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Association of High Sensitivity C-Reactive Protein with Type II Diabetic Retinopathy

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ABSTRACT

Objectives: The objective of this study is to find out the association of diabetic retinopathy with high sensitivity (hs) CRP levels.

Methodology: It was a descriptive cross-sectional study. The study was conducted at the Army Medical College's Department of Physiology and Centre for Research in Experimental and Applied Medicine (CREAM), Rawalpindi, in partnership with the Armed Forces Institute of Ophthalmology (AFIO), Rawalpindi for a tenure of 12 months. Ninety subjects were included in the study, thirty in each group: controls, diabetic subjects, and patients suffering from diabetic retinopathy (DR).

Results: Healthy controls, diabetic subjects, and patients with DR were found to have mean Fasting Plasma Glucose (FPG) values of 5.51 0.34 (mmol/l), 8.11+/- 0.67 (mmol/l), and 8.73+/- 0.90 (mmol/l), respectively (p=0.001). The mean HbA1c level in normal subjects was noted as 5.08 +/- 0.27 in healthy controls as compared to 7.70 +/-0.89 (mmol/l)) in diabetic subjects and 9.02+/-1.76 (mmol/l) in patients with DR (p=0.001 by ANOVA). In normal subjects, the mean hs CRP levels were noted to be 3.74+/-1.97 (mg/l) in healthy controls as compared to 15.32 +/- 2.93 (mg/l) in diabetic subjects and 26.71 +/- 4.88 (mg/l) in patients with diabetic retinopathy (p=0.001 by ANOVA).

Conclusion: During the study, it was found that elevated levels of these inflammatory biomarkers can accurately predict the onset of diabetic retinopathy and that hs CRP levels are strongly related to the development of diabetic retinopathy. Monitoring this inflammatory marker in the serum can therefore help prevent diabetic microangiopathic consequences, including DR.

Key words: Diabetic retinopathy, Fasting Plasma glucose, hs-CRP

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,] Critical analysis and manuscript review; [1] Data analysis; Manuscript Editing.

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Introduction

Diabetes Mellitus (DM) is a worldwide health problem that is divided into two types with differences in the pathophysiology of type 1 and type DM.[1] It is a silent killer which affects almost all body parts. Eyes, blood vessels, nerves and kidneys all are affected. The diagnosis of T2DM depends upon Plasma glucose level. The criteria to diagnose diabetes mellitus is the level of fasting plasma glucose ≥126 mg/dL (7.0mmol/l). The test for fasting plasma glucose is performed after not eating or drinking anything but water for 8 hours.[2] Criteria for

Random plasma Glucose (RBG) are 2-hour oral glucose tolerance test ≥ 200 mg/dL (11.1mmol/l) and haemoglobin A1c ≥ 6.5%. If 2 hourly oral glucose tolerance test gives a result between 140mg/dl (7.8mmol/l) and 200mg/dl (11.1 mmol/l) then it is labelled as impaired glucose tolerance while levels of fasting plasma glucose 101mg/dl to 124mg/dl (5.6-6.9mmol/l) are considered as impaired fasting glucose (IFG).[3]

One of the most serious consequences in people with chronic hyperglycaemia is diabetic retinopathy (DR). Non-proliferative diabetic retinopathy (NPDR) and advanced-stage proliferative diabetic retinopathy (PDR) are the two stages of the disease. On clinical examination, signs of DR are micro-aneurysms, vitreous haemorrhage, retinal haemorrhage, exudates, cotton wool spots, neovascularization, and fibrosis. Diabetic Macular Edema (DME) is a condition in which the breakdown of the Blood Retinal Barrier (BRB) occurs which is responsible for fluid outflow and the proteins circulating into the neural retina.[4]

In 1930, the high-sensitivity C- C-reactive protein (hs-CRP) was discovered. It is an acute phase protein that hepatocytes produce in response to inflammation. hs-CRP possesses anti-inflammatory and pro-inflammatory properties. By binding to phospholipids, phosphocholine, chromatin, histone, and fibronectin, it serves an anti-inflammatory role in the identification and disposal of foreign particles and wounded cells.[5]

By triggering the traditional complement pathway and phagocytic cells via Fc receptors to hasten the removal of cellular waste, injured or apoptotic cells, and foreign particles, it causes inflammation. However, in ITP, hs-CRP can develop into a dangerous state when autoantibodies with the phosphocholine arm in auto-immune processes activate it. Through the activation of the complement system and inflammatory cytokines, it can occasionally make tissue injury worse.[6]

The hs-CRP levels respond to the onset and cessation of the inflammatory stimuli more accurately than the erythrocyte sedimentation rate (ESR), which is a proximate indicator of inflammation. Chronic inflammatory illness is indicated by persistently increased hs-CRP values. Hs CRP binds to phosphocholine, which is expressed on the surface of dead or damaged cells as well as some bacteria. This acts as a stimulant for the complement system, encouraging the phagocytosis of bacteria, necrotic, and apoptotic cells by macrophages. The term "acute phase response" refers to a rise in IL-6 concentrations produced by macrophages and adipocytes in response to a variety of acute and chronic inflammatory diseases such as bacterial, viral, or fungal infections.[7]Inflammation triggers the release of interleukin-6 and other cytokines, which stimulates hepatocytes to produce hs CRP and fibrinogen.

According to research, hs CRP levels are increased in both type 1 and type 2 diabetics. Many studies have found that high levels of hs CRP are linked to an increased risk of diabetic microvascular sequelae such as neuropathy, nephropathy, and retinopathy. Serum hs-CRP levels rise in diabetic retinopathy, although studies show that elevated serum hs-CRP levels are more common in patients with proliferative diabetic retinopathy than in those with non-proliferative diabetic retinopathy. According to research, serum hs CRP can be utilized as a biomarker of diabetic retinopathy.[8]

Blood flow to the retina is auto-regulated by means of many non-nervous mechanisms such as, endothelin-1. When the balance between endothelin-1 and another mediator such as nitric oxide (NO) is disturbed, it causes retinal hemodynamic impairment in diabetic retinopathy. Endothelin-1 has been implicated in the etiology of diabetic retinopathy, according to several studies. Furthermore, research points to the benefit of endothelin-1 antagonists in reducing diabetic complications. But the pathophysiology behind the association between high hs-CRP and diabetes sequelae, notably diabetic retinopathy, is still unknown.[9]

It has been believed that hs-CRP interacts with Fc receptors, specifically Fc receptor I (CD64) and Fc receptor II (CD32), to produce reactive oxygen species (ROS) and promote inflammation. As a result of hs-CRP's activation of the NF-B signalling pathway, pro-inflammatory molecules such as TNF and ROS are produced.[10] Hence we designed the study to investigate the correlation between diabetic retinopathy and elevated hs CRP. This study will help the ophthalmologist in detecting means of early non-invasive diagnosis of diabetic retinopathy.

Methodology

Healthy, diabetic, and diabetic retinopathic subjects were taken from the medical OPD of Pak Emirates Military Hospital (PEMH) and Armed Forces Institute of Ophthalmology, Rawalpindi (AFIO). Each group comprised of thirty subjects. Male, female, and their attendants visiting OPD from November 2019 to November 2020 were interviewed. Informed consent was taken from the subjects who were fulfilling the inclusion criteria and within the age ranges of 35-55 years. The subject's details were taken including medical history. The initial classification of subjects was carried out through a preliminary examination.

AFIO and Army Medical College's ethical review committee gave their formal clearance before the study could be officially conducted. From all of the patients and healthy volunteers, informed consent was obtained in writing as per Performa attached as annexure –I and II. Type 2 diabetes without complications and diabetic retinopathy patients confirmed by Ophthalmologists were enrolled in the study. After accomplishing their demographic details with history and clinical examination, Serum hs CRP levels were determined by drawing blood samples. Strict aseptic procedures were followed when drawing blood samples. Venepuncture was used to obtain 5ml of blood, of which 2.5ml was transferred to an EDTA tube for full blood analysis and the remaining 2.5ml was centrifuged for ten minutes at 2000-3000 revolutions per minute for separation of serum. For further examination at the CREAM laboratory, serum was pipetted out, transferred to Eppendorf tubes, and then stored at -80 c.

Results

Healthy controls, diabetic subjects, and patients with DR were found to have mean Fasting Plasma Glucose (FPG) values of 5.51 0.34 (mmol/l), 8.11+/- 0.67 (mmol/l), and 8.73+/- 0.90 (mmol/l), respectively (p=0.001). The mean HbA1c level in normal subjects was noted as 5.08 +/- 0.27 in healthy controls as compared to 7.70 +/-0.89 (mmol/l)) in diabetic subjects and 9.02+/-1.76 (mmol/l) in patients with DR (p=0.001 by ANOVA). In normal subjects, the mean hs CRP levels were noted to be 3.74+/-1.97 (mg/l) in healthy controls as compared to 15.32 +/- 2.93 (mg/l) in diabetic subjects and 26.71 +/- 4.88 (mg/l) in patients with diabetic retinopathy (p=0.001 by ANOVA).

Table 1: Age, FBG, RBG, HbA1c, serum homocysteine, hs CRP, and BMI compared by one-way ANOVA between the normal healthy, diabetic, and diabetic retinopathic groups

Variables	Group I Normoglycemic (n=30)	Group II DM (n=30)	Group III DR (n=30)	P Value
Age years	44.63 ± 4.88	44.90 ± 5.83	45.07 ± 5.73	0.954
FBG mmol/l	5.51 ± 0.34	8.11 ± 0.67	8.73 ± 0.90	**0.0001**
RBG mmol/l	6.55 ± 0.43	12.27 ± 0.76	12.84 ± 0.85	**0.0001**
HbA1c mmol/l	5.08 ± 0.27	7.70 ± 0.89	9.02 ± 1.76	**0.0001**
hs CRP mg/l	3.74 ± 1.97	15.32 ± 2.93	26.71 ± 4.88	**0.0001**
BMI kg/m²	27.64 ± 1.80	27.64 ± 1.80	28.24 ± 1.57	0.296

The significance level is set at P 0.05 and all values have been reported as Mean + SD.

FBG: Fasting Blood Glucose, RBG: Random Blood Glucose, HbA1c: Glycosylated haemoglobin, hs CRP: C - reactive protein, BMI: Body Mass Index

TABLE 2: Comparison of age, FBG, RBG, HbA1c, serum homocysteine, hs CRP and BMI between two groups by post-hoc Tukey test

Variables	Group 1 Vs Group II	Group 1 Vs Group III	Group II Vs Group III
FBG mmol/l	**0.0001**	**0.0001**	**0.002**
RBG mmol/l	**0.0001**	**0.0001**	**0.006**
HbA1c mmol/l	**0.0001**	**0.0001**	**0.0001**
hs CRP mg/l	**0.0001**	**0.0001**	**0.0001**

Group I= Normoglycemic, Group II= DM, Group III= DR

Table 3: Correlation of age, FBG, RBG, HbA1c, BMI with serum hs CRP

Variable	Parameters correlated	r-value	p-value
Serum hs CRP mg/l	Age	0.109	0.308
	FBG mmol/l	0.802	**0.0001**
	RBG mmol/l	0.837	**0.0001**
	HbA1c mmol/l	0.734	**0.0001**
	BMI kg/m^2	0.124	0.243

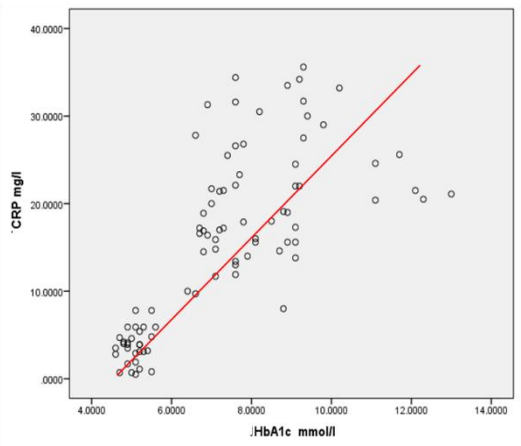

Figure 1: Correlation curve showing a statistically significant correlation between serum hs CRP and HbA1c

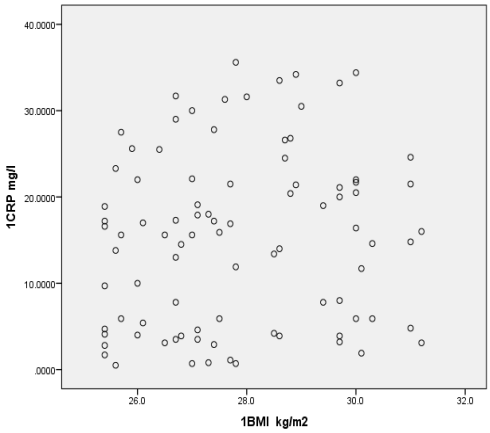

Figure 2: Correlation curve showing an insignificant correlation between serum hs CRP and BMI (n=90)

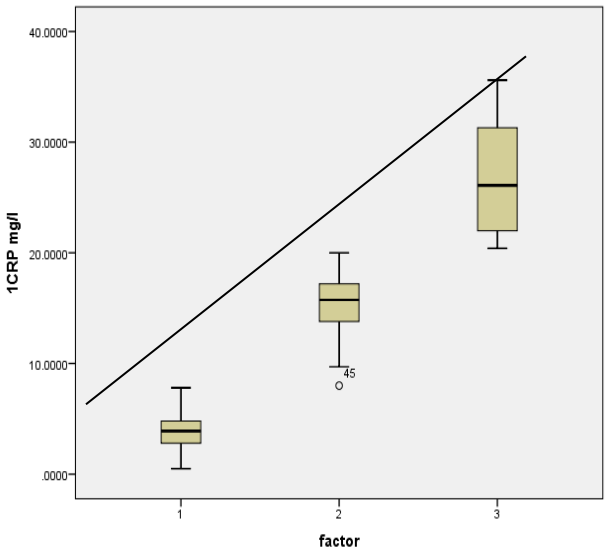

Figure 3: Boxplot showing a comparison of serum hs CRP levels between three study groups (n=90)

Discussion

The study aimed to compare blood homocysteine and high-sensitivity C-reactive protein levels between healthy normal controls and diabetic patients with and without DR. Diagnosing diabetes patients using WHO criteria is a common and usual practice. We measured everyone's HbA1c, fasting blood glucose (FBG) and random blood glucose (RBG), by these criteria. This standard criterion has been applied to several investigations of a similar sort.

This study consisted of healthy controls, diabetic patients without DR and patients with diabetic retinopathy. Each group consisted of thirty patients. Diabetic patients suffering from any systemic disease affecting retinal vasculature were excluded from the study. A similar inclusion and exclusion criteria were followed by relevant studies. [10,11] In the diabetic and diabetic retinopathy groups, the mean age was discovered to be 44.90 + 5.83 and 45.07 + 5.73, respectively. We chose this age range since numerous research indicate that hs CRP levels have risen with advancing years as a result of numerous inflammatory changes. Mean hs -CRP levels in normal, diabetic and DR subjects are 3.74 ± 1.97, 15.32 ± 2.93 and 26.71 ± 4.88 respectively. ANOVA is applied among three groups and a significant p-value (0.0001) is obtained. Comparison of hs-CRP levels were undertaken between two groups through the application of the post-hoc Tukey test and a significant p-value was obtained. When hs-CRP levels are compared with age, no significant results are obtained (Pearson correlation coefficient r=0.109 and p=0.308). Our study results contrast with a study conducted earlier in which serum levels of hs-CRP have shown an association with age-related macular degeneration.[12]They demonstrated that ageing causes a decline in liver and kidney function, which is a major contributor to elevated levels of hs-CRP.

Hassaan and coauthors discovered no discernible differences in hs-CRP levels between patients with DR and healthy controls at different stages of diabetic retinopathy.[11]This may indicate that this inflammatory marker may play a function in the detection of diabetic retinopathy but not in the monitoring and evaluation of the disease's progression or severity. Zaciragic and co-authors have shown similar results in their study, in which they compared hs-CRP levels between diabetic patients with NPDR and diabetic patients with PDR.[12] This is in contrast to a study carried out in which he showed that differences in hs-CRP levels between diabetic and DR patients exist but p=0.47 showing no significant difference and this may be due to pan-retinal photocoagulation which may reduce the levels of this inflammatory biomarker.[13,14]

When serum hs CRP levels and BMI were examined in our study, there was no discernible difference (Pearson correlation coefficient r=0.124 and p=0.243). Our research found no correlation between serum hs-CRP levels and BMI.

According to our research, there is a statistically significant positive link between fasting plasma glucose levels and serum levels of hs CRP (Pearson correlation coefficient: r=0.802; p0.0001). A previous study demonstrated a correlation between hs CRP and fasting blood glucose (r = 0.40 and p.0.05), providing support for ours.[15]

The pathophysiological basis of such a relationship is due to pro atherogenic effects of hs CRP. These effects of hs-CRP are responsible for endothelial dysfunction, decrease in nitric oxide production and increase in the formation of arteriogenic chemo-cytokines. [15]

Our study showed a positive association between hs CRP levels and HbA1c (r=0.734 and p=0.0001). Another study that was published revealed a relationship between CRP and HbA1c in females with diabetes mellitus type 2. This association was statistically significant and showed a positive correlation.[16]

Diabetic microangiopathic complications, diabetic retinopathy has caused significant social and economic burden.[17,18]The strength of this study is that we have focused on early detection methods of diabetic retinopathy. Early diagnosis of this life-limiting complication may help in improving the quality of life by reducing the chances of its occurrence. Hs-CRP is a simple and inexpensive biomarker. Therefore, levels of these biomarkers in predictions of DR may be clinically helpful since diabetic retinopathy in patients with T2DM was linked to raise serum hs-CRP levels in the present study. The limitation of this study is a small sample size, and we could not extend the scope of the study to multiple centres due to time constraints and budget limitations.

Conclusion

Serum hs-CRP and the onset of diabetic retinopathy are strongly correlated. This inflammatory biomarker's raised levels can be implemented to anticipate the onset of diabetic retinopathy. Therefore, keeping an eye on this inflammatory biomarker's blood levels will help prevent the emergence of diabetic microangiopathic sequelae, notably DR.

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