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Original Article

Evaluation of Monocyte Chemoattractant Protein-1 levels as diagnostic biomarker of Diabetic Retinopathy.

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Abstract

Background: Monocyte Chemoattractant Protein-1 (MCP-1) is an inflammatory bio-peptide which release into body circulation in specific response to retinal inflammation. The objective of the current study was to find the levels of monocyte chemoattractant protein-1 in diabetic patients with and without diabetic retinopathy and to compare the functional role of MCP-1 as a better diagnostic biomarker in the early phase of diabetic retinopathy with conventional methods.

Methodology: This case-control study was conducted at the Physiology Department of Basic Medical Science Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC)-Karachi, from April 2019 to October 2020. Total 100 participants between 40-65 years of age were separated into four groups: Group A (n=25) diabetic-patients with diabetes duration 5-7 years with no retinopathy; Group B (n=25) diabetic patient's diabetes duration 8-10 years with mild retinopathy, Group C (n=25) diabetic patient's diabetes duration 10-15 years with moderate retinopathy while Group D (n=25) healthy normal individuals. All study participants were gone through a detailed history and blood sugar levels, visual acuity, slit-lamp examination, and serum MCP-1 were estimated.

Results: The present study reported significantly elevated levels of MCP-1 among group-A (without retinopathy), group B (mild diabetic retinopathy), and Group- C (moderate retinopathy), while Group-D (healthy controls) had the least level of MCP-1 (p-value=0.001) that indicate the early phase of retinal inflammation. Current study has shown strong association between MCP-1 with duration of disease ($r=0.885$; $p < 0.01$), and HbA1c ($r =0.71$; $p <0.01$) in all study participants. While statistically significant and positive association and correlation were reported between MCP-1 with blood sugar parameters (fasting and random) ($r = 0.876$; $p< 0.01$) in all study participants.

Conclusion: Elevated levels of MCP-1 with the progression of early retinal inflammation diabetic retinopathy had been reported in the current study. These findings conceivably propose that MCP-1 could be an early diagnostic marker for an additional diagnostic tool for early detection of diabetic retinopathy compared with all conventional clinical findings.

Keywords

Diabetes Mellitus, Diabetic Retinopathy, Monocyte Chemoattractant Protein-1.

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Introduction

Diabetes Mellitus (DM) is an endocrine syndrome of persistent hyperglycemic condition in the absence of treatment[1,2]. Diabetic retinopathy (DR) is one of the severe complications of DM that causes visual impairment and is the chief source of irreversible blindness globally. Abnormal hyperglycemia disrupts the vasculature of the retina that causes macular degeneration and edema, retinal exfoliation, neovascularization, and ultimately glaucoma[3,4]. Clinically, DR is classified into two stages, i.e., non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).NPDR represents the beginning stage of DR, wherein penetrability in vessels and impediment of slender meshwork are the two significant pathological finding[5,6]. At this progression, retinal vascular pathologies, for example, microaneurysms, intraretinal drain, and hard exudates, become more prominent[6]. The significant regular beginning of visual misfortune in DR patients is macular edema instigated by diabetes. Diabetic Macular Edema (DME) is the thickening and swellings of the macula. DME can be introduced at any phase of DR and give ground to distortion of vision and abatement down visual sharpness[7,8]. The prevalence of NPDR is about 25.4%, and PDR is almost 1.4% globally[9,10]. The common symptoms of DR are visual impairment, dark black spots, and defects in the field of vision[7,8]. Pathologically, DR is associated with microvasculature damage in the retina, and hyperglycemia plays a significant role in the pathogenesis of retinal modifications, atherosclerotic dislocation in the retinal vein that with crucial findings as micro-aneurysm, retinal hemorrhages (RH), cotton wool spots, venous bleeding, and eventually retinal detachment[10,11]. Therefore, MCP-1 is the biomarker that can indicate the early phase of DR and could be reliable for patients for early diagnosis of DR[11]. Monocyte chemoattractant protein-1 (MCP-1) plays a crucial role in the pathogenesis of diabetic retinopathy in the early phase of DR.

The MCP-1 an inflammatory marker has a unique DR function to damage the retina and induce inflammation. It modifies the normal dynamics of the retinal barrier and allows inflammatory chemokines for angiogenesis in the retina[12]. MCP-1 is a vital and one of the fundamental chemokines that oversee migration and penetration of monocytes/macrophages and initiate retinal inflammation[13,14]. During hyperglycemia, pigmented cells of the retina, endothelial, and Muller's glial cells play a significant role in the overexpression of MCP-1[12]. One study revealed the association between MCP-1 and cathepsin-D in young adults suffering from DR[15]. Physiologically, a small amount of MCP-1 has been documented that escape to the exterior, but surprisingly its overexpression has been reported in retinal injury. The objective of the current study is to find out levels of monocyte chemoattractant protein-1 in diabetic patients with and without diabetic retinopathy and to compare the functional role of MCP-1 as a better diagnostic biomarker in early diabetic retinopathy with conventional methods.

Methodology

A case-control study was conducted at the Department of Physiology after the approval of Ethical Review Board (Ref No: F.2-81/2019-GENL/12732/JPMC). All 100 participants between 40-65 years of age were selected from the Ophthalmology department, Jinnah Postgraduate Medical Center (JPMC) Karachi, from April 2019 till October 2020. Diagnosis of diabetes as per according to the reference of American Diabetic Association 18 and diagnosis of DR was made by slit-lamp examination, and after that, all patients were placed according to particular groups. At the same time, patients who were suffering from retinal disease, vitreomacular traction, pan-retinal photocoagulation treatment, cataract, diabetes >15 years, proliferative DR, myocardial ischemia, deep vein thrombosis, stroke, lower extremity ischemia, hepatic and renal insufficiency, any blood disorder all were excluded from the present study.

Participants were informed regarding the study and had given written consent. All study participants were separated into four groups: Group A (n=25) comprised diabetic patients with diabetic duration 5-7 years without retinopathy; Group B (n=25) comprised diabetic patients and

disease duration 8-10 years with mild retinopathy sign, Group C (n=25) comprises diabetic patients with duration of disease 10-15 years with moderate retinopathy sign while Group D (n=25) were healthy individuals. Blood samples of all participants were collected in the early morning after overnight fasting of just about 8 hours at baseline.

A consultant examined patients thoroughly and took detailed history after slit-lamp examination and fundoscopy. Serums were separated and were kept stored at -80ºC and were then used to estimate blood sugar levels, HbA1c%, and levels of MCP-1 were estimated through ELISA sandwich procedure (Bioassay Technology Laboratory, Catalog no: E0124Hu).

For data analysis, SPSS version 21.0 software was used. For a continuous variable, descriptive analysis was done. For continuous variables data, i.e., anthropometric (age, height, weight, BMI) clinical variable (blood pressure), and biochemical (blood sugar levels and serum MCP-1, etc.) variables were shown as mean ± standard deviation (SD). One way ANONA with posthoc Tukey's and Scheffe test were applied for comparison among groups. Pearson-coefficient of correlation (r) had been applied to correlate the levels of MCP-1. And p-value <0.05 was taken significantly.

Results

Out of 100 individuals, 60% were male representing 15 individuals in each group, while the rest of the other 40% were female and represented 10 individuals in a single group. There mean age and weight of groups A, group B, and Group C were significantly higher (p<0.01), while the mean difference of height of all groups had not statistically significant (p>0.79).

Table 1: Baseline of study participants.

Variables	Groups (Mean ± SD)				p-value
	Group A (n=25)	Group B (n=25)	Group C (n=25)	Group D (n=25)	
Age (years)	52.7 ± 6.4	55.2 ± 3.5	58.7 ± 2.9	45.9 ± 3.6	<0.01*
Weight (kg)	76.7± 5.9	77.8 ± 4.6	76.7 ±5.2	74.4 ±5.9	0.12
Height (m)	6.0± 0.1	6.2± 0.6	6.0 ± 0.1	5.8 ± 0.4	0.25
BMI (kg/m^2)	24.0± 2.3	24.0 ± 1.4	24.1 ± 1.8	24.2 ± 1.8	0.97

Group-A: 5-7 years of diabetes duration without retinopathy; Group-B: 8-10 years of diabetes duration with mild retinopathy (1= microaneurysm); Group-C: 10-15 years duration of diabetes with moderate retinopathy (2-3 microaneurysms plus retinal and venous bleeding); Group-D: healthy controls
*p<0.05 is considered statistically significant.

Levels of FBG, RBG, and HbA1c were significantly elevated in diabetic patients compared to controls (p< 0.001). The mean difference of serum MCP-1 of study participants was shown in Table II. Higher levels of MCP-1 at baseline were reported in patients with 5-7 years of duration of diabetes as compared to controls and patients with 8-10 years diabetes duration (p<0.001). When we compared and correlated the levels of MCP-1 amongst group A, group B, and Group C: a growing trend has been seen for MCP-1with higher significant levels of blood glucose parameters. This shows that retinal function was continuously pretentious, and there was an injury to the retinal itself. Strong association were reported for MCP-1 with disease duration (r=0.885; p < 0.01), and HbA1c (r =0.71; p <0.01) in all study individuals. Whereas powerful and positive associations were seen for MCP-1 with blood glucose parameters (r = 0.876; p< 0.01) in all study individuals.

Table 2: Blood glucose parameters and MCP-1 of study participants.

Variables	Groups (Mean ± SD)				p-value
	Group A	Group B	Group C	Group D	
FBS (mg/dl)	118.3 ± 13.7	153.5 ± 15.0	230.1 ± 34.6	76.5 ±9.5	<0.01*
RBS (mg/dl)	179.0 ± 16.4	237.6 ± 26.1	287.4 ± 32.4	116.7 ±10.6	<0.01*
HbA1c (%)	6.4 ± 0.6	7.2 ±0.7	7.8 ±0.5	4.6 ±0.4	<0.01*
MCP-1 (ng/dl)	125.6 ± 14.2	343.4 ± 19.6	503.0 ± 43.7	18.6 ± 4.7	<0.01*

Group-A: 5-7 years of diabetes duration without retinopathy; Group-B: 8-10 years of diabetes duration with mild retinopathy (1= microaneurysm); Group-C: 10-15 years duration of diabetes with moderate retinopathy (2-3 microaneurysms plus retinal and venous bleeding); Group-D: healthy controls

*p<0.05 is considered statistically significant.

Discussion

DM is a severe metabolic disease with grave consequences and leads to DR. Importantly, DR is one of the ongoing complications of DM that leads to visual impairment universally. This present study revealed the clinical implication of serum MCP-1 as an early diagnostic biomarker and additionally assesses the affiliation of serum MCP-1, an early phase of diabetic retinopathy in a diabetic patient. Overexpression of MCP-1 in diabetic patients with a shorter diabetic duration in group A in the current study and more elevated levels of MCP-1 in group B and group C had shown that as the duration of disease progression, it causes overexpression of MCP-1 due to inflammation which results in the retinal integrity damage and escort to visual impairment.

The above statement is more endorsed by the fact that group C had a higher expression of MCP-1 as compared to group B. On the other side, group A has significantly higher levels of MCP-1 than group D. Taghavi et al.[16] and Reddy et al.[17] have recommended that as the retinal injury advanced, the overproduction of MCP-1 inclining. Additionally, Behfar et al.[18] and Urbancic et al.[19] have explored the association of MCP-1 in the early clinical phase of DR, and its overexpression indicates premature retinal inflammation. Henceforth, MCP-1 levels could be utilized as an extra indicative tool to detect DR in the early phase in diabetic individuals.

Escalation of fasting and random glucose levels and raised HbA1c% uncovered significant increasing mean difference amongst all groups (p-value <0.01) and also revealed a positive and robust relationship of glucose parameters with an expression of MCP-1 (p-value <0.01). Mitra et al.[12] and Chang et al.[20] have postulated that diabetic retinopathy is recurrent in those individuals who had uncontrolled and poor hyperglycemia status in the body. The truth of the matter is additionally upheld by Deshmane et al.[21], that diabetes which expresses that endothelial and cell injury is because of uncontrolled hyperglycemia prompting cellular integrity of various essential organs, especially the retina. Poor glycemic control can escort the retina toward diabetic retinopathy.

The limitation of the study is that we have a small sample size which is not representative of a large population, and we could not intervene at any stage that gives more additional information about inflammation of the retina.

Conclusion

The current study has examined and reached the resolution that monocyte chemoattractant protein-1 (MCP-1) levels were higher in diabetic patients without retinopathy. As the disease duration increases, levels of MCP-1 become much higher than baseline in diabetic retinopathy patients. Based on the review, we can say that the MCP-1 is a compelling and novel biomarker for surveying inflammation in the early phase of diabetic retinopathy patients before clinical signs showing retinal inflammation. Along these lines, monocyte chemoattractant protein-1 can be an early and

additional diagnostic biomarker in diabetic patients without retinopathy despite any clinical sign.

Conflicts of Interest

The authors have declared that no competing interests exist.

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