

Original Article

ASSOCIATION OF SERUM C- REACTIVE PROTEIN (CRP), CYSTATIN C AND HOMOCYSTEINE WITH DIABETIC RETINOPATHY IN PATIENTS OF TYPE 2 DIABETES MELLITUS

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ABSTRACT:

Objective: The aim of this study was to investigate the association of levels of serum cystatin C (Cys C), CRP and Homocysteine (Hcy) with diabetic retinopathy (DR) associated with type 2 diabetes mellitus and elucidate their clinical prognostic significance.

Material and Methods: A total of 485 patients of type 2 diabetes were recruited, and their levels of serum cystatin C, Homocysteine (Hcy), and C-reactive protein were measured by using a high sensitivity latex-enhanced immunoturbidimetric method. Type 2 diabetes was diagnosed as per the criteria of the American Diabetes Association 2012 and 1999 World Health Organization. Venepuncture was performed after an overnight fast, and venous blood of all patients was drawn. Fundus fluorescein angiography (FFA) was performed on all patients, and the procedure was carried out by the ophthalmologist. Age-related macular degeneration (AMD) was diagnosed by FFA and optical coherence tomography (OCT). All participants were divided into four groups. Group 1 included patients with no Diabetic Retinopathy (NDR) and AMD (n=60, Age 55.1±9.11); Group 2 included patients suffering from Non-Proliferative Diabetic Retinopathy (NPDR) (n=180, Age 56.7±6.21); Group 3 comprised of patients suffering from Proliferative Diabetic Retinopathy (PDR) (n=160, Age 57.1±10.07) and Group 4 consisted of AMD (n=85, exclude patients with NPDR or PDR, Age 55.9±6.27).

Results: Levels of serum CRP, Cys C, and Hcy were significantly distinctive between specific groups. The levels of serum Cys C in the PDR and AMD groups were significantly elevated as compared to NPDR and control groups (p<0.05, p<0.01, respectively). The levels of serum CRP in the PDR and AMD group were elevated as compared to NPDR and control group (p<0.05, p<0.01 respectively) and the levels of serum Hcy in PDR and AMD group were elevated significantly as compared with NPDR (p<0.01) and control group (p<0.01).

Conclusion: It is concluded that during the clinical implications of diabetic retinopathy (DR) in patients with type 2 diabetes, the serum levels of C- reactive protein, Cystatin C, and Homocysteine play an important role.

Key Words: Cystatin C, Homocysteine, Macular degeneration

INTRODUCTION:

The most frequent complication of diabetes in microvascular disease is the Diabetic Retinopathy that affects nearly 93 million people¹ and causes leading numbers of blindness and poor vision worldwide.²

It is a global public health problem with physical, psychological, and socioeconomic consequences. In Asia itself, the prevalence of diabetes varies from 15.8-43.1% in different demographic studies.³⁻⁵ If the prevalence of DM continues to rise dramatically in Asian continent⁶ in concomitance with the aging population, the socioeconomic disease burden will likely increase exceptionally in the future.

Hence, the search for biomarkers and risk factors of DR is of paramount importance in order to prevent disease progression. Therefore, we investigated the role of

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Cystatin C, which is a member of cystatin superfamily, a family of cysteine proteinase inhibitors; nucleated cells mainly produced Cystatin C and is also present in urine, serum, cerebrospinal fluid, semen, colostrum, and semen in a relatively lower concentration.⁷ It has been proved that the levels of serum or plasma cystatin C are independent of weight, age, gender, dietary factors, liver diseases, and infections, but few studies discovered its possible relation with age. It was emphasized that levels of serum cystatin C are increased at 50 years of age⁸ and some studies have revealed that diabetes, inflammation, and BMI also affect Cystatin C levels irrespective of kidney functions.⁹

Recent clinical trials have proved that there is strong evidence of correlation of serum levels of Cystatin C and prognosis of Diabetic Retinopathy¹⁰ and serum Cystatin C levels were also well correlated in age-related macular degeneration (AMD).¹¹ The higher levels of serum cystatin C have also been correlated with prediabetes.¹² A recent clinical trial based in China has suggested that elevated levels of serum Cystatin C were associated with the severity of DR, along with diabetes duration, and HbA1c levels are an important risk factor.¹³ Therefore levels of serum Cystatin C can be useful as a prognostic factor in patients for higher risk of DR associated with type 2 diabetes mellitus (T2DM).

Among several markers of inflammation, the levels of serum C-reactive protein (CRP) were greatly elevated in patients with diabetes. CRP is an acute-phase reactant that is produced as a result of ongoing infection or inflammation. It also plays a unique role in innate immunity. Increased inflammatory activity in diabetic retinopathy, as reflected by significantly elevated levels of CRP, is associated with endothelial dysfunction. Moreover, CRP was also found to be elevated in patients with macular degeneration.¹⁴

Homocysteine (Hcy), which is formed by the demethylation of methionine, is a sulfur-containing amino acid. Several scientific

studies have proved the complex interaction between the levels of blood Hcy and DR prevalence, especially PDR. The incidence of DR is also high in diabetic patients with hyperhomocysteinemia. Therefore, the total plasma level of Hcy can be used as an important prognostic biomarker in patients of DR irrespective of other factors associated with T2DM.¹⁵

MATERIAL AND METHODS:

A total of 485 patients were recruited in this study from February 2014 to January 2018 at Jinnah hospital. All recruited patients were diagnosed with type 2 diabetes. The protocol of the study was approved by the respective research committee. All participating patients provided written informed consent. Patients with type 1 diabetes, patients with acute complications of diabetes, patients with diagnosed diabetic nephropathy or secondary nephropathy or complicated T2DM with infections such as rheumatoid arthritis, systemic lupus erythematosus (SLE), diabetic nephropathy, presence of any psychological or neurological disorder and the presence of ocular diseases like glaucoma, uveitis, pigment degeneration, tumor, and wet age-related macular degeneration were excluded from the study. All participants were subjected to standard interviewer questionnaires, systemic and ocular examinations along with standard baseline blood investigations.

The relevant data of age and diabetes duration of all participants was collected. Type 2 diabetes was diagnosed based on the American diabetes association criteria of 2012 and 1999 World Health Organization standards. Blood pressure was measured after the participants were seated and relaxed for at least 5 minutes. Venepuncture was used to draw venous blood of all patients after an overnight fast. Immunoturbidimetric method enhanced with high sensitive latex was used to measure the levels of serum C-reactive protein (CRP) cystatin C (Cys C) and homocysteine (Hcy). Fasting plasma glucose (FPG), glycosylated hemoglobin

(HbA1c) were also estimated by the name of the method by using age and sex-adjusted models.

Ophthalmologist performed fundus fluorescein angiography (FFA) on all participants. Optical coherence tomography (OCT) and FFA were used to diagnose diabetes-related macular degeneration. Participants were grouped into four groups. Group 1 patients with no AMD and no Diabetic retinopathy (NDR) (n=60, Age 55.1±9.11); Group 2 patients suffering from Non-Proliferative Diabetic Retinopathy (NPDR) (n=180, Age 56.7±6.21); Group 3 patients suffering from Proliferative Diabetic Retinopathy (PDR) (n=160, Age 57.1±10.07) and Group 4 AMD (n=85, exclude patients with NPDR or PDR, Age 55.9±6.27).

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 16.0 software package. The presentation of data was mean±standard deviation (SD). One-way ANOVA was used to make comparisons between pairs of groups. A two-tailed P value of <0.05 was regarded as statistically significant.

RESULTS:

We recruited a total of 485 patients and divided them into four groups. Table 1 presents the clinical features of all four groups. We observed no critical differences in the age, sex, duration of diabetes.

Table-1: Characteristics of the study population

| | G1 | G2 | G3 | G4 |
|------------------------------|-----------|-----------|------------|-----------|
| N | 60 | 180 | 160 | 85 |
| Mean±SD Age (years) | 55.1±9.11 | 56.7±6.21 | 57.1±10.07 | 55.9±6.27 |
| M/F ratio | 30/30 | 101/89 | 85/75 | 40/45 |
| Duration of diabetes (years) | 13.1 | 13.7 | 14.5 | 14.1 |
| HbA1c (%) | 8.1 | 7.9 | 8.2 | 8 |

Serum CRP, Cys C, and Hcy levels among the following groups are presented in figures 1-3.

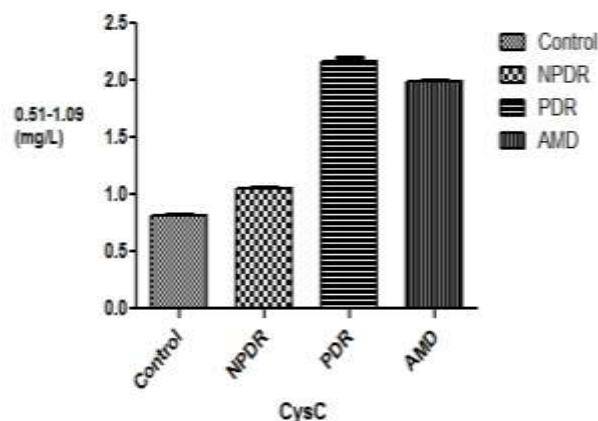


Fig-1: The serum levels of Cys C in four groups

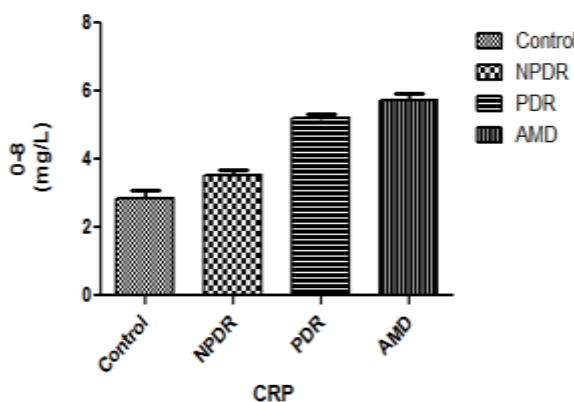


Fig-2: The serum levels of CRP C in four groups

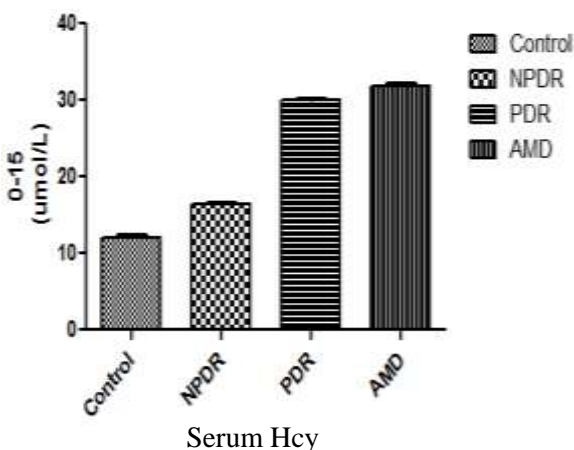


Fig-3: The serum levels of Hcy in four groups

Regarding the serum levels of Cys C in the four groups, there were no differences between the NPDR group and the control group. But the levels of serum Cys C were elevated in PDR and AMD groups compared with the control group ($p < 0.01$), but in comparison with the NPDR group, we also found the levels of Cys C in PDR and AMD were increased significantly ($p < 0.01$, $p < 0.05$, respectively), but we did not find any differences among the PDR and AMD groups ($p > 0.05$). (Table – 2)

The levels of serum CRP in the NPDR and control groups showed no differences. Serum CRP levels were remarkably elevated in the PDR and AMD group than the control group ($p < 0.01$). In comparison with NPDR group, serum levels of CRP in PDR and AMD were found to be elevated ($p < 0.05$, $p < 0.01$, respectively), but no statistical difference was found among the PDR and AMD groups ($p > 0.05$) (Table – 2). There was no difference in serum Hcy between control and NPDR groups. The levels of Hcy were considerably higher in the PDR and AMD groups than the control ($p < 0.01$). In comparison with the NPDR group, we also found the levels of Hcy in PDR and AMD significantly higher than the NPDR group ($p < 0.01$), but there was no statistically significant difference among PDR and AMD groups ($p > 0.05$) (Table – 2).

Table-2: Comparison of serum cystatin-C, C-reactive protein and homocysteine levels between four groups

| Parameter | Comparison between groups | | | | | |
|------------------|---------------------------|----------------|---------------|------------|------------|------------|
| | Control & AMD | Control & NPDR | Control & PDR | AMD & PDR | AMD & NPDR | PDR & NPDR |
| Serum cystatin C | $p < 0.01$ | $p > 0.05$ | $p < 0.05$ | $p > 0.05$ | $p < 0.05$ | $p < 0.01$ |
| Serum CRP | $p < 0.01$ | $p > 0.05$ | $p < 0.01$ | $p > 0.05$ | $p < 0.01$ | $p < 0.05$ |
| Serum Hcy | $p < 0.01$ | $p > 0.05$ | $p < 0.01$ | $p > 0.05$ | $p < 0.01$ | $p < 0.01$ |

$p < 0.05$ Significant

$p > 0.05$ Insignificant

DISCUSSION:

Serum levels of CRP, Cys C, and Hcy in patients were found to be elevated with advanced stages of Diabetic Retinopathy (PDR and AMD), which implies a common pathophysiological mechanism consisting of an interplay of chronic inflammation, cell injury and degenerative neuronal pathology.¹⁶ This further implies that Diabetic Retinopathy is not just a vascular disease but a neurovascular disease.¹⁷

Cystatin C is a strong inhibitor of cysteine proteinase of lysosomal and extracellular substances and is expressed invariably in a wide variety of human cells such as fibroblasts, glial cells, pancreatic islet cells, and endothelial cells. The major site for Cystatin C secretion is retinal pigment epithelium that is located in the posterior eye and has a crucial role during the process of macular degeneration.

Moreover, it was hypothesized that Cystatin C proved to be an important factor during the remodeling of arterial blood vessels wall, neovascularization, inflammatory, and degenerative neuronal pathology.¹⁸ Similarly, the DR results in abnormal pathophysiological accumulation of fluid and subsequent edema of the macula, which causes inflammation, optic neuropathy, retinal neovascularization, and excessive expression of glial cells.¹⁹ Furthermore, Cystatin C and DR share common mechanisms, and that explains their close connection.¹¹

C- Reactive Protein (CRP) is considered as a chronic marker of several pathological conditions such as preclinical atherosclerosis, arterial wall inflammation, systemic endothelial dysfunction, impaired fibrinolysis and subclinical inflammation that leads to further progression of diabetic retinopathy that may exacerbate further tissue damage.^{19,20}

The pathogenesis of DR includes the hyperhomocysteinemia which causes accelerated cell injury in retinal capillaries²¹ by producing reactive oxygen species (ROS) and inducing oxidative stress and

abnormal generation of nitric oxide species that leads to abnormal proliferation of smooth muscle cells of the vessels and alteration of vasomotor activity.^{22,23}

Considering the fact that there are many factors that have a role in the progression and development of DR, instead few still remain unclear. As we had explained the data analysis and mechanisms above, we believe that elevated Cystatin C, CRP, and Homocysteine are strong causative factors for DR, mainly in the progression of retinopathy not subjected to other factors.

CONCLUSION:

Serum Cystatin C, CRP, and Homocysteine levels are potential and useful biomarkers in predicting and analyzing the progression of DR. Taking into consideration the ever-increasing financial burden of diabetes, CRP, Cystatin C, and Homocysteine assays can prove to be more beneficial to estimate the progression and prognosis of DR.

RECOMMENDATIONS:

Further studies should incorporate possible thresholds and common pathophysiological mechanisms between the blood levels of these biomarkers and the separate degrees of DR in a larger number of patients. Besides, these markers are easy and more economical to measure as compared to optical coherence tomography (OCT), and high-quality fundus fluorescein angiograms.

AUTHOR'S CONTRIBUTION:

MQ: Conception of idea and article writing
MG: Data collection and revision of article
HJQ: Review critically
MSA: Data analysis and editing
AF: Data collection
SM: Data analysis

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