ORIGINAL ARTICLE

Measurement of Inflammation-Related Biomarkers in Different Chronic Kidney Diseases in Humans: Role of Aging and Gender?

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ABSTRACT

INTRODUCTION: Chronic kidney disease (CKD) is a worldwide health problem that can be associated with a considerable degree of inflammation. The inflammation can result from different mechanisms in different kidney diseases including the imbalance of pro-inflammatory/anti-inflammatory biomarkers levels. This study aimed to determine the level of physiological bioactive inflammation-related biomarkers (gelatinase-associated lipocalin (NGAL), monocyte chemoattractant protein 1 (MCP1), and clusterin (CLU)) in different chronic kidney diseases (CKDs) and to investigate whether gender or aging is critical in these measurements. **MATERIALS AND METHODS:** 84 individuals (19 healthy, 29 chronic glomerulonephritis, 26 diabetic nephropathies, 6 benign nephroscleroses, 4 lupus nephritis) were enrolled in this study. The inflammation progression degree in CKD was estimated by measuring the plasma level of NGAL, MCP1, and CLU using ELISA. Serum total protein, urea, and creatinine were measured using an automatic analyzer. **RESULTS:** The plasma level of urea and creatinine was increased while total protein level was decreased in all the patients compared to control participants. The level of NGAL, MCP1, and CLU was significantly increased in all the kidney diseases compared to controls. In addition, there were no differences in the level of inflammation-related markers between women and men. Moreover, the levels of inflammatory markers were increased in the kidney diseases regardless of the age difference. **CONCLUSIONS:** This study showed that the physiological bioactive substances NGAL, MCP1, and CLU can be increased in renal pathologies and considered as good indicators of the progression of inflammation in CKDs, with no role of gender and age in their increment plasma levels.

Keywords
Chronic Kidney Diseases, Inflammation, Neutrophil Gelatinase-Associated Lipocalin, Monocyte Chemoattractant Protein 1, Clusterin.

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INTRODUCTION

Inflammation plays a pivotal role in chronic kidney disease (CKD) pathogenesis. This critical role was first recognised in the late twentieth century and still attracts researchers to study its role in the progression of kidney diseases and increasing the mortality rate.[1]

The systemic or intrarenal inflammation in the microvasculature is associated with the production of a group of tubular toxins which leads to nephron dropout, tubular injury, and provoking of CKD. In addition, inflammation can induce alterations in kidney function (e.g., compromised receptor-mediated vasoreactivity, and the coagulation system activation) which can lead to irreversible tubular injury and nephron failure.[2] Moreover, another study revealed the inverse relationship between the inflammation biomarkers and the measurement of kidney function in CKD patients. The plasma levels of IL-6, CRP, fibrinogen, and soluble TNF -α, and consequently the inflammation, were attributed to the progression of chronic kidney disease.[3]

In addition to the well-known biomarkers of kidney function (e.g., urea, creatinine), researchers are trying to investigate the importance of utilising new bioactive signaling markers in the identification of the severity of CKD including those inflammation-related biomarkers. Neutrophil gelatinase -associated lipocalin (NGAL) is encountered in different parts of the human body like the loop of Henle, the collecting ducts, and white blood cells.4 NGAL expression increases cell proliferation, cytogenesis, and damage to the kidney.[5] Coppolino et al. (2020) have suggested NGAL as a promising marker for a decline in renal function in patients with glomerular diseases.[6]

Monocyte chemoattractant protein 1 (MCP-1) is the first member of the C-C chemokines family in humans. Its molecular weight is 13 kDa and it serves as a strong chemotactic factor for monocytes.[7] Tubular epithelial cells of the kidneys are the main producers of the MCP-1, and it is engaged in the interstitial inflammation development and fibrosis, a process invariably present in kidney diseases.[8] Clusterin (CLU) is a 75 kD heterodimeric glycoprotein and has multiple biological functions such as adhesion of cells, DNA repair, tissue remodelling, apoptosis, and membrane recycling. This bioactive substance is highly expressed in the renal tubular epithelial after renal injury and can be deposited in the kidney as an immune deposit.[2] Its increased levels in the urine have been reported after renal ischaemia or exposure to various nephrotoxins, which might indicate damage in the proximal and distal tubules.[9]

Aging has been considered as one of the predisposing risk factors to CKD. Most studies revealed that the majority of patients with microalbuminuria or gradual reduced glomerular filtration rate are among the elderly population.[10–12] In aging, it is hypothesised that there is a direct effect of inflammation on the development of both chronic kidney diseases and cardiovascular diseases. It was found that the deterioration in kidney function was linked to the high level of inflammatory biomarkers in elderly individuals.13 On the other hand, recent studies suggested that CKD condition can induce premature aging through varied mechanisms, including inflammation.[14]

Gender differences have been recognised in the field of nephrology. CKD appears to be more prevalent in women than men.[15] In contrast, another study showed a higher incidence of ESRD in males compared to females.[16] Additionally, a recent study showed no sex difference in the haemodialysis practices and treatment targets.[17]

This study is conducted to measure the level of physiological bioactive substances NGAL, MCP1, and CLU as inflammation-related biomarkers in different kidney diseases and, to investigate whether gender or aging is critical in these measurements.

MATERIALS AND METHODS

Blood samples were collected from Salah Al-Din General Hospital in Tikrit, Iraq by specialised doctors. 70 blood samples were collected from patients suffering from various types of chronic kidney diseases who did not reach the stage of dialysis. 20 healthy individuals were used as the control sample. 10 ml of blood was collected from the volunteers for the experiment (patients and control). The serum was separated by using a centrifuge at 3,000 rpm for five minutes, then the serum was isolated in different tubes and kept in the freezer at -20 °C until use.

Ethical approval was obtained to conduct the experiments by the local ethical committee. The participants were informed of the details of the experiment, and written consent was obtained by each participant separately.

Patients were divided into groups based on the type of chronic disease. Sandwich ELISA technique was used to measure the serum concentration of MCP-1, CLU, and NGAL in serum of patients according to the manufacturer's instructions of the kits used (Thermofisher-Germany were used for MCP-1, PicoKine-USA for CLU, and BioPorto Diagnostics for NGAL).

The concentration of urea, creatinine, and total protein in the serum of the participants were estimated using an automatic analyzer (SK3002B) according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Data are expressed as mean ± SD where n is the number of different participants. Differences between the groups were assessed using a one-way ANOVA in conjunction with Tukey's post hoc test to assess possible differences at different conditions. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed by GraphPad Prism.

RESULTS

Serum urea, creatinine, and total protein in chronic kidney diseases.

Serum urea, creatinine, and total protein are measured to investigate the kidney functions in all the samples of controls and patients. Serum urea and creatinine concentrations were significantly elevated in all the pathological conditions compared to healthy controls while serum total protein was significantly reduced in the patients with different kidney diseases compared to controls (Figure 1).

Plasma level of CLU, MCP1, and NGAL in chronic kidney diseases.

In order to determine the level of inflammation in kidney diseases, CLU, MCP1, and NGAL are measured in healthy individuals and patients with chronic glomerulonephritis, diabetic nephropathy, benign nephrosclerosis, and lupus

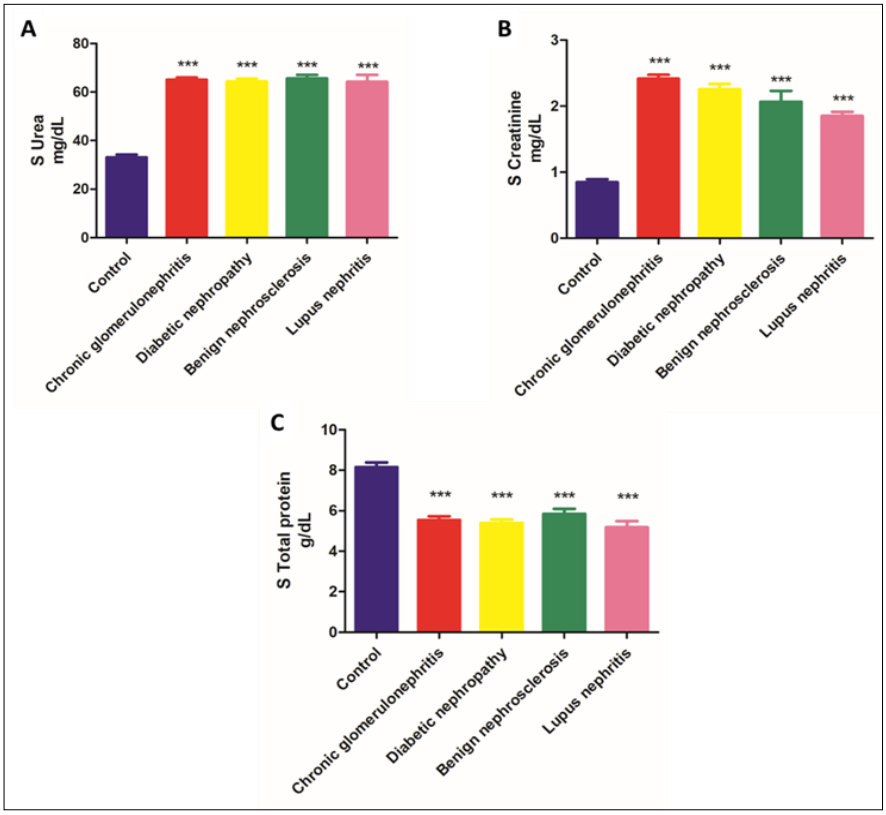

Figure 1: Serum level of urea, creatinine, and total protein in chronic kidney diseases. Data are expressed as mean ± SD for comparison of urea (A), creatinine (B), and total protein (C) levels in chronic glomerulonephritis (n=29), diabetic nephropathy (n=26), benign nephrosclerosis (n=6), and lupus nephritis (n=4) versus control patients. *** indicates p-value < 0.0001.

nephritis. The plasma levels of all biomarkers are significantly increased in all kidney diseases in comparison to control individuals (Figure 2).

Sex differences of levels of CLU, MCP1, and NGAL in chronic kidney diseases.

Plasma levels of the CLU, MCP1, and NGAL are measured in both males and females in patients with chronic glomerulonephritis and diabetic nephropathy. This is to investigate if there is an effect of sex difference on the level of the inflammatory markers in these chronic kidney diseases. There was no effect of sex differences on the plasma levels of the inflammatory markers in chronic kidney diseases. In addition, the baseline levels of CLU, MCP1, and NGAL in healthy participants were nearly the same in both sexes (Figure 3).

Aging effect on the levels of CLU, MCP1, and NGAL in chronic kidney diseases.

To investigate the effect of aging on the level of the inflammatory markers in chronic kidney diseases, patients with chronic glomerulonephritis and diabetic nephropathy are divided into two groups according to their age, either in the 5th or 6th decade of life; and their plasma levels of CLU, MCP1 and NGAL are measured. There was no difference in the inflammatory markers in the different age groups of the patients with both diseases (Figure 4).

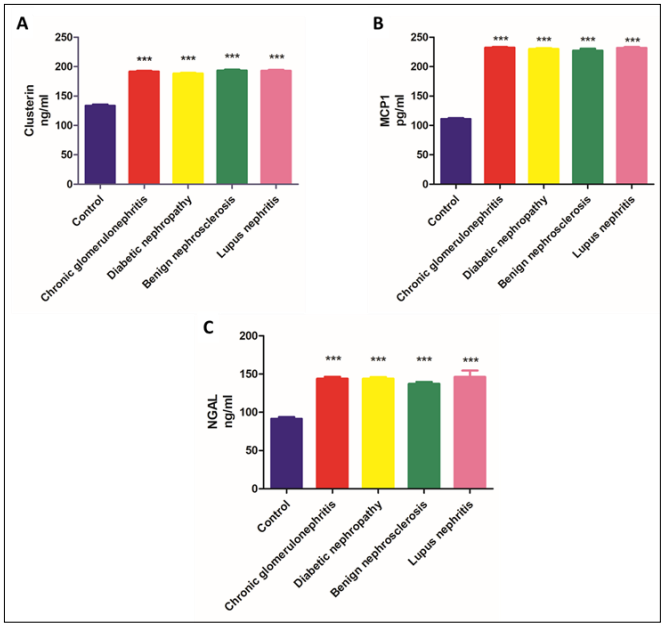

Figure 2: Plasma level of clusterin, MCP1, and NGAL in chronic kidney diseases. Data are expressed as mean ± SD for comparison of clusterin (A), MCP1 (B), and NGAL (C) levels in chronic glomerulonephritis (n=29), diabetic nephropathy (n=26), benign nephrosclerosis (n=6), and lupus nephritis (n=4) versus control patients. *** indicates p-value < 0.0001.

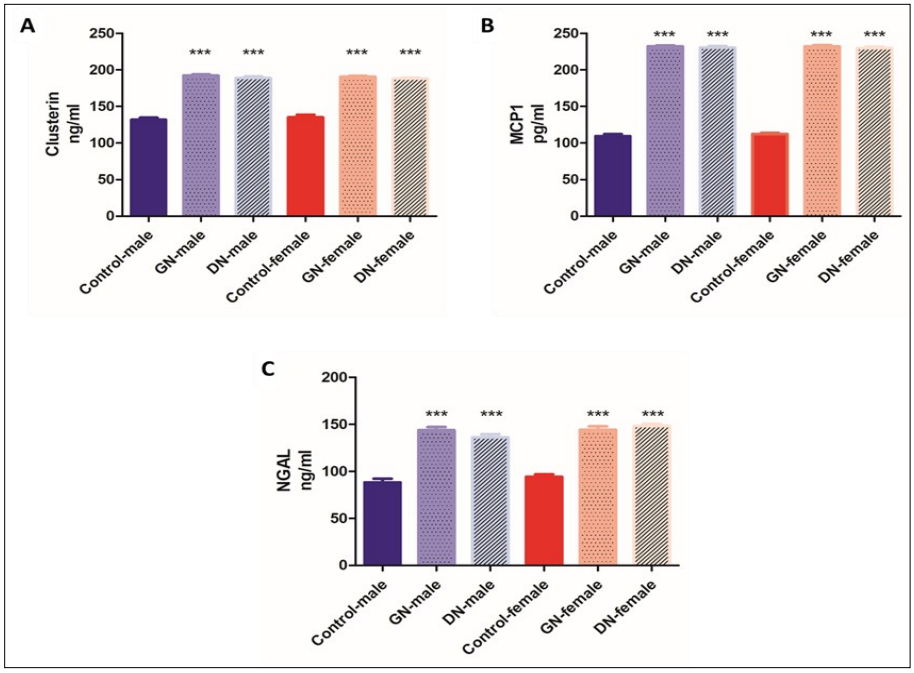

Figure 3: Sex differences in the plasma level of clusterin, MCP1, and NGAL in chronic kidney diseases. Data are expressed as mean ± SD for comparison of clusterin (A), MCP1 (B), and NGAL (C) levels in chronic glomerulonephritis (n=18 males, 11 males), diabetic nephropathy (n=9 males, 17 females) versus control patients. *** indicates p-value < 0.0001.

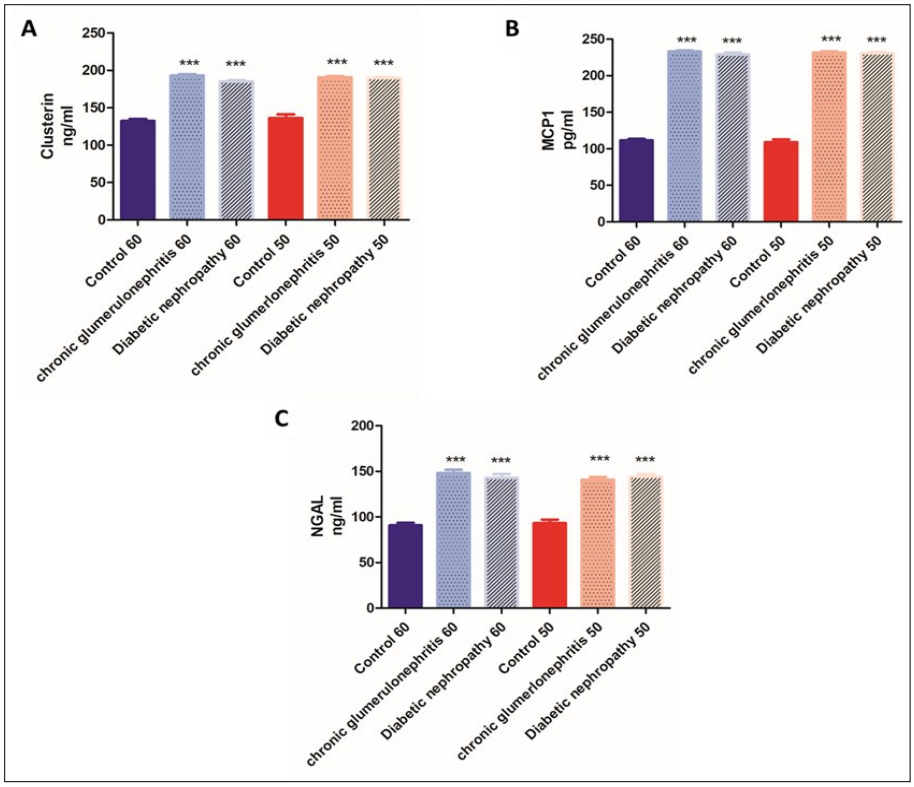

Figure 4: Aging effect on the plasma level of clusterin, MCP1, and NGAL in chronic kidney diseases. Data are expressed as mean ± SD for comparison of clusterin (A), MCP1 (B) and NGAL (C) levels in chronic glomerulonephritis (n=12, 60yr; n=17, 50yr), diabetic nephropathy (n=8, 60yr; n=18, 50yr) versus control patients. *** indicates p-value < 0.0001

DISCUSSION

For many years, a lot of scientific findings were conducted to search for an alarming sign for kidney function deterioration, especially in the early stages of the diseases that affect kidney functions.

Although the current routine renal tests which may include total protein, urea, and creatinine are used frequently to assess kidney function. All these tests have their drawbacks. Their levels were altered only after a loss of more than 50% of kidney function, and their relation to GFR is not a straight line but rather a parabolic curve.[18] Their levels change at the later stage of the disease progression which could results in a false sense of security in the early stages of kidney diseases. Serum creatinine level is changed due to renal and extrarenal factors. Concerning renal factors, the tubular secretion of creatinine is increased in CKD which leads to an unpredictable overestimation of GFR. On the other hand, the most important extrarenal factor is muscle mass, which is varied in different ages, gender, ethnicity.[20]

Urea is an end product of protein metabolism, and its level can vary due to many reasons such as a high or low protein diet, catabolic state, major gastrointestinal haemorrhage, and dehydration. In addition, 40–50% of the filtered urea can be reabsorbed by the tubules which makes it a less accurate indicator of glomerular or tubular function.[21] With regard to serum protein concentration, its measurement remains a central element of experimental medicine as proteins perform and modulate most metabolic processes in living organisms. However, apart from renal factors, serum total protein can be affected by many non-renal factors, for instance, malnutrition, dehydration, infection, and hepatic failure.[22]

The present study aimed at comparing the measurement of nontraditional biomarkers (NGAL, CLU, and MCP-1) with the routine traditional tests to detect kidney function in selected groups of kidney diseases patients in Mosul city. These studied kidney diseases are chronic glomerulonephritis, diabetic nephropathy, benign nephrosclerosis, and lupus nephritis.

The results of measurement of both serum urea and creatinine showed significantly higher levels in all patients in comparison to the control group indicating a deteriorated kidney function. These results reflect the inability to filtrate and excrete these metabolic products in comparison with normal individuals in the control group. These results are inconsistent with previous studies conducted on renal diseases as in this present study.[23]

On the other hand, serum total protein level in all patients' groups was significantly reduced compared to the control group. This result reveals a significant loss of kidney conservative function in retaining body proteins. Both proteinuria and albuminuria are considered important indicators of CKDs. Glomerulonephritis or nephritis which involve inflammation or swelling of the kidney filters are among the most common causes of hypoproteinaemia and proteinuria.[24]

Several inflammatory cytokines are released which can be measured to assess the level of progression of inflammation. In the present study, CLU, NGAL, and MCP-1 are chosen and measured to determine if they can be considered as alternative efficient markers of inflammation in patients with CKD. Secreted CLU is released in response to cell stress and acts as cytoprotective by reducing oxidative stress and binding to misfolded proteins.[25] Our study showed a significant increase in the level of CLU in all CKDs. This result is in line with Musial et al., (2020) study which found increased both serum and urinary clusterin as indices of kidney injury after allogeneic haematopoietic stem cell transplantation and concluded that clusterin is a useful marker in the assessment of subclinical acute kidney injury.[26] In addition, another study showed that renal inflammation and tissue fibrosis are worsening due to CLU deficiency after ischaemia reperfusion injury in the kidney.[27] With regard to NGAL, the NGAL is increased significantly in the CKD patients in comparison with controls. This result is supported by another study (Guo et al., 2018) in that NGAL can reflect the entity of renal impairment and be considered as a risk marker for the progression of chronic kidney diseases.[28] Regarding MCP-1, it was significantly augmented in patients with CKD compared to controls. In line with this study, other researches showed that MCP-1 levels increased in chronic kidney diseases in children[29] and adults[30]. In addition, deletion of MCP-1 has reduced glomerular and interstitial infiltration of macrophages and histological damage in several models of renal diseases.[31] These findings collectively suggest a potential role of MCP-1 and its inhibition in diagnosing and treating patients with kidney inflammatory disease.

This study also investigated whether these inflammation-related biomarkers are altered in different physiological conditions such as different sex and age. To the best of our knowledge, this is the first study that addressed the effect of sex and age differences in NGAL, CLU, and MCP-1 circulating serum levels in different chronic kidney diseases in adults. The present study showed that there is no effect of sex difference on the level of these inflammation-related

biomarkers. This result could indicate the non-significant interference of sex hormones with these biomarkers. This result is consistent with a recent study that determined the level of NGAL in patients with asymptomatic carotid artery stenosis and found that there is no sex difference in NGAL levels in women versus men.[32] In contrast, a study conducted on children with lupus nephritis found that there is no sex difference in MCP-1 while females have a higher level of NGAL than males.[33] However, the limitation of the present study is that the females were post-menopausal women which could explain the insignificant variance. This is because of the loss of the possible protective influence of estrogen, which is seen in pre-menopausal women. Further study is required to check the impact of sex variance on these biomarkers in young patients with CKD.

The prevalence of elderly people is increased universally due to change from the pattern of high birth rate and high mortality to a reduction of birth rate and delayed mortality. The National Kidney Foundation (NKF) urges people over the age of 60 to check for kidney diseases as reports showed that Kidney disease can occur at any time, but those over the age of 60 are more liable to kidney diseases.[34] There is an increasing prevalence of comorbidities associated with a high burden of CKD in the elderly population. A review clarified that although a significant proportion of the elderly population has a decline in renal function, many longitudinal studies showed that normal adults have an intact renal function with age.[35] In the present study, the aging effect, as another physiological aspect, was assessed on the level of the inflammatory biomarkers in kidney diseases. Depending on their age, subjects are chosen and arranged into two groups: 50's and 60's decades. The present study showed that age variance, at least between these two decades the '50s and '60s, has no significant impact on the levels of the inflammation-related biomarker, which could indicate that the biomarkers are related to the inflammatory process and the correlated decline in kidney function rather than the age. This result is in line with a study by Khawaja et al., (2019) who found that age does not affect NGAL in patients with and without acute kidney injury.[36] A study recommended that the kidney function of people should be classified according to their age.[37] However, the researchers clarified that pathologies affecting the kidney such as nephrotic syndrome fall outside the scope of the kidney aging term as kidney disease remain an important health concern.

CONCLUSIONS

The present study illustrated that the physiological bioactive substances NGAL, MCP-1, and CLU are indicative markers for the inflammatory process that is continuously and progressively affecting kidney function. The loss of kidney function is related to the elevation in the tested inflammation -related biomarkers. In addition, this is the first study to demonstrate that the increase in NGAL, MCP-1, and CLU level in chronic kidney diseases is sex and age-independent. Understanding the role of these markers in the progression of inflammation, and the implications of their inhibition in chronic kidney diseases will facilitate developing better diagnostic and therapeutic strategies in patients with inflammatory kidney disease.

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CONFLICT OF INTEREST

There is no conflict of interest.

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