

The Oxidative Effect Of Nlrp3 And Cyp2e1 In Development Of Renal Failure Associated With Hypertension And Diabetes Mellitus Diseases

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Abstract: Renal Failure (RF) is devastating pathology with several causative factors, resulting in dialysis or kidney transplantation needed by end-stage renal disease (ESRD). The progression of RF is closely related to systemic oxidative stress, which causes various complications such as hypertension and diabetic mellitus . The current study was focusing on the oxidative effects of NLRP3 and CYP2E1 gene expression in the pathological development of renal failure disease with hypertension and diabetic mellitus as complications associated diseases. This study involved 100 patients diagnosed clinically and seriologicaly with renal failure 52 males and 48 females, their age from 17 to 80 years . Also the study comprise (18 males and 12 females) healthy volunteers as control group. RNA extracted from blood samples and converted to cDNA which subjected to real-time PCR for detection gene expression . The results detected that Hypertension and diabetic mellitus are the important risk factors that causes and advances renal failure disease . NLRP3 and CYP2E1 have significantly overexpression (1.60 folds and 5.37 folds) respectively in RF patients compared with healthy group ($p < 0.05$). However, the expression of NLRP3 and CYP2E1 increase in diabetic mellitus (2.16 and 1.58) respectively than hypertension in RF patients with and control group ($p < 0.05$). Diabetic mellitus generate free radical consequently causes ROS, hence elevated expression in oxidative NLRP3 and CYP2E1 genes in diabetic RF patients .

KEYWORDS: Oxidative stress, NLRP3, CYP2E1, Renal failure, Diabetes, Hypertension.

INTRODUCTION

Today, more than 2 million people around the world suffer from renal failure (RF), with most of them undergoing dialysis or other forms of renal replacement therapy . Oxidative stress is one of the risk factors for renal injury , which referred to an imbalance between generation of (ROS) reactive oxygen species and natural antioxidant potential (Nasri, N.,2013). The essential reactive oxygen species are hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻), and Superoxide (O₂⁻) (Cadenas,E., and Davies,K., 2000) . Notably, reactive oxygen species react with all biomolecules in the cells through inactivating cellular components and oxidizing the nucleic acids . Thus, oxidative stress causes some diseases such as cancer, vascular disease, diabetes, and chronic kidney disease (Nasri,H., 2017). Several kidney disorders and disease are induced by oxidative stress , also its mediated sevrsal complications of these diseases .Some systemic diseases including hypertension and diabetes mellitus can induce oxidative stress in kidney (Ozbek,E., 2012).

NLR pyrin domain-containing protein 3 (NLRP3) inflammasome, a multiprotein complex mediated pro-inflammatory cytokines. The NLRP3 inflammasome released from damaged and dying cells in the recognition of endogenous danger signals. The NLRP3 inflammasome was affected in several renal diseases such as the acute kidney disease (AKD) and the chronic kidney disease (CKD) (Wu ,M.,*et al.*, 2018) . The cellular damage and inflammation coexistence indicate that the NLRP3 inflammasome has critical role in inflammation and oxidative stress in RF disease (Vilaysane,A., *et al.*, 2010). Inflammasome-independent NLRP3 interacts with mitochondria and intervening mitochondrial reactive oxygen species generation and mitophagy (Kim ,Y.,*et al.*, 2019). Recent study , reported oxidation of some biomolecules , leading to various changes in structure and function for these molecules. The oxidation process is mainly induced in the mitochondria with the help of mitochondrial oxidative enzymes such as (CYP2E1) and that the production of ROS from this process may lead to the occurrence of kidney failure. (Ling,X., and Kuo, K.,2018).

Cytochrome P2E1 is one type of CYP450 is a superfamily of hemoproteins important for the drugs biotransformation . It is found primarily in the liver and also expressed in kidney. CYP2E1 is metabolizing many small molecules including ethanol , acetaminophens and pro-carcinogens nitrosamines and azo compound (Gonzalez, F.,2005) . Metabolism of these compounds mediated by CYP2E1 produces toxic intermediates and excessive quantities of ROS. The main causes of various chronic diseases are elevated levels of ROS and thus oxidative stress due to elevated levels of CYP2E1 protein and induced enzymatic activity. (Leung ,T.,*et al.*, 2013) . The current study was performing to detect the oxidative effect of NLRP3 and CYP2E1 in progression of renal failure disease and healthy individuals and with complications associated diseases

MATERIALS AND METHODS

Study subjects

The total of 100 random patients has participated in this study (48 females and 52 males) . Also, the study comprises (18 males and 12 females) healthy volunteers as control group, who had no pathological state at the time of the study . All individuals in the control group were matched to patients in gender and age groups. Blood samples and their sera were taken from hospitalized RF patients, some of them with different associated diseases (hypertension and diabetics mellitus). The average measurement of creatinine and urea was used as a gold standard for the diagnosis as a renal failure. Creatinine and urea levels for patients and control group were examined according the methods proposed by Amin.N. *et al.*,(2014) .The study protocol in agreement with ethics of Al- Zahraa Teaching Hospital, and all subjects gave written informed consent.

Molecular study

NLRP3 and CYP2E1 genes expression were performing using reverse transcription-quantitative Real-time polymerase chain reaction . Following the manufacturer 's protocol, total RNA was collected from blood samples using the AccuZol reagent.Reversely, the extracted RNA was converted to the complementary DNA (cDNA) using (СИТТОЛ Company / Russia). The procedure was conducted in 25 µl volume reaction following the manufacturer's instructions. Real-time PCR quantitative was applied in this study by using EVA green. The fluorescent dye has detected any double-stranded DNA including cDNA and the folding was documented as a cycle threshold (Ct) value. The mRNA of endogenous control gene *B- actin* was amplified and used to standardize the mRNA levels of NLRP3 and CYP2E1 genes. Primers that used in RT-qPCR are listed in table (2)

Subjects	Patients Group	Control group	Reference range	P-value
Creatinine Total Mean (mg/dl) ±SD	4.68±9.09	0.93± 0.19	0.6 -1.2	<0.001
Urea				
Total Mean (mg/dl) ± SD	113.27±47. 9	38.62± 7.15	15-40	< 0.001

Table (2): The primers sequences used in RT-qPCR

Genes	Primer	Sequence (5' →3') direction	References
NLRP3	Forward	5'-CTCCTTTACGC CAGGGTGAG-3'	(Sayanthooran,S., <i>et al.</i> , 2016)
	Reverse	5'-AGATAGCGGGAATGATGATATGAG-3'	
CYP2E1	Forward	5'-TTGGTTGACTCACTCTTTCCTTT-3'	(Christina, G., <i>et al.</i> , 2013)
	Reverse	5'-TGATGTCTGATGAGGAGGTTT G-3'	
B- actin Refe-rence gene	Forward	5' CTGGAACGGTGA AGGTGACA-3'	(Panarina,M., <i>et al.</i> , 2014)
	Reverse	5' CGGCCACATTGTGAACTTTG '3	

Statistical analysis

Data were translated into a computerized structure . the data are presented as means ± standard deviation (SD). SPSS version 25 and Microsoft Excel 2010 computer softwares were used for conducted statistical analysis. Prevalence of variable as gender, age is detected by approximate percent . Data were normally distributed ,an unpaired t-test was used to compare the difference between patients with RF and controls. An estimate was considered statistically if its P value was less than 0.05.

RESULTS

The results of serum examination reveal significantly elevated both creatinine and urea mean concentration for all patients (4.684±9.09 mg/dl and 113.27± 47.90 mg/dl) respectively , when compared with healthy control group (0.93± 0.19 mg/dl, and 38.62± 7.15 mg/dl) respectively and with the reference range for creatinine and urea (up to 1.2 mg/dl and 15-40 mg/dl) respectively (p <0.001) table (2).

Table (2):The levels measurement of creatinine ,urea of RF patients

In this study, we concerned about oxidataive role for NLRP3 and CYP2E1 genes expression. RT-qPCR revealed that mRNA of NLRP3, CYP2E1 genes expression were higher (1.60 and 1.38, respectively) in blood isolated from RF patients compared to normal control subject. According to gender , the same result indicated non-difference in NLRP3 gene expression between males and females of RF patients (1.11, 1.32) respectively . In contrast , CYP2E1 showed significant fold value overexpression in males RF patients (1.77) compared with females (0.99) , table (3).

Table(3): mRNA expression of NLRP3 and CYP2E1 in RF patients and control subjects

Subjects	Mean Ct gene	Mean Ct actin	Mean Ct test Δ	Mean Ct $\Delta\Delta$	Folding
NLRP3					
Patients	18.27	18.42	-0.15	-0.68	1.60*
Control group	18.99	18.46	0.52	0.00	1.00
Males	17.64	18.41	-0.77	0.15	1.11**
Females	18.93	18.41	0.51	-0.40	1.32
CYP2E1					
Patients	18.23	18.12	0.11	-0.47	1.38*
Control	18.82	18.12	0.70	0.00	1.00
Males	17.79	18.12	-0.33	-0.83	1.77*
Females	18.66	18.12	0.54	0.01	0.99

*=significant : **= non-significant

The patients with RF are associated with multiple related disease complications, such as hypertension (HTN) and diabetic mellitus (DM) which is lead to increase the morbidity and mortality ,thus the oxidative stress plays a key roles in these complications (Rapa,S., *et al.*, 2020) .

Our patients included 15 renal failure patients with diabetes(RF-DM) ,and 29 renal failure patients with hypertension(RF-HTN) .We investigated the of NLRP3 and CYP1E2 gene expression in renal failure(RF) patients with RF-DM and RF-HTN. NLRP3 appeared significant difference in gene expression between two associated disease hypertension and diabetic mellitus (1.21 , 2.16) respectively and control (1.00) ($p < 0.05$). Furthermore , NLRP3 showed overexpression in patients with DM disease(2.16) than HTN. Moreover , the gene expression of CYP1E2 showed (1.58 fold) in RF-DM patients ,while RF- HTN appeared down expression (0.65) as in figure (1-A and 1-B)

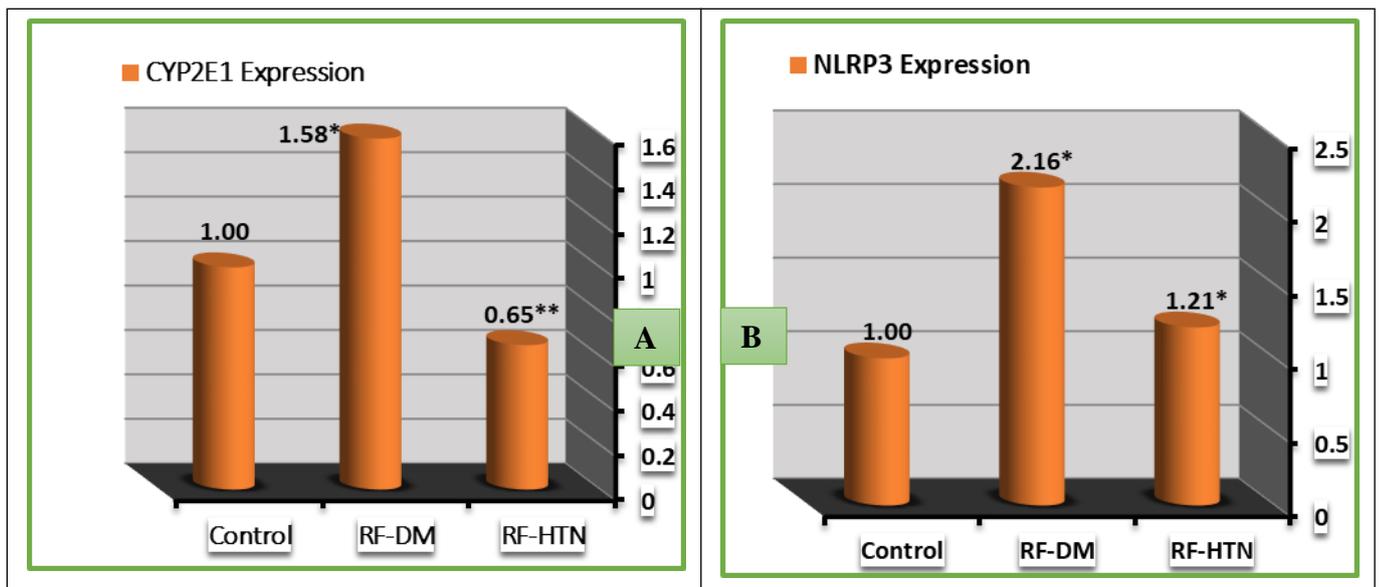


Figure (1-A): NLRP3 and associated diseases and control ; (1-B) CYP2E1 and associated diseases and control

RF-HTN : Renal failure with Hypertension , RF-DM :Renal failure with Diabetic Mellitus , significant :*($p < 0.05$) ; non-significant :**($p > 0.05$)

DISCUSSION

Both creatinine and urea are toxic substances .The results in table (2) demonstrated significant increase both creatinine and urea levels comparing with control and reference range . Our findings are consistent with previous studies for Aljebory,A. *et al.*, (2019) who found that serum creatinine and urea showed high levels in pre-hemodialysis patients when compared with that found in healthy volunteers for both genders. Every day , about 2% of the creatine in the body is transformed into creatinine, which results in a relatively constant daily production of creatinine.(Rajendran,N.*et al.*, 2017) .

The increase in urea levels is because urea is one of the protein metabolism by-products, that is produced mainly in the liver and then excreted via urine . In pathological circumstance of kidney f it is accumulated in patients' blood, and induces uremia (Entedhar,R. *et al*, 2016).

In current study, we focused on NLRP3 and CYP2E1 gene expression induced oxidative stress . The results determined high expression for both genes compared with healthy control .This study was consistent with Vilaysane,A., *et al.*,(2010) ; Granata,S., *et al.*, (2015) and Sun,R., *et al.*,(2020). Additionally, this result disagree with results of Déri,M., *et al.*, (2020) .

According to gender in table (3) , we detected there is no variance in mRNA NLRP3 expression between males and females , the result coming in agreement with published observations by El-Horany,H., *et al.*, (2017) . CYP2E1 gene showed overexpression in male RF patients compared with females, this finding in line with result of Rinn,J., *et al.*, (2004)who showed the CYP2E1 gene are preferably more expressed in males than females, which mean that CYP2 E1 gene differentially affected by sex in renal disease .Consistency , These findings have major impacts on the differential metabolism of men and women and on how they react to medication and oxidative stress.

According to the previous study, the progress of renal failure is implicated of mitochondrial dysfunction (MTD) as principle factor. The major cellular metabolism and energy production are happened in mitochondria .MTD also implicated to increased inflammation ,oxidative stress , reduce production of ATP , as well as , ROS generation , and release production of proapoptotic, such as mtDNA) and cytochrome CYP2E1 .

NLRP3 inflammasome is activated by MTD in kidney damage (Zhuang,Y., *et al.*, 2015) . Besides , mitochondrial ROS inhibitors, considerably reduced the activation of NLRP3 inflammasome (Ding,W., *et al.*, 2016) . Other research shows, that NLRP3 inflammasome mediates renal damage through mitochondrial dysfunction, which involves the presence of regulatory positive feedback among mitochondrial dysfunction and NLRP3 inflammasome activation. The mitochondrial respiratory chain consists of three complexes I, II and III , and establish that ROS was derived from complex I and complex III, (but not that derivative from complex II) promotes the activation of NLRP3 inflammasome and release of IL-1 β . As ROS producing mitochondria are generally eliminated by mitophagy (a specialized branch of mitochondrial autophagy) . The mitochondrial diseases , cellular ageing and environmental stress, have all been linked with mitophagy suppression and the gathering of injured mitochondria and increase ROS level (Kepp, O., *et al* ., 2011).

Several studies have found that the iron rich CYP2E1 may play a significant role in RF disease, like nephrotoxic chronic kidney disease (Wang,Z., *et al.*, 2014). CYP2E1 is an effective ROS generator such as superoxide anion and hydrogen peroxide, producing powerful oxidants such as hydroxyl radicals in the presence of catalytic iron. ROS levels elevation, consequently oxidative stress related to high levels of CYP2E1 protein and encouraged enzymatic activity, are the primary causes of numerous liver disorders associated with chronic kidney disease and many other pathophysiological conditions, involving type II diabetes (Leung ,T.,*et al.*, 2013). The CYP2E1 activation is well established as oxidative stress, lipid peroxides and antioxidant defences in the liver and kidneys, a member of the CYP450 mixed-function oxidase system in the hepatic and extrahepatic tissues such as the kidney constitutively expressed (Yan,J., *et al.*, 2018) . During the catalytic cycle, the cytochrome 2E1 serves as the central pathway for the production of high levels of ROS through the metabolism of hepatotoxins and nephrotoxins.(Sun,R., *et al.*, 2020).

Regarding associated diseases in RF patients figure (1). Our results reveal that the significant prevalence of RF associated with HTN was about third (29 %) of total patients, whereas DM patients represent 15%. So this study demonstrated that both HTN and DM are major risk factors to causes and progress of RF disease. Consistently, Renal Failure with DM patients has overexpression in NLRP3 and CYP2E1 genes than RF-HTN patients. These findings compatible with findings of Fan,J., *et al.*, (2019) who stated: When RF patients are exposed to hyperglycemia conditions, podocytes have considerably generated ROS, that is the key to activating NLRP3 inflammasomes. Besides, we consistent with Kim ,Y.,*et al.*, (2019), they indicated the systemic activation of the NLRP3 might play a major role in the progression and development of diabetic in RF patients. Shahzad,K., *et al.*, (2015) clarifies the significance of the NLRP3 in pathogenesis of diabetic patients in kidney disease, and they showed the circulating levels of IL-1b and IL-18 as well as NLRP3 renal expression elevated in DM patients, and those rises preceded accumulation of albuminuria and glomerular extracellular matrix, suggesting that NLRP3 inflammasome activation causes the onset of DM. Christina, G.,*et al.*, (2013) detected the relation of DM with elevated expression of CYP2E1 and they explained this fact as the earliest case in the Diabetic nephropathy is the direct effect of elevated levels of glucose in the systemic circulation. Glucose is metabolized to pyruvate upon entering the cells via glycolysis, which leads to the production of NADH. Pyruvate and NADH enter the mitochondria through metabolism, and pyruvate is converted into CO₂ and water with additional donor electrons(FADH₂ ,NADH). This increase in electron donors disrupts the respiratory chain by producing reactive oxygen species (ROS). ROS are produced in abundance to generate oxidative stress. This mediator will stimulate CYP2E1 gene expression in peripheral blood lymphocyte cells.

CONCLUSION

NLRP3 and CYP2E1 genes have significantly upregulation and induced oxidative stress resulting in progression of RF disease. Hypertension and diabetic mellitus are the important risk factors that causes and advances renal failure disease. The significant prevalence of RF associated with hypertension was about third of total patients. Diabetic mellitus generate free radical consequently causes ROS, hence elevated expression in oxidative NLRP3 and CYP2E1 genes in diabetic RF patients.

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