Monocyte Chemotactic Protein -1, Nitrite and C - reactive protein in Diabetic Retinopathy

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ABSTRACT Background: Diabetic retinopathy (DR) is a major cause of visual loss worldwide. DR presents in 10% of persons with diabetes Although hyperglycemia and hypertension are cleary involved in the pathogenesis of DR , other risk factors and pathogenetic pathways are not fully elucidated despite substantial research. **Objective:** This study was planned to look for a relationship between moncyte chemotactic protein- 1(MCP-1) levels in the aqueous humor and serum and diabetic retinopathy (DR) , also to evaluate the possible role of serum nitrite and C-reactive protein (CRP) in the development of DR. **subjects& methods**: The study included 90 type-2 diabetic patients, 45 of them complicated with non proliferative diabetic retinopathy(NPDR)(group1) and 45 patients had proliferative diabetic retinopathy (PDR) (group 2), as well as 42 healthy subjects served as control group (group 3) . All subjects were subjected to complete clinical examination, thorough ocular examination including ophthalmoscope and proper investigations with stress on lipid profile , fasting, 2hour postprandial blood glucose, HbA1c , serum MCP-1, serum CRP, serum nitrite and aqueous MCP-1 **Results**:A significant increase in the aqueousMCP-1 level was detected in patients with diabetic retinopathy when compared with control group (P=0.014). There was nonsignificant difference in aqueous MCP-1 in – between both groups of DR (P=0.624). On the other hand , there was nonsignificant difference in serum MCP-1 between patients with DR and control group (P=0.086). There was a very highly significant increase in the serum nitrite level in patients with DR when compared with control group (P<0.001), also a very highly significant increase in the sprum nitrite level was detected in PDR group compared with NPDR group (P<0.001). On the other hand, there was nonsignificant difference in serum CRR level between patients with DR and control group (P=0.86). There were significant increases in the serum cholesterol , serum triglyceride, serum LDL levels while there was significant decrease in serum HDL level in patients with diabetic retinopathy when compared with the control group. Also , a highly significant positive correlation was found between serum MCP-1 and HbA1c level in patients with DR. **conclusion**:MCP-1 in the aqueous humor as a marker is diagnostic for DR rather than its severity , also the role of nitric oxide in DR not only confined to the development but also to the progression of the disease.

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1. Introduction

Cardiovascular Complications are the leading cause of morbidity and mortality in patients with diabetes mellitus; up to 80% of deaths in patients with diabetes are closely associated with vascular disease affecting microcirculation or macrocirculation. (1)

Diabetic retinopathy (DR) is one of the most common complications of diabetes. This devastating disease is a leading cause of blindness in people of working age in industrialized countries and affects the daily lives of millions of people. Despite tight glycemic control, blood pressure control and lipid-lowering therapy, the number of DR patients keeps growing and therapeutic approaches are limited. (2) Moreover, there are significant limitations and side effects associated with the current therapies. Thus there is a great need for development of new strategies for prevention and treatment of DR. Studies have shown that DR has prominent features of chronic, subclinical inflammation. (2)

It was found by fudus examination and specific cytokines level assessment that cytokines directly take part in pathogenesis of retinopathy before clinical manifestation. (3)

Monocyte chemotactic protein-1 (MCP-1), also known as monocyte chemoattractant and activating factor (MCAF), is one of chemokines which initially identified as the most important chemoattractant for monocytes and macrophages. (4) It has been shown now to attract as well activated T cells, natural killer (NK) cells and basophiles. MCP-1 is produced and secreted by a variety of cells such as vascular endothelial cells, vascular smooth muscle cells, monocytes, and fibroblasts in response to specific

stimuli. MCP-1 mediates its function through interacting with chemokine receptor CCR2 [4,5]

Retinal ischemia due to capillary occlusion plays a crucial role in pathogenesis of DR. One of the possible mechanisms of capillary occlusion is an increase in adhesion of macrophages to the endothelium. Experimental studies have shown association between macrophages activation and retinal angiogenesis [6] In addition, immunohistochemical analysis determined the presence of intraocular macrophages in the human proliferative epiretinal membrane secondary to DR [7]. Also, hypoxia was found to increase MCP-1 mRNA and protein expression in a mouse model with hypoxia-induced ocular neovascularization, and that the injection of anti-MCP-1 antibodies depressed the inflammatory neovascularization in this model[8]

Human retinal pigment epithelial cells have been shown to express chemokines including MCP-1, accounting for the majority of monocyte chemotactic activity in supernatants of human retinal pigment epithelial cells which are stimulated with IL-l and TNF-α suggesting the possibility that MCP-1 is involved in pathogenesis of DR [9].

Nitric oxide (NO) is a free radical of low molecular weight with an unshared electron that can regulate an ever-growing list of biological processes. It is present practically in all tissues. It defuses easily across cell membranes because its lipophilic properties [10].

NO is extremely unstable and easily oxidized causing difficult in vivo measurement, so evaluation of the stable NO end products nitrite ($N02^-$) and nitrate ($N03^-$) collectively in biologic fluid is used to estimate NO production [11].

Endothelial dysfunction with increased generation of oxygen-derived free radicals is a critical factor, and endothelium-dependent vasodilatation is impaired in both types of DM [12].

Vascular endothelial dysfunction develops in large part as a consequence of acquired defects of nitric oxide (NO) signaling [13].

NO plays at least two critical and competing roles: vasoregulatory signaling and detoxification of ROS " Reactive oxygen species". As hyperglycemia induces the generation of ROS by several mechanisms including formation of AGE " advanced glycation end products", lipid peroxidation and also dysregulation of transition metals that serve as catalysts for autooxidation, this large amount of ROS alters the utilization of NO from vasoregulatory tasks such as vasodilation and thrombolysis to detoxification. This shift results in local oxidative tissue injury and impaired NO mediated vasodilation [13].

Therefore, measurement of serum NOx (stable oxidized metabolites) in patients may be useful for understanding the status and pathophysiology of inflammatory diseases and those in which inflammation is a component[14].

Elevated intraocular nitric oxide (NO) has been reported in DR [15]. Also the activity of nitric oxide synthase (NOS) has been documented to be increased in the retina of diabetic rats compared with normal rats[16].

Chronic inflammation emerges as a potential mediator of microvascular complications of diabetes including DR[17]. As a marker of systemic inflammation C-reactive protein (CRP) is produced by hepatocytes under the influence of cytokines especially IL-6 which is a proinflammatory cytokine proved to be increased in DR [18].

CRP has pleiotropic effects, both "pro-inflammatory" and "antiinflammatory" activities have been described. In addition to the antiinflammatory effects, CRP has been shown to induce the expression of interleukin-1 receptor antagonist and increase release of the antiinflammatory cytokine interleukin-10 while repressing synthesis of interferon- gamma ([19].

CRP also activates complement, enhances phagocytosis, up-regulates the expression of adhesion molecules in endothelial cells, inhibits endothelial nitric-oxide synthase expression in aortic endothelial cells [20].

The net effect of CRP-mediated complement activation is that CRP can participate in host defense systems while limiting the potentially damaging inflammatory effects of the late stage complement components [21].

CRP as an inflammatory biomarker was found to be involved in endothelial dysfunction and atherogenesis and has been associated with macrovascular disease and the nonocular microvascular complications of diabetes [22]. Data on a possible association of CRP with DR, however, are sparse, and results from limited studies have been inconsistent [23].

So this study was planned to look for a relationship between MCP-1 levels in the aqueous humor and serum and diabetic retinopathy, also to evaluate the possible role of serum nitrite and CRP in the development of diabetic retinopathy.

2. Subjects and Methods:

This study was carried out in the departments of Internal medicine, Ophthalmology and Biochemistry, Faculty of medicine, Zagazig University.

The study was conducted on 132 subjects with immature cataract (54 males and 78 females) including 90 patients with type 2 DM complicated by diabetic retinopathy (DR) and 42 healthy subjects.

They were divided into the following groups :

Group 1 : It included 45 patients of non proliferative diabetic retinopathy (NPDR), 18 males and 27 females . The duration of diabetes was between 9-14 years with a mean value ± SD of (3.15 ± 1.66), their ages were between 52-75 years with a mean value ± SD of (60.7 ± 6).

Group 2 : It comprised 45 patients of proliferative diabetic retinopathy (PDP), 24 males and 21 females. The duration of diabetes was between 10-16 years with a mean value ± SD of (12.72 ± 3.23) , their ages range from 54-73 years with a mean value ± SD of (63 ± 5.2).

Group 3: It included 42 healthy subjects with immature cataract , 12 males and 30 females, of matched age as diabetic patients with a mean value ± SD (61.57 ± 9.6).

Patients were randomly recruited from those attending the diabetes outpatient clinic of Zagazig University hospitals.

After being informed on the purpose and procedures of the study, all subjects signed an informed consent form.

Type 2 DM was diagnosed according to American Diabetes Association Guidelines for diagnosis and classification of DM [24] .

Diabetic retinopathy was diagnosed according to the history and ophthalmoscopic examination and fluorescine angiography after pupillary dilatation, retinopathy was scored into non proliferate and proliferate types [25] .

The following criteria were considered as exclusion criteria : prior ocular surgery , history of intraocular inflammation, history of intraocular ischemia due to causes other than diabetic retinopathy , hypertension, hepatic and renal diseases.

All patients and control subjects were submitted to :

* Thorough history taking with special stress on age, sex, duration of diabetes and type of treatment.
* Proper clinical examination with special stress on signs of liver diseases, Body mass index (BMI) determination and waist circumference, blood pressure determination and signs of diabetic complications.
* Thorough complete ocular examination including visual acuity by Landolt chart , slit lamp biomicroscopy, applanation tonometry and ophthalmoscopy.
*Cataract surgery by extracapsular cataract extraction and phacoemulsification
* Laboratory investigations including :
- CBC
- Liver functions tests
- Blood urea and serum creatinine
- Lipid profile (HDL, LDL, triglycerides and total cholesterol)
- Fasting and 2 hour postprandial blood glucose.
- Glycosylated haemoglobin ($HbA1_c$)
- Serum MCP-1 by ELISA (enzyme linked immunosorbant assay),serum nitrite by colorimetric assay using Griess reaction and serum CRP by turbidimetry ; all of these markers measured from fasting venous blood samples taken in the morning after 12 hours fast.

-Aqueous MCP-1 : 100 ul of aqueous humor samples were aspirated at the start of cataract surgery and they were kept frozen at - 20 ^{0}C until the time of estimation of MCP-1 by ELISA according to the method of **Akoun etal** [26]

Statistical analysis:

Data analyzed by WINPEP statistical program. All data are expressed as means ± SD. Analysis of trends was performed using linear regression.

When comparing two groups, a student's test was used , and to analyze data among groups of three or more , a one way ANOVA was performed and secondary analysis was performed with the student's test with Bonferroni correction .

3. Results

Table (1): Comparison between aqueous MCP-1 levels in different groups of the study:

There is a significant increase in the aqueous MCP-1 level in patients with diabetic retinopathy when compared with control group (P=0.014). Also a significant increase in the aqueous MCP-1 level in both groups of diabetic retinopathy when compared with control, as patients with NPDR have a significantly higher aqueous MCP-1 levels compared with controls (P=0.042). Also patients with PDR have significant increase in aqueous MCP-1 levels compared with controls (P=0.027). On the other hand, there is non significant increase in aqueous MCP-1 level in PDR compared with NPDR (P=0.624).

Table (2): Comparison between serum MCP-1 levels in different groups of the study:

There is non significant difference between serum MCP-1 in the 3 studied groups when compared with each others

Table (3): Comparison between serum nitrite levels in the studied groups :

There is a very highly significant increase in the serum nitrite level in patients with diabetic retinopathy when compared with control (P<0.001). Also a significant increase in the serum nitrite level in both groups of diabetic retinopathy when compared with control, as patients with NPDR have significantly higher serum nitrite levels compared

with control (P=0.004). Also patients with PDR have significantly very higher serum nitrite levels compared with control (P<0.001). There is also a very highly significant increase in serum nitrite level in PDR compared with NPDR groups (P<0.001).

Table (4): Comparison between CRP levels in the studied groups:

There is non significant difference between serum CRP level in the 3 studied groups when compared with each others.

Table (5) : Comparison between lipid profile levels in different groups of the study :

There is a significant increase in the serum cholesterol level, serum TG level and serum LDL level in patients with diabetic retinopathy when compared with control.

There is significant decrease in serum HDL levels in patients with diabetic retinopathy when compared with control (P=0.06).

Also, there is highly significant increase in serum TG levels in patients with NPDR when compared with control (P=0.008) .

In addition there is a significant increase in the serum cholesterol levels, serum TG levels and serum LDL levels in patients with PDR when compared with control.

There is highly significant decrease in serum HDL levels in patients with PDR when compared with control (P=0.008).

There is non significant increase in the serum cholesterol level, serum TG level and serum LDL level in patients with PDR when compared with NPDR . Although, there is significant decrease in serum HDL level in PDR when compared with NPDR (P=0.041).

Table (6): Correlation between serum MCP-1 levels , HbA1c levels and fasting blood glucose levels in the studied groups :

There is a highly significant positive correlation between serum MCP-1 levels and HbAlc in patients with DR (P=0.002) but there is no correlation between serum MCP-1 levels and fasting blood glucose in all studied groups

Table (1): Comparison between aqueous MCR-1 levels(pg/ml) in each two groups by Mann Whitney test:

Group	Median	95% CI	Z	P
Control	64.405	44.2- 132.3	2.445	**0.014***
DR	106.86	16-783.3		
Control	64.405	44.2- 132.3	2.03	**0.042***
NPDR	97.402	65.73- 280.24		
Control	64.405	44.2- 132.3	2.206	**0.027***
PDR	112.52	95.54- 223.6		
NPDR	97.402	65.73- 280.24	0.518	**0.624**
PDR	112.52	95.54- 223.6		

Table (2): Comparison between serum MCP-1 levels (pg/ml) in each two groups by Student's t test:

Group	Mean	SEM	t	P
Control	61.69	11.36	1.7	0.086
DR	45.96	3.14		
Control	61.69	11.36	1.175	0.250
NPDR	47.44	4.99		
Control	61.69	11.36	1.47	0.153
PDR	44.47	3.95		
NPDR	47.44	4.99	0.465	0.646
PDR	44.48	3.95		

Table (3): Comparison between serum nitrite levels (µmol/l)in each two groups by Mann Whitney test:

Group	*Median*	*95% CI*	*Z*	*P*
Control	9.39	7.57-10.72	4.297	***<0.001******
DR	21.8	12.05-46.41		
Control	9.39	7.57-10.72	2.86	***0.004****
NPDR	14.35	12.05-21.41		
Control	9.39	7.57-10.72	4.583	***<0.001******
PDR	38.25	25.94 -46.41		
NPDR	14.35	12.05-21.41	3.443	***<0.001******
PDR	38.25	25.94 -46.41		

Table (4): Comparison between serum CRP levels(mg/l) in each two groups by Student'st test:

Group	*Mean*	*SEM*	*t*	*P*
Control	10.43	2.183	0.171	***0.86***
DR	9.94	1.66		
Control	10.43	2.183	0.849	***0.403***
NPDR	7.846	2.125		
Control	10.43	2.183	0.481	***0.634***
PDR	12.05	2.508		
NPDR	7.85	2.125	1.277	***0.212***
PDR	12.05	2.508		

Table (5): Comparison between lipid profile levels (mg/dl) in each two groups by Student's t test:

	Cholesterol			*Triglycerides*			*HDL*			*LDL*		
Group	*Mean*	*SEM*	*P*	*Mean*	*SEM*	*P*	*Mean*	*SEM*	*P*	*Mean*	*SEM*	*P*
Control	181.28	9.45	*0.042**	123.07	8.35	*0.04**	54.92	3.75	*0.05**	105.21	10.75	*0.04**
DR	230.8	15.39		177.46	17.5		46.3	2.35		145.7	12.16	
Control	181.28	9.4	*0.166*	123.07	8.35	*0.008***	54.92	3.75	*0.454*	105.21	10.75	*0.122*
NPDR	204.86	13.32		149.13	16.08		51.06	2.83		129.53	10.77	
Control	181.28	9.45	*0.015**	123.07	8.35	*0.016**	54.928	3.75	*0.008**	105.21	10.75	*0.029**
PDR	256.73	26.62		205.8	29.99		41.53	3.43		161.86	21.43	
NPDR	204.86	13.32	*0.092*	149.13	16.08	*0.107*	51.06	2.83	*0.041**	129.53	10.77	*0.189*
PDR	256.73	26.62		205.8	29.99		41.53	3.43		161.86	21.43	

Table(6): Correlation between serum MCP-1 levels (pg/ml, HbAlc levels (%) and fasting blood glucose levels (mg/dl) in all studied groups by Pearson correlation Coefficient:

	Serum MCP-1							
	Control group		*Diabetic retinopathy*		*NPDR*		*PDR*	
	rho	*P*	*rho*	*P*	*rho*	*P*	*rho*	*P*
HbAlc (%)	0.172	*0.556*	0.817	*0.002***	-0.304	*0.27*	0.28	*0.313*
blood glucose	0.236	*0.208*	0.082	*0.655*	0.36	*0.094*	-0.015	*0.296*

4. DISCUSSION

Diabetic retinopathy, a principal cause of blindness, is characterized by increased retinal vascular permeability and progressive retinal vascular closure, resulting in tissue hypoxia and neovascularization [(27).] Although the precise mechanisms are not fully understood, components of inflammation and endothelial dysfunction have been demonstrated to be involved in the pathogenesis and progression, especially in PDR[(15)].

Based on the results of this study, there was a statistically significant increase in MCP-1 levels in the aqueous humor of patients with diabetic retinopathy in comparison with control group; and a non significant increase with the progression of NPDR into PDR (table1) .This finding is in

consistency with that obtained by a study done by **Tashimo et al[28].** which reported that aqueous MCP-1 levels were significantly higher in patients with DR than non diabetic patients with the highest levels in PDR. Chemokines that induce chemotaxis of particular leucocytes populations such as IL-8 and MCP-1, have been identified and elevated in vitreous samples of patients of DR [29,30].

Our findings are consistent with prior studies that reported intraocular MCP-1 concentrations were correlated with the retinal neovascularization and the amount of macular edema [31].

Our results were also supported by those obtained by **Wakabayashi etal. [32]** who reported that vitreous concentration of MCP-1 was increased significantly in patients with both active and inactive DR compared with control subjects and that MCP-1 may play an important role in the pathogenesis of DR and works in consort with VEGF (Vascular Endothelial Growth Factor) in the progression of pathological angiogenesis in DR .

The increased MCP-1 in the aqueous could be explained by the fact that hyperglycemia, a feature of diabetes mellitus, increases the expression of MCP-1 by vascular endothelial cells [33], monocytic ,retinal-pigmented epithelial cells and the Muller glial cell which is a component of epiretinal membrane formed in PDR. This expression of MCP-1 is under the control of NF-$_k$B [34]. High levels of glucose and VEGF were found to induce NF-$_k$B activation and this was followed by upregulation of MCP-1 promotor [35].

Also, hypoxia which is a characteristic event in retinopathy was found to increase MCP-1 mRNA and protein expression in a mouse model with hypoxia-induced ocular neovascularization, and that the injection of anti-MCP-1 antibodies depressed the inflammatory neovascularization in this model [8]. In addition, abundant macrophages have been observed in the retina and vitreous of diabetic patients and these infiltrating macrophages produce MCP-I [36] which by turn diffuses into the aqueous under the effect of the anterior/ posterior gradient of the eye [37].

The results of the present study also revealed that there was nonsignificant decrease of serum MCP-1 measured between patient with DR and control group(table 2) and this was in agreement with the results of **Meleth et al. [38]** and Mine et al. [39] This could be due to the possibility that the inflammatory process of retinopathy may reflect mostly local changes within the ocular tissues [38].

On the other hand**, Zietz et al.[40]** and **Chacon and Fernandez [41]** reported a significant increase of serum MCP-1 in diabetic patients compared with non-diabetic control .

Also, **Morita et al.[5]** found that circulating MCP-1 level was higher in proliferative retinopathy patients than in non-retinopathy patients in type 2 diabetics.

This controversy with the findings of the present study could be due to the possible subclinical underlying atherosclerosis and risk factors of cardiovascular disease like morbid obesity that could be present in the diabetic patients in these studies. These factors by turn might significantly increase serum MCP-1 concentrations [42] .

There was a positive correlation between HbAlc level and serum MCP-1(Table 6) and this is in agreement with the findings reported by **Kiyici et al. [43]** and **Mine et al. [39]** who found that the serum MCP-1 level is affected by the glycemic control in diabetic patients. This could be explained by the fact that HbAlc is one of AGEs formed during the course of diabetes and MCP-1 is one of the key proinflammatory mediators whose expression is stimulated by AGE &/ or high glucose signaling pathways [44] .

Our study showed a very highly significant increase in serum nitrite level in patients with diabetic retinopathy in comparison with non diabetic controls with a very highly significant increase as the disease advanced from NPDR to PDR (Table 3). This is in agreement with the studies of Doganay et al. [15] ,Ozden et al. [45] and Izumi et al. who reported similar results.

Also, our results go in harmony with those obtained by Sandra etal. [46] where they found that total nitrite and nitrate concentrations in serum in type 2 diabetic patients were significantly higher than those in the control group and they concluded that total nitrite and nitrate levels in the serum could be parameters that play relevant role in diabetes development and even suggested as markers for prognosis of diabetes.

This increased serum nitrite level in DR patients may be due to several factors including hyperglycemia which was found to increase NO production both directly by increased iNOS (inducible nitric oxide synthetase) expression and indirectly by generating AGEs which have been reported to depress NO activity , resulting in secondary upregulation of NO production due to this chronic inactivation [47]. Also, cytokines such as IL-1, TNF-a and interferon which have been reported to be increased in diabetes, induce the expression of iNOS increasing NO production [15]. In addition to excessive production by the NO-generating cells such as endothelial cells, polymorphic neutrophils and macrophages,which are known to be involved in the pathogenesis of diabetes[48].

Furthermore, increased serum NO levels are assumed to reflect NO in both the retina and other organs which may be affected in DM [11], especially that increased intraocular NO was found to cause

overproduction of peroxynitrite, a free radical which causes breakdown of the BRB, allowing diffusion of NO to systemic circulation [49].

On the other hand , **Amrita et al. [50]** found serum NO was significantly low in diabetic participants as compared to control . These different results could be due to unstability of NO and easy oxidization causing difficulty in its measurements, so it is better to measure stable NO end products as nitrite to estimate NO production as in our study . Another explanation may be the better glycemic control in the above mentioned study making oxidizing stresses less than in our study .

Our results revealed statistically nonsignificant decrease in serum CRP levels among patients with DR as compared to controls (Table 4). This coincides with the findings of **Tsunoda et al.[51]** who reported that the levels of CRP were lower in DR in comparison with control, and explaining that by the possibility that some humoral factors related to retinopathy such as insulin growth factor-1 negatively modulates the CRP production. Our findings are consistent with a number of studies which reported that CRP levels were rarely raised and did not correlate with diabetic microvascular complications especially DR[52,53,54] . Although other inflammatory markers such as von Willebrand factor (vWF) and sialic acid showed a significant increase among patients with DR. This could be explained by the inherent inflammatory character of diabetes [55].

Also, in harmony with our study, **Spijkerman etal. [56], le etal .[57]** who reported that CRP levels were not associated with DR progression, while others didn't find any associations between CRP and DR [58,59] .

On the other hand, **Van Hecke et al.[17] ,Izuora et al.[60] and Ling et al.[61]** reported that there was a significant correlation between CRP and DR and this controversy with the results of the present study could be explained by the fact that there is a racial variation in the CRP gene and it was found to affect the serum level of CRP in the general population[62] and also by the fact that hypertension present in the diabetic patients included in these studies was found to be a positive modulator of serum CRP [51].

Meanwhile , **Laurence etal . [23]** reported an inverse (or protective) association of high CRP levels with low prevalence of DR .

A possible explanation for this result is that CRP has proangiogenic properties and stimulates monocytic cells to unregulate expression of vascular endothelial growth factor.[63] Thus, elevated CRP levels may be beneficial in the preproliferative stages of DR by increasing retinal perfusion and relieving ischemia. Also CRP has also been reported to have antiinflammatory effects in monocytes through downregulation of α2-macroglobulin expression and upregulation of liver X receptor α expression [64].

The present study showed a significant increase in serum levels of cholesterol, triglycerides, LDL-cholesterol with a significant decrease in HDL-cholesterol level in patients with DR compared with non-diabetic controls (Table 5).This concides with the results of **Van Leiden et al [65].** who reported that DR was positively correlated with elevated serum cholesterol and serum TG levels.

Both type 1 and type 2 DM lead to multiple abnormalities of lipid and lipoprotein metabolism. These involve both the exogenous pathway of lipid metabolism which deals with diet-derived lipids, and the endogenous pathway which begins with the hepatic production of TG rich very low density lipoproteins (VLDLs) [66]. Also, hyperlipidaemia in diabetic patients may be due to non-hyperglycemic factors. For example, diabetes may occur in the presence of a familial syndrome of abnormal TG or LDL metabolism and secondary causes of hyperlipidaemia [67].

In contrary with these results**, Nayak and Roberts [54].** showed nonsignificant difference in the cholesterol, LDL-c and HDL-c levels in between DR patients and controls. They explained that by the unsual increase in the cholesterol and LDL-c levels in controls, which may be due to the unrestricted diets as well as possible inactive lifestyle.

From the above mentioned discussion we can reach to a conclusion that MCP-1 seems to have a strong relation to the pathogenesis of diabetic retinopathy and that MCP-1 in the aqueous humor as a marker is diagnostic for the presence of DR rather than its severity. Also, the role of nitric oxide in DR not only confined to the development but also to the progression of the disease; thus serum nitrite could be a useful marker in the diagnosis and prognosis of DR, meanwhile serum CRP level has no significant relation to the process of retinopathy. Finally, dyslipidemia is high risk factor for development and progression of DR.

Recommendation

We suggest that, in addition to lines of treatment that have shown benefits in reducing incidence and progression of DR as tight glycemic control, blood pressure control, lipid lowering therapy, laser photocoagulation, vitrectomy and VEGF blockers, anti-MCP-1 and chemokine receptors may be potential targets for therapeutic intervention to prevent or slow down the disease. In addition, diabetic patients may be benefited by antioxidants therapies that have impelling punch of pro-oxidants.

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