 mg/dL). VLDL values for [NL] group was also lower in AA (20.0 ± 6.55 mg/dL) patients in relation to MM and MC. MI patients had significantly higher L-LDL (26.1 ± 5.43 mg/dL) with respect to AA in the [NL] group.

Figure 4-5: Dyslipidemic in lipoprotein subfractions in the overall study cohort.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Table 4-7: Dyslipidemia in lipoprotein subfractions amongst ethnicities.

Lipoprotein Subfractions	AA	MM	MC	MI
[D] (n=27) [1]	(n = 9)	(n = 8)	(n = 7)	(n = 3)
L-HDL (mg/dL)	8.56 ± 3.84	6.75 ± 2.82	7.00 ± 3.96	8.00 ± 5.29
I-HDL (mg/dL)	17.4 ± 1.81	19.6 ± 2.87	18.0 ± 2.00	16.3 ± 6.66
S-HDL (mg/dL)	7.56 ± 1.88	7.13 ± 2.53	10.1 ± 3.29	7.67 ± 6.51
VLDL (mg/dL)	40.3 ± 11.2	48.6 ± 9.58	53.0 ± 4.08	45.3 ± 11.1
IDL (mg/dL)	71.0 ± 6.04[a]	56.4 ± 16.9 [b]	59.1 ± 5.93[ab]	65.0 ± 4.00 [ab]
L-LDL (mg/dL)	26.9 ± 8.57	26.4 ± 10.2	23.4 ± 9.95	24.3 ± 4.62
I-LDL (mg/dL)	20.8 ± 7.12	29.0 ± 8.82	20.6 ± 4.08	26.3 ± 9.02
S-LDL (mg/dL)	11.4 ± 13.5	17.4 ± 7.11	18.4 ± 9.85	23.7 ± 16.3
Mean LDL size (Å)	265.0 ± 6.18	262.1 ± 3.76	261.0 ± 5.23	259.0 ± 8.54
[MD] (n= 181) [1]	(n = 55)	(n = 39)	(n = 56)	(n = 31)
L-HDL (mg/dL)	13.8 ± 8.93	11.3 ± 6.29	13.2 ± 7.49	11.1 ± 5.31
I-HDL (mg/dL)	19.7 ± 5.09 [a]	21.5 ± 3.82 [ab]	21.9 ± 5.08 [ab]	22.5 ± 4.6 [b]
S-HDL (mg/dL)	6.64 ± 3.18 [ab]	7.54 ± 3.83 [ab]	8.04 ± 3.62 [a]	5.81 ± 2.06 [b]
VLDL (mg/dL)	28.8 ± 9.40 [a]	33.8 ± 8.14 [bc]	37.1 ± 9.03 [b]	31.8 ± 6.35 [c]
IDL (mg/dL)	51.4 ± 15.1	48.3 ± 13.0	47.8 ± 15.4	51.5 ± 15.4
L-LDL (mg/dL)	24.6 ± 10.6	22.6 ± 8.19	23.8 ± 11.2	25.5 ± 9.45
I-LDL (mg/dL)	15.8 ± 8.47	14.3 ± 6.53	13.7 ± 6.58	14.2 ± 6.89
S-LDL (mg/dL)	5.22 ± 5.83	5.46 ± 6.84	5.27 ± 6.59	4.42 ± 8.31
Mean LDL size (Å)	268.1 ± 4.45	267.6 ± 6.2	267.9 ± 5.74	269.3 ± 5.78
[NL] (n=140) [1]	(n = 59)	(n = 22)	(n = 47)	(n = 12)
L-HDL (mg/dL)	31.7 ± 16.3 [a]	20.0 ± 9.42 [b]	24.1 ± 13.3 [b]	26.5 ± 9.39 [ab]
I-HDL (mg/dL)	25.1 ± 5.16	25.5 ± 3.92	25.7 ± 5.39	24.6 ± 2.78
S-HDL (mg/dL)	5.05 ± 3.14	6.36 ± 3.67	6.02 ± 3.00	4.17 ± 2.37
VLDL (mg/dL)	20.0 ± 6.55 [a]	28.2 ± 6.19 [b]	29.3 ± 7.36 [b]	22.4 ± 5.62 [ab]
IDL (mg/dL)	38.7 ± 10.4	41.4 ± 7.61	41.8 ± 9.49	43.4 ± 11.3
L-LDL (mg/dL)	19.4 ± 7.74 [a]	22.5 ± 7.20 [ab]	22.6 ± 5.31 [ab]	26.1 ± 5.43 [b]
I-LDL (mg/dL)	8.17 ± 4.57	8.46 ± 6.12	9.72 ± 6.33	9.67 ± 4.07
S-LDL (mg/dL)	1.41 ± 2.26	0.96 ± 1.99	1.32 ± 2.62	0.67 ± 1.23
Mean LDL size (Å)	271.4 ± 3.60	272.4 ± 3.50	271.7 ± 3.46	272.3 ± 2.39

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.*****All lipoprotein values, in the [D] group were significantly different from the corresponding values in [MD and NL] groups; similarly, there were differences between [MD] and [NL] groups.

Figure 4-6 shows the effect of D on particle size and LDL phenotype. Analyses show that dyslipidemic [D] subjects are typified by a 63% chance in having a pattern “B” phenotype LDL particle. Type “B” has been associated with the smaller and dense LDL lipoproteins which have been linked as a high risk for mortality. Additionally, a type “B” has aa smaller particle size (258.9 ± 3.4Å). It is worth noting, that in whether [D] group, [MD], or [NL] the difference between the particle size is significant, mainly due to the association with the phenotype of the LDL particle. No major differences were observed within ethnicities. Analysis shows that, as the cohort shifts from [D] subjects (63% and a type “B”) into [MD] the phenotype shifts into a type “A” 56% also which is associated with higher particle size (272.0 ± 2.4 Å). A further shift in the cohort [NL] (type A becomes more prominent and has a higher chance of 84% with an increase in particle size (272.9 ± 2.2 Å).

Figure 4-6: Dyslipidemia in mean LDL particle size within ethnicities.

Phenotype	Particle (Å)	**AA**	**MM**	**MC**	**MI**
[D]	Total (n = 27) [1]	(n = 9)	(n =8)	(n = 7)	(n= 3)
A (n, %)	5 (18.5)	3 (33)	1 (13)	1 (14)	0 (-)
Particle Size	270.6 ± 0.9 [a]	270.7 ± 1.2	270.0 ± 0.0	271 ± 0.0	-
B	17 (63)	3 (33)	7 (88)	5 (71)	2 (67)
Particle Size	258.9 ± 3.4 [b]	257.7 ± 4.2	261.0 ± 2.2	258.2 ± 1.5	255.0 ± 7.1
AB	5 (18.5)	3 (33)	0 (-)	1 (14)	1 (33)
Particle Size	266.4 ± 1.1 [c]	266.7 ± 1.2	-	265.0 ± 0	267.0 ± 0.0
[MD]	Total (n = 181) [1]	(n = 55)	(n = 39)	(n= 56)	(n = 31)
A (n, %)	101 (56)	29 (53)	22 (56)	30 (54)	21 (68)
Particle Size	272.0 ± 2.4 [a]	271.6 ± 2.2	271.7 ± 2.7	272.4 ± 2.2	272.1 ± 2.6
B	47 (26)	13 (24)	13 (33)	15 (27)	6 (19)
Particle Size	260.9 ± 3.8 [b]	262.0 ± 2.2	260.8 ± 4.5	260.1 ± 2.8	260.7 ± 6.8
AB	33 (18)	13 (24)	4 (10)	11 (20)	4 (13)
Particle Size	266.7 ± 0.8 [c]	266.5 ± 1.1	267.3 ± 0.5	266.5 ± 0.5	267.0 ± 0.8
[NL]	Total (n = 140) [1]	(n = 59)	(n = 22)	(n = 47)	(n = 12)
A (n, %)	117 (84)	48 (81)	19 (86)	39 (83)	11 (92)
Particle Size	272.9 ± 2.2 [a]	272.7 ± 2.2	273.5 ± 2.3	272.9 ± 2.4	272.8 ± 1.8
B	7 (5)	3 (5)	2 (9)	2 (4)	0
Particle Size	263.1 ± 1.9 [b]	262.0 ± 2.0	265.0 ± 0.0	263.0 ± 1.4	-
AB	16 (11)	8 (14)	1 (5)	6 (13)	1 (8)
Particle Size	267.1 ± 0.93 [c]	267.0 ± 0.9	267.0 ± 0.0	267.2 ± 1.2	267.0 ± 0.0

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given column with different superscripts[abc] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

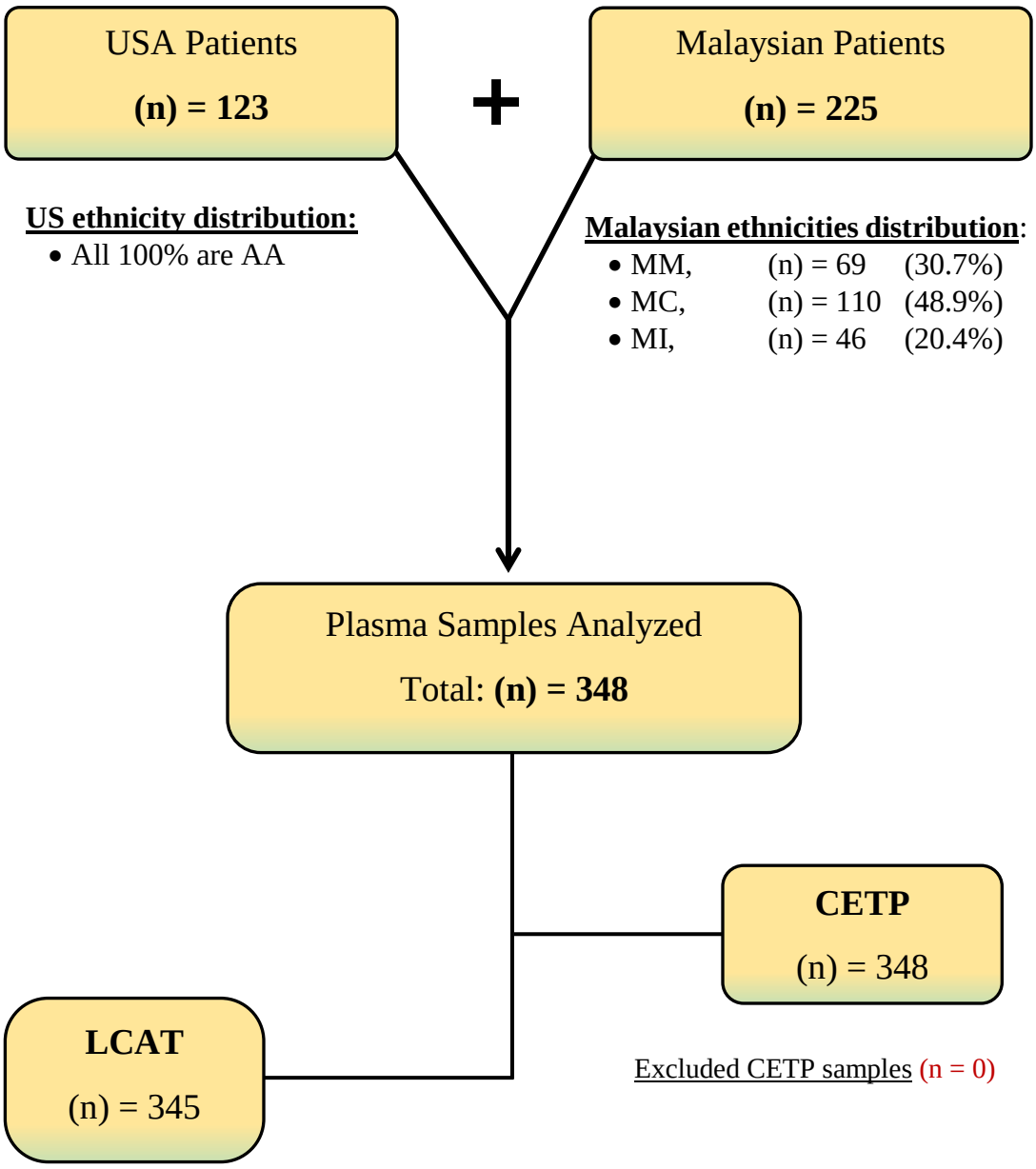
CHAPTER 5: RESULTS – SPECIFIC AIM 3: TO DOCUMENT THE EXTENT TO WHICH HDL ENZYMES CONTRIBUTE TO DYSLIPIDEMIA IN MHD PATIENTS.

The study flow is shown in **Figure 5-1.** The study was the collective analysis of RCT enzymes CETP and LCAT. Samples from the PATCH (USA) (n = 123), additionally to the PATCH Malaysia (n = 225), were used. From n =348 samples, three were excluded during LCAT measurements (samples were beyond the detection range). Since this investigation was an extension of subset inquiry from the joint PATCH studies, no additional approvals were required.

Figure 5-2 shows the CETP activity measured in 348 participating patients. Analyses showed that the mean collective cohort for CETP activity was (41.0 ± 18.6 nmol/mL/hr). Results showed that MC subjects (35.8 ± 17.0 nmol/mL/hrs.) had a significantly lower CETP activity than MM (45.8 ± 19 nmol/mL/hrs.) and MI (44.7 ± 18.9 nmol/mL/hrs.) patients. No difference was recorded amongst AA (41.6 ± 18.7 nmol/mL/hrs.) patients and the other ethnicities. CETP is known to facilitate the exchange between CE and TAG amongst HDL and VLDL/LDL particles, and within this study, we recorded a strong inverse correlation ($r = -0.201$) ($p<0.001$) between CETP activity and TAG levels (**Appendix Figure 2**).

Figure 5-3. CETP activity was analyzed amongst ethnic patients. To examine the effect of D on CETP activity, we separated data into two groups $\geq$ or $<$ 3.8 TAG/HDL ratio. Analyses showed that AA patients had significantly lower CETP activity in subjects allocated into the $\geq$ 3.8 TAG/HDL-C ratio (32.1 ± 14.0 vs. 43.1 ± 19.0 nmol/mL/hrs.) in relation to AA patients with < 3.8 TAG/HDL-C ratio. No other differences were recorded amongst MM, MC and MI patients. Additional analyses found a correlation between CETP and TAG/HDL-C ratio, which was noted to be a strong inverse association ($r = -0.153$) ($p<0.004$) (**Appendix Figure 3**).

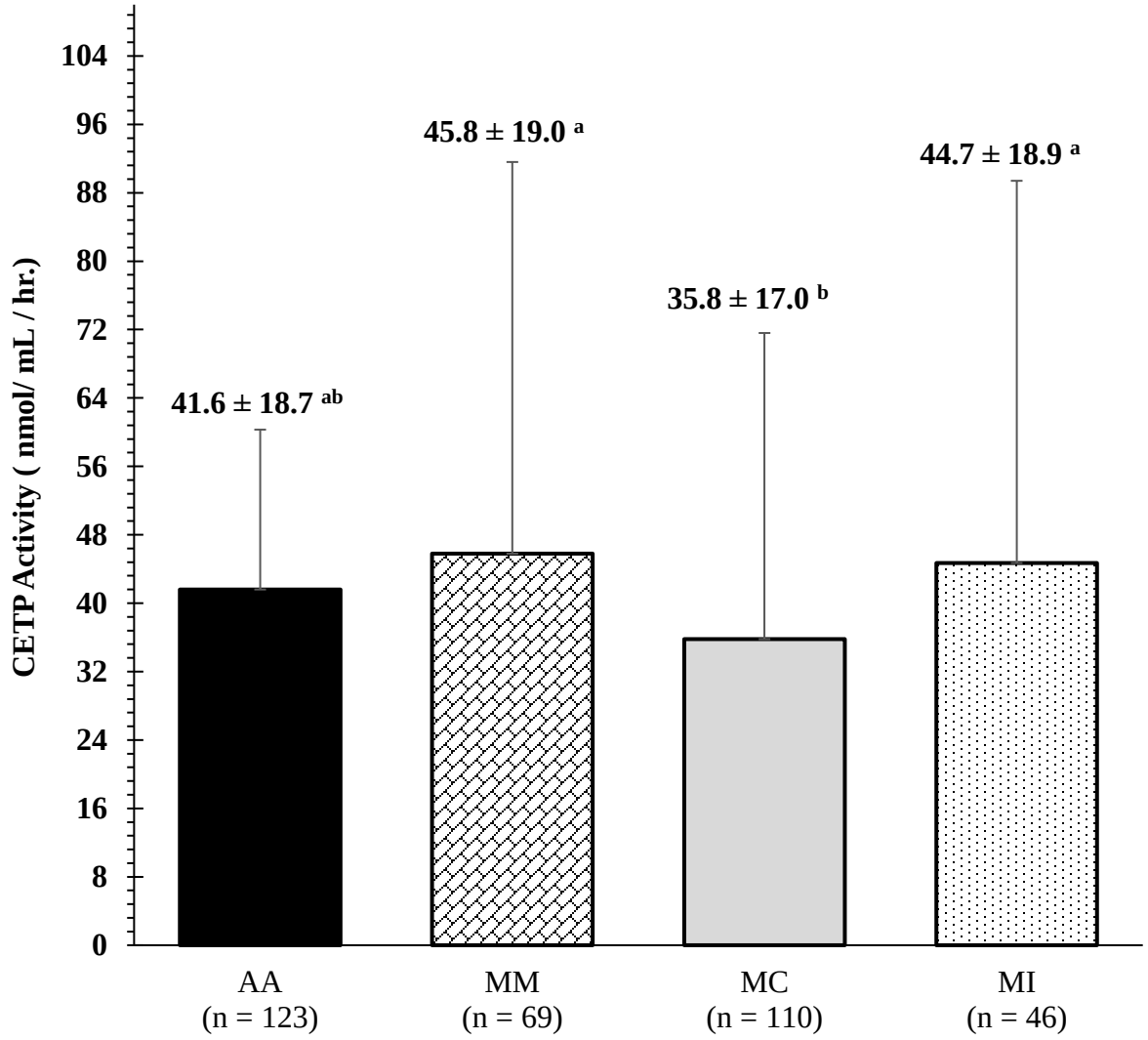
Figure 5-1: Aim 3 study flow.



Excluded LCAT samples (n =3) (Beyond the detection range)

- (1) A.A., patient – US cohort (n = 122)
- (1) Mal., patient – Malaysian cohort (n = 68)
- (1) Chin., patient – Malaysian cohort (n = 109
- (0) Ind., patients – Malaysian cohort (n = 46)

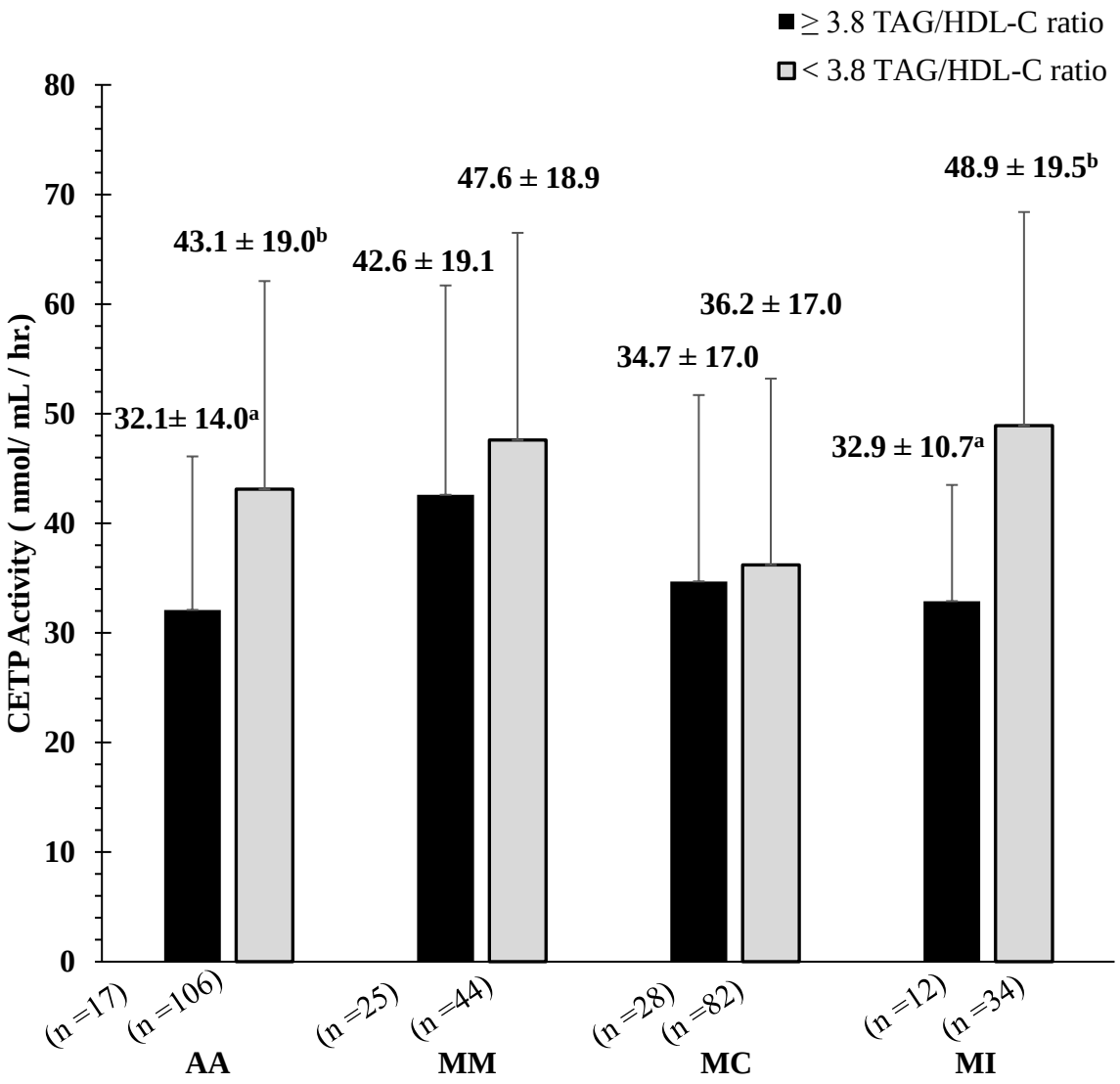
Figure 5-2: CETP activity amongst the ethnicities in the study cohort.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

Average CETP activity for all (n=348) patients: 41.0 ± 18.6 nmol/mL/hr.

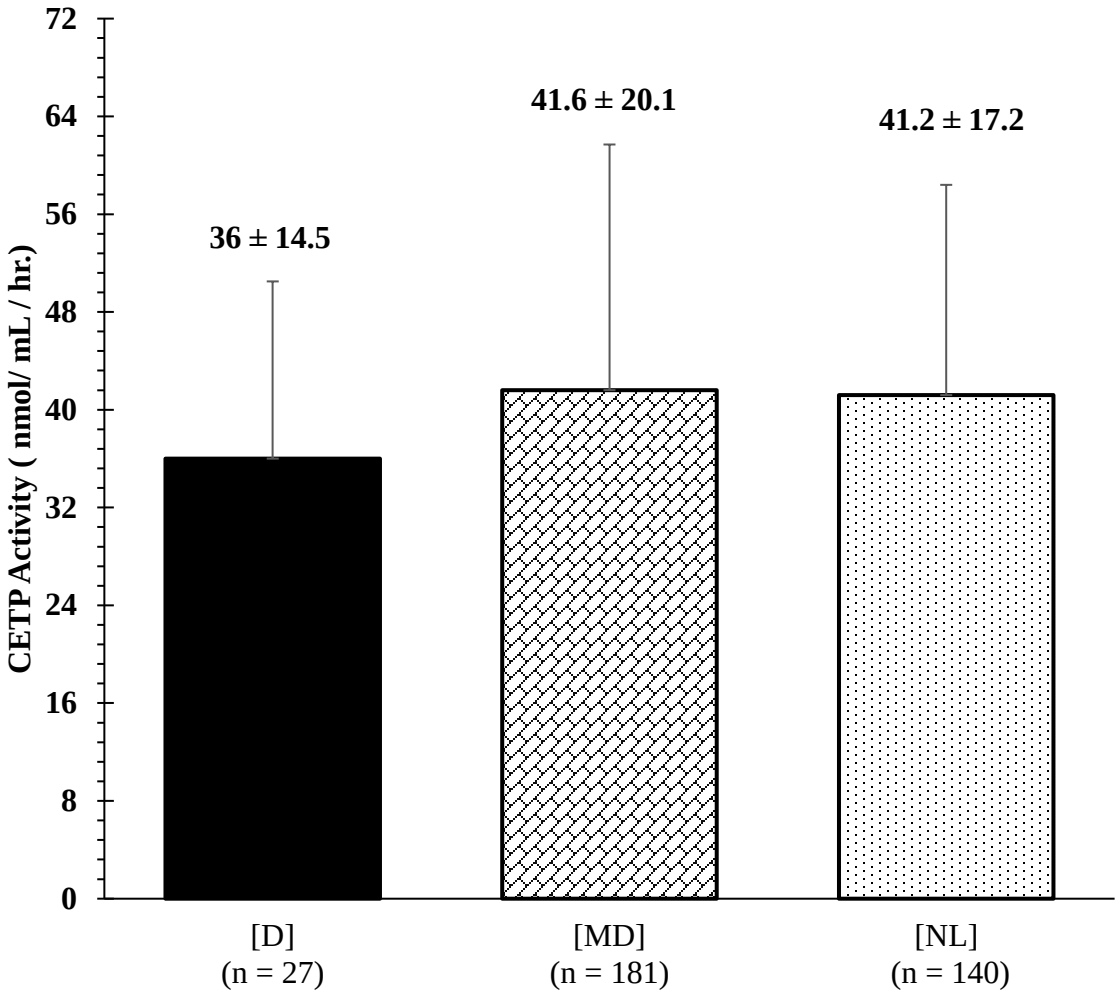
Figure 5-3: CETP activity based on categorical TAG/HDL-C ratio (≥ or < 3.8).



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

Figure 5-4 shows CETP activity analyzed based on the dyslipidemic criteria. Results showed that [D] (36.0 ± 14.5 nmol/mL/hrs.), [MD] (41.6 ± 20.1 nmol/mL/hrs.) and [NL] (41.2 ± 17.2 nmol/mL/hrs.) patients showed no differences in the activity.

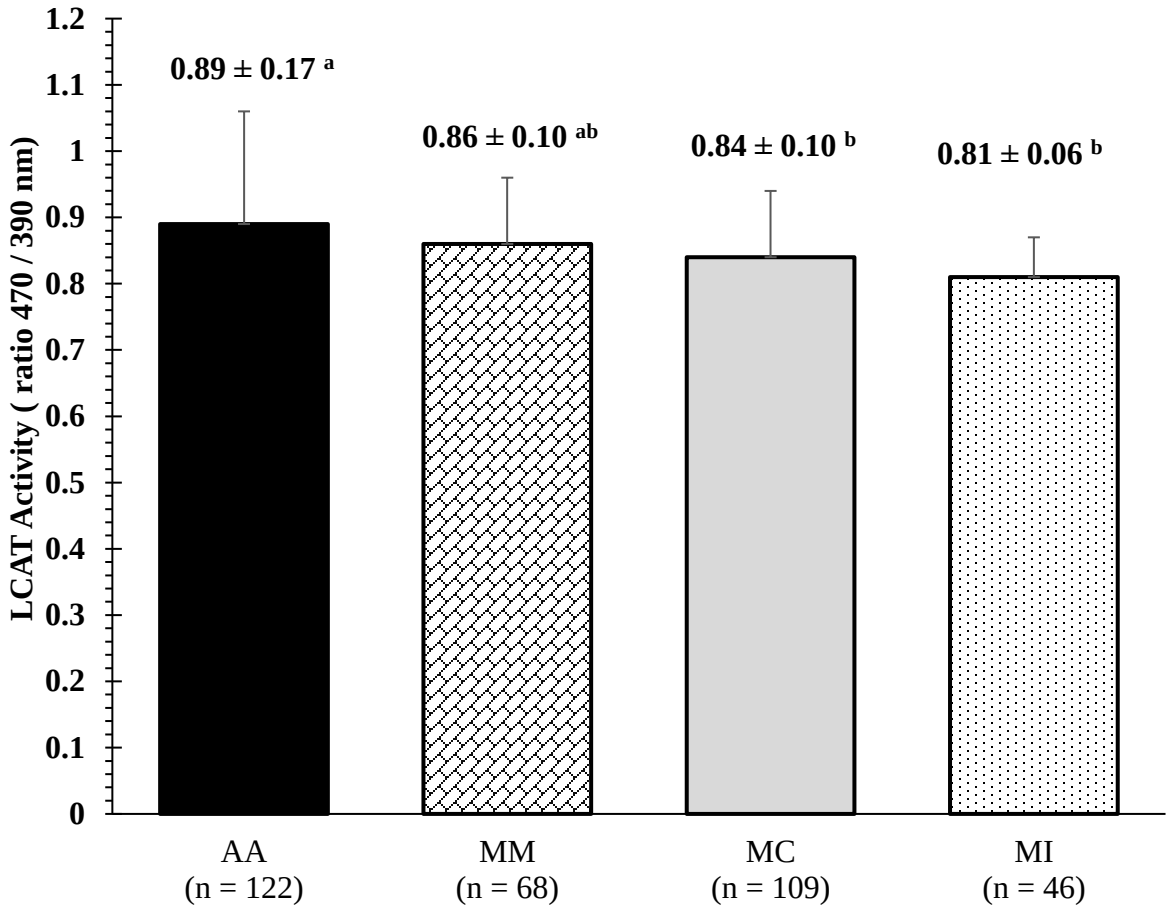
Figure 5-4: CETP activity between dyslipidemic groups.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA. Classification: [NL]: TAG < 150 mg/dL, HDL-C > 40 mg/dL and LDL-C < 100 m/dL; [D]: TAG > 150 gm/dL, HDL-C < 40 mg/dL and LDL-C >100 mg/dL; [MD]: at least one of D and /orNL group.

Figure 5-5 shows LCAT activity amongst the four ethnicities. In a collective cohort of 345 patients, the mean average LCAT activity was (0.86 ± 0.13 ratio of 470/390 nm). Analyses showed, that AA patients had significantly higher LCAT activity (0.89 ± 0.17 ratio of 470/390 nm) when compared to MC (0.84 ± 0.10 ratio of 470/390 nm) subjects and MI patients (0.81 ± 0.06 ratio of 470/390 nm.)

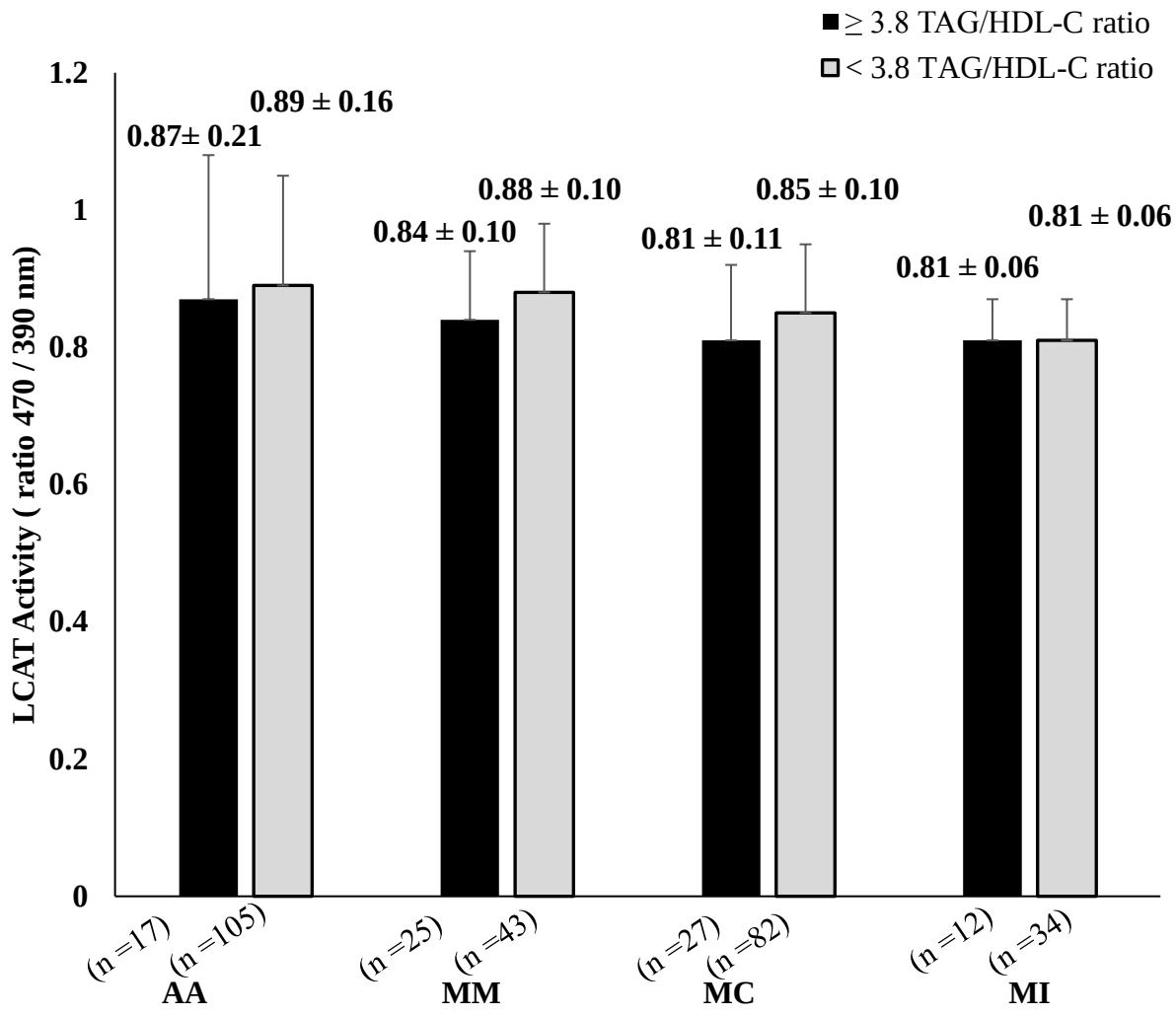
Figure 5-5: LCAT activity between the ethnicities.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA. Total of (n = 345) MHD plasma samples were analyzed, for a cohort average of (0.86 ± 0.13 ratio of 470/390 nm)

Figure 5-6: shows LCAT activity measured between the four ethnic patients. Analyzed samples were separated into two groups ≥ or < 3.8 TAG/HDL ratios. Results showed that there were no differences between the ethnicities. Additionally, a correlation between LCAT activity and TAG levels was conducted, (r = -0141) (p<0.009) (**Appendix Figure 4**).

Figure 5-6: LCAT activity based on the categorical TAG/HDL-C ratio (≥ or < 3.8).

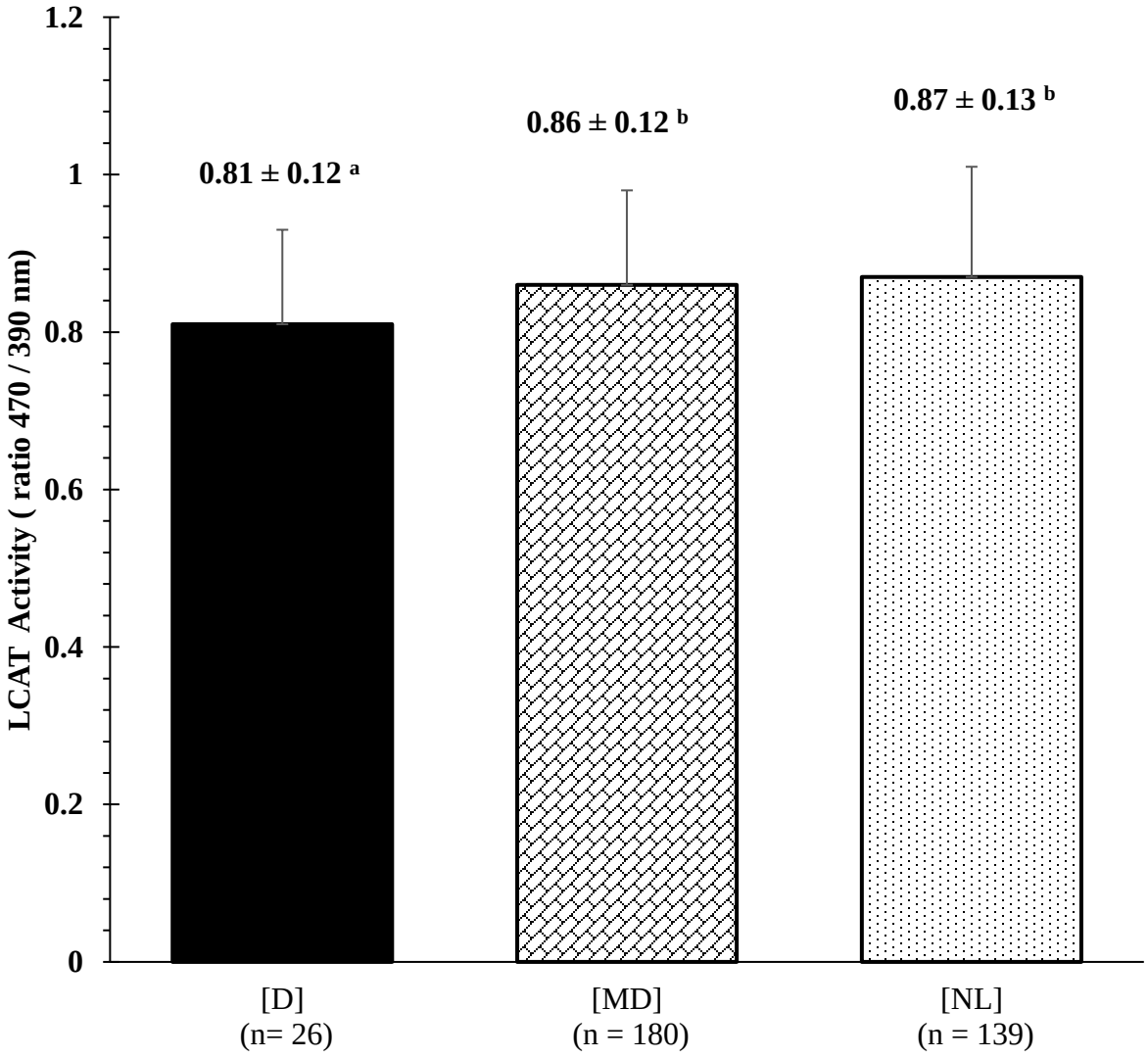


Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

NS, values were recorded between LCAT activities amongst ethnicities.

Figure 5-7 shows the analyses of LCAT activity between D on the bases of [D, MD, NL]. We noted that LCAT activity was significantly lower in [D] patient (0.81 ± 0.12 ratio of 470/390 nm) when compared against [MD] (0.86 ± 0.12 ratio of 470/390 nm) and [NL] (0.87 ± 0.12 470/390 nm).

Figure 5-7: LCAT activity between dyslipidemic groups.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA.

Figure 5-8 shows the overall summary of samples analyzed in the investigation including CRP (n = 324) and AGE-CML, a subset from the AA cohort (n = 59). In the CRP analyses, while the complete set was measured, n = 24 samples were excluded (plasma samples were beyond the detection range). Similarly, n = 1 in the AGE-CML analyses was excluded due to the sample being beyond the detection range.

Figure 5-8: Overall summary of the samples measured in the overall investigation.

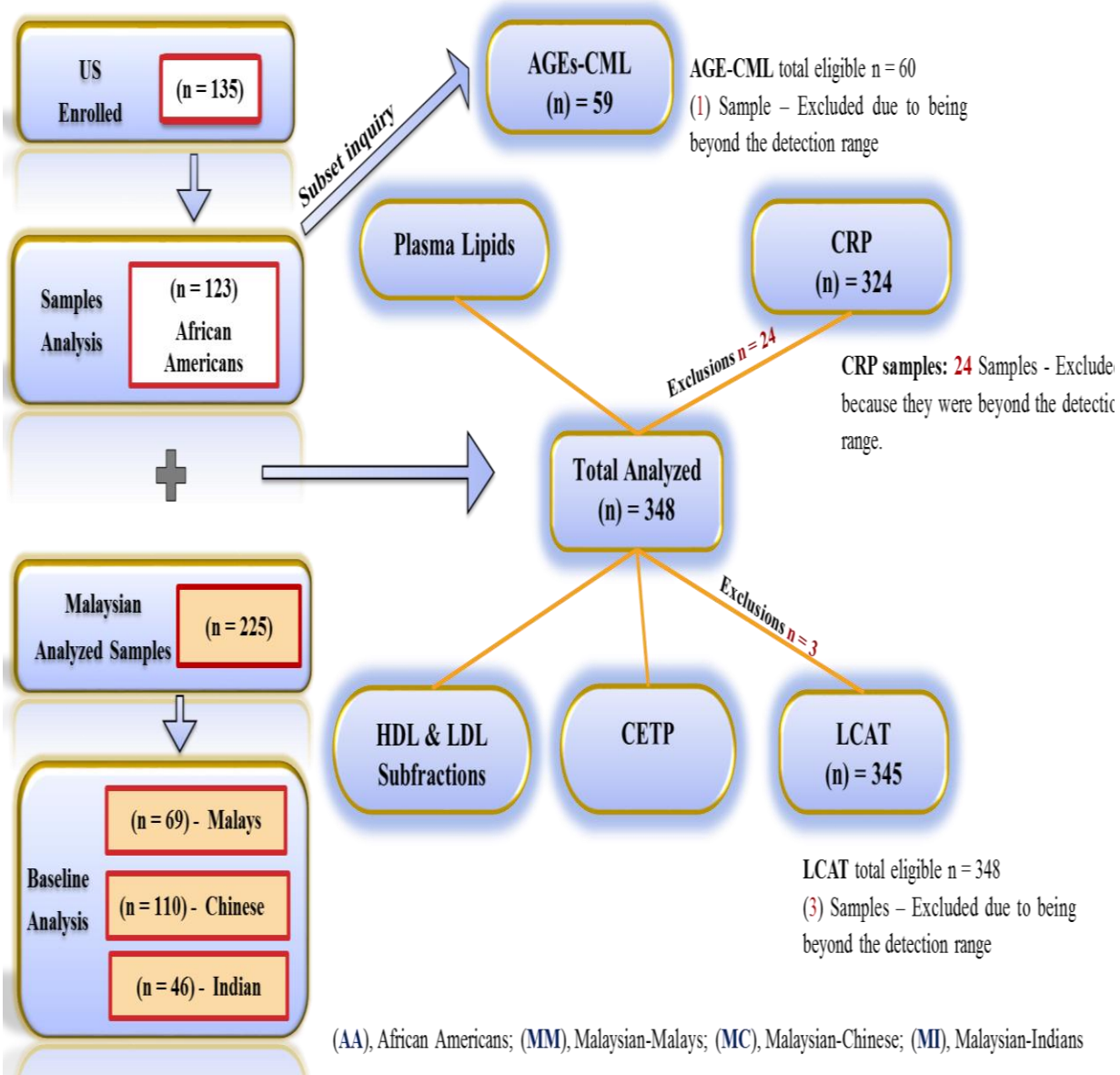
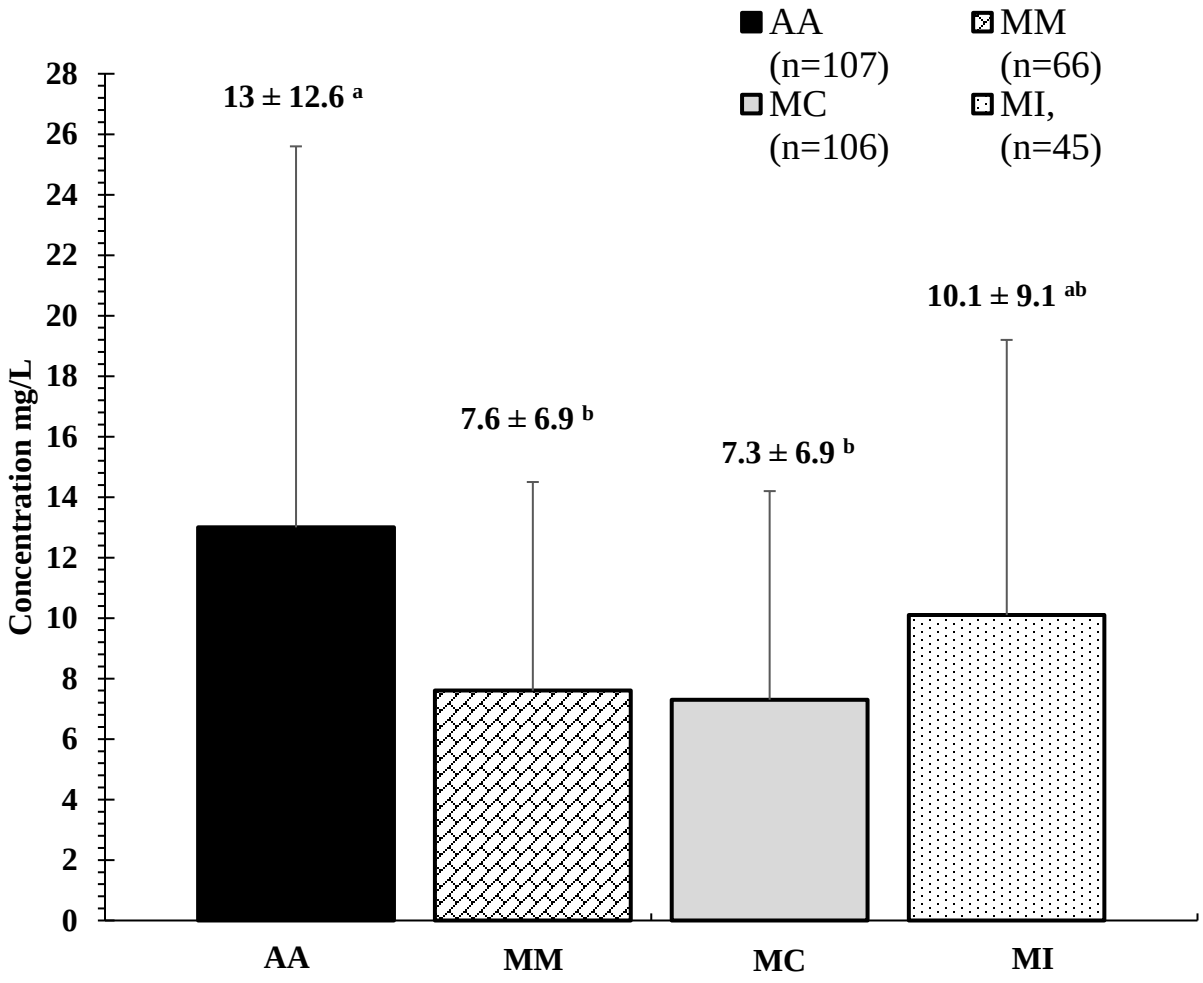


Figure 5-9 shows, analyses of the baseline CRP for the collective PATCH trial. The study measured (n = 324) plasma samples. The analysis showed elevated CRP levels in both cohorts for an average mean 9.61 ± 9.72 mg/L. CRP concentration amongst ethnicities was noted to be statistically higher in AA patients (13.0 ± 12.6 mg/L) when compared to MM (7.6 ± 6.9 mg/L), and MC (7.3 ± 6.9 mg/L), however not the MI (10.1 ± 9.1 mg/L) patients.

Figure 5-9: C-reactive protein levels between the ethnicities.



Values are mean ± SD. Values with different superscripts[ab] were significantly different from each other ($p < 0.05$) using one-way ANOVA. Overall study CRP plasma levels for (n) = 324 subjects at baseline: 9.61 ± 9.72 mg/L. Data were adjusted using ANCOVA, for the covariate factor such as BMI.

Table 3-13 shows the analyses of advanced glycation products. AGEs-CML were measured in the plasma from a subset of AA patients (n = 59). The overall analyses measured an average AGE-CML concentration of 4.2 ± 2.4 ng/ml. There were no differences in AGE-CML amongst gender groups; no difference were observed in the DM versus non-DM group. The lipid ratios of TAG/HDL-C ratios and [D, MD, NL], also recorded no significant change amongst the groups in AGE-CML levels.

Table 5-1: AGEs-CML analyses in the US cohort.

Parameter	**AGEs – CML (ng/mL)** (n = 59)[1]	**P-values**
Total concentration levels	4.2 ± 2.4	-
Men (n = 37)	4.5 ± 2.4	
Female (n =22)	3.6 ± 2.2	0.134
DM (n = 40)	4.3 ± 2.6	
Non-DM (n =19)	3.9 ± 1.9	0.584
TAG/HDL-C ratio ≥ 3.8 (n=12)	4.2 ± 2.2	
TAG/HDL-C ratio < 3.8 (12 = 47)	4.2 ± 2.5	0.972
[D] (n = 6)	4.7 ± 2.7	
[MD] (n = 31)	3.6 ± 2.1	
[NL] (n = 22)	4.7 ± 2.6	0.217

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given group with different superscripts[ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA. Type-II DM patients had higher CML in the plasma (4.3 ± 2.6 ng/mL) vs. Non-DM (3.9 ± 1.9 ng/mL) and higher CRP (14.2 ± 13.7 mg/L vs 11.9 ± 10.9 mg/L), but, the patterns did not show a correlation based on Person R which recorded a p=-0.107. Abbreviation: AGEs-CML, Advanced glycation end-products- Nε-carboxymethyl-lysine

Additional analyses and results

Appendix B Figure 1 shows AGE-CML plasma analyses in relation to BMI. It was observed that lower BMI had higher CML levels (6.37 ± 0.61 ng/mL). As BMI increased, CML levels declined gradually, with a higher BMI of ≥45 having the lowest CML levels (0.92 ± 0.5 ng/ML) in plasma. These findings were not significant, and the sample size was not large.

Appendix B Figure 2 shows the percent risk fact and the atherogenic index were evaluated for the collective cohort. Data showed that AA patients were at a higher risk (23%) when compared to MM, MC, and MI. The findings were compared with the atherogenic index of plasma, in which case, MM, MC, and MI patients of the Malaysian cohort were at higher risk and not AA. These two contradictory findings are in dispute and need to be further evaluated on a future study when mortality rate data is available.

Appendix B Figure 3 shows the analyses of CRP in relation to dyslipidemia. Data shows a decreasing trend from [D] to [MD] and [NL]; however, these changes did not achieve significance.

Appendix B Figure 4 shows the analyses of CRP amongst the four ethnicities, when analyzed by TAG/HDL-C ratio ≥ or < 3.8. No significant differences were observed between the two groups within the respective ethnicities. However, a trend is seen, in which case, MHD patients allocated in the ≥ 3.8 ratio group had higher CRP levels when compared to their counterparts of the < 3.8 ratio group.

Appendix B Table 1 shows the demographics of the collective cohort in the US and Malaysian cohort. Noted differences are MM patients have are significantly younger than AA, MC, and MI. Roughly 83% of the collective cohort are non-smokers, 48% of the combined studies have DM, and 85% are hypertensive. While in the US, many physicians have begun to scale back

on prescribing statin since various studies have found that LDL is not a primary issue in ESRD patients; in Malaysia statin use is still considered. BMI was significantly different due to AA patients being obsessed with a BMI of 30, whereas the Malaysians had a lower BMI of 25. Other differences were observed in albumin concentration, systolic B/P and Kt/V.

Appendix B Table 2 shows the lipoprotein composition between gender and differences between ethnicities. Analyses show that females overall have higher L-HDL, I-HDL, and S-HDL, in addition to VLDL and IDL subfractions. Males have a higher composition of the S-LDL.

Appendix B Table 3 shows lipid analyses in statin versus the non-statin group. The statin group overall has higher levels of TAG (129 ± 72 vs. 105 ± 52 mg/dL) and higher nonHDL cholesterol. Analyses show differences between the ethnicities within the statin or non-statin groups; of which the differences are observed between AA patients and the Malaysian cohort.

Appendix B Table 4 shows lipoprotein subfraction analyses composition with respect to statin and non-stain use. No significant difference due to statin use was observed in lipoprotein analyses. Only differences shown are between ethnicities in either the statin and/or nonstatin group; thus, the use of statin is not a factor in subfraction composition.

CHAPTER 6: DISCUSSION

The US is the most industrialize and developed nation in the world, although, despite the advancements in science and medicine in the past 20 years has yet to provide answers on how to slow the development and progression of diseases such as CKD and ESRD. More disappointing is the fact that both diseases CKD and ESRD, cost of care are on the rise and expected to reach $100 billion ($34 billion just for ESRD) [126], which is an unsustainable development. Moreover, this burden is shared with similar parallels across the world. Malaysia, one of the fastest developing countries, composed of multiple ethnicities (Malays, Chinese, Indian), has had an upsurge in the incidence of ESRD due to DM nephropathy, which has nearly reached a seven-fold increase and continue to rise [221, 222]. Moreover, progression into ESRD has been increasing at an estimated rate of 6-9%, which as of late has catapulted the country in the 8th place as industrialized nations, which will face the peril of the disease, and economic burden [16].

Moreover, the significant increases in CVD and the associated mortality within the population, cannot be explained by the traditional risk factors such as elevated LDL-C serum and low levels of HDL-C [78]. Usually, ESRD patients present with declined HDL-C and increases in TAG levels [223]. However, these patients are affected by a phenomenon known as “reverse epidemiology,” in which parameters such as high HDL-C and low LDL-C plasma concentration may not be as protected when compared with their healthy counterpart [81]. Moreover, both HDL and LDL lipoproteins consist of multiple subfractions, composed of different sizes, different densities, that appear to exhibit different metabolic behaviors. Numerous studies have indicated that the quality of these subfractions was maybe more important than quantity, which leads us to believe in the possibility of lipoprotein subfraction dysfunction as a major issue [224, 225]. HDL in specific has been an area of interest due to the anti-inflammatory and antioxidant properties it

exhibits. Thus, many studies and clinical interventions have focused on raising the HDL with inconclusive results. It has been suggested that dysfunctional HDL is caused by exposure to prolonged inflammation and oxidative stress; moreover, this may mitigate cholesterol efflux in ESRD patients. [65]. Numerous investigations have also looked at the RCT pathway, and the associated enzyme CETP, LCAT, or LPL, but studies have had inconclusive results.

ApoA-I has been another area of investigation, since it is the most abundant protein on the HDL particles, wherein various reports have shown a decrease in ApoA-I concentration, which has been linked with the downregulation of the ApoA-I gene [66, 226]. ESRD is a multifaceted process, which involves various confounding factors over time. The patients experience a series of abnormal disorders such as inflammation, OS, D, malnutrition, and endothelial dysfunction. D is primarily the result of decreased HDL and increased TAG levels, followed by normal and/or increased LDL. However, the contribution of each of these parameters to D is poorly understood; moreover, the contribution of the following parameters to D in different ethnicities is unknown. Additionally, ESRD patients present with the accumulation of oxidized lipids, lipoproteins followed with low plasma HDL-C along with impaired HDL maturation and function [78].

This investigation was developed in order to shed light and understand the complexity of inflammation and D in a group of multi-ethnic cohorts of MHD patients. Subjects in this study are part of the “PATCH” USA, and Malaysia, where patients were provided with a TRF supplement or placebo for the duration of 12-months. Specifically, this dissertation focuses on the baseline analyses of the multi-ethnic MHD patients, with the primary objective of characterizing and describing dyslipidemia through lipids, lipoproteins, reverse cholesterol transport key enzymes, and other markers of inflammation and or OS.

The central hypothesis of this study is that dyslipidemia amongst MHD patients of different ethnicities will reflect underlying alterations in lipoproteins and enzymes of lipid metabolism. The rationale for the proposed study is that once a dyslipidemic MHD profile is determined, interventions could be used to manage dyslipidemia.

The study dissertation is composed of four ethnicities, AA, MM, MC, and MI, with an average age of 56 ± 13 years old. Age was noted to be significantly higher (older) in AA patients compared to their counterparts. The majority of the MHD cohort is composed of 61%, males, whereas 39% are females. Collectively US and Malaysian cohort was more hypertensive 85% and 48% DM. This was a surprising finding in this study since the leading cause, and the most prominent contributor to disease development is primarily DM followed by HTN. Another confounding factor was BMI. Analyses showed a difference between the ethnicities, with AA having the highest BMI. At 30.0 ± 7.5 kg/m^2, it is classified as obese, whereas the Malaysian cohort had a BMI of 25 ± 4.1 kg/m^2 which is overweight. To the generally healthy population, a higher BMI has been linked to being unhealthy and prone to disease. However, this is not the case for MHD population; a high BMI considered is protective due to the obesity paradox in the context of reverse epidemiology [227, 228]. An investigation by Kalantar-Zadeh et al (2014) postulated the U-shaped BMI, in CKD patients between <25 kg/m^2 and/or ≥ 35 kg/m^2 were associated with worse outcomes in all patients; this happens to be independent of severity of CKD [229], thus based on the study cohort, AA subjects may be more protected than their Malaysian counterparts.

Lipids in the AA and the Malaysian cohort were recorded to be within the normal range: TAG <150 mg/dL, HDL-C was greater 40-60, TC < 200 and, LDL-C <100mg/dL. Nonetheless, a difference between the two cohorts was recorded. Results showed AA patients having different values in lipids when compared with MM, MC, and MI.

The lipid profile for AA subjects was characterized by significantly lower TAG, TC, LDL-C, NonHDL, TAG/HDL ratio, TC/HDL ratio, LDL/HDL ratio, and higher HDL-C when compared with the Malaysian patients. Furthermore, the study did not observe any differences within the Malaysian ethnicities. Other lipid analyses based on the genders, showed that irrespective of the ethnicity, females overall had higher TAG levels compared to men. Women also had higher HDL-C, higher TC, LDL-C, NonHDL, in addition to the lipid ratios. Dyslipidemia was also assessed amongst the complete cohort (n=348) and ethnicities based on the TAG/HDL-C ratio ($\geq$ or $<$ 3.8). The $\geq$ 3.8 TAG/HDL-C ratio group, was significantly higher in TAG, TC, LDL-C, NonHDL, and lower HDL-C lipid values. Similar results were attained when dyslipidemic grouping ($\geq$ 3.8) were analyzed for the four ethnicities AA, MM, MC and MI. Moreover, another investigator Moradi et al. (2014) [83], applied the lipid ratios, obtaining similar results in lipid profiles.

Characterizing HDL and LDL lipoprotein subfractions distribution was one of the primary goals of this investigation. However, unlike lipid analyses, data on lipoproteins and their distribution, especially in a multiethnic cohort of MHD patients, lacks results to compare findings, in addition to uncertainties on how to interpret results in such a dynamic population. Advancements in molecular techniques have made it possible to assess the concentration of individual HDL and LDL lipoprotein subfractions. Using the Lipoprint by Quantimetrix gel electrophoresis as the basis to separate the lipoproteins into different subfractions. HDL Lipoprint analysis can generate ten fractionated lipoproteins (HDL1-10); additionally, it combines the particles into three classes creating the L-HDL (HDL1-3), I-HDL (HDL4-7) and S-HDL (8-10). Few studies in the field of cardiovascular disease have attempted to understand HDL functionality, as well as the three subfractions, large, intermediate and small, on what they may represent in CVD. Even less is known concerning the field of MHD on what the subfractions represent. Using

the Lipoprint method, the understanding used to be that the L-HDL was similar to HDL2 which meant was protective. However it is not the case, because HDL_2 is different species of the lipoprotein; moreover, it was attained via ultracentrifugation, a different technique thus, not possible to compare at this time [176]. I-HDL was also shown to be on the protective part of the HDL spectrum; and, the S-HDL was interpreted and identified to be the atherogenic part of the HDL family depictive of HDL_3, again not the same lipoprotein species [230, 231]. However, the above premise that L-HDL is protective, and S-HDL was not has been a heated debate and a new shift in different thinking. The ***IDEAL*** and ***EPIC*** case-control studies have shown contrary evidence to the old belief, that the L-HDL was protective. Analyses showed that elevated plasma HDL-C levels (≥70 mg/dL) and the L-HDL particles were associated with a higher risk for CVD. In contrast, the S-HDL particle has shown that it may be more functional thus, more efficient in promoting cholesterol efflux from macrophage to remove excess cholesterol [108].

The LDL-subfraction analysis has been a robust understanding; using the same method, it can generate seven LDL subfractions [LDL1-7], as well as measuring concentrations of VLDL subfraction, followed by IDL. The seven LDL-subfraction are further combined into three groups: L-LDL (LDL1), I-LDL (LDL2), and S-LDL (LDL3-7). This analysis can also provide the patients mean LDL particle size (diameter) of the lipoprotein for which three-phenotype profiles are generated A, B, and Intermediate [AB]. Suggested evidence points to the importance of this feature since it can predict the development and progression of coronary heart disease (CHD) [232]. More studies have looked at the importance of these phenotypes, that were established based on cholesterol LDL subfractions, for which a “pattern-A” corresponds to the larger and more buoyant (LDL1) lipoprotein, speculated to be protective. The other profile is “pattern-B” corresponds to the smaller, denser, and more atherogenic LDL-[3-7] lipoproteins, which bears a higher risk for

oxidation. It has been recognized through various investigations that the small denser LDL lipoprotein has the potency of being uptake more freely by arterial tissues where they can oxidize during disease physiology.

Additionally, they decrease the receptor-mediated uptake and increase the proteoglycan binding, thus decreased anti-oxidant concentration [78]. The least described profile, which is in the middle between "A & B" is "pattern-Intermediate" corresponds to (LDL2), also known as [AB] profile. It is a very fluid and influx profile for which dependent on the different stages of the disease the subject could be under; it can shift to either side "A" or "B" if there are improvements or unfavorable regression [233, 234]. This [AB] phenotype is highly prevalent in patients who are presented with lipid disorders and additionally have been associated with significantly elevated intermediate-density lipoprotein (IDL), an increased risk that has been linked to atherosclerosis and heart disease in hemodialysis (HD) patients [235].

Lipoprotein subfraction analyses showed in the Malaysian cohort that I-HDL was the prominent HDL lipoprotein, followed by L-HDL and least represent S-HDL. Analyses also revealed that there were differences in the L-HDL between the three ethnicities, MC had the highest values in L-HDL, followed by MI and MM. Changes were also observed in S-HDL, while not very pronounced MC subjects had the highest content of the S-HDL, followed by MM and MI. In all three ethnicities, IDL showed to have a significantly higher content than VLDL. No major differences were recorded in LDL analyses. When the lipoprotein subfraction was compared with the AA cohort, there were significant differences in the L-HDL and S-HDL. AA patients had significantly higher L-HDL, and lowest VLDL, whereas MM had the highest content of the S-HDL, and MC had a higher value in VLDL. No changes were recorded in LDL analyses.

Subfraction data was analyzed for the two groups; TAG/HDL-C ratio (≥ or < 3.8). Patients with ≥ a 3.8 TAG/HDL-C ratio observed a significant decrease in L-HDL and an increased in the S-HDL and S-LDL, whereas <3.8 ratios observed a rise in the L-HDL, and I-HDL whereas S-LDL decreased. A study by Gluba-Brzozka et al. (2017) looked and analyzed the lipoprotein subfraction, in CKD and a healthy control group. Their analyses found that CKD patients all had more abundant L-HDL (similar what our data shows), whereas the healthy subjects had abundant of the S-HDL, and it was hypothesized that the S-HDL might be a better HDL lipoprotein to facilitate cholesterol efflux. [236]. In another investigation, a retrospective analysis in a Caucasians group of Polish ESRD patients Gluba-Brzozka et al. (2017), found a notable difference in both HDL and LDL subfractions. Polished ESRD patients were characterized by significantly higher L-[HDL and L-LDL] countered by the lower composition of small-[HDL and LDL] which was concluded that the L-HDL maybe a contributor to impairment of RCT [78]. In an earlier publication by our group Tashkandi. B et al., (2019) [237] we investigated a cohort of Saudi Arabic patients, finding different results in their HDL distribution, unlike the US and Malaysian cohorts who had higher components of large and small. Their L-HDL (30%) and S-HDL (14%) distribution were relatively equal throughout the 3-time point measured, with 55% of the particles account for I-HDL.

CETP was measured and showed a significant decrease in MC and increases in MM and MI patients. Moreover, the dyslipidemic patients had lower activity of CETP activity versus its counterpart. LCAT activity was higher in AA concerning MC and MI. Additionally, we measured CRP in ESRD subjects regardless of sex, age, and ethnicity experience above the normal inflammation (extremely high CRP). Although plasma CRP is elevated for all four patients, African Americans expressed a 2X-fold increase in levels of inflammation than their counterparts,

which could place AA at a much higher risk for a cardiac event. Other analyses, for oxidation was analyses of AGEs-CML in plasma. Results were inconclusive. Even on DM patients whom theoretically and based on various publication should have expressed a higher level; thus it will be an area which needs further future analyses [238, 239]

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

Summary conclusion

The first part of this study was to document the degree of dyslipidemia (D) in a cohort of African American (AA) MHD patients, and characterize lipid, lipoprotein composition, and associated enzymes. Additionally, we wanted to document the status of the cohort inflammation by measuring the C-reactive protein (CRP) in the plasma; moreover, on a subset of this cohort, we measured the advanced glycation end-products- Nε-carboxymethyl-lysine (AGEs-CML) in the plasma an oxidation marker.

Lipid and lipid ratios for the 123 AA MHD patients showed to be within the normal range. TAG concentrations were lower than 150 mg/dL, HDL was within the desirable range 40 – 60 mg/dL, TC was <200 mg/dL and LDL-C was < 100 mg/dL. To assess for dyslipidemia in the AA cohort, the Adult Treatment Panel (ATP III) guidelines were utilized. Results showed that 14% of the population with ≥ a 3.8 TAG/HDL-C ratio was dyslipidemic, which was characterized by elevated TAG, TC, LDL-C, and lipid ratios, whereas HDL-C had significantly declined. Lipoprotein analyses revealed that AA subjects had borderline equal L-HDL and I-HDL, which were the prominent HDL lipoproteins, and significantly higher when compared to the distribution of the S-HDL. AA patients also had significantly more IDL than VLDL; furthermore, the L-LDL was the prominent lipoprotein with regards to I-LDL and S-LDL which was least represented. Overall, AA patients had 65% of phenotype “A” in LDL particle type. Other analyses within dyslipidemia showed a decrease in the L-HDL and I-HDL; consequently S-HDL increased two-fold. LDL analyses revealed a significant rise in the atherogenic S-LDL and a rise in I-LDL. Adding to the case was LDL particle phenotype “B” was present in 47% of dyslipidemic patients, in line with the rise of the atherogenic particles.

Dyslipidemic AA patients had a significant decline in CETP active; however, no changes in the LCAT activity. Collective the cohort had significantly elevated CRP levels, irrespective of whether the patients were D or not. AGEs-CML analyses, in the AA subgroup was inconclusive in the finding, any difference, amongst genders, diabetics as well as dyslipidemic MHD subjects.

The second aim of the study was to document dyslipidemia in a multi-ethnic cohort of MHD patients, composed of Malaysian-Malay (MM), Malaysian-Chinese (MC) and Malaysian-Indian (MI). Lipid analyses showed that parameters were all within normal range. The only noted difference was MM, and MI had significantly lower levels of HDL-C when compared to MC. Overall the Malaysian cohort was 29% D, for subjects in a $\geq$ 3.8 TAG/HDL-C ratio group, characterized by the significant increase in TAG and the decreases of the HDL-C level. HDL lipoprotein analyses, collectively revealed that I-HDL was the most pronounced subfraction, followed by L-HDL, and least shown was the S-HDL. Changes in both L-HDL and S-HDL were documented amongst ethnicities, leading us to deduce that most changes in HDL lipoproteins seemed to be driven by the fluctuations among the two subfractions. No significant changes were observed in the LDL subfraction composition.

An overall analysis and comparison between the four ethnicities revealed that AA patients had lower TAG, TC, LDL-C, and higher HDL-C when compared with Malaysian patients. Combined, the two cohorts showed to have 24% of patients being D, with results depictive of elevated TAG and significantly decreased HDL-C. In HDL lipoprotein analyses, data showed that AA patients had a significantly higher content of the L-HDL subfraction, whereas MC, MM had a higher composition of the S-HDL. VLDL analyses were observed to be significantly higher in Malaysian patients with respect to AA subjects, and no changes were observed in LDL lipoproteins. Moreover, a thorough analysis of dyslipidemia concerning subfractions, revealed a

pattern in which L-HDL and I-HDL had significantly decreased, whereas the S-HDL and S-LDL had increased.

CETP activity in Malaysian patients showed a decrease in its activity; however, it did not reach significance. Analyses also uncovered differences between the four ethnicities, mainly, depictive of AA patients having the lower activity within this study cohort. LCAT activity was significantly higher in AA patients, while no other changes were recorded with respect to dyslipidemia. Inflammation in the Malaysian cohort revealed to be substantial. Analyses between the four ethnicities showed a remarkable difference between AA, who had significantly higher CRP levels when compared to MM and MC.

In this dissertation, we were able to fulfill our primary objective in characterizing dyslipidemia and lipoprotein subfraction composition in a cohort multi-ethnic MHD set, which to our knowledge is the first study of its kind to tackle such task. Furthermore, the data in this study were also supportive of the proposed hypothesis, in associating dyslipidemia in MHD to abnormal lipoprotein particles, and higher inflammation was recorded in all four ethnic patients.

Recommendation and future directions

For future directions: One key area to investigate is to explore and measure ApoA-1 protein. This inquiry should be conducted in order to help facilitate a better understanding of the reverse cholesterol transport (RCT) mechanism and look into the effect of dyslipidemia that may have on this protein.

Secondarily, PON1 should be investigated, since it is considered to be the antiatherogenic component of the HDL lipoprotein.

Thirdly, a future investigation should examine the functionality of the HDL lipoprotein; with the purpose of understanding which of (L-HDL vs.S-HDL and/or L-LDL vs. S-LDL) maybe protect and ones that could contribute to disease in MHD subjects.

Furthermore, a follow up into the PATCH clinical trial should be, to measuring after 12-month of intervention all of the lipoproteins, CETP, LCAT, LPL (part of RCT), and CRP, and attempt to answer the questions on how did TRF supplementation affect these parameters in MHD patients, and whether the intervention affect dyslipidemia.

APPENDIX A

Appendix Tables 1: Dyslipidemic profile criteria assessed based Adult treatment panel III (ATP III) guidelines.

Dyslipidemia Categories	**TAG**	**HDL-C**	**LDL-C**
Lipid Concentrations	*(mg/dL)*	*(mg/dL)*	*(mg/dL)*
Dyslipidemic group [**D**]	Higher >150	Lower HDL < 40	Higher > 100
Mixed Dyslipidemic group [**MD**]	*At least one criteria of for dyslipidemia*		
Normo-lipidemic group [**NL**]	Lower < 150	Higher > 40	Lower < 100

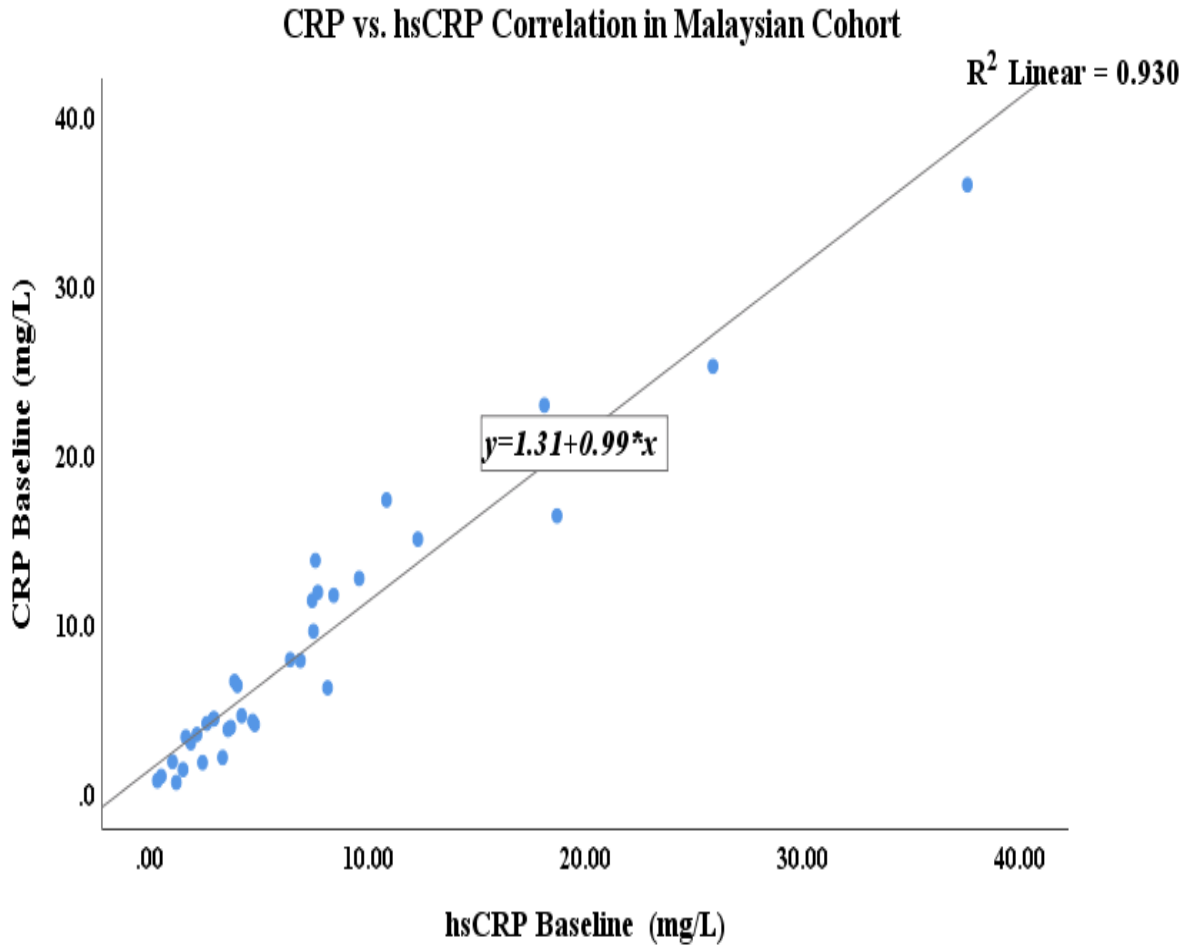
[**D**], the dyslipidemic group; [**NL**] normo-lipidemic group; [**MD**], mixed dyslipidemic group. Criteria based on the Adult Treatment Panel (ATP III) guidelines [240-242]

Appendix Tables 2: Recommended lipid ranges.

Lipid Parameters	Low	Normal/ Desirable	Borderline High	Very High	Extremely High
TAG (mg/dL)	---	< 150	150 – 199	200 – 499	≥ 500 Hypertriglycerimia domain
HDL-C (mg/dL)	< 40	40 – 60	≥ 60	---	---
TC (mg/dL)	---	< 200	200 – 239	≥ 240	Hypercholesterolemic domain
LDL-C (mg/dL)	---	< 100	130 – 159	160 – 189	≥ 190
NonHDL-C (mg/dL)	---	< 130	130 – 159	≥ 159	---
TAG/HDL-C ratio	---	< 2	2 – 3.8	≥ 3.8	---
TC/HDL-C ratio (Men)	---	< 3.5	3.5 – 5.0	≥ 5.0	---
TC/HDL-C ratio (Women)	---	< 3.0	3.0 – 4.4	≥ 4.4	---
LDL/HDL ratio	---	< 2.5	2.5 – 3.3	≥ 3.3	---

Lipid parameters are recommendations based on ATP-III guidelines; [243], ACC/AHA [242] and KDIGO [9, 244].

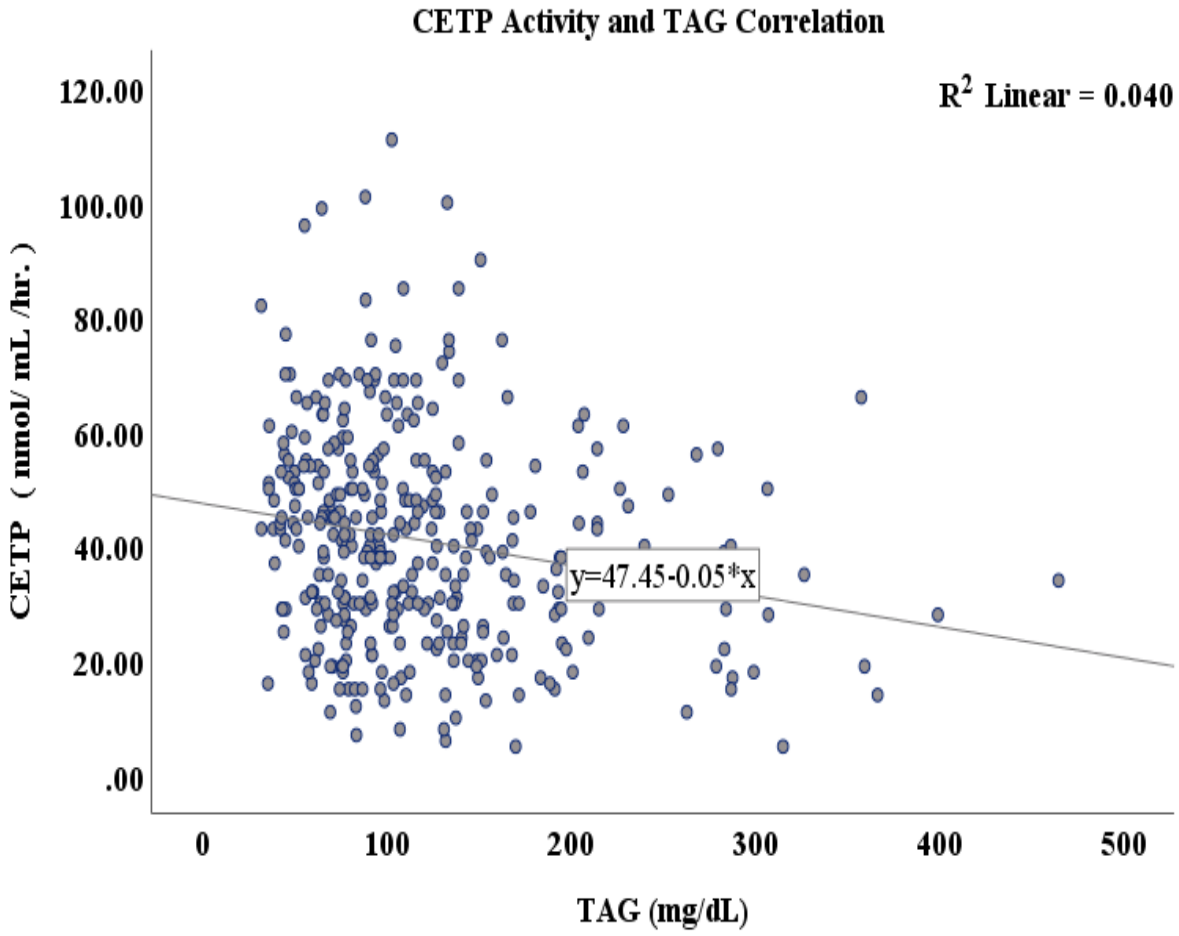
Appendix Figures 1: CRP versus hs-CRP in the Malaysian plasma samples.



Samples analized for CRP and hsCRP in order to standarized the two experiments was (n) = 36. The adjustment formula was: [y = 1.31 + 0.99x] with a R^2 = linerar = 0.930

Additional analyses of the Pearson Correlation showed an r = 0.965 ($p < 0.001$) a significant association between the two assays.

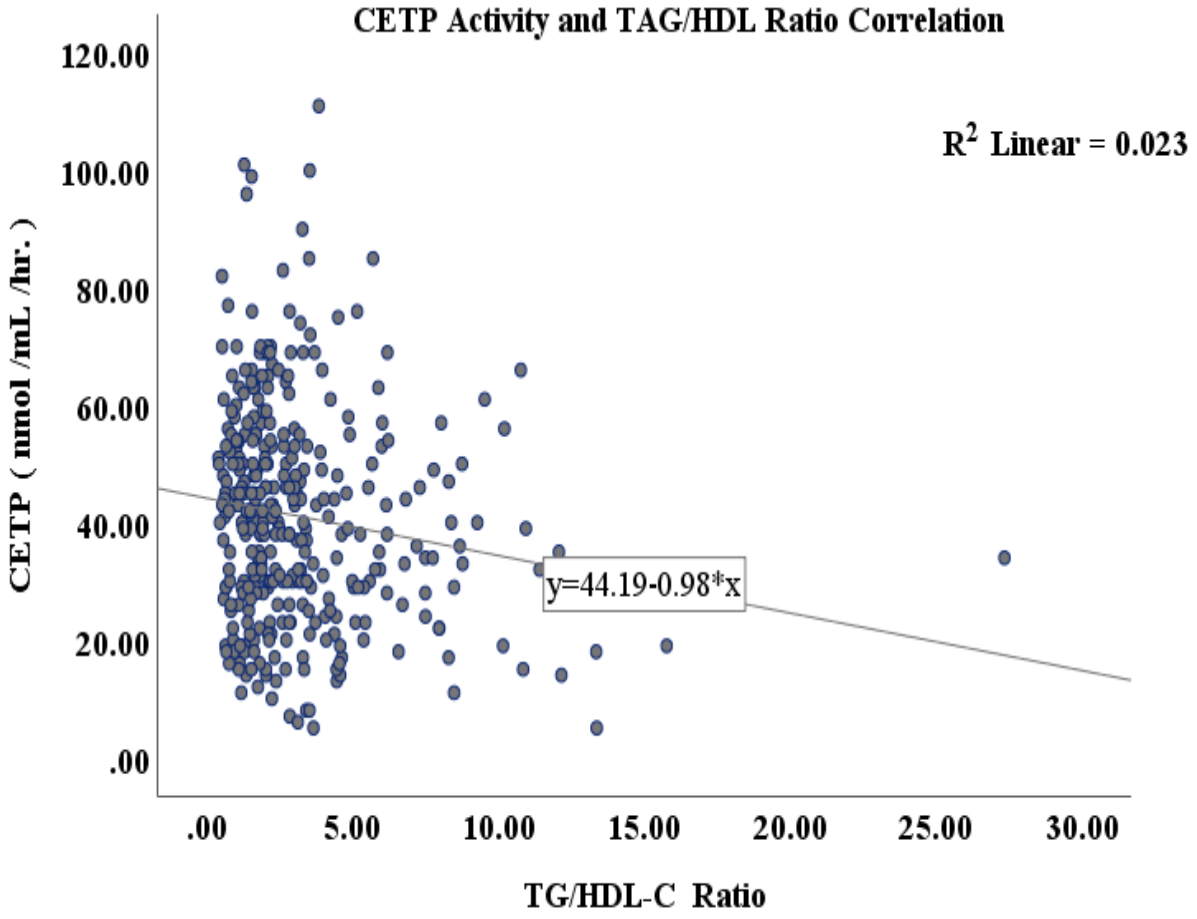
Appendix Figures 2: CETP activity and TAG concentration correlation for all samples.



CETP activity (nmol/mL hr.) has an inverse correlation with TAG concentration (mg/dL) in (n = 348) plasma sample from MHD patients. $R^2 = 0.040$ with a y = 47.45 - 0.05x equation.

Pearson Correlation showed a strong association between parameters with an r = -0.201 which is inversely significantly associated ($p<0.001$).

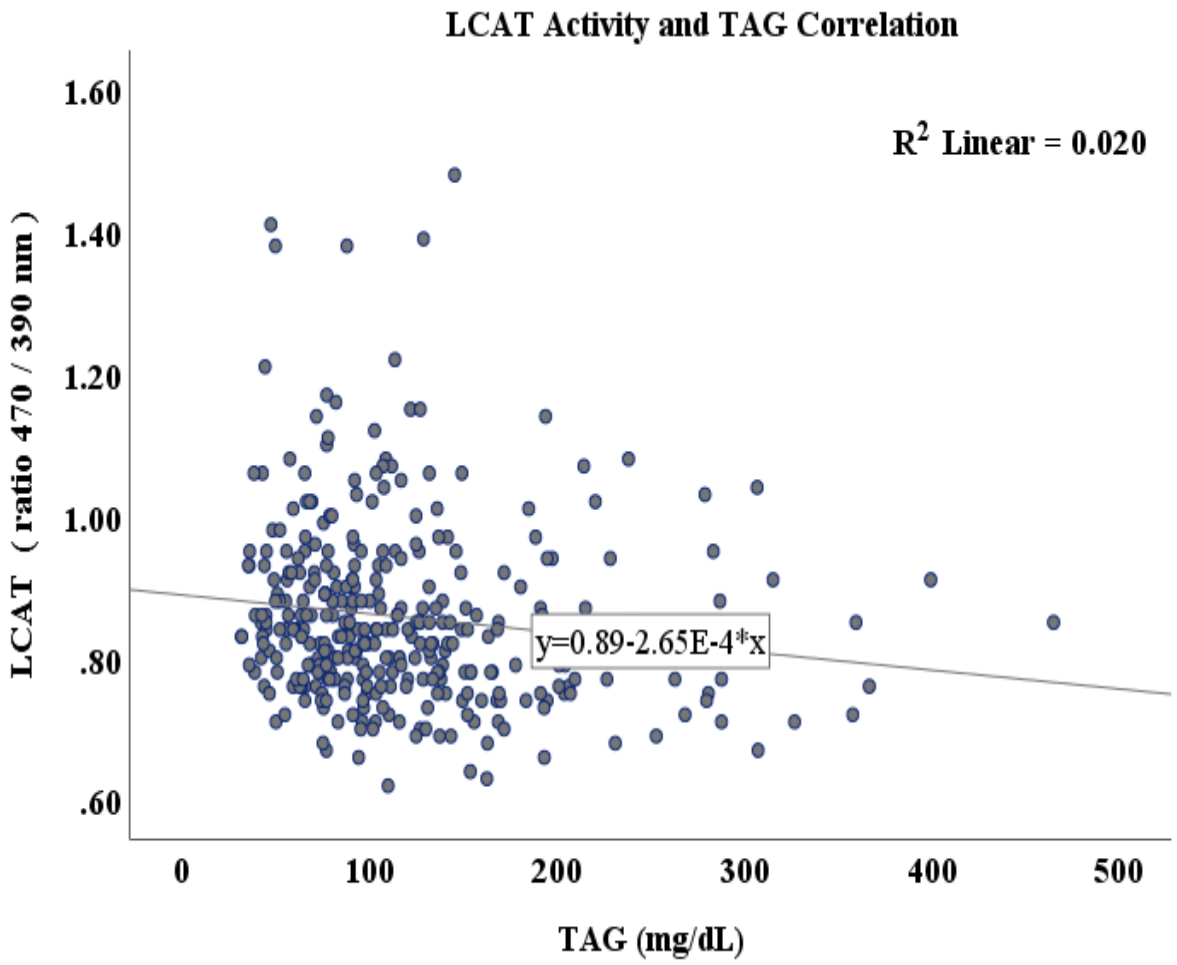
Appendix Figures 3: CETP activity and TAG/HDL-C ratio correlation for all samples.



CETP activity (nmol/mL hr.) has an inverse correlation with TAG/HDL-C ratio in (n = 348) plasma sample from MHD patients. $R^2 = 0.023$ with a y = 44.19 – 0.98x equation.

Pearson Correlation showed a strong association between parameters with an r = -0.153 which is inversely significantly associated ($p<0.004$).

Appendix Figures 4: LCAT activity and TAG concentration correlation for all samples.

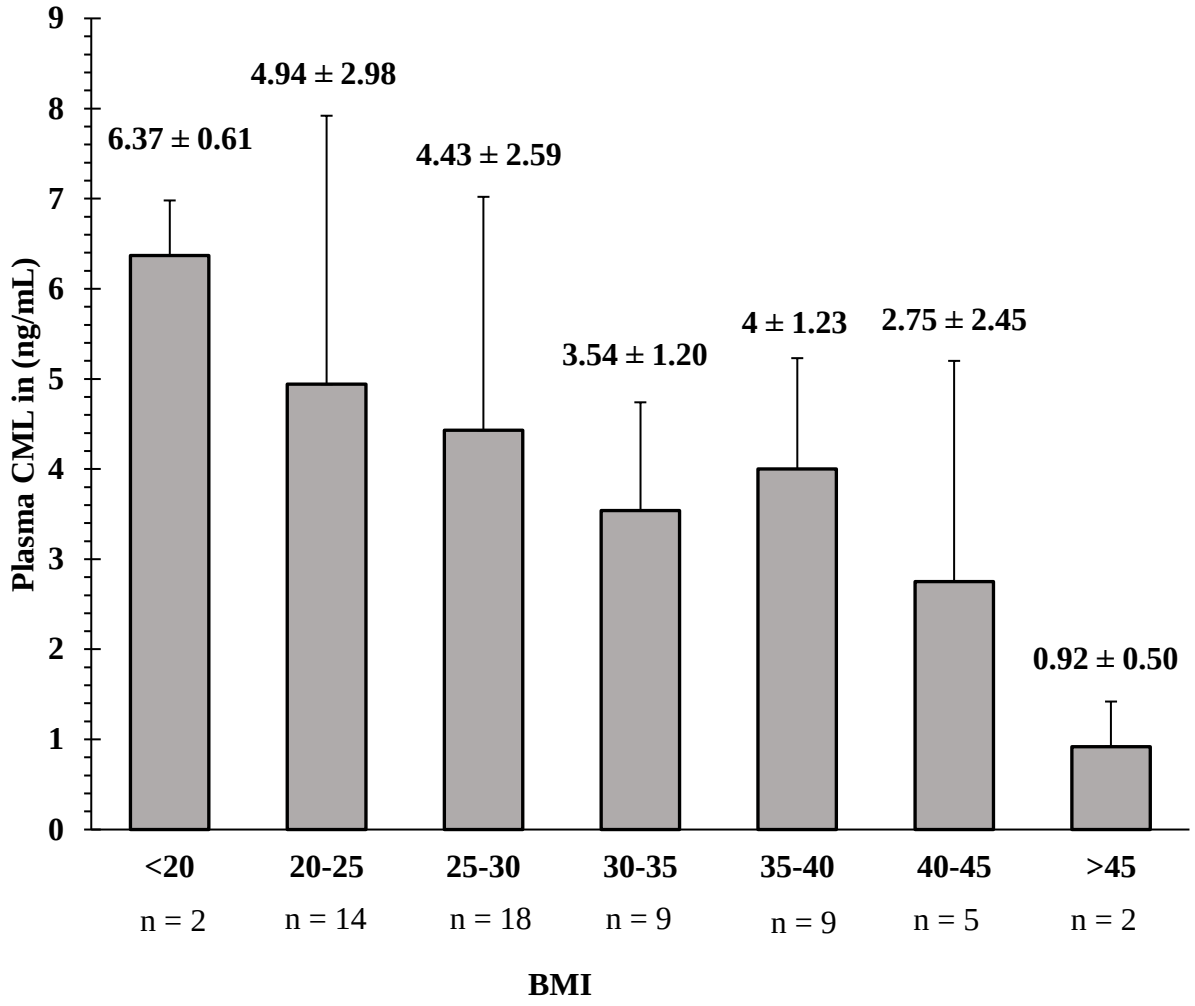


LCAT activity a ration between (470/390 FI) has an inverse correlation with TAG concentration levels in (n = 345) plasma sample from MHD patients. $R^2 = 0.020$ with a $y = 0.89 – 2.65E^{-4}x$ equation.

Furthermore, Pearson Correlation showed a strong association between parameters with an r = -0.141 which is inversely significantly associated ($p<0.009$).

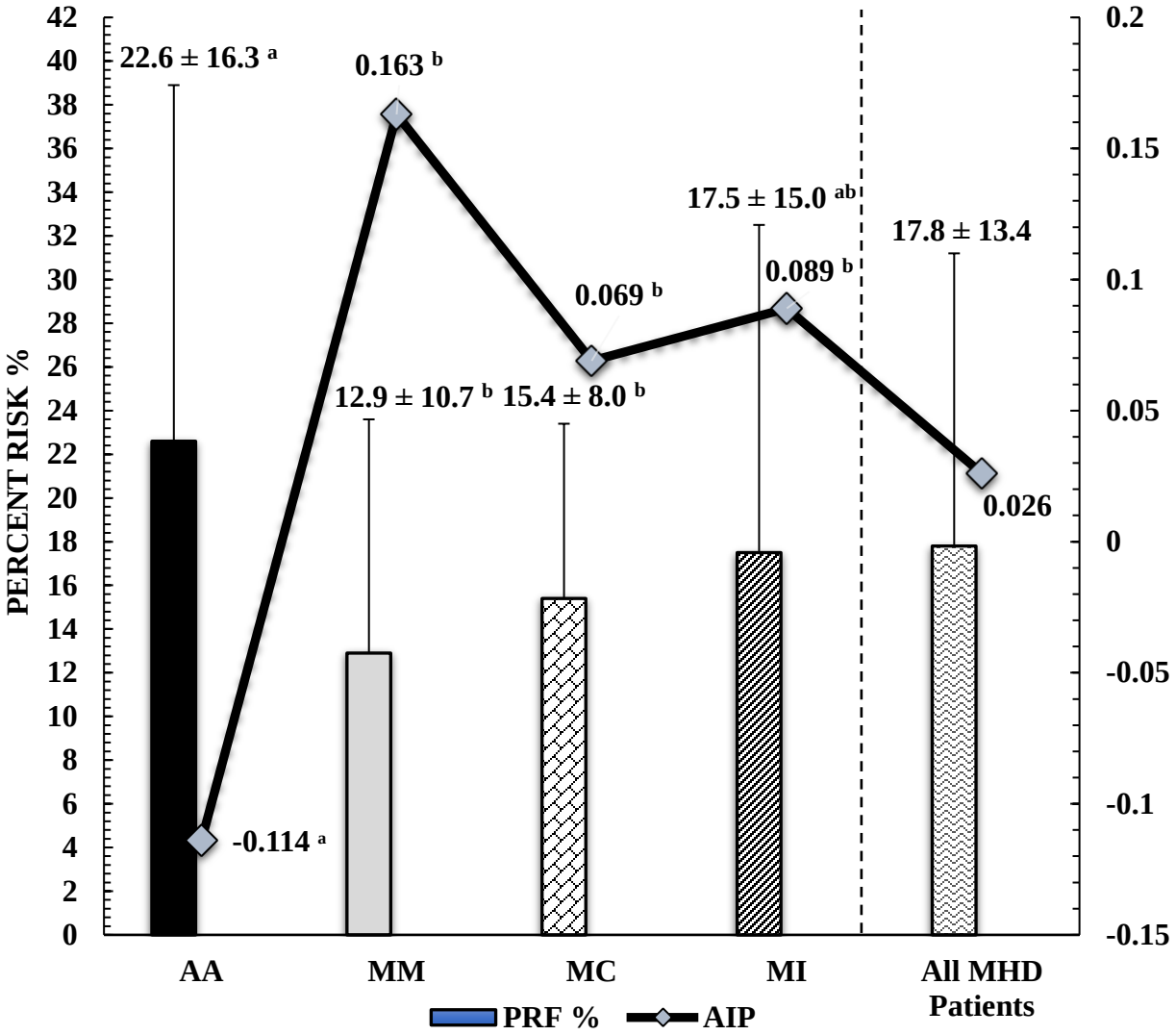
APPENDIX B: Other Analyses

Appendix B Figures 1: AGEs-CML based on BMI analyses in the US cohort.



Values are reported as mean ± SD. The values with different superscript[abc] represent significant differences from each other ($p < 0.05$) when using One-Way ANOVA. Statistics showed, **N.S.,** between the seven subgroups p-value = 0.141.

Appendix B Figures 2: Percent risk factor and atherogenic index of plasma in MHD ethnic patients.



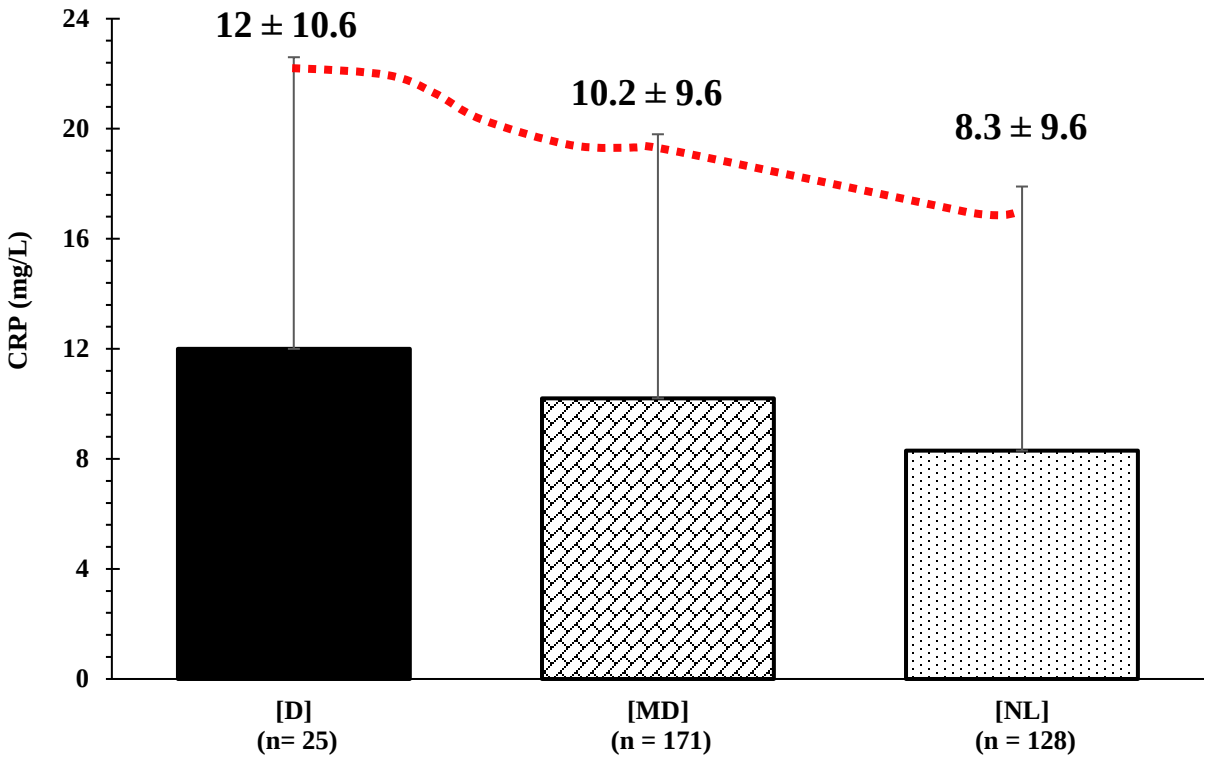
Values are reported as mean ± SD. The values with different superscript[ab] represent significant differences from each other ($p < 0.05$) when using One-Way ANOVA. Data were adjusted using ANCOVA, for fixed variables: gender, DM, HTN, and Tabaco in relation to covariate factors.

PRF%: < 5% low risk; 5-7.4% borderline risk; 7.5-19.9% intermediate risk; ≥ 20% high risk.

AIP: 0.11 - low risk; 0.11 - 0.21 intermediate risk; ≥ 0.21 increased risk.

Abbreviations: AA, African American (n = 123); MC, Malaysian-Chinese (n = 110); MM, Malays (n = 69); MI., Malaysian-Indian (n = 46); PRF%, percent risk factor; AIP, Atherogenic Index of Plasma.

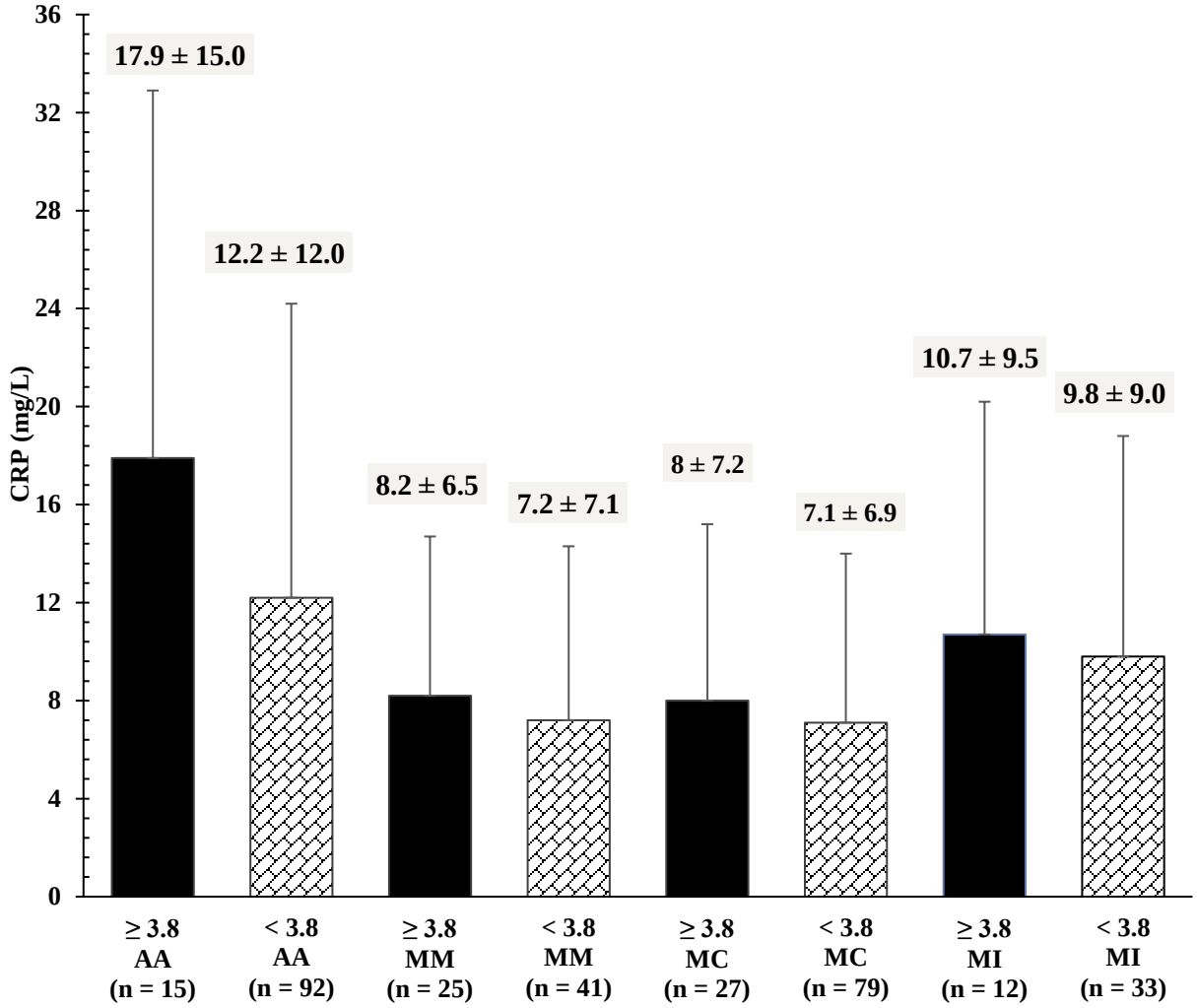
Appendix B Figures 3: CRP analyses based on dyslipidemia.



Values are reported as mean ± SD. No difference but a decreasing trend.

Abbreviation: D, dyslipidemic; MD, mixed dyslipidemic; NL, normo-lipidemic.

Appendix B Figures 4: CRP among ethnicities according to TAG/HDL-C ratios ≥ or < 3.8.



Values are reported as mean ± SD.

Appendix B Table 1: Demographics of the collective in the US and Malaysian cohort.

Demographics	**Total** (n = 348)	**AA** (n = 123)	**MM** (n = 69)	**MC** (n = 110)	**MI** (n = 46)	*P-value*
Age (yrs.)	56 ± 13	59 ± 13[a]	48 ± 14[b]	57 ± 11[a]	55 ± 12 [a]	**<0.001***
Gender (n, %)						
Males	211 (60.6)	77 (62.6)	37 (53.6)	65 (59.1)	32 (69.6)	NS
Females	137 (39.4)	46 (37.4)	32 (46.4)	45 (40.9)	14 (30.4)	
Tobacco Smokers (n, %)						
Smokers	57 (16.4)	36 (29.3)	9 (13)	10 (9.1)	2 (4.3)	**<0.001***
Non-Smokers	289 (83)	87 (70.7)	59 (85.5)	99 (90)	44 (95.7)	
Other-unknown	2 (0.60)	-	1 (1.4)	1 (0.90)	-	
Diabetes (DM) (n, %)						
DM	167 (48)	68 (55.3)	27 (39.1)	48 (43.6)	24 (52.2)	NS
Non-DM	181 (52)	55 (44.7)	42 (60.9)	62 (56.4)	22 (47.8)	
Hypertension (n, %)						
HTN	296 (85.1)	108 (87.8)	57 (82.6)	96 (87.3)	35 (76.1)	NS
Non-HTN	52 (14.9)	15 (12.2)	12 (17.4)	14 (12.7)	11 (23.9)	
Statin (n, %)						
User	190 (54.6)	55 (44.7)	37 (53.6)	73 (66.4)	25 (54.3)	**0.011***
Non-User	158 (45.4)	68 (55.3)	32 (46.4)	37 (33.6)	21 (45.7)	
BMI (kg/m^2)	27.0 ± 6.0	30 ± 7.5 [a]	26 ± 4.6[b]	25 ± 4.2[b]	26 ± 3.2[b]	**<0.001***
Albumin (g/dL)	3.9 ± 0.43	3.8 ± 0.3[a]	3.8 ± 0.4[a]	4.1 ± 0.4 [b]	4.0 ± 0.4[b]	**<0.001***
Systolic B/P (mmHg)	150 ± 23	140 ± 21[a]	151 ± 21[b]	158 ± 21[b]	159 ± 24[b]	**<0.001***
Diastolic B/P (mmHg)	79 ± 15	79 ± 15	78 ± 16	79 ± 14	77 ± 16	0.807
Dialysis/Vintage (months)	73 ± 61	66 ± 65	84 ± 66	76 ± 55	71 ± 56	0.285
Kt/V	1.6 ± 0.3	1.5 ± 0.2 [a]	1.6 ± 0.3[ab]	1.6 ± 0.3[b]	1.6 ± 0.3[ab]	**0.030***

Values are reported as mean ± SD. The values in a given row with different superscript[**abc**] represent significant differences from each other ($p < 0.05$) when using One-Way ANOVA.

Data were adjusted using ANCOVA, in relation to covariate factors such as age, gender, tobacco, statin, BMI, Albumin, systolic, and diastolic as well as Kt/V.

Appendix B Table 2: Lipoprotein composition between genders and ethnicities.

Lipoproteins in Men (n, %) (n=211)	**AA** 77 (36)	**MM** 37 (18)	**MC** 65 (31)	**MI** 32 (15)
L-HDL (mg/dL)	18.9 ± 12.5 [a]	10.8 ± 5.90 [b]	16.0 ± 9.90 [ab]	12.7 ± 7.0 [b]
I-HDL (mg/dL)	21.0 ± 5.36	22.0 ± 3.72	22.0 ± 5.25	22.1 ± 4.04
S-HDL (mg/dL)	5.86 ± 3.00 [a]	7.43 ± 3.47 [b]	6.74 ± 2.94 [ab]	5.31 ± 2.13 [a]
VLDL (mg/dL)	24.7 ± 10.7 [a]	33.9 ± 10.6 [b]	32.7 ± 9.21 [b]	30.8 ± 7.32 [b]
IDL (mg/dL)	44.9 ± 14.6	46.5 ± 12.8	43.1 ± 10.8	49.4 ± 12.7
L-LDL (mg/dL)	20.4 ± 8.89 [a]	23.4 ± 8.37 [ab]	23.5 ± 8.77 [ab]	26.0 ± 8.36 [b]
I-LDL (mg/dL)	11.8 ± 7.00	14.8 ± 8.17	12.8 ± 6.36	15.1 ± 7.64
S-LDL (mg/dL)	4.26 ± 6.29	6.19 ± 8.10	4.31 ± 6.67	4.87 ± 9.08
Lipoprotein in Women (n, %) (n=137)	**AA** 46 (34)	**MM** 32 (23)	**MC** 45 (33)	**MI** 14 (10)
L-HDL (mg/dL)	27.3 ± 19.2 [a]	16.8 ± 9.90 [b]	19.6 ± 13.9 [ab]	20.0 ± 12.5 [ab]
I-HDL (mg/dL)	24.0 ± 5.92	23.2 ± 4.80	25.1 ± 5.51	24.1 ± 5.80
S-HDL (mg/dL)	6.09 ± 3.52	6.75 ± 3.87	8.13 ± 4.16	5.92 ± 3.58
VLDL (mg/dL)	26.6 ± 9.17 [a]	33.6 ± 8.72 [b]	37.7 ± 10.7 [b]	28.8 ± 11.2 [ab]
IDL (mg/dL)	49.9 ± 16.4	47.7 ± 12.9	50.2 ± 15.6	52.2 ± 19.0
L-LDL (mg/dL)	25.4 ± 9.84	22.7 ± 7.92	22.9 ± 9.43	24.7 ± 8.19
I-LDL (mg/dL)	13.7 ± 9.41	13.4 ± 9.82	11.9 ± 7.71	10.9 ± 6.03
S-LDL (mg/dL)	3.15 ± 5.82	4.50 ± 6.70	4.58 ± 7.23	4.29 ± 10.1

Values are reported as mean ± SD. The values in a given row with different superscript[abc] represent significant differences from each other ($p < 0.05$) when using One-Way ANOVA.

Appendix B Table 3: Lipid analyses in statin analyses.

Lipid **Statin group**	**Total** (n = 190)[1]	**AA** (n = 55)	**MM** (n = 37)	**MC** (n = 73)	**MI** (n = 25)
TAG (mg/dL)	129 ± 72 [a]	94 ± 50 [a]	146 ± 73 [b]	139 ± 68 [b]	145 ± 96 [b]
HDL (mg/dL)	47 ± 15	51 ± 19	45 ± 11	47 ± 13	43 ± 12
TC (mg/dL)	160 ± 39	148 ± 46	165 ± 33	165 ± 35	162 ± 38
LDL (mg/dL)	88 ± 33	79 ± 42	92 ± 30	91 ± 28	90 ± 26
NonHDL (mg/dL)	113 ± 41	98 ± 48 [a]	120 ± 35 [b]	119 ± 34 [b]	118 ± 39 [ab]
TAG/HDL- Ratio	3.19 ± 2.45 [a]	2.21 ± 1.66 [a]	3.71 ± 2.46 [b]	3.38 ± 2.24 [b]	3.97 ± 3.72 [b]
TC/HDL-C Ratio	3.68 ± 1.37	3.28 ± 1.59	3.90 ± 1.17	3.75 ± 1.12	4.02 ± 1.63
LDL/HDL-C Ratio	2.05 ± 1.02	1.84 ± 1.34	2.17 ± 0.87	2.08 ± 0.81	2.25 ± 0.95
Lipid **Non-Statin group**	Total (n = 158) [1]	**AA** (n = 68)	**MM** (n = 32)	**MC** (n = 37)	**MI** (n = 21)
TAG (mg/dL)	105 ± 52 [b]	93 ± 44 [a]	138 ± 75 [b]	104 ± 41 [a]	100 ± 33 [a]
HDL-C (mg/dL)	47 ± 16	50 ± 19 [ab]	41 ± 9 [a]	51 ± 17 [b]	43 ± 11 [ab]
TC (mg/dL)	156 ± 35	148 ± 38	162 ± 36	160 ± 34	165 ± 22
LDL-C (mg/dL)	88 ± 32	80 ± 36 [a]	95 ± 29 [ab]	88 ± 28 [ab]	103 ± 23 [b]
NonHDL (mg/dL)	109 ± 38	99 ± 42 [a]	121 ± 37 [b]	109 ± 30 [ab]	122 ± 25 [ab]
TAG/HDL-C Ratio	2.60 ± 1.87 [b]	2.27 ± 1.55 [a]	3.76 ± 2.76 [b]	2.26 ± 1.32[ac]	2.54 ± 1.26 [abc]
TC/HDL-C Ratio	3.60 ± 1.31	3.38 ± 1.45 [a]	4.15 ± 1.42 [b]	3.30 ± 0.84[ac]	4.04 ± 1.04 [abc]
LDL/HDL-C Ratio	2.10 ± 1.05	1.92 ± 1.20	2.44 ± 1.01	1.87 ± 0.72	2.56 ± 0.86

Values are mean ± SD for the numbers indicated in parentheses[1]. Values in a given row with different superscripts [ab] were significantly different from each other ($p < 0.05$), using one-way ANOVA.

Overall: Statin patients (n = 190) vs. Non-statin (n = 158) patients showed a significant difference (P<0.001) in TAG composition Statin (128 ± 72 mg/dL) vs. Non-Statin (105 ± 52 mg/dL) and in TAG/HDL-C ratio (P=0.015) (3.19 ± 2.45 vs. 2.60 ± 1.87).

Appendix B Table 4: Subfraction composition in the statin analyses.

Lipoproteins Statin group (n=190)	**AA** 55 (29)	**MM** 37 (19)	**MC** 73 (38)	**MI** 25 (13)
L-HDL (mg/dL)	22.0 ± 16.2 [a]	14.9 ± 9.43 [b]	15.5 ± 10.2 [b]	15.2 ± 9.56 [ab]
I-HDL (mg/dL)	22.5 ± 5.46	23.5 ± 4.05	23.1 ± 5.35	22.1 ± 5.51
S-HDL (mg/dL)	6.20 ± 3.52 [a]	7.00 ± 3.73 [ab]	8.01 ± 3.69 [b]	5.96 ± 2.92 [ab]
VLDL (mg/dL)	25.5 ± 11.3[ac]	33.0 ± 8.27 [bc]	36.6 ± 10.3 [b]	30.1 ± 9.98 [c]
IDL (mg/dL)	46.8 ± 16.1	46.9 ± 13.4	46.6 ± 12.5	48.4 ± 13.5
L-LDL (mg/dL)	22.2 ± 10.3	23.0 ± 8.04	22.5 ± 8.63	24.1 ± 7.42
I-LDL (mg/dL)	12.8 ± 8.75	14.8 ± 8.56	12.9 ± 6.70	14.4 ± 7.54
S-LDL (mg/dL)	4.02 ± 6.97	5.22 ± 7.28	5.16 ± 7.14	6.44 ± 11.8
Lipoproteins Non-Statin group (n=158)	**AA** 68 (43)	**MM** 32 (20)	**MC** 37 (24)	**MI** 21 (13)
L-HDL (mg/dL)	22.0 ± 15.7 [a]	12.0 ± 7.11 [b]	21.3 ± 13.8 [ab]	14.7 ± 9.68 [a]
I-HDL (mg/dL)	21.8 ± 5.98	21.4 ± 4.31	23.5 ± 5.98	23.3 ± 3.44
S-HDL (mg/dL)	5.74 ± 2.90[ab]	7.25 ± 3.60 [a]	5.92 ± 2.76 [ab]	4.95 ± 2.18 [b]
VLDL (mg/dL)	25.3 ± 9.29 [a]	34.6 ± 11.2 [b]	31.3 ± 8.89 [b]	30.3 ± 6.84 [ab]
IDL (mg/dL)	46.7 ± 15.0	47.3 ± 12.3	44.8 ± 15.2	52.5 ± 16.2
L-LDL (mg/dL)	22.4 ± 8.96	23.1 ± 8.32	24.9 ± 9.62	27.4 ± 8.96
I-LDL (mg/dL)	12.3 ± 7.40	13.4 ± 9.44	11.5 ± 7.34	13.1 ± 7.30
S-LDL (mg/dL)	3.71 ± 5.37	5.62 ± 7.80	2.95 ± 6.14	2.62 ± 4.35

Values are reported as mean ± SD. The values with different superscript[abc] represent significant differences from each other ($p < 0.05$) when using One-Way ANOVA.

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ABSTRACT

ROLE OF DYSLIPIDEMIA ON LIPID METABOLISM IN MAINTENANCE HEMODIALYSIS PATIENTS

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December 2019

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Degree : Doctor of Philosophy

Maintenance hemodialysis (MHD) patients experience various abnormalities such as systemic inflammation (SI), oxidative stress (OS), and dyslipidemia (D). Defined as an imbalance of plasma lipids, lipoproteins, and lipid metabolism enzymes, D has been associated with a rise in morbidity and mortality within ESRD patients due to cardiovascular disease (CVD). However, the contribution of each of these parameters to D is poorly understood; moreover, the impact of the following parameters on dyslipidemia in different ethnicities is unknown. Hence, the objective of this study was to characterize D in a multi-ethnic cohort of ESRD patients. We hypothesized that dyslipidemia amongst MHD patients of different ethnicities will reflect underlying alterations in lipoproteins and enzymes of lipid metabolism. The rationale for the proposed study is that once a dyslipidemic MHD profile is determined, targeted interventions could be used to manage it. The degree of D was investigated in a cohort comprised of African American (AA), Malaysian-Malays (MM), Malaysian-Chinese (MC), and Malaysian-Indians (MI), of whom were enrolled in the PATCH clinical trial USA and/or Malaysia.

Lipids, lipoprotein particles, associated enzymes cholesteryl ester transfer protein (CETP), Lecithin-cholesterol acyltransferase (LCAT), as well as an inflammatory marker C-reactive

protein (CRP), were measured. Assessment of dyslipidemia was based on the criteria from the Adult Treatment Panel (ATP III) guidelines. TAG/HDL ratios (≥ or < 3.8) were utilized to ascertain LDL phenotypes.

All lipid parameters in both groups were within the normal range; however, AA subjects had lower triglycerides (TAG) levels and higher high-density lipoprotein – cholesterol (HDL-C) when compared to Malaysian patients. Additionally, a biphasic response was recognized with regards to the relationship between TAG and HDL; as one rises, the other falls. Dyslipidemic analyses showed that all lipid values, in the TAG/HDL-C ratio ≥ 3.8 group were significantly different from the corresponding values in the < 3.8 TAG/HDL ratio group. The effect of dyslipidemia in the subfractions showed a decline in L-HDL and I-HDL consequently, S-HDL increased. Also, D increased VLDL and IDL subfractions, as well as I-LDL and S-LDL. Due to dyslipidemia, the mean LDL particle size decreased significantly in diameter, wherein 63% of patients were more likely to have a phenotype "B" LDL particle; which has been linked to the atherogenic small dense LDL. CETP activity was different amongst ethnicities and declined in the ≥ 3.8 TAG/HDL ratio group. LCAT activity was higher in AA with respect to Malaysians patients and was not affected by dyslipidemia. All patients had significantly elevated CRP levels, of which AA was the highest.

In conclusion, we analyzed lipids, lipoproteins, CETP, and LCAT activities, for a multi-ethnic cohort study, in addition to measuring CRP. For future directions, it would be ideal to investigate the effect of D on Apolipoprotein-A1, PON1, and, most important, test for HDL functionality.

AUTOBIOGRAPHICAL STATEMENT

The author Mr. Eno Latifi received his Bachelor and Science Degree from Wayne State University in Detroit, Michigan, as part of the May 2012 graduating class. Throughout the time as an undergraduate, he volunteered and conducted research at the Nutrition Food and Science department under Dr. Pramod Khosla's guidance. Then after graduating, he pursued a Master of Science degree, which he obtained in May 2014, with a foundation and focused on clinical research and biochemistry. He later returned and pursued a Doctor of Philosophy, with a focus in the area of clinical trial, biochemistry, and biostatics. During this time, he was clinical investigatory in addition to graduate teaching assistant, where he was a lecturer for various upper and lower undergraduate courses at the Nutrition and Food Science department.