Oxidant and Antioxidant
In Smoking versus non Smoking Haemodialysis Patient

Thesis

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Rationale and Background:

Premature atherosclerosis is one of the primary causes of morbidity and mortality in patients with chronic renal insufficiency although the mechanisms responsible for this excessive cardiovascular risk are not completely understood ***(Jungers et al.,2000)***

Patients with chronic renal failure (CRF) have three to five times higher likelihood of having a cardiovascular event than the general population ***(Locatelli F, et al.,2000)*** and this likelihood is 3.5 to 50 times in hemodialysis patients. ***(Brunner FP, Selwood NH .,1992)***

Recent evidence from different non-renal patient groups, such as patients with burn injury ***(Ritter et al., 2003),*** coronary artery disease ***(Heitzer et al.,2001)*** and congestive heart failure (***Tsutsui et al.,2002),*** demonstrates that various surrogate markers of oxidative stress parameters predict mortality, but data from the renal patient population are scarce

Much recent evidence suggests that ESRD is a disease state characterized by increased oxidative stress, and it has been speculated that oxidative stress may be a potentially important source of cardiovascular morbidity and mortality in this patient population ***(Himmelfarb et al.,2002, Locatelli et al.,2003)***

Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient anti-oxidant defence mechanisms ***(Sies ,1997)***

Generation of oxidative compounds is physiologically relevant as an important step in inflammation and tissue repair processes. Therefore, it represents part of the defense mechanisms against invading microorganisms and malignant cells, as well as of tissue healing and remodelling. On the other hand, an improper, or maladaptive, activation of oxidative processes may be chronically present in pathological situations, such as uraemia, contributing to cell and tissue injury ***(Handelman .,2000)***

ESRD patients are characterized by an imbalance between pro-oxidant and anti-oxidant factors, and increased oxidant stress has been associated with typical

complications of ESRD such as atherosclerosis and ß$_2$-microglobulin amyloidosis ***(Descamps et al.,2001).*** Previous studies showed that hemodialysis (HD) patients are in a state of increased oxidative stress which may constitute a pathway for accelerated atherosclerosis in this group ***(Pawlak et al.,2004; Pawlak et al.,2003)***

Cigarette smoke is a potent exogenous source of oxidative stress in humans because of the inflammatory response it induces and the free radicals present in smoke ***(Alberg .,2002).*** In fact, a puff of cigarette smoke contains 10^{14} and 10^{15} free radicals in the tar and gas phases, respectively ***(Church and & Pryor 1985)***

Vitro investigations have consistently demonstrated that cigarette smoke depletes plasma of vitamin E as well as other antioxidants ***(Leonard et al., 2003)***

Smoking might theoretically initiate renal disease because it adversely affects renal function in healthy subjects ***(Tozawa et al.,2002, Ritz et al.,2000).***There is a growing consensus that vascular risk factors such as smoking, hypertension, and hyperlipidemia act as promoters of preexisting renal disease regardless of the underlying cause ***(Ruggenenti et al., 2001)***. Smoking induces both systemic and intrarenal hemodynamic alterations that can be significant for renal disease progression. Smoking may also injure the kidneys by damaging the renal microvasculature through oxidative stress, reduced nitric oxide generation, and increased endothelin plasma concentration. Smoking-induced tubular cell dysfunction may further contribute to tubulointerstitial injury and progression of CRF (***Orth ,2000).***

Increased oxidative stress can also cause oxidation of proteins, lipids, and DNA, and peroxynitrite can nitrosylate tyrosine residues in proteins, all of which may lead to molecular and cellular dysfunction ***(Wattanapitayakul et al.,2000)***

Of the compounds with terminal carbonyl groups that result from lipid peroxidation, malondialdehyde (MDA) is widely used as an index of oxidative damage***.(Draper and Hadley .,1990)***

Oxidants in cigarette smoke extract can pass through the pulmonary alveolar wall into the blood and induce systemic oxidative stress, which most likely facilitates oxidative modification of LDL and endothelial dysfunction, explaining early key events in the development of atherosclerosis ***(Yu Yamaguchi et al.,2007)***

Healthy people are protected against free radicals by several defense mechanisms, of which GSH (glutathione) is the most important intracellular scavenger of free radicals. GSH serves as a reductant in oxidation reactions resulting in the formation of oxidized GSH. Therefore decreased GSH levels and increased oxidized GSH levels may reflect depletion of the antioxidant reserve ***(Halliwell and Gutteridge, 1999)*** GSH is involved in the protection from dangerous effects of free radicals ***(Yoshida et al 1995)*** .Hence , GSH is emerging as one of the fundamental antioxidant defense mechanisms in oxidant-induced injury and inflammation***(Halil et al.,2006)***

Among insulin-and non–insulin-dependent patients with diabetes, smoking seems to be an independent risk factor for nephropathy and accelerates the rate of progression of renal failure ***(Orth ,2000).***

In hypertensive patients, smoking independently increases the risk for albuminuria and may cause decline of renal function ***(Regalado et al.,2000).*** The role of smoking in primary renal diseases is less known, but studies have indicated a relation with the development of proteinuria in patients with polycystic kidney disease ***(Chapman et al.,1994)*** and deterioration of renal function in patients with lupus nephritis, polycystic kidney disease, and glomerulonephritis ***(Orth et al.,1998 and Stengel et al.,2000).***

The mechanisms of smoking-induced renal damage are only partly understood and comprise acute hemodynamic (*e.g.*, increase in BP and presumably intraglomerular pressure) and chronic effects (*e.g.*, endothelial cell dysfunction). Renal failure *perse* leads to an increased cardiovascular risk. The latter is further aggravated by smoking. ***(Orth., 2004)***

Aim of Work:

The present study will compare the effect on plasma antioxidant and oxidant concentrations in two groups of renal failure patients on dialysis; one group includes the active smokers and the other one includes the non–smokers patients.

Another control group will be included in the study those are non-smoker healthy volunteers.

Subjects and Methods:

The present study is planned to assess the blood levels of the water soluble antioxidant (glutathione peroxidase) and the oxidant (malondialdehyde) in the following groups:

Group (1): 15 male subjects who are active smokers with chronic renal failure on regular haemodialysis

Group (2): 15 male subjects who are non-smokes with chronic renal failure on regular haemodialysis

Group (3): 15 male subjects who are non smokers and not exposed to other people's smoke and serves as controls

In a trial to find any alteration in the levels or activities of glutathione peroxidase and malondialdehyde

Criteria of selection:

- All patients will be non diabetic
- All patients will be having a 4-hourly haemodialysis sessions thrice weekly using polysulfone membranes for at least 6 months prior to the study
- All subjects are going to be males in order to avoid the effect of gender on some of the parameters that will be investigated e.g. glutathione peroxidase (GPX) which is higher in females than males ***(Andersen et al., 1997).***
- All patients and controls will not be receiving antioxidant therapy

Methods:

All the patients and control individuals will be subjected to the following**:**

1. Detailed history.
2. Complete physical examination.
3. Routine laboratory investigations.

4. Subjects will be asked to fill in a self-completion questionnaire giving details about their smoking habits and to provide blood samples. Free consent for the biochemical tests will be obtained.

5. Blood samples of each participant will be analyzed after an overnight fasting and abstinence from smoking to assess the plasma concentration of malondialdehyde (oxidant) and glutathione peroxidase (antioxidant).

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الملخص العربى

الشوارد الحره فى دخان السجائر تسببب ضررا تاكسديا للجزيئات الكبيره مما قد يؤدى الى أمراض مختلفه كذلك يشارك الغسيل الدموى فى مرضى الفشل الكلوى المزمن فى التأثيرعلى المواد المؤكسده و المضادة للأكسدة.
يقارن البحث الحالى تركيز مضادات الأكسدة فى بلازما الدم و نشاط الأنزيمات المضادة للأكسده فى كرات الدم الحمراء للمدخنين والغير مدخنين من مرضى الفشل الكلوى تحت جلسات الغسيل الدموى الكلوى , مع مثيلاتها فى غير المدخنين و لا يعانوا من الفشل الكلوى
سوف يتم اختيار ثلاث مجموعات من الذكور :-
المجموعه الأولى :سوف تشمل 15 فرد من المدخنين و يعانوا من مرض الفشل الكلوى تحت جلسات الأستصفاء الدموى.
المجموعه الثانيه :تشمل 15 فرد لا يدخنون و لكن يعانوا من الفشل الكلوى و تحت جلسات الأستصفاء الدموى.
المجموعة ا لثالثة :تشمل 15 فرد أصحاء لا يعانوا من الفشل الكلوى و غير مدخنين (المجموعه الضابطه) وذلك لمحاولة ايجاد علاقه بين الجلوتاثيون بيروكسيديز و المالونديالدهيد فى كل من المجموعات الثلاثة و سوف يتم اختيارهم بالشروط التالية:-

(1) جميعهم غير مرضى بمرض البول السكرى.
(2) مريض الفشل الكلوى يغسل 4 ساعات ثلاث جلسات أسبوعيه على الأقل لمدة 6 أشهر سابقه.
(3) الثلاث مجموعات من الذكور و ذلك لتثبيت عامل الجنس لانه وجد أن الجلوتاثيون بيروكسيديزأكثر فى النساء عنه فى الرجال.
(4) المجموعات الثلاثه لا يتعاطى أى منهم أية أدويه مضاده للأكسده .

وسوف يتم عمل تحاليل روتينيه لكافة المجموعات الثلاثه و يتم عمل تحاليل الجلوتاثيون بيروكسيديزو المالونديالدهيد بعد صيام من السجائر و قبل جلسة الغسيل الدموى صباحا يوميا .

الهدف من الرساله-

محاولة وجود علاقه أو تاثير المواد المؤكسده و المضادة للأكسده بالتدخين فى المجموعتين الأولى والثانية لمرضى الفشل الكلوى و تحت جلسات الأستصفاء الدموى والمجوعة الثالثة الغير مدخنة ولا تعاني من الفشل الكلوى المزمن.

العوامل المؤكسدة و المضادة للأكسدة فى المدخنين وغير المدخنين من مرضى الفشل الكلوي تحت جلسات الأستصفاء الدموي.

رسالة
مقدمة توطئة للحصول على ماجستير الكلى من
كلية الطب – جامعة عين شمس

من
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Contents

List of Tables

List of Figures

List of Abbreviations

Abbrev	
ACS	Active cigarette smoking
AM	Alveolar macrophage
BALF	bronchoalveolar lavage fluid
BH_4	Tetrahydrobiopterin
CHD	Congestive heart disease
CRF	Chronic renal failure
CRI	Chronic renal insufficiency
CS	Cigarette Smoking.
CVD	Cardiovascular
DHBA	2,3-dihydorxybenzoic acid
ELF	Epithelial lining fluid
Epo	Erythropoietin
ERK	extracellular-signal regulated kinases (Erk1/2)
ESKD	End stage kidney disease
ESRD	End stage renal disease
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
GPx	Glutathione peroxidase
GSH	Glutathione
GSSG	Glutathione disulfide
HD	Hemodialysis
HMG	3-hydroxy-3-methylglutaryl.
IL-6	INTERLEUKIN-6
IFN-γ	Interferon gamma.
JNK	Jun N-terminal kinase
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide
LTB_4	leukotriene B_4 (, D_4, E_4)
MAPK	mitogen-activated protein kinases
MDA	Malondialdehyde
MPO	Myeloperoxidase.
MPT	Mitochondrial permeability transition

NADPH	Nicotinamide Adenine Dinucleotide PHosphate
NE	Neutrophil elastase
NO	Nitric oxide
NOS	Nitric oxide synthase
NXY-059	(disodium4-[(tert-butylimino)-methyl]benzene-1,3-disulfonate N-oxide)
PARG	Poly(ADP-ribose) glycohydrolase
PBN	A-phenyl-N-t-butyl-nitrone
PMNs	polymorphonuclear neutrophils
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RRT	Renal replacement therapy
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TEAC	Trolox equivalent antioxidant capacity
TNF-α	Tumor necrosis factor-alpha
UCP	Uncoupling proteins
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase