OXIDATIVE STRESS STATUS IN RENAL DISEASE PATIENT

SUWIPAR DEEBUKKHUM

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Biomedical Sciences
January 2012

OXIDATIVE STRESS STATUS IN RENAL DISEASE PATIENT

SUWIPAR DEEBUKKHUM

A Thesis Submitted to the Graduate School of Naresuan University

in Partial Fulfillment of the Requirements

for theMaster of Science Degree in Biomedical Sciences

January 2012

This thesis entitled "Oxidative stress status in renal disease patient" submitted by Suwipar Deebukkhum in partial fulfillment of the requirements for the Master of Science Degree in Biomedical Sciences is here by approved.

Patcharin Pingmuangkaew .....Chair

(Patcharin Pingmuangkaew, Dr.)

S. Tangvarasittichai .....Committee

(Assistant Professor Surapon Tangvarasittichai, Ph.D.)

Orathai Tangvarasittichai .....Committee

(Associate Professor Orathai Tangvarasittichai, Ph.D.)

Weerawut Mingkuan .....Committee

(Weerawut Mingkuan, Dr.)

Approved

K. Pupatwibul

.....

(Assistant Professor Kanungnit Pupatwibul, Ph.D.)

Dean of the Graduate School

25 January 2012

ACKNOWLEDGEMENT

There are many contributions and supports from many people in the completion of this research. I would like to take this opportunity to express my appreciation to them.

First, I would like to express my deep appreciation and gratitude to Assistant Professor Dr. Surapon Tangvarasittichai, my advisor, for providing beneficial advices even during the busy time and for his kindness throughout my study. He was always there to listen and to give advice. He is responsible for involving me in my research. He taught me how to ask questions, read literature, and express my ideas. He needs to be persistent to accomplish any goal. Thus, I would like to thank again from my heart.

Besides my advisors, I would like to thank Dr. Adisorn Boonnark, Miss. Sukuman Niyomtam, and Mr. Komsit Sathapanapitukkij, for her friendship, encouragement, helper, and supporter.

I would like to thank all staff of Renal Disease Department, and all staff of Clinical Pathology Department, Uttaradit Hospital for all help and technical services. I would like to thanks to all peoples, who inspired me but are not names in this acknowledgement. Last, I thank my family to encouragement until this research had finished.

Suwipar Deebukkhum

Title	OXIDATIVE STRESS STATUS IN RENAL DISEASE PATIENT
Author	Suwipar Deebukkhum
Advisor	Assitant Professor Surapon Tangvarasittichai, Ph.D.
Co-Advisor	Associate Professor Orathai Tangvarasittichai, Ph.D.
Academic Paper	Thesis M.S. in Biomedical Sciences, Naresuan University, 2011
Keywords	Oxidative stress, lipid hydroperoxide, malondialdehyde, total antioxidant capacity, Chronic kidney disease (CKD)

ABSTRACT

Diabetic nephropathy (DN) is currently the leading cause of end stage renal disease. Over the past several researchs much progress has been made our understanding of the pathogenesis of DN. Genetic, interactive of glycemic and blood pressure control to reyard the progression of nephropathy. Chronic kidney disease (CKD) is defined by estimated GFR (eGFR). Historically, serum urea and creatinine will be elevated and particular help in confirming that this is due to CKD. Reversible causes of renal impairment should plays be considered, and it is critical to identify patients with progressive decline in renal function over only a few month, and whose underlying disease is most likely to be treatable. Chronic kidney disease is defined by eGFR and stages of CKD; stage 1 has eGFR > 90 ml/min/1.73 m^2, stage 2 has eGFR 60-89 ml/min/1.73 m^2, stage 3 has eGFR 30-59 ml/min/1.73 m^2, stage 4 has eGFR 15-29 ml/min/1.73 m^2, and stage 5 (ESRD) has eGFR <15 ml/min/1.73 m^2. However, it is common for the condition to be deteced when routine blood testing reveals unexpected renal impairment. The early stages of CKD (stages 1-3) are asymptomatic. However, as renal function declines a constellation of non specific symptoms become apparent. Those with CKD stage 4 and 5 may complain of uraemic symptoms. This study measure correlation among of NAG activity was positive correlation between microalbumin, $r = 0.333$, $p<0.001$. And eCrCl was negative correlaton between microalbumin, $r = -0.315$, $p<0.001$. These equations are now being reported routinely by biochemistry laboratories, resulting were increase in the recognition of previously

inflammatory marker is also significantly elevated in renal disease patients in the present study. One possible cause may be from the glomerulosclerosis that results from the influx and accumulation of inflammatory cells (monocytes and macrophages), with mesangial cells responding in the similar manner to vascular smooth muscle cells. Then, renal disease and cardiovascular disease should share similar risk factors. NAG activity is marker widely distributed lysosomal enzyme located in the renal proximal tubules. Our data showed that increase of NAG activity levels were significantly associated with the decline in eCrCl and GFR

Type 2 diabetes mellitus (T2D) was well known as a chronic disease and increased risk to develop cardiovascular disease. Hyperglycemia was a major factor of the excess production of free radical. Free radical was any molecule capable of independent existence that contains one or more unpaired electrons in orbital and caused free radical were very reactive. The major compounds of arterial cell membrane were phospholipid with consist of polyunsaturated fatty acid (PUFAs) their conjugated double bonds were sensitive to free radical damage and produced biochemical products such as lipid hydroperoxide (LOOH) and malondialdehyde (MDA). Living organism produced antioxidant system to protected organism from free radical damage. Antioxidant in the body worked together against free radical then the studied of network of antioxidant can provide interesting data than any antioxidant alone. This study measure two oxidative stress biomarkers, lipid hydroperoxide (LOOH) and malondialdehyde (MDA) in addition this study will measure total antioxidant.

The results showed that both 2 oxidative stress biomarker (lipid hydroperoxide and malondialdehyde) of renal kidney failure (ESRD; n=73) were significantly higher than healthy control (n = 89, $P < 0.001$) for both oxidative stress biomarker and total antioxidant capacity of renal kidney patient (n = 203) was significantly lower than healthy control (n = 89, $P < 0.001$). The result of bivariate correlation among oxidative stress biomarker and total antioxidant capacity showed positive correlation between oxidative stress biomarker, lipid hydroperoxide (LOOH) and malondialdehyde (MDA) both renal kidney patients and healthy control subjects ($r = 0.250$, $P < 0.0010$), negative correlation between oxidative stress biomarker with total antioxidant capacity renal kidney patients and healthy control subjects (r = -

0.065, p = 0.359), positive correlation between MDA and NAG activity of renal kidney patients (r = 0.189, p = 0.012), and negative correlation between MDA and eCrCl of renal kidney patients (r = -0.142, p = 0.043). In conclusion patients with renal kidney patients had increased in oxidative stress biomarker indicated by elevated lipid hydroperoxide and malondialdehyde. The total antioxidant capacity levels of renal kidney patients were decreased this may be cause by the counter action for the oxidative stress in renal kidney patients.

LIST OF CONTENTS (CONT.)

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

WHO	= World Health Organization
HDL -C	= High-density lipoproteins cholesterol
LDL -C	= Low density lipoprotein cholesterol
LOOH	= Lipid hydroperoxide
MDA	= Malondialdehyde
DM	= Diabetes mellitus
ESRD	= End-stage renal disease
NAG activity	= N-acetyl –β-D-glucosamunidase activity
GPx	= Glutathiona peroxidase
AGEs	= Advanced glycation end-products
ROS	= Reactive oxygen species
COX	= Cyclooxygenase
PUFAs	= Polyunsaturated fatty acids
L·	= Carbon centered radical
LOO·	= Lipid peroxyl radical
ROO·	= Peroxyl radicals
R-O·	= Akoxyl radicals
NO	= Nitric oxide
SOD	= Superoxide dismutase
OH•	= Hydroxyl radicals
ONOO-	= Peroxynitrite radicals
O2•-	= Superoxide
$NADP^+$	= Nicotinamide adenine dinucleotide phosphate
Fe^{2+}	= Ferrous ions
Fe^{3+}	= Ferric ions
LPO	= Lipid peroxidation
$-CH_2-$	= Methylene group

ABBREVIATIONS (CONT.)

GSH	= Glutathione
Gred	= Glutathione reductase
GSSG	= Oxidized glutathione, Glutathione disulfide
LH	= Lipid hydroxide
GC	= Gas chromatography
HPLC	= High performance liquid chromatography
NAD(P)H	= Nicotinamide adenine dinucleotide phosphate (H+)
TBARS	= Thiobarbituric acid reactive substance
TBA	= Thiobarbituric acid
BMI	= Body Mass Index
FOX	= Ferrous oxidation xylenol orange assay
MS	= Mass spectrometry
WC	= Waist circumstance
CHD	= Coronary heart disease