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Association of C-Reactive Protein with Risk of Complications of diabetic nephropathy

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Abstract

Background: Diabetic nephropathy become the main cause of chronic renal diseases in the world, that have been demonstrated high mortality rate and disability in patients with diabetes mellitus. Many recent studies demonstrate the associations of CRP and development of renal impairment in type two diabetic patients demonstrating the relation between systemic inflammation and glycemic control and consequently with severity of diabetic complications. **Aim of study:** evaluate the role of CRP and to analyze any correlation of CRP with nephropathy complications of diabetes. **Material:** the study was a cross sectional study of 62 type tow diabetic patients attended to Kirkuk general Hospital and 28 healthy subjects. Patient group was separated into two groups first include those have diabetic nephropathy and the second group include patient s without nephropathy. CRP level in serum sample was evaluated and albumin– creatinine ratio in random urine samples were calculated. **Results:** the present study revealed that the CRP serum concentration was significantly high in patient with nephropathy than those without nephropathy ($P< 0.01$). Also, demonstrated that the urine albumin–creatinine ratio was significantly increased in patients with nephropathy as compared with those without nephropathy as well as there is a strong positive correlation between CRP and urine albumin– creatinine ratio. **Conclusion**: A positive correlation of CRP with albumin– creatinine ration indicating the role of CRP in development of DN and possibility of used CRP measurement to predicate the DN development in type two diabetic patients. also supports the treatment targeting inflammatory mediators in improving diabetic complication.

Keywords: C-reactive proteins , albumin creatinine ratio, diabetic nephropathy

1. Introduction

Diabetes mellitus (DM), an endocrinemetabolic disorder of multiple etiologies manifested by consistent elevated levels of glucose in the blood. It occurs either due to the destruction of beta-cells of pancreas or due to the development of resistance towards insulin and leads to various metabolic dysfunctions in the body which critically affect numerous regions of the body, secondarily [1,2]. Diabetic nephropathy (DN) is become the main cause of chronic renal diseases in the world, that have been demonstrated high mortality rate and disability in patients with diabetes mellitus. Recently developing of diabetic nephropathy in patients with diabetes mellitus has been increasing dramatically [3], the reason is not cleared yet but many complex factors involved in it. Since not all diabetic patients develop all complications, general screening of occurrence of different complications has become an important part of diabetes care today. Determination of early complication stage permits for more focused treatment to prevention and delay the worsts complications [4]. C- reactive protein (CRP) is one of the most proinflammatory mediator produced by the monocytes of the tissue factor that have an important role in inflammatory process. Even though the role of CRP in development of diabetic complications remain unclear, many recent studies confirm the correlation between the level of CRP and HbA_{1c} suggesting an association between systemic inflammation and glycemic control and consequently with severity of diabetes [5-7]. Another study suggests that metabolic and inflammatory factors associated with diabetes, such metabolic factors include hyperglycemia, modified lipoproteins and high level of free fatty acid may stimulate CRP production by monocyte, macrophage and endothelial cells. CRP stimulates immune system by binding to phosphocholine on the damaged cell surface inducing classical complement cascade that regulate the activity of macrophage and monocytes, suggestion the role of CRP in the opsonization [8]. Providing

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new evidence suggested that type 2 diabetes mellitus (T2DM) is a mild inflammatory disease.

Chronic mild inflammation with high levels of inflammatory proteins production has been demonstrated in the development of T2DM. CRP is considered by many researchers to be a strong inflammatory marker of T2DM, that produced by liver cells, and totally regulated by interleukin 6 (IL-6) and TNF-α, which are produced by adipose tissues. Also, high levels of CRP in chronic inflammation have been associated with hypertension, heavy drinking, obesity smoking, and low physical activity. [9,10].

In diabetic patients a prevalence of diabetic nephropathy and end-stage kidney disease has been increased recently, the reason behind these abnormalities have not recognized yet but may be due to a complex influence of environmental, social with effect of genetic factors. The most common mechanism that has been adapted for the development of DN is the relation between the phagocytes cell and cytokines and chemokines, increase level of the cytokines has been confirmed in diabetic patients and also in experimental animal model of DN [11]. Elevated CRP serum levels in patients with T2DM, mostly associated with an increase in microalbuminuria and renal impairment, suggesting a positive relation between CRP serum level and the development of DN. the inflammatory agents, CRP can stimulate IL-6 secretion by inducing a NF-κB-dependent mechanism [12].

During an inflammatory process state, CRP blood levels increase sharply within the first 6 hours reaching a peak after 48 hours with the levels of up to 350–400 mg/L. also CRP may influence atherosclerosis directly by inhibiting prostacyclin synthesis in endothelial cells and stimulating adhesion molecule secretion, resulting in endothelial dysfunction [13,14]. Another study found that CRP activates a CD32b-Smad3-mTOR pathway by blocking mTOR signalling and collagen secretion inducing renal fibrosis in T2DN [15]. CRP is inflammatory protein secreted in response to inflammatory stimulus, activates the complement promoting opsonization after binding with pathogen, then followed by production of specific immunoglobulin [16]. Diabetic nephropathy (DN) has been defined by the presence of albumin in urine, increased levels of urine albumin is associated with an increased risk of both nephropathy and cardiovascular disease in patients with type 2 diabetes mellitus. Several studies suggested the correlation between urinary albumin excretion and hyperglycemia as well as insulin resistance. Albumin- creatinine ratios more reliable to indicate the presence of nephropathy because it excludes other renal disease [17]. This study aims at evaluation of CRP role and analyze any correlation of CRP with nephropathy complications of diabetes.

2. Methods:

Objectives of the study:

1- Measure C-reactive protein serum level for patients with type tow diabetes mellitus and the control group.
2- Measure urine albumin-creatinine ratio.

This study is cross sectional study include 62 patients attended with type tow Diabetes mellitus in Kirkuk general Hospital between February 2021 to April 2021. Subjects who were critically suffer from other disease were excluded from the study. The study was approved according to ethical approval that was adapted by College of Medicine, Tikrit university and informed consent form was obtained from all participants. A 5 ml of blood sample was drawn then serum sample separated to be used in in laboratory investigations. Urine sample was collected to determine the albumin-creatinine ratio.

Measurement of CRP level in serum sample and albumin– creatinine ratio was calculated. CRP Test Kit (Immunofluorescence Assay) was intended for quantitative determination of C-reactive protein (CRP) in serum (Human C-Reactive Protein ELISA Kit. Merck. company). First morning urine samples of patients were collected to be used for measuring the urine albumin and creatinine levels.

Statistical analysis

The data was performed using SPSS software (version 20) by mean and standard deviation for quantitative variables and frequency. The values were grouped into two categories. The association between blood CRP levels and urine albumin creatinine ratio was done by cross comparison of percentages using Chi-square test.

3. Results

A sixty-two individuals of type two diabetic patients and twenty-eight healthy subjects were included in this study, The present study display that the CRP blood level was significantly high in patients' groups as compared with control group. As shown in the Table (1).

Table 1: levels of CRP in diabetic patients and healthy control group.

Groups	CRP levels mg/L			P value
	No.	Mean	SD.	
Patients	62	8.25	2.24	< 0.01
Control	28	4.46	0.78	

Regarding the blood level of CRP this study revealed that the CRP serum concentration was significantly high in patient with nephropathy comparing with those without nephropathy (P< 0.01) as shown in Table (2). Also, the present study demonstrated that the urine albumin– creatinine ratio was significantly

increased in patients with nephropathy in comparison with those without nephropathy. As shown in Table (3).

Table 2: Levels of CRP in diabetic patients (with and without nephropathy) and the control group

Groups	CRP levels mg/L			P value
	No.	Mean	SD.	
Diabetic Nephropathy	35	10.05	1.72	< 0.01
Diabetic Non-Nephropathy	27	6.44	0.75	
Control	28	4.46	0.78	

Table 3: Levels of UACR in diabetic patients (with and without nephropathy) and the control group

Groups	UACR			P value
	No.	Mean	SD.	
Diabetic Nephropathy	35	178.45	35.87	< 0.01
Diabetic Non-Nephropathy	27	48.65	18.9	
Control	28	28.45	11.68	

The study revealed that, the highest mean of HbA1c was recorded in DN group (10.68±1.87%) compared with diabetic non-nephropathy (8.34±2.9%) and the control group (4.83±1.12 %) (P<0.001), Table 4.

Table 4: Levels of HbA1c in Diabetic Patients (with and without Nephropathy) and the Control Group

Groups	HbA1c			P value
	No.	Mean	SD.	
Diabetic Nephropathy	35	10.68	1.87	< 0.01
Diabetic Non-Nephropathy	27	8.34	2.9	
Control	28	4.83	1.12	

This study demonstrated that there is a significant positive relation between CRP serum levels of type tow diabetic patients and urine albumin– creatinine ratio as shown in Figure (1).

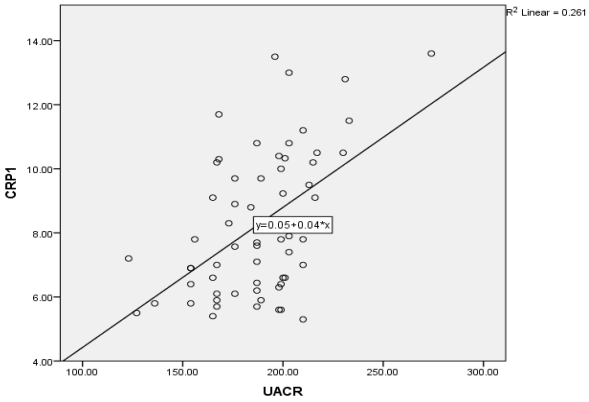

Figure 1: show the relation between serum level of CRP and UACR in diabetic patients.

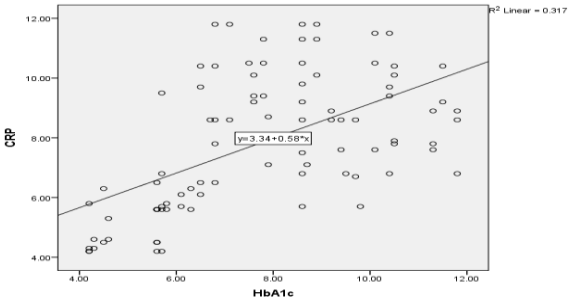

Figure 2: show the relation between serum level of CRP and HbA1c in diabetic patients

4. Discussion

The measurement of CRP level in patients' group with T2D was realized that there is a significant higher CRP level in patients group than that of healthy control group (P=0.01) , in agreement with many recent studies who conducted that CRP levels are higher in patients with type two diabetes mellitus comparing with healthy subjects and this finding may be due to the role of CRP as proinflammatory agent that increase release of proinflammatory like IL-6 and TNF-alpha an addition to its role in activation of NLRP3 inflammasomes which have important role in developing diabetes. Also, they found other evidences indicate that the levels CRP in peripheral blood are strongly associated with glucose levels and HbA1c in addition to confirm the relation between the CRP levels and glucose in pre-diabetic patients. The present study found that the concentration of CRP in serum samples was higher in diabetic patients with nephropathy than those without nephropathy as discussed elsewhere [10,14].they found that CRP play important role in developing of many diabetic complications include nephropathy this finding may belong to the effect of CRP inducing renal inflammation through induction of CD32b-NF-κB-dependent mechanism, resulting increase in T cell and macrophage filtration and stimulation of IL-1bata , TNF-α, and MCP-1 in diabetic renal tubules. Also because CRP stimulated renal fibrosis by activation of CD32b-Smad3-mTOR signaling pathway as well as promoting TGF-β/Smad signaling which aggregate extracellular matrix accumulation causing renal fibrosis in T2DP , other evidence demonstrating that CRP is involved in the development of DN by stimulating epithelial-mesenchymal transition via the induction of apoptosis and promoting an inflammatory microenvironment[18,19]. By all these previous mechanisms CRP participate in the development of DN. The present study demonstrated that the CRP level was significantly associated with HbA1c level in diabetic patients' group. Also, the present study to confirm that elevated CRP levels were significantly associated with a higher incidence of DN, irrespective to the duration of DM suggesting the involvement of CRP in development of DN. An

agreement with many other studies who indicating a strong association between baseline CRP serum level and occurrence of DN [20,21]. In contrast with another study suggested there is no relation between CRP base line level and development of DN[22].Our study demonstrated the a significant positive relation between the CRP serum level and Urine Albumin-creatinine ratio (UACR) in type tow diabetic patients which is consider as a marker for the development of DN this finding confirmed the involvement of CRP in development of DN an agreement with many previous studies who concluded the association of CRP and Urine albumin-creatinine ratio [23,24].This finding was consistent with a known mechanism of CRP as a strong proinflammatory agent that act to increase release of other inflammatory mediator as well as activate migration of macrophage and monocyte to the kidney which secret free radical and release of inflammatory gents these chemicals promote cells injury by amplifying inflammatory response. Also, CRP paly important role in mesenchymal cell proliferation by release of proinflammatory cytokines in addition to increase glomerular albumin permeability which finally lead to albuminuria [9]. In response to inflammatory activation of CRP inducing cascade activation of IL-6 to IL-1, increasing the availability of proinflammatory mediators inhibits the activation of NLRP3 inflammasomes [25]. As well as a recent study revealed that CRP could exacerbate epithelial–mesenchymal transformation after binding with apoptotic cells via the activation of the Wnt/β-catenin and ERK1/2 which act to regulated kinase (ERK) pathways play important roles in the incidence of diabetic renal. disease [26].

5. Conclusions

The increased CRP level in diabetic nephropathy and a positive correlation with albumin – creatinine ratio and HbA1c indicating the role of CRP in development of DN and possibility of using CRP measurement to predect the DN development in type two diabetic patients. Also supports the treatment targeting inflammatory mediators in improving diabetic complication.

6. Recommendations.

Further studies required to established the mechanism by which CRP involve in development of DN and the effect of proinflammatory inhibitors as a possible treatment improving DN.

7. Conflicts of interest

No potential conflicts of interest are disclosed.

8. Acknowledgments

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