ORIGINAL ARTICLE

ASSOCIATION OF SERUM FERRITIN AND CRP LEVELS WITH DEVELOPMENT AND SEVERITY OF DIABETIC RETINOPATHY

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Background: Diabetes mellitus is a global health issue. Chronic hyperglycaemia induces endothelial dysfunction and metabolic derangements which are postulated to be the cornerstone for the pathogenesis of diabetic microangiopathic complications. The interplay of oxidative stress, endothelial dysfunction and inflammation in this regard has led to investigation of role of inflammatory biomarkers as adjuncts to routine diagnostic testing. This study was designed to elucidate the association between serum ferritin and high sensitivity CRP levels and diabetic retinopathy. **Methods:** The study was carried out at Department of Physiology and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, in collaboration with Armed Forces Institute of Ophthalmology, Rawalpindi for a period of one year. A total of 90 subjects were recruited into three equal groups; healthy subjects, diabetics and patients with diabetic retinopathy. Serum high sensitivity C-reactive protein (hs-CRP) was estimated by Enzyme Immunoassay (EIA) and serum ferritin levels were carried out by enzyme linked immunosorbent assay. **Results:** The mean age was 48.50±5.32, 50.90±3.83 and 50.83±3.54 years respectively. Insignificant differences in height, weight and BMI were found across the groups. Highly significant difference was noted in the mean CRP levels in normal subjects. The mean ferritin levels were also found to be significantly different. Univariate multinomial regression revealed that variance in CRP levels could account for 91.1% of variance in status of patients and that in ferritin levels could account for 96.7%. **Conclusion:** These prognostic markers correlate well with diabetic retinopathy and have an independent predictive ability as well. This may in future be inculcated into screening and surveillance of diabetic microangiopathic complications.

Keywords: Ferritin, CRP, Diabetic Retinopathy

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INTRODUCTION

Diabetes Mellitus is a major global health threat.[1] It is known to be a silent killer and is associated with severe organ dysfunction. Approximately 415 million people around the world are known to be affected with diabetes mellitus. The soaring numbers have been projected to reach 642 million by 2040. It is estimated that 193 million people with diabetes mellitus are still undiagnosed and are therefore at a greater risk of developing complications on account of being untreated.[1] Type 2 diabetes mellitus (T2DM) is mainly characterized by insulin resistance followed initially by an increase in insulin secretion and in later stages, exhaustion of β cells with subsequent hyperglycaemia. Genetic predisposition and environmental factors like sedentary life-style and obesity play a major role in pathogenesis of diabetes melliuts.[2] Long term complications of T2DM include nephropathy which may lead to renal failure, neuropathy with risk of foot ulcers and gangrene and retinopathy with potential blindness and features of autonomic dysfunction including gastrointestinal, cardiovascular symptoms and sexual dysfunction.[3] Retinopathy is one of the common microvascular complications of T2DM, resulting in blindness in over 10,000 people per year. Individuals with T2DM are 25 times more likely to have retinopathic changes than individuals without this disease.[4] In Pakistan, it has been estimated that the prevalence of diabetic retinopathy is 21% among newly diagnosed type 2 diabetic patients with potential visual impairment.[5] Diabetic retinopathy is characterized by micro-aneurysms, dot/blot haemorrhages cotton-wool spots, hard exudates, retinal oedema, neo-vascularization, vitreous haemorrhages and retinal detachment.[6] C-reactive protein (CRP) is a well-established marker of inflammation and is produced by liver cells in response to a variety of stimuli where it plays a role in angiogenesis and endothelial dysfunction.[7] This makes the cornerstone of the role of CRP in the pathogenesis of diabetic retinopathy and has been studied extensively.[8,9] Lesser is known about its associations with microangiopathic diabetic complications but some studies have demonstrated its association with microalbuminuria[10], diabetic retinopathy[11], and atherosclerotic cardiovascular disease[12]. Ferritin, the multimeric iron storage protein is made up of a light and heavy chain. The ratio between these two is tissue specific and determined by availability of iron. Alterations in this ratio lead to a change in iron storage, ferritin turnover and synthesis of Vascular Endothelial Growth Factor (VEGF).[13] Elevated ferritin levels also pose an oxidative threat. This effect is more marked in beta cells of pancreas and

hepatocytes where insulin secretion and gluconeogenesis may be adversely affected. Additionally, end organ sensitivity to insulin may also be modulated by ferritin stores.[14] Some investigators have studied the association of ferritin with angiogenesis[15] but a proven association with diabetic retinopathy has not been demonstrated. This study was undertaken to delineate the association of serum ferritin and high sensitivity CRP (hs-CRP) levels with diabetic retinopathy and their predictive ability in occurrence of diabetic retinopathy which may pave the way for their usefulness as a predictive marker for this debilitating diabetic microvascular complication in future.

This study aimed to compare the levels of serum ferritin and hs-CRP in patients with and without diabetic retinopathy and to determine their association with diabetic retinopathy.

PATIENTS AND METHODS

This cross-sectional comparative study was carried out for a period of one year at Department of Physiology, and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi in collaboration with Armed Forces Institute of Ophthalmology, Rawalpindi. Non-probability convenience sampling was carried out. Individuals aged 35–55 years, not taking any antioxidant or antihypertensives for at least two weeks were included in the study after their informed consent.

A total of ninety patients were taken and divided into 3 groups by using WHO sample size calculator. Group I comprised of 30 healthy controls, Group II of 30 diabetics without diabetic retinopathy and Group III of 30 patients with diabetic retinopathy. The study was carried out after formal approval from ethical review committee of Army Medical College and Armed Forces Institute of Ophthalmology (AFIO). All the patients who were hypertensive or diabetics having other complications except retinopathy, e.g., diabetic neuropathy, nephropathy and renal failure, ischemic heart disease were excluded from the study. Five ml of blood was withdrawn using strict aseptic measures and was added to gel activating tubes for serum extraction. Blood glucose was estimated on a glucometer (Akon Lab). Glycosylated haemoglobin (HbA1c) was determined. Serum hs-CRP was estimated with Enzyme Immunoassay (EIA). Serum ferritin levels were obtained by enzyme linked immunosorbent assay.[16] Data was collected and analysed using SPSS-21.

RESULTS

The groups were similar with regard to age, weight, height and BMI (p>0.05) (Table-1). Also shown is comparison of mean values of blood sugar fasting, HBA1c and CRP across the three groups along with the p-values. Table-2 shows pair-wise comparison of the study variables with p-values.

Table-1: Comparison of study variables among healthy, diabetic and diabetic retinopathy groups

Variables	Group I Normoglycemic (n=30)	Group II DM (n=30)	Group III DR (n=30)	*p*
Age (years)	48.50±5.32	50.90±3.83	50.83±3.54	0.054
Weight	78.24±19.21	71.83±10.82	76.89±19.68	0.313
Height (m)	1.69±0.17	1.65±0.07	1.69±0.05	0.315
BMI (Kg/m^2)	25.33±2.09	26.24±3.20	25.86±2.40	0.401
BSF	90.87±8.14	172.68±75.74	217.37±89.27	**0.0001**
HBA1c	5.87±0.7	7.74±1.85	7.72±1.48	**0.0001**
CRP	1.68±0.75	3.16±1.23	5.87±1.67	**0.0001**
Serum Ferritin	125.99±75.52	118.74±99.12	284.82±168.5	**0.0001**

Table-2: Pairwise comparison of BSF, HbA1c, CRP & serum ferritin

Variable	Group I vs Group II	Group I vs Group III	Group II vs Group III
BSF	**0.0001**	**0.0001**	**0.033**
HBA1c	**0.0001**	**0.0001**	0.998
CRP	**0.0001**	**0.0001**	**0.0001**
Ferritin	0.971	**0.0001**	**0.0001**

DISCUSSION

This study was conceived with the aim of comparing the high sensitivity CRP and ferritin levels in healthy controls with those of diabetic subjects and those having diabetic retinopathy. Serum ferritin levels were found to correlate significantly with fasting blood sugars as was also shown by Acton[17] the consistent difference between serum ferritin in diabetic and healthy individuals. The lack of existence of a statistically significant difference in HbA1c of diabetic and diabetic retinopathy subjects (with higher mean values in diabetic subjects) calls into question the importance of adequate diabetes controls in delaying the onset and progression of diabetic microvascular complications. This is consistent with the observation of subjects with good glycaemic controls but a genetic susceptibility to DR developing DR and similar results have been shown by Kenneth[18] as well, but is in contrast to a difference in mean HbA1c in diabetics and DR subjects respectively (p=0.0003) demonstrated by Peng[19]. A similar lack of difference between HbA1c across different severities of diabetic retinopathy was also shown in study done by Blum[20] supporting the plausibility of occurrence of DR independent to the adequacy of glycaemic controls achieved. Similar correlation between DR and CRP has also been demonstrated by a study done by Kenneth[18]. Blum[20] also showed consistent differences in CRP levels between healthy controls and DR patients but no significant difference across different stages of DR was found. This may raise the possibility of this inflammatory marker having a role in screening for DR but not in monitoring the progression and assessing its severity. Budak[21] compared CRP levels

between healthy controls and DR subjects and yielded significant differences. Jia ZT[22] supported this observation but in addition also demonstrated a difference in levels across different severities. A statistically significant positive correlation between fasting blood sugars and CRP were seen and is also supported by findings of Fröhlich[23]. Significant difference in CRP was observed between patients with DR and Diabetes but not between DR and healthy controls but no significant difference in BMI was observed by a similar study by Sen[24]. As in our study the patients with diabetic retinopathy were not stratified according to severity, so such comparisons of mean CRP levels with increasing severity of DR cannot be made.

Consistent difference between serum ferritin in diabetic and non-diabetic subjects was shown by Acton[17] but in female gender only and no postulated explanation can be provided for lack of such an effect in male gender. Similar correlation between ferritin levels and diabetic retinopathy was established across both genders by Canturk[25].

CONCLUSION

Significant economic and social burden posed by diabetic microangiopathic complications, diabetic retinopathy in particular, calls for increasing emphasis and commitment towards primordial and primary prevention. The importance of an early diagnosis of this debilitating complication by effective screening can thus, not be undermined. CRP, an inflammatory biomarker has a promising association with diabetic retinopathy.

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