ORIGINAL RESEARCH PAPER

General Medicine

A STUDY OF SERUM CRP IN PATIENTS OF TYPE-2 DIABETES MELLITUS AND ITS CORRELATION WITH DIABETIC NEPHROPATHY

KEY WORDS:

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Introduction

Inflammation is thought to be a critical regulator in type-2 diabetes mellitus development. In type-2 diabetes mellitus, several proinflammatory cytokines generate low-grade inflammation, which is a frequent occurrence in most people. Diabetes patients have greater C-reactive Protein (CRP) levels than those who do not have diabetes. [1,2,3]

CRP is a significant inflammatory markers. A category of non-enzymatic protein cytokines triggers the body's inflammatory and immunological responses. [7] Type-2 diabetes has been linked to a higher incidence of low-grade inflammation. CRP and other inflammatory markers have been linked to DM and diabetic nephropathy. [6,7,8,9] Inflammation has a key role in the onset and progression of renal disease in diabetic patients. [10]

By this study, we will try to assess the levels of serum CRP in type-2 diabetes mellitus patients and thereby see whether there is any correlation with the stage of diabetic nephropathy, and if these markers can be used to detect the presence of nephropathy.

AIMS AND OBJECTIVES

1. To study serum levels of CRP in patients of type-2 diabetes mellitus,
2. To study the correlation of serum CRP with diabetic nephropathy.

Review of Literature

In 1998, a concept was postulated that long-term activation of the innate immune system, resulting in chronic inflammation, triggered illness rather than repair, leading to the development of type-2 diabetes. Since then, numerous studies have been conducted to determine the relationship between inflammatory markers such as CRP and atherosclerosis and microvascular consequences of diabetes mellitus, such as nephropathy. [11]

C-REACTIVE PROTEIN

C-reactive Protein (CRP) is a multifunctional molecule that is produced by the human innate immune system. [12,13,14,14] CRP is a non-covalently linked cyclic pentameric protein with five identical subunits. Each subunit is 23 kDa in size and possesses an intra-disulfide bond. [69,70] On the same face of each homopentamer subunit is a phosphocholine (PCh)-binding site.

The CRP was identified at Rockefeller University in 1930. Patients with acute pneumococcal pneumonia had this protein in their blood. Unlike antibody, CRP was detectable from the beginning of the illness and decreased significantly as the disease progressed. These modifications happened before the immunoglobulin IgG response was present. The CRP was named after its reaction with the pneumococcal cell wall's C-polysaccharide. [16]

CRP is a pattern recognition molecule that activates the adaptive immune response as part of the innate immune system. In humans, CRP is the prototypical acute phase reactant. Acute phase reactants are proteins that the liver produces in reaction to a number of clinical situations, such as infection, inflammation, and trauma. Opsonization is one way in which CRP plays an important role in host defense from infection. [16]

Another important activity of CRP is its ability to activate the complement system. [17] Hepatocytes produce the majority of circulating CRP, which is controlled by IL-6. [13] CRP production begins quickly in response to an inciting stimulus, with a peak in serum levels arriving in about 48 hours. C-Reactive Protein is the principal downstream mediator of the acute phase response and is primarily derived via IL-6 dependent hepatic biosynthesis.

CRP binds to the phosphorylcholine of cell-wall polysaccharides from a wide spectrum of bacteria and fungi and interacts with phagocytic cells at an injury site. When CRP attaches to a bacterium, it makes the pathogen vulnerable to phagocytosis and activates the immune complement cascade. CRP and other acute-phase reactants, unlike antibodies, have no structural diversity and are not released or targeted specifically. CRP can be retained in the hepatic endoplasmic reticulum in addition to the regular hepatic storage and release, and this storage declines during inflammation, resulting in more effective CRP secretion. CRP can also be released by atherosclerotic plaques, vascular tissue, lymphocytes, and monocytes, among other things. Because of its quick (24–28 h) and strong response to a wide array of inflammatory situations, CRP is a major marker of the acute-phase responses. CRP is thus a component of the nonadaptive (innate) host response to infection and other diseases. [18]

Adipocytes release a variety of biologic products, including nonesterified free fatty acids, retinol-binding protein-4, leptin, TNF-alpha, resistin, IL-6, and adiponectin. Insulin sensitivity is modulated by adipokines, which also regulate body weight, hunger, and energy expenditure. Insulin resistance in skeletal muscle and liver is caused by an

increase in adipokines and free fatty acids. Adipocyte products and adipokines trigger an inflammatory response, which explains why inflammatory markers like CRP rise in type-2 diabetes mellitus. Inflammatory cells have also been discovered infiltrating fat tissues.

circulating concentrations of markers and mediators of inflammation, as well as acute-phase reactants such as fibrinogen, CRP, IL-6, plasminogen activator inhibitor-1, sialic acid, and white cells, were shown to be elevated in these conditions, epidemiologic associations of inflammation with obesity and T2DM were made. [24-28] Several studies on human and animal models during the next few decades added to the body of evidence showing the involvement of inflammation in the onset and progression of diabetes. [19-29] Chronic activation of pro-inflammatory pathways in insulin action target cells, according to accumulating data, may lead to obesity, insulin resistance, and related metabolic diseases, including T2DM. [29] Obesity and metabolic syndrome are a group of diseases linked to a diet high in fat and low in exercise, conditions in which sub-acute chronic inflammation is a common and potentially unifying cause, accompanied by activation of at least two major inflammatory pathways, stress-activated Jun N-terminal kinases (JNK) and the transcription factor NF-kappaB. [19,23,30-34] Although a number of studies have shown that adipokines generate extra inflammatory responses in obesity and worsen obesity-induced metabolic and cardiovascular disorders, this inflammatory state is further amplified by adipokines via the generation of pro-inflammatory cytokines. [71] Brown adipose tissue (BAT) has been linked to peripheral insulin resistance and glucose levels in animal studies, indicating that it plays a significant role in energy and glucose homeostasis. [35-37] White adipose tissue (WAT), particularly visceral WAT (around the trunk, upper body, or belly), appears to be a primary source of inflammatory indicators in T2DM, as well as a target of the inflammatory process in diabetics. TNF- , IL-1, IL-6, IL-10, leptin, adiponectin, monocyte chemoattractant protein, angiotensinogen, resistin, chemokines, serum amyloid protein, and a variety of other bioactive substances involved in the inflammatory pathways are among the cytokines and other bioactive compounds produced. [38-41]

DIABETES MELLITUS: EVOLVING FROM A METABOLIC DISORDER TO AN INFLAMMATORY CONDITION [42]

Although diabetic nephropathy has long been thought to be a non-immune illness, mounting data now suggests that chronic low-grade inflammation and innate immunity activation play a role in the pathogenesis of type-2 diabetes. Inflammatory and pro-inflammatory markers such as CRP, sialic acid, tumour necrosis factor-alpha, and IL-6 have been shown to be positively correlated with measures of insulin resistance in non-diabetic patients with impaired glucose tolerance or impaired fasting glucose, as well as diabetic patients. Furthermore, diabetic patients had higher levels of inflammatory parameters than non-diabetics. Various inflammatory indicators, adhesion molecules, and proinflammmatory molecules may also have a role in the development of microvascular diabetic complications, such as nephropathy. Additional evidence comes from the fact that anti-inflammatory medications can diminish the acute phase response and may lower the chance of developing type-2 diabetes mellitus.

Renal dysfunction and microalbuminuria in type-2 diabetes mellitus patients are closely related to raised levels of serum CRP. [43,44], which is indicative of a close link between serum levels of CRP and the development of diabetic nephropathy. IL-6 in the inflammatory cascade is induced by CRP through the NF- B-dependent mechanism. [72] In a study published in 2016 , it was demonstrated that C-Reactive Protein Promotes Diabetic Kidney Disease in db/db Mice via the CD32b-Smad3-mTOR signaling Pathway. They found that CRP promoted renal inflammation via the CD32b-NF- B-dependent mechanism, resulting in upregulation of TNF- , IL-1 and MCP-1 and an increase in CD3+ T cell and F4/80+ macrophage infiltration in the diabetic kidney of CRPtg-db/db mice, revealing a role for CD32b-NF- B signaling in CRP-mediated renal inflammation. This finding was consistent with a known mechanism of NF- B in diabetic renal inflammation [43-50].

A novel and significant finding in this study was that CRP mediated renal fibrosis via the CD32b-Smad3-mTOR signaling pathway. Increasing evidence shows that activation of TGF- /Smad signaling contributes to accumulation of the extracellular matrix, resulting in progressive renal fibrosis in both animal and human diabetic kidneys[50-57].

DN has been shown to show indications of inflammation in a number of experimental and clinical trials. Three recent studies support the notion that inflammation plays a key role in the development of DN. Patients with type-2 diabetes and overt nephropathy have the greatest levels of many acute-phase indicators of inflammation, such as CRP (CRP), serum amyloid A (SAA), fibrinogen, and IL-6, according to Dalla Vestra and colleagues[73]. Furthermore, participants with a wider glomerular basement membrane (GBM) had greater levels of CRP, SAA, and IL-6. More importantly, the scientists established a link between GBM thickening, a critical diabetic disease, and in addition, two experimental studies provide light on how diabetes-related kidney impairment is linked to inflammation. Chow et al.[74] found that db/db mice, a model of type-2 diabetes and DN, had higher levels of intracellular adhesion molecule-1 (ICAM-1), which promotes inflammation by increasing leukocyte infiltration and adherence, in the glomeruli and tubules, as well as a significant increase in macrophage infiltration. These findings strongly involve ICAM-1-induced inflammation in the development of renal injury in diabetes. In the second study, Kelly et al.[75], demonstrated in a model of diabetes and hypertension that, despite hyperglycemia and elevated blood pressure, albuminuria was reduced and renal function was preserved in rats treated with ruboxistaurin, an inhibitor of protein-kinase C-ß. Protein-kinase C contains several isoforms that are active in diabetes and signal a variety of cellular responses, including inflammatory mediator activation and production, such as pro-inflammatory cytokines. Interleukin-6 regulates extracellular matrix dynamics in mesangial and podocyte cells, stimulates mesangial cell proliferation, boosts fibronectin expression, and improves endothelial permeability. A link between pro-inflammatory cytokines and DN has been suggested in a number of clinical investigations. Pro-inflammatory cytokines can be produced by endothelial, mesangial, glomerular, and tubular epithelial cells. These compounds have also been linked to substantial renal consequences. Among the inflammatory markers CRP has been widely studied and used as marker of underlying inflammation in pathogenesis of nephropathy [4,60].

1. Satyesh K Sinha et al. discovered a link between serum hsCRP and the incidence of diabetic nephropathy in the Jackson Heart Study(JHS). They recruited 5,306 persons from the city of Jackson, Minnesota, who self-identified as African Americans or black adults with a baseline age of 21-84 years between 2000 and 2004 (exam 1). JHS 2 data was collected from 2005 to 2008, whereas JHS 3 data was collected from 2009 to 2013. Patients with Diabetic Nephropathy or no information on hs-CRP at exam 1 were excluded from the JHS 3 trial to investigate the link between hs-CRP and incident instances of Diabetic Nephropathy. There were a total of 4,043 persons detected after all of the exclusions. Those who developed diabetic nephropathy had significantly higher baseline values of hsCRP, age, fasting plasma glucose, serum lipids, systolic blood pressure, waist circumference, and diabetes mellitus duration (p 0.05) than those who did not. The overall incidence rate of DN was reported to be 7.9%. Participants

with high tertile hs-CRP (>4.24 mg/L) took less time to develop Diabetic nephropathy than those with low tertile hs-CRP (1.461 mg/L); P 0.001. Participants with increased hs-CRP had a higher incidence of Diabetic Nephropathy (HR 2.34, 95 percent CI 1.04–5.24) when compared to the control group. [76].

2. Abid K Shaheer, et al. published A Comparative Study of High Sensitivity C-Reactive Protein and Metabolic Variables in Type-2 Diabetes Mellitus with and without Nephropathy. The study comprised 96 people divided into three groups: healthy non-diabetic controls, type-2 diabetic patients with no problems, and type-2 diabetes mellitus patients with nephropathy. The findings a statistically significant ($p < 0.05$) link between rising blood hsCRP levels and the degree of microalbuminuria, as well as the severity of diabetic nephropathy. The result showed a significant ($p<0.05$) relationship between hs-CRP and the different metabolic variables like Fasting Blood Glucose (FBG), Post Prandial Blood Glucose (PPBG), Total Cholesterol (TC), Triglycerides (TG), LDL-Cholesterol (LDL-C), TC:HDL-Cholesterol (HDL-C) ratio and estimated Glomerular Filtration Rate (eGFR)[61].

3. Study of C-Reactive Protein in Type-2 Diabetes and its Relation with Various Complications from Eastern India done by Ramtanu Bandyopadhyay, et al selected a total of 80 patients (M:F:: 47:33), found that patients with the complications of diabetes, like nephropathy had significantly higher levels of serum CRP[62].

4. In a study on the elevated Serum C-Reactive Protein Level and Microalbuminuria in Patients With Type-2 Diabetes Mellitus done by Mohammad Javad Mojahedi et al. They measured serum levels of HS-CRP in 87 patient with type 2 diabetes mellitus. They were divided into a microalbuminuric group (n = 45) and normoalbuminuric group ,i.e. those with a 24-hour urine albumin less than 30 mg/d (n = 42). Patients with microalbuminuria were significantly older and affected by diabetes mellitus longer than those without microalbuminuria. Also, their mean HS-CRP was significantly higher (4.98 ± 1.45 mg/L versus 2.82 ± 2.10 mg/L; P < .001). The Pearson correlation test showed a significant correlation between HS-CRP level and urine albumin level (r = 0.43; P < .001). The specificity and sensitivity of HS-CRP for detection of microalbuminuria in were 78.5% and 68.8%, respectively, and the positive and negative predictive values were 77.5% and 70.2%, respectively. In conclusion type 2 diabetic patients, microalbuminuria is accompanied by elevated hs-CRP, suggesting activation of inflammatory pathways in progression of renal and cardiovascular atherosclerotic disease[63].

In type-2 diabetic nephropathy , high-sensitive CRP tests were performed. Lan Liao et al used an ELISA to measure blood hs-CRP levels in 55 healthy controls, 66 Type-2 diabetes patients without DN, and 68 Type 2 diabetic patients with DN. In 58 diabetic patients without DN at the baseline, a 5-year prospective study was conducted to assess changes under urine albumin excretion (UAE), serum hs-CRP concentration, and kidney function in targeted intervention comprising blood glucose and blood pressure. Results: Type-2 diabetes patients with DN had the highest baseline hs-CRP concentration, followed by Type-2 diabetic patients without DN, while normal controls had the lowest. In patients who did not have DN at the end of the 5-year intervention, hs-CRP concentration at the end was considerably lower than at the start ($p< 0.05$). However, towards the end of the study, the patients with DN had a slightly lower hs-CRP concentration than at the start . The hs-CRP concentrations in individuals with DN at the endline were substantially greater than those without DN at the endline at both baseline and endpoint. In conclusion, a higher blood hs-CRP concentration in Type-2 diabetes patients may be a risk factor for DN. In Type-2 diabetes individuals, hs-CRP can predict the onset of DN to some extent. [64]

In a study done by Nikhil Choudhary and Ravinder S Ahlawat on the role of IL-6 and CRP in pathogenesis of diabetic nephropathy, 60 patients were taken with type-2 diabetes mellitus, who were then further divided into 3 groups of 20 each – normoalbuminuria, microalbuminuria and macroalbuminuria. They found that there was a positive correlation between the urinary albumin excretion and levels of hsCRP ($p<0.001$), and interleukin-6 ($p<0.001$); which showed that inflammatory markers were elevated in diabetic nephropathy and an association with urinary albumin excretion was also present.[11]

In a related study on CRP and its significance in CKD, done by PC Lalramenga et al, on 140 subjects with CKD, they found that high serum CRP levels are associated with lower eGFR.[65]

In another study done by V. Panichi et al on the levels of CRP in CKD patients, they found that CRP (as well as IL-6) levels were related with renal function ($p<0.001$).[66]

MATERIALS AND METHODS

Estimation of C-reactive Protein:

C-reactive Protein was estimated by the turbidometric method.

Specimen: Fresh serum specimen should be preferred. Samples with presence of fibrin should be centrifuged before testing. CRP in serum is stable for 7 days at 2-8°C and for 3 months at -20°C. Sample should be brought to room temperature before testing. Hemolysed sample should not be used.

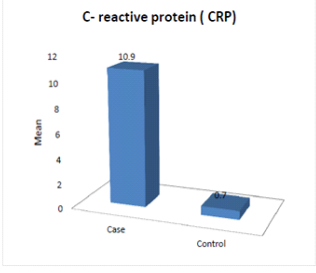
Reagent: Turbilatex CRP is a reagent set for quantitative determination of CRP in human serum based on turbidimetric method. Turbilatex CRP is a two liquid reagent using one step procedure. By this method, CRP can be determined within 2 minutes. Turbilatex CRP can be used on any spectrophotometer, discrete semi automated and automated analyser.

Principle: Turbilatex CRP contain latex particles coated with specific antihuman CRP which reacts with CRP in the sample resulting in agglutination. Agglutination causes change in absorbance, measured at 540nm (530-550nm) and is proportional to the concentration of CRP in the sample.

RESULTS AND OBSERVATIONS

Comparison of C-Reactive Protein, CRP values between case and control (mg/dl)

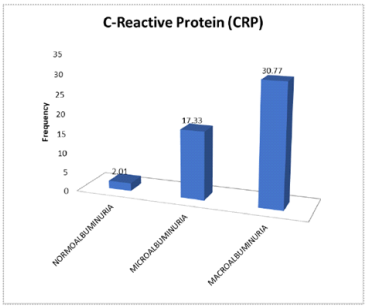
CRP	Case	Control	p-value
Mean ± SD	10.9±14.0	0.7±0.1	0.0001



The mean CRP of the subjects in Case group was 10.9 mg/dl & in control group was 0.7mg/dl, The case group had significantly higher ($p <0.0001$) mean value than the control group.

:Mean values of C-Reactive Protein (CRP) (mg/dl) in relation to urinary albumin excretion

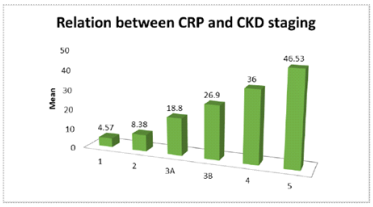
CRP	NORMOALBUMINURIA	MICROALBUMINURIA	MACROALBUMINURIA	*p*-value
Mean±SD	2.01±2.73	17.33±14.84	30.77±9.63	<0.0001



The highest mean serum CRP 30.77±9.63 mg/dl was noticed in macroalbuminuria group and the lowest mean serum CRP 2.01±2.73mg/dl was noticed in normoalbuminuria group.There was significant (<0.0001) difference between the group

Relation between C-Reactive Protein (CRP) in mg/dl and CKD staging

CRP	CKD Staging						*p*-value
	1	2	3A	3B	4	5	
Mean±SD	4.57±8.22	8.38±10.03	18.8±6.83	26.9±8.45	36±7.14	46.53±12.11	0.0011



he mean values of CRP as per CKD stage were- 4.57+8.22 mg/dl, 8.38+10.03mg/dl, 18.8+6.83mg/dl, 26.9+8.45mg/dl, 36+7.14mg/dl, 46.53+12.11 mg/dl in the stage 1, 2, 3A, 3B, 4, and 5 respectively. There was significant positive correlation between the values of CRP and CKD stage , with the progression of different stages of nephropathy.

DISCUSSION

In the present study, mean levels of CRP in the case group is 10.9 mg/dl and control group was 0.7 mg/dl, which is statistically significant. In a study by Mohammed Javad Mojahedi et al, published in 2009, mean hsCRP was found to be significantly higher in diabetic patients[63]. In a study published by R Tabassum et al, the mean CRP levels were found to be significantly higher in the diabetic patients when compared to non diabetic patients[68]. In the study done by Ritu Gupta et al, they found that the mean values of hsCRP in the control group was 0.93+0.81 mg/l, whereas 4.06+2.59 mg/l in the diabetic patients. [67] In a followup study conducted by Barbara Thorand et al, they found that the mean values of CRP were 16.48+12.69 mg/l and <6.00+0.00 mg/l in the diabetic and non-diabetic groups at the end of study period.[77]

In this present study, the highest serum CRP levels were noted in the macroalbuminuria group and lowest in the normoalbuminuria group, which was statistically significant. This was consistent with the results of the study done by Nikhil Choudhary et al[11]. Another study by Fatima El Boukhrissi et al, found significantly higher levels of hsCRP in microalbuminuria compared to diabetes mellitus patients without albuminuria[78]. In the study done by Mohammad Javad Mojahedi, they showed a significant correlation between hsCRP and urinary albumin levels, p value <0.001.[63]

In the current study, serum CRP levels showed positive correlation with CKD stages, consistent with the study done by V Panichi et al which demonstrated that CRP levels increased as renal function decreased[66]. Another study by PC Lalramenga et al showed that higher levels of CRP were associated with CKD stage 4 and 5 (i.e.lower eGFR)[79]. In a study by Tri Asih Imro'ati et al results of hsCRP data description as per the CKD stages revealed highest mean values of hsCRP in CKD stage 4 as compared with stage 3 and stage5. hsCRP levels in this study did not represent CKD stages due to insufficient samples[80].

SUMMARY

In the present study, mean CRP in the case group is 10.9 mg/dl and control group was 0.7 mg/dl, which is statistically significant.

In this present study, the highest serum CRP levels were noted in the macroalbuminuria group and lowest in the normoalbuminuria group, which was statistically significant.

In the current study, serum CRP levels showed positive correlation with CKD stages,

CONCLUSION

In conclusion, the chief observation seen in our present study was that there is a significant difference between the inflammatory parameter,CRP between diabetic and non diabetic groups, and also a significant relation between the inflammatory parameters and urinary albumin excretion as well as severity of diabetic nephropathy.

The values of CRP in type-2 diabetes mellitus and control was 10.9 mg/dl and control group was 0.7 mg/dl respectively, which was statistically significant. The values of CRP showed positive correlation with urinary albumin excretion and a negative correlation with estimated GFR. A decrease in estimated GFR signifies a decrease in renal function, denoting severity of diabetic nephropathy.

Similar to several studies, this present study suggests the fact that there is a significant correlation between CRP with diabetic nephropathy, which indicates that inflammation may play a significant role in the pathogenetic mechanism of type-2 diabetes mellitus and its complications, one of them being diabetic nephropathy.

Since inflammatory markers, CRP showed significant rise in the diabetic group when compared to the non diabetic control.group and also showed positive correlation with diabetic nephropathy, CRP estimation at earlier stages of type-2 diabetes mellitus can help to detect risk for nephropathy in the early phase and help prevent development and progression of diabetic nephropathy by taking appropriate measures at the earliest

REFERENCES

1. Ford ES. Body mass index, diabetes, and C-reactive protein among US adults. Diabetes care. 1999 Dec 1;22(12):1971-7.
2. Grau AJ, Buggle F, Becher H, Werle E, Hacke W. The association of leukocyte count, fibrinogen and C-reactive protein with vascular risk factors and ischemic vascular diseases. Thrombosis research. 1996 May 1;82(3):245-55.
3. Goldberg RB. Cardiovascular disease in diabetic patients. Medical Clinics of North America. 2000 Jan 1;84(1):81-93.
4. Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. Diabetic Medicine. 2004 Mar;21(3):203-7.
5. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, Plebani M, Fioretto P. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. Journal of the American Society of Nephrology. 2005 Mar 1;16(3 suppl 1):S78-82.
6. Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, Rose-John S, Fuller GM, Topley N, Jones SA. Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. Immunity. 2001 Jun 1;14(6):705-14.

7. Pop awska-Kita A, Siewko K, Szpak P, Król B, Telejko B, Klimiuk PA, Stokowska W, Górska M, Szelachowska M. Association between type 1 diabetes and periodontal health. Advances in medical sciences. 2014 Mar 1;59(1):126-31.
8. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M. Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney international. 1991 Dec 1;40(6):1007-12.
9. Navarro JF, Mora C, Rivero A, Gallego E, Chahin J, Macía M, Méndez ML, García J. Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: effects of pentoxifylline administration. American Journal of Kidney Diseases. 1999 Mar 1;33(3):458-63.
10. Navarro JF, Mora C, Macıéa M, Garcıéa J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. American Journal of Kidney Diseases. 2003 Jul 1;42(1):53-61.
11. Choudhary N, Ahlawat RS. Interleukin-6 and C-Reactive protein in pathogenesis of diabetic nephropathy new evidence linking inflammation, glycemic control, and microalbuminuria. IJKD 2008;2:72-9
12. Agrawal A, Suresh MV, Singh SK, Ferguson Jr DA. The protective function of human C-reactive protein in mouse models of Streptococcus pneumoniae infection. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2008 Dec 1;8(4):231-7.
13. Agrawal A, Gang TB, Rusiñol AE. Recognition functions of pentameric C-reactive protein in cardiovascular disease. Mediators of inflammation. 2014 Oct;2014.
14. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu. Rev. Immunol.. 2005 Apr 23;23:337-66.
15. Vilahur G, Badimon L. Biological actions of pentraxins. Vascular pharmacology. 2015 Oct 1;73:38-44.
16. Du Clos TW. Function of C-reactive protein. Annals of medicine. 2000 Jan 1;32(4):274-8.
17. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system: I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. The Journal of Immunology. 1974 Jun 1;112(6):2135-47.
18. Kop WJ, Weinstein AA, Deuster PA, Whittaker KS, Tracy RP. Inflammatory markers and negative mood symptoms following exercise withdrawal. Brain, behavior, and immunity. 2008 Nov 1;22(8):1190-6.
19. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. The Journal of clinical investigation. 2006 Jul 3;116(7):1793-801.
20. Williamson RT. On the treatment of glycosuria and diabetes mellitus with sodium salicylate. British medical journal. 1901 Mar 30;1(2100):760.
21. Reid J, Macdougall AI, Andrews MM. Aspirin and diabetes mellitus. British medical journal. 1957 Nov 9;2(5053):1071.
22. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. Physiology. 2004 Aug;19(4):183-90.
23. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993 Jan 1;259(5091):87-91.
24. Ogston D, McAndrew GM. Fibrinolysis in obesity. Lancet. 1964;2:1205-7.
25. GR F, CT V. Reduction of blood fibrinolytic activity in diabetes mellitus by insulin. Lancet (London, England). 1959 Dec 1;2(7111):1067-.
26. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. Emerging Risk Factors Collaboration C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010;375(9709):132-40.
27. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. New England journal of medicine. 1997 Apr 3;336(14):973-9.
28. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2003 Jul 1;52(7):1799-805.
29. Marques-Vidal P, Schmid R, Bochud M, Bastardot F, Von Känel R, Paccaud F, Glaus J, Preisig M, Waeber G, Vollenweider P. Adipocytokines, hepatic and inflammatory biomarkers and incidence of type 2 diabetes. the CoLaus study. PloS one. 2012 Dec 12;7(12):e51768.
30. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 Dec;444(7121):860-7.
31. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annual review of physiology. 2010 Mar 17;72:219-46.
32. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation. 2011 Jun 1;121(6):2111-7.
33. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation. 2007 Jan 2;117(1):175-84.
34. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nature medicine. 2009 Aug;15(8):914-20.
35. Sacks H, Symonds ME. Anatomical locations of human brown adipose tissue: functional relevance and implications in obesity and type 2 diabetes. Diabetes. 2013 Jun 1;62(6):1783-90.
36. Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. The Journal of clinical investigation. 2015 Feb 2;125(2):478-86.
37. Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. American Journal of Physiology-Endocrinology and Metabolism. 2010 Oct;299(4):E601-6.
38. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007 May 1;132(6):2169-80.
39. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa KI, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of clinical investigation. 2006 Jun 1;116(6):1494-505.
40. Antonopoulos AS, Margaritis M, Coutinho P, Shirodaria C, Psarros C, Herdman L, Sanna F, De Silva R, Petrou M, Sayeed R, Krasopoulos G. Adiponectin as a link between type 2 diabetes and vascular NADPH oxidase activity in the human arterial wall: the regulatory role of perivascular adipose tissue. Diabetes. 2015 Jun 1;64(6):2207-19.
41. Antoniades C, Antonopoulos AS, Tousoulis D, Stefanadis C. Adiponectin: from obesity to cardiovascular disease. obesity reviews. 2009 May;10(3):269-79.
42. Navarro-González J, Mora-Fernández C, de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy: Abstract: Nature reviews Nephrology. Nature Reviews Nephrology. 2011 May 3;7(6):327-40.
43. Wang C, Yatsuya H, Tamakoshi K, Uemura M, Li Y, Wada K, Yamashita K, Kawaguchi L, Toyoshima H, Aoyama A. Positive association between high-sensitivity C-reactive protein and incidence of type 2 diabetes mellitus in Japanese workers: 6-year follow-up. Diabetes/metabolism research and reviews. 2013 Jul;29(5):398-405.
44. Overgaard AJ, McGuire JN, Hovind P, Parving HH, Rossing P, Pociot F. Serum amyloid A and C-reactive protein levels may predict microalbuminuria and macroalbuminuria in newly diagnosed type 1 diabetic patients. Journal of Diabetes and its Complications. 2013 Jan 1;27(1):59-63.
45. Liu F, Chen HY, Huang XR, Chung AC, Zhou L, Fu P, Szalai AJ, Lan HY. C-reactive protein promotes diabetic kidney disease in a mouse model of type 1 diabetes. Diabetologia. 2011 Oct;54(10):2713-23.
46. Mezzano S, Aros C, Droguett A, Burgos ME, Ardiles L, Flores C, Schneider H, Ruiz-Ortega M, Egido J. NF- B activation and overexpression of regulated genes in human diabetic nephropathy. Nephrology Dialysis Transplantation. 2004 Oct 1;19(10):2505-12.
47. Lee FT, Cao Z, Long DM, Panagiotopoulos S, Jerums G, Cooper ME, Forbes JM. Interactions between angiotensin II and NF- B–dependent pathways in modulating macrophage infiltration in experimental diabetic nephropathy. Journal of the American Society of Nephrology. 2004 Aug 1;15(8):2139-51.
48. Ka SM, Yeh YC, Huang XR, Chao TK, Hung YJ, Yu CP, Lin TJ, Wu CC, Lan HY, Chen A. Kidney-targeting Smad7 gene transfer inhibits renal TGF- /MAD homologue (SMAD) and nuclear factor B (NF- B) signalling pathways, and improves diabetic nephropathy in mice. Diabetologia. 2012 Feb;55(2):509-19.
49. Lazaro I, Oguiza A, Recio C, Mallavia B, Madrigal-Matute J, Blanco J, Egido J, Martin-Ventura JL, Gomez-Guerrero C. Targeting HSP90 ameliorates nephropathy and atherosclerosis through suppression of NF- B and STAT signaling pathways in diabetic mice. Diabetes. 2015 Oct 1;64(10):3600-13.
50. Yang SM, Ka SM, Wu HL, Yeh YC, Kuo CH, Hua KF, Shi GY, Hung YJ, Hsiao FC, Yang SS, Shieh YS. Thrombomodulin domain 1 ameliorates diabetic nephropathy in mice via anti-NF- B/NLRP3 inflammasome-mediated inflammation, enhancement of NRF2 antioxidant activity and inhibition of apoptosis. Diabetologia. 2014 Feb;57(2):424-34.
51. Liang YJ, Shyu KG, Wang BW, Lai LP. C-reactive protein activates the nuclear factor- B pathway and induces vascular cell adhesion molecule-1 expression through CD32 in human umbilical vein endothelial cells and aortic endothelial cells. Journal of molecular and cellular cardiology. 2006 Mar 1;40(3):412-20.
52. Jialal I, Devaraj S. The role of C-reactive protein activation of nuclear factor kappa-B in the pathogenesis of unstable angina. 2007:195–197.
53. Hong SW, Isono M, Chen S, Iglesias-De La Cruz MC, Han DC, Ziyadeh FN. Increased glomerular and tubular expression of transforming growth factor- 1,
its type II receptor, and activation of the Smad signaling pathway in the db/db mouse. The American journal of pathology. 2001 May 1;158(5):1653-63.
54. Li JH, Huang XR, Zhu HJ, Oldfield M, Cooper M, Truong LD, Johnson RJ, Lan HY. Advanced glycation end products activate Smad signaling via TGF- -dependent and-independent mechanisms: implications for diabetic renal and vascular disease. The FASEB Journal. 2004 Jan;18(1):176-8.
55. Fujimoto M, Maezawa Y, Yokote K, Joh K, Kobayashi K, Kawamura H, Nishimura M, Roberts AB, Saito Y, Mori S. Mice lacking Smad3 are protected against streptozotocin-induced diabetic glomerulopathy. Biochemical and biophysical research communications. 2003 Jun 13;305(4):1002-7.
56. Tan CK, Leuenberger N, Tan MJ, Yan YW, Chen Y, Kambadur R, Wahli W, Tan NS. Smad3 deficiency in mice protects against insulin resistance and obesity induced by a high-fat diet. Diabetes. 2011 Feb 1;60(2):464-76.
57. Sun YB, Qu X, Howard V, Dai L, Jiang X, Ren Y, Fu P, Puelles VG, Nikolic-Paterson DJ, Caruana G, Bertram JF. Smad3 deficiency protects mice from obesity-induced podocyte injury that precedes insulin resistance. Kidney international. 2015 Aug 1;88(2):286-98.
58. Chow FY, Nikolic-Paterson DJ, Ozols E, Atkins RC, Tesch GH. Intercellular adhesion molecule-1 deficiency is protective against nephropathy in type 2 diabetic db/db mice. Journal of the American Society of Nephrology. 2005 Jun 1;16(6):1711-22.
59. Kelly DJ, Chanty A, Gow RM, Zhang Y, Gilbert RE. Protein kinase C inhibition attenuates osteopontin expression, macrophage recruitment, and tubulointerstitial injury in advanced experimental diabetic nephropathy. Journal of the American Society of Nephrology. 2005 Jun 1;16(6):1654-60.
60. Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspects of interleukin 6. Immunology today. 1990 Jan 1;11:443-9.
61. Shaheer AK, Tharayil JK, Krishna PW. A comparative study of high sensitivity C-reactive protein and metabolic variables in type 2 diabetes mellitus with and without nephropathy. Journal of clinical and diagnostic research: JCDR. 2017 Sep;11(9):BC01.
62. Bandyopadhyay R, Paul R, Basu AK, Chakraborty PP, Mitra S. Study of c reactive protein in type 2 diabetes and its relation with various complications from Eastern India. Journal of Applied Pharmaceutical Science. 2013 Jul 1;3(7):156.
63. Mojahedi MJ, BONAKDARAN S, Hami M, Sheykhiani M, Shakeri MT, AYAT EH. Elevated serum C-reactive protein level and microalbuminuria in patients with type 2 diabetes mellitus. 2009 Jan;3(1):12-6.
64. Liao L, Lei MX, Chen HL, Wu J, Guo LJ. High-sensitive C-reactive protein and Type 2 diabetic nephropathy. Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical Sciences. 2004 Dec 1;29(6):627-30.
65. Lalramenga PC, Gupta S. Study of C-reactive protein significance in chronic kidney disease.
66. Panichi V, Migliori M, De Pietro S, Taccola D, Bianchi AM, Norpoth M, Metelli MR, Giovannini L, Tetta C, Palla R. C reactive protein in patients with chronic

renal diseases. Renal failure. 2001 Jan 1;23(3-4):551-62.
67. Gupta R, Pamecha H. To Study Relationship of Serum hsCRP with Type 2 Diabetes Mellitus, its Vascular Complications and Non-Diabetics-Case Control Study. The Journal of the Association of Physicians of India. 2020 Aug 1;68(8):25-9.
68. Tabassum R, Mia AR, Reza-Ul-Haq KM, Yesmin M, Faruqui JM. C-reactive Protein Level in Type-2 Diabetic Patients Attending Mymensingh Medical College Hospital, Mymensingh. Mymensingh medical journal: MMJ. 2017 Jan 1;26(1):56-60.
69. Shrive AK, Gheetham GM, Holden D, Myles DA, Turnell WG, Volanakis JE, Pepys MB, Bloomer AC, Greenhough TJ. Three dimensional structure of human C-reactive protein. Nature structural biology. 1996 Apr;3(4):346-54.
70. Lv JM, Lü SQ, Liu ZP, Zhang J, Gao BX, Yao ZY, Wu YX, Potempa LA, Ji SR, Long M, Wu Y. Conformational folding and disulfide bonding drive distinct stages of protein structure formation. Scientific reports. 2018 Jan 24;8(1):1-0.
71. Takaoka M, Nagata D, Kihara S, Shimomura I, Kimura Y, Tabata Y, Saito Y, Nagai R, Sata M. Periadventitial adipose tissue plays a critical role in vascular remodeling. Circulation research. 2009 Oct 23;105(9):906-11.
72. Wang HR, Chen DL, Zhao M, Shu SW, Xiong SX, Gan XD, Chao SP, Cao JL. C-reactive protein induces interleukin-6 and thrombospondin-1 protein and mRNA expression through activation of nuclear factor- B in HK-2 cells. Kidney and Blood Pressure Research. 2012;35(4):211-9.
73. Kahn R, Alperin P, Eddy D, Borch-Johnsen K, Buse J, Feigelman J, Gregg E, Holman RR, Kirkman MS, Stern M, Tuomilehto J. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. The Lancet. 2010 Apr 17;375(9723):1365-74.
74. Zilberstein A, Ruggieri R, Korn JH, Revel M. Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. The EMBO journal. 1986 Oct;5(10):2529-37.
75. Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura SI, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 1986 Nov;324(6092):73-6.
76. Sinha SK, Nicholas SB, Sung JH, Correa A, Rajavashisth TB, Norris KC, Lee JE. hs-CRP is associated with incident diabetic nephropathy: findings from the Jackson heart study. Diabetes Care. 2019 Nov 1;42(11):2083-9.
77. Thorand B, Löwel H, Schneider A, Kolb H, Meisinger C, Fröhlich M, Koenig W. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. Archives of internal medicine. 2003 Jan 13;163(1):93-9.
78. El Boukhrissi F, Benbella I, Ouleghzal H, Safi S, Bamou Y, Balouch L. Evaluation of ultrasensitive CRP and microalbuminuria as cardiovascular risk markers in type 2 diabetic moroccan patients. La Tunisie medicale. 2017 Nov 1;95(11):982-7.
79. Sanyal M, Hossain MZ, Khan MA, Rahman F, Ali MH. Correlation Between Serum Creatinine & Urine Albumin in Diabetic Nephropathy Patients. Journal of Dhaka Medical College. 2018;27(2):141-7.
80. Imro'ati TA, Thaha M, Irwanadi C. Comparison of high-sensitivity C-reactive Protein Level between chronic kidney disease stage. Biomolecular and Health Science. 2018;1(1):1-7.