

STUDY PROTOCOL

LIPID - LOWERING REGIMES IMPROVE OXIDATIVE STRESS, TRYPTOPHAN DEGRADATION IN HYPERCHOLESTEROLEMIA CKD PATIENTS

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Brief Title:

**LIPID-LOWERING THERAPIES IN VIETNAMESE CHRONIC KIDNEY
DISEASE POPULATION**

I. BACKGROUND - ABSTRACT/SUMMARY STATEMENT OF THE RESEARCH PROJECT:

The prevalence of chronic kidney disease (CKD) in Vietnamese population is increasing along with hypertension and diabetes. 3.1% of the population diagnosed as CKD (stages 3-5) with positive findings in urine test (Ito et al., 2008). In CKD patient, cardiovascular disease (CVD) is the leading cause of mortality. Kidney Disease Statistics for the United States showed that the prevalence of CVD is 69.6 percent among person ages 66 and older who have CKD, compared to 34.7 percent among those who do not have CKD (Health, 2012).

The lipidemic disorder is one of the cardiovascular risk factors in CKD but it was not fully concerned. Highest cholesterol in CKD patient is the total or low-density lipoprotein (LDL).

A one-year prospective study of stage 3 or 4 CKD patients with dyslipidemia Siriraj Hospital showed that the percentage of patients with hypercholesterolemia was 78%, hypertriglyceridemia 54%, and low high-density lipoprotein-C 36% (Sangswang & Sriwijitkamol, 2015). Two-thirds of CKD patients with hyperlipidemia had mixed hyperlipidemia. Despite the high frequency of statin treatment, only one-third of CKD patients achieved the LDL-C goal (Sangswang & Sriwijitkamol, 2015). A question whether high-dose of statins monotherapy is more effective in LDL cholesterol lowering is still unclear, but high doses are associated with a high rate of hepatotoxicity, myopathy. Thus, we need to explore more advanced lipid-lowering therapies to manage dyslipidemia in patients with CKD.

Dyslipidemia has been proposed an independent risk factor in accelerating renal injury (Trevisan, Dodesini, & Lepore, 2006). Statin and Statin/ezetimibe were evidenced to prevent cardiovascular complications in CKD patient. Co-administration of ezetimibe the first time enhanced proteinuria-lowering effects of pitavastatin in non-diabetic CKD patients partly via a cholesterol-independent manner. Ezetimibe may have pleiotropic actions that could contribute to renoprotective properties of this lipid-lowering agent

(Nakamura, T., et al. (2010). However, it is still unclear if Statin and Statin/ezetimibe therapies can improve the renal function by proteinuria.

Because of the high prevalence of cardiovascular morbidity and mortality in CKD patient, we need a better understanding of the mechanism for treatment strategy. Metabolomics was used in identifying new biomarkers of CKD such as acylcarnitines, glycerolipids, dimethylarginines and metabolites of tryptophan. C-mannosyl tryptophan and pseudouridine have better performance than creatinine in CKD stratification (Hocher & Adamski, 2017). Inflammation status was significantly reduced by treatments. There was no significant difference between treatments (Moutzouri et al., 2013). Endothelial dysfunction secondary to the pro-inflammatory and pro-oxidative state caused by Oxidative stress (OS) causing endothelial dysfunction secondary to the pro-inflammatory and pro-oxidative was observed in CKD. However, how hypolipidemic agents impact on Oxidative stress is still not clear. Tryptophan (Trp) degradation via indoleamine (2,3)-dioxygenase (IDO), with consequent increased in kynurene (Kyn) concentrations, has been considered as a marker of immune system activation (Schefold et al., 2009). The effect cholesterol-lowering treatment on oxidative and inflammation parameters in CKD might be mediated by restoration of antioxidant taurine concentrations, suggesting that amelioration of both oxidative and inflammation status in CKD patients could be partly explained by the cholesterol-lowering effects (Zinelli et al., 2015).

In clinical practice, physicians always concern the effects and safety before giving the prescription. However, despite the high frequency of statin treatment, only 1/3 of CKD patients achieved the LDL-C goal. Whether high-dose of statins mono-therapy is more effective in LDL-C lowering is still unclear, but are associated with a high rate of hepatotoxicity, myopathy.

Lowering LDL-C with statin mono-therapy and statin/ezetimibe combination reduces the risk of CVD in population without kidney disease. Which Cholesterol-lowering therapies are suitable for stage 3,4 CKD patients in term of e-GFR reduction and side effects? There is no data related to this field in the Vietnamese CKD population. Thus, more advanced lipid-lowering therapies and a better understanding of the mechanism is needed for treatment strategy of hyperlipidemia in Vietnamese patients with CKD. The following research questions need to be addressed:

(1) Do different lipid-lowering drugs (simvastatin monotherapy and ezetimibe/simvastatin combination therapy) have a different impact on the lipid profile, oxidative stress indices, Tryptophan degradation?

(2) Does statin monotherapy or co-administration of ezetimibe ameliorate the renal dysfunction in CKD patients?

(3) How do lipid-lowering therapy effect on lipidemia disorders in patients with CKD by mechanism involved in oxidative stress and tryptophan?

(4) Are the unexpected effects on muscle problem and hepatic enzyme profile of long-term statin monotherapy and statin/ezetimibe combination therapy a big concern?

II. REVIEW OF RESEARCH LITERATURE

2.1. Criteria for Chronic Kidney Disease

- Improving Global Outcomes 2012 Clinical Practice Guideline (KDIGO 2013) (Stevens, Levin, & for the Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group, 2013).

- Either of the following present for >3 months. Markers of kidney damage (one or more)

+ Albuminuria (AER \geq 30 mg/24 hours; ACR \geq 30 mg/g)

+ Urine sediment abnormalities Electrolyte and other abnormalities due to tubular disorders Abnormalities detected by histology Structural abnormalities detected by imaging

+ History of kidney transplantation

+ Decreased GFR GFR $<$ 60 ml/min/1.73 m² (GFR categories G3a–G5)

2.2. Chronic Kidney Disease stages (Stevens et al., 2013)

Stage	GFR	Treatment
1	90+	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3A	45-59	Moderately reduced kidney function
3B	30-44	
4	15-29	Severely reduced kidney function
5	<15 or on dialysis	Very severe, or end-stage kidney failure (sometimes call established renal failure)

2.3. Dyslipidemia in chronic disease

Dyslipidemia is associated with atherosclerotic vascular disease and an increased risk of CVD events, including acute myocardial infarction (AMI). Guidelines recommended aggressive lipid-lowering therapies in patients at high risk of CVD. Although patients with severe CKD are at high risk of CV events, the use of statins was limited for many reasons. Unlike individuals in the general population, patients with CKD are at risk of malnutrition and inflammation that have a cholesterol-lowering effect. In 42 cases of CVD in CKD patients, cholesterol levels, and mortality risk inversely correlated while there was a strong and positive correlation between serum cholesterol and CVD mortality in the absence of inflammation and malnutrition. Because of the risk of rhabdomyolysis, fibrates are contraindicated in patients with renal failure. That was one of the reasons why lipid-lowering agents is not popularly use in patients with CKD ("<CKD machenism.pdf>,")

2.4. Oxidative stress, inflammation and cardiovascular disease in chronic renal failure

Traditional risk factors, as well as additional nontraditional risk factors, can directly damage the kidney. Some studies have shown the relationship between oxidative stress and inflammatory biomarkers. Some clinical studies indicated an inverse

correlation between oxidative stress status and estimated glomerular filtration rate (eGFR) (Stevens et al., 2013).

Different mechanisms could explain the oxidative stress increase in CKD. Some conditions of CKD patients such as advanced age, diabetes, hypertension, lower levels intake of the antioxidant vitamin, and failure of ROS clearance in renal function are related to the increase of oxidative stress and progression of CKD (Meenakshi Sundaram, Nagarajan, & Manjula Devi, 2014)

2.5. Tryptophan and oxidative stress in chronic disease

Tryptophan (Trp) plays a role of a precursor of several metabolic pathways involving different end products, such as proteins, serotonin, melatonin, and kynurenone (Ruddick, 2006). Tryptophan (Trp) degradation via indoleamine (2,3)-dioxygenase (IDO) has been proposed as an immune system activation marker (Schefold et al., 2009). The effect cholesterol-lowering treatment on oxidative and inflammation parameters in CKD might be mediated by restoration of antioxidant taurine concentrations, suggesting that amelioration of both oxidative and inflammation status in CKD patients could be partly explained by the cholesterol-lowering effects (Zinelli et al., 2015)

2.6. Guidelines for the management of Dyslipidaemias ("Ten Commandments" from the 2016 ESC/EAS Guidelines for the management of dyslipidaemias, 2016)

(1) Total risk estimation using a risk estimation systemsuch as SCORE is recommended for asymptomatic adults>40 years of age without evidence of chronic cardiovascular disease (CVD), diabetes, chronic kidney disease (CKD) or familial hypercholesterolaemia.

(2) High- and very high-risk individuals can be detected on the basis of documented CVD, diabetes mellitus,moderate to severe renal disease, very highlevels of individual risk factors, familial hypercholesterolaemia or a high SCORE risk and are a high priority for intensive advice with regards to all risk factors.

(3) LDL-Cholesterol (LDL-C) should be used as the primary lipid analysis for screening, risk estimation, diagnosis andmanagement. HDL-C is an independent risk factor and is included in the electronic version of SCORE,HeartScore.

(4) LDL-Cis recommended as the primary target for treatment.

(5) In patients at VERY HIGH CV risk, an LDL-C goal of <1.8mmol/L (70mg/dL), or a reduction of at least 50% if the baseline LDL-C is between 1.8 and 3.5mmol/L (70 and 135mg/dL) is recommended.

(6) In patients at HIGH CV risk, an LDL-C goal of <2.6mmol/L (100mg/dL), or a reduction of at least 50% if the baseline LDL-Ce is between 2.6 and 5.1mmol/L (100 and 200 mg/dL) is recommended.

(7) A statin is the usual first line treatment to reach the LDL-C goal and should be used up to the highest recommended dose or highest tolerable dose to reach the goal.

(8) Familial Hyperlipidaemia should be suspected in patients with CHD before the age of 55 years for men and 60 years for women, in subjects with relatives with premature fatal or non-fatal CVD, in subjects with relatives having tendon xanthomas, and in subjects with severely elevated LDL-C

(in adults >5 mmol/L [190 mg/dL], in children >4 mmol/L [150 mg/dL]).

(9) Treatment with statins is recommended for older adults with established CVD in the

Same way as for younger patients.

(10) High dose statins should be given early after admission in all acute coronary syndrome patients without contraindication or a history of intolerance, regardless of initial LDL-C values.

2.7. Total cardiovascular risk estimation (Catapano et al., 2016)

Very high risk	Subjects with any of the following: <ul style="list-style-type: none">• Documented cardiovascular disease (CVD), clinical or unequivocal on imaging. Documented CVD includes previous myocardial infarction (MI), acute coronary syndrome (ACS), coronary revascularization (percutaneous coronary intervention (PCI), coronary artery bypass graft surgery (CABG)) and other arterial revascularization procedures, stroke and transient ischaemic attack (TIA), and peripheral arterial disease (PAD). Unequivocally documented CVD on imaging is what has been shown to be strongly predisposed to clinical events, such as significant plaque on coronary angiography or carotid ultrasound.• DM with target organ damage such as proteinuria or with a major risk factor such as smoking, hypertension or dyslipidemia.• Severe CKD (GFR <30 mL/min/1.73 m²).• A calculated SCORE $\geq 10\%$ for 10-year risk of fatal CVD.
High risk	Subjects with: <ul style="list-style-type: none">• Markedly elevated single risk factors, in particular cholesterol >8 mmol/L (>310 mg/dL) (e.g. in familial hypercholesterolaemia) or BP $\geq 180/110$ mmHg.• Most other people with DM (some young people with type 1 diabetes may be at low or moderate risk).• Moderate CKD (GFR 30–59 mL/min/1.73 m²).• A calculated SCORE $\geq 5\%$ and <10% for 10-year risk of fatal CVD.
Moderate risk	Subjects are considered to be at moderate risk when their SCORE is $\geq 1\%$ and <5% at 10 years.
Low risk	The low-risk category applies to individuals with SCORE <1% for 10-year risk of fatal CVD.

2.8. Recommendations for lipid management in patients with moderate to severe chronic kidney disease (Catapano et al., 2016). 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias

Recommendations	Class	Level
Patients with stage 3–5 CKD have to be considered at high or very high CV risk.	I	A
The use of statins or statin/ ezetimibe combination is indicated in patients with non-dialysis dependent CKD.	I	A
In patients with dialysis-dependent CKD and free of atherosclerotic CVD, statins should not be initiated.	III	A
In patients already on statins, ezetimibe or a statin/ezetimibe combination at the time of dialysis initiation, these drugs should be continued, particularly in patients with CVD.	IIb	C
In adult kidney transplant recipients treated with statins may be considered.	IIb	C

2.9. Recommendations for treatment goals for low-density lipoprotein-cholesterol (Catapano et al., 2016)

Recommendation	Class of recommendation	Level of evidence
In patients at VERY HIGH CV risk, an LDL-C goal of <1.8 mmol/L (70 mg/dL) or a reduction of at least 50% if the baseline LDL-C is between 1.8 and 3.5 mmol/L (70 and 135 mg/dL) is recommended.	I	B
In patients at HIGH CV risk, an LDL-C goal of <2.6 mmol/L (100 mg/dL), or a reduction of at least 50% if the baseline LDL-C is between 2.6 and 5.2 mmol/L (100 and 200 mg/dL) is recommended.	I	B
In subjects at LOW or MODERATE risk, an LDL-C goal of <3.0 mmol/L (<115 mg/dL) should be considered	IIa	C

2.10. Lipid Measurement in Adults with CKD. (Sarnak et al., 2015). 2013 KDIGO Clinical Practice Guidline for Lipid management in CKD

1.1. In adults with newly identified CKD (including those treated with chronic dialysis or kidney transplantation), we recommend an evaluation with a lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides). (1C)

1.2. In adults with CKD (including those treated with chronic dialysis or kidney transplantation), follow-up measurement of lipid levels is not required for the majority of patients. (Not Graded)

2.1.1. In adults aged \geq 50 years with eGFR $<$ 60 ml/min/1.73 m² but not treated with chronic dialysis or kidney transplantation (GFR categories G3a-G5), we recommend treatment with a statin or statin/ezetimibe combination. (1A)

2.1.2: In adults aged \geq 50 years with CKD and eGFR \geq 60 ml/min/1.73 m² (GFR categories G1-G2), we recommend treatment with a statin. (1B)

2.2: In adults aged 18-49 years with CKD but not treated with chronic dialysis or kidney transplantation, we suggest statin treatment in people with one or more of the following (2A):

- known coronary disease (myocardial infarction or coronary revascularization)
- diabetes mellitus
- prior ischemic stroke
- estimated 10-year incidence of coronary death or non-fatal myocardial infarction > 10%

2.3.1: In adults with dialysis-dependent CKD, we suggest that statins or statin/ezetimibe combination not be initiated. (2A)

2.3.2: In patients already receiving statins or statin/ezetimibe combination at the time of dialysis initiation, we suggest that these agents be continued. (2C)

2.4: In adult kidney transplant recipients, we suggest treatment with a statin. (2B)

2.11. Recommended doses of statins in adults

Recommended doses of statins in adults			
	ACC/AHA Recommendations for eGFR > 60 mL/min/1.73 m ²		KDIGO Recommendations for e-GFR < 60 mL/min/1.73 m ²
	High-Intensity Statin	Moderate-Intensity Statin	
Atorvastatin	40-80 mg	10-20 mg	20 mg
Fluvastatin	—	80 mg	80 mg
Lovastatin	—	40 mg	Not studied
Pravastatin	—	40-80 mg	40 mg
Rosuvastatin	20-40 mg	5-10 mg	10 mg
Simvastatin	— ^a	20-40 mg	40 mg
Simvastatin/ezetimibe	—	Not mentioned in ACC/AHA guidelines	20 mg/10 mg

2.12. Simvastatin

- Treat with statins for 1-2 weeks, up to 4-6 weeks, and maintain during long-term treatment.
- Simvastatin largely excreted in the feces. Approximately 10-15% of the drug excreted by the kidney
- Dosage is 5-80 mg/day, in the evening.
- No dosage adjustment is needed in the elderly
- **Contraindications of Simvastatin:**
 - + Active liver disease or unexplained persistent elevations of serum transaminases

- + Pregnancy and lactation
- + Concomitant administration of potent CYP3A4 inhibitors (e.g. itraconazole, ketoconazole, posaconazole, HIV protease inhibitors (e.g. nelfinavir), erythromycin, clarithromycin, telithromycin, and nefazodone)
- + Concomitant administration of gemfibrozil, ciclosporin, or danazol.
- **Unexpected effects of Simvastatin and how to manage unexpected effects**
 - + **Myopathy:** The risk of myopathy is related to dose. In a clinical trial of 41,413 patients treated with simvastatin, 24,747 patients (about 60%) with an average follow-up of at least 4 years, the incidence of myopathy was approximately 0.03%, 0.08% and 0, 61% at doses of 20, 40 and 80 mg/day, respectively.
 - If the patients have muscle aches, weakness or cramps, do CK test. If CK increases (> 5 times) discontinue treatment, re-assess levels within 5 to 7 days.
 - If the muscular symptoms are severe and cause daily discomfort, even if the CK levels are < 5 times normal, consider stopping treatment.
 - + **Elevated liver enzymes:** Continuous elevations (up to > 3 times) liver enzymes have occurred in some patients receiving simvastatin.
 - When simvastatin is discontinued, the level of transaminase usually decreases gradually down to the pre-treatment level.

+ **Diabetes Mellitus**

Some evidence suggests that statins as a class raise blood glucose and in some patients, at high risk of future diabetes, may produce a level of hyperglycemia where formal diabetes care is appropriate. This risk, however, is outweighed by the reduction in vascular risk with statins and therefore should not be a reason for stopping statin treatment.

+ Other side effects: Dizziness, fainting, fast or irregular heartbeat,...

2.13. Ezetimibe

- Ezetimibe inhibits the intestinal absorption of cholesterol, which reduces cholesterol from the small intestine into the liver.
- Dose may be 10-40mg / day
- Ezetimibe is metabolized mainly in the small intestine and liver, excreted in the bile.
- No dosage adjustment in elderly patients, patients with CKD
- Hepatic impairment: EZE is not known in patients with moderate or severe liver failure, should not be used.
- Patients with chronic kidney disease / renal disease
 - + Monotherapy: No need to adjust the dose of Ezetimibe.
 - + Combination with Simvastatin:
 - No dose adjustment of Ezetimibe or simvastatin in patients with GFR ≥ 60 mL/min / 1.73m².
 - In patients with GFR < 60 mL / min / 1.73m²), the dose of Ezetimibe is 10 mg and simvastatin is 20 mg once daily, taken in the evening. Patients should be monitored if higher doses of simvastatin are used.

2.14. New York Heart Association class III (McMurray et al., 2012)

Classification of heart failure symptoms

Class I	People whose physical activity is not limited. Ordinary physical activity does not cause undue fatigue, heart palpitations, trouble breathing, or chest pain.
Class II	People who have some limitation on physical activity. They are comfortable at rest, but ordinary physical activity causes fatigue, heart palpitations, trouble breathing, or chest pain.
Class III	People who have a marked limitation on physical activity. They are comfortable at rest, but less-than-ordinary physical activity causes fatigue, heart palpitations, trouble breathing, or chest pain.
Class IV	People who are unable to carry on any physical activity without discomfort. Symptoms may be present even at rest. If any physical activity is done, discomfort increases.

2.15. 2017 Taiwan lipid guidelines for high-risk patients

LDL-C increased CV risk in patients with CKD. In adults with glomerular filtration rate (GFR) < 60 mL/min/1.73m² without chronic dialysis (CKD stage 3-5), statin therapy should be initiated if LDL-C ≥ 100 mg/dL. Ezetimibe can be added to a statin to consolidate the CV protection in CKD patients (Li et al., 2017)

3. Objective of the research

The target of this study is to focus on mechanisms underlying dyslipidemia in CKD and clinical trial evidence for lipid lowering therapy in patients with CKD via parameters of lipid, oxidative stress, tryptophan degradation as well as renal function, in comparison to statin/ezetimibe therapies in CKD patients with dyslipidemia.

- 1) To verify the effect of lipid lowering therapies on lipid profile and inflammatory status in Vietnamese CKD population. [Time Frame: at the baseline and at 4th month, and one-year following].
- 2) To compare the effect of lipid-lowering regimes (simvastatin mono-therapy and EZE/simvastatin 10/20, EZE /simvastatin 10/40 combination therapy) on lipid profile, oxidative stress indices, Tryptophan degradation, renal parameter, and side effect in Vietnamese CKD patient. [Time Frame: after the 4th month, 8th month and one-year following].

We hope with scientific evidence, doctors will well understand the mechanism, effect, and safety of potential and existing therapies. Therefore, the prescription rate of lipid-lowering drugs and control rate of hyperlipidemia could be increased in CKD

patients. The major purpose is to improve clinical outcomes of CKD patients through the control of hyperlipidemia.

4. METHODOLOGY

4.1. Study design: A prospectively study with clinical trials, 12 months follow up.

4.2. Subject: 30 patients with hypercholesterolemia (LDL-C), stage 3-4 Chronic Kidney Disease, not receiving dialysis and 30 control participants (sex, age-matched)

4.2.1. Inclusion Criteria:

30 patients with following criteria

- CKD in the 3,4 stage (e-GFR: 15-60 ml/min/1.73 m²)
- Presence of proteinuric CKD defined as creatinine clearance >20 ml/min/1.73 m² combined with urinary protein excretion rate >0.3 g/24 h
- LDL cholesterol concentration > 100 mg/dl (2,59 mmol/l)
- Age:
 - + ≥ 50 years old but not treated with chronic dialysis or kidney transplantation
 - + In adults aged 18-49 years with CKD but not treated with chronic dialysis or kidney transplantation, we suggest statin treatment in people with one or more of the following:
 - known coronary disease (myocardial infarction or coronary revascularization)
 - diabetes mellitus
 - prior ischemic stroke
 - estimated 10-year incidence of coronary death or non-fatal myocardial infarction > 10%

4.2.2. Exclusion Criteria

- In adults with dialysis-dependent CKD
- Heart failure (New York Heart Association class III or more)
- Previous or concomitant treatment with corticoids, statin, immunosuppressive agents, vitamin B6, B12, folate
- Pregnancy
- Patients who do not agree to participate the research
- Patients are unable to fully understand the purposes/risks of the study and to provide a written informed consent

4.2.3. Criteria for control

- 30 aged, sex-matched subject
- Do not have history of diabetes, hypertension, cardiovascular or cerebrovascular disease, renal failure, blood dyscrasias, cancer, retinal vascular disorders
- Current medication with vitamin B6, B12, or folic acid

4.3. Place:

- In Viet Nam: Internal examination room, internal department, Department of Biochemistry of Hue University Hospital.

- In Italy: Department of Biochemistry of Sassari University Hospital

4.4. Time: 3 years (From November 2017 to November 2020)

4.5. Medication:

- Group1: simvastatin 40mg/day 12 months
- Group2: EZE/simvastatin (10/20) mg/day 12 months
- Group3: EZE/simvastatin (10/40) mg/day 12 months

4.6. Procedure

- 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 will be taken for local laboratory assays lipid profile, proteinuria, urea and creatinine, creatine kinase, SGOT, SGPT, total blood count, total haemostasis test

- If the patient satisfies the including criteria, written informed consent obtained 30 enrolled patients will be randomized into 3 groups and will receive one of 3 lipid-lowering therapies at the baseline and continue for 12 months

Group 1: (10 participants) receive 40 mg/day simvastatin (patient No 1, 4, 7...)

Group 2: (10 participants) receive ezetimibe/simvastatin 10/20 mg/day: (patient No 2, 5, 8...)

Group 3: (10 participants) receive ezetimibe/simvastatin 10/40 mg/day: 10/40: (patient No 3, 6, 9...)

- Patients will follow the treatment for 12 months and will be seen in the study clinics for routine follow-up checks and blood test at 4, 8, and 12 months.

- At each follow-up time, study treatment will be continued, unexplained muscle pain and non-study treatment recorded.

Patients are asked for an unscheduled visit if they want to have any additional review.

- If patients are unable or unwilling to attend the follow-up checks, ask them if they have any serious adverse events.

- The blood samples will be sent to Biochemical Department of Sassari University for oxidative stress indices and Tryptophan degradation indices every 4 months.

5. Biochemical analysis

- Serum Creatinine, GFR, urine protein, lipid profile, SGOT, SGPT, CK (Total Cholesterol, LDL-C, HDL-C, TG): Will be done in Hue University hospital/Hue Central Hospital.

+ Total Cholesterol, LDL-C, HDL-C, and TG were measured by enzymatic methods using commercial kits (Boehringer-Mannheim, Mannheim, Germany): Patients must fast for at least 12 hours before the test

+ Serum CK levels were measured by using a fully automated biochemical analyzer (Abbot).

+ Proteinuria

+ Serum urea, creatinine

+ Total cells blood count

+ Total hemostasis test

[Time Frame: at baseline and 4, 8, 12 months of treatment]

- Serum concentrations of Oxidative stress indices (MDA, All/UA, Taurine), Trp degradation indices (Kyn, Trp, Kyn/Trp ratio): Will be done in Sassari University hospital.

MDA, All/UA ratio, taurine, Kyn, Trp were determined by capillary electrophoresis UV detection.

Blood samples for Oxidative stress indices and Trp degradation indices will be collected, frozen, and sent to Sassari University hospital.

[Time Frame: at baseline and 4, 8, 12 months of treatment]

- Total Genome DNA-Methylation

7. References

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