ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Original Research Article

"A COMPARATIVE STUDY OF IRONSTATUS AND GLYCATED HEMOGLOBIN IN TYPE 2 DIABETES MELLITUS IN A TERTIARY CARE HOSPITAL"

Dr. K. Ravi Sankar[1], *Dr. Sumangala M Kadi2

1. **Assistant Professor, Department of Biochemistry, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry – 605502.**
2. **Associate Professor, Department of Biochemistry, Bhaarath Medical College and Hospital, Chennai, Tamilnadu.**

***Corresponding Author: Dr. Sumangala M Kadi, Associate Professor, Department of Biochemistry, Bhaarath Medical College and Hospital, Chennai, Tamilnadu.**

ABSTRACT

Background: Diabetes Mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism, due to defects in either insulin secretion or insulin action or both.

AIM: To compare and correlate the levels of serum Ferritin, serum iron, total iron binding capacity and Glycated Hemoglobin in Type 2 Diabetic patients and Normal controls.

Material & Methods:Study Design: Case – Control study.**Study area:** The study was done in the Dept. of. General Medicine & Bio chemistry inSri Lakshmi Narayana Institute of Medical Sciences, Puducherry.**Study Period:**Oct. 2021 – March 2022.**Study population:** Type 2 diabetic patients and

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

controls attending General Medicine Out Patient Department. **Sample size:** A total of 150 (50 cases & 100 controls) were included in the study.**Sampling method:** Simple Random sampling method. **Study tools and Data collection procedure:**We have measured Fasting plasma glucose, serum Ferritin, Iron, TIBC, HbA1c and Hb in the present study in 50 cases and 100 controls. **Statistical Analysis:**The data was collected, compiled and compared statistically by frequency distribution and percentage proportion. Quantitative data variables were expressed by using Descriptive statistics (Mean ± SD). Qualitative data variables were expressed by using frequency and Percentage (%).

Results: Mean±SDforGlycatedhemoglobinforcasesis8.94±1.84andforcontrolis5.23± 0.45andthedifferenceofmeanofcasesandcontrolis3.7,withZ-scorevaluebeing 10.3. Mean ± SD of Glycated hemoglobin is higher in cases than in controls and the mean difference is statistically significant($p<0.01$).

CONCLUSION: A highly significant correlation was found between fasting plasma glucose, Ferritin and glycated hemoglobin in type 2 diabetic subjects. The present study suggests that estimating iron and ferritin levels provides a sensitive prediction of cardiovascular complications in type 2 diabetic subjects such that early precautions could be taken thus preventing cardiovascular complications.

Key words: fasting plasma glucose, Ferritin and glycated haemoglobin, Type 2 Diabetes mellitus

INTRODUCTION:

Diabetes Mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism, due to defects in either insulin secretion or insulin action or both.[1] The chronic hyperglycemia in Diabetes mellitus is associated with long-term macro and micro vascular complications like coronary artery disease, neuropathy, retinopathy and

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

nephropathy.[2] These complications can progress to end stage outcomes such as, end stage renal disease, blindness and amputation.[3]

With an increasing incidence worldwide, Diabetes mellitus will be the 7th leading cause of morbidity and mortality in 2030.[4] The prevalence of diabetes is increasing globally and the maximum increase will be in developing countries like India. The global prevalence of Diabetes mellitus is around 8.8% with total number of diabetic subjects around 415 million; and it is predicted that by 2040, the burden of this disease will further increase with estimated prevalence up to 10.4% and 642 million subjects affected worldwide. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.[5]

Iron is a transitional metal and micronutrient which is essential for several physiological functions in the body. Iron is also a pro-oxidant known to catalyze the formation of reactive oxygen species (ROS). Hyperglycemia causes increasedGlycation of hemoglobin which in turn increases the release of free iron and stimulate Ferritin synthesis. [6,7] Free iron will enhance the generation of oxygen free radicals, results in increased oxidative stress, stimulates the production of circulating inflammatory markers and pro inflammatory cytokines leading to diabetic vascular complications.[8,9] Recent studies have shown that diabetes mellitus is associated with increased oxidative stress. Studies have shown that increased Serum Iron could be associated with higher incidence of Type 2 Diabetes mellitus.

Ferritin is an index of body iron stores, also an inflammatory marker. Increased Ferritin levels in the blood reflect both the involvement of inflammation and independent actions of excess iron. Recent Studies have demonstrated that elevated levels of Ferritin are associated with Glucose intolerance and insulin resistance suggesting that inflammation is also involved in the etiology of Type 2

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Diabetes.[10,11]Glycated hemoglobin indicates an average blood glucose levels over the past 3 months (6 -8 weeks).[12] Amongst the various markers of Glycemic control, HbA1c is considered as gold standard for monitoring long-term glucose control in people with diabetes mellitus , especially with Type 2 Diabetes mellitus(T2DM) patients.[13]

Therefore the present study was carried out to evaluate the serum levels of Ferritin, serum Iron, TIBC and Glycated hemoglobin in controls and Type 2 diabetic subjects.

AIM: To compare and correlate the levels of serum Ferritin, serum iron, total iron binding capacity and Glycated Hemoglobin in Type 2 Diabetic patients and Normal controls.

Material & Methods:

Study Design: Case – Control study.

Study area: The study was done in the Dept. of. General Medicine &Bio chemistry in Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry.

Study Period: June 2021 – March 2022.

Study population: Type 2 diabetic patients and controls attending General Medicine Out Patient Department.

Sample size: A total of 150 (50 cases & 100 controls) were included in the study.

Sampling method: Simple Random sampling method.

Inclusion Criteria: Clinically diagnosed cases of type 2 diabetes between the age group of 40-60 years of either sex. The diagnosis of type 2 diabetes mellitus wasestablished in accordance with the recommended criteria of American Diabetes Association and based on detail clinical history.

Exclusion criteria:

- Type 1 diabetes mellitus

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

- Patients on repeated blood transfusion
- Patients on corticosteroid therapy and iron supplementation
- Hemoglobinopathies
- Chronic alcoholics , chronic smokers
- Chronic kidney disease
- Pregnant women

Study tools and Data collection procedure:

We have measured Fasting plasma glucose, serum Ferritin, Iron, TIBC, HbA1c and Hb in the present study in 50 cases and 100 controls by the following methods:

1. Fasting Glucose : Glucose Oxidase - Peroxidase (GOD POD) method.
2. Serum Ferritin : Chemiluminescent Microparticle Immunoassay (CMIA)
3. Serum iron : Ferrozine method
4. TIBC : Ferrozine method
5. Hb A 1 c : Cation - exchange HPLC
6. Hemoglobin : Modified Cyanmethemoglobin method

Patients were advised to take normal diet the day before sampling. After overnight fast for 8-10 hrs, venous blood was drawn from the type 2 Diabetes mellitus patients and controls. It was distributed in three tubes – EDTA, Sodium fluoride and Plain tubes. The sample was centrifuged at 3000Rpm for 5 min, plasma and serum was separated and stored in refrigerator at 2-8 °C and were analyzed in batches.

Statistical Analysis:

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

The data was collected, compiled and compared statistically by frequency distribution and percentage proportion. Quantitative data variables were expressed by using Descriptive statistics (Mean ± SD). Qualitative data variables were expressed by using frequency and Percentage (%).

OBSERVATION & RESULTS:

Table 1: Gender distribution among cases and controls

Gender	Cases		Controls		Total
	No	%	No	%	
Male	36	30	66	66	102
Female	14	24	34	34	48
Total	50		100		150

Of the 50 type 2 diabetic subjects, 36 were males and 14 were females and among controls, 66 were males and 34 were females.

Table 2: Sample Distribution According to Age Group, N =150

Age Group (Yrs)	Cases		Controls		Total
	X	%	Y	%	
40-45	15	30	31	31	46
46-50	12	24	25	25	37
51-55	18	36	33	33	51
56-60	5	10	11	11	16
Total	50		100		150

X: Number of cases. **Y:** Number of controls. **N:** Total cohort

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Group	Mean Age	SD	p-Value
Cases (X = 50)	49.12	5.41	> 0.05
Controls (Y=100)	48.93	5.34	

The mean age of cases was 49.12 years and mean age of the controls was 48.93 years with Standard Deviation (SD) 5.41 and 5.34 respectively. The p-value obtained on comparing the mean age of cases and controls was not significant (p-value > 0.05)

Table 3: Distribution of Serum Ferritin in Controls and Cases. N=150

Ferritin	Controls		Cases	
	No.	%	No.	%
M(22-275) F(20-204)	95	95	8	16
> 275	5	5	42	84
Total	100	100	50	100

Group	Mean	SD	p-Value
Controls(100)	78.7	56.1	<0.01
Cases(50)	280	77.3	

Mean ± SD of Serum Ferritin for cases is 280.4 ± 77.26 and for controls is 78.66 ± 56.10 and difference of means between cases and controls is 201.74 with Z - score value being 16.4. Mean ± SD of Ferritin between the two groups is statistically significant (P<0.01).

Table 4: Comparison of Mean ± SD of Blood Glucose levels between two Groups

Group	Mean	SD	p-Value

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Controls(100)	85.3	11.4	<0.01
Cases(50)	174.1	43.7	

Mean ± SD for plasma Glucose levels for cases is 174.14 ± 43.72 and for controlis85.27±11.40andthedifferenceofmeanofcasesandcontrolis88.87,withZ-score value being 14.1. Mean ± SD of Blood Glucose is higher in cases than in controls and the mean difference is statistically significant ($p < 0.01$).

Table 5: Comparison of Serum Ferritin levels in relation to Blood Glucose Levels

Parameters	**Cases**		**Controls**		**p-Value**
	Mean	**SD**	**Mean**	**SD**	
Ferritin	280	77.26	78.66	56.1	<0.01
Glucose	174	43.72	85.27	11.4	<0.01

Mean±SDforGlycatedhemoglobinforcasesis8.94±1.84andforcontrolis5.23± 0.45andthedifferenceofmeanofcasesandcontrolis3.7,withZ-scorevaluebeing 10.3. Mean ± SD of Glycated hemoglobin is higher in cases than in controls and the mean difference is statistically significant($p<0.01$).

Table 6: Mean ± SD of Glycated hemoglobin (%) and Blood Glucose (mg/dl) among cases and controls

Parameters	**Cases**		**Controls**		**p-Value**
	Mean	**SD**	**Mean**	**SD**	
Glycated hemoglobin	8.98	1.84	5.23	0.45	< 0.01
Glucose	174.14	43.72	85.27	11.4	< 0.01

Table 7: Comparison of serum Ferritin, Iron, TIBC and Hb in cases and controls

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Parameters	Cases		Controls	
	Mean	SD	Mean	SD
Ferritin (ng/dl)	266.8	84.6	72.3	36.3
Iron(μg/dl)	85.7	29.4	69.8	19.3
TIBC(μg/dl)	296.9	40.2	298.4	39.1
Hb (g/dl)	13.68	0.88	13.59	0.78
P-Value	< 0.01	<0.05	>0.05	>0.05

The cases were divided into 3 groups based on glycated hemoglobin (HbA1c). There were 7 cases with good Glycemic control (< 7 %), 24 cases with moderate Glycemic control (7-9%) and 19 cases with poor Glycemic control (> 9%), and then compared the serum ferritin and iron levels within threegroups.

Table 8: **Correlation between Ferritin and Glycated hemoglobin among cases**

Parameters	Mean	SD	r value	p value
Ferritin	280.4	77.26	0.76	<0.001
Glycated hemoglobin	8.98	1.84		

There is positive correlation between Ferritin and HbA1c levels among Cases. The correlation coefficient (r) of serum ferritin and HbA1c is 0.76; and their relation is statistically significant, p < 0.001.

Table 9: **Correlation between Iron and Glycated hemoglobin amongcases**

Parameters	Mean	SD	r value	p value
Iron	85.7	29.4	0.47	<0.05
Glycated hemoglobin	8.98	1.84		

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

There is positive correlation between Iron and Glycated hemoglobin levels among Cases. The correlation coefficient (r) of serum iron and HbA1c is 0.47; and their relation is statistically significant, $p < 0.05$.

Table 10: **Correlation between FPG and Glycated hemoglobin amongcases**

Parameters	Mean	SD	r value	p value
FPG	174.1	43.72	0.86	<0.001
Glycated hemoglobin	8.98	1.84		

There is positive correlation between FPG and Glycatedhemoglobin levels among Cases. The correlation coefficient (r) of serum FPG and HbA1c is 0.86; and their relation is statistically significant, $p < 0.01$.

DISCUSSION:

Diabetes mellitus is the most common endocrine metabolic disease is characterized by high levels of blood glucose due to defects either in insulin secretion, or insulin action, or in both.[92] Long-term hyperglycemia is involved in the pathogenesis of many diabetic micro vascular and macro-vascular complications. The ultimate causes of micro vascular complications are chronic hyperglycemia, active polyol pathway and formation of advanced glycation end products that damage blood vessels, leading to vascular complications. Systemic inflammation and oxidative stress plays a key role in the pathogenesis of insulin resistance and Type 2 Diabetes mellitus. Inflammatory biomarkers may be of valuable tool for risk evaluation.

Apart from the elevation of inflammatory markers, direct association of trace elements in diabetes mellitus has been observed in many research studies. Of these minerals, Iron was found

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

to be having important association with diabetes mellitus especially Type 2 Diabetes mellitus and its complications.

The statistical analysis of the obtained values showed that the Serum Ferritin values are significantly higher in type 2 diabetic cases (280.4 ± 77.26 ng/ml) compared to controls (78.66±56.10 ng/ml). The mean difference was significant at p- value <0.01.

Pramiladevi et al[14] observed that serum ferritin levels in the diabetic cases was found to be higher than in the control group and it was statistically highly significant ($p<0.01$), and suggested in pancreas it may cause damage to pancreatic beta cell and decreased insulin secretion and in liver it may cause insulin resistance.

Ford et al also observed similar findings. It found that elevated serum ferritin concentration was associated with increased risk of diabetes.[15]

Kim et al[16] showed that the value of serum ferritin was higher in the type 2 diabetes patients than the control subjects. They concluded that serum ferritin can be employed as a marker of not only glucose homeostasis but also insulin resistance both in type 2 diabetic and control subjects.

Pratik et al[17] found that there is significant increase in serum ferritin in diabetes mellitus compared to control group and hyperferritinemia may be one of the causes for decreased insulin production and development of insulin resistance in diabetes mellitus.

Ferritin is one of the key proteins regulating iron homeostasis, is widely available clinical biomarker to evaluate iron status. However, growing evidence has shown that even moderately increased iron stores represented by high-normal ferritin concentrations are associated with diabetes.

All the cases are type 2 diabetic and all the controls are normal without diabetes. The statistical

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

analysis of the obtained values showed that Mean ± SD for Blood Glucose levels for cases is 174.14 ± 43.72 and for controls (non-diabetic) is 85.27 ±11.40 and difference of means of cases and controls is 88.87, with Z-score value being14.1. Mean ± SD of plasma Glucose is higher in cases than in controls and the p-value being < 0.01.

The increase is found to be statistically highly significant (p <0.01) which is in accordance with Amanullah S et al [18], Mahajan A et al [19]& Meshram A et al.[20]Song Y et al**,** suggested that baseline levels of fasting glucose, insulin, and HOMA-IR were significantly higher among diabetic patients compared with healthy controls.[21]

The statistical analysis of the obtained values showed that the Mean ± SD of Ferritin and Blood Glucose levels for cases (280.4 ± 77.26) and (174.14 ± 43.72) and the Mean ± SD of Ferritin and Blood Glucose levels for controls (78.66 ± 56.10) and (85.27 ± 11.40). The mean difference was significant at P value < 0.01. Meghna Borah, et al, observed that Serum ferritin levels also had a positive correlation with fasting blood glucose levels with a Pearson correlation coefficient "r" of0.75 that is statistically highly significant (p < 0.01).[22]

All the cases are with type 2 diabetes and all the controls are normal without diabetes. Mean ± SD for serum iron levels for cases is 85.7 ± 29.4 and for control is69.8 ± 19.30 and the difference of mean of cases and control is 15.9, with Z-score valuebeing 3.46. Mean ± SD of serum iron is higher in cases than in controls and the mean difference is statistically significant (p < 0.05).Sudhakar et al, observed the serum iron level in the diabetic cases was found to be higher than that in the control group and it was statistically highly significant (p < 0.01).[23]

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

Both serum ferritin, serum iron have shown positive correlation with HbA1c and there correlation coefficient (r) values + 0.76 and + 0.47 and there was statistically significant (p-value = 0.01). Fasting plasma glucose also has positive correlation with HbA1c and

Correlationcoefficient (r) is + 0.86 and p - value = 0.01. Sudhakar et al. reported that Serum level of free iron concentration and serum ferritin was higher in patients with type 2 Diabetes mellitus with poor Glycemic control. Also there was a positive correlation with serum free iron concentration, serum ferritin and Glycemic control. These suggest important role of iron in metabolic derangement in diabetic patients and itscomplications.[23]

Mukesh Gohel et al observed a significant positive correlation between free iron concentration and FBS, PLBS and Glycated Hemoglobin which measures short and long term Glycemic control in type 2 diabetes mellitus patients. These relationship is statically significant (p value < 0.01).[24]

CONCLUSION:

A highly significant correlation was found between fasting plasma glucose, Ferritin and glycated hemoglobin in type 2 diabetic subjects. The present study suggests that estimating iron and ferritin levels provides a sensitive prediction of cardiovascular complications in type 2 diabetic subjects such that early precautions could be taken thus preventing cardiovascular complications.

REFERENCES:

1. Valdez R, Liu T, Yoon PW, Khoury MJ. Family history and prevalence of diabetesin the U.S. population .Diabetes care .2007;30:2517-22.

2. American Diabetes Association (2008): National diabetes fact sheet. 2007,

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

3. Zimmet PZ, Kelly west lecture 1991.Challenges in diabetes epidemiology fromwest to the rest. Diabetes Care 1992;15 :232-52.

4. Mathers CD, Loncar D. Projections of global mortality and burden of disease from2002 to 2030__PLoS Med, 2006, 3(11):e442.

5. International Diabetes Federation. IDF Diabetic Atlas 7th Edition.

6. Bertelsen M, A¨ nggard EE, Carrier MJ: Oxidative stress impairs insulininternalization in endothelial cells in vitro. *Diabetologia* 44:605–613, 2001

7. Fujimoto S, Kawakami N, Ohara A. Non-enzymatic glycation of transferrin:decrease of iron binding capacity and increase of oxygen radical production. BiolPharm Bull. 1995; 18: 396 – 400.

8. Shah S, Iqbal M, Karam J, Salifu M, McFarlane SI. Oxidative stress, glucosemetabolism, and the prevention of type 2 diabetes: pathophysiologicalinsights. Antioxid Redox Signal. 2007;9:911–929.

9. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y,Yao P, Liu LG. Inflammatory markers and risk of type 2 diabetes: a systematicreview and meta-analysis. Diabetes Care. 2013;36:166–175.

10. Jiang, R., Manson, J. A. E., Meigs, J. B., MA, J., Rifai, N. & Hu, F. B. 2004. Bodyiron stores in relation to risk of type 2 diabetes in apparently healthy women.*JAMA: the journal of the American Medical Association*, 291, 711.

11. Suarez-Ortegon MF, Arbelaez A, Mosquera M, Mendez F, Aguilar-de PC. Bodyiron stores as predictors of insulin resistance in apparently healthy urbanColombian men. Biol Trace Elem Res. 2012;145(3):283–5.

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

12. American Diabetes Association. Standards of Medical care in Diabetes- 2011.Diabetes Care. 2011;34(1):S11-61.

13. Glycosylated hemoglobin [Online]. 2007 August 16

14. Pramiladevi R, Umakanth B, Shreeram K. Serum Ferritin Levels In Type IIDiabetes Mellitus, Sch. J. App. Med. Sci., 2013; 1(5):472-5.

15. Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among U.Sadults. Diabetes Care 1999;22(12):1978-983

16. Kim NH, Oh JH, Choi KM, Kim YH, Baik SH, Choi DS, et al. Serum ferritin inhealthy subjects and type 2 diabetes mellitus. Yonsei Med J. 2000;41(3):387-92.

17. Pratik H. Raghavani and Habibunnisa Sirajwala Serum ferritin level in patientswith type-2 diabetes mellitus . IJBAR (2014) 05 (06) . ISSN: 2229-3809 (Online).

18. Amanullah S, Jarari A, Govindin M, Basha MI, Khateeja. Association of hs-CRPwith diabetic and non-diabetic individuals. Jordon Journal of Biological Sciences.2010;3(1):7-12

19. Mahajan A et al. High-Sensitivity C-reactive protein levels and type 2 diabetes inurban north Indians. J Clin Endocrinol Metab.2009;94(6):2123-127

20. Meshram A et al. HbA1c, hs-CRP and anthropometric parameters evaluation in the

patients of diabetes mellitus of central rural india. Int J Med Sci PublicHealth2013;2(2):291-94

21. Song Y et al. Insulin sensitivity and insulin secretion determined by homeostasismodel assessment (HOMA) and risk of diabetes in a multiethnic cohort of women:The women's health initiative observational study. Diabetes Care 2007; 30(7):1747-752.

ISSN:0975-3583,0976-2833 VOL13,ISSUE04,2022

22. Meghna Borah, Rohini K. Goswami et al. Evaluation of serum ferritin in in type IIdiabetes mellitus: Borah M et al. Int J Res Med Sci. 2016 Nov;4(11):4916-4921)

23. Sudhakar B, Toshinwal P, Shah RM, Toshinwal S (May 2014). Elevated serumferritin and serum free iron – a novel marker for pre diabetes type 2 in relationshipwith HbA1C. Jour of Med Sc & Tech; 3(2); Page No: 61 – 66.

24. Mukesh Gohel, H. B. Sirajwala, Anusha Chacko. Serum Free Iron Concentrationin Patients with Type 2 Diabetes Mellitus with Good and Poor Control and ItsCorrelation with Glycemic Control. International Journal of Diabetes Research2013, 2(2): 33-38.