ORIGINAL RESEARCH PAPER

Biochemistry

A COMPARATIVE STUDY OF IRON PROFILE AND SERUM ELECTROLYTES IN TYPE 2 DIABETIC PATIENTS AND HEALTHY CONTROLS"

KEY WORDS: Serum Iron, serum electrolytes.

Dr. Subhash Chandra Bhaskar Junior resident, Dept of Biochemistry, SMS Medical College, Jaipur

Dr Naval Kishor Sharma Junior Resident, Dept of Biochemistry, SMS Medical College Jaipur

Dr. Harjeet Singh Senior Professor &HOD, Dept of Biochemistry, SMS Medical College, Jaipur

ABSTRACT

Introduction: Diabetes mellitus (DM) has become one of the common and challenging health- related problems in the 20th century. Aim of this study was to assess the levels of serum electrolytes and Iron profile (Iron, TIBC, Ferritin) among type II diabetic individuals in comparison with healthy controls. **Methodology-** This cross sectional study was conducted in department of biochemistry and endocrinology in SMS Medical College and hospital Jaipur. 75 cases of type II DM and 75 healthy controls were included. Serum electrolytes and Iron profile calculated and compaired between cases and healthy controls. **Result-** Significantly high ($p<0.01$) levels of Serum Iron, Ferritin, potassium was seen in cases in comparison to healthy controls, Significantly low ($p<0.01$) levels of TIBC, Sodium was seen in cases in comparison to healthy controls. **Conclusion-** Alterations of serum electrolytes and Iron profile may be pathophysiologic and clinically characteristics of diabetes. Hence periodic monitoring of serum iron profile and electrolytes may be needed among those with diabetes mellitus.

INTRODUCTION

Diabetes mellitus type 2 (T2DM) is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Typically it begins in middle or older age, although rates of type 2 diabetes are increasing in young people.

The classic symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss. T2DM is typically a chronic disease associated with a ten-year-shorter life expectancy.

In the developed world, T2DM is the largest cause of non traumatic blindness and kidney failure. The development of T2DM is caused by a combination of lifestyle and genetic factors.

T2DM is due to insufficient insulin production from beta cells in the setting of insulin resistance.

Mineral elements role is well documented in insulin yield, retention and release, along with maintaining its configurational coherence. Insulin resistance heralds the onset of T2DM and various studies point to its connection with iron overload in the body. Being a transition element, iron has marked redox activity, and any potential harm targeted at the body is prevented by its binding with transport or storage proteins.

Enzyme activities and intermediatory metabolism are being carried over by major macro minerals like Sodium, Potassium, Chloride. Derangements in the serum electrolyte levels may be related with Diabetes Mellitus. Electrolyte derangement that is occurring as a result of complications of diabetes are fatal in severe from and thus requires immediate and urgent management.

There are very few studies have done in the past which showed status of Iron profile and levels of serum electrolytes in DM type 2 patient and results of those studies are also inconsistent. Hence we planned this study to assess the levels of serum electrolytes and Iron profile (Iron, TIBC, Ferritin) among type II diabetic individuals in comparison with healthy controls.

AIM AND OBJECTIVES:

To study and compare the levels of iron profile (Serum Iron, TIBC, Ferritin), serum electrolytes in type 2 diabetic patients and healthy controls.

Review Of Literature

Epidemiology: Global Burden of T2DM:

Pacific Islanders, Asian Indians, and Native Americans have a significantly higher risk of developing the disorder. According to the International Diabetes Federation (IDF), in 2019, three countries with the highest number of diabetes patients are China (116.4 million), India (77.0 million), and the United States of America (31.0 million).

Iron And T2dm:

The role of iron in the pathogenesis of diabetes is suggested by 1) an increased incidence of type 2 diabetes in diverse causes of iron overload. 2) reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy.

Elevated serum ferritin levels were independently predicted incident of type 2 diabetes in prospective studies among apparently healthy men and women. Ferritin is an iron-phosphorus-protein complex that is a biomarker for evaluating body iron contents. Tissue and organ damage occurs when iron concentrations are elevated. Increased accumulation of iron affects insulin synthesis and its secretion from the pancreas and interferes with the insulin-extracting capacity of the liver. Iron deposition in muscle decreases glucose uptake because of muscle damage. Increase in Serum Iron level contribute to macro vascular disease as iron has an adverse effect on endothelium and accelerates the development of atherosclerosis. During the course of atherosclerotic plaque formation, ferritin gene expression increases.

Serum Sodium And T2dm:

It is proposed that the correlation between diabetes mellitus and decreased serum sodium may be due to the altered vasopressin regulation.[5] Insulin stimulates the expression of vasopressin-induced aquaporin AQP-2 water channels. The absorption of water in the intestinal tract is increased due to slower stomach emptying that may play a role in hyponatraemia. Hyperglycaemia leads to an increase in serum osmolality, which results in water movement to the extra cellular compartment, out of the cells, and reduces

serum sodium levels by dilution. Hyperglycaemic status also induces hypovolemic-hyponatraemia due to osmotic dieresis.

Serum Potassium And T2dm:
Probable explanation for Hyperkalemia in diabetes may be Exogenous insulin which can induce mild hyperkalaemia as it promotes the potassium influx into the hepatic cells and skeletal muscle cells, thereby increasing the activity of Na+ and K+ ATPase pump. Also, hyperkalaemia is associated with impaired insulin secretion, leading to decreased glucose utilization in the peripheral tissues. This results in carbohydrate intolerance and hyperglycaemia.

MATERIALS AND METHODS :
Approval was taken from -The Ethical Committee, Research Review Board, Department of Endocrinology

Place of study:
Department of Biochemistry and Central Lab, in association with Department of Gastroenterology, SMS Medical College and Hospital, Jaipur.

Study Type, Design:
Hospital based comparative observational study, Cross sectional study.

Study Period: - August 2021 to Nov 2022.

Sample size:-
A sample size of 75 cases in each group is calculated at 95% confidence interval and power of 80% to verify expected difference of 8.4 in mean and 18.5 SD for serum iron level .

Inclusion Criteria: Cases:
Diagnosed type 2 diabetes mellitus patients fasting blood sugar ≥ 126mg/dl, 2-hr plasma sugar ≥ 200mg/dl or HbA1c of more than 6.5% who had given written informed consent and age between 30-60 years.

Control:-
Demographically matched healthy individuals who were willing to participate in the study and had given written consent.

Exclusion criteria:-
1. Any subject with recent history of fever, infections and chronic illness with diabeties mellitus.
2. Pregnant women.
3. Patients having haemoglobinopathies.
4. Type 1 DM, type 2DM on insulin therapy, endocrine disorders (other than type 2 DM), patients with cardiovascular disease, electrolyte disturbances.
5. Patients who were on antipsychotic drugs.

Sample collection and preparation-
After obtaining informed consent from the study subjects, the venous blood was collected under aseptic standard protocols from outdoor & indoor in morning after overnight(12hr) fasting in plain and EDTA vials. The plain vials were left standing for 30 minutes. Then samples were centrifuged with 3000 rpm for 10 minutes to separate serum. Serum iron, TIBC, ferritin and other biochemical parameters were estimated from plain vial. HbA1c estimated from EDTA vial.

Principle assays
1. HbA1c level by latex immune turbid metric assay in fully automated chemistry analyser AU680(BECKMAN COULTER)
2. Serum levels ofElectrolytes(selectivemethod)inf u l l y Automated chemistry analyzerAU680 (BECKMAN COULTER).
3. Serum iron (ferrozine colorimetric method) and TIBC level (saturation- precipitation method)inA u t o m a t e d chemistryanalyzerAU680 (BECKMAN COULTER).
4. SerumFerritinlevel(chemiluminescenceassay)i n Automated immunochemistry analyzer ADIVA Centaur XP (SIMENS).

Estimation Of Glycated Hemoglobin :
After preparing the hemolysate, the Glycated Hemoglobin (HbA1c) concentration is quantified by a latex turbidimetric assay. The addition of an anti-human HbA1c antibody causes agglutination that is proportional to the concentration of HbAlc and can be measured by turbidimetry.

Reference range:
< 6% for non-diabetic
< 7% for glycemic control of person with diabetes

Estimation Of Iron (calorimetric):
Transferrin (Fe^{+2}) + e - $\xrightarrow{\text{Ascorbic Acid}}$ 2 Fe^{+2} + Transferrin

$_{Fe}$+2 $\xrightarrow{\text{Ferrozine}}$ Coloured Complex

Intensity of the colour is proportional to the iron concentration in sample.

Reagent Contents:
- R.1 (Buffer)
- R.2 (Reductant)
- R.3 (Colour)

Reagent Preparation:
Working reagent (WR): Dissolve one tube of R.2 (reductant) in a bottle of R.3 (buffer). Cap and mix gently to dissolve contents.

Calculations :
Iron (ug/dl) = $\frac{\text{(A) Sample} - \text{(A) Sample Blank X 100}}{\text{(A) Standard}}$

Normal value:
Male:65 -175µg/dl
Female: 40-150 µg /dl

Estimation Of Tibc :
Serum transferrin is saturated with an excess of Fe^{+2} and the unbound portion is precipitated with magnesium carbonate.

Calculations:
TIBC = iron conc. in the supernatant x 3 (dilution factor)

Normal value: 200 – 400 ug / dl

Estimations Of Ferritin
Principle:
The ADVIA Centaur Ferritin assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti-ferritin antibodies. The first antibody, in the Lite Reagent, is a polyclonal goat anti-ferritin antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-ferritin antibody, which is covalently coupled to paramagnetic particle.

Expected value

Category	N	Mean (ng/mL)	Mean (pmol/L)	95th Percentile Range (ng/mL)	95th Percentile Range (pmol/L)
Normal Males	142	94	207	22–322	48–708
Normal Females	134	46	101	10–291	22–640

Estimation Of Electrolytes (ion Selecting Electrode Method)
The ISE module for Na+, K+, and Cl- employs crown ether membrane electrodes for sodium and potassium and a molecular oriented PVC membrane for chloride those are

specific for each ion of interest in the sample. An electrical potential is developed according to the Nernst Equation for a specific ion. When compared to the Internal Reference Solution, this electrical potential is translated into voltage and then into the ion concentration of the sample.

Procedure and calculation:

The intensity of the light emitted could be described by the following equation:

$I = k \times c^n$

I= Intensity of emitted light, c= the concentration of the element k = constant of proportionality

$n \sim 1$

(at the linear part of the calibration curve)

Then,

That is the intensity of emitted light is directly related to the concentration of the sample. $I = k \times c$

Expected values

Serum: Na+: 136 - 145 mEq/L

K+: 3.5 - 5.1 mEq/L

Cl-: 98 - 107 mEq/L

OBSERVATIONS AND RESULTS

*P-value as obtained on applying students' "t"-test

Table 1: Comparison of Mean Iron Levels between T2DM & controls

Test/ Parameters	Controls (n=75)	CASES (n=75)	P-value
Serum Iron (µg/dl)	70.67 ± 11.61	96.85 ± 15.60	< 0.01 (S)

Table 2: Comparison of Mean S. TIBC Levels between T2DM & controls

Test/ Parameters	Controls (n=75)	CASES (n=75)	P-value
Serum TIBC (µg/dl)	304.59 ± 58.97	258.15 ± 32.93	< 0.01 (S)

Table 3: Comparison of Mean S. Ferritin between T2 DM & controls

Test/ Parameters	Controls (n=75)	CASES (n=75)	P-value
Serum Ferritin (ng/dl)	56.39 ± 21.17	112.57 ± 30.72	< 0.01 (S)

Table 4: Comparison of Mean S. Sodium between T2DM & controls

Test/ Parameters	Controls (n=75)	CASES (n=75)	P-value
Sodium (mmol/L)	144.35 ± 2.82	139.97 ± 2.54	< 0.01 (S)

Table 5: Comparison of Mean Potassium between T2DM & controls

Test/ Parameters	Controls (n=75)	CASES (n=75)	P-value
Potassium (mmol/L)	4.55 ± 0.52	4.82 ± 0.55	0.0011 (S)

Table 6: Correlation between Serum HBA1c and Serum IRON TYPE 2 DM

Parameter	P value	R Score	R2	Significance
HBA1c vs IRON	<0.0001	0.7977	0.6363	S

*Data analysis using Pearson correlation analysis

DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder with heterogeneity that is characterized by the common feature of chronic hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. In this study we included 75 patients of T2DM and 75 healthy controls. Cases and controls were demographically similar. This study shows significantly higher HbA1c level in diabetic patients in comparison to healthy controls (p<0.01).

In this study mean serum Iron levels were significantly higher in diabetic patients in comparison to healthy controls (p<0.01). We also found significant positive correlation(R= 0.7977) between HbA1c and Serum iron levels in patients with Diabetes (p<0.0001). It shows Serum iron concentration increases with the HbA1c levels in diabetic patients.

In this study serum TIBC was significantly lower in diabetic patients in comparison to healthy controls (p<0.01) which indicates significant negative correlation(R= -0.6506) between HbA1c and Serum TIBC levels. It shows Serum TIBC concentration decreases with the HbA1c levels in diabetic patients.

There was significantly higher mean serum Ferritin level in diabetic patients in comparison to healthy controls (p<0.01) with positive correlation (R= 0.6457) between HbA1c and Serum Ferritin levels. It shows Serum Ferritin concentration increases with the HbA1c levels in diabetic patients.

Our results matches with the study conducted by Dr. Anand et al in 2019[6] they found an increase in the levels of serum free iron concentration and serum transferrin saturation levels with poor glycaemic control.

Our results also correlates with the study conducted by Faridullah Shah et al in 2020[7] they found decrease in TBIC,UBIC whereas an increase was noticed in % saturation, HbA1c, serum iron and fasting blood glucose levels. A study in Iran, by Atari Hajipirloo S et al in 2016[8] has pointed out that elevated levels of iron in first-degree relatives of T2DM patients might be a predisposing factor for them towards the development of diabetes in future.

In a study by Nan Hee Kim *et al*[9], the serum ferritin had a positive correlation with fasting plasma glucose. Ferritin levels were positively correlated with FBS, PP2BS and HbA1c. Similar study conducted by Jeevan K. Shetty et al[10] reported that diabetics with increased level of Serum Ferritin had significantly poor glycaemic control reflected by higher levels of HbA1c as compared to diabetes cases under good glycaemic control and healthy controls.

In our study serum sodium levels were significantly lower in diabetic patients in comparison to healthy controls (p<0.01).Our results correlates with the study conducted by Sreenivasulu Uppara et al[11]in 2020 they found the mean value of serum sodium in diabetic patients is significantly lower compared to controls. Rajagamberan R et al in 2020 also showed significantly low levels of sodium in patients of diabetes in comparison to healthy controls.

Our study shows significantly high Potassium level in diabetic patients in comparison to healthy controls (p=0.011) which correlates with the study conducted by Sreenivasulu Uppara et alin 2020 they found the mean value of serum potassium was higher in diabetes patients compared to controls (p < 0.0001). Rajagambeeeran R et al in 2020 also showed significantly (p<0.001) high potassium in patients of diabetes in comparison to healthy controls. Our results were in concordance in the study conducted by Sarguru Datchinamoorthi et al in 2016 showed in diabetic patients Pottasium levels were found to be high compared with controls and it is statistically significant (P<0.05).

CONCLUSION

This study proves that estimation of iron profile and serum electrolytes levels can be useful to understand patho physiology and complications of T2DM. Hence periodic monitoring of serum iron profile and electrolytes required in diabetes mellitus.

REFERENCES

1. Liamis G, Liberopoulos E, Barkes F: Diabetes mellitus and electrolyte disorders. World J Clin Cases 2014;2(10):488-4
2. Dr. Anand, Dr. Sharmila Dev: Comparative assessment of iron profile in diabetes and control patients. International Journal of Medical and Health Research Volume 5; Issue 1; 2019; 277-280
3. Faridullah Shah, Naseeb UR, Zakia Khan, Rafat Ullah: biochemical assessment of iron profile in type 2 diabetes patients. The Professional Medical Journal Professional Med J 2020;27(8):1590-1594.
4. Nan Hee Kim, Kyung M, Sei Hyun Baik, Sang Jin Kim. Serum ferritin in healthy subjects and Type 2 diabetes patients. Yonsei Medical Journal. 2000; 41(3):387-392.
5. Jeevan K Shetty, Mungli Prakash, Mohammad S. Ibrahim: Relationship between free iron and glycated hemoglobin in uncontrolled type 2 diabetes patients associated with complications. Indian J Clin Biochem. 2008; 23(1):6770.
6. Sreenivasulu Uppara, Bhagyamma Sollapurapp, Rama Kishore Akula, Thanuja Ramanna: A Study on Serum Electrolyte Imbalance in Type-2 Diabetes Mellitus - A Hospital-Based StudyJ Evid Based Med Healthc, 2020/ Vol. 7 / Issue 48 / 2349-2562
7. Rajagambeeram R, Malik I, Vijayam M: Evaluation of serum electrolytes and their relation to glycemic status in patients with type 2 diabetes mellitus. International Journal of Clinical Biochemistry and Research 2020; 7(1):130133.
8. Thivyahprabha AG. Relation between serum electrolytes and serum creatinine levels in diabetes mellitus. Int J Clin Biochem Res 2017: 4(3):257260.
9. Datchanamurthi S, Vanja R, Rajagopalan B. Evaluation of serum electrolytes in type 2 Diabetes Mellitus. Int J Pharm Sci Rev Res 2016;40(1):251-253.
10. Sarguru Datchinamoorthi, Vanaja R, Balaji Rajagopalan: Evaluation of Serum Electrolytes in Type II Diabetes Mellitus Int. J. Pharm. Sci. Rev. Res., 40(1), 2016; Article No. 45, Pages: 251-253.