# UNIVERSITÀ DEGLI STUDI DI SASSARI

**INTERNATIONAL Ph.D SCHOOL IN**
**LIFE SCIENCES AND BIOTECHNOLOGIES**

# LIPID - LOWERING REGIMES IMPROVE OXIDATIVE STRESS, TRYPTOPHAN DEGRADATION IN HYPERCHOLESTEROLEMIA CHRONIC KIDNEY DISEASE PATIENT

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**Ph.D Thesis**

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## ATTESTATION OF AUTHORSHIP

I hereby declare that this study is my own work to the best of my knowledge. It doesn't contain any publication of other previous authors except contents appearing in the citations.

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| | |
|---|---|
| ACR | Albumin-to-creatinine ratio |
| AER | Albumin excretion rate |
| ANOVA | Analysis of variance |
| BMI | Body mass index |
| BP | Blood pressure |
| CE | Capillary electrophoresis |
| CK | Creatine phosphokinase |
| CKD | Chronic kidney disease |
| CVD | Cardiovascular disease |
| eGFR | Estimated glomerular filtration rate |
| ESRD | End-stage renal disease |
| GSH | Glutathione peroxidase |
| H2O2 | Hydrogen peroxide |
| HDL-C | High-density lipoprotein-cholesterol |
| IDO | Indoleamine 2,3 dioxygenase |
| IFN ɣ | Interferon gamma |
| IL | Interleukin |
| KDIGO | Kidney Disease Improving Global Outcomes |
| LDL-C | low-density lipoprotein-cholesterol |
| MDA | Malondialdehyde |
| MDRD | Modification of Diet in Renal Disease |
| NO | Nitric oxide |
| OS | Oxidative stress |
| OxLDL | Oxidized low-density lipoproteins |
| SGOT | Serum glutamic oxaloacetic transaminase |
| SGPT | Serum Glutamic Pyruvic Transaminase |

| | |
|---|---|
| ROS | Reactive oxygen species |
| TBARS | Thiobarbituric acid reactive substances |
| TC | Total cholesterol |
| TDO | Tryptophan 2,3 dioxygenase |
| TG | Triglyceride |
| TNF α | Tumour necrosis factor alpha |

## ABSTRACT

Chronic kidney disease (CKD) is a global health problem, in which cardiovascular disease (CVD) is the leading cause of death. The prevalence of CKD in the Vietnamese population is increasing along with hypertension and diabetes, lipid disorder. The lipidemic disorder was evidenced as one of the cardiovascular risk factors in CKD patients, but it was not fully concerned. Oxidative stress indices, tryptophan degradation indices are endothelial biomarkers that have a relationship with CKD. Treatment of hyperlipidemia CKD patient reduced CVD events. Statins have been shown to improve lipidemia in patients with chronic kidney disease. Ezetimibe, a cholesterol absorption inhibitor, plays an important role in reducing LDL- C. The combination of EZE and statin is more effective in lipid disorders treatment than Statin monotherapy was also recorded. However, the impact mechanism of two therapies related to oxidative stress, plasma kynurenine, and tryptophan concentrations during the treatment was not well-known. And is there any clinical problems related to side effect occurred during treatment? Therefore, we conducted the study to discover the relationship between lipid-lowering regimes and oxidative stress, as well as tryptophan degradation improvement in CKD patients with hypercholesterolemia. With 12 months of treatment and monitoring through clinical signs as well as laboratory indicators, in which, patients were monitored every four months, we have the following results:

Patients with CKD stage 3-4 have demonstrated dyslipidemia, increased oxidation status, decreased antioxidant levels, and increased inflammation. We found that treatment with lipid-lowering therapies regardless of simvastatin alone or simvastatin/ezetimibe combination significantly improved lipid profiles (decreased TC, TG, LDL-C and increased HDL-C), ameliorated oxidative stress status (decreased MDA, increased PSH, PON and TEAC levels) and decreased inflammatory markers (Kyn concentration and Kyn/Trp ratio) in all patients. Even though there was no statically critical contrast between the distinctive treatment regimens, we found an improvement in lipidemia parameters, oxidative stress, and inflammation, in the group treated by Simvastatin 40 mg/day + Ezetimibe 10 mg/day appeared to be better in the other 2 groups. While analyzing treatment-related relationships, we found

that diminishing LDL-C levels simultaneously decreases oxidative stress as well as improves plasma inflammation. Dislipidemia treatment diminished MDA concomitant decline in Kyn/Tryp was likewise recognized. This shows the relationship between LDL-C, MDA, and Kyn, Kyn / Tryp. The improvement in inflammation in CKD patients was partly explained by a decrease in LDL-C through a decrease in oxidative stress. Lipid-lowering treatment increases the concentration of antioxidants such as PSH, TEAC, PON, especially the concentration of these substances increases more when treated by Simvastatin 40mg/Ezetimibe 10mg therapy. Meanwhile, PSH was inversely correlated with Kyn, Kyn / Tryp, proposing that the reduction in inflammation in CKD patients may be due to the recovery of antioxidants, including PSH or vice versa. All patients did not show clinical signs of jaundice, myalgia, rhabdomyolysis as well as unchanged in SGOT, SGPT, and CK levels. Renal function did not change during treatment, suggests that lipid-improving therapies do not improve kidney function nor worsen it. Treatment for inflammation was associated with an improvement in eGFR, although this correlation had a low correlation coefficient. This sets a new research direction in the future with more patients.

In summary, for CKD patients stage 3-4, who do not achieve an LDL-C target, Simvastatin / Ezetimibe at a dose of 40mg and 10mg respectively daily is used to inhibit cholesterol absorption and synthesis. This protocol may induce more lipid-lowering effects, allowing more patients to achieve LDL-C goals, subsequently, reduce oxidative stress as well as inflammation, but without causing dose-related side effects amount.

# 1. INTRODUCTION

## 1.1. Chronic kidney disease

Chronic kidney disease (CKD) is a long-standing illness, gradually decreased kidney function. Symptoms that develop slowly and in a severe stage include loss of appetite, nausea, vomiting, nocturia, confusion, fatigue, pruritus, fluid retention... Diagnosis is mainly based on laboratory testing of renal function. Chronic kidney disease (CKD) could be a major open wellbeing risk within the world, related to a disturbing increment in its frequency and predominance. CKD spent a burden of economic cost to health systems. Known as an asymptomatic infection, CKD can cause many complications counting cardiovascular disease (CVD), and result in diminishing life expectancy.

CKD is characterized as the presence of kidney damage or an assessed glomerular filtration rate (eGFR) less than 60 ml/min/1.73 $m^2$, persisting for at least 3 months, independent of the cause. It is a state of progressive loss of kidney function ultimately resulting in the requirement for renal replacement therapy (dialysis or transplantation). Kidney damage refers to pathologic abnormalities either recommended by imaging studies or renal biopsy, abnormalities in urinary sediment, or increased urinary albumin excretion rates (Inker et al., 2014).

Studies recommend that the pathophysiology of dyslipidemia in those with CKD may be distinctive from that of the common population. Changes in some key enzymes and receptors in CKD result predominantly in dysregulation of high-density lipoprotein (HDL) and triglyceride metabolism (Vaziri, 2006).

Increasing oxidative stress has been described in cardiovascular events in CKD patients. Cardiovascular morbidity and cardiovascular mortality are the leading causes of death in stages 3 to 4 of chronic kidney disease instead of progression to ERSD. Oxidative stress is increased in conjunction with increasing renal dysfunction (N. R. Hill et al., 2016). Oxidative stress, results trigger endothelial dysfunction and left ventricular hypertrophy to develop cardiovascular disease. Inflammation in CKD promotes the oxidant generation process. Endothelial dysfunction and left ventricular hypertrophy are the two clinical sequelae

of oxidative stress. (Kao, Ang, Pall, & Struthers, 2010). Understanding the role of oxidative stress in CKD, it is important to consider treatment options in the CKD population.

### 1.2. Epidemiology

It is too difficult to determine the true incidence and prevalence of CKD because of the asymptomatic nature of early to moderate stages in CKD. Within the general population, the prevalence of CKD is around 10% to 14%. Prevalence estimates might be biased by restriction of the markers and methods that are used to estimate GFR and to define kidney damage of the markers and methods. Based on albuminuria (microalbuminuria or A2) and eGFR, its prevalence is of 7% and 3% to 5%, respectively (Coresh, Astor, Greene, Eknoyan, & Levey, 2003). In the USA, the estimated prevalence of CKD from 1999 to 2006 was almost 11, 5% (4, 8% in stages 1 - 2 and 6, 7% in stages 3 - 5), in which, 47% older than 70 years. Prevalence of CKD increased in the elderly and may be companioned by an increase in diabetes and hypertension (Levey & Coresh, 2012).

The prevalence of prior stages of CKD was 14.8% in 2015, recommended that an expected 30 million American grown-ups have CKD, with millions of others at increased risk (Saran et al., 2019).

Data from a systematic review and meta-analysis of Hill, R.N in 2016 showed that worldwide predominance of CKD is around 11% to 13%, is considered as high around the world predominance, with the larger part stage 3 (N. R. Hill, Fatoba, S. T., Oke, J. L., Hirst, J. A., O'Callaghan, C. A., Lasserson, D. S., & Hobbs, F. D., 2016).

Chronic kidney disease is an around world wellbeing issue with a high frequency not as it were in Western nations but too in Asia as well.

In Indonesia, the rate of CKD per million populations in 2002 was 14,5% and expanded to 30,7% in 2006 (Prodjosudjadi & Suhardjono, 2009). A cross-sectional survey from adult Chinese showed that the prevalence of CKD was 10.8% (Xu et al., 2015). In the Vietnamese population, the prevalence of CKD is increasing along with hypertension and diabetes. 3.1% of the population diagnosed with CKD stages 3-5 (Ito, Dung, Vuong, Tuyen do, et al., 2008). (Ito, Dung, Vuong, Tuyen, et al., 2008).

### 1.3. CKD Pathology

The pathophysiology of CKD involves two mechanisms. The first one is related to underlying etiology (immune complex glomerulonephritis and inflammatory mediators to a specific type, or renal tubules toxins and interstitial disease) and the second one, is involving hyperfiltration and hypertrophy of remaining nephrons. Besides that, vasoactive hormones, cytokines, growth factors, and increased activity of the renin-angiotensin system in the kidney induced hyperfiltration and hypertrophy, and therefore cause an increase in intrarenal pressure and consequently result in nephrosclerosis (Putri & Thaha, 2014).

In developed nations, CKD is related to the elderly, diabetes, hypertension, obesity, and CVD, with diabetic glomerulosclerosis and hypertensive nephrosclerosis; in any case, it is often difficult to diagnose. In developing countries, common causes of CKD also include glomerular and tubulointerstitial diseases coming about from infections and introduction to drugs and toxins. Diabetic glomerulosclerosis is characterized by slowly increasing albuminuria, hypertension, and declining in GFR. Nephrosclerosis caused by hypertension has no clear markers of kidney damage, but albuminuria concentrations can occur after the onset of GFR decline (Levey & Coresh, 2012).

CKD is initially determined as diminished renal function, which may progress to renal failure (ESRD). Initially, when renal tissue loses function, only a few abnormalities is presented because of the renal adaptation of the remaining tissue. Later, kidney function deteriorated significantly, consequently, greatly impaired renal function impair the kidney's ability to maintain fluid and electrolyte homeostasis. The capacity to concentrate urine decreases early and is followed by a diminish in the capacity to excrete excess phosphate, acid, and potassium. When renal failure is progressed (eGFR $\leq$ 15 mL/min/1.73 $m^2$), the capacity to successfully dilute or concentrate urine is lost; hence, urine osmolality is usually close to that of plasma, and urinary volume does not react promptly to varieties in water intake.

## 1.4. Comorbidities

CKD is often considered associated with the old population. However, in the CKD population, hypertension, diabetes mellitus, obesity, are often occurred (Gansevoort et al., 2013). Known as non-modifiable CKD chance calculate Elderly populace are male, non-Caucasian ethnicity; whereas hypertension, proteinuria, and metabolic factors are respected as modifiable CKD risk factors that decline CKD function (Levey & Coresh, 2012).

Obesity and smoking have been identified with the development and progression of CKD. Likewise, metabolic factors, for example, insulin resistance, dyslipidemia, and hyperuricemia have been implicated in CKD progression (Johnson et al., 2013). Decline in kidney function resulted in blood pressure elevation, and persistently increase in blood pressure rapidly decreases kidney function (Bakris et al., 2000). Hence, CKD and hypertension express a close interrelation, as cause and effect association. Besides hypertension, diabetes mellitus is regarded as the most common cause of end-stage CKD. Diabetes mellitus causes renal failure; while the increase of uremia and parathyroid hormone levels in CKD patients cause insulin resistance in tissue, induce the disturbance of glucose metabolism and glycogen production (Andrianesis & Doupis, 2013). CVD and kidney disease interact with each other. CKD is believed to increase the risk of cardiovascular disease (CVD) and is considered as an independent CVD events risk factor. CVD is often contributed by CKD; while ERSD is regarded at higher CVD related mortality risk. Impairment of this organ will contribute to the dysfunction of another organ (Liu et al., 2014).

## 1.5. Diagnosis and classification of CKD

### 1.5.1. Criteria for CKD diagnosis

The definition of CKD of the K/DOQI was acknowledged. CKD is defined as kidney damage or GFR < 60 mL/min/1,73m$^2$ for at least 3 months, not concerning of cause. Kidney damage can be determined by albuminuria presence, assessed by albumin/creatinine ratio >30 mg/g in two of three spot urine specimens. GFR can be estimated from calibrated serum creatinine and estimating equations, such as the Modification of Diet in Renal Disease (MDRD) Study equation or the Cockcroft-Gault

formula. The severity of CKD is classified into five stages by the degree of eGFR (Inker et al., 2014).

### 1.5.2. Classification of CKD

As classified by the 2012 KDIGO CKD, 6 categories were obtained based on glomerular filtration rate, G1 to G5 in which G3 was split into 3a and 3b. The stage of CKD is also categorized into categorized three levels, based on albuminuria (A1, A2, and A3). Besides, each stage being sub-categorized according to the urinary albumin-creatinine ratio (ACR).

**Based on GFR, 6 categories were classified as bellowed:**

G1:90 ml/min per 1.73 m2 and above

G2: 60 to 89 ml/min per 1.73 $m^2$

G3a: 45 to 59 ml/min per 1.73 $m^2$

G3b: 30 to 44 ml/min per 1.73 $m^2$

G4: 15 to 29 ml/min per 1.73 $m^2$

G5: less than 15 ml/min per 1.73 m2 or treatment by dialysis.

**The three levels of albuminuria based on the albumin-creatinine ratio (ACR)**

A1: ACR less than 30 mg/gm (less than 3.4 mg/mmol)

A2: ACR 30 to 299 mg/gm (3.4 to 34 mg/mmol)

A3: ACR greater than 300 mg/gm (greater than 34 mg/mmol).

This classification has been useful in recognizing prognostic signs related to diminished function and increased albuminuria. However, the limitation of applying this classification is the overdiagnosis of CKD, particularly within the elderly.

## 1.6. CKD treatment

Treatments in CKD aim to prevent development, slow progression, restrict complications caused by low GFR, reduce risk of cardiovascular disease, and hence, improve survival with high life quality. The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guideline for CKD management in CKD was published in 2003, thereby, CKD management is based on clinical diagnosis and stage of CKD as well.

Clinical diagnosis allows doctors to choose specific therapies that are directed to the cause and pathophysiology. Thereafter, the stage classification can be utilized to direct non-specific treatments to slow progression and diminish the risk of complications (C. A. Johnson et al., 2004). Since then, with evidence from clinical trials of statin therapy in adults with CKD, the KDOQI guideline has supplied dyslipidemia treatment to manage dyslipidemia in patients with CKD. KDIGO recommends dyslipidemia therapy in CKD patients based on risk for CVD. Statin initiation is not recommended in CKD patients treated by dialysis (Sarnak et al., 2015).

Within the early stages of CKD, preservation treatment was applied, including supportive care referred to treatment without having dialysis or transplant. The main treatments in this stage include diet, medication, and cause based treatment. In the last stage of CKD, advanced treatment was applied such as peritoneal dialysis, artificial kidney or kidney transplant, considered impaired renal replacement therapies. However, conservative treatment is still necessary while waiting for renal replacement therapies. Conservative therapy aims to make sure patients keep up remaining kidney function as long as possible, by maintaining homeostasis even though renal function has decreased. Below are some goals of conservative treatment:

- Slow down the progression of CKD.
- Minimize the accumulation of urea and urea toxins.
- Good control of hypertension.
- Prevent atherosclerosis and cardiovascular complications.
- Keep the electrolyte balance and calcium, phosphorus.
- Avoid malnutrition and preserve patient life's quality.

Treatment of hypertension, treatment of anemia, prevention of calcium-phosphorus disorders, treatment of hyperuricemia and prophylaxis of drug-induced complications, use of drugs to slow down the progression of renal fibrosis,… are considered as preservation treatment (Inker et al., 2014)

## 1.7. Dyslipidemia in chronic kidney disease

The lipidemia disorder is one of the cardiovascular risk factors in CKD but it was not fully concerned. High low-density lipoprotein (LDL-C) levels can lead to artery damage and consequently prompt cardiac complications and stroke. In CKD patients, cardiovascular disease (CVD) is the leading cause of mortality. This affiliation is because CKD is frequently connected with dyslipidemia, which is considered a risk factor of CKD. In CKD patients, dyslipidemia was happened at the beginning phases of renal dysfunction and will in general advance with the lessening of kidney function. Dyslipidemia in CKD is generally because of increasing triglyceride, LDL-C levels, and diminished HDL-C. Statins were evidenced to be safe and effective in both prevention and lipid-lowering of CVD in the early stage of CKD as well as post-transplant (Hager, Narla, & Tannock, 2017).

In the United State, the prevalence of CVD was 69.6% among CKD patients age ≥ 66 compared to 34.7% among persons without CKD (Health, 2012). TC and LDL-C are the highest cholesterol in CKD patients. Two-thirds of cases of hyperlipidemia in CKD patients were mixed hyperlipidemia. Only one-third of CKD patients under treatment of statin achieved the LDL-C goal (Sangsawang & Sriwijitkamol, 2015).

### 1.7.1. Guidelines for the management of dyslipidemia

Dyslipidemia in CKD was recommended with pharmacological cholesterol-lowering treatment in adults as bellowed: (Wanner & Tonelli, 2014).

- Persons ≥ 50 years old with eGFR <60 ml/min/1.73 $m^2$, recommended a statin or statin/ezetimibe combination therapy.

- Persons ≥50 years old with eGFR ≥60 ml/min/1.73 $m^2$, recommended treatment with a statin.

- Persons aged 18 - 49 years diagnosed CKD, not treated with chronic dialysis or kidney transplantation, suggested statin treatment with one or more of the following conditions: diabetes mellitus, known coronary disease, prior ischemic stroke, estimated 10-year incidence of coronary death or non-fatal myocardial infarction >10%.

### 1.7.2. Recommendations for lipid management in patients with moderate to severe chronic kidney disease

***Table 1.1. Guidelines for the management of Dyslipidaemias***

Guideline for dyslipidemia presented in bellowed table (Catapano et al., 2017)

| Recommendations | Class | Level |
|---|---|---|
| CKD patients stages 3 - 5 are considered at high or very high CV risk. | I | A |
| Single-use statins or statin and ezetimibe combination is indicated in CKD patients not treated by dialysis. | I | A |
| Statins should not be prescribed in CKD patients treated by dialysis and without atherosclerotic CVD. | III | A |
| In CKD patients previously treated with statins, ezetimibe, or statin/ezetimibe combination at the dialysis initiation time, these drugs should be continued, especially in patients with CVD. | IIb | C |
| Statin therapy should be considered in adults with kidney transplantation. | IIb | C |

- Using SCORE risk for adults > 40 years old not diagnosed as chronic CVD, CKD, diabetes, or familial cholesterolemia.

- High and very high-risk persons: evidenced CVD, diabetes mellitus, renal disease (moderate to severe), very high-risk or high SCORE risk, and the high priority for intensive advice for all risk factors.

- LDL-Cholesterol should be suggested to be the primary lipid analysis for screening, diagnosis, and management of dyslipidemia. HDL-Cholesterol is considered an independent risk factor.

- LDL-Cholesterol is suggested to be the primary target for treatment.

- It is recommended in patients at very high CV risk, LDL-C objective of 70 mg/dL, or a reduction of at least 50% (if the baseline LDL-Cholesterol is between 70 and 135 mg/dL).

- It is suggested in patients at high CV hazard, an LDL-Cholesterol objective of 100 mg/dL, or a decrease of at least 50% (if the baseline LDL-Cholesterol is between 100 and 200 mg/dL).

- A statin is usually the first-line treatment to reach the LDL-C goal and should be prescribed up to the highest recommended dose to obtain the goal.

#### 1.7.3. Recommendations for treatment goals for LDL-C

***Table 1.2. Treatment goals for LDL-C***

***Treatment goals for LDL-C are recommended as bellowed (Catapano et al., 2017)***

| Recommendation | Class | Level |
|---|---|---|
| In patients at exceptionally high CV hazard, an LDL-C objective of 70 mg/dL) or a decrease of in any event half if the baseline LDL-C is somewhere in the range of 70 and 135 mg/dL is suggested. | I | B |
| In patients at high CV hazard, an LDL-C objective of 100 mg/dL, or a decrease of at any rate half if the baseline LDL-C is somewhere in the range of 100 and 200 mg/dL is suggested. | I | B |
| For persons at low or moderate risk, an LDL-C goal of <115 mg/dL should be considered | IIa | C |

According to the guideline of Li et al. (2017), LDL-C increased cardiovascular risk in patients with CKD. Therefore, in adults having LDL-C concentration ≥ 100 mg/dL with GFR < 60 mL/min/1.73m2 without dialysis (CKD stages 3-5), statin therapy should be initiated. Ezetimibe can combine with a statin to strengthening CV protection in CKD patients (Li et al., 2017).

## 1.8. Lipid lowering drugs in CKD

### 1.8.1. Simvastatin

Simvastatin, an HMG-CoA reductase inhibitor, is a semi-synthetic derivative of lovastatin, the first statin approved by the FDA. Simvastatin has been shown to diminishes coronary dismalness, general mortality, and cardiovascular mortality also. Results from a large double-blind study with coronary heart disease patients demonstrated that simvastatin

fundamentally diminished coronary events from 28% to 19% and reduced deaths from 12% to 8% and in 5-year follow up (Talreja O, 2020).

**Mechanism of Action:** Biosynthesis of cholesterol is comprised of a multi-step pathway, in which the rate-limiting step involves the (HMG-CoA) reductase enzyme. Mevalonic acid is formed under the action of Acetyl-CoA, a substrate, and next reactions result in cholesterol formation. Simvastatin decreased cholesterol concentrations by inhibitor HMG-CoA reductase. By lowering the synthesis of cholesterol in the liver through competitively 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA reductase), simvastatin help to reduce cholesterol. In response to the decrease in hepatic LDL-C, hepatic LDL receptors will increase as a result of a compensatory upregulation, and consequently, LDL-C being taken up from the blood into the liver. Simvastatin also reduces dyslipidemia associated complications (Talreja O, 2020).

**Administration:** Simvastatin is available in 5 mg, 10 mg, 20 mg, 40 mg, and 80 mg tablets. The initial dose for hypertriglyceridemia treatment is recommended from 10 to 20 mg daily, and the maximum is 40 mg. The dose is adjusted to target goal LDL levels (Talreja O, 2020).

**Indications**: Simvastatin is indicated for patients with high cholesterol levels and other coronary risk factors. It has been evidenced to be an effective lipid-lowering drug and it is utilized in all types of extreme essential hypercholesterolemia. Also, Simvastatin is indicated as an adjunct to diet.

**Contraindications:** Patients who have active liver disease including elevated hepatic enzymes, pregnancy, or breastfeeding are contraindicated to simvastatin (Talreja O, 2020).

**Adverse Effects**: headache, myalgia, abdominal pain, constipation, and upper respiratory infections are common adverse effects. Rarely are atrial fibrillation, hepatic abnormalities including hepatitis with jaundice, more than 3-fold increase in transaminases, and potential liver failure. Greater than a three-fold elevation in creatine phosphokinase (CPK) levels, rhabdomyolysis, and compartment syndrome in the lower legs is considered an adverse musculoskeletal effect (Talreja O, 2020).

**Monitoring:** continuing laboratory monitoring for lipid profile is not necessary for patients on simvastatin therapy. It should be evaluated 4 weeks after initiation and then adjust the dose. Transaminase tests are performed at baseline and then, depending on clinical

symptoms such as abdominal pain, jaundice, fatigue, and loss of appetite. Creatine phosphokinase levels are also needed to be evaluated at baseline and periodically, especially in high-risk patients such as renal insufficiency. Patients with medication combination should be closely monitored for musculoskeletal and hepatic unexpected effects may be caused by potential simvastatin toxicities (Talreja O, 2020).

**Toxicity:** in practice, patients with mild musculoskeletal symptoms, temporary stop simvastatin, and then re-challenge at a lower dose. If a lower dose also leads to similar adverse effects, discontinue and replace with an alternative statin. Discontinuation is warranted if the patient presents severe musculoskeletal symptoms (Taylor, Panza, & Thompson, 2016), severe hepatotoxicity, hyperbilirubinemia, or jaundice (Baber & Muntner, 2014).

### 1.8.2. Ezetimibe

Ezetimibe is a lipid-lowering agent, approved by the FDA in 2002. Ezetimibe acts as an intestinal cholesterol absorption inhibitor and is indicated in reducing TC, LDL, apolipoprotein B, HDL-C in patients with primary hyperlipidemia, mixed hyperlipidemia, familial hypercholesterolemia, and homozygous sitosterolemia. Ezetimibe can be as monotherapy, in combination with fenofibrate or with HMG-CoA reductase inhibitors (Sizar O, Updated 2020 Apr 21).

**Mechanism of Action**: Ezetimibe, an inhibitor, inhibits cholesterol absorption at the brush border of the small intestine by the sterol transporter. Declining cholesterol absorption in the intestine results in the decrement of cholesterol to the liver and the increase of cholesterol from the blood. Play the role of an inhibitor of intestinal cholesterol absorption, ezetimibe diminishes hepatic cholesterol stores. Diminishing TC, TG, LDL-C, and increasing HDL-C are consequences of the reduction in cholesterol absorption (Sizar O, Updated 2020 Apr 21).

Ezetimibe metabolism occurred in the small intestine and the liver; and then, via bile, it is excreted back into the gastrointestinal tract, and once again inhibits the absorption of cholesterol. By reducing cholesterol intake, ezetimibe causes a depletion of LDL-C stores in the liver and leads to upregulation of hepatic LDL receptors, result in taking LDL-C to the

liver from the blood (Phan, Dayspring, & Toth, 2012). Different from other cholesterol-lowering drugs, ezetimibe neither increases bile acid excretion nor inhibits cholesterol synthesis in the liver.

**Administration:** Ezetimibe is orally administered. With a 22 hours half-life, it is recommended 10 mg once daily with or without meals and is often in adjunctive with a cholesterol-lowering diet. Ezetimibe is regularly used in combination with fenofibrate or statin. It could be used at the same time with fenofibrate or statin, but it is better to be taken 2 hours before or 4 hours after the administration of bile acid equestrian drugs (Sizar O, Updated 2020 Apr 21).

**Adverse effects**: Headache, runny nose, and sore throat are the most common adverse effects. Less common symptoms are body aches, back pain, chest pain, diarrhea, joint pain, fatigue, and weakness.

**Contraindications:** concomitant use with an HMG-CoA reductase inhibitor in patients with active hepatic disease, unexplained persistent elevations in serum transaminases, or hypersensitivity to any component of the formulation, are contraindications of ezetimibe. In pregnancy or breastfeeding, it is not prescribed in combination with an HMG-CoA reductase inhibitor. It is not necessary to adjust the dosage for patients with renal impairment if it is used as a monotherapy. Ezetimibe is not suggested in patients with moderate to severe hepatic impairment. Ezetimibe given with a statin is contraindicated in patients with hepatic impairment (Sizar O, Updated 2020 Apr 21).

**Monitoring:** it is necessary to follow a lipid profile at baseline and as clinically indicated after that. If using a combination agent that contains a statin, liver function tests also need to be obtained at baseline (Sizar O, Updated 2020 Apr 21).

**Toxicity:** hypothyroidism, renal impairment, or skeletal muscle toxicity are risks of concomitant use of statin. This toxic will increase with age over 65 years old (Sizar O, Updated 2020 Apr 21).

## 1.9. Oxidative stress and inflammation

Systemic inflammation and oxidative stress occurred in CKD patients. By damaging to molecular components of the kidney, oxidative stress and inflammation promote renal damage. In CKD, oxidative stress and inflammation have cyclical relationships. The inflammatory process in CKD helps to repair radical-mediated damage but it is also a source of other free radicals that lead to further damage to renal tissue. (Tucker, Scanlan, & Dalbo, 2015). Results from Xu et al. study demonstrated that oxidative stress and inflammation interacted with each other to play an important role in the progression of CKD. Oxidative stress and inflammation changed parallel when CKD was developing. During an exacerbation, these markers increase higher. (Xu et al., 2015).

### 1.9.1. Oxidative stress

Oxidation is a process that happened in the body to eliminate pathogens and toxic metabolites in physiological conditions. Oxidative stress occurred when reactive oxygen species (ROS) are delivered more than antioxidants. This will harm cell structures, including lipids, proteins, and DNA. In other words, oxidative stress is characterized by an imbalance between the production of free radicals and antioxidant capacity. Lipid peroxidation is the noticeable consequence of free radical damage (Valko et al., 2007). Free radical is characterized by one or more unpaired electron(s) in its external shell and oxygen that has a vital role in their formation. The term ROS refers to the reactive radicals of oxygen. ROS represents a large variety of oxygen free radicals, such as superoxide anion (O2-), hydroxyl radical (OH-), and hydrogen peroxide (H2O2). In normal physiological conditions, ROS level is regulated by cells via balancing the production and the elimination of ROS. But under oxidative stress conditions, excessive production of ROS can result in fatal damage in the cells (Chandrasekaran, Idelchik, & Melendez, 2017).

In CKD patients, structural change, loss of renal energy, and high uremia levels are considered to result in an imbalance between free radicals and antioxidants. Besides that, accompanied by CKD are usually hypertension, diabetes mellitus, dyslipidemia, and known cardiovascular risk factors. These factors are evidenced to associate with oxidative stress that

can trigger the inflammatory process and promote the progression of renal injury. Oxidative stress is involved in the acceleration in CKD progression, directly through glomerular damage and renal ischemia or indirectly through inflammation, hypertension, and endothelial dysfunction. Related factors of oxidative stress status in CKD include malnutrition, inflammation, increased oxidase activity, or a decrease in oxidative defenses (Modaresi, Nafar, & Sahraei, 2015).

There are some clinical biomarkers to detect oxidative stress and antioxidant status in CKD patients. Oxidant has a very short half-life so it is difficult to directly measure oxidative stress with precision. Therefore, indirect measurement of oxidative stress through their oxidation products was applied, such as lipid peroxidation end products, oxidized proteins, and antioxidant molecules (Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2006). Since oxidants can oxidize a variety of components such as lipids, proteins, carbohydrates, and nucleic acids; hence, the oxidation products have a longer half-life, from hours to weeks. Consequently, they can be used as oxidative stress markers (Locatelli et al., 2003). In lipids, oxidative stress causes lipid peroxidation and leads to produce malondialdehyde (MDA)(Niki, 2014).

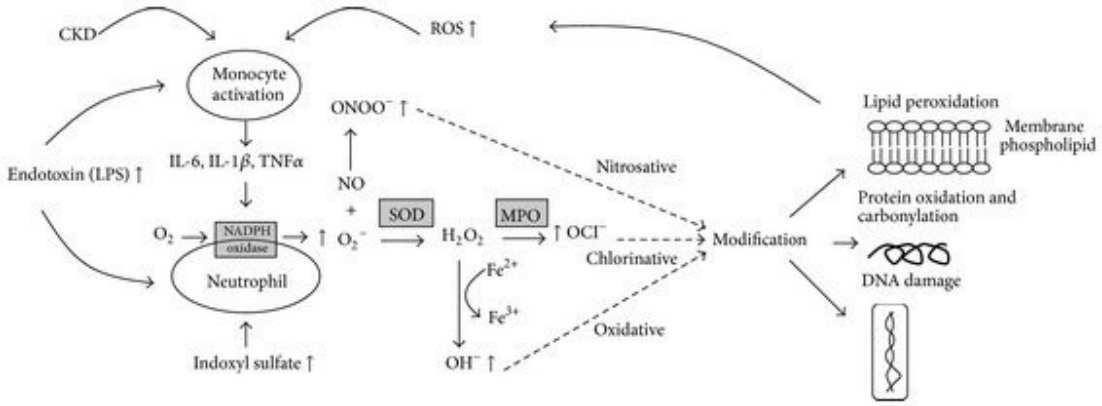



***Figure 1.1. The synthesis of reactive oxygen species in patients with CKD.***

Excessive reactive ROS including ONOO−, OH−, and OCl− is generated from oxygen through several main enzymes (NADPH oxidase, superoxide dismutase (SOD), and myeloperoxidase (MPO)). Several factors can also increase ROS generation, including

cytokines (IL-8, IL-1$\beta$, and TNF-$\alpha$) released from activated monocytes, uremic toxin (indoxyl sulfate), and endotoxin (LPS) from the HC procedure. The resulting excessive ROS can lead to nitrosative (ONOO−), chlorinative (OCl− ), and oxidative (OH−) modifications to lipids, proteins, and DNA (Sung et al., 2013).

There are many biological oxidative stress markers, but contributed to the pathogenesis of inflammation in CKD patients are the oxidation product of LDL (ox-LDL), advanced glycosylation end products (AGEs), and oxidized thiol components. Thiobarbituric acid-reactive substance (TBARS), a production of lipid oxidation, increases in CKD patients and is associated with endothelial dysfunction. Whereas, as a result of protein oxidation, advanced oxidation protein products (AOPPs), increases in the uremic state, and their level rises in accordance with the decline of renal function. By interacting with nucleic acids of cell, oxidants inactive mitochondrial enzymes, and directly cause DNA damage. As a result, DNA repairs enzymes and transcription factors, thereafter resulting in cell death (Modlinger, Wilcox, & Aslam, 2004). 8-hydroxy-2'deoxyguanosine (8-OHdG) was found an increase in CKD patients is a nucleic acid oxidation product. Therefore, AOPPs and 8-OHdG are also often used as markers of oxidative stress in patients with CKD (Locatelli et al., 2003).

CKD patient with uremia and leukocyte produces superoxide radical. Kidney normally excretes indoxyl sulphate (IS), an organic anion, known as a form of the urea toxin. In CKD patients, these anions will accumulate in the blood, exacerbate inflammation, and impairs vascular endothelial function, thereby aggravate the progression of CKD (Sung, Hsu, Chen, Lin, & Wu, 2013).

Risk factors of uremia in CKD patients, including volume increase, chronic inflammation, anemia, are associated with systemic oxidative stress that can cause inflammation and additional tissue damage (Locatelli et al., 2003). Therefore, oxidative stress can affect the progression of kidney disease in a two-way direction. It can exacerbate inflammation, which contributes to the development of fibrosis and inflammation through enhanced and activated the signaling pathway results in cell death of renal tubular. Fibrosis and inflammation may increase ROS formation (Hosohata, 2016). According to the studies

above, oxidative stress is demonstrated closely related to CKD and its complications.

Among the numerous biological targets of oxidative stress, lipids are the most involved in biological molecules. Lipid oxidation generates some secondary products, which are mainly aldehydes, with the possibility to exasperate the unsafe impacts of oxidation. The aldehydes are relatively stable and have been proven to be genotoxic by reaction with proteins and nucleic acids. MDA is one of the final products of intracellular polyunsaturated fatty acid peroxidation. The increase in free radicals causes MDA overproduction. MDA level is regularly known as an oxidative stress marker and antioxidant status in CKD patients. (Del Rio, Stewart, & Pellegrini, 2005).

### 1.9.2. Inflammation

Inflammation as a part of CKD was recognized in the late 1990s when it was demonstrated to be linked to cardiovascular disease and mortality (Stenvinkel et al., 1999). Over the years, there has been increasing exponentially in inflammation in CKD and resulted in the growth in the awareness of our inflammation. Today, inflammation is considered a risk factor of traditional morbidity and mortality in CKD.

The inflammatory cytokine in CKD not as it was delivered by lymphocytes, but too by different tissue, such as adipose tissue, become dysfunctional in CKD (Iglesias & Díez, 2010). Consequently, visceral adipose tissue in ESRD exhibits high mRNA expression of proinflammatory cytokines, such as TNF-α, CD68, adiponectin-1 receptor, and chemoattractant monocyte protein. In stages 3-5 of CKD, visceral fat volume correlates with circulating IL-6 (Kerr et al., 2013).

Increasing plasma concentrations of fibrinogen, TNF-α, and diminishing serum albumin are related to rapid loss of renal function in CKD patients (Amdur et al., 2016).

The renin-angiotensin system plays a critical part in blood pressure regulation. This system also plays a part in the pathogenesis of inflammation and the progression of CKD. T cells, known as natural killer cells, and monocytes are formed when multiple inflammatory processes are capable of expressing angiotensin II and angiotensin type 1 (AT1) receptors. Through the AT1 receptor, Angiotensin II can stimulate oxidative stress through oxidative

bursts, capable of stimulating phagocytic and chemical activity. So, in addition to the effect of inflammation on its formation, angiotensin II also induces inflammation and aggravates the process itself (Cohen & Hörl, 2012).

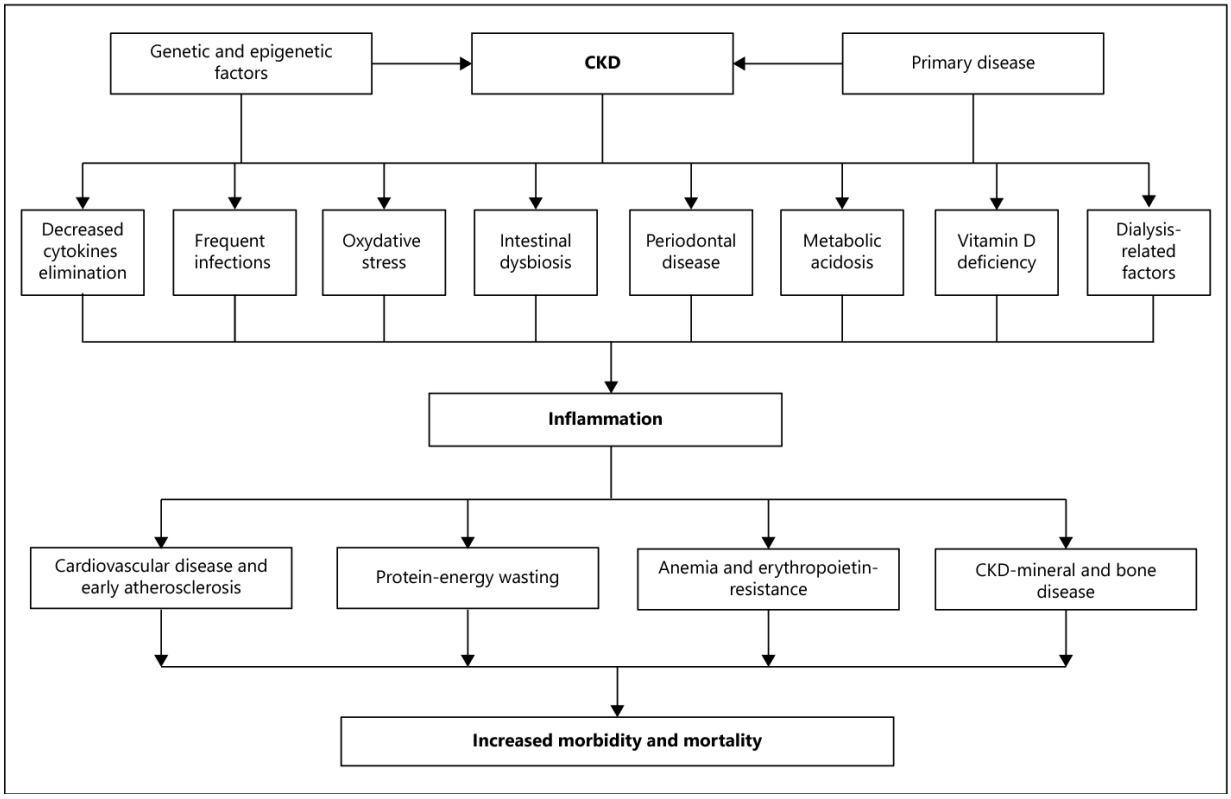



***Figure 1.2. The causes and consequences of inflammation in chronic kidney disease***

Above figure expressed the causes and consequences of inflammation in CKD (Akchurin & Kaskel, 2015). Tryptophan, the essential amino acid, acts as a precursor to several metabolic pathways involved in various end products, such as proteins, serotonin, melatonin, and kynurenines (Kyn). Tryptophan (Trp) is oxidized by indole ring cleavage by tryptophan 2,3-dioxygenase (TDO) and/or indoleamine 2, 3-dioxygenase (IDO). TDO is expressed primarily in the liver and is induced by Trp or corticosteroids. IDO, on the other hand, is overwhelming in extra-hepatic cells, counting macrophages, microglia, neurons, and astrocytes. Within the liver, about 99% of Trp is metabolized to Kyn by TDO via the kynurenine pathway under physiological conditions. The first rate-limiting enzymatic step involving IDO is activated in inflammatory conditions such as infections or oxidative stress. IDO activity is

upgraded by pro-inflammatory cytokines, such as IFNγ, IL-1, IL-2, IL-6, and TNFα, and is inhibited by the anti-inflammatory cytokine IL-4. (Capuron et al., 2011), (Chen & Guillemin, 2009). In this way, when the immune response is activated, the Kyn pathway is systemically up-regulated. Subsequently, the Kyn to Trp concentrations ratio reflects the Trp breakdown linked with inflammation conditions (Chen & Guillemin, 2009). Inflammation is a critical burden on patients with chronic kidney disease. The persistent rise of inflammatory markers appears to be exacerbated by disease progression. Inflammation may support a decrease in renal function by promoting endothelial dysfunction, atherosclerosis, and glomerular damage. Even though the mechanisms responsible for inflammation in CKD are not completely clear, oxidative stress has been proposed as a likely supporter of inflammation in renal disease. Oxidative stress and inflammation are inseparably linked as they form a vicious cycle, in which oxidative stress provokes inflammation by several mechanisms including activation of NF-κB, with consequent activation and recruitment of immune cells.

Inflammation is a noteworthy burden for patients with CKD. The constant increment in inflammatory markers shows up to be exacerbated by the progression of the disease. Inflammation can impair kidney function by promoting endothelial dysfunction, atherosclerosis, and glomerular damage. Inflammation can impair kidney function by advancing endothelial brokenness, atherosclerosis, and glomerular harm. In spite of the fact that the inflammation mechanisms in CKD are not completely clear, oxidative stress has been suggested to be the underlying cause of inflammation in kidney disease. Oxidative stress and inflammation have ties inseparable since they frame a vicious circle, in which oxidative stress stimulates inflammation by several mechanisms counting activation of NF-κB, in this manner enacting and recruiting immune cells (A. Zinellu et al., 2015).

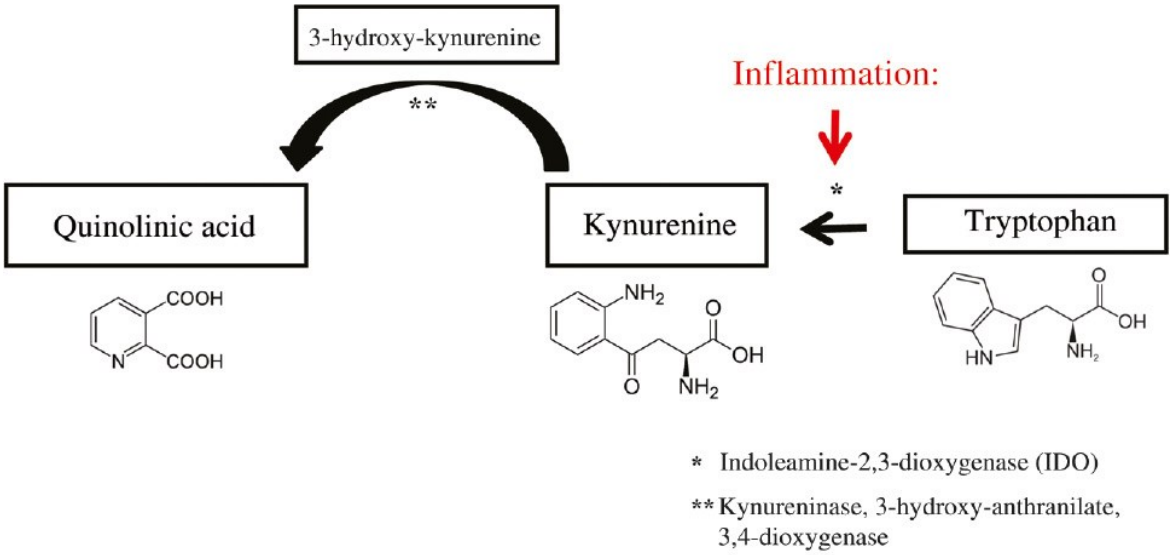



***Figure 1.3. Schematic overview on the Tryp-Kyn pathway (Verheyen et al., 2017).***

*Inflammation factors (e.g. interferon gamma, IL-1, IL2, IL-6, TNF – α) induce the expression of the enzyme IDO in monocytes/macrophages and dendritic cells.*

## 2. RESEARCH OBJECTIVES

In clinical practice, doctors consistently concern about the impacts and security before giving the medicine. Despite the high frequency of statin treatment, only 1/3 of CKD patients accomplished the LDL-C aim. The effect of a higher dose of mono-therapy statin in LDL-C lowering is still unclear, but its side effect is associated with a high incidence of hepatotoxicity or myopathy. Lowering LDL-C with statin monotherapy or statin/ezetimibe combination reduces the risk of CVD in the population without kidney disease. Which cholesterol-lowering therapies are suitable for stage 3, 4 CKD patients in terms of eGFR reduction and side effects? There is no information identified with this field in the Vietnamese CKD population. Therefore, further advanced lipid-lowering therapies and a superior comprehension of the mechanism are needed for the treatment strategy of hyperlipidemia in Vietnamese patients with CKD. The accompanying exploration questions were tended to: (1) Do distinctive lipid-lowering regimes (simvastatin monotherapy and ezetimibe/simvastatin combination therapy) differently affect the lipid profile, oxidative stress indices, Tryptophan degradation? (2) Does statin monotherapy or co-administration of ezetimibe improve the renal dysfunction in CKD patients? (3) How does lipid-lowering therapy impact lipidemia disorders in patients with CKD by mechanism involved in oxidative stress and tryptophan? (4) Are the unexpected consequences for muscle issues and hepatic chemical profile of long-term statin monotherapy and statin/ezetimibe combination therapy a major concern?

We hope with scientific evidence, doctors will surely know the mechanism, impact, and safety of potential and existing therapies. Along these lines, the prescription rate of lipid-lowering medications and control rate of hyperlipidemia could be expanded in CKD patients. The significant reason for existing is to improve clinical outcomes of CKD patients through the control of hyperlipidemia. With that point, we directed this exploration with the accompanying objectives:

1. To evaluate the effect of lipid-lowering regimes (simvastatin monotherapy and EZE/simvastatin 10/20 and EZE /simvastatin 10/40 combination therapy) on lipid profiles, oxidative stress indices, Tryptophan degradation in CKD patient.

2. To evaluate the proteinuria-lowering effects of cholesterol-lowering therapies.
3. To understand the relationship between oxidative stress, tryptophan degradation during treatment with lipid-lowering regimes.
4. To describing the muscle problems (muscle pain, CK concentration) and hepatic enzyme profile during treatment.

# 3. MATERIAL AND METHODS

## 3.1. Study design

This is a prospective study with clinical trials of 12 months follow-up was conducted between October 2017 and September 2020.

## 3.2. Subject recruitment

30 patients and 30 control participants were recruited for the study (with sex, age-matched). This study included patients who were admitted to Family Medical Centre, and Department of General Medicine - Endocrinology, HUMP, Viet Nam. The patients and control participants were chosen based on the following criteria:

### 3.2.1. Inclusion criteria

We enlisted patients with all of the following criteria into the study

- Patient diagnosed with CKD in the 3-4 stage (e-GFR: 15-60 ml/min/1.73 m2)

- Presence of proteinuria CKD defined as creatinine clearance >20 ml/min/1.73m2 combined with urinary protein excretion rate >0.3 g/24 h

- LDL cholesterol concentration > 100 mg/dl (2.59 mmol/l).

- Age: ≥ 50 or from 18-49 years old with one or more of the following: diabetes mellitus, known coronary disease, prior ischemic stroke, estimated 10-year incidence of coronary death or non-fatal myocardial infarction >10%.

#### *3.2.1.1. Diagnosis of chronic kidney disease:*

Patients diagnosed with chronic kidney disease based on the National Kidney Foundation/ the 2012 Kidney Disease: Improving Global Outcomes (NKF/KDIGO-2012) (Inker et al., 2014), including:

- Signs of kidney damage (lasting for more than 3 months):

+ Albuminuria (albuminuria ≥ 30 mg/24h).

+ Hematuria.

+ Electrolyte abnormalities due to renal tubular dysfunction.

+ Abnormalities detected through prehistoric exploitation.

+ Abnormalities were detected through kidney-urinary ultrasound examination (the size of the kidneys may be smaller than normal, the hyper parathyroid tissue may be inferior, and the bone marrow distinction will be poor).

- And / or glomerular filtration rate is below 60 ml/min/1,73m$^2$ for 3 months or more.

#### *3.2.1.2. Stage of chronic kidney disease*

According to NKF / KDIGO-2012, chronic kidney disease is classified into 5 stages based on eGFR as follows (Inker et al., 2014):

***Table 3.1. Stages of chronic kidney disease***

| **Stage** | **Terms** | **eGFR** (ml/min/1,73m$^2$) |
|---|---|---|
| 1 | Normal or high | ≥ 90 |
| 2 | Mildly decreased | 60 - 89 |
| 3a | Mildly to moderately decreased | 45 - 59 |
| 3b | Moderately to severed decreased | 30 - 44 |
| 4 | Severely decreased | 15 - 29 |
| 5 | Kidney failure | < 15 |

Diagnosis of the stage of chronic kidney disease is based on estimated Glomerular Filtration Rate. In this study, eGFR was calculated by the Modification of Diet in Renal Disease (MDRD 4-variable GFR Equation) 2007 formula (National kidney foundation)

eGFR = 175 x sCr$^{-1,154}$ x age$^{-0,203}$ x 1,212 (if patient is black) x 0,742 (if female).

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m$^2$

sCr (standardized serum creatinine) = mg/dL.

µmol/L x 0,0113 = mg/dL.

age = years

### 3.2.2. Exclusion criteria

These patients did not recruit into the study:

- Patients who refuse to join in this study

- CKD patient who dialysis-dependent

- CKD patients with heart failure (New York Heart Association class III or more)

- CKD patients that previous or concomitant taking corticoids, statin, immunosuppressive agents, vitamin B6, B12, folate

- Pregnancy

- CKD patients do not agree to participate in the study

- CKD patients are unable to understand the risks of the study, or patients can not provide a written informed consent form.

#### 3.2.3. Criteria control group

The control group was recruited from healthy check-up subjects. The subjects were considered as normal through comprehensive clinical examination and conducting tests, including urea, serum creatinine, fasting serum glucose, aminotransferases in normal limits.

- Aged, sex-matched subjects

- Persons do not have a history of diabetes, hypertension, cardiovascular or cerebrovascular disease, renal failure, blood dyscrasias, cancer, and retinal vascular disorders.

Persons do not currently use medication with vitamin B6, B12, or folic acid.

### 3.3. Study place

- In Viet Nam: Internal examination room, Family Medicine Centre, Department of General Internal medicine & endocrinology, Department of Biochemistry, Medical Genetics Department of Hue University of Medicine and Pharmacy

- In Italy: Department of Biomedical Science, Sassari University.

### 3.4. Data collection

The data collected in the study included clinical and demographical information, as age, gender, weight, height, body mass index (BMI), education level, systolic blood pressure, diastolic blood pressure, and smoking status; subclinical characteristics such as TC, TG, LDL-C, HDL-C, SGOT, SGPT, CK, urea, creatinine, estimated glomerular filtration rate, MDA, PSH, kynurenine, PON, TEAC, kynurenine, tryptophan, kynurenine to tryptophan ratio were collected using a structured questionnaire.

- Potentially eligible patients attended a screening visit in an internal examination room or internal department of Hue University Hospital for medical history and other eligibility criteria checked.

- Fasting blood samples were taken to analyzed conventional lipid profiles (Total C, LDL- C, DHL-C, and Triglyceride), proteinuria, urea and creatinine, creatine kinase, SGOT, SGPT, total blood cell count, and total homeostasis test.

- Patient satisfied with the including criteria wrote the informed consent form.

- 36 enrolled patients were randomized into 3 groups and received different lipid-lowering therapies at the baseline and continue for 12 months.

- The patient underwent 12 months of treatment, clinical routine monthly follow-up, and blood test at 4, 8, and 12 months.

- At each follow-up time, study treatment was continued, clinical characteristics, unexplained muscle pain, and non-study treatment were recorded. Patients were asked for an unscheduled visit if they want to have any additional review.

- Patients were unable or unwilling to attend the follow-up checks, asked if they have any serious adverse events.

- The blood samples were sent to the Department of Biomedical Science of Sassari University for analyzing oxidative stress indices and tryptophan degradation indices every 4 months.

- 30 patients were included. The following procedure is shown in the bellowed chart:

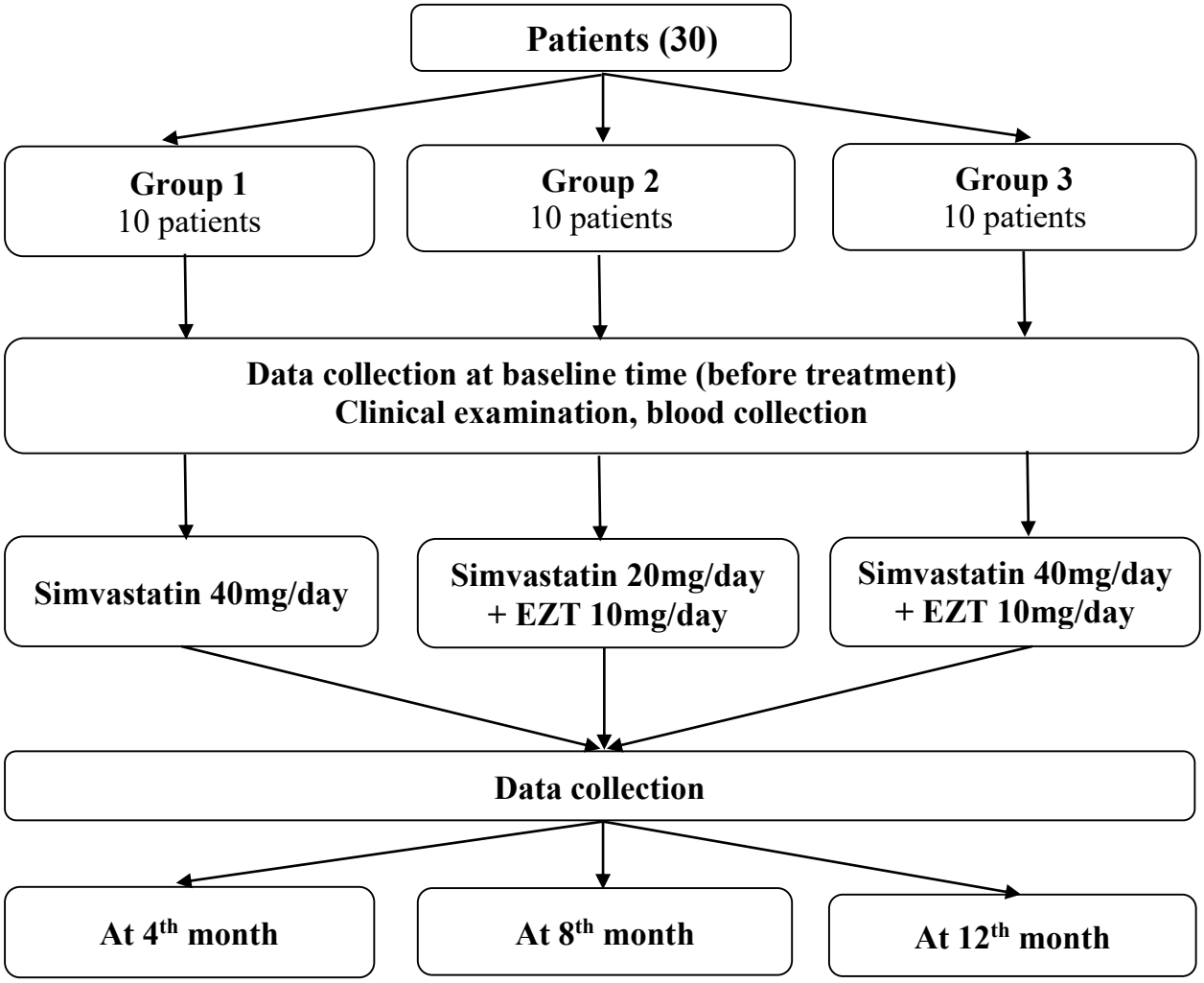



***Figure 3.1. Workflow of study***

### 3.5. Treatment therapies in the study

- 30 enrolled patients were randomized into 3 groups and received one of 3 lipid-lowering therapies at the baseline and continue for 12 months.

+ Group 1: (10 participants) received 40 mg/day simvastatin

+ Group 2: (10 participants) receive ezetimibe/simvastatin 10/20 mg/day

+ Group 3: (10 participants) receive ezetimibe/simvastatin 10/40 mg/day.

### 3.6. Biochemical analysis

#### 3.6.1. Routine Laboratory Analyses

TC, TG, HDL-C, creatinine, SGOT, SGPT, CK, proteinuria, and urine creatinine levels were measured spectrophotometrically using a Cobas c501 autoanalyzer (Roche Diagnostic, USA). LDL levels were determined by utilizing the Friedewald equation. HbA1c was measured by HPLC (high-pressure liquid chromatography) method on the Tosoh G8 Analyzer (Tosoh Bioscience, South San Francisco, CA) (Roche, 2020).

3.6.2. **Oxidative stress indexes**

**- Malondialdehyde measurement**

MDA level was measured according to the spectrophotometric measurement of the color that occurred during the reaction of thiobarbituric acid with MDA (Esterbauer & Cheeseman, 1990). Reset the spectrophotometer reading blank sample at 535 nm, then read first the standard curve and then all the samples (Niki, 2014). MDA measurement was performed in the clinical biochemistry laboratory in Sassari, Italy.

**- Protein thiols - SH (PSH)**

Protein - SH was determined in plasma protein and was performed by the spectrophotometric using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as the titrating agent by measuring the absorbance of conjugate at 405 nm. Moreover, the GSH standard curve was used to determine sample concentration. PSH Level is normalized with the quantity of plasma proteins measured by Lowry's method (Ellman, 1959). PSH measurement was performed in the clinical biochemistry laboratory in Sassari, Italy.

**- Paraoxonase (PON)**

The activity of PON is determined by measuring the increase in absorbance at 412 nm, which is due to the formation of 4 nitrophenols used as substrate the paraoxon (O, O-diethyl-O-p-nitrophenyl phosphate). Paraoxonase activity was assessed using the molar extinction coefficient of 17 100 / Mcm, and a unit of enzyme activity is defined as 1 nmol 4-nitropherol formed in a minute. Paraoxonase activity is calculated in U/L. (Gan, Smolen, Eckerson, & La Du, 1991). PON measurement was performed in the clinical biochemistry laboratory in Sassari, Italy.

**- Trolox equivalent antioxidant capacity (TEAC)**: One of the most regularly utilized direct tests is the Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) with modifications predominantly dependent on the time used for measurement, and radical formed (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993).

#### 3.6.3. Tryptophan degradation indexes

**Tryptophan and kynurenine measurement**

Tryptophan and kynurenine quantification of was dictated by capillary electrophoresis equipped with a UV detector. Collect blood into evacuated tubes containing EDTA, and then centrifuged at 3000 × g for 5 min at 4°C. Mix 100 µl of obtained plasma with 50 µl of methyltryptophan in internal standard (50 µmol/l) and 1 ml of cold acetonitrile. After that, we evaporated 1 ml of supernatant under vacuum and redissolved the residue with 80 µl of water. Then, the sample was injected in capillary electrophoresis (A. Zinellu et al., 2012). This technique was performed in the clinical biochemistry laboratory in Sassari, Italy.

### 3.7. Ethical approval

The explanation about the study and informed consent form was obtained from patients and the controllers. This study project was approved by the Ethical Committee of Hue University of Medicine and Pharmacy.

The study complied with the standards of the Helsinki Declaration and was enrolled with clinical trials, gov (NCT03543774).

### 3.8. Statistical analysis

SPSS software, version 20.0 64 bit (IBM Corporation, NY, USA) was applied to do statistical analysis. Continuous data were expressed as mean ± standard deviation and the categorical data were expressed as percentages. Comparisons between the control and CKD group were analyzed by the Student's t-test. One way ANOVA was used to compare the difference in 3 treatment therapies. The Levene's test was used to assess the equality of error variances and the Student-Newman-Keuls was used to access pairwise comparisons. The effect of the drug treatments on continuous variables by time was evaluated by one-way

repeated measures ANOVA. Interaction between different drug therapies and time of treatment on variables were analyzed by MIX REPEAT ANOVA. Correlation analysis between the pair of variables was performed by Pearson's correlation. Statistical significance was declared if the P-value was less than 0.05.

# 4. RESULTS

## 4.1. Demographic and clinical characteristics of patients and controls at baseline

### 4.1.1. Demographic and clinical characteristics of patients and controls at baseline

***Table 4.1. Demographic and clinical characteristics of patients and controls at baseline***

| **Demographic and clinical characteristics** | | **Controls** | **CKD patient** | **p-value** |
|---|---|---|---|---|
| | | **Mean ± SD** | **Mean ± SD** | |
| **Gender** | Male | 12 (40%) | 13 (43.3%) | 0.071[b] |
| | Female | 18 (60%) | 19 (56.7%) | |
| **Age** | | 65.80 ± 10.62 | 65.28 ± 10.57 | 0.793[a] |
| **BMI** | | 22.09 ± 2.1 | 22.81 ± 1.32 | 0.117 [a] |
| **Blood pressure** | Systolic BP, mmHg | 122.5 ± 11.12 | 150.78 ± 19.39 | <0.001 [a] |
| | Diastolic BP, mmHg | 74.33 ± 6.66 | 85.16 ± 11.53 | <0.001 [a] |
| **Kidney profile** | Creatinine, mg/dL | 0.77 ± 0.16 | 2.06 ± 0.64 | <0.001 [a] |
| | eGFR, ml/min/1.73m$^2$ | 86.18 ± 25.35 | 31.76 ± 11.18 | <0.001 [a] |
| | Proteinuria, g/24h | 0.1 ± 0.31 | 1.41 ± 1.48 | <0.001 [a] |
| **CRP (mg/l)** | | 5.83 ± 11.8 | 10.38 ± 7.13 | 0.059 [a] |
| **SGOT (U/L)** | | 25.57 ± 9.97 | 27.22 ± 11.95 | 0.558 [a] |
| **SGPT (U/L)** | | 23.97 ± 7.48 | 27.57 ± 12.65 | 0.119 [a] |
| **CK (U/L)** | | 53.39 ± 18.48 | 56.67 ± 22.26 | 0.379 [a] |
| **HbA1C (%)** | | 5.5 ± 0.63 | 6.47 ± 1.72 | 0.013 [a] |
| TC, mg/dL | | 156.37 ± 32.18 | 242.67 ± 25.95 | <0.001 [a] |
| TG, mg/dL | | 126.40 ± 78.46 | 214.87 ± 96.69 | <0.001 [a] |
| HDL-C, mg/dL | | 43.80 ± 13.43 | 37.20 ± 7.89 | 0.025 [a] |
| LDL | | 88.79 ± 23.18 | 160.21 ± 21.59 | <0.001 [a] |
| LDL/HDL ratio | | 2.5 ± 2.41 | 4.48 ± 1.06 | <0.001 [a] |
| **Oxidative stress indexes** | | | | |
| MDA (µmol/L) | | 2.15 ± 0.96 | 2.77 ± 1.15 | 0.031 [a] |

| | | | |
|---|---|---|---|
| PSH (µmol/L) | 4.82 ± 1.47 | 4.28 ± 0.98 | 0.108 [a] |
| TEAC (mM) | 5124.50 ± 455.91 | 4943.89 ± 401.09 | 0.000 [a] |
| PON (U/L) | 69.42 ± 42.47 | 34.13 ± 17.72 | 0.000 [a] |
| **Tryptophan degradation indexes** | | | |
| Kyn (µmol/L) | 2.25 ± 1.06 | 3.44 ± 1.49 | 0.001 [a] |
| Tryp (µmol/L) | 53.48 ± 16.69 | 41.24 ± 14.94 | 0.006 [a] |
| Kyn/Trp ratio | 0.043 ± 0.015 | 1 ± 0.07 | 0.001 [a] |

*[*] P-value for comparing between healthy controls and CKD group, [a]n Independent Samples T-Test, [b] Chi-square test of homogeneity.*

The study was conducted on 30 CKD subjects and 30 healthy controls. Demographic, clinical characteristics, oxidative stress indexes, and inflammation biomarkers of CKD patients, as well as healthy controls at baseline, are reported in Table 4.1. CKD patients and healthy controls were matched on some characteristics, such as age, gender, BMI, SGOT, SGPT, and CK. Nonetheless, CKD patients introduced higher systolic, diastolic blood pressure than controls ($p < 0.001$). As expected, results obtained show that CKD patients presented worse results of kidney profiles of urea, creatinine, eGFR, and Proteinuria in comparing healthy controls ($p < 0.001$). As revealed in another study, total cholesterol, TG, LDL-C, HDL-C, LDL/HDL ratio concentrations of CKD patients demonstrated higher versus controls ($p <0.001$) (A. Zinellu et al., 2015). CRP increased in CKD patients, even though not statistically significant ($p = 0.059$). Our results were similar to the results of Zinellu (A. Zinellu et al., 2015), concentrations of oxidative stress indexes obtained in our study showed that plasma levels of MDA were higher in CKD patients than in controls ($p =0.031$) while TSH, TEAC, PON concentration obtained in the study showed lower in CKD population compare with healthy controls but the significant difference only revealed in TEAC, PON concentration ($p < 0.001$). As displayed in Table 4.1, CKD patients present higher plasma levels of Kyn (3.44 ± 1.49 µmol/L) vs (2.25 ± 1.06 µmol/L); lower plasma levels of Tryp (41.24 ± 14.94) vs (53.48 ± 16.69) and, therefore, higher Kyn/Trp ratio (1 ± 0.07 µmol/L) vs (0.043 ± 0.015 µmol/L) contrasted with controls.

### 4.1.2. Demographic and clinical characteristics of CKD randomized groups at baseline

***Table 4.2. Demographic and clinical characteristics of CKD groups after randomized at baseline***

| Demographic and clinical characteristics | All patient | Group 1 n = 10 (Simvastatin 40 mg/day) Mean ± SD | Group 2 n = 10 Eze/Simva 10/20 mg/day Mean ± SD | Group 3 n = 10 Eze/Simva 10/40 mg/day Mean ± SD | p value |
|---|---|---|---|---|---|
| Male | 19 (59.38%) | 7 (63.63%) | 5 (50%) | 7 (63.63%) | 0.767 |
| Female | 13 (40.62%) | 4 (36.37%) | 5 (50%) | 4 (36.37%) | |
| Age | 65.07 ± 10.89 | 61.80 ± 6.6 | 66 ± 11.04 | 67.40 ± 14.07 | 0.505 |
| BMI | 22.81 ± 1.32 | 23 ± 1.3 | 21 ± 0.75 | 23.35 ± 1.67 | 0.903 |
| Systolic BP, mmHg | 150.78 ± 19.39 | 148.18 ± 22.28 | 151.5 ± 19.3 | 152.73 ± 19.94 | 0.859 |
| Diastolic BP, mmHg | 85.16 ± 11.53 | 84.55 ± 12.93 | 85 ± 12.02 | 85.91 ± 10.68 | 0.963 |
| Creatinine, mg/dL | 2.05 ± 0.64 | 1.97 ± 0.52 | 1.89 ± 0.39 | 2.29 ± 0.9 | 0.340 |
| eGFR, ml/min/1.73m$^2$ | 32.42 ± 11.14 | 34.46 ± 12.29 | 32.03 ± 7.72 | 30.77 ± 13.48 | 0.766 |
| Proteinuria, g/24 h | 1.43 ± 1.52 | 1.40 ± 1.35 | 1.40 ± 2.01 | 1.43 ± 1.52 | 0.987 |
| CRP (mg/l) | 10.70 ± 7.26 | 9.10 ± 5.63 | 13.2 ± 8.77 | 9.8 ± 7.10 | 0.415 |
| NRL | 2.43 ± 2.02 | 3.54 ± 3.11 | 1.89 ± 0.71 | 1.85 ± 0.92 | 0.291 |
| SGOT | 27.37 ± 12.33 | 23.90 ± 12.65 | 32.2 ± 15.06 | 26.00 ± 7.86 | 0.304 |
| SGPT | 27.57 ± 12.88 | 25.40 ± 15.78 | 32.8 ± 9.21 | 24.50 ± 12.42 | 0.296 |
| CK | 56.67 ± 22.26 | 52.60 ± 19.22 | 59.6 ± 29.4 | 57.80 ± 18.26 | 0.778 |
| HbA1C (%) | 6.37 ± 1.71 | 6.20 ± 1.55 | 6.3 ± 2.11 | 6.60 ± 1.58 | 0.871 |
| TC, mg/dL | 242.67 ± 25.95 | 244.00 ± 22.3 | 228.80 ±16.01 | 255.20 ± 31.57 | 0.069 |

| | | | | | |
|---|---|---|---|---|---|
| TG, mg/dL | 214.87 ± 96.16 | 232.70 ± 69.25 | 184.10 ± 88.54 | 227.80 ± 26.09 | 0.481 |
| HDL-C, mg/dL | 37.20 ± 7.89 | 37.60 ± 9.85 | 38.20 ± 5.81 | 35.80 ± 8.15 | 0.790 |
| LDL, mg/dL | 160.21 ± 21.6 | 159.14 ± 22.58 | 153.62 ± 17.75 | 167.86 ± 23.72 | 0.343 |
| **Oxidative stress indexes** | | | | | |
| MDA (µmol/L) | 2.77 ± 1.15 | 3.04 ± 1.19 | 2.50 ± 0.98 | 1.78 ± 1.31 | 0.588 |
| PSH (µmol/L) | 4.28 ± 0.98 | 4.38 ± 0.82 | 3.83 ± 0.61 | 4.65 ± 1.3 | 0.128 |
| TEAC (mM) | 4943.89 ± 401.09 | 5105.05 ± 362.43 | 4856.96 ± 389.42 | 4869.66 ± 438.16 | 0.307 |
| PON (U/L) | 34.13 ± 17.71 | 38.13 ± 17.8 | 32.41 ± 21.92 | 31.86 ± 13.72 | 0.686 |
| **Tryptophan degradation indexes** | | | | | |
| Kyn (µmol/L) | 3.44 ± 1.49 | 3.2 ± 1.26 | 3.83 ± 1.61 | 3.34 ± 1.68 | 0.685 |
| Tryp (µmol/L) | 41.24 ± 14.94 | 31.81 ± 11.40 | 48.67 ± 12.41 | 43.78 ± 16.26 | 0.174 |
| Kyn/Trp ratio | 0.10 ± .07 | 0.12 ± 0.10 | 0.09± 0.05 | 0.09 ± 0.06 | 0.554 |

*P values for comparing between 3 groups, evaluated by one way ANOVA with Bonferroni correction.*

30 patients were randomized into 3 different groups and received 3 different treatment therapies to ameliorate lipid concentration. After randomization, no significant contrasts were found among the three treatment groups (Table 4.2)

## 4.2. The change of lipid profile, oxidative stress indices, Tryptophan degradation during treatment of three lipid – lowering therapies.

### 4.2.1. The effect treatment on lipid profiles during treatment

#### *4.2.1.1. The effect of treatment on Total Cholesterol during treatment*

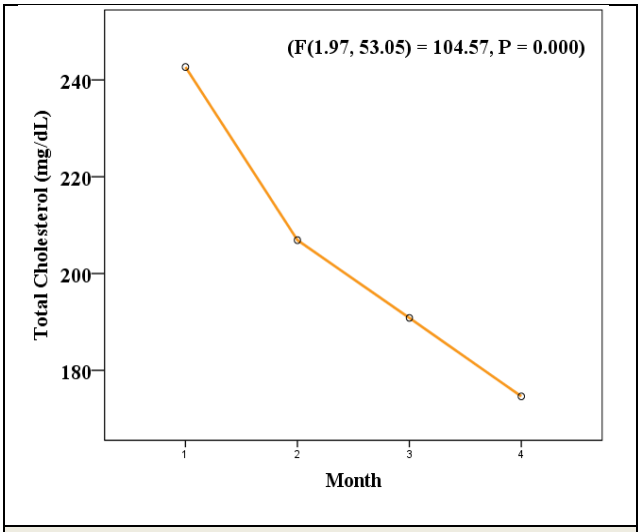



**Figure 4.1 A**

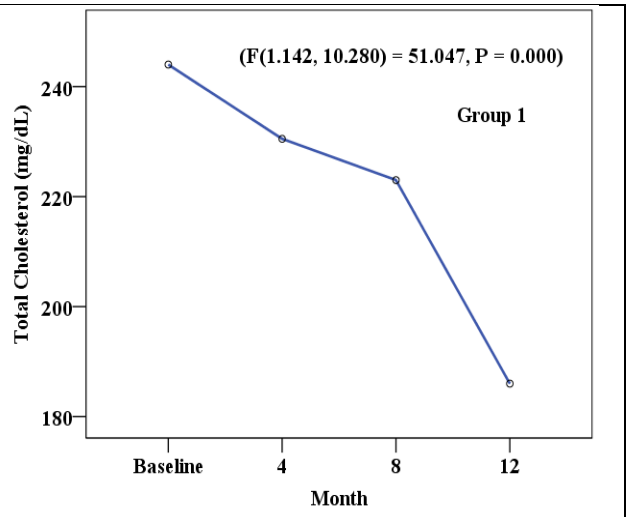



**Figure 4.1 B**

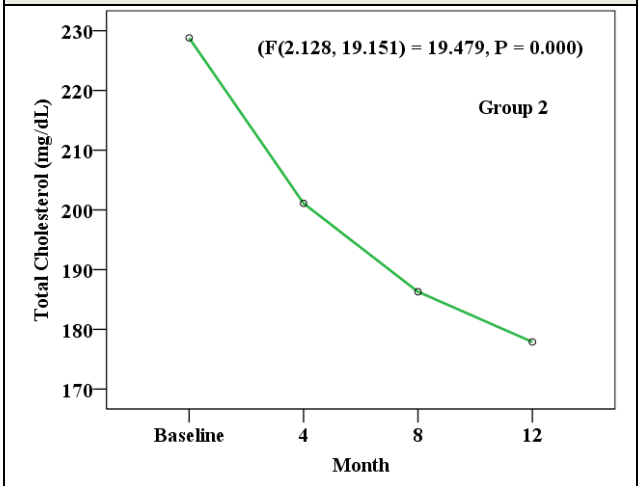



**Figure 4.1 C**

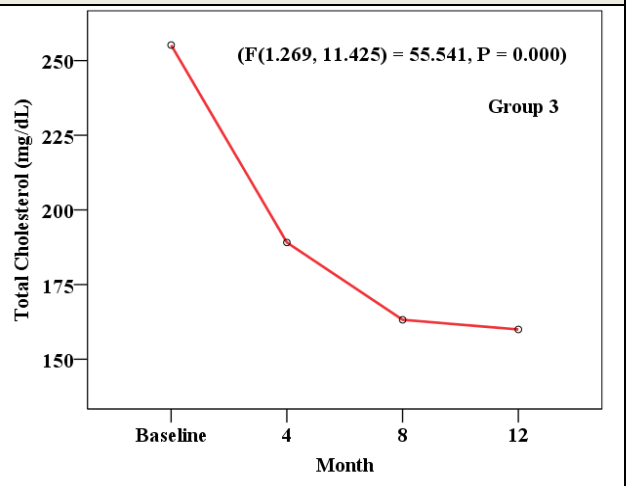



**Figure 4.1 D**

***Figure 4.1. The linear trend for Total Cholesterol during treatment***

*Figure 4.1 A: In all patients; Figure 4.1 B: In group 1; Figure 4.1 C: In group 2; Figure 4.1 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

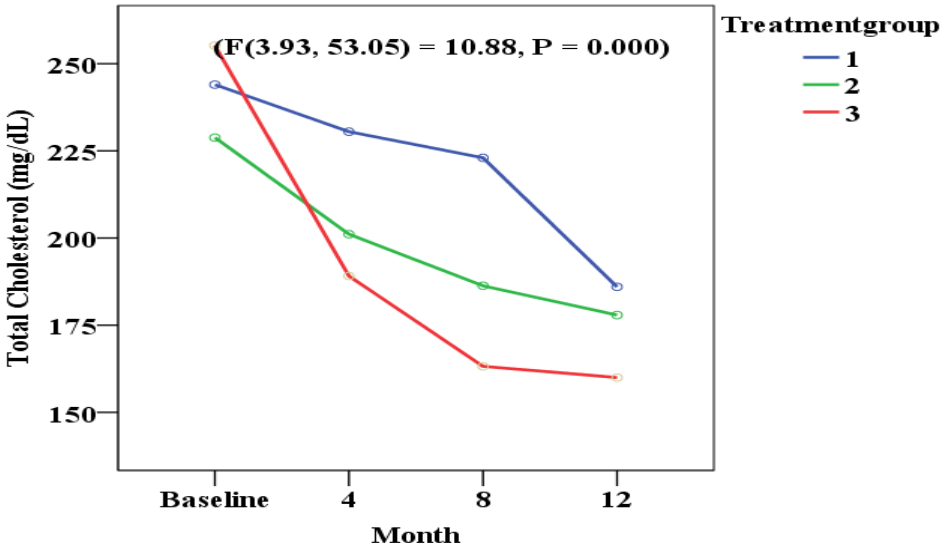



***Figure 4.2. The linear trend for Total Cholesterol during treatment between 3 groups***

*P-values have been evaluated by one way repeated measures ANOVA with Bonferroni correction*

We analyzed data by using repeated-measures ANOVA to investigate the changes in mean scores over four-time points, and differences in mean scores under three different treatment therapies. A mixed ANOVA technique was used to understand if there was an interaction between the time of treatment and the type of treatments on the dependent variable. Treatment times are considered as variables within the subject and the treatment subgroups are determined as between the subject's variables.

TC gradually decreased during treatment and there was a significant difference between the time points ($F(1.97, 53.05) = 104.57$, P = 0.000) in all patients (Figure 4.1 A) and in each group ($p<0.05$) (Figure 4.1 B,C,D). TC decreased by 28% after 12 months of treatment, of which, group 1, group 2, group 3 decreased 23%, 22% and 37%, respectively. Results from Mix ANOVA showed that there is a difference between 3 groups ($F(2, 27) = 7.656$, $P = 0.002$) (Figure 4.2). However, in multi comparison, significant difference only observed between group 1 vs. group 2 (p = 0.023), group 1 vs. group 3 (p = 0.003), no difference between group 2 vs. group 3 was seen (p = > 0.05).

### *4.2.1.2. The effect of treatment on Triglyceride during treatment*

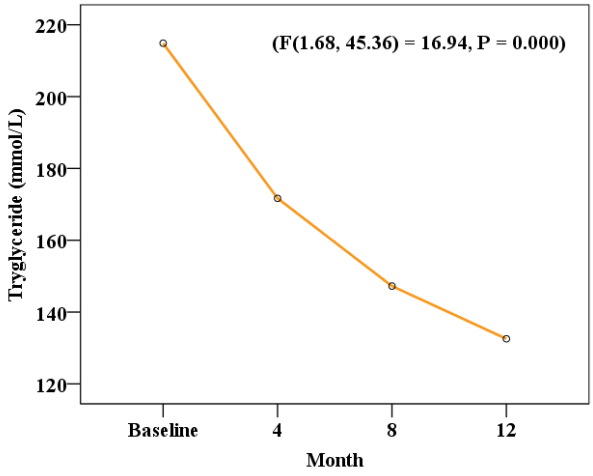



**Figure 4.3 A**

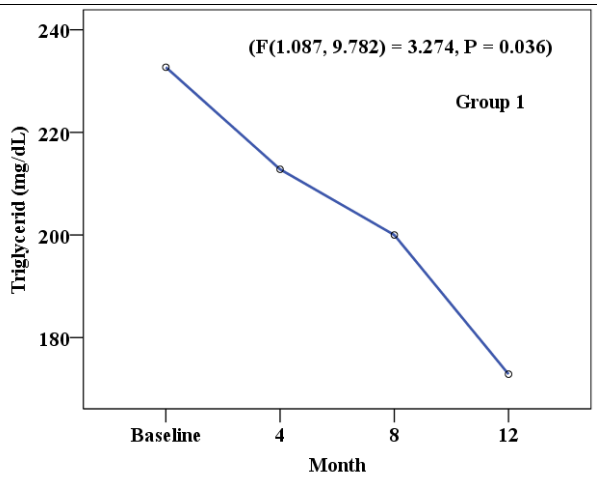



**Figure 4.3 B**

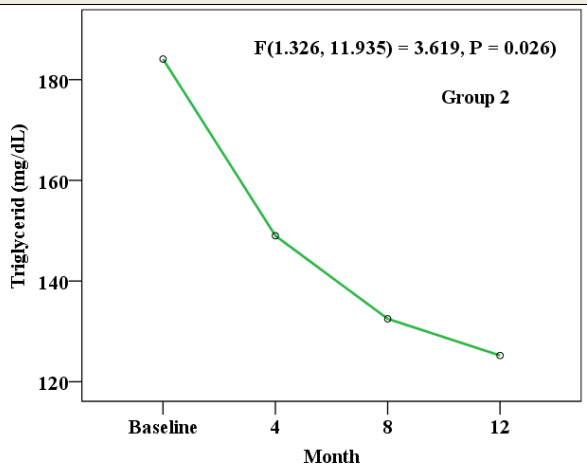



**Figure 4.3 C**

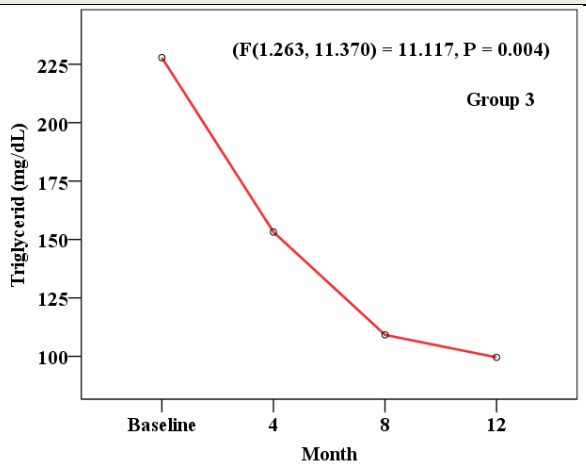



**Figure 4.3 D**

***Figure 4.3. The linear trend for Triglyceride during treatment***

*Figure 4.3 A: In all patients; Figure 4.3 B: In group 1; Figure 4.3 C: In group 2; Figure 4.3 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

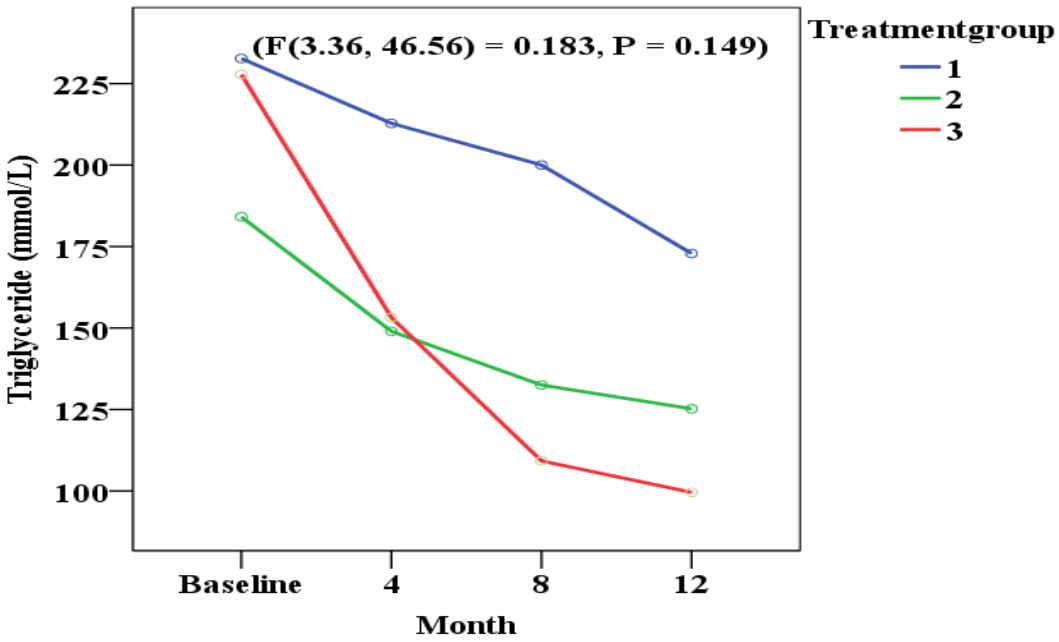



***Figure 4.4. The linear trend for Triglyceride during treatment between 3 groups***

In all patients, TG decreased gradually during treatment and there was a statistically significant difference between the time points ($F(1.68, 45.36) = 16.94$, $P < 0.001$) (Figure 4.3 A**)** and in each groups ($p< 0.05$) (Figure 4.3 B,C,D**)**. TG decreased by 38% after 12 months of treatment, of which, group 1 decreased 25%, group 2 decreased 32% and group 3 decreased 56%. However, there was no difference in TG concentration when comparing between 3 different regimens over time (F (3.36, 46.56) = 0.183, P = 0.149). (F(3.36, 46.56) = 0.183, P = 0.149) (Figure 4.4**).**

### *4.2.1.3. The effect of treatment on LDL Cholesterol during treatment*

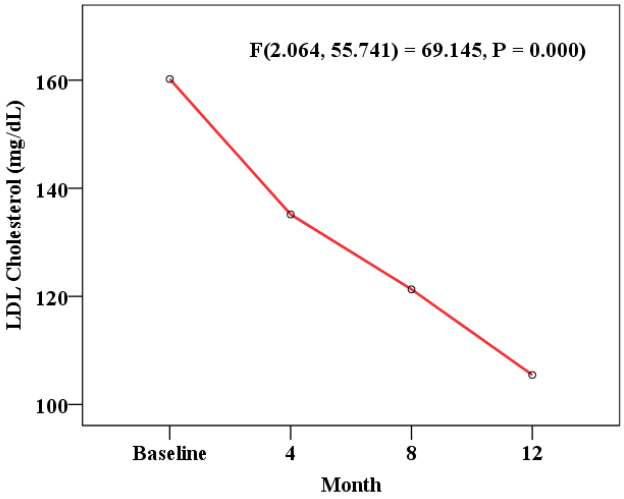



**Figure 4.5 A**

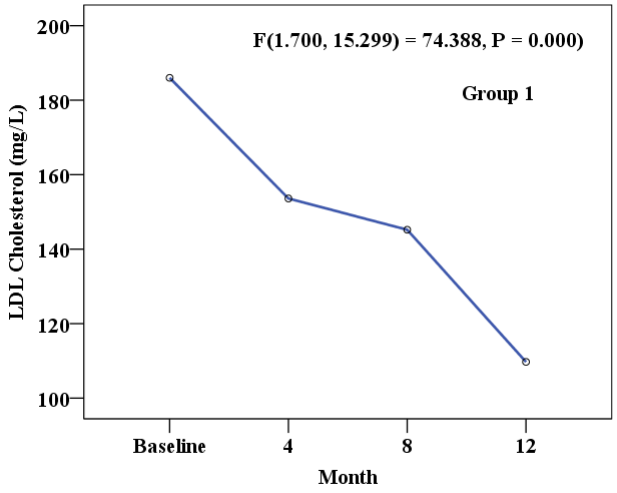



**Figure 4.5 B**

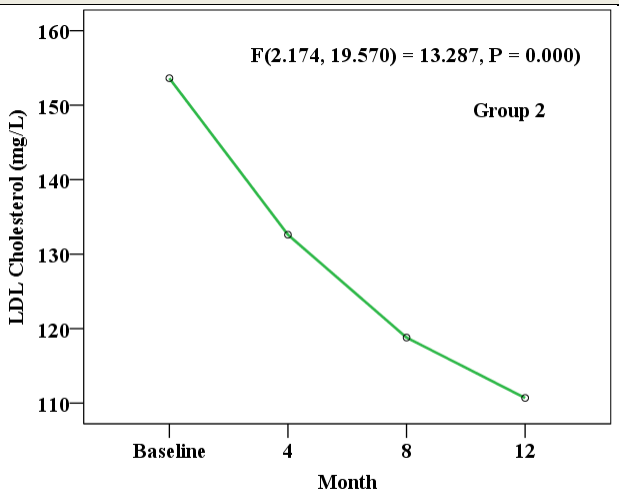



**Figure 4.5 C**

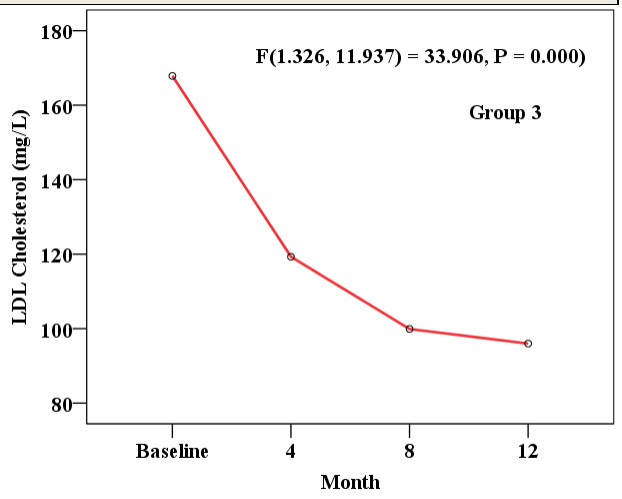



**Figure 4.5 D**

***Figure 4.5. The linear trend for LDL-C during treatment***

*Figure 4.5 A: In all patients; Figure 4.5 B: In group 1; Figure 4.5 C: In group 2; Figure 4.5 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

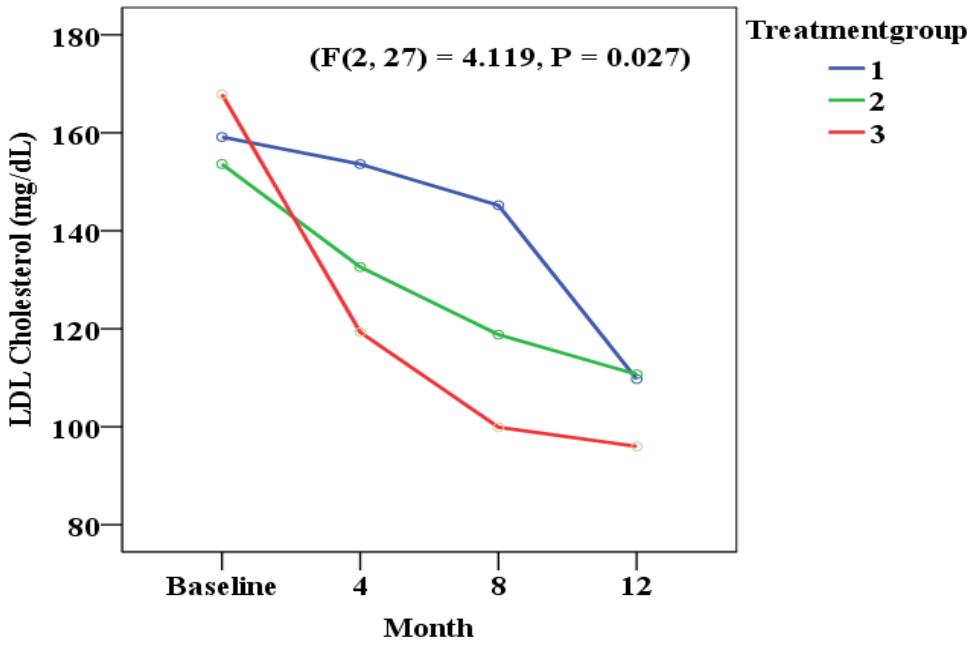



***Figure 4.6. The linear trend for LDL-C during treatment between 3 groups***

LDL-C decreased during treatment in all patients with significant difference between the time points ($F(2.064, 55.741) = 69.145$, $P = 0.000$) (Figure 4.5 A) and the improvement also demonstrated in each groups ($p<0.05$) (Figure 4.5 B,C,D) . LDL-C decreased by 34% after 12 months of treatment, of which, group 1, group 2 and group 3 decreased 31%, 28% and 43%, respectively. Mean LDL-C concentration differed statistically significantly between 3 groups ($F(2, 27) = 4.119$, $P = 0.027$) (Figure 4.6), but in multiple comparison, only significant difference was obtained between group 1&3 ($p = 0.025$).

### *4.2.1.4. The effect of treatment on HDL Cholesterol during treatment*

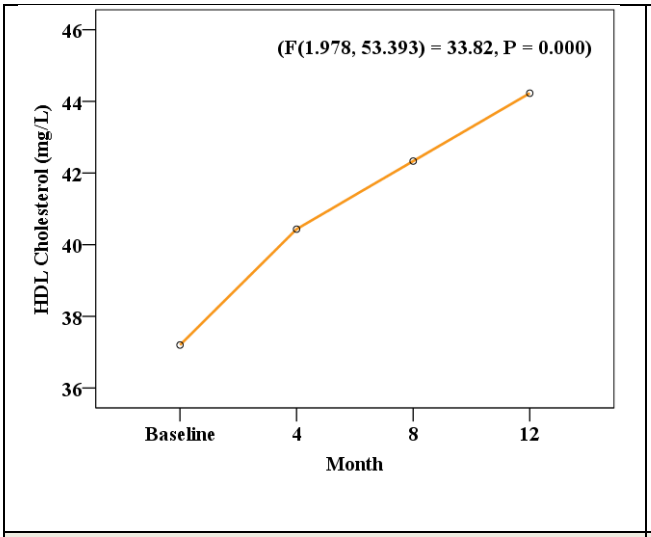



**Figure 4.7 A**

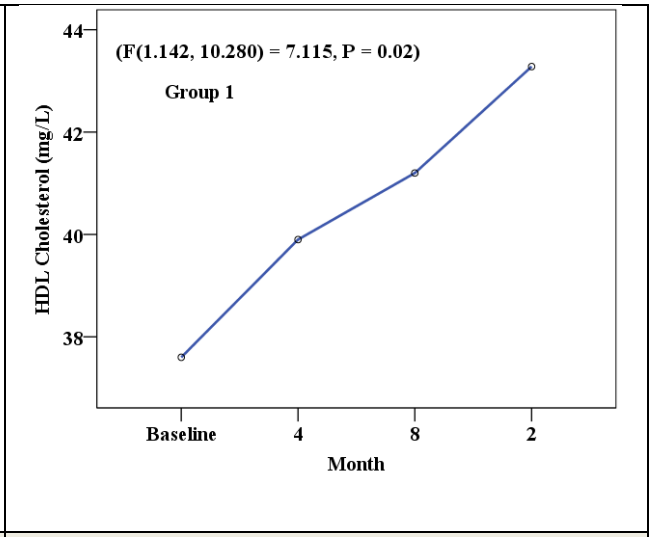



**Figure 4.7 B**

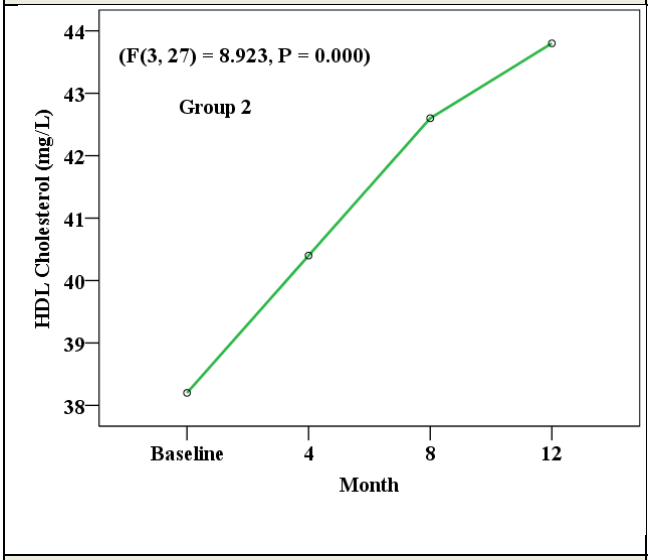



**Figure 4.7 C**

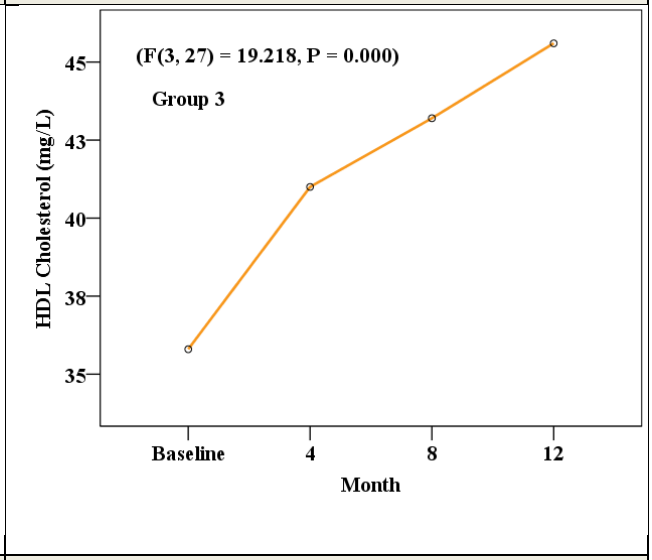



**Figure 4.7 D**

***Figure 4.7. The linear trend for HDL-C during treatment***

*Figure 4.7 A: In all patients; Figure 4.7 B: In group 1; Figure 4.7 C: In group 2; Figure 4.7 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

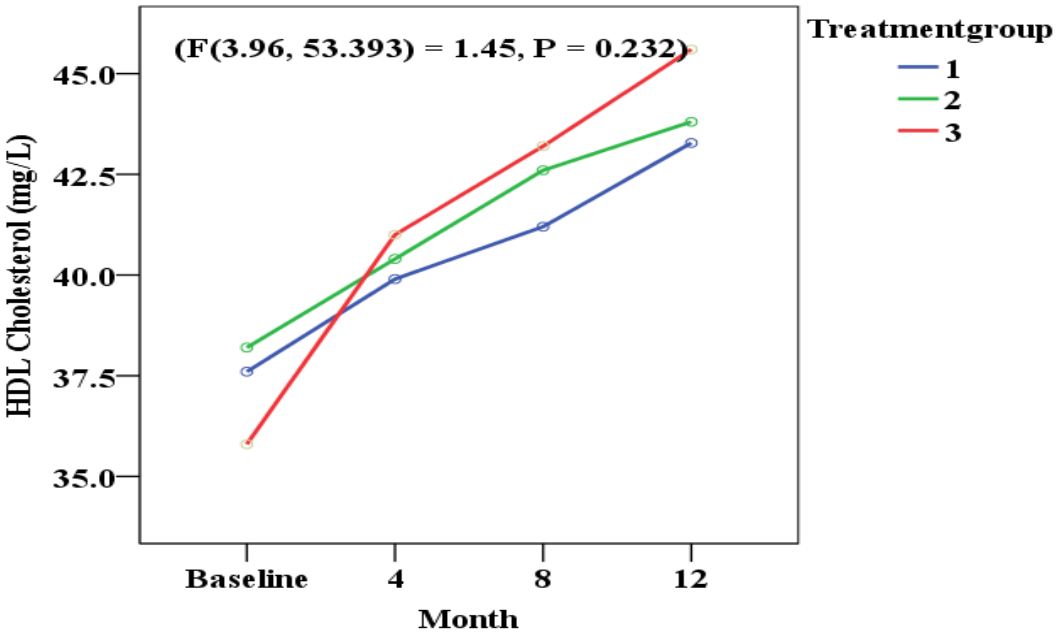



***Figure 4.8. The linear trend for HDL-C during treatment between 3 groups***

HDL-C increased during treatment in all patients with significant difference between the time points (F(1.978, 53.393) = 33.82, P = 0.000) (Figure 4.7 A) and the improvement also demonstrated in each groups ($p<0.05$) (Figure 4.7 B,C,D). HDL-C increased by 14%, 13% and 22% after 12 months in group 1, group 2 and group 3, respectively. Between 3 therapies, we did not find significant difference on mean HDL - C concentration (F(3.96, 53.393) = 1.45, P = 0.232) (Figure 4.8).

### 4.2.2. The effect of lipid-lowering regimes on oxidative stress indices

#### *4.2.2.1. The effect of treatment on MDA during treatment*

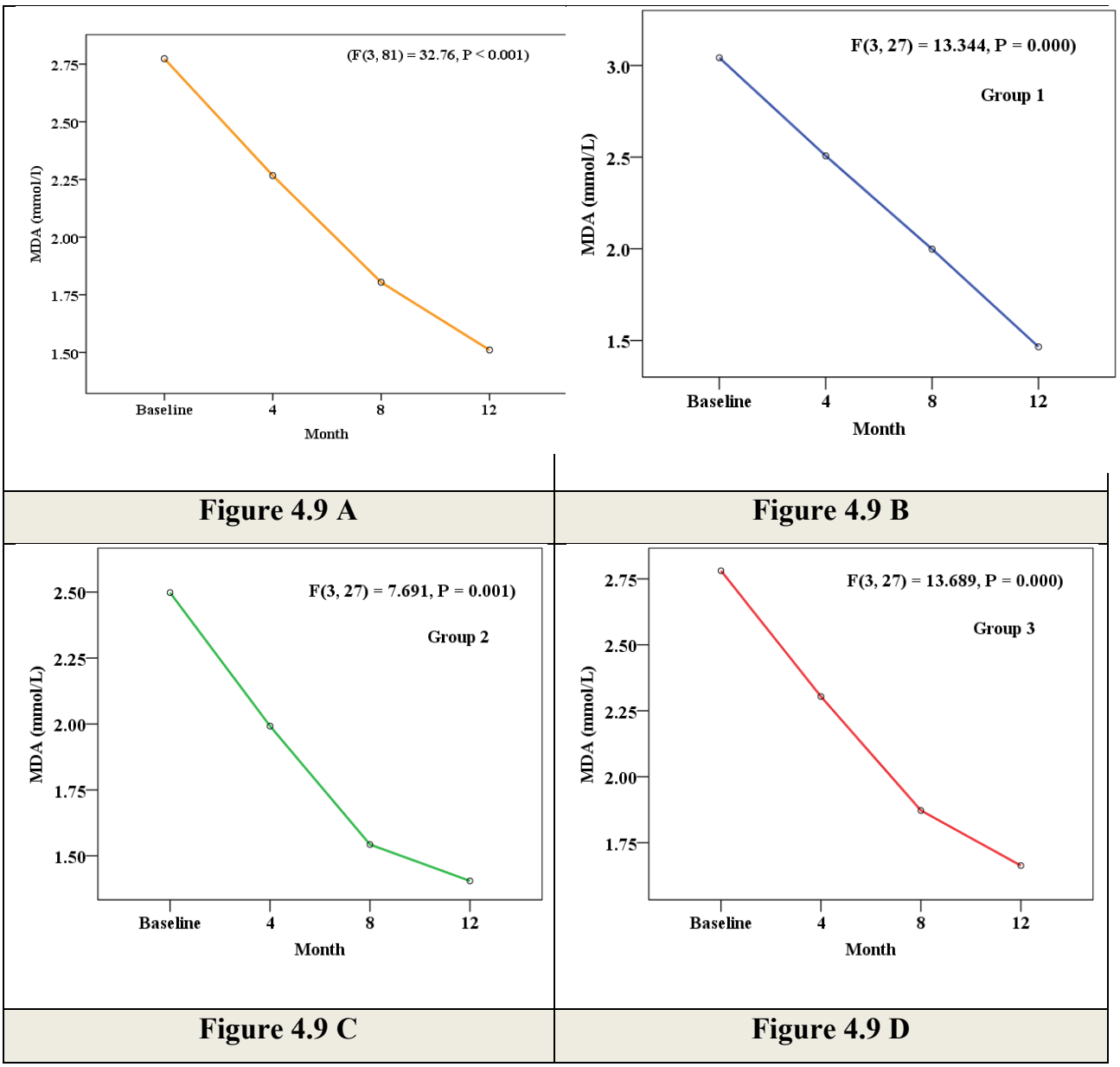



**Figure 4.9 A** | **Figure 4.9 B**

**Figure 4.9 C** | **Figure 4.9 D**

***Figure 4.9. The linear trend for MDA during treatment***

*Figure 4.9 A: In all patients; Figure 4.9 A B: In group 1; Figure 4.9 A C: In group 2; Figure 4.9 A D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

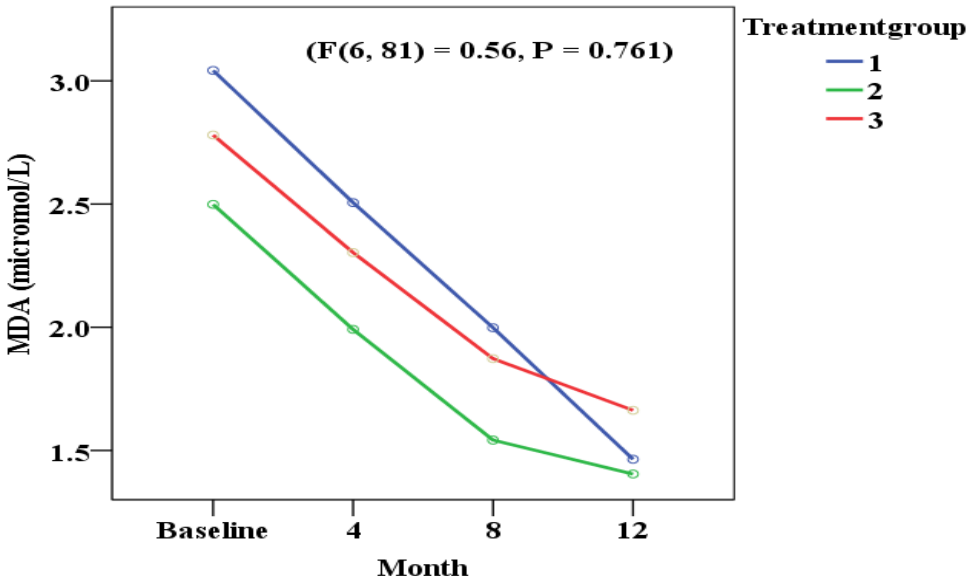



***Figure 4.10. The linear trend for MDA during treatment between 3 groups***

A (time) x (treatment group) mixed-model ANOVA revealed that the main effect for time was significant. MDA concentration differed statistically significantly between time points in all patients ($F(3, 81) = 32.76$, $P < 0.001$) (Figure 4.9 A). MDA concentration also gradually decreased from baseline to 4$^{th}$ month, 8$^{th}$ month and 12$^{th}$ month of treatment with $F(3, 27) = 13.344$, $P = 0.000$), $F(3, 27) = 7.691$, $P = 0.001$) and $F(3, 27) = 13.689$, $P = 0.000$), respectively (Figure 4.9 B,C,D). A marked decrease in MDA was observed (45% after 12 months) with a trend for a greater effect in patients of group 1. However, different lipid-lowering therapies did not show a different effect on the change of MDA concentration during treatment ($F(6, 81) = 0.56$, $P = 0.761$) (Figure 4.10). A significant decrease in MDA was observed with a trend for a greater effect in patients of group 3. (A. Zinellu et al., 2015).

### *4.2.2.2. The effect of lipid-lowering regimes on PSH*

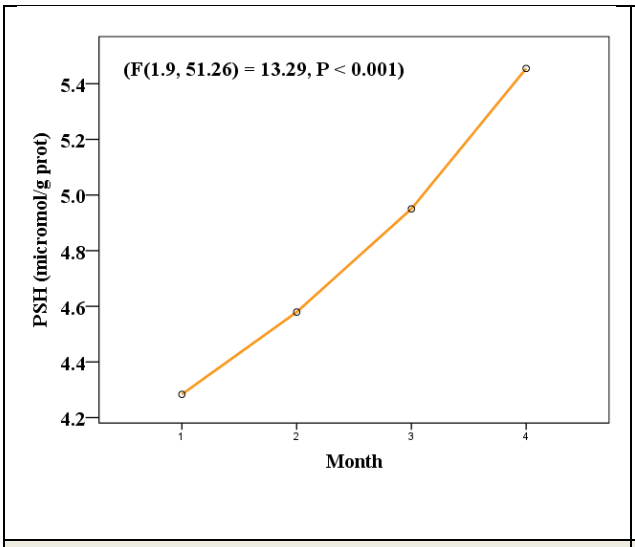



**Figure 4.11 A**

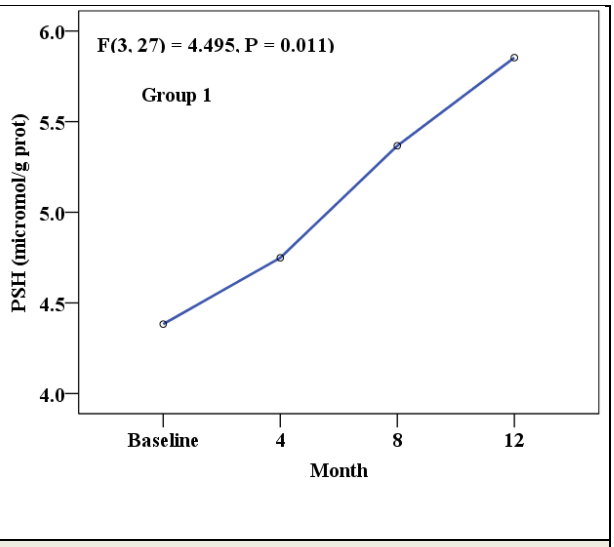



**Figure 4.11 B**

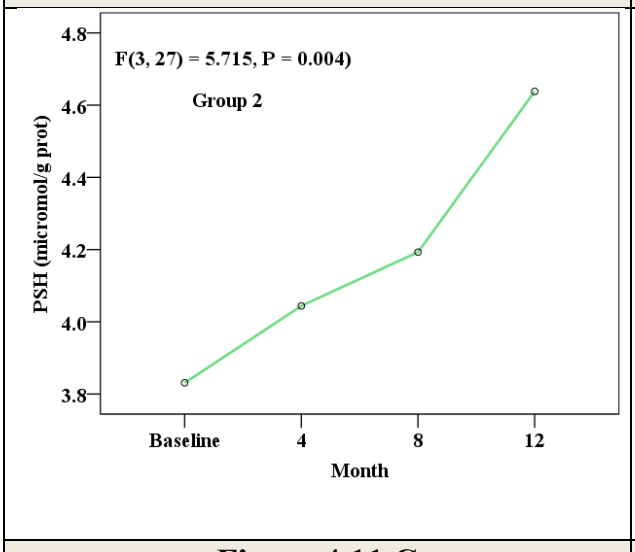



**Figure 4.11 C**

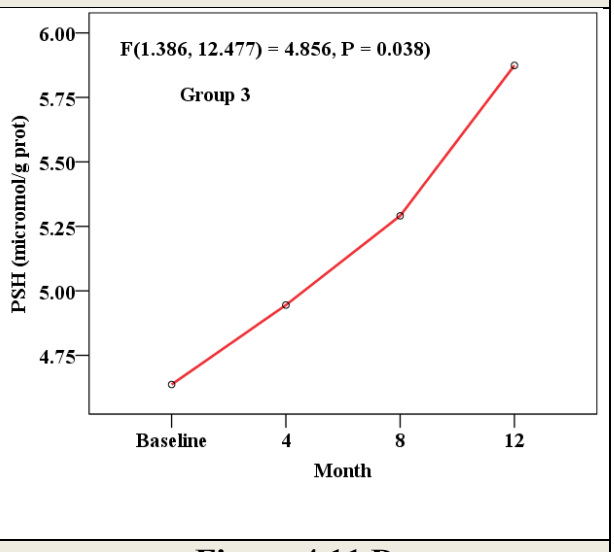



**Figure 4.11 D**

***Figure 4.11. The linear trend for PSH during treatment***

*Figure 4.11 A: In all patients; Figure 4.11 B: In group 1; Figure 4.11 C: In group 2; Figure 4.11 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

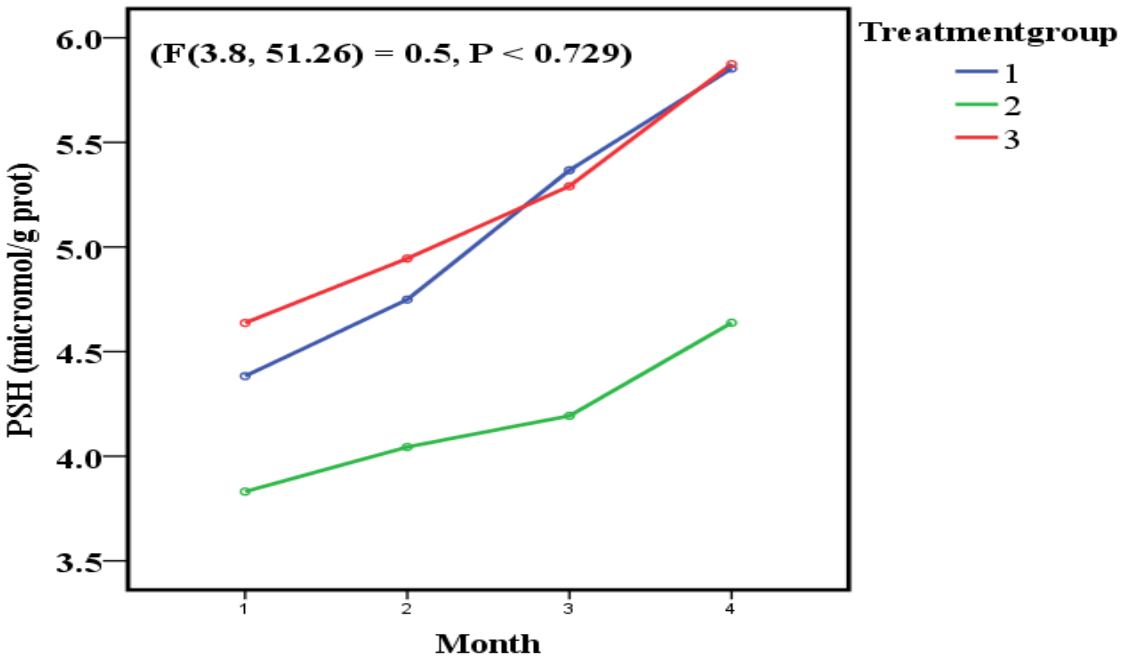



***Figure 4.12. The linear trend for PSH during treatment between 3 groups***

PSH concentration ameliorate significantly during treatment contrasted with baseline in all patients ($F(1.9, 51.26) = 13.29$, $P < 0.001$) (Figure 4.11 A) and in individual groups with $F(3, 27) = 4.495$, $P = 0.011$), $F(3, 27) = 5.715$, $P = 0.004$), and $F(1.386, 12.477) = 4.856$, $P = 0.038$) (Figure 4.11 B,C,D) for group 1,2, and 3, respectively. Anyway, in multiple comparison, PSH level between baseline and at 4th month did not differ significantly ($p = 0.111$). PSH increased in 3 groups after 12 months, 34% (group 1), 21% (group 1), and 27% (group 3).

While analyzing the distinction of mean PSH between the 3 groups, no statistically significant difference was observed, ($F(3.8, 51.26) = 0.5$, $P < 0.729$) (Figure 4.12).

### *4.2.2.3. The effect of treatment on TEAC during treatment*

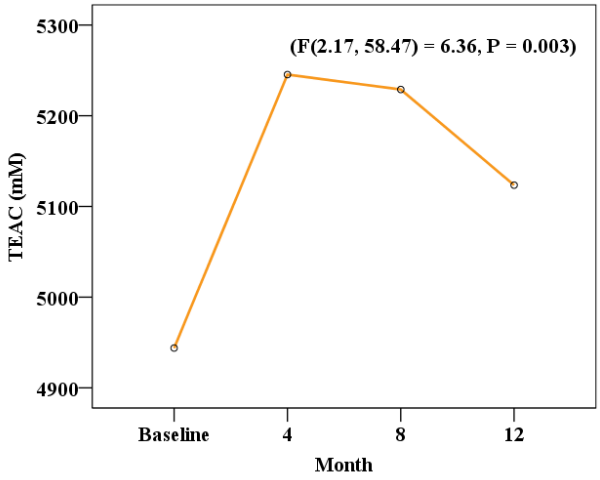



**Figure 4.12 A**

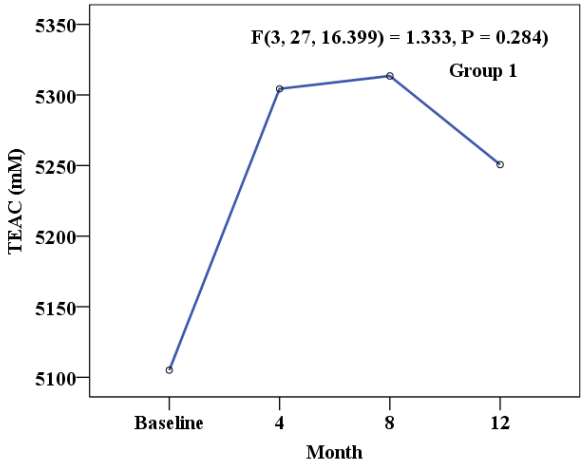



**Figure 4.12 B**

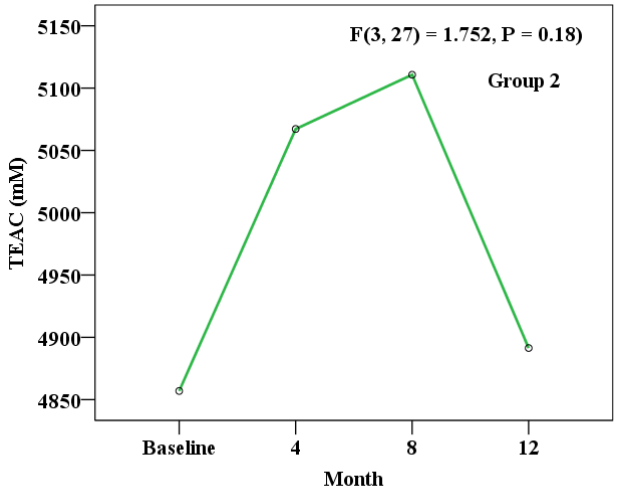



**Figure 4.12 C**

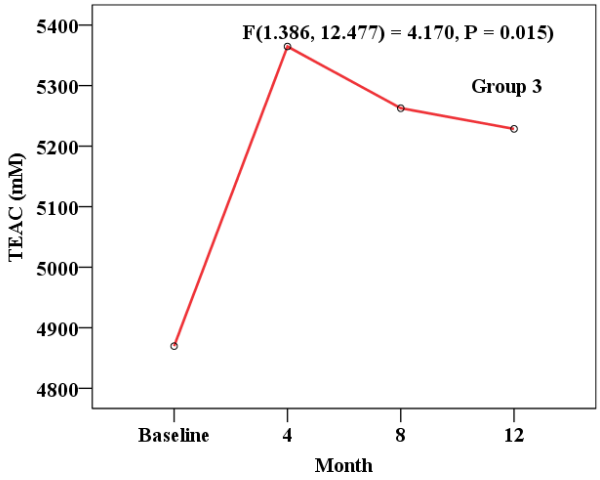



**Figure 4.12 D**

***Figure 4.12. The linear trend for TEAC during treatment***

*Figure 4.12 A: In all patients; Figure 4.12 B: In group 1; Figure 4.12 C: In group 2; Figure 4.12 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

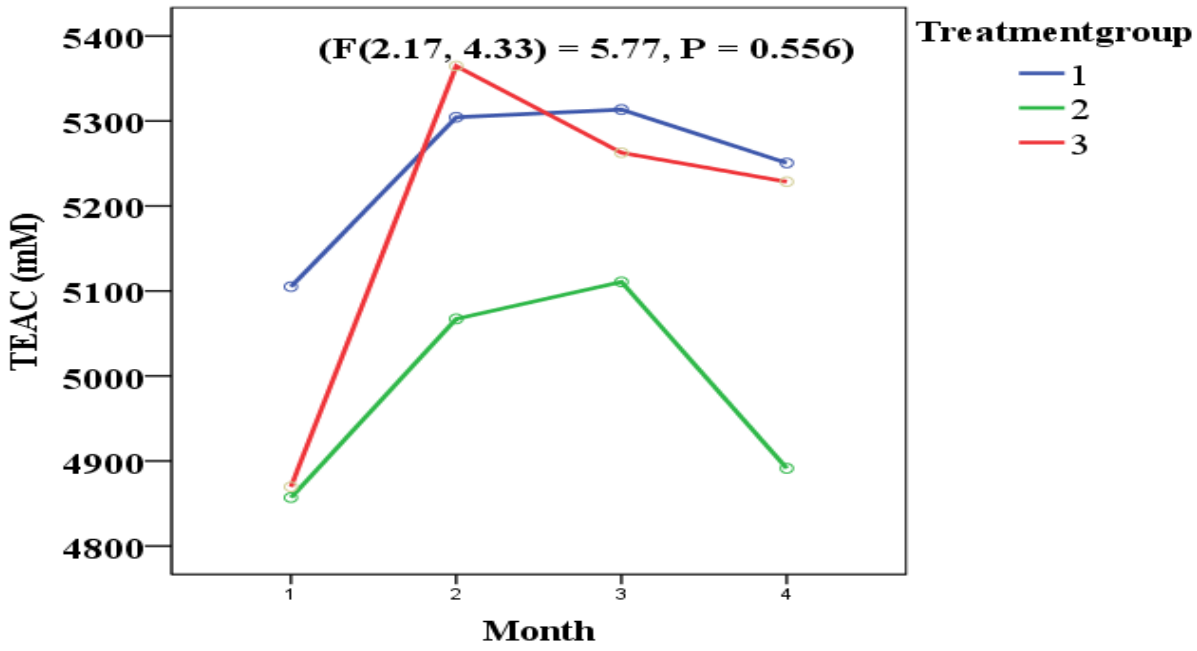



***Figure 4.13. The linear trend for TEAC during treatment between 3 groups***

TEAC concentration different between time points (F (2.17, 58.47) = 6.36, P = 0.003) (Figure 4.12 A). But in multi comparison, results demonstrated only the difference between before and after 4,8, and 12 months of treatment ($p<0.05$), while no significant difference was observed during treatment. When considering each group separately, the difference in TEAC was statistically significant in group 3, F(1.386, 12.477) = 4.170, P = 0.015) (Figure 4.12 C), of which only a difference between baseline and at 4th month was obtained ($p<0.05$) (Figure 4.13). And also, no significant interaction was determined by MIX ANOVA between time points and groups on TEAC concentration (F(2.17, 4.33) = 5.77, P = 0.556).

### *4.2.2.4. The effect of treatment on PON during treatment*

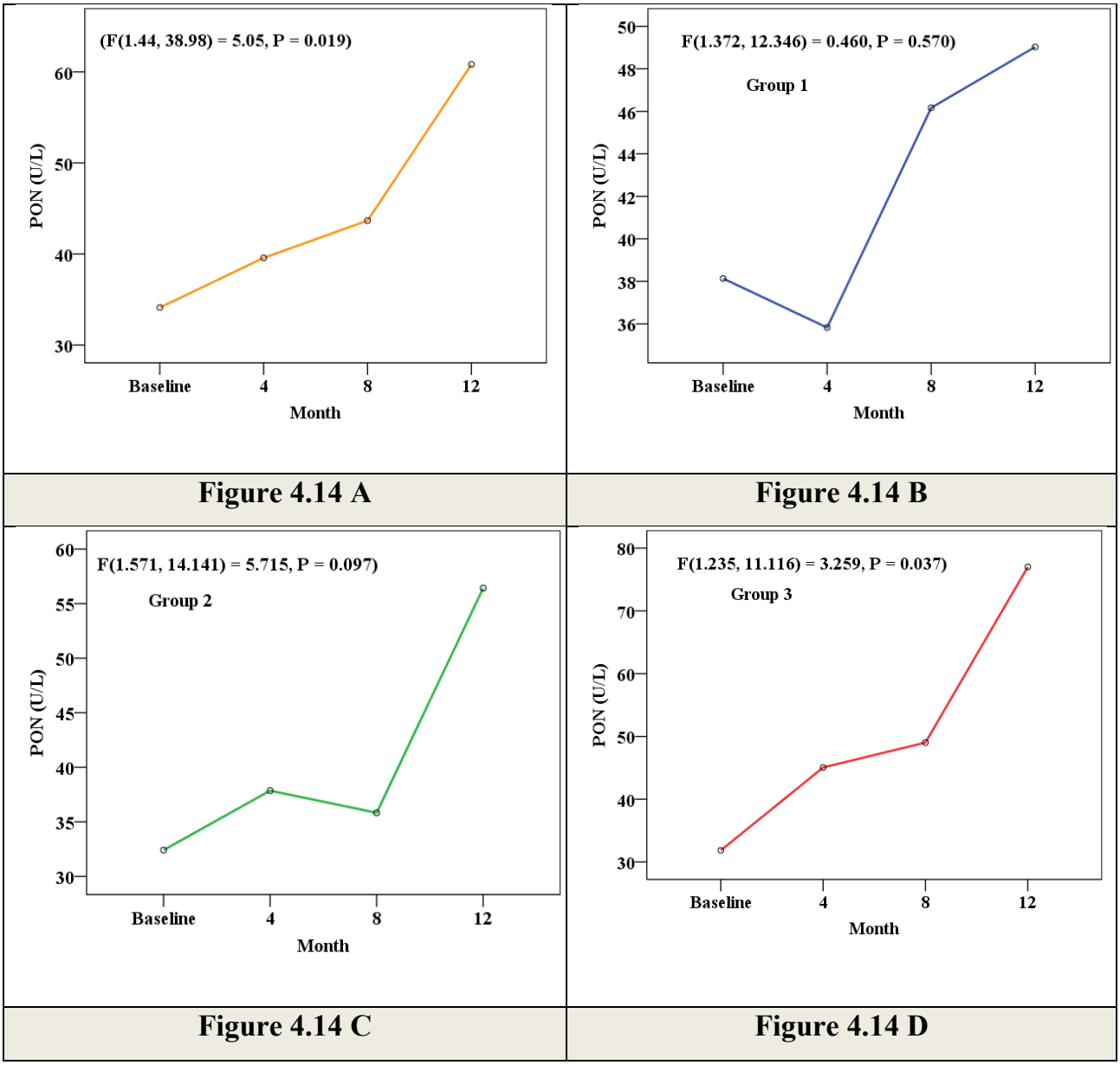



**Figure 4.14 A** | **Figure 4.14 B**

**Figure 4.14 C** | **Figure 4.14 D**

***Figure 4.14. The linear trend for PON during treatment***

*Figure 4.14 A: In all patients; Figure 4.14 B: In group 1; Figure 4.14 C: In group 2; Figure 4.14 D: In group 3. P-values have been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

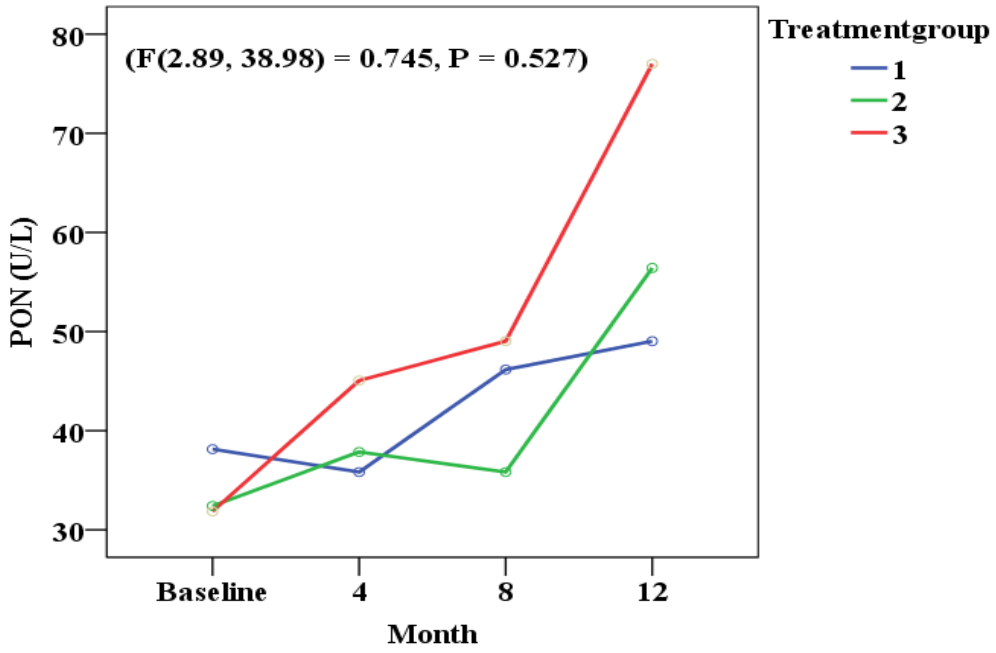



***Figure 4.15. The linear trend for TEAC during treatment between 3 groups***

As it is displayed in Figure 4.14, PON concentration significant changed between time points ($F(1.44, 38.98) = 5.05$, $P = 0.019$), but only between before treatment and after 12 months. PON concentration increased with a trend for a greater effect of group 3 with F(1.235, 11.116) = 3.259, P = 0.037), and increased only after 12 months of treatment, meanwhile, PON did not change significantly in group 1 and in group 2 as well with p> 0.05 (Figure 4.14 B,C,D). Interaction between time points and groups did not significant change PON concentration ($F(2.89, 38.98) = 0.745$, $P = 0.527$) (Figure 4.15).

### 4.2.3. The effect of Tryptophan degradation indexes during treatment

#### *4.2.3.1. The effect of treatment on Kynurenine during treatment*

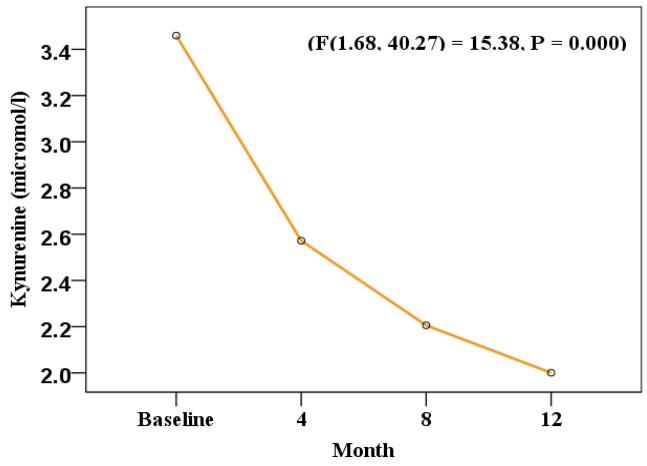



**Figure 4.16 A**

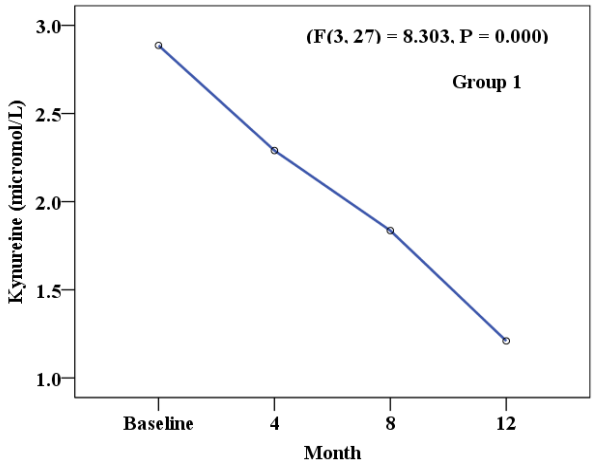



**Figure 4.16 B**

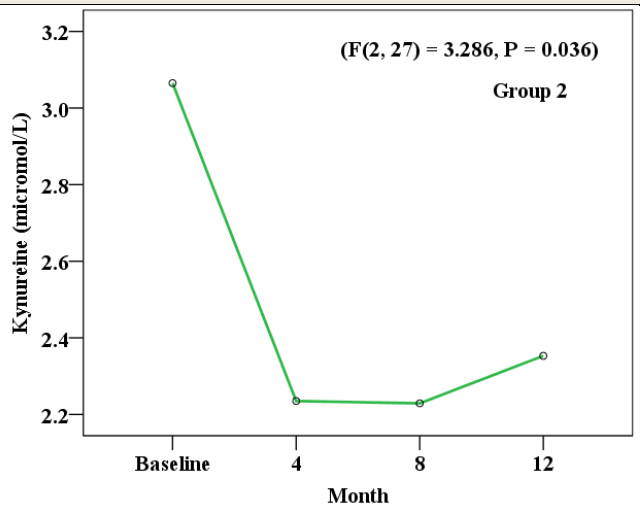



**Figure 4.16 C**

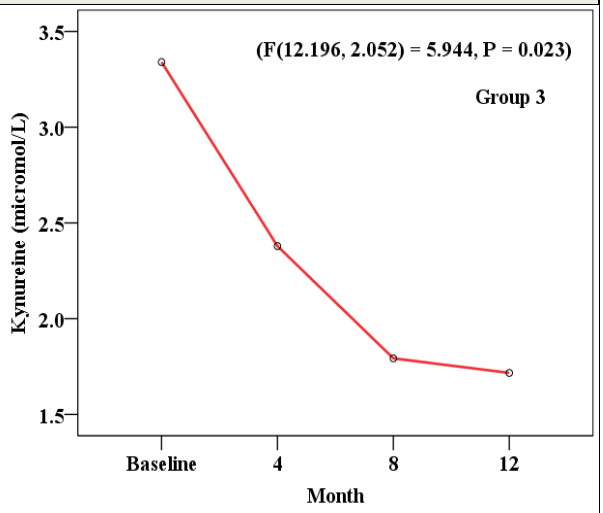



**Figure 4.16 D**

***Figure 4.16. The linear trend for Kynurenine during treatment***

*Figure 4.16 A: In all patients; Figure 4.16 B: In group 1; Figure 4.16 C: In group 2; Figure 4.16 D: In group 3. P-values have been evaluated by one way repeated measures ANOVA with Bonferroni correction*

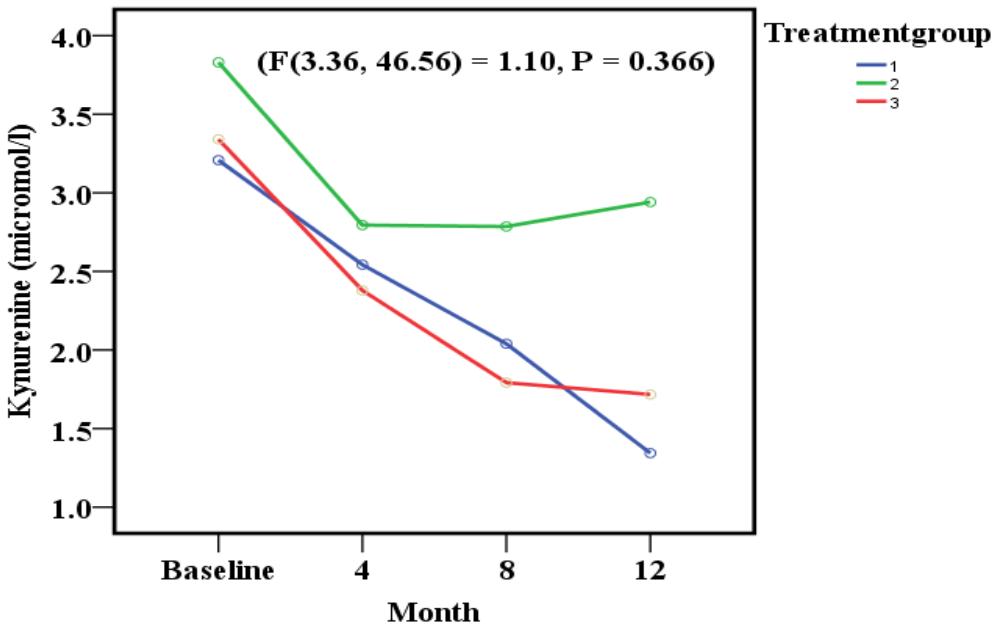



***Figure 4.17. Linear trend for TEAC during treatment between 3 groups***

As showed in figure 4.16 A, Kyn significantly differed between time points (F(1.68, 40.27) = 15.38, P = 0.000). Lipid-lowering therapies significantly reduced 36% of Kyn concentrations in all patients as well as in individual groups ($p<0.05$), with a greater effect in patients of group 3 (46%) at the 8$^{th}$ month of treatment. Nonetheless, in each group, the decrease of Kyn was not statistically significant in the 4$^{th}$ month (p = 0.467) until the 8th month (p = 0.049) and the 12th month (p = 0.047). At the 12$^{th}$ month, kyn increase 42% compared to baseline, and the decrease appeared to be prominent in group 2. And no difference on Kyn concentration was evidenced between the 3 groups during treatment ($F$(3.36, 46.56) = 1.10, P = 0.366) (Figure 4.17).

### *4.2.3.2. The effect of treatment on Tryptophan during treament*

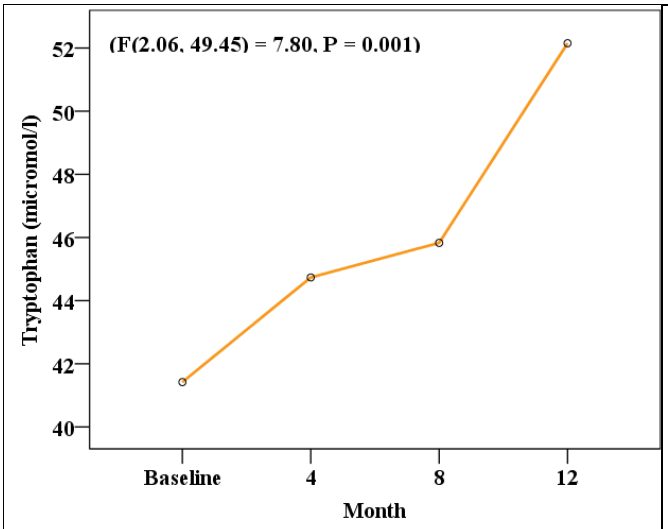



**Figure 4.18 A**

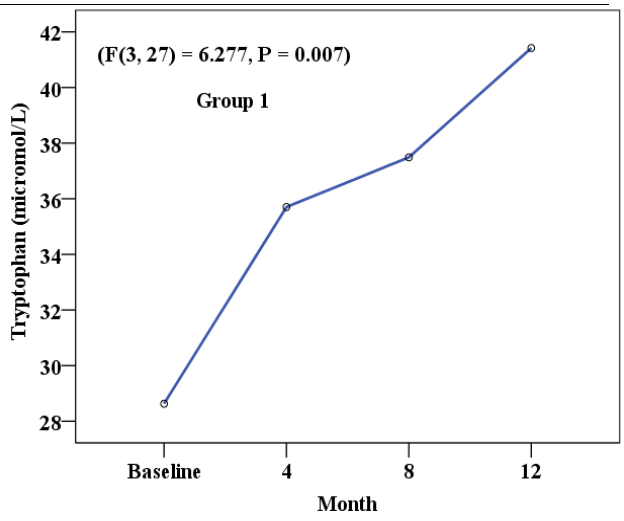



**Figure 4.18 B**

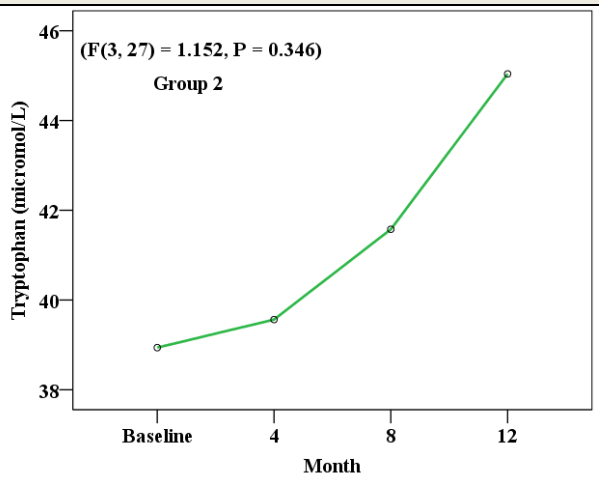



**Figure 4.18 C**

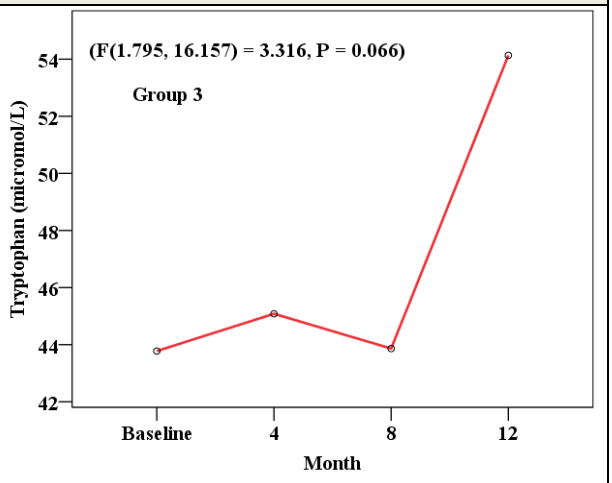



**Figure 4.18 D**

***Figure 4.18. The linear trend for Tryptophan during treatment***

*Figure 4.18 A: In all patients; Figure 4.18 A B: In group 1; Figure 4.18 A C: In group 2; Figure 4.18 A D: In group 3. P-values have been evaluated by one way repeated measures ANOVA with Bonferroni correction*

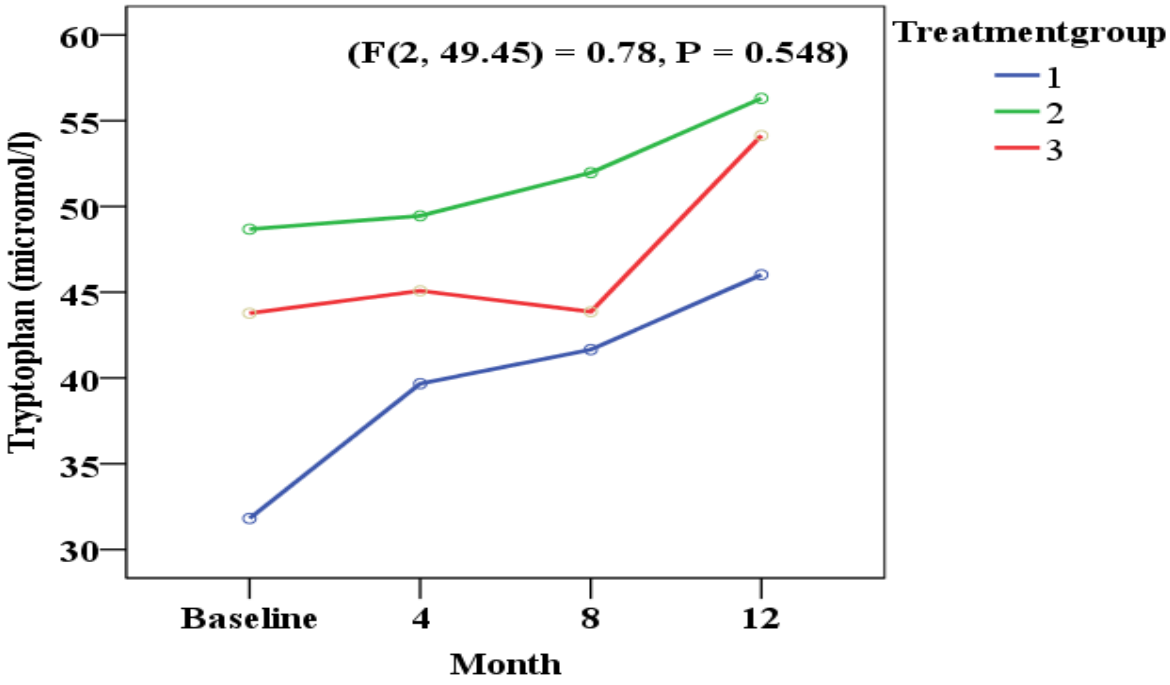



***Figure 4.19. Linear trend for Tryptophan during treatment between 3 groups***

Mean Tryptophan concentration in all patients increased 26% in 12$^{th}$ month compare with baseline ($F$(2.06, 49.45) = 7.80, P = 0.001) (Figure 4.18 A). However, for each group, the increase in Tryp concentration was only statistically significant in group 1 compared to pre-treatment (p = 0.036) (Figure 4.18 B). Meanwhile, Tryp also tended to increase after treatment in group 2 and group 3 although the difference was not statistically significant with p = 0.346 and p = 0.066, respectively (Figure 4.18 C,D). Results from a MIX repeated measures ANOVA determined that the change of Tryptophan concentration not differed statistically significantly between 3 groups ($F$(2, 49.45) = 0.78, P = 0.548) (Figure 4.19).

### *4.2.3.3. The effect of treatment on Kyn/Trp ratio during treament*

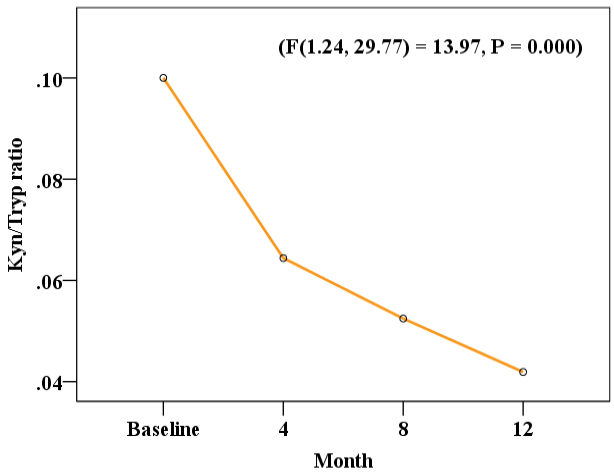



**Figure 4.20 A**

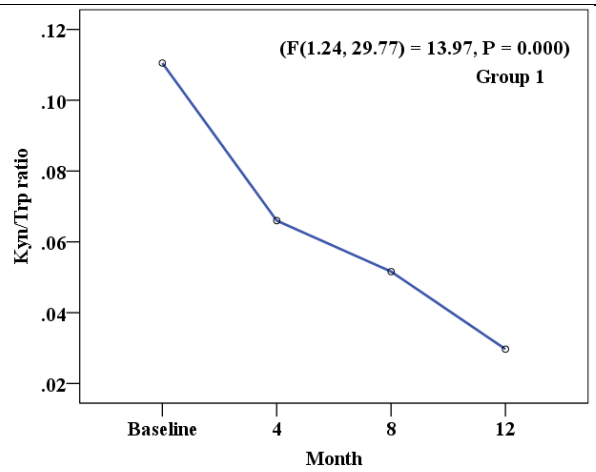



**Figure 4.20 B**

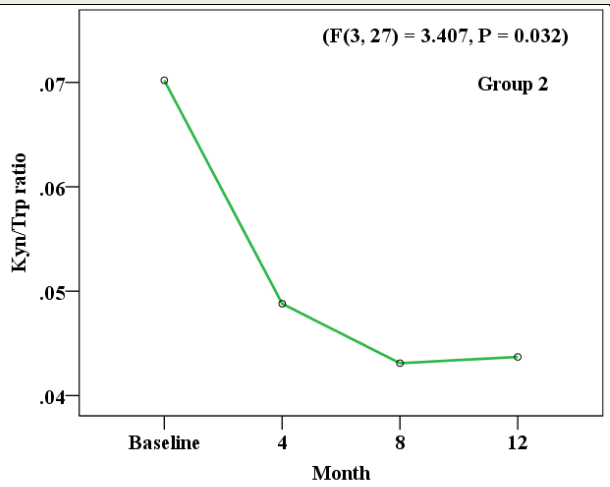



**Figure 4.20 C**

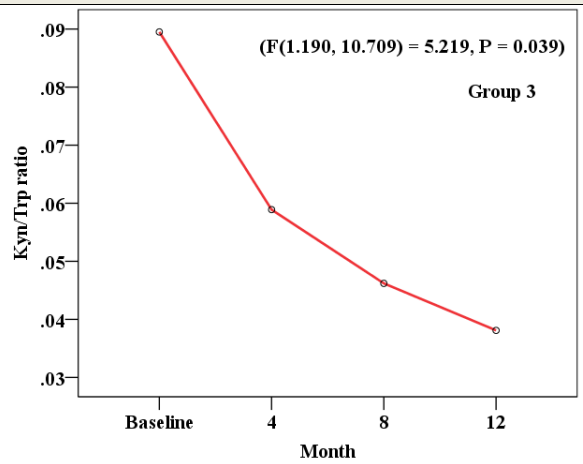



**Figure 4.20 D**

***Figure 4.20. The linear trend for Kynurenine/Tryptophan during treatment***

*Figure 4.20 A: In all patients; Figure 4.20 B: In group 1; Figure 4.20 C: In group 2; Figure 4.20 D: In group 3. P-values have been evaluated by one way repeated measures ANOVA with Bonferroni correction*

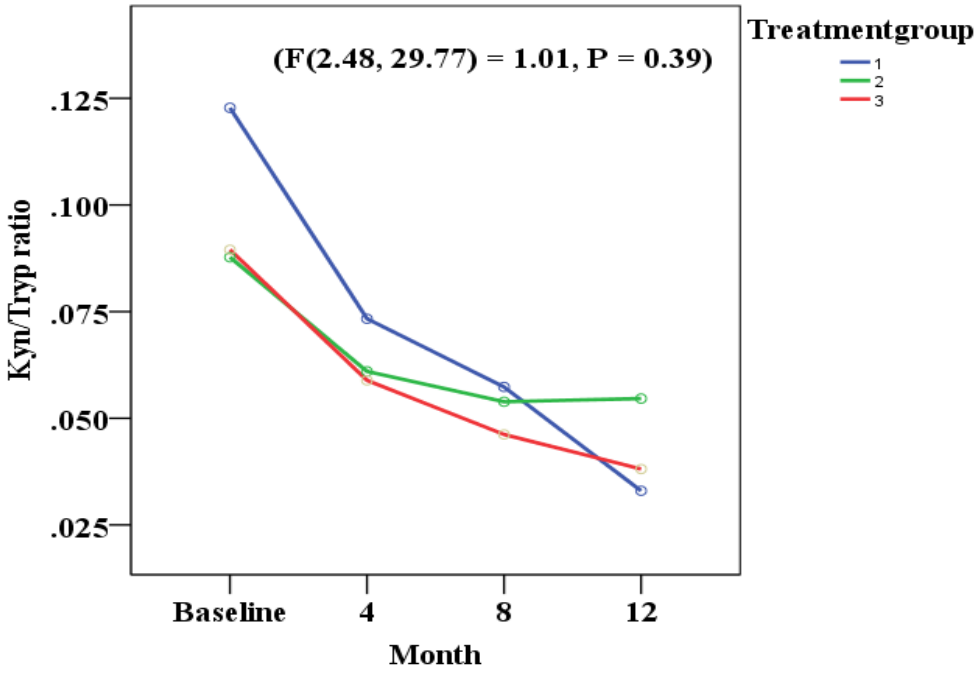
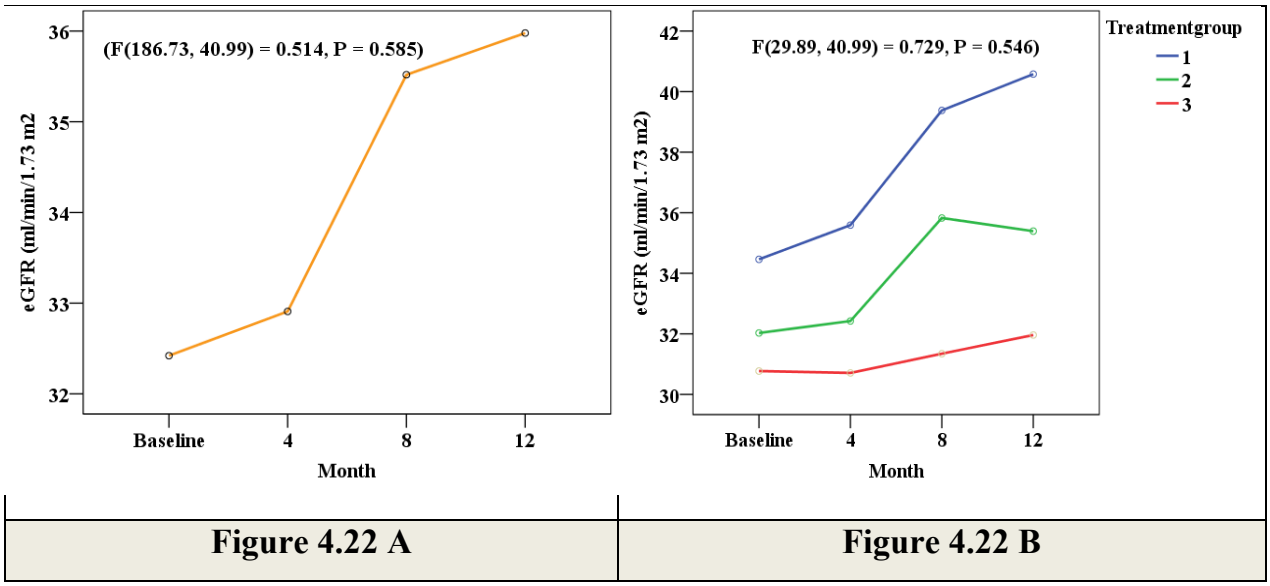



***Figure 4.21. The linear trend for Kyn/Tryp ratio during treatment between 3 groups***

As reported in Figure 4.20A, mean Kyn/Trp concentration differed statistically significantly between time points when analyzed in all patients and also in the separated group ($p < 0.05$) (Figure 4.20 B, C, D). But there was also no difference between the 3 groups during treatment ($F(2.48, 29.77) = 1.01$, $P = 0.39$) (Figure 4.21). Results from the research of Angelo Zinellu demonstrated that drug treatment significantly reduced Kyn concentrations in all patients (21%) as well as in individual treatment groups, with a greater effect in patients of the group (23%).

## 4.3. The effect of lipid-lowering regimes on kidney profiles.

### 4.3.1. The effect of lipid-lowering regimes on eGFR




| Figure 4.22 A | Figure 4.22 B |
|---|---|

***Figure 4.22. Distribution of eGFR by time in all patients and by group***

*Figure 4.22 A: in all patients by time; Figure 4.22 B in all patients by time and group. P values as been evaluated by one way repeated measures ANOVA with Bonferroni correction*

As reported in figure 4.22 A, an incremental trend of eGFR by time was obtained in all patients and each group, but not statistically differed between time points, (F(186.73, 40.99) = 0.514, P = 0.585). There was also no difference in the variation of eGFR during the treatment of the 3 groups, (F(29.89, 40.99) = 0.729, P = 0.546) (figure 4.22 B). Lipid-lowering drugs did not significantly affect eGFR after 12 months of treatment in all groups.

### 4.3.2. The effect of lipid-lowering regimes on Proteinuria

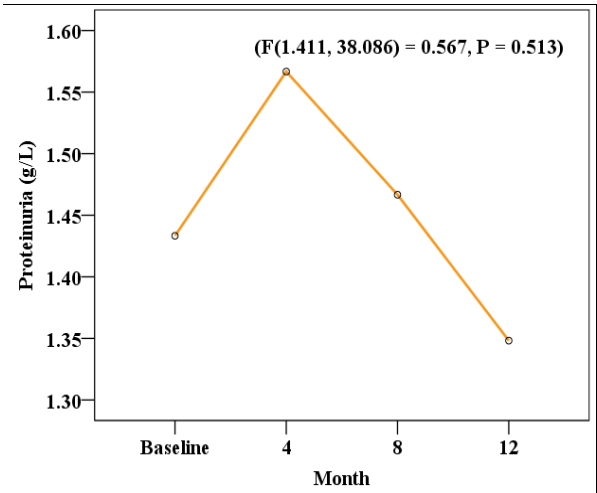



**Figure 4.23 A**

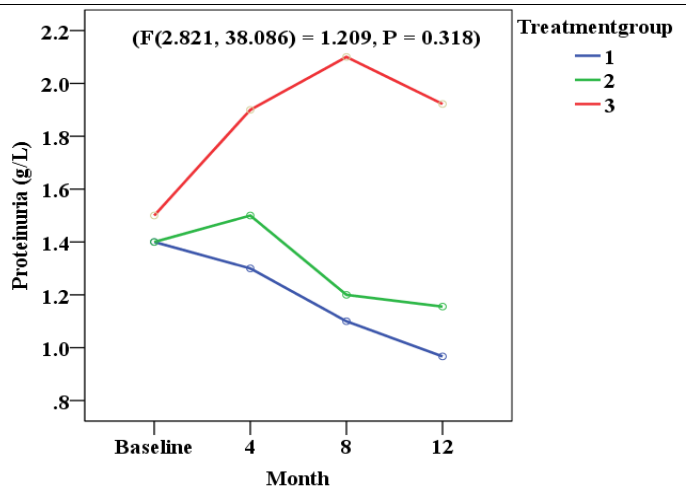



**Figure 4.23 B**

***Figure 4.23. Distribution of Proteinuria by time in all patients and by group***

***Figure 4.23 A: in all patients by time; Figure 4.23 B in all patients by time and group. P values as been evaluated by one-way repeated measures ANOVA with Bonferroni correction.***

Proteinuria concentration did not change by time (F(1.411, 38.086) = 0.567, P = 0.513) in all patients (Figure 4.23 A) and 3 different lipid lowering therapies did not differ significantly effect on mean proteinuria concentration (F(2.821, 38.086) = 1.209, P = 0.318) Figure 4.23 B.

### 4.3.3. The effect of lipid-lowering regimes on Creatinine

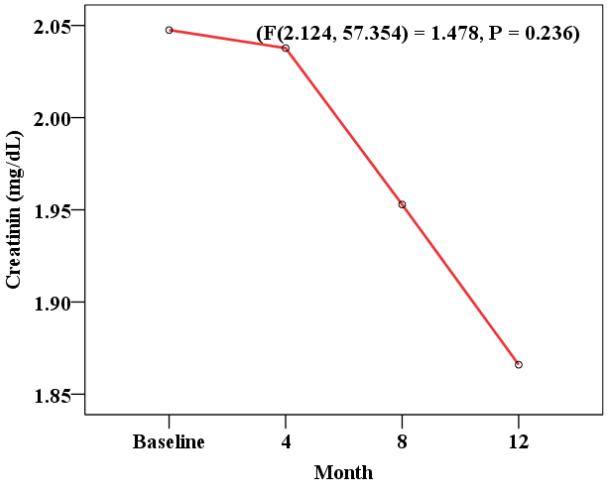



Figure 4.24 A

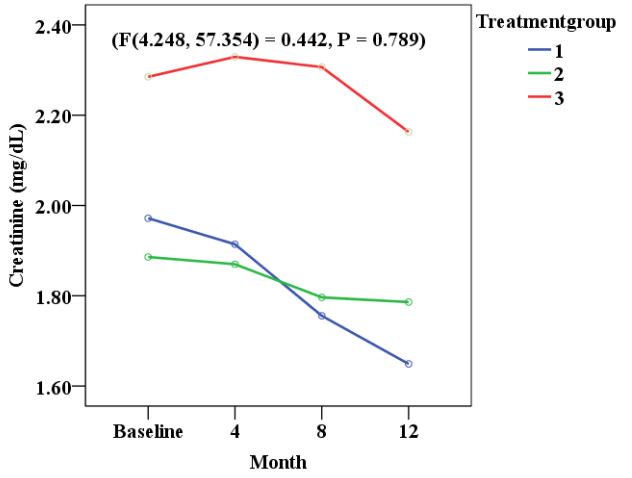



Figure 4.24 B

***Figure 4.24. Distribution of Creatinine by time in all patients and by group***

*Figure 4.24 A: in all patients by time; Figure 4.24 B in all patients by time and group. P values as been evaluated by one-way repeated measures ANOVA with Bonferroni correction.*

Mean Creatinine concentration not differ statistically significantly between time points in all patients (F(2.124, 57.354) = 1.478, P = 0.236) (Figure 4.24 A) even when it seem to be ameliorated by time. Besides that, no difference between 3 groups was observed (F(4.248, 57.354) = 0.442, P = 0.789) (Figure 4.24 B).

## 4.4. The relationship between lipid profiles, oxidative stress, tryptophan degradation during treatment

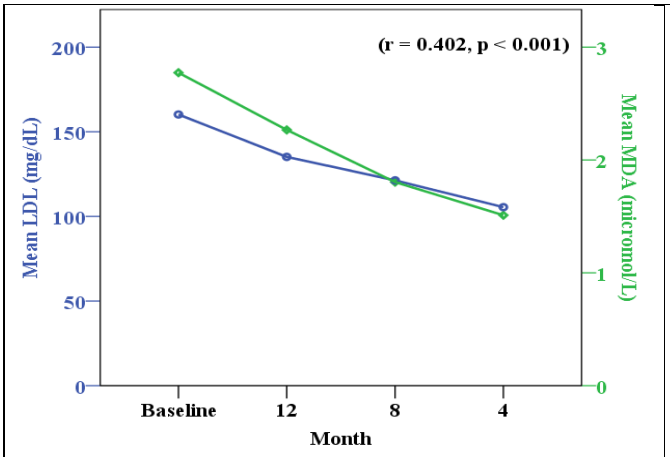



**Figure 4.25 A**

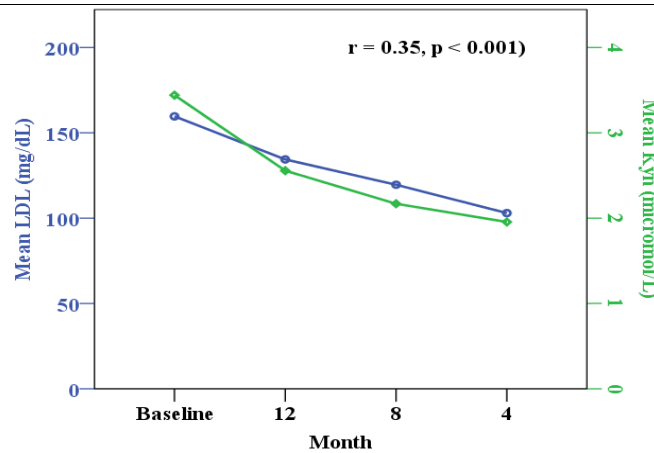



**Figure 4.25 B**

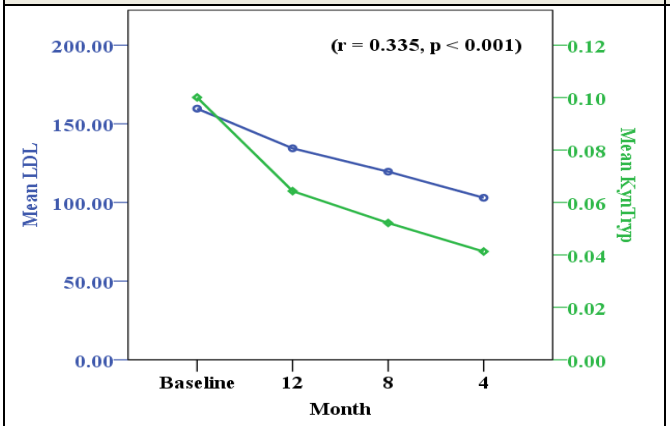



**Figure 4.25 C**

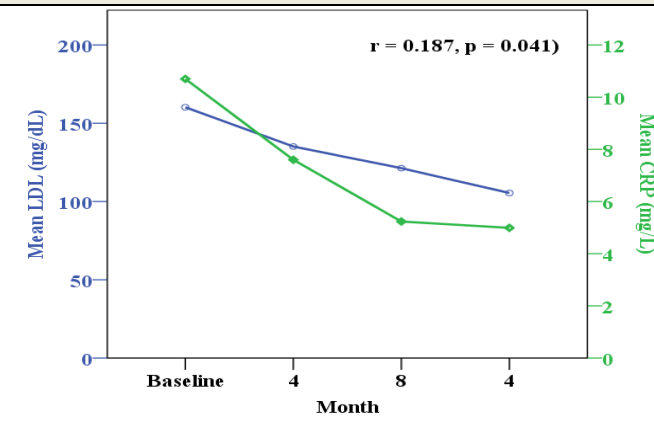



**Figure 4.25 D**

***Figure 4.25. Correlation between mean LDL-C and other variables during treatment***

*Correlation between mean LDL-C and mean MDA (Figure 4.25 A); mean LDL-C and mean Kyn (Figure 4.25 B); mean LDL-C and mean Kyn/Tryp ratio (Figure 4.25 C); mean LDL-C and mean CRP (Figure 4.25 D). Pearson Correlation. r is the correlation coefficient.*

Figure 4.25 showed that during treatment with lipid-lowering drugs, a downtrend line of LDL-C leads to a decrease trend in MDA ($r = 0.402$, $p < 0.001$), as well as in inflammatory markers such as Kyn ($r = 0.35$, $p < 0.001$), Tryn / Tryp ($r = 0.335$, $p < 0.001$).

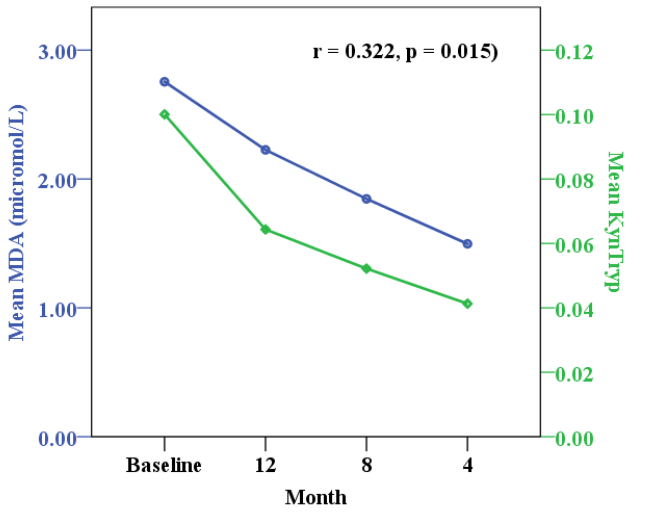



**Figure 4.26 A**

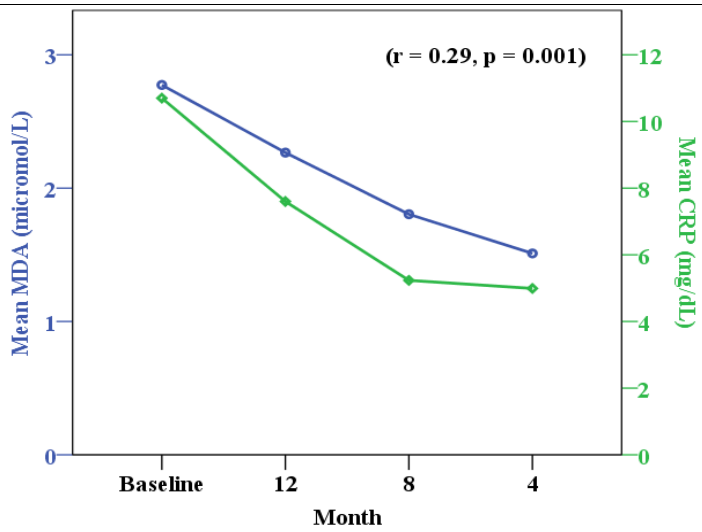



**Figure 4.26 B**

***Figure 4.26. Correlation between MDA and other variables during treatment***

*Correlation between mean MDA and mean Kyn/Tryp ratio (Figure 4.26 A); Correlation between mean MDA and mean CRP (Figure 4.26 B). Pearson Correlation. r is correlation coefficient.*

Lipid-lowering drugs decreased MDA in parallel with decreased Kyn / Tryp (r = 0.322, p = 0.015) (4.26 B). Another inflammatory marker that also had a statistically significant decrease according to MDA was CRP, although the correlation was not high (r = 0.29, p = 0.001) (Fig. 4.26 B).

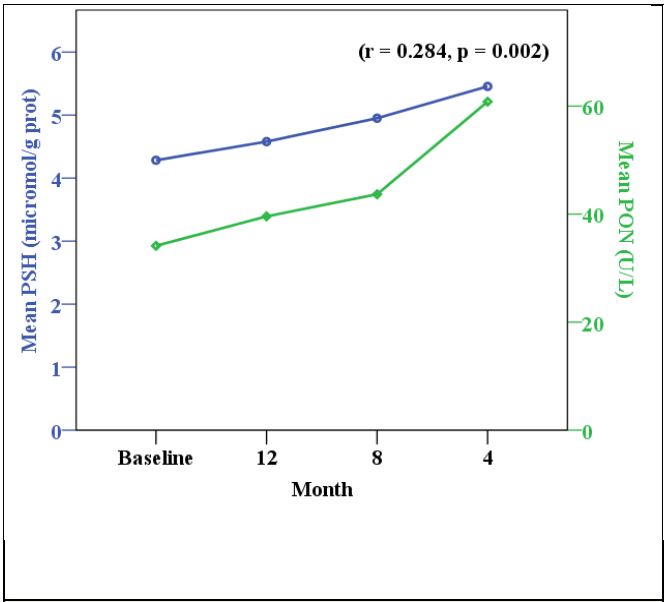



**Figure 4.27 A**

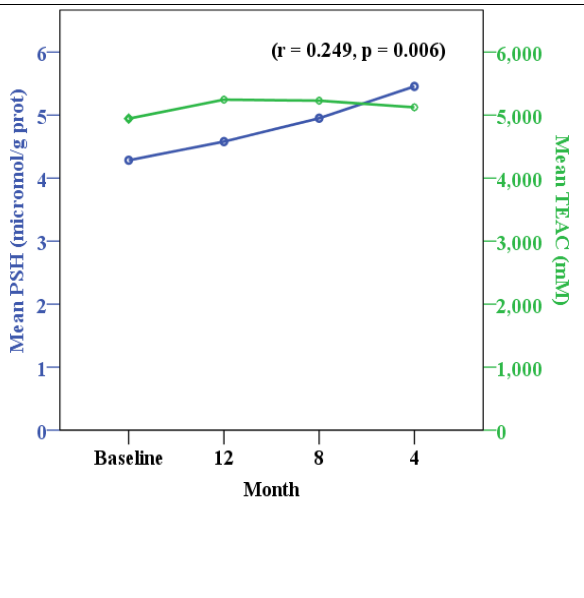



**Figure 4.27 B**

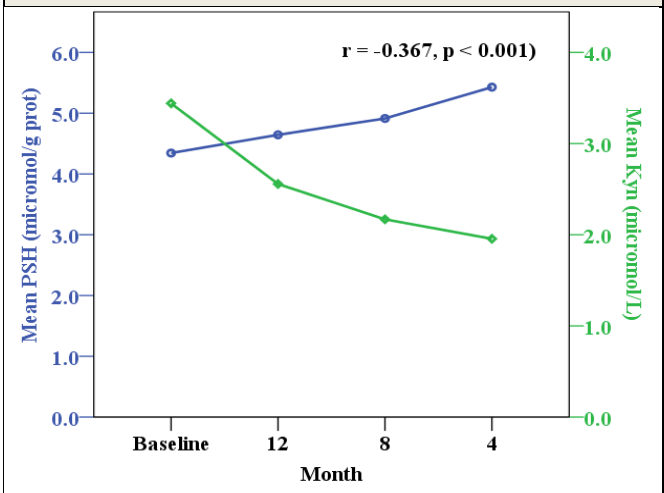



**Figure 4.27 C**

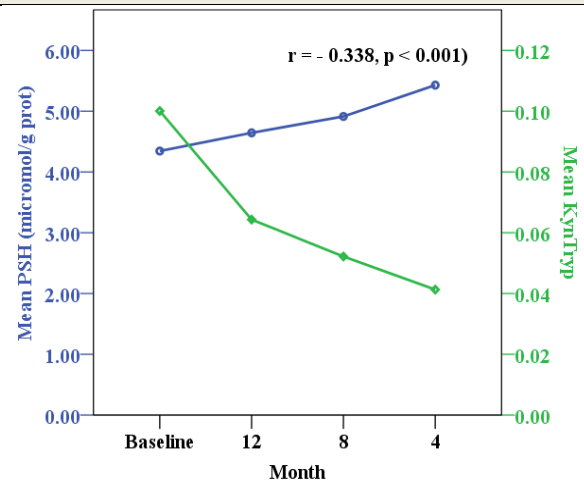



**Figure 4.27 D**

***Figure 4.27. Correlation between PSH with other variables during treatment***

*Correlation between mean PSH and mean PON (Figure 4.27 A); Correlation between mean PSH and mean TEAC (Figure 4.27 B); Correlation between mean PSH and mean Kyn (Figure 4.27 C); Correlation between mean PSH and mean Kyn/Tryp ratio (Figure 4.27 D). Pearson Correlation. r is the correlation coefficient.*

The treatment process increased PSH concentration, leading to a statistically significant increase in PON (r = 0.284, p = 0.002) (Fig. *4.27 A*), TEAC (r = 0.249, p = 0.006) (Fig. 4.27 B). In addition, increasing PSH also reduces inflammation, manifested in concentration of Kyn (r = -0.367, p <0.001) (Fig. 4.27 C) as well as Tryn/Tryp (r = -0.338, p <0.001) (Fig. 4.27 D).

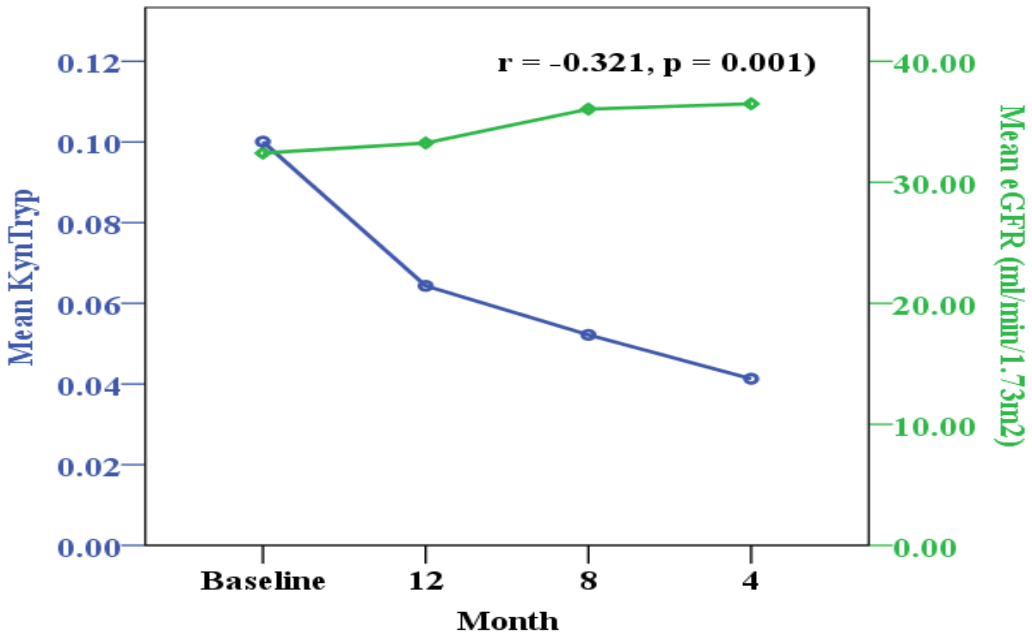



***Figure 4.28. Correlation between Kyn/Tryp with eGFR during treatment***

*Pearson Correlation. r is the correlation coefficient.*

Although in our study, the treatment did not significantly alter the mean eGFR, when considering the correlation relationship may be seen in fig. 4.28, increasing inflammation such as Kyn/Tryp ratio will result in decrease in eGFR (r = 0.321, p = 0.001).

## 4.5. The unexpected effect of treatment on muscle problems and hepatic enzyme profile during treatment.

### 4.5.1. Clinical symptoms

No patients showed clinical problems related to muscle weakness or jaundice during treatment and follow-up time.

### 4.5.2. The change of SGPT, SGOT, CK during treatment

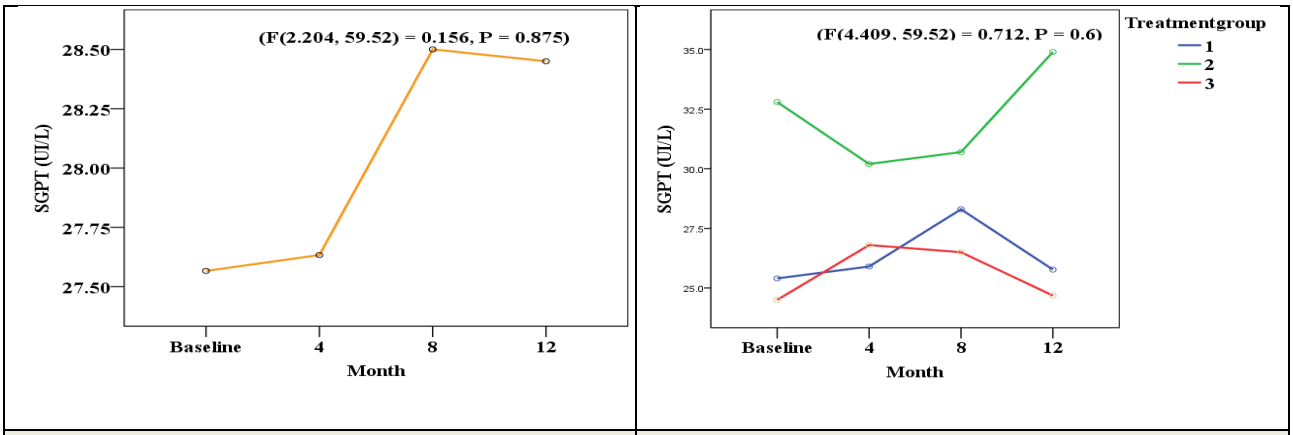



**Figure 4.29 A**




**Figure 4.29 B**

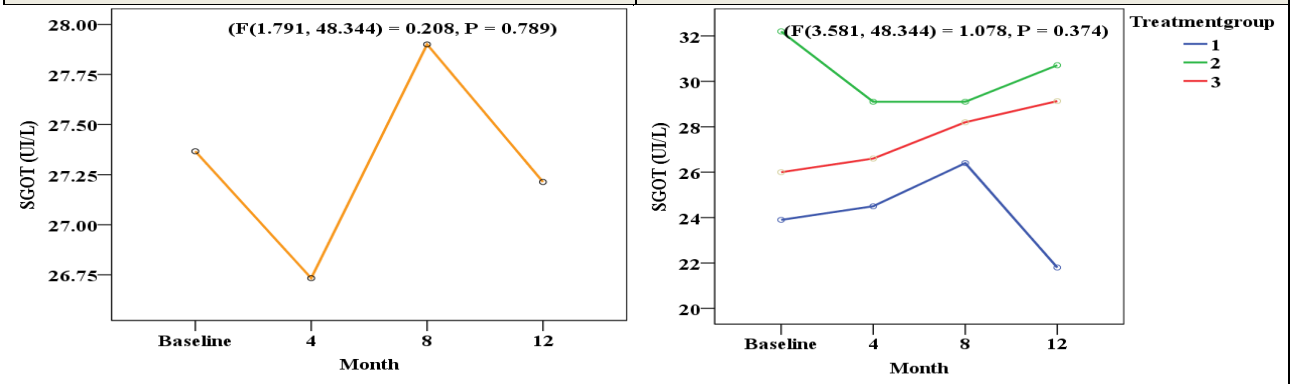



**Figure 4.29 C**




**Figure 4.29 D**

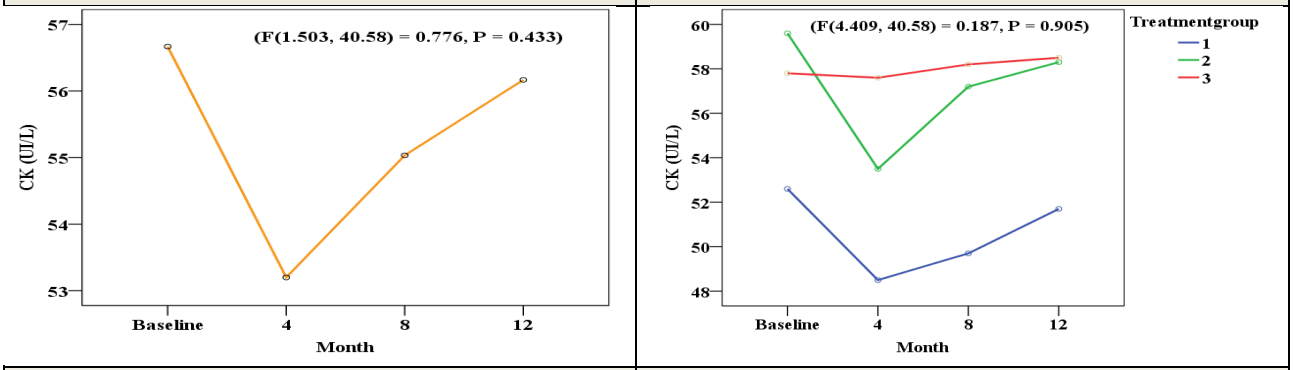



**Figure 4.29 E**




**Figure 4.29 F**

***Figure 4.29. Distribution of SGPT, SGOT, CK by time and group during treatment***

*Distribution of SGPT (Fig. 4.29 A,B); Distribution of SGOT (Fig 4.29 C,D; Distribution of CK (Figure 4.29 E,F);Pearson Correlation. r is correlation coeffient.*

Liver enzymes, including SGOT, and SGPT were tested repeatedly every four months to assess liver side effects while creatine kinase (CK) was monitored during treatment to assess muscle injury. According to figure 4.29, SGOT, SGPT, and CK did not change statistically over time nor did there were any differences between the three groups during treatment, $p > 0.05$.

# 5. DISCUSSION

## 5.1. Characteristics of study subjects

Patients with CKD stage 3-4 have high incidence rates of CVD that is the leading cause of death, rather than progression to ERSD. It is because CKD patients often suffer from other diseases such as diabetes, hypertension, and hyperlipidemia, or old age. These conditions are evidenced associated with oxidative stress, inflammation that promote renal injury. Our study was conducted on 30 healthy controls and 30 CKD patients. CKD patients and healthy controls were chosen individually matched on some characteristics: age, gender, weight, height, BMI to have a better comparison between the 2 groups. CKD patients presented significantly higher systolic and diastolic blood pressure than controls. Both hypertension (HTN) and CKD are truly interrelated worldwide open wellbeing issues. U.S. adults have high blood pressure and CKD accounts for about 30% and 15%, respectively. (Horowitz, Miskulin, & Zager, 2015). CKD and hypertension are associated with an interlocking causal relationship. Impaired renal function is often associated with hypertension, and persistent increases in blood pressure accelerate the progression of renal damage. This interaction was observed in the Chronic Renal Insufficiency Cohort (CRIC) clinical trials by Lash, Go et al. In particular, the prevalence of hypertension within 3612 adults with moderate stage CKD was 86% compared with 29% in the general population (Lash et al., 2009).

As displayed in Table 4.1, CKD patients presented a lipid profile disorder compared to the control group, in which, TC, TG, LDL-C increased while HDL-C decreased. This result is also matched with the results of Angelo Zinellu (A. Zinellu et al., 2015). A new study conducted in the Saudi population with CKD once again confirmed the high level of TC, LDL, TG, and low HDL levels in this population than controls (Aljabri KS, 2019). Moreover, TC/HDL, LDL/HDL, and TG/HDL ratios were significantly high in patients with CKD. Thus, confirming the presence of atherogenic lipid profile needing early

Interventions were applied to prevent cardiovascular complications in chronic kidney disease are accompanied by characteristic abnormalities of lipid metabolism, which appear as a result of nephrotic syndrome or renal failure and are reflected in altered apolipoprotein

profile as well as hyperlipidemia. The correlation between the progression of chronic kidney disease and dyslipidemia has been proven in clinical studies. High levels of cholesterol and triglycerides have been identified as independent risk factors for the progression of kidney disease. Many theories showed physiological mechanisms related to this relationship, however, currently, oxidative stress and insulin resistance are the best known (Trevisan, Dodesini, & Lepore, 2006).

In addition to the hyperlipidemia, CKD patients in our study also showed a significant increase in MDA and decrement trend of PSH, PON, TEAC. The same results are also reported by Gaosi Xu et al, in which, with the development of CKD, serum levels of MDA were significantly increased, and the serum levels of SOD (superoxide dismutase) and GSH-PX (glutathione peroxidase) were significantly decreased in these participants (Xu et al., 2015). Increased oxidative stress has been found in CKD patients via some markers such as lipid oxidized lipid products and oxidized LDL-enhanced protein precursors, F2 isoprostanes, and the 8-hydroxyl 2-deoxyguanosine oxidized DNA damage (Cachofeiro et al., 2008). On the other hand, mitochondria are involved in ROS production. Impairment in the mitochondrial respiratory system in patients with CKD is said to be a consequence and cause of enhanced oxidative stress (Modaresi et al., 2015). ROS can affect cellular function and damage proteins, lipids, and nucleic acids, while also inhibiting the enzymatic activities of the respiratory chain. Progression of CKD to advanced stages is associated with a significant increase in ROS formation (Granata et al., 2009). The impaired oxidation balance may result from a combination of increased production and decreased clearance of ROS as well as unsuccessful antioxidant protection. (Cachofeiro et al., 2008).

Paraoxonase 1 (PON1) is a high-density lipoprotein esterase and plays a role in several human diseases including diabetes and atherosclerosis. Low PON1 activity is associated with an increased risk of major cardiovascular events. PON 1 is considered as a multifunctional antioxidant enzyme because it has PON, arylesterase (ARE), and homocysteine thiolactonase (HTLase) activity (Shunmoogam, Naidoo, & Chilton, 2018). PON-1 enzyme detoxifies homocysteine thiolactone (HTL) as well as breaks down oxidized LDL molecules. HTL was

evidenced as a metabolite that is more active than homocysteine (Hcy), which mediates the toxic and harmful effects of Hcy. HTLase is a component of HDL that hydrolyzes HTL to Hcy. Endothelial damage, especially due to LDL homocysteinylation, is prevented by performing HTL detox. HTLase is a product of the polymorphic PON-1 gene. High HTLase activity has been reported to provide better protection against protein homocysteinylation compared to low HTLase activity. TEAC, antioxidant was conclude to represent a mixed antioxidant reaction, rather than a single antioxidant. While responding to oxidative stress, the mechanism of the response may vary between clinical situations, so the clinical significance of changes in plasma TEAC remains to be determined. TEAC was positively associated with the renal decline (Chrzanowska, Kamińska, Głyda, Duda, & Makowska, 2010).

Via the kynurenine pathway, dietary tryptophan is catabolized by tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase to N-formylkynurenine and then converted to Kyn (Salter & Pogson, 1985). In normal conditions, the activity of IDO that happened during Trp catabolism was low. In response to infection and tissue inflammation, IDO was induced by pro-inflammatory cytokines interferon-g modified. The Kyn/Tryp ratio has been suggested as an index to monitor the IDO activation status (Capuron et al., 2011).

Results in Table 4.1 demonstrated that, in CKD patients, plasma kynurenine concentration is higher, and tryptophan concentration is lower than in controls. Kynurenine concentration increased, while tryptophan concentration decreased significantly, and thus, the Kyn/Trp ratio also increased. This finding was similar to the previous result of Angelo Zinellu in 2015 (A. Zinellu et al., 2015). According to an observation, there was a higher plasma kynurenine concentration and chronic inflammatory in CKD. (Zhao, 2013). These results suggest that IDO activity increased may be due to inflammatory processes in CKD. Due to IDO activation, serum tryptophan reduces, and kynurenine increase in parallel.

## 5.2. The effect of lipid-lowering regimes on lipid profile, oxidative stress indices, Tryptophan degradation.

CKD is frequently present with dyslipidemia, known as a risk factor for cardiovascular disease. Among that, TC, TG, and LDL-C concentrations increase while the HDL-C concentration decreases. Patients with stages 3-5 CKD are considered high or very high CV risk because of the high prevalence of the cardiovascular disease. LDL-C levels are considered a therapeutic target for hypolipidemia in CKD patients.

Single-use of statin or statin/ ezetimibe combination is indicated in CKD patients not dependent on dialysis. Based on these reasons, we have designed a study using lipid-lowering drugs to evaluate the effectiveness of the drugs on oxidative markers and inflammatory markers. We randomized patients into three different groups to receive three different treatment regimens. Group 1 was treated with 20mg of Simvastatin, group 2 was prescribed with a combination of 10 mg EZT and 20mg Simvastatin, while group 3 was given 10 mg EZT and 20mg.

Our results have demonstrated the role of lipid-lowering drugs in improving lipidemia profiles over the treatment time. TC, TG, LDL-C decreased while HDL-C gradually increased over time in all patients as well as in different treatment groups. Although there was no statistically significant difference between the 3 groups during treatment, after 12 months, the improvement in blood lipid was found to be greater in group 3 compared to the other 2 groups. As anticipated, three lipid-lowering therapies significantly improved lipid profile in all patients, in which, better results were showed in patients treated with ezetimibe/simvastatin 10/40 mg daily. Compared to all other lipid-lowering agents available, the HMG-CoA reductase inhibitor not only lowers LDL cholesterol more effectively but may also be better tolerated. The usual therapeutic dose lowers total cholesterol by 25 to 30%, LDL cholesterol by 30 to 40%, and triglycerides by 10 to 15%. HDL cholesterol increases by about 5 to 10%. Simvastatin has been reported to have a beneficial effect on cardiovascular mortality and also on coronary artery diseasu. In a large double-blind study in patients with coronary heart

disease, simvastatin significantly reduces from 12% to 8% mortality rate and from 28% to 19% coronary events within five years (Talreja O, 2020).

Ezetimibe inhibits the cholesterol absorption from the small intestine, results in the decrease of cholesterol level in the blood (Brar, 2004). The decrease of cholesterol absorption leads to the decrease of cholesterol to the liver, therefore increased cholesterol clearance from the blood, and consequently, reduces cholesterol stores in the liver. The improvement of total cholesterol, triglycerides, LDL-C, and HDL-C are results of the reduction in cholesterol absorption (Sizar O, Updated 2020 Apr 21). Simvastatin plus ezetimibe allows clinicians to simultaneously inhibit two cholesterol metabolisms: cholesterol biosynthesis in the liver and cholesterol absorption in the small intestine. This dual inhibitory mechanism has a substantially better performance in reducing serum low-density lipoprotein and increasing high-density lipoprotein, compared with the mechanism observed with drug alone. This combination increases an opportunity to have a successful treatment in patients with dyslipidemia (Kei, Filippatos, & Elisaf, 2016).

Patients with CKD have high cardiovascular morbidity and mortality, as a consequence of an aggregation of cardiovascular risk factors in this population. Kidney disease increased gradually the signs of oxidative stress even in the early stages of CKD. This may be the result of an increase in reactive oxygen types as well as reduce the likelihood of anti-oxidants. This oxidative stress can accelerate the progression of kidney damage. Inflammation markers, such as C-reactive protein and cytokines increase when kidney function decline suggests that CKD is a low-grade inflammatory process. Oxidative stress is one of the factors that hat might be engaged with the activation of the inflammatory process in CKD. The utilization of statins to reduce the risk of major vascular events in CKD patients is essential because CKD is considered a high risk of the cardiovascular subject. This beneficial effect of statins is not only on lipid-lowering but especially concerning the regulation of oxidative stress and inflammation (Cachofeiro et al., 2008).

MDA level in our study decreased in all 3 treatment regimens. This result is similar to the results from a systematic review and meta-analysis journal when the patient is on statin

treatment (Angelo Zinellu, Paliogiannis, Usai, Carru, & Mangoni, 2019). Statin therapy appears to increase glutathione reductase activity and induction of heme-oxygenase system 1. Furthermore, the result of the antioxidant effect seems related to its ability to inhibit oxidative enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and regulates antioxidant enzymes such as catalase (Hsu et al., 2006)

PSH, PON1, TEAC, are antioxidants, improved during treatment with lipid-lowering drugs. However, this reduction occurred only in group 3 (treated with Simvastatin/EZE at 40/10mg/day) and not in group 1 (treated with simvastatin 40mg alone) nor in group 2 (treated with Simvastatin/EZE dose 20/10mg/day). CKD is related to relatively high plasma concentrations of both total Cys and Hcy. Simvastatin / EZE combination therapy with 40 / 10mg therapy significantly increased PSH levels. The PSH value represents the total number of sulfhydryl protein groups in plasma. The most reduced -SH group in plasma is that of human serum albumin, with its high concentration. The single free cysteinyl of HSA, Cys34, accounts for about 80% of the reduced thiol content in human plasma and is an important collector of reactive oxygen species and vascular nitrogen, thus an important redox buffer of blood. Therefore, when reactive oxygen species are present, the PSH concentration decreases, while the increase in PSH concentration indicates a decrease in oxidative stress (Conard et al., 2010). Similar to our results, in a study that treated Simvastatin alone in coronary artery disease, PON1 levels did not change (Januszek, 2016). PON1 deficiency is associated with increased oxidation of low-density lipoprotein and the development of atherosclerosis. Recently it has been proven that decreased PON1 activity predicts higher risks of major adverse effects cardiac events in patients with CKD and low serum PON1 levels may be an independent predictor of cardiovascular mortality in HD patients (David J. Kennedy & al, 2017). This indicates the need to combine Ezetimibe 10mg with Simvastatin 40mg in treatment to increase the antioxidant capacity of PON1, TEAC, and PSH.

### 5.3. The relationship between lipid profiles, oxidative stress, tryptophan degradation during treatment.

When examining the correlation between the plasma lipid profiles, oxidative index, and inflammatory indices during treatment by lipid-lowering regimens, our results demonstrated that a decrease in LDL-C level leads to a statistically significant parallel reduction of MDA, Kyn, and Kyn/Tryp ratio. Besides, the LDL-C reduction treatment also significantly reduced the CRP concentration, although the correlation coefficient was not high ($r = 0.187$, $p = 0.041$). Moreover, the results from Fig. 4.26A and Fig. 4.26B demonstrated that, during 12 months of treatment, the reduction of MDA was accompanied by a significant reduction of Kyn/Tryp as well as a decrease of CRP. This shows the relationship between LDL-C, MDA, and Kyn, Kyn/Tryp. The improvement of inflammation in CKD patients stage 3-4 is explained by the decrease in LDL-C through improved oxidative stress. Results obtained in our study when analyzing the correlation of one antioxidant, PSH, with other variables showed that LDL-C decrease during treatment will significantly increase PSH ($r = 0.179$, $p = 0.05$) and treatment with an increase in PSH results in a marked decrease in inflammation, manifested in decreased Kyn, Kyn/Tryp. Moreover, PSH also showed a statistically significant positive correlation with other antioxidants such as PON1, TEAC. Unfortunately, the correlation coefficient between them is not high. These results suggest that 12 months of lipid-lowering therapy reduces inflammation in CKD patients possibly through an increase in antioxidants such as PSH as well as a decrease in MDA, as a result of a decrease in LDL-C. Our results are similar to our results of Angelo Zinellu in 2015, drug treatment significantly improved the lipid profile in all patients and this was associated with a consistent reduction in Kyn and Kyn/Trp ratios regardless of treatment groups. The improvement of both oxidation and inflammation after cholesterol-lowering treatment in CKD can be mediated by restoring antioxidant taurine levels during treatment, which has been shown to improve oxidative stress status. The inflammation in CKD patients may be explained by cholesterol-lowering effects. (A. Zinellu et al., 2015).

Our data are reliable with the results of another author when studying the effects of atorvastatin, a type of statins, on the inflammatory parameters of CKD (Goicoechea et al., 2006). This author demonstrated that, in addition to its lipid-lowering effect, atorvastatin also significantly reduces the plasma concentrations of CRP, IL-1, and TNF-a. Kyn is thought to be excreted by the kidneys, so increasing glomerular filtration rate during treatment with lipid-lowering drugs may also help improve Kyn. This is also proven in my study when there is an inverse correlation between eGFR with Kyn and with Kyn/Tryp ratio. However, all three lipid-lowering therapies in our study did not significantly improve eGFR. So the reduction of Kyn, Kyn/Tryp ratio can be explained by other factors. The significant relationship between a parallel reduction in Kyn concentration and a Kyn / Trp ratio with a decrease in MDA suggests a close interaction between inflammation and oxidation in this context. The decrease in plasma Kyn that occurs during cholesterol-lowering therapy may be due to decreased IDO regulation as a result of reduced oxidative stress. A pre-inflammatory state is characterized in the CKD population. It is considered an important indicator of patient health and outcome. Although the exact biological mechanisms that contribute to the high rates of infection in chronic kidney disease are not well defined, ROS may have an important role to play, especially when kidney function is impaired. Tryp degradation via indoleamine (2,3) - dioxygenase (IDO), thereby increasing Kyn concentrations, has been suggested as an immune system activation marker. Inflammation is a mechanism that is sensitive to redox. Oxidative stress may contribute to a pro-inflammatory state in CKD through the activation of NF-kB, which in turn activates and recruit immune cells. (Cachofeiro et al., 2008).

### 5.4. The effect of lipid-lowering regimes on renal function and undesirable side effect during treatment.

The effectiveness of statin-based therapies in reducing cardiovascular mortality in people with CKD appears to decrease as eGFR decreases. The strongest evidence to support the cardiovascular benefits of statins in people with chronic kidney disease is shown when taking ezetimibe plus simvastatin compared with placebo. The Heart and Kidney Protection Study (SHARP) is the first randomized controlled trial (RCT) to evaluate the effect of CV

outcome in CKD patients. The study did not show any significant difference in the progression of end-stage kidney disease in non-dialysis patients ((Pandya, Rao, & Chaudhary, 2015))
It is evidenced that the beneficial effects of statins are not only lipid-lowering and may include anti-inflammatory, antioxidant, and immunomodulatory effects. ((Kassimatis & Goldsmith, 2014)). Plasma KYNA/TRP ratio was sensitive and reliable to indicate renal function and could be used as a new biomarker to assess the risk or presence of kidney disease (Zhao 2013). The use of statins to reduce proteinuria and improve renal outcome in CKD patients has been reported but no RCTs or meta-analysis showed statins delay the progression of CKD ((Kassimatis & Goldsmith, 2014),(Palmer et al., 2014), (Upadhyay, 2014)), (Baigent et al., 2011).

Similar to the above studies, the results of our study, also found no significant effects of lipid-lowering drugs on renal function. Our results showed an inverse correlation between decreased inflammation and increased renal function, considering the Kyn/Tryp ratio with eGFR. However, all 3 groups treated with 3 different lipid-lowering regimens showed no statistically significant benefit on eGFR even though eGFR tended to increase in all 3 groups. Therefore, the goal of improving renal function through the use of lipid-lowering drugs is not suggested in CKD patients. However, on the contrary, treatment didn't worsen kidney function anyhow.

Our clinical trial also evaluated the effects of treatment regimens on liver and muscle damage. The results obtained in the study demonstrated that the combination of simvastatin with ezetimibe as well as the use of statin alone for 12 months in patients with non-dialysis CKD does not significantly increase CK levels. No patients with muscle manifestations nor cases of rhabdomyolysis have been reported. Also, there was no patient with transaminase increase to 3 times the upper limit, or jaundice. Results from SHARP research (Baigent et al., 2011) that performed on 6247 CKD patients without dialysis as well as the study of Angelo Zinelu also have the same findings as us (A. Zinellu et al., 2015).

# CONCLUSION

CKD patients are considered a high or very high-risk factor for cardiovascular disease. In the general population, there is a strong association between dyslipidemia and the risk of coronary artery disease. Results from clinical trials have demonstrated that statins, drugs that reduce cholesterol in the blood, can reduce the risk of stroke. Through inhibition of cholesterol synthesis by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase, statins are widely used in current clinical indications to improve primary and secondary CVD prevention outcomes. However, in CKD patients, characterized by an increase in oxidation and inflammation compared to the general population, statin alone improves blood lipids, as well as oxidative stress or inflammation. Whether or not is still unknown. On the other hand, the administration of high doses of statins in patients with decreased eGFR may cause complications related to liver function, kidney function, or diseases related to muscle damage. Therefore, we combined statin with ezetimibe, a drug that strongly inhibits intestinal cholesterol absorption from food and bile. Since oxidative stress and inflammation play an essential role in the pathogenesis of CKD, our study evaluated the effect of lipid-lowering therapies on oxidative stress as well as inflammation in CKD patients. Through the results achieved during 12-month treatment, we have the following conclusions:

First, all three hypolipidemia therapies used to treat dyslipidemia in 12 months in patients with CKD stage 3-4 significantly improved lipid profiles (decrease TC, TG, LDL-C, and increased HDL-C), and oxidation stress status (decreased MDA levels, increased concentrations of PSH, PON, and TEAC) and decreased inflammation markers (Kyn concentration, and Kyn/Trp ratio) in all patients. However, despite the potential for improvement in inflammatory and oxidative stress parameters, it appeared to be better in the group treated with Simvastatin 40 mg/day + Ezetimibe 10 mg/day, but this difference was not statistically significant.

Second, when investigating the correlation between LDL-C, oxidative stress indicators, and inflammatory indicators, we found that there is a positive correlation between

LDL-C and MDA, Kyn concentration, and Kyn/Tryp ratio during treatment. On the other hand, an increase in MDA in parallel with an increase in Kyn / Tryp was also discovered. This suggests the relationship between LDL-C, MDA, Kyn, and Kyn / Tryp. The improvement in inflammation in CKD patients is explained by a decrease in LDL-C through a decrease in oxidative stress. Meanwhile, PSH, a known antioxidant, is inversely related to Kyn, Kyn/Tryp. This result suggests that the reduction in inflammation in CKD patients may be due to an increase in antioxidants such as PSH.

Third, the results of our study showed that, during 12 months of treatment and follow-up, all patients did not have clinical manifestations of jaundice, muscle aches, or rhabdomyolysis. This has also been demonstrated by our SGOT, SGPT, and CK test results that have been monitored every 4 months.

Last, renal function, as assessed by eGFR, has also been monitored to assess the effect of lipid-lowering therapies on chronic renal progression. The results showed no significant change during treatment, meaning that the drug-lowering therapy did not improve kidney function nor worsen it. However, the first time we noticed a negative correlation between eGFR and inflammation during 12 months of treatment, presented in concentrations Kyn and eGFR correlation as well as the Kyn/Tryp ratio and eGFR correlation, although the correlation is not very high. This poses a new research direction in the future with more patients.

In summary, patients with stage 3-4 CKD do not meet LDL-C targets, the Simvastatin / Ezetimibe combination at 40m, 10mg daily doses are used to inhibit cholesterol absorption, and synthesis thereby may induce more lipid-lowering effects, allowing more patients to achieve LDL-C targets, subsequently reducing oxidative stress as well as inflammation, but without causing dose-related adverse effects amount.

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