ISSN: 2755-1059

Journal of Nephrology & Endocrinology Research

SCIENTIFIC Research and Community

Research Article

Open Access

# Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) and Nuclear Factor-Kappa B (NF- κB) Expression in Chronic Kidney Disease Patients (CKD) from South India

**Vandit Sevak[1], Rathika Chinniah[2], Sasiharan Pandi[1], Padma Malini Ravi[1], Radha Venkatesan[4], Sampathkumar K[3], Dinakaran T[2] and Balakrishnan Karuppiah[2]***

[1]Department of Immunology, Madurai Kamaraj University, Madurai, India

[2]Madurai Kidney Centre & Transplantation Research Institute, Madurai, India

[3]Meenakshi Mission Hospital & Research Institute, Madurai, India

[4]Department of Animal Science, Bharathidasan University, Trichy, India

## ABSTRACT

**Aim:** The present study elucidated the expression level of Nrf2 and NF-κB, in chronic kidney disease (CKD) patients from South India. Further, the expression levels of these genes are analyzed for cytokines TNF-α and IFN-γ and various subgroups of cases including dialysis, non-dialysis, diabetic and non-diabetic CKD patients, and correlation analysis performed.

**Methods:** 133 CKD patients and 133 healthy individuals were enrolled for the evaluation of NF-κB and Nrf2 expression by qPCR.

**Results:** Upregulated Nrf2 mRNA expression (as fold change) was observed in diabetic (Fc: 1.13), and non-diabetic (Fc: 1.30) patients. Inversely, Nrf2 mRNA expression was down regulated in non-dialysis groups (Fc: 0.80). NF-κB expression was significantly upregulated in non-diabetic (Fc:1.92), non-dialysis (Fc:1.59), dialysis (Fc: 1.36) and CKD patients (pooled) (Fc: 1.27). A dysregulated NF-κB expression was observed in diabetic groups (Fc: 0.69). Nrf2 mRNA expression were positively correlated with IFN-γ expression in diabetes ($r = 0.28$, $p = 0.05$) and non-diabetic CKD patients ($r = 0.37$, $p < 0.002$). Likewise, TNF-α expression was positively correlated with Nrf2 expression in CKD (pooled) patients ($r = 0.33$, $p < 0.000$). A negative correlation was observed in Nrf2 and NF-κB expression in CKD (pooled) ($r = -0.028$, $p = 0.833$).

**Conclusion:** The study concluded the down regulated Nrf2 and upregulated NF-κB expressions in non-dialysis patients. On the contrary, in diabetic CKD patients, the expression level of NF-κB was downregulated and Nrf2 was upregulated. Thus, a negative correlation was observed between Nrf2 and NF-κB genes with respect to their expression patterns.

***Corresponding author**

Balakrishnan Karuppiah, Professor (Retd), Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India. Tel: +91 9842114117; Email:immunobala@mkuniversity.org

**Received:** November 21, 2022; **Accepted:** November 24, 2022; **Published:** December 09, 2022

**Keywords:** Nrf2, NF-κB, Gene Expression, Cytokines, Glomerular Filtration

## Introduction

Globally Chronic Kidney Disease (CKD) is a major health problem [1, 2]. WHO estimates CKD as the 10th leading cause of death [3]. Recently, the International Society of Nephrology's Kidney Disease Data Center Study reported that the CKD prevalence in India is approximately15-17%. The prevalence rate of CKD from different regions of India varies from <1% to 13% [4, 5]. The age-adjusted end stage kidney disease (ESKD) incidence in urban cohort estimates around 232 pmp and unfortunately India lacks a national registry for ESKD [6-8]. The growing burden of hypertension and diabetes mellitus was one of the leading causes of CKD in Western countries [9]. In India, diabetes and hypertension account for 40–60% of cases of CKD [7]. Apart from this, water contamination, overcrowding, poor sanitation, pollutants, poverty, known and unknown nephrotoxins (heavy metals and plant toxins in indigenous remedies) lead to glomerular and interstitial kidney diseases. The States of Andhra Pradesh, Odisha and Goa have high levels of CKD of unknown etiology (CKDu), of suspected agents such as cadmium, fluoride, arsenic, and pesticides and leads to chronic interstitial nephropathy with insidious onset and slow progression [10]. Genetic inbreeding and consanguinity may increase the risk of congenital anomalies of the kidney and urinary tract and obstructive or reflux nephropathy.

Chronic inflammation is a consequence of glomerular and tubulointerstitial pathology [11-15]. Patients with asymptomatic proteinuria exhibit low-grade inflammation linked to endothelial dysfunction [16]. In many forms of CKD, proteinuria is a well-recognized predictor of disease progression [17]. Previous results

have shown that oxidative stress is a major aggravating factor for the progression and pathogenesis of kidney diseases [18-20]. Tumor necrosis factor (TNF) plays a significant role in the progression of renal disorders, interacting with the kidney in a paracrine or autocrine manner [21-25]. Cytokine TNF is mainly produced by monocytes but also, to a lesser extent, by renal cells (endothelial, epithelial, mesangial, and tubular cells) [25-27]. Inflammation and oxidative stress are the main drivers of CKD progression and lead to cardiovascular and other complications [28-30].

The nuclear factor erythroid-2-related factor 2 (Nrf2), activates the transcription of 500 genes in the human genome, mainly for cytoprotective functions. Nrf2 is the master regulator of genes encoding antioxidant and detoxifying enzymes (catalase, SOD, UDP-glucuronosyltransferase, NAD(P)H: quinine oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), glutamate-cysteine ligase, glutathione S-transferase, glutathione peroxidase, and thioredoxin), and related proteins [31, 32]. Nrf2 is held in the cytoplasm as an inactive complex bound to Keap1 (Kelch-like ECH-associated protein 1), a repressor molecule that facilitates Nrf2 ubiquitination [33]. Once the cells are exposed to electrophiles or ROS, Keap1 releases the Nrf2 in the cytoplasm. The limitation in the proteasomal degradation of Nrf2in the cytoplasm results in the accumulation of the de novo synthesized Nrf2 and in turn gets translocated to the nucleus [34-36]. The activation of Nrf2 leads to the production of anti-inflammatory effects by activating NF-κB.

NF-κB is activated through oxidative stress which in turn regulates the transcription of several genes encoding pro-inflammatory cytokines, chemokines, and leukocyte adhesion molecules [37, 38]. Progression of renal diseases involves mainly NF-κB proteins mediating renal inflammation by promoting gene expression in different cell types, including renal cells, innate immune cells, and lymphocytes [39,40]. The members of TNF cytokines were the main contributors of renal damage on glomerular and tubular cells [41,42]. The data availability on the expression of Nrf2 and NF-κB, two key target gene products in CKD patients is limited. The current study was aimed to identify the expression dynamics of Nrf2 and NF-κB in CKD patients and their role in aetiopathogenesis.

## Material and Methods

### Enrolment of Samples

A total of 133 CKD patients (24 females with mean age: 48.41 ± 15.53 yrs; 109 males with mean age: 50.69 ± 15.29 yrs) and 133 healthy volunteers (60 females with mean age: 43.34 ± 13.11 yrs; 73 males with mean age: 40.06 ± 13.15 yrs) were enrolled. Case selection for CKD was defined by the trained physician based on Kidney Disease Quality Outcome Initiative (K/DOQI). Generally, CKD cases with kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m2 for 3 months or more, were included. Kidney disease severity is classified into five stages according to the level of GFR. The present study included CKD subjects under different category: dialysis, non-dialysis, diabetic and non-diabetics (with >18 years of age). Patients with malignancies, pediatric kidney failure, autoimmune disease and AIDS were excluded. The gene expression analysis for Nrf2 and NF-κB, IFN-γ and TNF-α genes were performed by qPCR methods. IFN-γ and TNF-α cytokine expression data were used only for correlation analysis (expression data not shown). A detailed questionnaire and written informed consent was collected from all participants and the study was approved by Institutional Ethical Committee.

### RNA Extraction and cDNA Synthesis

The total RNA was extracted from PBMCs using the Trizol reagent (Invitrogen Life Technologies, Germany) followed by incubation with DNAase (Qiagen) for 15 min. The RNA pellet was solubilized in Nuclease-Free Water and Optical Density was measured at 260/280 with a UV spectrophotometer. cDNA was synthesized followed by reverse transcription using an oligo (dt) primer and M-MuLV reverse transcriptase (Thermo Scientific kit). cDNA synthesis was performed using 20 µl reaction (2 µl template RNA (0.1 ng - 5 µg), 1 µl oligodT and 9ul, nuclease-free Water) and reaction mix 8 µl (1 µl reverse transcriptase, 1 µl RiboLock RNase Inhibitor, 1 µl dNTPs, 4 µl RT buffer) according to standard procedures. The cDNA of the samples were aliquoted and stored at -80°C.

### Quantitative Real-Time PCR (qPCR)

The expression levels of Nrf2 and NF-κB genes were performed with quantitative real-time polymerase chain reaction (qRT-PCR) (Rotar-Gene Q, Qiagen). The Nrf2 and NF-κB genes at transcript level were checked with the following primers - Nrf2: F-5′- TCCAGTCAGAAACCAGTGGAT-3′ and R-5′ GAATGTCTGCGCCAAAAGCTG-3′; and NF-κB: F-5′-CAGATGGCCCATACCTTCAAAT-3’ R-5′CGGAAACGAAATCCTCTCTGTT and endogenous control, β-ACTIN F-5′-CAGTCGGTTGGAGCGAGCAT-3′ and R-5′ GGACTTCCTGTAACAACGCATCT-3′ [43, 44]. The total reaction was performed in a 20µl volume with 11 µL of 2×SYBR Green Master Mix (Qiagen); 0.5 µL of 10 µM forward primer; 0.5 µL of 10 uM reverse primer; 2 µL of a 1:10 dilution of cDNA template and 6 µl RNase free water. The thermal cycle conditions were: 95oC for 2 min (hold), 40 cycles at 95oC for 10s, 53oC for 15s, 72oC for 25s. The relative gene expression of Nrf2 and NF-κB gene between patients vs. controls was calculated and normalized against β-ACTIN using the ΔCt method. The appropriate positive and negative controls and NTC were included in each run. The specificity of amplification was confirmed by PCR product run on agarose gel electrophoresis and melt curve analysis. The difference of mean Ct values between test and control was determined. 2-ΔΔCt was used to calculate the Fold Change (FC). Fold change of <1 indicates a down-regulation, while fold change of >1 indicates upregulation.

### Statistical Analysis

Data were expressed as mean ± SEM. The data were analyzed with GraphPad Prism version 8.0.2. (GraphPad Software, Inc., San Diego, CA, USA.). The differences between independent groups were assessed by Mann Whitney U test and one-way ANOVA. Pearson’s correlation coefficient was calculated to examine the correlations between variables. Statistical significance was accepted at $p<0.05$.

## Results

### Relative Expression of Nrf2 and NF-κB Genes

The relative mRNA expressions of Nrf2 and NF-κB gene were analyzed separately for CKD (pooled), dialysis, non-dialysis, diabetic and non-diabetic CKD patients and compared with respective controls. The levels of different genes were represented in Fold Change (Table 1, Figure 1A-1E). No significant level of expression was observed for Nrf2 in CKD (pooled) (Fc:1.01±0.17) and dialysis patients (Fc:1.01±0.18). However, a significant down regulation of Nrf2 was observed in non-dialysis patients (Fc:0.80 ±0.29). The significant up regulation of Nrf2 were observed in diabetic (Fc:1.13±0.28) and non-diabetic (Fc:1.30±0.29) CKD patients.

**Table1: Relative gene expression of Nrf2 and NF-κB in CKD patients**

| S. No | CKD | Nrf2 | NF-kb | *p* Value |
|---|---|---|---|---|
| 1 | Pooled | 1.01±0.17 | 1.27±0.28 | 0.0001 |
| 2 | Dialysis | 1.01±0.18 | 1.36±0.39 | 0.0001 |
| 3 | Non-Dialysis | 0.80±0.29 | 1.59±0.74 | 0.041 |
| 4 | Diabetic | 1.13±0.28 | 0.69±0.34 | 0.002 |
| 5 | Non-Diabetic | 1.30±0.29 | 1.92±0.48 | 0.037 |

*P* significance < 0.05

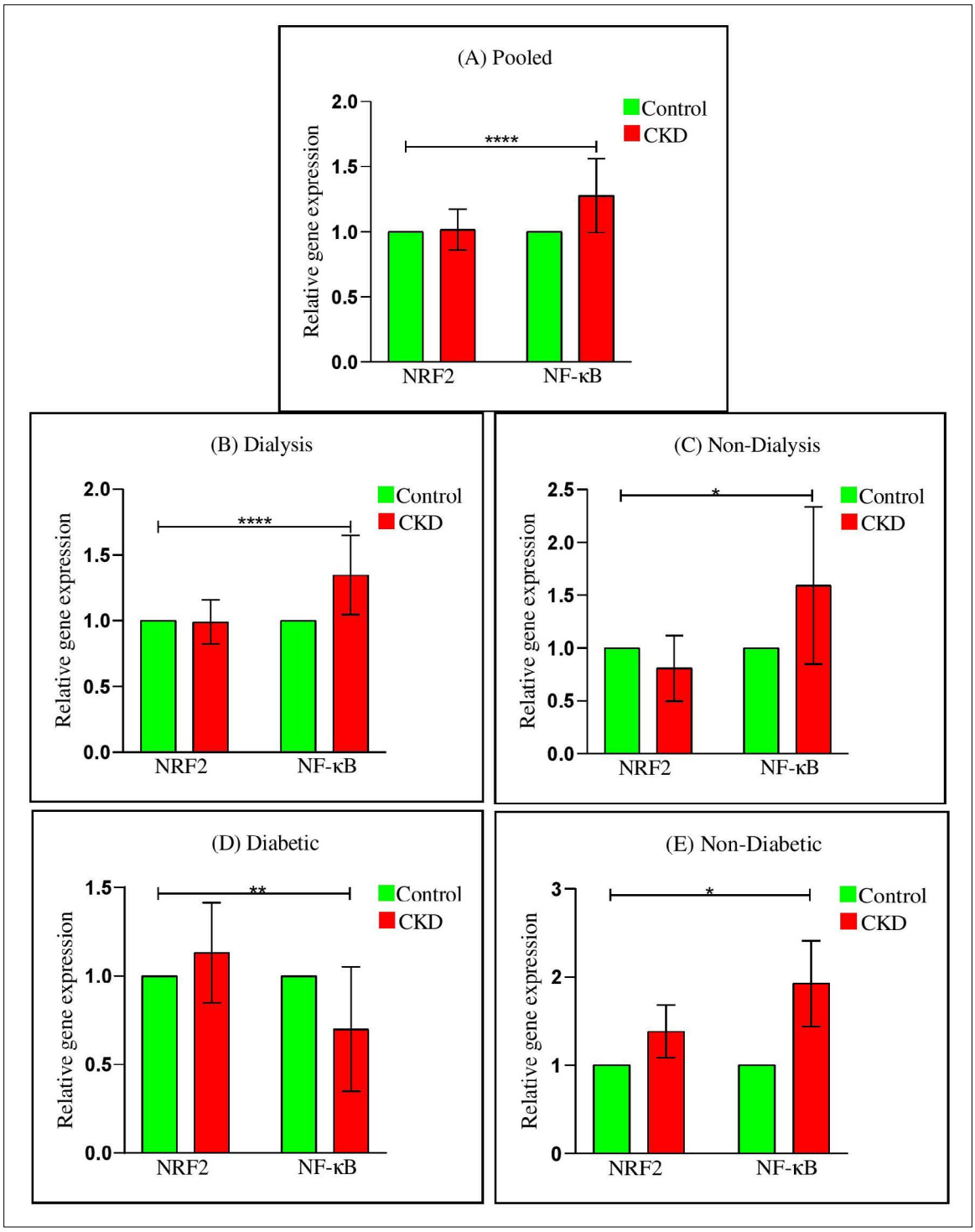


**Figure 1:** Relative mRNA Expression of NRF2 and NF- κB in CKD patients (A) Pooled (B)Dialysis (C) non-Dialysis (D) Diabetic (E) Non-Diabetic

Similarly, a significantly up-regulated expression was observed for NF-κB gene in CKD (pooled) (Fc:1.27±0.28), dialysis (Fc:1.36±0.39), non-dialysis (Fc:1.59±0.74) and non-diabetic patients (Fc:1.92±0.48). However, in diabetes patients, a significant down-regulation for NF-κB gene was observed (Fc:0.69 ±0.34).

**Correlation between Nrf2 and NF- κB**

A significant positive correlation was observed for Nrf2 and NF-κB gene in dialysis patients (r=0.172; p<0.04). However, a negative correlation was observed in non-dialysis patients (r=-0.28; p<0.015). A positive correlation was observed in diabetic patients (r=0.128; p<0.548), however, without statistical significance. No significant correlation was observed in non-diabetic patients (r=0.001; p<0.99). However, a negative correlation was observed for Nrf2 and NF-κB genes in CKD (pooled) (r =-0.028; p<0.83) patients (Table 2; Figure 2).

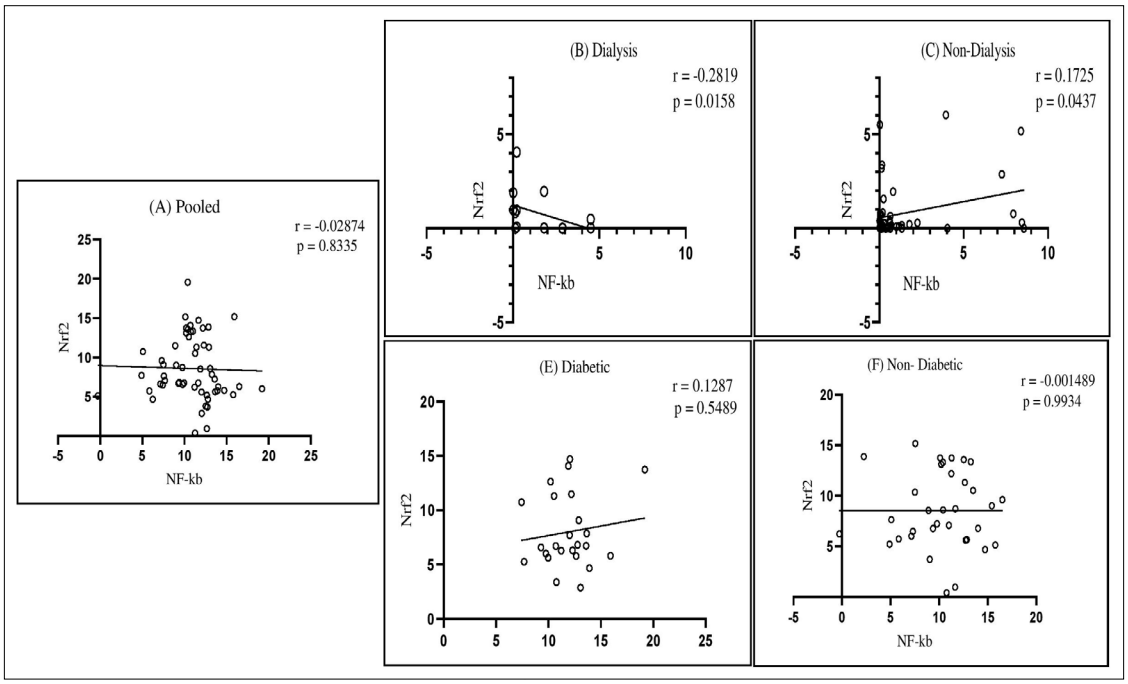


**Figure 2:** Correlation between Nrf2 and NF-κB expression among CKD patients. (A) Pooled (B) Dialysis (C) non-Dialysis (D) Diabetic and (E) Non-Diabetic.

**Correlation between Nrf2 and NF-κB with IFN-γ Cytokines**

The correlation in the levels of Nrf2 and NF-κB with IFN-γ cytokine in CKD patients was studied and presented (Table 2, Figure 3 and 4). Significant positive correlations were observed for Nrf2 with IFN-γ in diabetes (r=0.28; p=0.05) and non-diabetic (r=0.37; p=0.002) CKD patients. A weakly positive correlation was observed in non-dialysis (r=0.50; p=0.09) CKD patients. No correlation was observed in dialysis patients. In the analysis for NF-κB with IFN-γ, a positive correlation was observed in diabetic (r=0.350; p=0.100) and non-diabetic (r=0.112; p=0.525), however, without statistical significance. Further, no correlation was observed for CKD (pooled) (r=0.003; p=0.977), dialysis (r=-0.04; p=0.73) and non-dialysis (r=0.08; p=0.80) CKD cases.

**Table 2: Correlations analysis of Nrf2 , NF-kB , IFN-γ and TNF-α in different subgroups of CKD patients**

| Correlation | Pooled | | Dialysis | | Non-Dialysis | | Diabetic | | Non-Diabetic | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *r* value | *p* value | *r* value | *p* value | *r* value | *p* value | *r* value | *p* value | *r* value | *p* value |
| Nrf2 vs NF-kB | -0.028 | 0.833 | -0.281 | 0.01 | 0.172 | 0.04 | 0.12 | 0.54 | 0.001 | 0.99 |
| IFN-γ vs NF-kB | 0.003 | 0.97 | -0.04 | 0.73 | 0.08 | 0.80 | 0.35 | 0.10 | 0.11 | 0.525 |
| IFN-γ vs Nrf2 | 0.07 | 0.50 | -0.07 | 0.43 | 0.50 | 0.09 | 0.283 | 0.05 | 0.372 | 0.002 |
| TNF-α vs NF-kB | 0.282 | 0.09 | -0.01 | 0.89 | -0.16 | 0.61 | 0.36 | 0.30 | 0.55 | 0.77 |
| TNF-α vs Nrf2 | 0.338 | < 0.0001 | -0.02 | 0.83 | 0.42 | 0.14 | 0.25 | 0.09 | 0.1322 | 0.26 |

*P* significance < 0.05

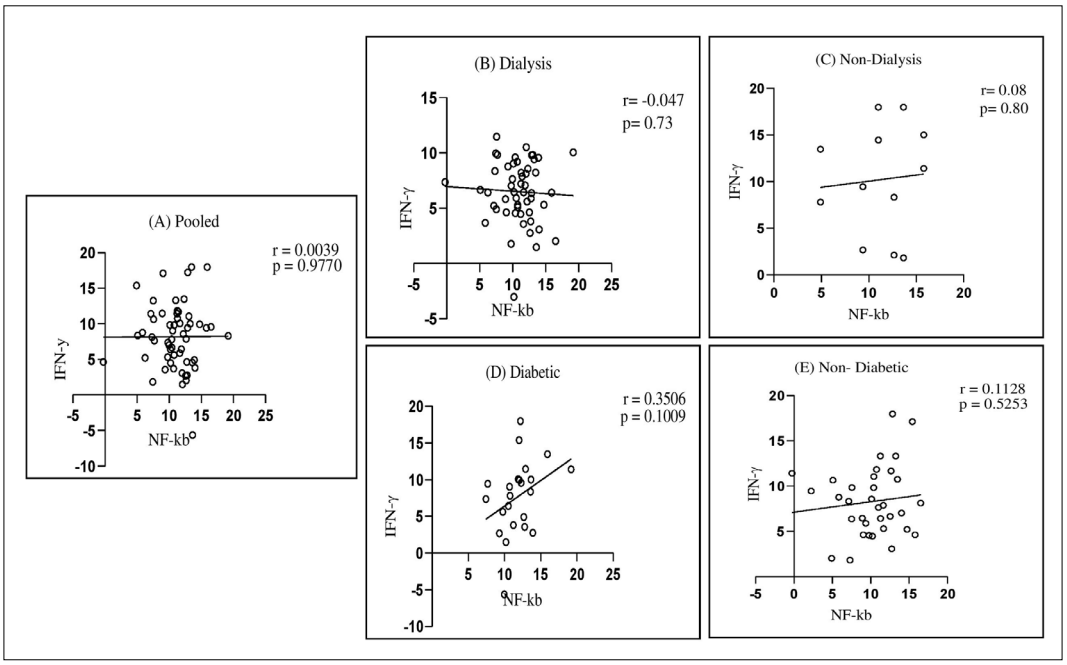


**Figure 3:** Correlation between IFN- γ and NF-kB expression among CKD patients. (A) Pooled (B) Dialysis (C) non-Dialysis (D) Diabetic and (E) Non-Diabetic

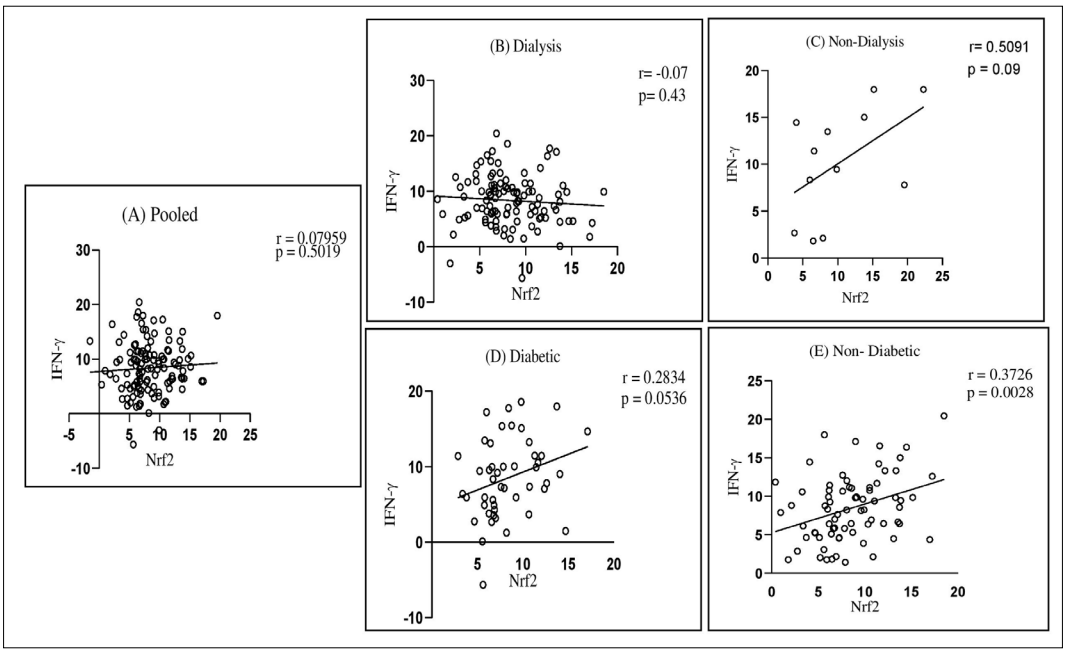


**Figure 4:** Correlation between IFN-γ and Nrf2 expression among CKD patients. (A) Pooled (B) Dialysis (C) non-Dialysis (D) Diabetic and (E) Non-Diabetic

**Correlation between Nrf2 and NF-κB with TNF-α cytokines**

The correlations between Nrf2 and NF-κB with TNF-α in CKD patients were analyzed and presented (Table 2 and Figures 5 and 6). A significant positive correlation was observed for Nrf2 with TNF-α in CKD (pooled) (r=0.33; p=0.000) CKD patients. Positive correlations were observed for non-diabetic (r=0.132; p=0.26) and non-dialysis CKD cases (r=0.42; p=0.14), however, without statistical significance. However, a weakly significant positive correlation was observed in diabetes (r=0.257; p =0.09) patients. No correlation was observed in dialysis patients. Similarly, a weakly positive correlation for NF-κB with TNF-α in CKD (pooled) (r=0.282; p<0.09) and an insignificant positive correlation in diabetic (r=0.36; p<0.30) and non-diabetic (r=0.55; p<0.77) CKD patients were observed. No correlations were observed for dialysis and non-dialysis patients.

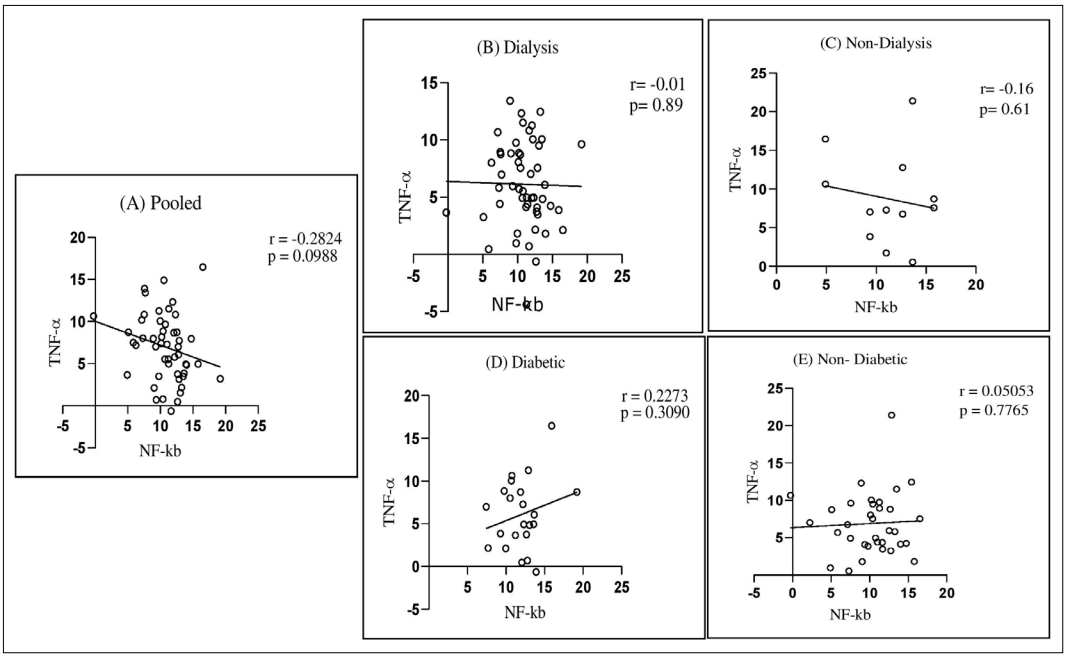


**Figure 5:** Correlation between TNF- α and NF-kB expression among CKD patients. (A) Pooled (B) Dialysis (C) non-Dialysis (D) Diabetic and (E) Non-Diabetic

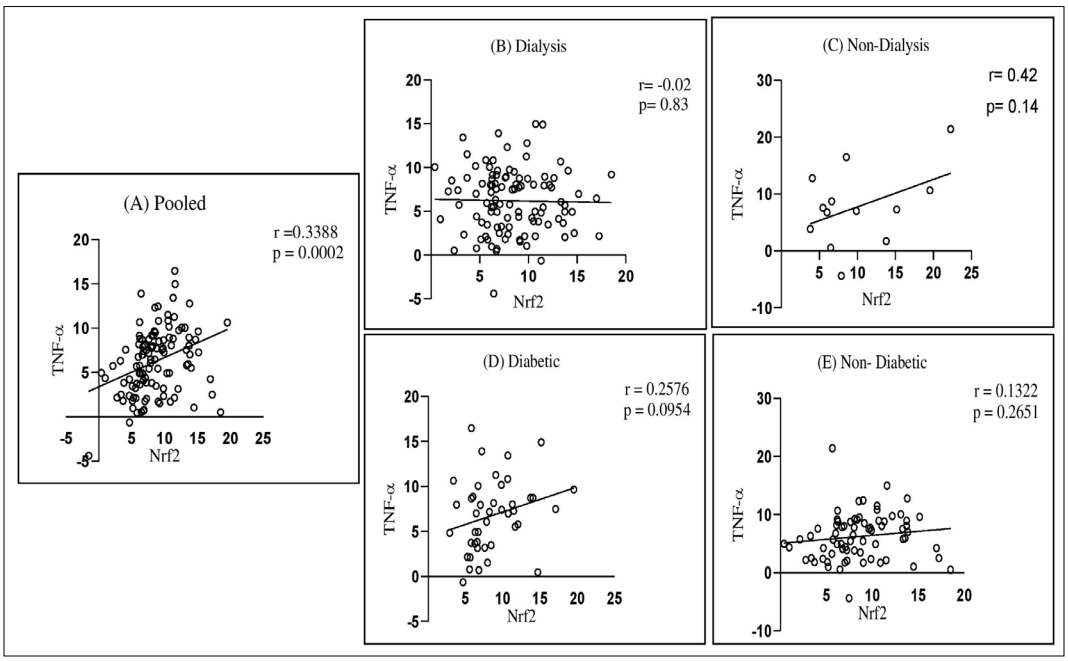


**Figure 6:** Correlation between TNF- α and Nrf2 expression among CKD patients. (A) Pooled (B) Dialysis (C) non-Dialysis (D) Diabetic and (E) Non-Diabetic

## Discussion

Oxidative stress in CKD leads to the increased production of ROS and deficiency of the endogenous antioxidant capacity. Several mechanisms contribute to the onset and pathogenesis of diabetic nephropathy, including genetic and hemodynamic factors, inflammation, oxidative stress, fibrosis, and cytokine signaling [45,46]. Generally, Nrf2 maintains cellular microenvironmental homeostasis [47]. The increased Nrf2 activity may provide cytoprotection against various chronic and inflammatory conditions [48,49]. Nrf2, an anti-inflammatory pathway, served as a link between inflammatory and metabolic pathways activated across multiple etiologies of CKD [50]. Genomic analyses have suggested that the expression of Nrf2 can be affecting genes involved in cytokines mediated inflammation [51].

We observed that the Nrf2 expression was downregulated in non-dialysis patients. It has been documented that high salt concentration in the cell leads to the downregulation of Nrf2 mainly through a sodium-dependent mechanism in kidney collecting duct cells, which might contribute to the excessive renal oxidative stress and CKD progression [47]. The downregulation of Nrf2 mRNA could mainly depend on the effect of Na+ but not Cl– or high osmolality. On the contrary, it was reported earlier that Nrf2 gene expression was decreased in hemodialysis (HD), in CKD patients with stages 3b–5, 4/5, and 5 [52,53]. Our present data are in concordance with a previous report stating that Nrf2 gene expression in CKD stage 3-5 groups was similar to healthy controls [54]. The impaired activation of Nrf2 is mainly responsible for antioxidant capacity, which is the master regulator of antioxidant

and phase-2 detoxifying enzymes and related proteins [55-57].

NF-κB is highly expressed in CKD patients, playing a pathogenic role in mediating chronic inflammation [58]. Our data revealed the up-regulation in the expression of NF-κB in CKD patients, dialysis, non-dialysis and non-diabetes groups. However, in diabetes groups, dysregulation of NF-κB was seen. It has been documented that, NF-κB is activated in patients with kidney disease such as diabetic nephropathy, glomerular disease, and animal models of renal inflammation and injury [59-60,39]. The mRNA expression profiles across multiple etiologies of CKD revealed up-regulation of pro-inflammatory genes, including human leukocyte antigen isoforms, toll-like receptors 1 and 3, and NF-κB [50].

Understanding the association between Nrf2 and NF-κB is very complex and not completely elucidated [61]. A previous study revealed enhanced NF-κB activity and expression of inflammatory genes in response to different insults in Nrf2 knockout mice than the corresponding wild-type mice. Our data documented that the expression of Nrf2 was negatively correlated with NF-κB expression in dialysis patients. It has also been documented that activation of Nrf2 produces a wide variety of anti-inflammatory effects, including lowered NF-κB pathway [62]. In concordance with a previous report, Nrf2 was positively correlated with NF-κB expression in non-dialysis patients [63].

NF-κB is a potent mediator stimulated by several cytokines, such as TNF-α and IL-1, involved in various inflammatory diseases [64]. Our study reported a positive correlation of TNF-α and NF-κB in CKD patients (pooled). Earlier studies have revealed that ischemia-reperfusion stimulate the production of TNF-α in an NF-κB-dependent manner, and TNF-α binds to its receptor to stimulate NF-κB activation, thereby providing a positive feedback mechanism of NF-κB regulation [65]. NF-κB is having opposite roles at different stages of inflammation [66]. During initiation, NF-κB stimulates inflammatory mediators such as TNF-α, angiotensin II and monocyte chemoattractant protein-1 (MCP-1). In contrast during resolution, NF-κB down-regulates inflammatory genes up-regulate anti-inflammatory genes and induce apoptosis of leukocytes. Thus, the NF-κB dynamics is a complex process in inflammatory events.

TNF-α has been demonstrated to be cytotoxic to glomerular mesangial and epithelial cells, to induce kidney damage [67,68], and to affect glomerular hemodynamics and GFR [69]. Previous studies have documented in experimental models of DKD, an increased level of TNF expression in glomerular and proximal tubular epithelial cells and renal interstitial fluid, leading to an increase in urinary albumin excretion [70-72]. TNF-α has been involved in the development of renal hypertrophy and glomerular hyperfiltration in early DKD [73,74]. In the present study, we reported a positive correlation of NF-κB and Nrf2 with TNF-α in diabetic and non-diabetic CKD patients. It has been documented that upon NF-κB signaling, TNF-α can induce transcription of cytokines that influence cell survival, proliferation, adhesion, inflammatory response and apoptosis [75,76]. Further, to understand the mechanism of Nrf2 and NF-κB gene research is needed for gene expression analysis together with cytokine dynamics to understand the impact of Nrf2 and NF-κB in CKD pathogensis.

## Conclusion

The study confirmed the down regulation of master regulator factor Nrf2 and upregulation of NF-κB in non–dialysis patients. However, in diabetic CKD patients, expression levels were just reversed. Correlation analysis between these transcription factors and cytokines such as IFN-γ and TNF-α revealed significant association between Nrf2 and IFN-γ in diabetic, non-diabetic and Nrf2 and TNF-α in CKD (pooled) patients. Thus, the study clearly revealed opposing tendencies in CKD aetiopathogenesis. Further, to understand the regulatory mechanisms of Nrf2 and NF-κB, a multicentric research is needed to completely elucidate the molecular aetiopathogenesis events leading to CKD.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgment

The authors were thankful to various central facilities such as Tissue Typing Services (KB), UGC and DST-WoSA (CR) at Madurai, Madurai Kamaraj University, Madurai.

## References

1. Couser WG, Remuzzi G, Mendis S, Tonelli M (2011) The contribution of chronic kidney disease to the global burden of major non-communicable diseases. Kidney Int 80: 1258-1270.
2. Luyckx V, Tonelli M, Stanifer J (2018) The global burden of kidney disease and the sustainable development goals. Bull World Health Organ 96: 414-422.
3. The top 10 causes of death (2020) WHO https://www.who.int/newsroom/fact-sheets/detail/the-top-10-causes-of-death.
4. Singh AK, Farag YM, Mittal BV (2013) Epidemiology and risk factors of chronic kidney disease in India – Results from the SEEK (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrol 14:114.
5. Ene-Iordache B, Perico N, Bikbov B, Carminati S, Remuzzi A, et al. (2016) chronic kidney disease and cardiovascular risk in six regions of the world (ISN-KDDC): A cross-sectional study. Lancet Glob Health 4: 307-319.
6. Modi GK, Jha V (2006) The incidence of end-stage renal disease in India: a population-based study. Kidney Int 70: 2131-2133.
7. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, et al. (2012) What do we know about chronic kidney disease in India: the first report of the Indian CKD registry. BMC Nephrol 13:10.
8. Varughese S, Abraham G (2018) chronic kidney disease in India: A clarion call for Change. Clin J Am Soc Nephrol 13: 802-804.
9. Snyder S, Pendergraph B (2005) Detection and evaluation of chronic kidney disease. Am Fam Physician 72: 1723-1732.
10. Jayasekara KB, Dissanayake DM, Sivakanesan R, Ranasinghe A, Karunarathna RH, (2015) Epidemiology of chronic kidney disease, with special emphasis on chronic kidney disease of uncertain etiology, in the north-central region of Sri Lanka. J Epidemiol 25: 275-280.
11. Kim HJ, Vaziri ND (2010) Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. Am J Physiol Ren Physiol 298: 662-671.
12. Imig JD, Ryan MJ (2013) Immune and inflammatory role in renal disease. ComprPhysiol 3: 957-976.
13. Impellizzeri D, Esposito E, Attley J, Cuzzocrea S (2014) Targeting inflammation: new therapeutic approaches in chronic kidney disease (CKD). Pharmacol Res 81: 91-102.
14. Meng XM, Nikolic-Paterson DJ, Lan HY (2014) Inflammatory processes in renal fibrosis. Nat Rev Nephro l10: 493-503.
15. Akchurin OM, Kaskel F (2015) Update on inflammation in chronic kidney disease. Blood Purif 39: 84-92.

16. Paisley KE, Beaman M, Tooke JE (2003) Endothelial dysfunction and inflammation in asymptomatic proteinuria. Kidney Int 63: 624-633.
17. Ying T, Clayton P, Naresh C, Chadban S (2018) Predictive value of spot versus 24-hour measures of proteinuria for death, end-stage kidney disease or chronic kidney disease progression. BMC Nephrol 19: 55.
18. Kim J, Seok YM, Jung KJ, Park KM (2009) Reactive oxygen species/oxidative stress contributes to the progression of kidney fibrosis following transient ischemic injury in mice. Am J Physiol Renal Physiol 297: 461-470.
19. Basile DP, Leonard EC, Beal AG, Schleuter D, Friedrich J (2012) Persistent oxidative stress following renal ischemia-reperfusion injury increases ANG II hemodynamic and fibrotic activity. Am J Physiol Renal Physiol 302: 1494-1502.
20. Basile DP, Bonventre JV, Mehta R, Nangaku M, Unwin R, et al. (2016) Progression after AKI: understanding maladaptive repair processes to predict and identify therapeutic treatments. J Am Soc Nephrol 27: 687-697.
21. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath, KA, et al. (2007) Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int 71: 619-628.
22. Noronha IL, Niemir Z, Stein H, Waldherr R (1995) Cytokines and growth factors in renal disease. Nephrol Dial Transplant 10: 775-786.
23. Ostendorf T, Burg M, Floege J (1996) Cytokines and Glomerular Injury. Kidney Blood Press Res 19: 281-289.
24. Mariano F, Bussolati, B, Piccoli, G Camussi, G (1997) Renal Vascular Effects of Cytokines. Blood Purif 15: 262-272.
25. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD (2007) Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int 71: 619-628.
26. Jevnikar AM, Brennan DC, Singer GG, Heng JE, Maslinski W, et al. (1991) Stimulated kidney tubular epithelial cells express membrane-associated and secreted TNFα. Kidney Int 40: 203-211.
27. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H (1999) Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: Aminoguanidine ameliorates the overexpression of tumour necrosis factor-α and inducible nitric oxide synthase in diabetic rat glomeruli. Diabetologia 42: 878-886.
28. Himmelfarb J, Stenvinkel P, Ikizler TA (2002) The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 62: 1524-1538.
29. Himmelfarb J, Hakim RM (2003) Oxidative stress in uremia. Curr Opin Nephrol Hypertens 12: 593-598.
30. Vaziri ND (2004) Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. Curr Opin Nephrol Hypertens 13: 93-99.
31. Li W, Khor TO, Xu C (2008) Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. BiochemPharmacol 76: 1485-1489.
32. Wakabayashi N, Slocum SL, Skoko JJ (2010) When NRF2 talks, who's listening? Antioxid Redox Signal 13: 1649-1663.
33. Suzuki T, Yamamoto M (2015) Molecular basis of the KEAP1-NRF2 system. Free Radic Biol Med 88: 93-100.
34. Kobayashi A, Kang MI, Watai Y (2006) Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol Cell Biol 26: 221-229.
35. Sykiotis GP, Bohmann D (2010) Stress-activated cap'n'collar transcription factors in aging and human disease. Sci Signal 3: 3.
36. Uruno A, Motohashi H (2011) The Keap1-Nrf2 system as an in vivo sensor for electrophiles. Nitric Oxide 25: 153-160.
37. Antunes F, Han D (2009) Redox regulation of NF-kappaB: from basic to clinical research. Antioxid Redox Signal 11: 2055-2056.
38. Kim J, Cha YN, Surh YJ (2010) A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutat Res 690: 12-23.
39. Sanz AB, Sanchez-Nino MD, Ramos AM, Moreno JA, Santamaria B, et al. (2010) NF-kappaB in renal inflammation. J Am Soc Nephrol 21: 1254-1262.
40. Zhang H, Sun SC (2015) NF-kappaB in inflammation and renal diseases. Cell Biosci 5: 63.
41. Ortiz A, Bustos C, Alonso J, Alcazar R, Lopez-Armada MJ, et al. (1995) Involvement of tumor necrosis factor-alpha in the pathogenesis of experimental and human glomerulonephritis. Adv Nephrol Necker Hosp 24: 53-77.
42. Justo P, Sanz A, Lorz C, Gomez-Garre D, Mezzano S, et al. (2003) Expression of Smac/Diablo in tubular epithelial cells and during acute renal failure. Kidney Int Suppl 86: 52-56.
43. Kostyuk SV, Porokhovnik LN, Ershova ES (2018) Changesof KEAP1/NRF2 and IKB/NF-κB Expression Levels Induced by Cell-Free DNA in Different Cell Types. Oxid Med Cell Longev 2018: 1052413.
44. Zong W, Ge Y, Han Y, Yang X, Li Q, et al. (2017) Hypomethylation of HLA-DRB1 and its clinical significance in psoriasis. Oncotarget 8: 12323-12332.
45. Steinberg EP (2003) Improving the quality of care—Can we practice what we preach? N Engl J Med 348: 2681-2683.
46. Dronavalli S, Duka I, Bakris GL (2008) The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab 4: 444-452.
47. Wada J, Makino H (2013) Inflammation and the pathogenesis of diabetic nephropathy. Clin Sci 124: 139-152.
48. Tonelli C, Chio, IIC DA (2018) Tuveson, Transcriptional regulation by Nrf2, Antioxidants Redox Signal 29: 1727-1745.
49. Keleku-Lukwete N, Suzuki M, Yamamoto M ((2018) An overview of the advantages of KEAP1-NRF2 system Activation during inflammatory disease treatment, Antioxidants Redox Signal 29: 1746-1755.
50. Cuadrado G, Manda A, Hassan MJ, Alcaraz C, Barbas A, et al. (2018) Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach, Pharmacol Rev 70: 348-383.
51. Martini S, Nair V, Keller BJ (2014) Integrative biology identifies shared transcriptional networks in CKD. J Am Soc Nephrol 25: 2559-2572.
52. Wakabayashi N, Slocum SL, Skoko JJ (2010) When NRF2 talks, who's listening? Antioxid Redox Signal 13: 1649-1663.
53. Liu M, Deng M, Luo Q, Dou X, Jia Z (2020) High-Salt Loading Downregulates Nrf2 Expression in a Sodium-Dependent Manner in Renal Collecting Duct Cells. Front Physiol 10: 1565.
54. Chen DQ, Cao G, Chen H (2017) Gene and protein expressions and metabolomics exhibit activated redox signaling and wnt/b-catenin pathway are associated with metabolite dysfunction in patients with chronic kidney disease. Redox Biol 12: 505-521.
55. Leal VO, Saldanha JF, Stockler-Pinto MB (2015) NRF2 and NF-jB mRNA expression in chronic kidney disease: a focus on nondialysis patients. Int Urol Nephrol 47: 1985-1991.
56. Kensler TW, Wakabayashi N, Biswal S (2007) Cell survival

responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev PharmacolToxicol 47: 89-116.

57. Saito H (2013) Toxico-pharmacological perspective of the Nrf2-Keap1 defense system against oxidative stress in kidney diseases. BiochemPharmacol 85: 865-872.
58. Ruiz S, Pergola PE, Zager RA, Vaziri ND (2013) Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int 83: 1029-1041.
59. Rangan G, Wang Y, Harris D (2009) NF-kappaBsignalling in chronic kidney disease. Front Biosci J Virtual Libr 14: 3496-3522.
60. Mezzano S, Aros C, Droguett A, Burgos ME, Ardiles L, et al. (2004) NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. Nephrol Dial Transplant 19: 2505-2512.
61. Sakai N, Wada T, Furuichi K, Iwata Y, Yoshimoto K, et al. (2002) p38 MAPK phosphorylation and NF-kappa B activation in human crescentic glomerulonephritis. Nephrol Dial Transplant 17: 998-1004.
62. Buelna-Chontal M, Zazueta C (2013) Redox activation of Nrf2 and NF-KB: a double end sword? Cell Signal 25: 2548-2557.
63. Zoja C, Benigni A, Remuzzi G (2014) The Nrf2 pathway in the progression of renal disease. Nephrol Dial Transplant 1: 19-24.
64. Leal VO, Saldanha JF, Stockler-Pinto MB (2015) NRF2 and NF-jB mRNA expression in chronic kidney disease: a focus on nondialysis patients. Int Urol Nephrol 47: 1985-1991.
65. Tak PP, Firestein GS (2001) NF-kappaB: a key role in inflammatory diseases. J Clin Invest 107: 7-11.
66. Donnahoo KK, Shames BD, Harken AH, Meldrum DR (1999) Review article: the role of tumor necrosis factor in renal ischemia-reperfusion injury. J Urol 162: 196-203.
67. Lawrence T, Fong C (2010) The resolution of inflammation: anti-inflammatory roles for NF-kappaB. nt J Biochem Cell Biol 42: 519-523.
68. Bertani T, Abbate M, Zoja C, Corna D, Perico N, et al. (1989) Tumor necrosis factor induces glomerular damage in the rabbit. Am J Pathol 134: 419-430.
69. McCarthy ET, Sharma R, Sharma M, Li JZ, Ge XL, et al. (1998) TNF-alpha increases albumin permeability of isolated rat glomeruli through the generation of superoxide. J Am Soc Nephrol 9: 433-438.
70. Baud L, Perez J, Friedlander G, Ardaillou R (1988) Tumor necrosis factor stimulates prostaglandin production and cyclic AMP levels in rat cultured mesangial cells. FEBS Lett 239: 50-54.
71. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, et al. (1993) mRNA expression of growth factors in glomeruli from diabetic rats. Diabetes 42: 450-456.
72. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H (1999) Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumour necrosis factor-alpha and inducible nitric oxide synthase in diabetic rat glomeruli. Diabetologia 42: 878-886.
73. Kalantarinia K, Awad AS, Siragy HM (2003) Urinary and renal interstitial concentrations of TNF-alpha increase prior to the rise in albuminuria in diabetic rats. Kidney Int 64: 1208-1213.
74. DiPetrillo K, Coutermarsh B, Gesek FA (2003) Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. Am J Physiol Renal Physiol 284: 113-121.
75. DiPetrillo K, Gesek FA (2004) Pentoxifylline ameliorates renal tumor necrosis factor expression, sodium retention, and renal hypertrophy in diabetic rats. Am J Nephrol 24: 352-359.
76. Luo SF, Fang RY, Hsieh HL, Chi PL, Lin CC, Hsiao LD, Wu CC, Wang JS, Yang CM (2010) Involvement of MAPKs and NF-kappaB in tumor necrosis factor alpha-induced vascular cell adhesion molecule 1 expression in human rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 62: 105-116.