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Evaluation of Serum Levels of Interleukin-18, and C-reactive Protein in Iraqi Patient With Diabetic Nephropathy

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Abstract

Background: Chronic renal failure is a public health issue worldwide. It is arising due to various factors, including genetic predisposition, increased life expectancy and lifestyle-related elements. Diabetes is the primary cause of end-stage renal disease, and diabetic nephropathy occurs in 30–40% of patients.

Aims: The objective of this research is to provide insight into the evaluation of the serum levels of IL18 and CRP in Iraqi patient with diabetic nephropathy.

Procedure: This study conducted as a case-control study of Iraqi patients with diabetic nephropathy, the study comprised 40 patients with an equal number of healthy age-and sex-matched individuals in the control group. The blood samples collected and the levels of interleukin-18 and C-reactive protein were measured in both groups.

Results: The study found a significant increase ($p \leq 0.05$) in the serum levels of IL-18 and CRP in diabetic nephropathy patients when compared to the control group. The mean IL-18 level in DN patients was 53.787 ± 1.639 pg/ml, significantly higher than the control group's 26.818 ± 1.751 pg/ml. Whereas, the mean CRP level in DN patients was 8.908 ± 0.658 mg/ml, compared to 3.675 ± 0.360 mg/ml in the control group. There were significant positive correlations between IL-18 and CRP levels ($r = 0.348$, $p = 0.006$), and between IL-18 and HbA1c levels ($r = 0.429$, $p = 0.001$).

Conclusion: Elevated IL-18 is associated with increased CRP and HbA1c levels, suggesting a link between inflammation and glycemic control in diabetic nephropathy.

Keywords: Diabetic nephropathy, Cytokine, Creatinine, Urea, CRP

Introduction

Chronic kidney disease (CKD) is a gradually disease of over 10% of the global population worldwide, which equals more than 800 million individuals (Kovesdy, 2022). Advanced economies have notable percentage of lifestyle-related illness, including obesity, hypertension and diabetes mellitus (T2D), CVD, and smoking, all of which have proven to be robust risk factors for CKD alongside genetic polymorphism (Hamrahan & Falkner, 2017; Aziz et al., 2019; Friedman et al., 2021; Jankowski et al., 2021). Diabetic nephropathy (DN) is a significant healthcare challenge, affecting up to 50% of individuals with diabetes. It is a leading reason of end-stage kidney disease, necessitating dialysis or

kidney transplantation (Selby & Taal, 2020). HbA1c is a crucial marker for long-term glycemic control, capable of reflecting the accumulating glucose level of the past three months (Sherwani et al., 2016). Elevated instability in HbA1c levels is linked to an increased threat of diabetic problems and cardiovascular complications (Lee et al., 2021). The rising global incidence of T2D and CKD has spurred research endeavors to address the growing epidemic of diabetic kidney disease (DKD) (Anders et al., 2018). Cytokines support the immune system's response to tissue damage or invasion of the body by infectious or non-infectious bodies (Majeed, 2022; Yuan et al., 2023) investigated that the levels of NLRP3, IL-1 β , and IL-18 were significantly elevated in all DN observation groups compared to the

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control group. C-reactive protein is a nonspecific indicator of inflammation that mediates and regulates innate immune signaling (Yao et al., 2019). CRP is synthesized by the liver (Zackiewicz et al., 2024). CRP can be used as a marker to determine the response to treatment and indicator of systemic inflammation (Abdel-Messeih et al., 2020).

The aim of current study is to assess alterations in the serum levels of IL-18 and CRP in Iraqi dialysis patients with diabetic nephropathy.

Material and method

Ethical approval

The study protocol received ethical approval from the University of Kufa, and each participant provided written consent following a concise overview of the project.

Samples of study

The present research included 60 blood samples (male and female) which were collected from patients diagnosed as CKD with T2DM who attending AL-Hussein teaching dialysis center and AL-Nasiriyah teaching hospital in Nasiriyah city south of Iraq period from (October 2023 to end of December 2023). Diabetic nephropathy was diagnosed in patients with prolonged history of diabetes mellitus, notable proteinuria as well as presence of other complications of diabetes mellitus.

Measuring of serum Interleukin-18 and other parameters

Two types of tubes were used for blood collection: clotting activator tube and EDTA tube. All samples of collected blood were kept for 30 min in a cold room before centrifugation for 10 min. The serum samples were frozen and stored at -20°C until testing. All samples were processed similarly according to standardized protocol which were human IL18 ELISA Kit product of Sunlong Biotech Com (china), CRP ELISA kit was product of CALBIOTECH Com. (CA, USA), and biochemical parameters which were analyzed using an autoanalyzer. For the EDTA tubes, the blood samples were gently mixed by inversion to ensure even distribution of the anticoagulant and used directly for complete blood count analysis using standardized automated hematology analyzer.

Statistical analysis

SPSS version 26 and Software Excel 2019 were used in this study. The mean and standard deviation

for each parameter were assigned. Statistical significance is allocated if P value ≤ 0.05 between groups using student t-test. And the correlations were analyzed by Pearson's correlation.

Results

Following the application of the inclusion and exclusion criteria and considering the medical history summary of each of the patient, seventeen patients with DN were excluded. Therefore, the total number of patients who met the criteria of the inclusion and were enrolled in this study accounts for sixty. Table 1 represented a comparative analysis of demographic and clinical parameters in DN patients and healthy individuals, showing that the DN group leaned towards middle age, and the mean of BMI within the high range. The patient group was composed of more males than females. A significant proportion of this group had hypertension. The majority of the individuals resided in urban areas, while a smaller portion lived in rural areas. The mean value urea and creatinine levels were calculated and it was above the normal range. Statistical analysis in this research showed that IL18 of patients with DN (53.787 ± 1.639 pg/ml) were significantly higher compared to the control group (26.818 ± 1.751 pg/ml) ($p = 0.001$), while C-reactive protein were also higher significantly DN group (8.908 ± 0.658 mg/ml) compared to the control group (3.675 ± 0.360 mg/ml) ($p: 0.001$) (see Tables 2, 3). Fig. 1 displays a significant

Table 1. Clinical and demographic details of participants.

Variables	Control group	DKD group
Total Number	40 (%)	60 (%)
Age (years)	36.125 ± 5.37	54.766 ± 15.021
Sex		
Male	21	39
Female	19	21
BMI (Kg/m^{-2})	23.990 ± 3.2	27.389 ± 5.45
Smoking		
Yes	18 (45%)	33 (55%)
No	22 (55%)	27 (45%)
Hypertension		
Present	0	36 (60%)
Absent	0	24 (40%)
Diabetes Mellitus		
Present	0	100 (100%)
Absent	0	0 (0%)
Living		
Rural	15 (37.5%)	21 (35%)
Urban	25 (62.5%)	39 (65%)
Family history	10%	30%
Urea (mg/dL^{-1})	24.312 ± 1.388	97.25 ± 5.033
Creatinine ($\mu\text{mol}/\text{L}^{-1}$)	0.865 ± 0.043	5.681 ± 0.317
RBC ($\times 10^{12} \setminus \text{L}$)	5.46 ± 0.78	4.13 ± 1.19
WBC ($\times 10^9 \setminus \text{L}$)	7.47 ± 1.77	12.39 ± 2.41
HB ($\text{g} \setminus \text{dl}$)	12.46 ± 0.74	9.88 ± 1.98

Table 2. Diabetic criteria in patient with diabetic nephropathy.

Diabetic Criteria	(Mean \pm SD)
HbA1c	0.413 \pm 11.180
Albumin	2.627 \pm 0.084
RBS	217.272 \pm 13.579
Creatinine	5.350 \pm 1.820
Urea	42.367 \pm 4.906
GFR	4.906 \pm 42.367

Table 3. IL 18 and CRP levels in participants.

Marker	Controls (Mean \pm SD)	CKD (Mean \pm SD)	P Value
IL 18 (pg\ml)	26.818 \pm 1.751	1.639 \pm 53.787	0.001
CRP (mg\ml)	3.675 \pm 0.360	8.908 \pm 0.658	0.001

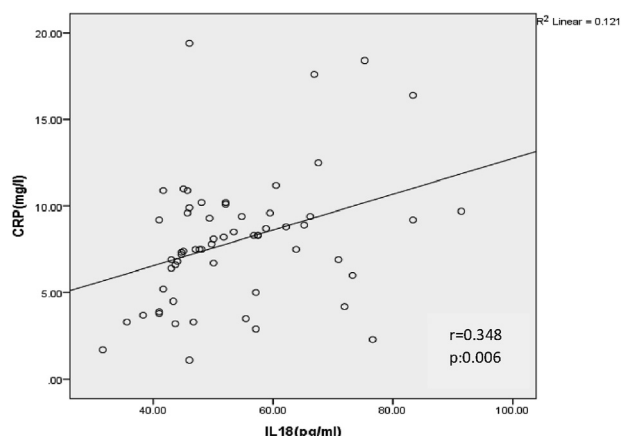


Fig. 1. Correlation analysis of IL-18 and CRP.

positive weak relationship between IL-18 and CRP, with $r = 0.348$, $p = 0.006$, also Fig. 2 displays a significant positive weak relationship between IL-18 and HbA1c, with correlation coefficient $r = 0.429$, $p < 0.001$. While Fig. 3 displays non-significant

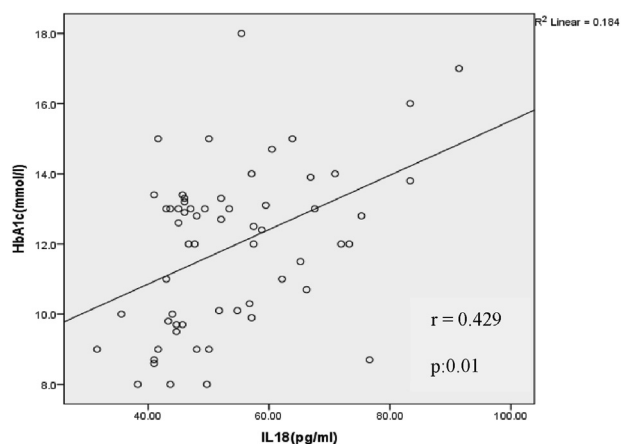


Fig. 2. Correlation analysis of IL-18 and HbA1c.

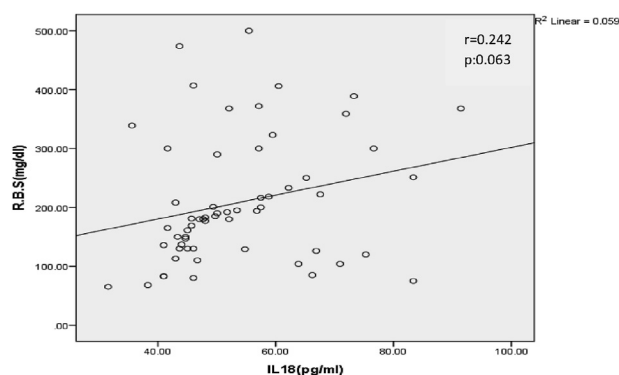


Fig. 3. Correlation analysis of IL-18 and RBS.

positive weak relationships between IL-18 and HbA1c with correlation coefficient $r = 0.242$, $p: 0.063$.

Discussion

According to clinical and demographic data for both groups the current study suggests a potential link between diabetic nephropathy and various variables including age, BMI, sex, hypertension, and family history. The increased mean age, higher occurrence of hypertension, as well as elevated urea and creatinine levels within the patient group in contrast to the healthy controls may suggest a plausible connection with the illness. Sex hormones may play a crucial role in the increased vulnerability of males to progressive kidney damage. Sex hormones have the ability to influence a variety of cellular mechanisms by regulating the production of different cytokines, growth factors, and vasoactive substances (Neugarten & Golestaneh, 2013). High level of patients is older than the members of the control group and that may be related to age-related reductions in kidney function as well as kidneys may sustain damages from injuries due to many illnesses, such as diabetes mellitus or hypertension. Glomerular and tubulointerstitial patterns are part of the spectrum of both glomerular and diffuse chronic transformations. Many people of older age are developing end-stage renal illness and need renal replacement therapy (Hansberry et al., 2005). Due to the fact that reduced Hb levels are strongly associated with the diminished production of erythropoietin that happens to be critical for maintaining erythropoiesis and that may develop anemia of CKD (Jing et al., 2012).

The current study found a significant increase in IL-18 levels in the DN group compared with controls. IL-18 is known to be a potent pro-inflammatory cytokine and plays a key role in the immune response and host defense against infection (Ihim et al., 2022). Notably, pro-inflammatory cytokines such as IL-18

have been linked to podocyte injury and proximal tubule dysfunction in early DKD, which may contribute to the formation of the disease before the development of albuminuria (Milas et al., 2020). The association between IL-18 level and renal dysfunction and damage incidence in DN were examined, and a positive result was found (Liu et al., 2022). Moreover, the authors Sueud et al. (2019) noted that patients with elevated levels of IL-18 in their serum had the probability to develop albuminuria, which considered as an early sign of the disease, at a higher rate. The current study finding was confirmed by the researchers in the work of Yuan et al. (2023), who detected higher levels of IL-18 in DN subjects relative to non-diabetic healthy subjects. The presence of CRP is usually referred to as “conventional inflammatory marker” which is high in situations where body is in inflammatory stress. The creation of C-reactive protein in different tissue components is most often observed at significant and chronic prevalent inflammations and it can be associated with the serum levels used to track the treatment course (Paust et al., 2009). A CRP level that is high in those with DN could be a sign of the inflammatory reaction that is chronic, which could have an effect on the process by which a disease develops. The last few years have seen the interest in IL-18 and CRP rise cautiously in respect to complications grounded in diabetes. Zhao et al. (2018) search for IL-18 as a potential indicator of the risk of glucose tolerance for women destined to develop gestational diabetes mellitus (GDM) during pregnancy. Steadily, it has been found that both IL-18 and CRP are types of inflammation markers that can detect inflammatory conditions in patients with Covid-19 (Karampoor et al., 2021). The current study, might actually insight a significant correlation between IL-18 and CRP levels in the blood flow, which could imply that both are the useful inflammation indicators. Fig. 2 demonstrates slight and modest corroborations between the serum IL-18 and the HbA1c with significance type. The results obtained now are in harmony with the statements about IL-18 endowed with other serum markers, that have been made by Maftai et al. (2021), and they assess that IL-18 is an impacting component that is involved in elevate HbA1c in DN. On the other side, the serum level of IL-18 and its Random blood sugar that has revealed a weak positive correlation (Fig. 3), although there is no statistically significant value, additionally depicts possibility that this kind of cytokine may also contribute to glucose metabolism and the regulation of blood sugar. The current study achieved a wider understanding of the connection between these two factors.

Conclusion

The study revealed increasing of IL-18, and CRP titrating patients compared to controls, implying their role in prolonged inflammation. A positive link was found between IL-18 and HbA1c levels, indicating their cooperation in immune response and diabetes disease. Positive associations were also noted between IL-18 and CRP, and IL-18 and RBS test, hinting at a connection to systemic inflammation. These findings may guide future studies on the roles of these markers in chronic inflammatory conditions and as potential treatment targets. Further research is necessary to confirm these initial findings.

Ethics information

The study protocol received ethical approval from the University of Kufa, and each participant provided written consent following a concise overview of the project.

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The current study is funded privately by the authors themselves.

Authors' contributions

Both authors equally contributed to the design and implementation of the study.

Conflicts of interest

There are no conflicts to report.

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