

Role of Increased Serum Ferritin, Insulin and Glycated Hemoglobin Levels On Retina in Diabetic Patients

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ABSTRACT

Background: Diabetes Mellitus type 2 is one of the most common endocrine disorders affecting more than 135 million people in the world¹. DM is an important health problem prevailing across the globe. Inflammation plays a significant role in the pathogenesis of diabetes and its associated complications. Evaluation of inflammatory biomarkers like serum ferritin, serum insulin and glycated hemoglobin helps in assessing diabetic complications like diabetic retinopathy (DR). DR is caused by damage to the blood vessels of the light-sensitive tissue at the back of the eye (retina). At first, diabetic retinopathy may cause no symptoms or only mild vision problems. Eventually, it can cause blindness. Poorly controlled patients of DM have hyperferritinemia which co-relates with diabetic retinopathy and vascular dysfunction.

Method: The present study was being conducted on 50 healthy controls and 100 type 2 diabetic subjects who were divided into two groups (HbA1C <6mg/dl and HbA1C >6mg/dl). In all the subjects fasting serum levels of glucose, serum ferritin, insulin and glycated hemoglobin were analyzed and correlation of HbA1c with different grades of Diabetic Retinopathy was determined.

Results: The mean concentrations of fasting serum glucose, ferritin, insulin and glycated hemoglobin were significantly increased in type 2 diabetic subjects when compared to controls. These mean concentrations were significantly increased in type 2 diabetic subjects with HbA1C >6mg/dl as compared to type 2 diabetics with HbA1C < 6mg/dl. A highly

significant correlation was found between fasting serum glucose and glycated hemoglobin, and serum ferritin and serum insulin levels in type 2 diabetic subjects. The glycemic status showed a rise in HbA1C levels with the increasing severity of diabetic retinopathy in a statistically significant manner.

Conclusion: There is a significant elevation and strong correlation found in the levels of ferritin, insulin, glycated hemoglobin, in type 2 diabetic subjects as compared to controls. Our study also found significant correlation between HbA1C levels and the different grades of diabetic retinopathy.

Keywords: Glycated Hemoglobin, Diabetic Retinopathy, Insulin, Ferritin.

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INTRODUCTION

Diabetes Mellitus type 2 is one of the most common endocrine disorders affecting more than 135 million people in the world.¹ Diabetes mellitus is a clinical syndrome characterised by hyperglycemia caused by absolute or relative deficiency of insulin. Inflammation has a fundamental role in the pathogenesis of diabetes and its associated complications and also contributes to reducing insulin sensitivity and the loss of insulin secretion by islet cells.

Hyperglycemia associated with diabetes can lead to modification of macromolecules, by forming advance glycation end products (AGE) and others, which can augment the production of

proinflammatory markers like cytokines in vascular endothelium causing Retinopathy. Diabetic retinopathy (DR) patients are 25 times more prone to become blind than non-diabetics.²

Diabetic Retinopathy is a complication that involves eyes.³ It is caused by damage to the blood vessels of the light-sensitive tissue at the back of the eye (retina). Initially diabetic retinopathy may cause no symptoms or only mild vision problems but later as the prognosis deteriorates it can cause blindness. Diabetic Retinopathy can develop in type1 or type2 diabetic patients. The longer you have diabetes and the less controlled your blood sugar is, the more likely you are to develop this eye complication.⁴

Diabetic retinopathy may even be present at the time of diagnosis of type 2 diabetic patients, consistent with the usually long duration of subclinical hyperglycemia in such patients and more than 60% of type 2 diabetic patients will have some degree of retinopathy after 20 years of onset of diabetes.

Worldwide, one-third of the estimated 285 million people with diabetes show signs of DR.⁵ It is estimated that diabetes mellitus affects 4 per cent of the world's population, almost half of whom have some degree of DR at any given time.⁶ India is already being termed as the "Diabetic capital of the world", with the number of patients with diabetes expected to rise from 40.9 million, at present, to a whopping 60.9 million by 2025.⁷

World Health Organization (WHO) has announced that currently India has 31.7 million diabetic subjects, and the number is expected to increase to a staggering 79.4 million by 2030.⁸

Ferritin has been known as an index of body iron store. Increased ferritin levels can cause pancreatic beta-cell dysfunction and increased insulin resistance, thereby leading to diabetes mellitus. Serum ferritin is the second-strongest determinant of blood glucose (after BMI) in regression models and the third strongest determinant of serum insulin.⁹ Serum Ferritin could be a marker of the insulin resistance syndrome and may also be an independent determinant of poor metabolic control in the diabetic patient.⁹

There is a bi-directional association between Diabetes mellitus and iron metabolism. Systemic iron overload contributes to abnormal glucose homeostasis by: (i) insulin deficiency as a result of oxidative stress on the pancreatic beta cells leading to cell death and decreased secretion of insulin. (ii) Insulin resistance caused directly by iron overload.¹⁰

Frequent blood donation lead to decrease iron stores, which in turn reduces postprandial hyperinsulinemia and improves insulin sensitivity.¹¹ Decrease in serum glucose, cholesterol, triglycerides and improvement in both beta cell secretion and peripheral insulin action in type-2 DM is seen after phlebotomy. Epidemiological studies also indicate the same correlation.¹¹ Poorly controlled patients of DM have hyperferritinemia which co-relates with diabetic retinopathy and vascular dysfunction.

Overall there is paucity of literature especially from India showing direct evidence that there is difficult control of DM in patients with iron overload. This study aims at estimating the levels of serum ferritin in patients of diabetes in order to find out its effect on retina and to establish its significance in the management and to prevent further complications.

MATERIALS AND METHODS

The study was conducted in collaboration of Department of Ophthalmology and Department of Biochemistry at Mahatma Gandhi Hospital & Medical College and attached group of hospitals, Jaipur between August 2018 to July 2019. People attending the outpatient department of Ophthalmology and Biochemistry laboratory of MGH Medical College and attached group hospitals were included in the study.

Study Design: It is a Prospective cross-sectional study.

Sample Size: We included Total 150 subjects out of which 100 subjects are diagnosed cases of Diabetes and 50 serve as control. The study will include 3 groups:

The Control Group:

Group A: Healthy adult subjects in the age group > 30 years of both the sexes.

The Case Group: 2 Groups

Group B: Hb1AC < 6mg/dl (50 cases)

Group C: Hb1AC > 6mg/dl (50 cases)

Inclusion Criteria: Cases of Type 2 Diabetes of both gender in the age group >40years attending Diabetic OPD, Ophthalmology OPD and Biochemistry lab in M.G.H. Medical college and attached group of hospitals, Jaipur.

Exclusion Criteria: Person on Thyroxine treatment, Pregnancy, Lactating mothers, Renal disease, Liver disease, Cardiovascular disease, Hypertension, Gout, Muscular disorders, Malignancy, Smoking, Alcoholism, Patients on drugs (Hypolipidemic drugs, Antihypertensives, steroids, probenacid, allopurinol etc.) and H/o chemotherapy or radiotherapy for malignancy are excluded.

Collection of Blood Samples: About 5 ml of venous blood sample was collected after a period of 12 hours overnight fasting. FBS, Glycated Hemoglobin, Serum Ferritin and Serum Insulin were estimated in the sample.

Ethical Committee: Approval obtained from the ethical committee of M.G.H. Medical College, Jaipur, Rajasthan.

Parameters Measured: In the present study following parameters were estimated:

1. **Fasting serum glucose** - by analytical kits from Erba Diagnostics Mannheim GmbH in semi-autoanalyzer (CHEM-5 Plus V2, Erba Mannheim).
2. **Serum Ferritin** - by Chemiluminiscence immunoassay (CLIA) kits from Acculite Monobind in Lumax CLIA strip reader.
3. **Serum Insulin** - by Chemiluminiscence immunoassay (CLIA) kits from Acculite Monobind in Lumax CLIA strip reader.
4. **Glycated Hemoglobin** - by Nephelometry kits from Agappe in MISPA-i card reader.
5. **Retinopathy** by -
 - Direct ophthalmoscopy
 - Indirect ophthalmoscopy
 - Slit lamp biomicroscopy using +90D lens
 - Suspected CSME by Fundus Fluorescein angiography
 - Optical coherence tomography (OCT)

RESULTS

A total number of 150 subjects have been studied. This includes 50 controls in Group A and 100 diabetic patients who were divided into 2 sub-groups.

Group B: HbA1C < 6 mg/dl; Group C: HbA1C > 6mg/dl.

In this study, the mean FBS level in Group A was 87.74 with a standard deviation of 9.4 mg/dl, in Group B was 140.7 with a standard deviation of 17.1 mg/dl and in Group C was 167.85 with a standard deviation of 26.24 mg/dl. This is statistically significant within the groups. (Table 1)

In this study, the mean HbA1C level in Group A was 5.01 with a standard deviation of 1.16, in Group B was 4.9 with a standard deviation of 0.62 and in Group C was 8.18 with a standard deviation of 0.66. This is statistically significant between the three groups and significant between Group B and C. (Table 2)

In this study, 28 subjects had ferritin levels between 0-100ng/dl, 15 had between 101-200ng/dl, 3 had between 201-300 ng/dl and 4 had between 301-400 ng/dl. The mean ferritin level was 112.08 with a standard deviation of 90.2 ngdl. Group B had 35 patients

with ferritin levels between 201-300ng/dl, 9 between 301-400 and 6 had > 400ng/dl. The mean ferritin level was 334.4 with a standard deviation of 39.4 ng/dl. Group C had 4 patients with ferritin levels between 201-300ng/dl, 28 between 301-400ng/dl and 18 had > 400 ng/dl. The mean ferritin level was 368.9 with a standard deviation of 46.5 ng/dl. This is statistically significant between groups and also within the groups. (Table 3)

In this study, in Group A 42 subjects had Insulin levels between 0-10µIU/L, 4 had between 11-20 µIU/L and 4 had between >20µIU/L. The mean insulin level was 8.4 with a standard deviation of 6.6 µIU/L. Group B had 8 patients with insulin levels between 11-20 µIU/L and 42 had >20 µIU/L. The mean insulin

level was 26.5 with a standard deviation of 6.4 µIU/L. Group C had 1 patient with insulin level between 0-10 µIU/L, 3 between 11-20 µIU/L and 46 had > 20 µIU/L. The mean insulin level was 31.7 with a standard deviation of 9.4 µIU/L. This is statistically significant between groups and also within the groups. (Table 4) In this study, a correlation is inferred for mild NPDR the range of HbA1c was 6.3% - 9.4%. Moderate NPDR the range was 6.7% - 10.3%. Severe NPDR the range was 8.5% – 11.3%. Very severe NPDR it was 10.2%-12.4%. PDR the range was 10.5%-13.4%. There was a statistically significant result observed with the values of glycosylated hemoglobin HbA1C, which were observed in different grades of diabetic retinopathy. (Table 6)

Table 1: Fasting Blood Sugar Levels (mg/dl)

FBS (mg/dl)	Group A	Group B	Group C
Mean ± S.D.	87.74 ± 9.4	140.7 ± 17.1	167.85 ± 26.24

P< 0.001

Table 2: HbA1C Levels

HbA1C Levels	Group A	Group B	Group C
Mean ± S.D	5.01 ± 1.16	4.9 ± 0.62	8.18 ± 0.66

P< 0.0001

Table 3: Serum Ferritin

S.Ferritin (ng/dl)	Group A	Group B	Group C
0-100	28	0	0
101-200	15	0	0
201-300	3	35	4
301-400	4	9	28
>400	0	6	18
Mean ± S.D	112.08 ± 90.2	334.4 ± 39.4	368.9 ± 46.5

P< 0.001

Table 4: Serum Insulin

S.Insulin (µIU/L)	Group A (n%)	Group B (n%)	Group C (n%)
0-10	42	0	1
11-20	4	8	3
>20	4	42	46
Mean ± S.D	8.4 ± 6.6	26.5 ± 6.4	31.7 ± 9.4

P< 0.0001

Table 5: Pearson's Correlation Co-efficient in Type 2 Diabetic subjects

Parameters	r - value	p value	Significance
Serum Ferritin and Serum Insulin	+0.3161	0.001	Highly significant

Karl Pearson's coefficient of correlation

p < 0.01, S (Significant)

p < 0.001, HS (Highly significant)

p > 0.05, NS (Not significant)

It is observed that there was a highly significant (p < 0.001) positive correlation existing between Serum Insulin and Serum Ferritin in Type 2 Diabetic subjects.

Table 6: Relationship between Grades of Diabetic Retinopathy & HbA1c levels

	N.	Mean	Std. Deviation	p – value
Mild	10	7.910	2.1984	0.000
Moderate	10	8.550	2.5173	HIGHLY
Severe	09	9.944	1.8325	SIGNIFICANT
Very Severe	09	11.356	1.4361	
PDR	12	12.017	2.2816	
Total	50	10.010	2.5997	

DISCUSSION

Diabetes mellitus is the commonest endocrine disorder. Systemic inflammatory activity plays a key role in the pathogenesis of vascular atherosclerosis, insulin resistance and type 2 diabetes mellitus. Ferritin is found to be an index for body iron stores and an inflammatory marker. It is the second strongest determinant of blood glucose (after BMI) and the third strongest determinant of serum insulin (after BMI and age).

This study was a descriptive correlative study, we had evaluated 150 subjects including 50 controls and 100 type 2 diabetic subjects. Of the 100 type 2 diabetic subjects, they were divided into two groups 50 with HbA1C levels <6 mg/dl and 50 with HbA1C levels >6mg/dl.

We studied Glycated hemoglobin levels, Serum ferritin and Serum insulin levels in controls and type 2 diabetic subjects as biochemical markers.

Identifying the patient who may be at high risk of severe retinopathy is important in advising ophthalmic care. The data are also helpful in planning future studies such as controlled clinical trials of treatment of diabetes and of diabetic retinopathy.

Fasting Serum Glucose

The mean value of fasting serum glucose was higher in type 2 diabetic subjects compared to controls and the mean value of fasting serum glucose was higher in type 2 diabetics with HbA1c <6mg/dl compared to type 2 diabetics with HbA1C > 6mg/dl. The increase is found to be statistically highly significant ($p < 0.001$) which is in accordance with Amanullah S et al¹², Mahajan A et al¹³ & Meshram A et al.¹⁴

Glycated Hemoglobin

The mean \pm SDs of HbA1c in controls was 5.01 ± 1.16 mg/dl & type 2 diabetes were in the range of 4.9 ± 0.62 mg/dl in diabetics with HbA1C <6mg/dl & mg/dl in 8.18 ± 0.66 mg/dl diabetics with HbA1C >6mg/dl respectively. The mean value of HbA1c was higher in type 2 diabetic subjects as compared to controls. The increase was statistically highly significant ($p < 0.001$). This is in accordance with Shetty J K¹⁵, Sathiyapriya V et al¹⁶ & Yan R et al.¹⁷

It was also observed that HbA1c level positively correlated with fasting serum glucose. This is in accordance with Meshram A et al¹⁴ & Shetty J K et al.¹⁵

We also found positive correlation existed between HbA1c and ferritin which was statistically significant. Glycated hemoglobin levels probably reflect the degree of glycemic control of the individual better than measuring fasting and post-prandial blood glucose levels. This is because glycated hemoglobin does not depend on variables such as patient co-operation, time of the day, stress, exercise, food intake or renal threshold. This makes attractive screening test in population studies.¹⁸

Serum Ferritin

The mean \pm SDs of ferritin in controls was 112.08 ± 90.2 ng/dl and type 2 diabetic subjects were in the range of 334.4 ± 39.4 ng/mL in diabetics with HbA1C < 6mg/dl and 368.9 ± 46.5 ng/ml in diabetics with HbA1C >6mg/dl respectively. The mean value of ferritin in type 2 diabetic subjects was higher when compared to controls. The increase was found to be statistically highly significant ($p < 0.001$). This is in accordance with the Ford E S¹⁹ & Shi Z.²⁰ In the present study we also found positive correlation existed between ferritin and HbA1c in type 2 a diabetic subject which was statistically significant. This is in accordance with Sumeet Smotra et al.¹¹

Fasting Insulin

The mean \pm SDs of fasting insulin levels in controls was 8.4 ± 6.6 μ U/mL & type 2 diabetes were in the range of 26.5 ± 6.4 μ U/mL in diabetics with HbA1C < 6mg/dl & 31.7 ± 9.4 μ U/mL in diabetics with HbA1C >6mg/dl respectively. The mean value of fasting insulin was higher in type 2 diabetic subjects as compared to controls. The increase was statistically significant ($p = 0.01$). This is in accordance with Nakanishi N et al.²¹ Measurement of the fasting insulin levels has long been considered as the most practical approach for the measurement of insulin resistance. It correlates well with insulin resistance.²²

Grades of Diabetic Retinopathy

The glycemic status of the patient was evaluated using HbA1C values which showed a rise in HbA1C levels with the increasing severity of diabetic retinopathy in a statistically significant manner. One of the studies conducted previously in this regard showed that the risk of PDR was six times higher among diabetics with poor glycemic control.²³ Another similar study showed that the HbA1C levels correlated with prevalence of retinopathy status in diabetic patients on insulin therapy.²⁴ Reductions in blood glucose or HbA1c 57 concentrations through tight blood glucose control in people with diabetes reduces the rate of progression microvascular complications such as DR, neuropathy and nephropathy.²⁵ In our study we found a cut off range of HbA1C for different grades of diabetic retinopathy above which retinopathy of that grade tended to manifest: in mild NPDR the range of HbA1c was found to be 6.3% - 9.4%, moderate NPDR - 6.7% -10.3%, severe NPDR - 8.5% – 11.3%, very severe NPDR - 10.2%-12.4% and for PDR -10.5%-13.4%.

CONCLUSION

Diabetes mellitus is an important health problem prevailing across the globe. Inflammation plays a significant role in the pathogenesis of diabetes and its associated complications. Evaluation of inflammatory biomarkers like glycated hemoglobin and ferritin helps in assessing diabetic complications.

There is significant elevation in the levels of ferritin, insulin, glycated hemoglobin, in type 2 diabetic subjects as compared to controls. There is strong association found between fasting serum glucose, serum ferritin, serum insulin and glycated hemoglobin.

According to our results together with previous other studies findings, we suggest that the quantitative determination of serum insulin and ferritin help in predicting type 2 diabetes mellitus associated complications. Poorly controlled patients of DM have hyperferritinemia which co-relates with diabetic retinopathy, diabetic nephropathy and vascular dysfunction.

A statistically significant correlation was found between HbA1C levels and the severity of diabetic retinopathy with more severe grades of diabetic retinopathy manifesting in patients with higher levels of HbA1C. Thus, the study concluded that inflammatory biomarkers like ferritin and insulin are strongly and independently associated with complications in diabetes like Retinopathy. In addition regular exercises and effective administration of anti-inflammatory agents may offer protection against type 2 diabetes mellitus associated complications.

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