Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Review Article

ROLE OF INFLAMMATION IN DEVELOPMENT OF DIABETIC COMPLICATIONS AND COMMONLY USED INFLAMMATORY MARKERS WITH RESPECT TO DIABETIC COMPLICATIONS

[a*]ARCHANA M. NAVALE, [b]DR. ARCHANA N. PARANJAPE

Department of Pharmacology, Parul Institute of Pharmacy, P.O. Limda, Ta. Waghodia, Dist.Vadodara-391760. Gujarat, India, Department of pharmacology, Parul Institute of Pharmacy and Research, P.O. Limda, Ta. Waghodia, Dist.Vadodara-391760. Gujarat, India.
Email: archanachavan_83@yahoo.co.in

Received: 26 Nov 2012, Revised and Accepted: 05 Jan 2013

ABSTRACT

Diabetes mellitus has been a major health concern since several past decades. Cases of Type 1 as well as types 2 diabetes are increasing day by day. With the immense advancement in pharmaceutical sciences the disease management has become fairly satisfactory. Good new drug therapeutic approaches are available, which makes it possible to keep the patient free from episodes of hyperglycemia or hypoglycemia for many years. With this the concern has shifted to the long term complications of diabetes. The major morbidity caused in the diabetic patients is due to dysfunction of several organ systems after a few years of having diabetes. Research has now clearly proved that these organ dysfunctions have their roots in diabetic pathophysiology. Many components of this pathophysiology have been identified. These components work interdependently with each other. One of the important factors identified is inflammation, which embraces the mechanisms like oxidative stress, neovascularization, apoptosis, cellular proliferation etc. It has been shown that inflammation plays an important role in microvascular as well as macrovascular complications of diabetes. There is a need of experimental work to be done for evaluating drugs for their effectiveness in protecting against inflammatory processes of diabetic complications. However, very less literature is available about methods used for evaluation of inflammation in diabetic complications. In this review we focus on role of inflammation in various diabetic complications followed by common inflammatory markers used by researchers in various studies on diabetic complications.

Keywords: Diabetic complications, Inflammation, Anti inflammatory activity

INTRODUCTION

Diabetes mellitus, especially type-2 diabetes, is a public health problem which has reached epidemic proportions due to the rapidly increasing rates of this disease worldwide. Target organ complications, secondary to diabetes, are one of the most important medical concerns of the present time.

Inflammation is a protective mechanism of body. However, in chronic diseases like, diabetes mellitus, hypertension, asthma etc. this protective mechanism becomes an important mechanism for progression of disease. Interleukin (IL)-1β is a pro-inflammatory cytokine involved in autoimmune process of Type 1 diabetes. In Type 2 diabetes mellitus the decreasing β cell mass is also associated with glucose toxicity mediated through IL-1β induced apoptosis. There are many studies emphasizing the presence and importance of inflammatory component in the pathogenesis of Diabetes mellitus. A very important role is played by adipose tissue, which releases various pro-inflammatory cytokines, such as, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) [1]. Recent studies have shown that inflammation, and more specifically pro-inflammatory cytokines, play a determinant role in the development of microvascular diabetic complications. This article takes an overview of importance of inflammation in various diabetic complications followed by a brief account of the commonly used inflammatory markers in various studies on diabetic complications.

INFLAMMATION IN RETINOPATHY

A major pathology of Diabetic retinopathy (DR) is microvascular complications such as non-perfused vessels, microaneurysms, dot/blot hemorrhages, cotton-wool spots, venous beading, vascular loops, vascular leakage and neovascularization. DR is characterized as a microvascular complication of diabetes [2, 3]. Alterations in blood flow, death of retinal pericytes (perivascular contractile cells) and basement membrane thickening accompanied by subtle increases in vascular permeability are the early events of this pathology. In later stages, further alterations in the vascular structure occur, such as, non-perfused vessels, microaneurysms, dot/blot hemorrhages, cotton-wool spots, venous beading, vascular loops and significant vascular leakage [4]. The non-proliferative stage of DR includes increased vascular permeability, macular edema and subsequent visual impairment. The proliferative stage of DR is characterized by neovascularization on the retinal surface because of impaired vascular function by capillary occlusion. In this stage, severe visual impairment or even blindness may be caused by bleeding, hemorrhage and subsequent retinal detachment because of the newly formed fragile vessels [5].

Inflammation is also a cause of retinal vascular leakage. The microvascular endothelium serves as a barrier to control the movement of blood, fluid and proteins across the vessel wall [6] due to its tight junctions and adhesion junctions that join endothelial cells to each other. Various signaling molecules such as, pro-inflammatory cytokines and chemokines induce disorganization/redistribution of junction proteins in the retinal endothelium. This leads to Structural and functional breakdown of this barrier, resulting in an abnormal extravasation of blood components causing retinal edema. TNF-α leads to downregulation of tight junction proteins mediated via PKCζ, resulting in significant retinal endothelial permeability within a few hours [7].

Legend: ICAM-1: intercellular adhesion molecule-1, VCAM-1: vascular cell adhesion molecule-1, iNOS: Inducible form of Nitric oxide synthase, TNF-α: Tumor necrosis factor α, IL-1β: Interluekin 1 β, IL-6: Interlukein 6, NF-kB: Nuclear factor-kB, AGE: advanced glycaton end products, ETC: mitochondrial electron transport chain, NADPH oxidase: nicotinamide adenine dinucleotide phosphate oxidase.

Mechanisms leading to capillary degeneration involve endothelial cell death induced by inflammatory cytokines such as, TNF-α and IL-1β. These cytokines increase caspase 3 activity inducing endothelial cell apoptosis [7,8]. Capillary occlusion in DR is mainly due to leukostasis. Leukocytes block blood flow because of their large cell volume and high rigidity. Several studies have shown that transient leukocyte attachment to the vessel wall correlates with capillary non-perfusion, and capillary reperfusion can occur when leukocytes detach and move on [9]. In addition to blocking blow flow, leukocytes induce endothelial cell death during leukostasis via Fas ligand (FasL) and Fas-mediated apoptosis [10].

Inflammation also has an important role in pathological neovascularization in DR. cytokines released in inflamed retinal tissue

recruits various leukocytes, which release various angiogenic factors and increase the activity of matrix metalloproteinase. This results in formation of new vasculature in inflamed retinal tissue [11]. Figure 1 summarizes the inflammatory process in Diabetic retinopathy (DR).

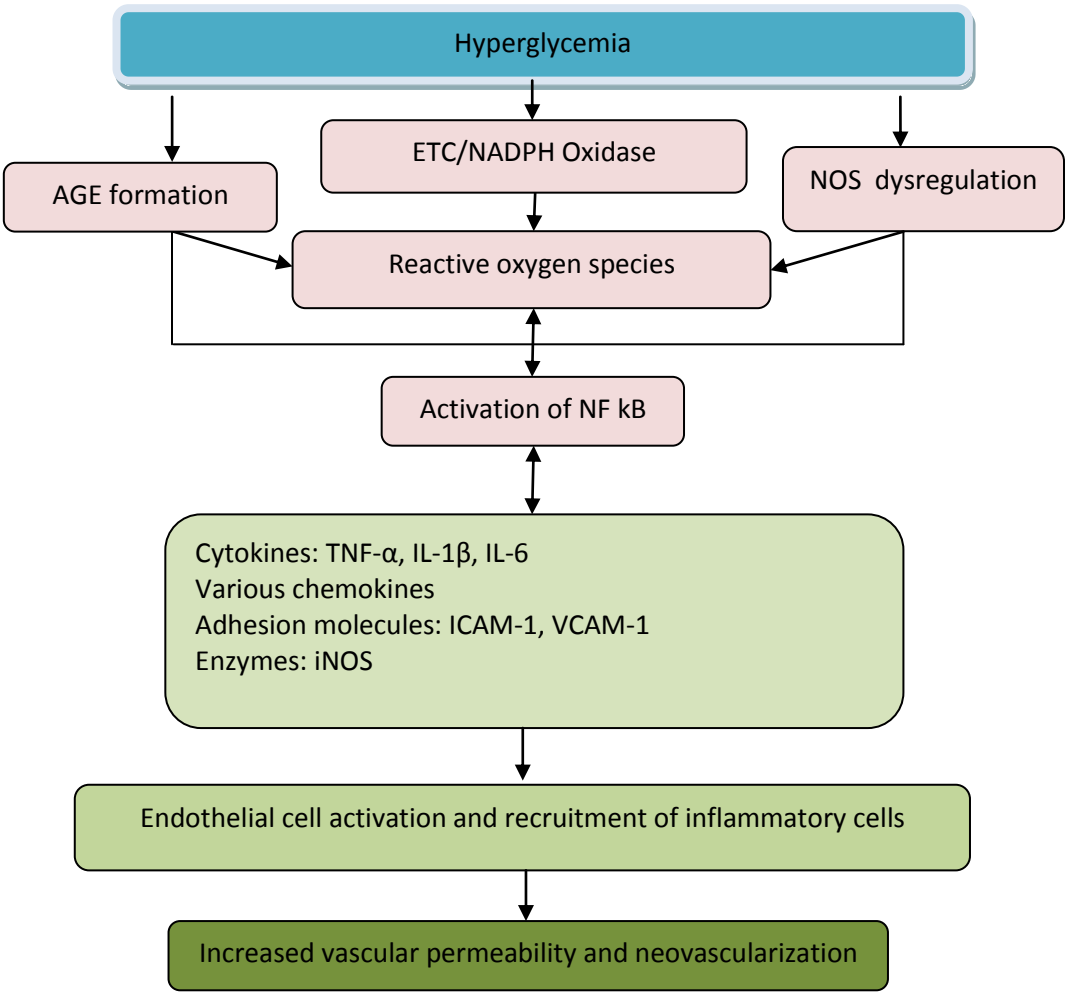

Fig. 1: It shows the cascade of events that contribute to the inflammatory response in diabetic retinopathy [4]**.**

INFLAMMATION IN DIABETIC NEPHROPATHY (DN)

Several studies have suggested a relationship between pro-inflammatory cytokines and DN mainly, IL-1, IL-6, IL-18, TNF-α and TGF-β1 [12, 13]. Endothelial, mesangial, glomerular and tubular epithelial cells synthesize various pro-inflammatory cytokines, which are implicated in pathologic processes of DR. Interleukin-1 enhances proliferation of mesangial cells and matrix synthesis, increases vascular permeability, leading to the development of intraglomerular microcirculatory abnormalities [14,15]. It also alters the expression of chemotactic factors and adhesion molecules. It alters intraglomerular hemodynamics by affecting mesangial cell prostaglandin synthesis [16]. Interleukin-6 has strong association with mesangial cell proliferation, increase in fibronectin expression and enhanced endothelial permeability [17, 18]. IL-18 induces the production of other inflammatory cytokines, such as IL-1, interferon γ and tumor necrosis factor, and might be associated with endothelial cell apoptosis [19].

TNF-α has important implications in inflammatory processes of DN. Studies have demonstrated enhanced expression of TNF-α mRNA in kidneys of diabetic rats [20, 21]. Due to its cytotoxic action towards glomerular, mesangial and epithelial cells, TNF- α may induce significant renal damage [22]. The harmful effect of TNF-α on the protein permeability barrier of the glomerulus is independent from alterations in haemodynamic factors or effects on recruited inflammatory cells [23]. TNF-α has been suggested as a critical factor for important renal alterations that occur during the initial stage of DN e.g. sodium retention and renal hypertrophy [24]. It has also been demonstrated that increased urinary and renal interstitial concentrations of TNF-α precede the occurrence of albuminuria [25]. A significant relationship has been observed between serum levels of TNF-α and urinary protein excretion in diabetic patients with normal renal function as well as in subjects with overt nephropathy and renal failure [12, 13]. This indicates that the deleterious effects of TNF-α begin very early in the process of diabetic nephropathy. Thus, it may serve as trigger for many inflammatory mechanisms. Apart from this it may also have a perpetuating role in the progression of pathology as it has been shown that there is a significant rise of urinary TNF-α excretion as DN progresses.

There is an increased expression of TGF β1 in glomeruli of streptozotocin induced diabetic animal [26]. There is clear evidence indicating the contribution of TGF β1 to cellular hypertrophy and increased synthesis of collagen, which have fibrogenic effects in kidneys leading to thickening of glomerular basement membrane observed in diabetic nephropathy [26-29]. The important role of TGF β1 is indicated by amelioration of DN in mice by administration of hepatocyte growth factor which specifically blocks profibrotic action of TGF β1 [30].

INFLAMMATION AND DIABETIC NEUROPATHY

Diabetic neuropathy develops as a result of local metabolic, enzymatic and microvascular changes induced by hyperglycaemia.

Polyneuropathy is mainly associated with two pathologies, axonal loss or demyelinative changes. Pro-inflammatory cytokines are produced locally by resident and infiltrating cells recruited by chemotaxis. These molecules exhibit multiple important effects on gial and neuronal homeostatic metabolic reactions. Chronic hyperglycaemia leads to dysregulation of various local cytokines, which may lead to activation of inflammatory effects of these cytokines. It has been shown that human peripheral diabetic neuropathy is associated with increased biochemical markers of inflammation and endothelial dysfunction. Moreover, painful neuropathy is associated with further increase in inflammation and markers of endothelial dysfunction and preservation of the nerve axon reflex [31]. It has been demonstrated that endogenous TNF-α production is accelerated in microvascular and neural tissues, which may lead to increased microvascular permeability, hypercoagulability and nerve damage. This initiates and promotes the development of characteristic lesions of diabetic polyneuropathy [32]. Infiltration with inflammatory leucocytes may lead to occlusion of perineural blood vessels. Mixed, axonal and demyelinative nerve lesions have been found associated with increased endoneurial infiltration with mononuclear cells associated with low-grade endoneurial inflammatory process. Persistent inflammation may lead to segmental demyelination and remyelination of nerve fibres and necrotizing vasculitis of perineurial and endoneurial blood vessels. This leads to ischemia and further generation of reactive oxygen species which enhances the ongoing inflammatory process [33]. Moreover, it has been shown that TNF-*α* promoter gene polymorphism, C(−857)T, is significantly associated with prolonged F-wave latency in the median nerve, that is a sensitive marker of peripheral nerve dysfunction, in patients with type 2 diabetes [32]. Michałowska-Wender G et al, did not find difference in the expression of TNF-α in blood serum of patients of diabetic polyneuropathy and control subjects. However, they observed higher levels of GRO-α in patients with subgroup of diabetic polyneuropathy with demyelinating changes [34]. GRO-α, the growth-regulated oncogene acting in some neoplastic and inflammatory processes, promotes tumour growth, metastases and infiltration by leukocytes. Uceyler et al, has also found association of various proinflammatory cytokine levels with pain in diabetic neuropathy [35].

INFLAMMATION AND MACROVASCULAR COMPLICATIONS

Several studies have shown an association between inflammatory markers and increased incidence of cardiovascular disease in diabetic patients. Extravascular or intravascular proinflammatory conditions such as oxidized LDL, advanced glycosylation end-products (AGE), or chronic infection lead to an increased secretion of proinflammatory cytokines such as interleukin-1 (IL-1), TNF, and IL-6. These in turn influence all processes of atherogenesis from increased monocyte adhesion to endothelial cells to increased risk of atherosclerotic plaque rupture. Insulin deficiency is also responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles [36]. AGE-modified proteins augment the production of proinflammatory cytokines and other inflammatory pathways in vascular endothelial cells by binding with surface receptors such as RAGE. Beyond the hyperglycemia, the diabetic state promotes oxidative stress mediated by reactive oxygen species and carbonyl groups. Thus there is a sizable amount of evidence suggesting that, inflammation links diabetes to atherosclerosis. A clinical study has shown that Phagocytic Index can be used as a marker to predict Ischaemic Heart Disease (IHD) events in DM patients, in whom lipid values are not predictive of IHD [37].

Hyperglycemia enhances Monocyte adhesion to endothelial cells. This serves as an early inflammatory stimulus leading to release of IL 8. IL-8 leads to chemotaxis of neutrophils and mediates monocyte–endothelial cell interactions. Glucose is known to regulate IL-8 production at the level of transcription. AGEs stimulate the production of IL-1, TNF-α, and granulocyte-macrophage colony-stimulating factor (GMC-SF) by macrophages. AGEs induce activation of the stress-sensitive NFκB pathway in vascular endothelial cells via generation of free radicals.

Monocyte–endothelial cell interactions are regulated by various adhesion molecules, such as, intercellular adhesion molecule-1 (ICAM), vascular cell adhesion molecule-1 (VCAM), E-selectin, P-selectin, and their ligands: LFA-1, Mac-1, VLA-4, and PSGL-1. Elevated levels of Soluble cell adhesion molecules (sCAMs) mainly ICAM, have been found in patients with type 2 diabetes [38].

METHODS USED IN VARIOUS STUDIES TO EVALUATE ANTI INFLAMMATORY ACTIVITY WITH RESPECT TO DIABETIC COMPLICATIONS

Determination of inflammatory markers is an important parameter used by various preclinical and even clinical studies. Estimation of various cytokine levels or their upstream or downstream mediators is the most widely used approach.

PKC-β1 and 2 are chiefly responsible for the deleterious effects on retinal, neural, and renal tissues. These isoforms impair retinal and renal blood flow and increase capillary leakage. PKC-induced increased extracellular matrix production and upregulation of various inflammatory cytokines further damage the macro and microvascular systems [39]. Tsai et al determined glomerular PKC activity, as a parameter for inflammatory nephropathic damage [40]. Shi et al., has measured the levels of IL-1β, TNF-α, IL-2, IL-6, and interferon (IFN)-γ as a measure of microvascular complications in target tissues in STZ induced diabetic rats [41]. Antioxidant enzyme activity plays a pivotal role in inflammatory processes associated with diabetic complications. For study of antioxidant enzyme activity, pancreas is immediately removed after termination of animals with STZ induced diabetes. Many researchers have determined the levels of reactive oxygen species (ROS), activity of glutathione peroxidase, superoxide dismutase (SOD), activity of glutathione reductase, and activity of catalase in diabetic animals as well as in clinical studies as marker for various microvascular complications [42-44]. RT PCR can be used to study expression of mRNA of various inflammatory mediators like, IL-1β, TNF-α, MCP-1, TGF-β1, fibronectin, NF-κB p50, NF-κB p65, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, the housekeeping gene) in target tissue [45]. Apart from this various inflammatory mediators can also be quantified by Western Blot Analysis from the tissue homogenate [40]. Fibrosis is one of the important processes of proliferative phase of chronic inflammation, involving mediators like TGF-β1, fibronectin and Type 4 collagen. This is an important pathologic process responsible for proliferative changes in Microvascular complications of diabetes. Many researchers have measured the levels of these cytokines in various target organs like renal cortex [40].

It has been clearly indicated that chronic subclinical inflammation is a part of insulin resistance syndrome [45]. Drugs which are effective in dampening such subclinical inflammation may provide protection against development of diabetic complications, which may be reflected partly as a protection provided by drug in experimental models of inflammation. Fatehi et al., has studied effects of Genistein, a tyrosine kinase Inhibitor on acute and chronic inflammation in STZ induced diabetic mice. Carrageenan-induced paw edema in mice is used as model of acute inflammation, while chronic inflammation is produced by cotton pellet induced granuloma in mouse [46]. Mice with no induced diabetes show increased inflammatory response as compared to normal mice because of ongoing inflammation in the tissue. Chronic treatment with drug hampers the process of inflammation, which may be reflected as effectiveness in these experimental models.

CONCLUSION

Diabetic complications are the concern for health system in today's scenario. Different diabetic complications have significantly higher prevalence in developing countries like India. As a need for developing antidiabetic drugs which may also protect against diabetic complications, it is necessary to understand pathophysiological mechanisms involved in them. Many factors have been identified and studied, of which inflammation is the major one. However, methods available for study of anti inflammatory activity with respect to diabetic complications are very few. There is a need to develop simple model which represents inflammation of diabetic complications to gear up drug discovery in this area.

REFERENCES

1. Navarro JF, Mora C. Role of inflammation in diabetic complications. Nephrol dial Transplant 2005; 20: 2601-4.
2. Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, et al. Diabetic retinopathy. Diabetes Care 1998; 21:143–56.
3. Klein R, Klein BE. Diabetic eye disease. Lancet 1997; 350:197–204.
4. Zhang W, Liu H, Al-Shabrawey M, Caldwell RW, Caldwell RB. Inflammation and diabetic retinal microvascular complications. J Cardiovasc Dis Res 2011; 2:96-103.
5. Davidson JA, Ciulla TA, McGill JB, Kles KA, Anderson PW. How the diabetic eye loses vision. Endocrine 2007; 32:107–16.
6. Yuan SY. Protein kinase signaling in the modulation of microvascular permeability. Vascul Pharmacol 2002; 39:213–23.
7. Aveleira CA, Lin CM, Abcouwer SF, AmbrÓsio AF, Antonetti DA. TNF-α signals through PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes 2010; 59:2872–82.
8. Kowluru RA, Odenbach S. Role of interleukin-1β in the pathogenesis of diabetic retinopathy. Br J Ophthalmol 2004; 88:1343–7.
9. Miyamoto K, Khosrof S, Bursell SE, Rohan R, Murata T, Clermont AC, et al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. Proc Natl Acad Sci USA 1999; 96:10836–41.
10. Joussen AM, Poulaki V, Mitsiades N, Cai WY, Suzuma I, Pak J, et al. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. FASEB J 2003; 17:76–8.
11. Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: Cause or consequence? Angiogenesis 2007; 10:149–66.
12. Navarro JF, Mora C, Rivero A, Gallego E, Chahin J, Macía M, et al. Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: Effects of pentoxifylline administration. Am J Kidney Dis 1999; 33: 458–63.
13. Navarro JF, Mora C, Macía M, García J. Inflammatory parameters are independently associated with urinary albumin excretion in type 2 diabetes mellitus. Am J Kidney Dis 2003*;* 42*: 53*–61.
14. Royall JA, Berkow RL, Beckman JS, Cunningham MK, Matalon S, Freeman BA. Tumor necrosis factor and interleukin 1 increase vascular endothelial permeability. Am J Physiol *1989*; *257*: *L339*–410.
15. Melcion C, Lachman L, Killen D, Morel-Maroger L, Striker GE. Mesangial cells, effect of monocyte products on proliferation, and matrix synthesis. Transplant Proc 1982*;* 14*:* 559–*64.*
16. Jones S, Jones S, Phillips AO. Regulation of renal proximal tubular epithelial cell hyaluronan generation: implications for diabetic nephropathy. Kidney Int 2001; 59:1739-49.
17. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigio AM, Saller A, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol 2005; 16: S78–S82.
18. Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspect of interleukin *6.* Immunol Today 1990*;* 11*:* 443-*9.*
19. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008; 19: 433-42.
20. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, *et al. mRNA* expression of growth factors in glomeruli from diabetic rats. Diabetes 1993*;* 42*:* 450-6.
21. Navarro JF, Milena FJ, Mora C, León C, Claverie F, Flores C, *et al.* Tumor necrosis factor-α gene express on in diabetic nephrophathy: relationship with urinary albumin excretion and effect of angiotensin-converting enzyme inhibition. Kidney Int 2005; 99 Suppl: 98-102.
22. Ortiz A, González-Cuadrado S, Bustos C, Alonso J, Gómez-Guerrero C, López-Armada MJ, et al. Tumor necrosis factor as a mediator of glomerular damage. J Nephrol 1995*;* 8*:* 27–34.
23. McCarthy ET, Sharma R, Sharma M, Li JZ, Ge XL, Dileepan KN, et al. *TNF-α* increases albumin permeability of isolated rat glomeruli through the generation of superoxide. J Am Soc Nephrol 1998*;* 9*:* 433–*8.*
24. Dipetrillo K, Coutermarsh B, Gesek FA. Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. Am J Physiol Renal Physiol 2003*;* 284*:* F113–F121.
25. Kalantarinia K, Ais AS, Siragy HM. Urinary and renal interstitial concentrations of TNF-α increase prior to the rise in albuminuria in diabetic rats. Kidney Int 2003*;* 64*:* 1208–*13.*
26. Sharma K, Eltayeb BO, McGowan TA, Dunn SR, Alzahabi B, Rohde R, Ziyadeh FN, Lewis EJ. Captopril-induced reduction of serum levels of transforming growth factor-beta1 correlates with long-term renoprotection in insulin-dependent diabetic patients. Am J Kidney Dis 1999; 34:818-23.
27. Sharma K, Ziyadeh FN. Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. Diabetes 1995; 44: 1139-46.
28. Janssen B, Hohenadel D, Brinkkoetter P, Peters V, Rind N, Fischer C, et al. Carnosine as a protective factor in diabetic nephropathy: association with a leucine repeat of the carnosinase gene CNDP1. Diabetes 2005; 54: 2320-7.
29. Isaka Y, Akagi Y, Ando Y, Imai E. Application of gene therapy to diabetic nephropathy. Kidney Int 1997; 60 Suppl: 100-3.
30. Dai C, Yang J, Bastacky S, Xia J, Li Y, Liu Y. Intravenous administration of hepatocyte growth factor gene ameliorates diabetic nephropathy in mice. J Am Soc Nephrol 2004; 15: 2637-47.
31. Doupis J, Lyons TE, Wu S, Gnardellis C, Dinh T, Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. J Clin Endocrinol Metab 2009; 94:2157–63.
32. Satoh J, Yagihashi S, Toyota T. The possible role of tumor necrosis factor-α in diabetic polyneuropathy. Exp Diabesity Res 2003; 4: 65–71.
33. Said G. Inflammation in Diabetic Neuropathies. Eur Neurol Rev 2006; 6:73-6.
34. Michałowska-Wender G, Adamcewicz G, Wender M. Impact of cytokines on the pathomechanism of diabetic and alcoholic neuropathies. Folia Neuropathol 2007; 45:78-81.
35. Uçeyler N, Rogausch JP, Toyka KV, Sommer C. Differential expression of cytokines in painful and painless neuropathies. Neurology 2007; 69:42-9.
36. Saklani A, Parcha V, Dhulia I, Kumar D. Combined effect of *Coccinia indica* (Wight & Arn) and *Salvadora oleoides* (Decne) on blood glucose level and other risk factors associated with type 2 diabetes mellitus in alloxan induced diabetic rats. Int J Pharm Pharm Sci 2012; 4: 79-84.
37. Bernhardt V, D'souza JRT, Shetty A, Shantaram M, Vaswani R. Evaluation of neutrophil function, opsonising capacity and lymphocyte proliferation for risk of developing ischemic heart disease in type 2 diabetes mellitus patients. Int J Pharm Pharm Sci 2012; 4:318-22.
38. Huerta MG, Nadler JL. Role of inflammatory pathways in the development and cardiovascular complications of type 2 diabetes. Curr Diabetes Rep 2002; 2:396–402.
39. Cumbie BC, Hemayer KL. Current concepts in targeted therapies for the pathophysiology of diabetic microvascular complications. Vasc Health Risk Manag 2007; 3: 823–32.
40. Tsai SJ, Huang CS, Mong MC, Kam WY, Huang HY, Yin MC. Anti-inflammatory and Antifibrotic Effects of Naringenin in Diabetic Mice. J Agric Food Chem 2012; 60: 514–21.
41. Shi YC, Liao JW, Pan T. Antihypertriglyceridemia and Anti-Inflammatory Activities of Monascus-Fermented Dioscorea in Streptozotocin-Induced Diabetic Rats. Exp Dia Res 2011; doi:10.1155/2011/710635
42. Abd Ellah MR, Niishimori K, Goryo M, Okada K, Yasuda J. Glutathion peroxidase and glucose-6-phosphate dehydrogenase activities in bovine blood and liver. J Vet Med Sci 2004; 66:1219-21.

43. Xie Z, Wu X, Gong Y, Song Y, Qiu Q, Li C. Intraperitoneal injection of Ginkgo biloba extract enhances antioxidation ability of retina and protects photoreceptors after light-induced retinal damage in rats. Curr Eye Res 2007; 32:471-9.
44. Yue WF, Li GL, Liu JM, Sun JT, Sun HX, Li XH, et al. Anti-oxidation and immune responses in mice upon exposure to manganese superoxide dismutase expressed in silkworm larvae, Bombyx mori L. Cell Biol Int 2007; 31:974-8.
45. Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000; 102: 42–7.
46. Fatehi M, Jafarzadeh M, Hassanabad ZF, Gholamnezhabad Z. The effect of Genistein, a tyrosine kinase Inhibitor on acute and chronic inflammation in Diabetic mice. DARU J Pharm Sci 2006; 14: 6-10.