***Original Article***

# *Association of C-Reactive Protein, Tumor Necrosis Factor-Alpha, and Interleukin-With Chronic Kidney Disease in Elderly.*

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**Abstract**

**Background:** Chronic kidney disease (CKD) is a significant decrease in kidney function that is long-lasting, irreversible, and typically progressive. It has been demonstrated that there is a correlation between the rates of morbidity and mortality of CKD and systemic inflammation.

**Objective:** This study's objective was to assess the correlation between C-reactive protein, interleukin-6, and tumor necrosis factor-alpha and chronic kidney disease in elderly patients.

**Material and methods:** Eighty participants 65 years old or older were included in the study, and they were split into 40 CKD patients(cases) and 40 subjects(controls). Every patient had a detailed medical history gathered, a clinical assessment, and abdominal and pelvic ultrasound to evaluate their kidney function. Laboratory investigations included serum of interleukin-6(IL-6), tumor necrosis factor-alpha(TNFα), highly sensitivity C-reactive protein (hs-CRP), electrolytes (Na, K, Ca, P), virology (HCV ab, HBVs Ag, HIV), CBC, postprandial blood glucose (PPS), fasting blood glucose (FBS), creatinine, urea, albumin /creatinine ratio in urine and lipid profile.

**Results:** In the CKD group, the mean value of IL-6 levels was 181.58 ± 130.63.among the control group the mean was 88.07 ± 86.90. The mean value of CRP among the cases group was 42.02 ± 24.85 and among the control group was 14.95 ± 11.70. The mean value of TNF alpha among the cases group was 358.34 ± 255.48 and among the control group was 188.99 ± 196.76.

**Conclusion:** The blood levels of tumor necrosis factor-alpha, interleukin 6, and C reactive protein varied statistically significantly in CKD patients and controls.

**Keywords:** C-reactive protein (CRP); Interleukin 6(IL-6); chronic kidney disease (CKD)

## INTRODUCTION

Chronic kidney disease (CKD) is a significant decrease in renal function that is long-lasting, irreversible, and typically progressive. The prevalence of CKD is ten times greater in those over 75 than in people under 40. Patients over 65 years old currently get half of all renal replacement treatment [1].

One of the primary organs targeted by the gradual, inflammatory biological process of aging is the kidney. As people age, their kidney function declines, with a gradual loss of nephrons and tubulointerstitial and glomerular scarring. Changes in glomerular and tubular function, systemic hemodynamics, and body homeostasis are the consequences of these changes, which begin in decade four of life and pick up speed during the fifth and sixth decades [2].

The occurrence of CKD worldwide is growing and is linked to a higher risk of cardiovascular disease (CVD), early death, and end-stage renal disease (ESRD) [3]. It's been established that there is a correlation between the mortality and morbidity rates of CKD and systemic inflammation [4]. For example, C-reactive protein (CRP), an acute phase reactant, is connected to CVD mortality in people with end-stage renal disease (ESRD) [5].

Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) are significant Cytokines that regulate inflammation, both acute and chronic. They are associated with CVD morbidity and mortality in the general population, dialysis patients, and predialysis patients. However, some epidemiology studies have revealed conflicting conclusions regarding the connection between CRP, IL-6, TNF-α, and CKD [6-8].

IL-6 is a multifaceted cytokine that controls immunity, inflammation, metabolism, and organ development in addition to hematopoiesis [9].

Patients with CKD frequently have raised serum IL-6 levels [10]; the main causes of this include elevated production brought on by fluid overload, chronic inflammation, and oxidative stress. Concurrently, the increase in IL-6 is additionally promoted by the decreased renal function-related clearance of IL-6. Hemodialysis for therapeutic purposes and peritoneal dialysis in general further elicit inflammatory responses and raise the IL-6 in those with advanced renal disease [11].

As earlier mentioned, interleukin-6 exacerbates renal damage and triggers the onset of chronic vascular disease, one of the principal consequences of CKD. Research shows that IL-6 primarily causes damage to the endothelial by downregulating adiponectin(an anti-atherogenic adipokine) expression and endothelial nitric oxide synthase [12]. Additionally, injecting exogenous IL-6 increases atherosclerosis; these results imply that IL-6 contributes to the elevated risk of CVD in individuals with chronic renal disease. Together, these findings indicate that increased IL-6 is not just a result of CKD but, more crucially, a trigger for the disease's progression and associated consequences [13].

After being cloned more than 20 years ago, TNFα was identified, which in turn prompted the TNF superfamily finding and their corresponding receptors. Via the transmembrane receptors TNFR1 and TNFR2, TNFα modulates several vital cellular functions, like division of cells, survival, proliferation, and death. TNFα

main source is macrophages, which remarkably have a strong sensitivity to TNFα. The development of several illnesses, such as obesity, diabetes, psoriasis, sepsis, atherosclerosis, rheumatoid arthritis, and Crohn's disease have been linked to abnormal TNFα production and TNF receptor activation [14].

Additionally, TNFα has been linked to illnesses affecting other systems, such as kidney failure, and there is proof that drugs that suppress TNFα might be useful in treating some of these diseases [15].

**The work objective** was to assess the correlation between c-reactive protein, interleukin-6, and tumor necrosis factor-alpha, and chronic kidney disease in elderly patients.

**Subject and methods**

This case-control study was carried out in the geriatric department and outpatient clinic at the Main Alexandria University Hospital. The Alexandria Faculty of Medicine's Medical Ethics Committee gave the required approval before the study started. The objectives of the study were explained to each participant, and all individuals signed informed written consent forms.

The study had 80 participants, 40 of whom were CKD patients (group 1) and 40 of whom were controls (group 2) with normal kidney function, aged 65 years or older. The participants were of both sexes. Either eGFR (estimated glomerular filtration rate) <60 ml/min/1.73 m2 or albuminuria > 30 mg/24 h for a minimum of three months have been used to diagnose CKD. CKD patients are stage 3,4 and 5 but not on dialysis.

**Table 1: Classification of chronic kidney disease according to eGFR**

| Stage | GFR status | eGFR (ml/min/1.73 m2) |
|---|---|---|
| 1 | Kidney disease with normal GFR | >90 |
| 2 | Mildly impaired GFR | 60-89 |
| 3 | Moderately impaired GFR | 30-59 |
| 4 | Severely impaired GFR | 15-29 |
| 5 | Kidney failure | <15 |

**Exclusion criteria:**

- Subjects who have undergone chemotherapy within the last two years or are currently enrolled in clinical trials that may affect chronic renal disease.
- Received a kidney transplant within the last six months or taking immunosuppressant medications.
- AIDS(acquired immune deficiency syndrome) and

HIV(human immune deficiency virus) history.

**Methods:**

1. Taking a proper history, which entails:
- Age, gender, job, marital status, and particular habits.
- The self-reported cardiovascular disease (CVD) history, diabetes, hypertension, high cholesterol, and usage of antidiabetic, lipid-lowering, and antihypertensive drugs.
2. Complete clinical evaluation.
3. Lab Analysis, consisting of:
- Complete blood count (CBC).
- Tests for renal function include full urine analysis, blood urea, and serum creatinine levels.
- Albumin /creatinine ratio in urine.
- Electrolytes: Phosphorous, Sodium, Potassium, Calcium.
- Lipid Profile.
- Blood glucose levels after a meal and fasting.
- Virology: HIV, HBsAg, and HCV antibodies.
- Serum hs-CRP values.
- Serum TNF-α values.
- Serum IL-6 values.

4. Abdomen and pelvic ultrasound for evaluation of the kidneys.

**Results:**

**The data Statistical analysis**

After data was entered into the computer, IBM SPSS software version 20.0 was used to analyze the results (IBM Corp, Armonk, NY). Numerical and percentage descriptions were used for qualitative data. To confirm the distribution's normality, **the Shapiro-Wilk test** was performed. The terms range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) were used to explain quantitative data. The findings were considered significant at a P-value less than 0.05.

**Table 2: Comparison of the two groups under study based on demographic data.**

| Demographic data | CKD patients (n = 40) | | Controls (n = 40) | | Test of sig. | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| Gender categories | | | | | | |
| Male | 21 | 52.5 | 21 | 52.5 | | 1.000 |
| Female | 19 | 47.5 | 19 | 47.5 | | |
| **Age in years** | | | | | | |
| Min. – Max. | 65.0 – 84.0 | | 65.0 – 89.0 | | | |
| Mean ± SD. | 70.48 ± 4.99 | | 71.0 ± 6.49 | | t=0.406 | 0.686 |
| Median (IQR) | 70.0 (66.0 – 74.0) | | 68.50 (66.0 – 74.0) | | | |
| **Marital status** | | | | | | |
| Married | 30 | 75.0 | 28 | 70.0 | | 0.617 |
| Widow | 10 | 25.0 | 12 | 30.0 | | |
| **Occupation** | | | | | | |
| Farmer | 3 | 7.5 | 5 | 12.5 | | |
| Guard | 0 | 0.0 | 1 | 2.5 | | |
| No work / Retired | 29 | 60.0 | 24 | 60.0 | | $^{MC}p=$ 0.608 |
| Physician | 0 | 0.0 | 1 | 2.5 | | |
| Technician | 0 | 0.0 | 1 | 2.5 | | |
| Worker | 8 | 20.0 | 8 | 20.0 | | |
| **Smoking** | | | | | | |
| No | 25 | 62.5 | 29 | 72.5 | | 0.340 |
| Yes | 15 | 37.5 | 11 | 27.5 | | |

p: p-value for comparing between Cases and Controls
$\chi^2$: **Chi-square test,**
IQR: **Inter quartile range**
**MC**: **Monte Carlo test,**
**t: Student t-test**
SD: **Standard deviation**

**Table 3: Comparison of the medical histories of the two groups under study.**

| Medical history | CKD patients (n = 40) | | Controls (n = 40) | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| CVD | 11 | 27.5 | 5 | 12.5 | 1.953 | 0.162 |
| Diabetes | 18 | 45.0 | 11 | 27.5 | 2.650 | 0.104 |
| Hypertension | 28 | 70.0 | 18 | 45.0 | 5.115* | 0.024* |

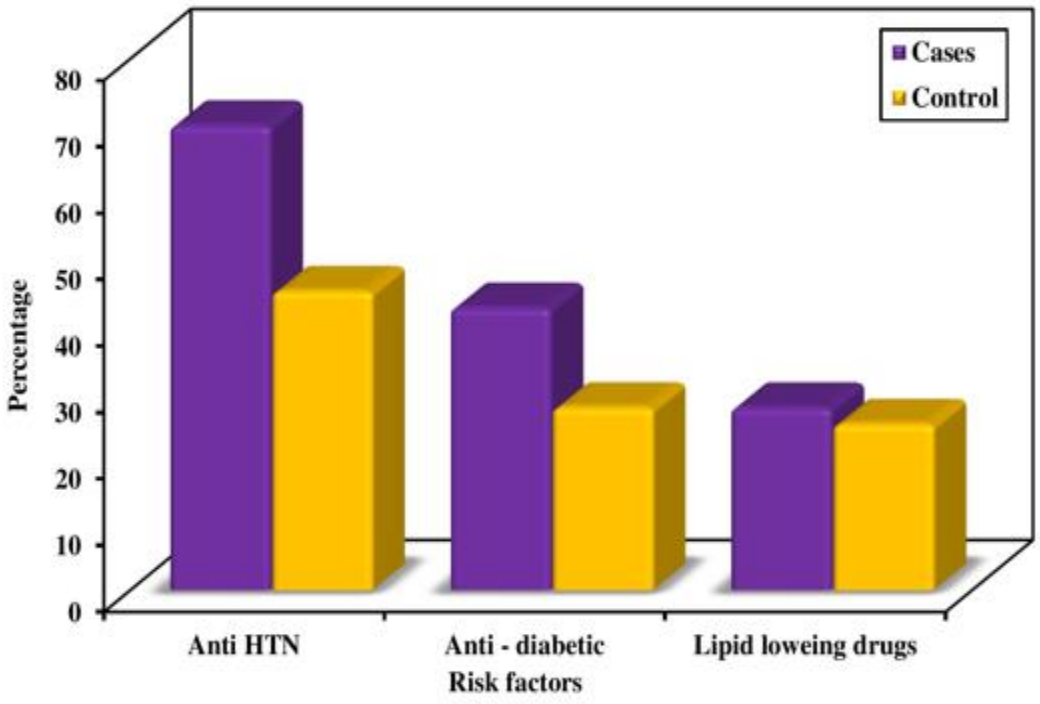


**Fig 1:** Comparison between the two studied groups according to drug history

**Table 4: Comparison between the two studied groups according to renal function**

| Renal function | CKD patients (n = 40) | Controls (n = 40) | U | p |
|---|---|---|---|---|
| **Urea** | | | | |
| Min. – Max. | 51.0 – 215.0 | 16.0 – 77.0 | | |
| Mean ± SD. | 121.05 ± 46.33 | 44.30 ± 13.69 | 44.0* | <0.001* |
| Median (IQR) | 125.0 (80.0 - 158) | 48.0 (37.5 – 50.5) | | |
| **Creatinine** | | | | |
| Min. – Max. | 1.10 – 14.0 | 0.30 – 1.20 | | |
| Mean ± SD. | 4.08 ± 2.63 | 0.86 ± 0.23 | 7.50* | <0.001* |
| Median (IQR) | 3.20 (2.1 – 5.55) | 0.90 (0.70 – 1.0) | | |

**U: Mann Whitney test**

**Table 5: Urine analysis was used to compare the two groups under study.**

| | CKD patients (n = 40) | | Controls (n = 40) | | Test of sig. | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **Urine analysis** | | | | | | |
| Free | 15 | 37.5 | 23 | 57.5 | | |
| Crystals | 1 | 2.5 | 8 | 20.0 | | |
| RBCs | 0 | 0 | 2 | 5.0 | | |
| RBCs & crystals | 2 | 5 | 0 | 0.0 | $\chi^2$= 23.616* | $^{MC}p$ <0.001* |
| PUS | 10 | 25 | 7 | 17.5 | | |
| Protein | 10 | 25 | 0 | 0 | | |
| Bilirubin | 2 | 5 | 0 | 0 | | |
| **Urinary albumin/creatinine ratio** | | | | | | |
| Min. – Max. | 15.0 – 390.0 | | 18.0 – 28.0 | | | |
| Mean ± SD. | 51.70 ± 63.26 | | 23.75 ± 2.76 | | U= 611.5 | 0.068 |
| Median (IQR) | 25.0 (21.5 – 70.0) | | 24.0 (22.0 – 25.5) | | | |

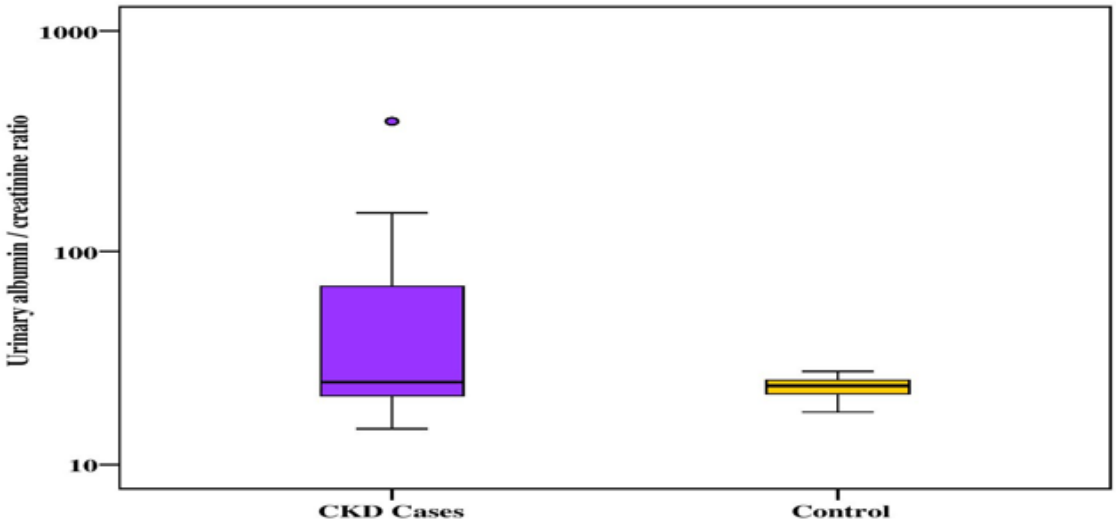


**Fig 2:** Comparing the two groups under study by Urinary albumin/creatinine ratio

**Table 6: Comparing the two groups under study by hs-CRP, TNF ALPHA, and IL-6**

| Inflammatory marker | CKD patients (n = 40) | Controls (n = 40) | U | p |
|---|---|---|---|---|
| **hs-CRP** | | | | |
| Min. – Max. | 6.60 – 97.0 | 3.0 – 48.0 | | |
| Mean ± SD. | 42.02 ± 24.85 | 14.95 ± 11.70 | 209.50* | <0.001* |
| Median (IQR) | 36.0 (19.5 – 60.0) | 10.0 (8.0 – 18.0) | | |
| **TNF ALPHA** | | | | |
| Min. – Max. | 29.47 – 950.0 | 15.0 – 840.0 | | |
| Mean ± SD. | 358.34 ± 255.48 | 188.99 ± 196.76 | 448.0* | 0.001* |
| Median (IQR) | 296.45 (181.13 – 568.48) | 153.09 (37.6 – 271.6) | | |
| **IL-6** | | | | |
| Min. – Max. | 16.73 – 480.0 | 7.0 – 430.0 | | |
| Mean ± SD. | 181.58 ± 130.63 | 88.07 ± 86.90 | 418.0* | <0.001* |
| Median (IQR) | 148.55 (91.68 – 284.13) | 76.87 (17.69 – 135.29) | | |

**According to Table 6,** the CKD patient's group CRP was substantially greater than the control group's ($p<0.001$). the CKD patient's group TNF alpha was substantially greater

than that of the control (p<0.001). **t**he CKD patients' group had considerably higher levels of IL6 than the control group (p<0.001).

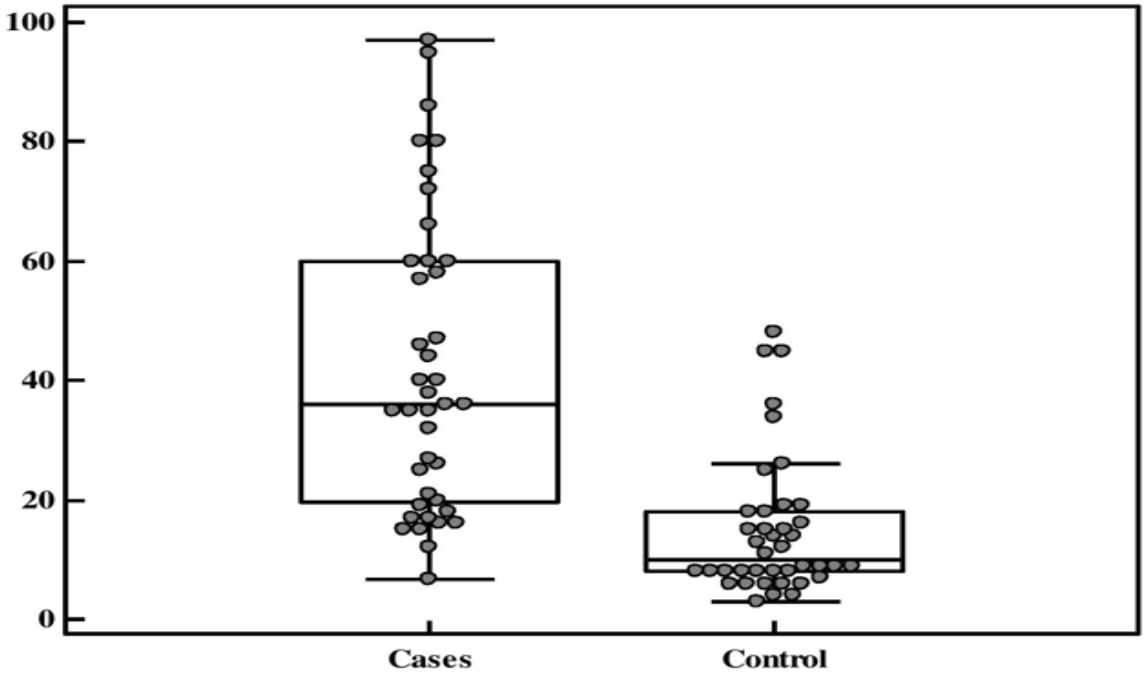


**Fig 3:** Comparing the two groups under study by hs-CRP

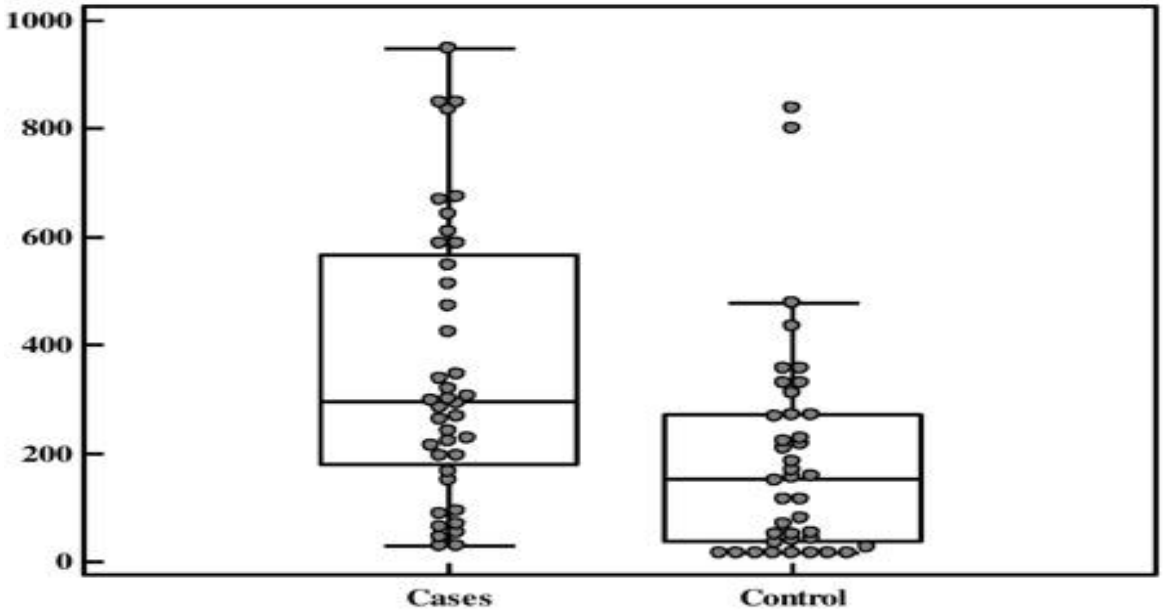


**Fig 4: Comparing the two groups under study by TNF ALPHA**

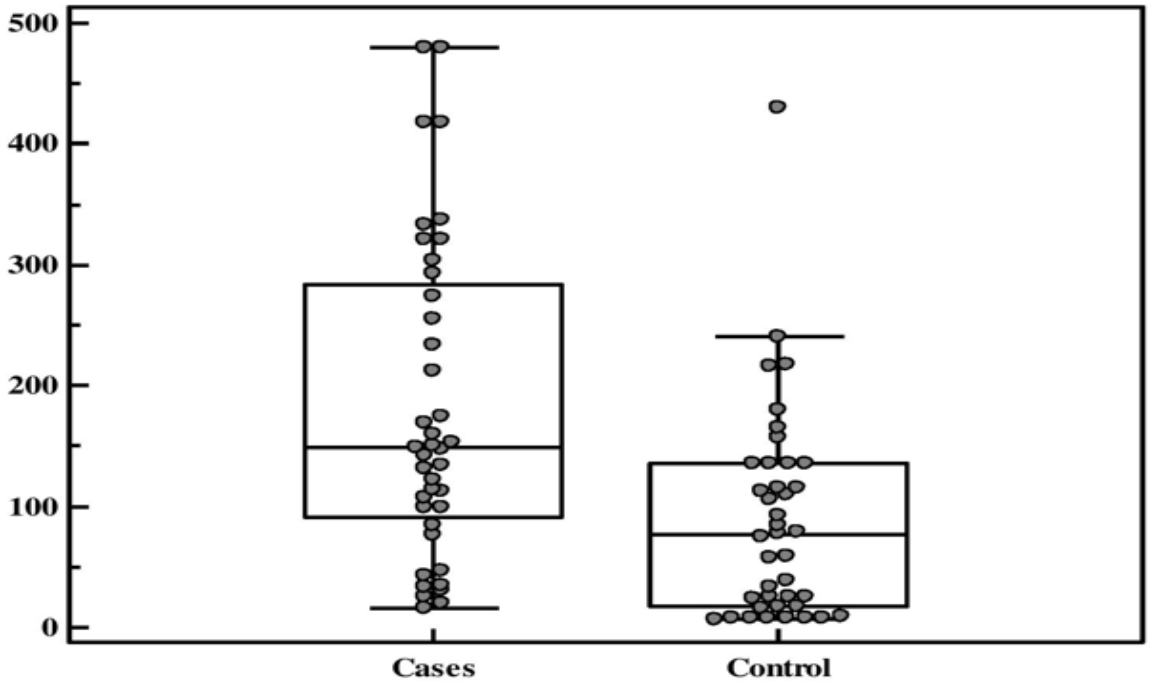


**Fig 5:Comparing the two groups under study by IL-6**

**Table 7: TNF serum levels in different comorbidities among cases.**

| | N | TNF ALPHA | | | U | p |
|---|---|---|---|---|---|---|
| | | Min. – Max. | Mean ± SD. | Median | | |
| **Hypertension** | | | | | | |
| No | **12** | 29.97 – 950.0 | 333.39 ± 285.75 | 296.45 | 153.50 | 0.673 |
| Yes | **28** | 29.47 – 850.0 | 369.04 ± 246.21 | 295.81 | | |
| **CVD** | | | | | | |
| No | **29** | 29.47 – 850.0 | 331.14 ± 251.81 | 284.23 | 124.0 | 0.294 |
| Yes | **11** | 69.44 – 950.0 | 430.05 ± 263.09 | 319.90 | | |
| **Diabetes** | | | | | | |
| No | **22** | 29.47 – 950.0 | 334.83 ± 241.65 | 296.45 | 183.50 | 0.697 |
| Yes | **18** | 29.97 – 850.0 | 387.08 ± 275.70 | 294.34 | | |

Table 7 showed that; among CKD patients TNF-Alpha levels demonstrated no statistically significant association with each Hypertension. (p=0.673), CVD. (p=0.294), and diabetes mellitus (p=0.697).

**Table 8: hs-CRP serum levels in different comorbidities among cases.**

| | N | hs-CRP | | | U | p |
|---|---|---|---|---|---|---|
| | | Min. – Max. | Mean ± SD. | Median | | |
| **Hypertension** | | | | | | |
| No | **12** | 6.60 – 95.0 | 39.13 ± 27.51 | 35.0 | 149.5 | 0.590 |
| Yes | **28** | 12.0 – 97.0 | 43.25 ± 24.05 | 38.0 | | |
| **CVD** | | | | | | |
| No | **29** | 6.60 – 97.0 | 40.40 ± 25.84 | 35.0 | 132.50 | 0.419 |
| Yes | **11** | 12.0 – 86.0 | 46.27 ± 22.61 | 36.0 | | |
| **Diabetes** | | | | | | |
| No | **22** | 15.0 – 97.0 | 42.86 ± 24.11 | 36.0 | 182.50 | 0.677 |
| Yes | **18** | 6.60 – 86.0 | 40.98 ± 26.38 | 39.50 | | |

Table 8 showed that; among CKD cases hs-CRP demonstrated no statistically significant association with each of hypertension. (p=0.590), CVD. (p=0.419), and diabetes mellitus (p=0.677).

**Table 9: IL-6 serum levels in different comorbidities among cases.**

| | N | IL-6 | | | U | p |
|---|---|---|---|---|---|---|
| | | Min. – Max. | Mean ± SD. | Median | | |
| **Hypertension** | | | | | | |
| No | **12** | 20.74 – 480.0 | 173.13 ± 152.02 | 148.55 | 155.0 | 0.716 |
| Yes | **28** | 16.73 – 418.43 | 185.19 ± 123.25 | 148.01 | | |
| **CVD** | | | | | | |
| No | **29** | 16.73 – 480.0 | 167.81 ± 129.04 | 142.26 | 121.5 | 0.254 |
| Yes | **11** | 34.42 – 480.0 | 217.87 ± 133.90 | 159.75 | | |
| **Diabetes** | | | | | | |
| No | **22** | 16.73 – 480.0 | 170.27 ± 127.20 | 148.55 | 181.50 | 0.657 |
| Yes | **18** | 25.23 – 418.43 | 195.40 ± 137.10 | 147.24 | | |

**U: The test of Mann Whitney**
p: Comparing a no to a yes using the p-value
SD: **The standard deviation**
Table (9) showed that IL-6 showed no statistically significant association with each of hypertension. (p=0.716), CVD. (p=0.254), and diabetes mellitus (p=0.657).

**Table 10: Correlation between kidney function (eGFR) and inflammatory markers levels among the whole participants.**

| | $r_s$ | p |
|---|---|---|
| **hs-CRP vs eGFR** | -0.618* | <0.001* |
| **Interleukin 6 vs eGFR** | -0.448* | <0.001* |
| **TNF ALPHA vs eGFR** | -0.408* | <0.001* |

$r_s$: coefficient of Spearman
*: significant analytically when $p < 0.05$

Table 10 showed a significant negative correlation between eGFR and each of hs-CRP, IL-6, and TNF-alpha among the total sample (n=80)

**Table 11:Correlation between different parameters for the total sample (n = 80)**

| | $r_s$ | p |
|---|---|---|
| **hs-CRP vs albumin/creatinine ratio in urine** | 0.160 | 0.158 |
| **IL-6 vs albumin/creatinine ratio in urine** | 0.059 | 0.601 |
| **TNF ALPHA vs albumin/creatinine ratio in urine** | 0.053 | 0.642 |

$r_s$: coefficient of Spearman

Table 11 showed no statistically important correlation between albumin/ creatinine ratio in urine and each of CRP, IL-6, and TNF-alpha serum levels, with p=0.158, p=0.601, and p=0.642 correspondingly.

**Table 12: Multivariate and univariate analysis for the parameters affecting CKD patients.**

| CKD cases | Univariate | | Multivariate | |
|---|---|---|---|---|
| | p | OR (95%C.I) | p | OR (95%C.I) |
| **Age in years** | **0.682** | 0.984 (0.911 – 1.063) | | |
| **CVD** | **0.101** | 2.655 (0.827 – 8.521) | | |
| **Diabetes** | **0.106** | 2.157 (0.849 – 5.481) | | |
| **Hypertension** | **0.025*** | 2.852* (1.137 - 7.152) | **0.160** | 2.409 (0.707 – 8.203) |
| **Antidiabetic** | **0.162** | 1.949 (0.765 – 4.965) | | |
| **Lipid-lowering drugs** | **0.799** | 1.138 (0.420 – 3.084) | | |
| **hs-CRP** | **<0.001*** | 1.093* (1.048 - 1.140) | **0.001*** | 1.127* (1.053 – 1.205) |
| **TNF ALPHA** | **0.004*** | 1.003* (1.001 – 1.006) | **0.261** | 0.980 (0.947 – 1.015) |
| **IL-6** | **0.002*** | 1.008* (1.003 – 1.014) | **0.276** | 1.038 (0.971 – 1.109) |

#: The multivariate analysis includes all factors with $p<0.05$.

OR: Odd`s ratio, C.I: Confidence interval,

LL: Lower limit UL: Upper Limit

*: Statistically important when $p \leq 0.05$

**Table 12** showed that Hypertension, hs-CRP, IL-6, and TNF-alpha are strongly correlated with the odds of CKD with ($p=0.025$), ($p< 0.001$), ($p=0.002$), and ($p=0.004$) correspondingly. The most independent variable connected with the chances of CKD was hs-CRP. In multivariate analysis, variables with $p<0.05$ in univariate analysis were included. ($p = 0.001$)

#: the three markers were adjusted with **CVD, Diabetes, Hypertension, and Anti-diabetic (**$p<0.2$ was included in the multivariate)

**Table 12** showed that after adjusted multivariate evaluation, there is a significant link between CKD and each of hs-CRP, TNG-alpha, and IL-6. with $p<0.001$, $p=0.010$ and $p=0.006$ respectively. This association is independent of other comorbidities like CVD, Diabetes, Hypertension, and anti-diabetic usage.

## Discussion

Our research revealed a substantial increase in hs-CRP between elderly CKD patients and CKD-free controls. In univariate regression analysis, hs-CRP was shown to be strongly correlated with chronic kidney disease (CKD). When doing multivariate regression evaluation, hs-CRP was discovered to be the most

important independent variable linked to CKD. Additionally, a strong negative relation was seen in our study between the level of hs-CRP and eGFR in all participants (n = 80).

In line with our research, Shlipak et al.[16] found that renal impairment was linked to an increase in CRP in a cohort study based on the population involving 5888 individuals over 65 years of age. In additional cross-sectional research, a substantial linear relationship between elderly CRP and cystatin-C was found. It's important to note that they employed cystatin C, which provides a more precise assessment of renal impairment in the elderly [17]. Also, Fried et al. claim that CRP and other inflammatory indicators are predictive of renal impairment in the elderly [18].

According to one study, which included 7317 non-diabetic participants, raised serum CRP levels were connected to worse creatinine clearance. This suggests that elevated CRP could put one at risk for kidney impairment [19]. In addition, Eun-Sil Yeo and colleagues suggested that among Korean type 2 diabetic patients, a high CRP level may be a separate hazard for CKD [20].

Babaei et al. discovered a substantial increase in hs-CRP in ESRD patients when contrasted to the controls, which is consistent with our study [21]. Furthermore, regardless of age or gender, it was stated that CKD patients had higher CRP levels [22]. Similarly to this, 103 chronic predialysis people in the Panichi et al. trial had increased CRP levels that were negatively linked with eGFR [23].

Shankar et al.[24]contradict our findings, stating that a long-term study examining the possibility of getting CKD in people who did not have CKD at the beginning of a cohort study with 4926 people and 15 years of follow-up discovered no link between CRP and CKD incidence. Additionally, Sarnak et al. discovered that the advancement of non-diabetic renal disease was not independently correlated with higher CRP levels [25].

However, after controlling for coexisting risk variables, Lee et al. reported that the relationship between CRP and CKD was no longer statistically significant. their study ranged from 21 to 74 years old for both cases and control with a mean of 55.9 ± 9.9 for cases and 52.5 ± 10.0 for control. However, in our research, the connection between CRP and CKD was still statistically considerable following modification of coexistent risk factors like DM, HTN, and CVD [26].

Our study found that TNF-alpha was markedly increased in elderly CKD patients compared to CKD-free controls, with p=0.001. Using univariate regression evaluation, increased serum levels of TNF-alpha were markedly linked to CKD, with (p=0.004). Our research showed a marked negative connection between the level of TNF-alpha and eGFR in the total sample (n=80), with p<0.001.

This aligns with studies conducted by Lee et al., which showed a negative link between creatinine clearance and TNF-alpha and its association with CKD [26]. In this regard, higher TNF-α levels in diabetic Korean patients could function as a separate risk factor for CKD, based on a study made by Eun-Sil Yeo et al. [20].

Also, Pruijm et al. found a significant relation between TNF-alpha with creatinine level, proposing a possible relation between inflammatory markers and the incidence of CKD [27]. According to Amdur et al, raised TNF-

alpha serum levels are linked to rapid deterioration of renal functions in a longitudinal study of CKD individuals [28].

In the same line with our study, TNF-alpha receptor 2 was positively connected to CKD incidence, according to Shankar et al in-follow-up research investigating the risk of incidence of CKD between individuals who were non-CKD-free at the start [24].

However, Conversely, Babaei, et al. concluded that TNF-alpha levels were elevated in ESRD individuals in contrast to the group of control, yet no statistically significant variation was observed [21].

Our study found that IL-6 was markedly raised in elderly CKD patients compared to CKD-free controls. Using univariate regression evaluation, increased IL-6 levels were significantly connected to CKD. Our study also discovered a marked negative link between IL-6 levels and eGFR in the total sample (n=80), with $p<0.001$.

This is in line with Lee et al who suggested that IL-6 is linked to the frequency and intensity of CKD [26]. Compatible with our study, Shlipak et al. concluded that renal insufficiency was independently associated with elevation in IL-6 in a survey of 5888 individuals aged ≥65 years [17].

Similarly, Shankar et al revealed that interleukin-6 was significantly connected to CKD in a long-term study investigating the possibility of getting CKD [24]. Consistent with this study, Panichi et al found that IL-6 level was elevated and inversely correlated with creatinine clearance in chronic predialysis patients [23]. Also, Babaei et al. concluded that the serum IL-6 level was notably raised in ESRD individuals versus the control group [21].

On the other side, Pruijm et al. stated that IL-6 did not significantly correlate with renal impairment in the general population [27].

According to Eun-Sil Yeo et al, the link of IL-6 to CKD is not statistically significant in diabetic Korean patients [20].

According to our research, there was no statistically significant relationship seen between albumin/ creatinine ratio in urine (ACR) and inflammatory indicators including CRP, interleukin-6, and TNF-alpha. Also, there was a lack of important discrepancy between the studied two groups regarding the albumin/ creatinine ratio in urine.

Incompatible to our study, Pruijm found that there was a significant association between microalbuminuria and inflammation in the population with CKD [27]. Also, Gupta et al revealed that plasma, TNF-α, hs-CRP, and IL-6 were positively connected with albuminuria and negatively with creatinine clearance [29].

In our investigation, there was a substantial positive link between CRP, TNF-alpha, and IL-6 among the CKD patients' group. ($P<0.001$)**.** This correlation agrees with the pathophysiology of CRP production as it is secreted from hepatocytes stimulated with inflammatory cytokines including interleukin-6 and TNF-alpha which is a master controller of the inflammatory cascade [30,31].

The relation of inflammatory markers to CKD may be claimed to be indirectly caused by associated co-morbidities like diabetes and hypertension [32,33]. So, we adjusted our results with co-existent comorbidities such as CVD, Diabetes, Hypertension, and Anti-diabetic usage in a multivariate regression analysis, and we found that inflammatory markers

including CRP, interleukin 6, and TNF alpha were still significantly increased in elderly CKD patients, independent of established risk factors as CVD, DM, and HTN.

Compatible with our study, Keller C et al. found that partially adjusted correlations between cystatin C and indicators of inflammation including CRP, IL-6, and TNF-alpha were statistically significant, suggesting that elevated levels of CRP, IL-6, and TNF-α could be a separate risk factor for CKD [34]. Also, Pecoits-Filho et al measured plasma hs-CRP, IL-6, and TNF-alpha levels and revealed that low eGFR is linked to an inflammatory state [10]. The explanation of significant elevation of inflammatory markers in elderly patients with CKD could be explained by decreased renal clearance of these markers, the effect of uremia that leads to increased cytokines production, frequent infection in CKD and dialytic patients, intestinal dysbiosis due to fluid overload and intestinal congestion, adverse effect of inflammation on renal function or the consequences of vitamin D deficiency on immunity [35].

**As a conclusion,** our study found a statistically significant association between each of the inflammatory markers: CRP, IL-6, and TNF-alpha, and CKD in the elderly. A significant negative correlation was found between each CRP, IL-6, and TNF-alpha with eGFR in the total sample. No statistically important link was found between the albumin/ creatinine ratio in urine (ACR) and indicators of inflammation including CRP, TNF-alpha, and IL-6 in the elderly.

Univariate regression analysis showed that Hypertension, hs-CRP, IL-6, and TNF-alpha are strongly connected to CKD, while multivariate regression analysis showed that indicators of inflammation including CRP, TNF alpha, and IL-6 were still markedly increased in elderly CKD patients separate from established risk factors as CVD, DM, and HTN.

**We recommend that** more long-term follow-up studies are needed to verify the causality of inflammatory markers in the incidence and advancement of CKD in the elderly. Also, interventional studies are required to assess if reduction of the inflammatory markers would decrease the risk of developing CKD in the elderly.

## REFERENCES

*[1] Bowker, L., Price, J., & Smith, S. (2012). Oxford handbook of geriatric medicine. OUP Oxford.*

*[2] Pichler, R., Hugo, C., & Johnson, R. J. (2010). Geriatric Nephrology. In Comprehensive Clinical Nephrology (pp. 785-94). Mosby.*

*[3] Weiner, D. E., Tighiouart, H., Elsayed, E. F., Griffith, J. L., Salem, D. N., Levey, A. S., & Sarnak, M. J. (2008). Inflammation and cardiovascular events in individuals with and without chronic kidney disease. Kidney international, 73(12), 1406-1412.*

*[4] Zimmermann, J., Herrlinger, S., Pruy, A., Metzger, T., & Wanner, C. (1999). Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney international, 55(2), 648-658.*

*[5] Yeun, J. Y., Levine, R. A., Mantadilok, V., & Kaysen, G. A. (2000). C-reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. American journal of kidney diseases, 35(3), 469-476.*

*[6] Feghali, C. A., & Wright, T. M. (1997). Cytokines in acute and chronic inflammation. Front Biosci, 2(1), d12-d26.*

*[7] Ridker, P. M., Rifai, N., Pfeffer, M., Sacks, F., Lepage, S., & Braunwald, E. (2000). Elevation of tumor necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction. Circulation, 101(18), 2149-2153.*

*[8] Barreto, D. V., Barreto, F. C., Liabeuf, S., Temmar, M., Lemke, H. D., Tribouilloy, C., ... & European Uremic Toxin Work Group (EUTox. (2010). Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. Kidney international, 77(6), 550-556.*

*[9] Su, H., Lei, C. T., & Zhang, C. (2017). Interleukin-6 signaling pathway and its role in kidney disease: an update. Frontiers in immunology, 8, 405.*

*[10] Pecoits-Filho, R., Heimbürger, O., Bárány, P., Suliman, M., Fehrman-Ekholm, I., Lindholm, B., & Stenvinkel, P. (2003). Associations between circulating inflammatory markers and residual renal function in CRF patients. American journal of kidney diseases, 41(6), 1212-1218.*

*[11] Takahashi, T., Kubota, M., Nakamura, T., Ebihara, I., & Koide, H. (2000). Interleukin-6 gene expression in peripheral blood mononuclear cells from patients undergoing hemodialysis or continuous ambulatory peritoneal dialysis. Renal failure, 22(3), 345-354.*

*[12] Bruun, J. M., Lihn, A. S., Verdich, C., Pedersen, S. B., Toubro, S., Astrup, A., & Richelsen, B. (2003). Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. American journal of physiology-Endocrinology and metabolism, 285(3), E527-E533.*

*[13] Huber, S. A., Sakkinen, P., Conze, D., Hardin, N., & Tracy, R. (1999). Interleukin-6 exacerbates early atherosclerosis in mice. Arteriosclerosis, thrombosis, and vascular biology, 19(10), 2364-2367.*

*[14] Parameswaran, N., & Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. Critical Reviews™ in Eukaryotic Gene Expression, 20(2), 87-103.*

*[15] Lamprecht, P., Voswinkel, J., Lilienthal, T., Nolle, B., Heller, M., Gross, W. L., & Gause, A. (2002). Effectiveness of TNF-α blockade with infliximab in refractory Wegener's granulomatosis. Rheumatology, 41(11), 1303-1307.*

*[16] Shlipak, M. G., Fried, L. F., Crump, C., Bleyer, A. J., Manolio, T. A., Tracy, R. P., ... & Psaty, B. M. (2003). Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation, 107(1), 87-92.*

*[17] Shlipak, M. G., Katz, R., Cushman, M., Sarnak, M. J., Stehman-Breen, C., Psaty, B. M., ... & Fried, L. (2005). Cystatin-C and inflammatory markers in the ambulatory elderly. The American journal of medicine, 118(12), 1416-e25.*

*[18] Fried, L., Solomon, C., Shlipak, M., Seliger, S., Stehman-Breen, C., Bleyer, A. J., ... & Newman, A. (2004). Inflammatory and prothrombotic markers and the progression of renal disease in elderly individuals. Journal of the American Society of Nephrology, 15(12), 3184-3191.*

*[19] Stuveling, E. M., Hillege, H. L., Bakker, S. J., Gans, R. O., De Jong, P. E., & De Zeeuw, D. (2003). C-reactive protein is associated with renal function abnormalities in a non-diabetic population. Kidney international, 63(2), 654-661.*

*[20] Yeo, E. S., Hwang, J. Y., Park, J. E., Choi, Y. J., Huh, K. B., & Kim, W. Y. (2010). Tumor necrosis factor (TNF-α) and C-reactive protein (CRP) are positively associated with the risk of chronic kidney disease in patients with type 2 diabetes. Yonsei medical journal, 51(4), 519-525.*

*[21] Babaei, M., Dashti, N., Lamei, N., Abdi, K., Nazari, F., Abbasian, S., & Gerayeshnejad, S. (2014). Evaluation of plasma concentrations of homocysteine, IL-6, TNF-alpha, hs-CRP, and total antioxidant capacity in patients with*

*end-stage renal failure. Acta Medica Iranica, 893-898.*

*[22] Das, A., Barman, B., Bhattacharya, P., Lynrah, K. G., Tiewsoh, I., & Phukan, P. (2022). Inflammation and its determinants in patients with chronic kidney disease: A study from North Eastern region of India. Cureus, 14(1).*

*[23] Panichi, V., Migliori, M., De Pietro, S., Taccola, D., Bianchi, A. M., Giovannini, L., ... & Palla, R. (2002). C-reactive protein and interleukin-6 levels are related to renal function in predialytic chronic renal failure. Nephron, 91(4), 594-600.*

*[24] Shankar, A., Sun, L., Klein, B. E., Lee, K. E., Muntner, P., Javier, F. N., ... & Klein, R. (2011). Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study. Kidney international, 80(11), 1231-1238.*

*[25] Sarnak, M. J., Poindexter, A., Wang, S. R., Beck, G. J., Kusek, J. W., Marcovina, S. M., ... & Levey, A. S. (2002). Serum C-reactive protein and leptin as predictors of kidney disease progression in the Modification of Diet in Renal Disease Study. Kidney international, 62(6), 2208-2215.*

*[26] Lee, B. T., Ahmed, F. A., Hamm, L. L., Teran, F. J., Chen, C. S., Liu, Y., ... & Chen, J. (2015). Association of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease. BMC nephrology, 16, 1-6.*

*[27] Pruijm[a], M., Ponte, B., Vollenweider, P., Mooser, V., Paccaud, F., Waeber, G., ... & Bochud, M. (2012). Not all inflammatory markers are linked to kidney function: results from a population-based study. American journal of nephrology, 35(3), 288-294.*

*[28] Amdur, R. L., Feldman, H. I., Gupta, J., Yang, W., Kanetsky, P., Shlipak, M., ... & CRIC Study Investigators. (2016). Inflammation and progression of CKD: the CRIC study. Clinical journal of the American Society of Nephrology, 11(9), 1546-1556.*

*[29] Gupta, J., Mitra, N., Kanetsky, P. A., Devaney, J., Wing, M. R., Reilly, M., ... & CRIC Study Investigators. (2012). Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clinical journal of the American Society of Nephrology, 7(12), 1938-1946.*

*[30] Sheldon, J., Riches, P., Gooding, R., Soni, N., & Hobbs, J. R. (1993). C-reactive protein and its cytokine mediators in intensive-care patients. Clinical chemistry, 39(1), 147-150.*

*[31] Eklund, C. M. (2009). Proinflammatory cytokines in CRP baseline regulation. Advances in clinical chemistry, 48, 111-136.*

*[32] Schmidt, M. I., Duncan, B. B., Sharrett, A. R., Lindberg, G., Savage, P. J., Offenbacher, S., ... & Heiss, G. (1999). Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. The Lancet, 353(9165), 1649-1652.*

*[33] Shankar, A., Klein, B. E., & Klein, R. (2004). Relationship between white blood cell count and incident hypertension. American journal of hypertension, 17(3), 233-239.*

*[34] Keller, C., Katz, R., Cushman, M., Fried, L. F., & Shlipak, M. (2008). Association of kidney function with inflammatory and procoagulant markers in a diverse cohort: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis (MESA). BMC nephrology, 9, 1-8.*

*[35] Akchurin, O. M., & Kaskel, F. (2015). Update on inflammation in chronic kidney disease. Blood purification, 39(1-3), 84-92.*