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Evaluation of Iron Profile in Type II Diabetes Mellitus Cases

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ABSTRACT

Introduction: Type 2 diabetes mellitus is the most common metabolic disorder, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Iron, a transitional metal has been shown to play a major role in pathogenesis of T2DM with a bi-directional relationship where iron affects glucose metabolism, and glucose metabolism in turn impinges on several iron metabolic pathways.

Aims or Objectives: To estimate and compare the parameters related to iron metabolism viz. Serum Iron (Fe), Serum Ferritin, Serum TIBC (Total Iron Binding Capacity), Serum Transferrin and Transferrin Saturation with Fasting Blood Sugar (FBS) between T2DM patients and healthy controls and correlation of FBS with the above iron parameters.

Material and methods: A case control study was conducted between 41 cases of confirmed T2DM patients and 40 age & sex matched healthy controls. Iron profile parameters & FBS were estimated in both the groups and compared. Iron parameters were also correlated with FBS.

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Results: Serum ferritin, Serum iron & serum transferrin saturation were found to be significantly higher in patients with T2DM compared to control group (P<0.001). Serum transferrin & serum TIBC were found to be slightly lower in cases as compared to controls (P<0.001).

Conclusion: The hypothesis of the current study indicates that free iron, being extremely toxic, produces reactive oxygen species (ROS) by participating in Fenton reaction that causes oxidative stress and thereby plays a role in pathogenesis of diabetes. Hence, serum ferritin – a marker of iron overload that indirectly assess insulin resistance; serum transferrin saturation & serum iron and other iron profile parameters should routinely be estimated in all T2DM patients and may also be included in the diabetic profile. Based on the level of parameters in iron profile appropriate measures should be taken to decrease iron load in both prediabetic and diabetic patients to ensure a good glycemic control and to prevent further vascular complications.

Keywords: FBS; Ferritin; Iron; Oxidative stress; Reactive oxygen species; TIBC; Transferrin, Transferrin Saturation; Type 2 Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus is the most common metabolic disorder [1,2], etiopathology being multi-factorial, is characterized by chronic hyperglycemia that results from defects in insulin secretion, insulin action or both along with decreased glucose utilization and increased glucose production [1,3,4].

The essential trace element Iron (Fe) and parameters related to it viz. Serum ferritin, Serum transferrin, TIBC (Total Iron Binding Capacity), UIBC (Unsaturated Iron Binding Capacity), Hb (Hemoglobin) have been evaluated in T2DM patients earlier as these are believed to be closely related to glucose metabolism [5,6,7].

Although piecemeal attempts have been made with selected parameters to understand the role of iron metabolism in T2DM, it needs further elaboration and reiteration to come out with an encouraging conclusion when all the parameters of iron indices are evaluated under one study and under one population on T2DM patients. Iron profile estimation in a patient of T2DM is often neglected although it is important and in recent past sufficient studies on iron profile in T2DM is also missing.

The study undertaken herewith by us is to highlight the role of iron metabolism on impaired glucose metabolism and evaluate whether or not the parameters related to iron metabolism can also work as predictors of T2DM.

MATERIALS AND METHODS

This prospective study was done for a period or 1 year after the consent from the Ethics Committee of the institution was taken. 81 subjects were involved, in which 41 were chosen as T2DM patients and 40 as healthy controls. Diagnosis of T2DM was based on ADA criteria. Age group of the individuals was between 35 and 69.

Subjects on iron supplements, pregnant & lactating women were excluded from the study.

Serum ferritin was measured by CLIA, transferrin by turbidimetry, iron by manual (Bathophenanthroline sulphonate method) method and TIBC by Ion Exchange Resin Ferrozine method using Beckman Coulter equipments while transferrin saturation was calculated. All data was recorded on an excel sheet and analysed statistically.

Reference ranges were:

Serum Iron: 50 – 150 ug/dL for both male & female

Serum Ferritin: Male: 20 – 250 ng/mL; Female: 10 – 120 ng/mL

Serum TIBC: 250 – 400 ug/dL for both male & female

Serum Transferrin: 200 – 360 mg/dL for both male & female

Serum Transferrin Saturation: 30% – 38% for both male & female

RESULTS

Figs 1-6 show graphical representation of the mean ± SD values of iron, iron related parameters (serum ferritin, TIBC, transferrin, transferrin saturation) and FBS values in cases v/s controls.

Serum iron was found to be higher in cases (140.24 ± 30.02 µg/dl) as compared to controls (121.32 ± 14.50µg/dl). Serum ferritin was found to be significantly higher in cases (107.78± 120.06 ng/ml) as compared to controls (48.54 ± 34.22ng/ml) with a p value < 0.001. Serum TIBC was slightly lower in cases (335.84 ± 67.73 µg/dl) as compared to controls (367.77 ± 48.79µg/dl). Serum transferrin was also slightly lower in cases (235.296 ± 47.46 mg/dl) as compared to controls (257.87 ± 34.11 mg/dl). Serum transferrin saturation level was found to be significantly higher in cases (40.04 ± 15.13 %) as compared to controls (33.42 ± 4.51 %). Fasting blood sugar level was expectedly higher in confirmed cases of Type II DM (126.7 ± 67.06 mg/dl) as compared to controls (96.8 ± 16.47 mg/dl) who were healthy individuals (Table 1).

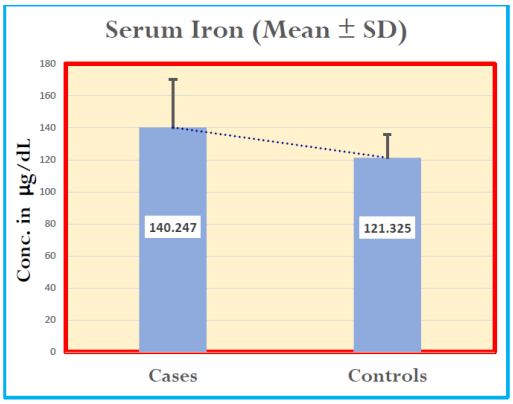
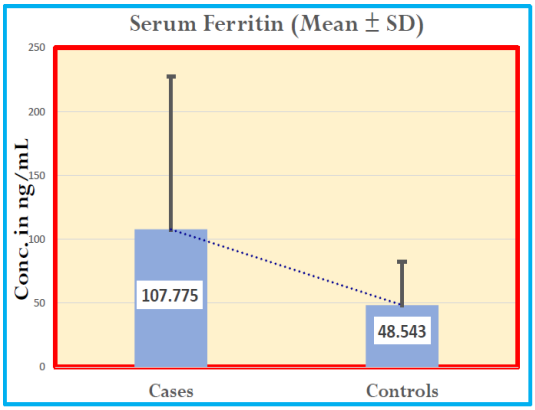


Fig 1.



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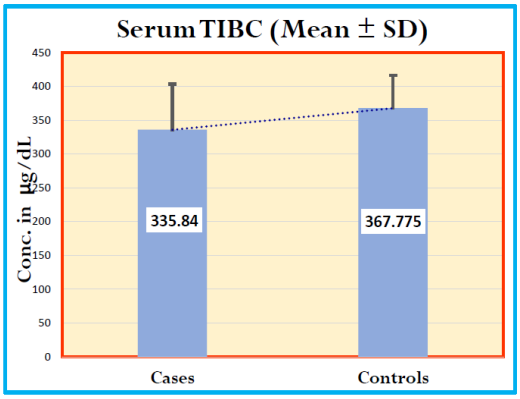

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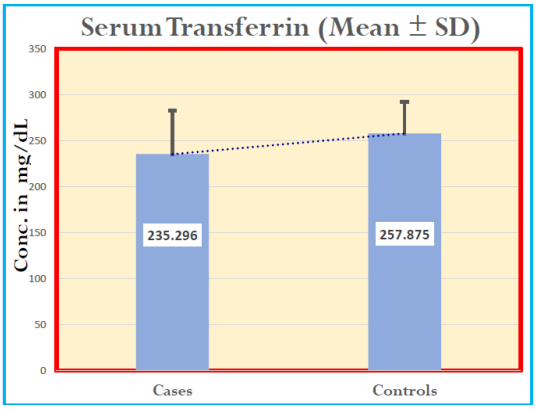

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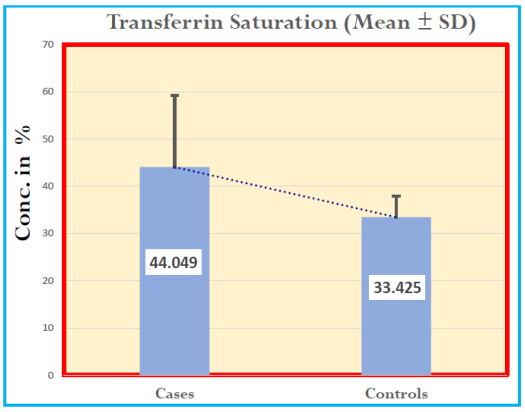

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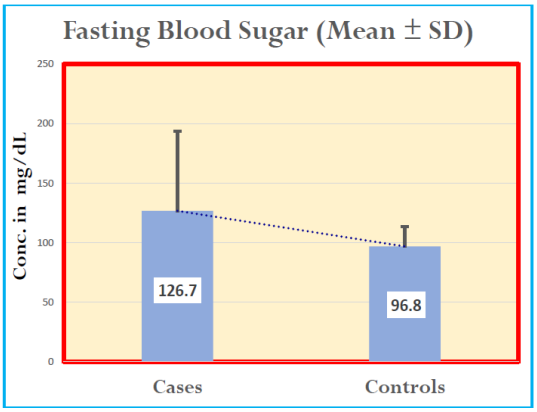

Fig 6.

Note: Mean has been shown as ***'blue bar'****, SD (Standard Deviation) is represented by* ***'black error bar'*** *while the difference in mean between two groups studied is shown as* ***'deep blue dotted trendline'****.*

Table 1: Bivariate Analysis

Variables	Cases		Controls		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Serum Iron	140.247	30.0273	121.325	14.504	< 0.001*
Serum Ferritin	107.775	120.0648	48.543	34.22	< 0.001*
Serum TIBC	335.840	67.7295	367.775	48.794	< 0.001#
Serum Transferrin	235.296	47.4604	257.875	34.118	< 0.001#
Transferrin Saturation	44.049	15.1335	33.425	4.5115	< 0.001*
Fasting Blood Sugar	126.7	67.06	96.8	16.47	< 0.001 #

*Mann-Whitney 'U' test # Independent Sample 't' test

Above table shows the bivariate analysis with comparison of all the parameters between cases and controls.

Each parameter that was expressed as continuous variable and compared between cases and controls during the bivariate analysis showed 'p value' = < 0.001 which was highly statistically significant.

A multivariate logistic regression analysis was done with all the above parameters (variables in the equation) to find out risk factor stratification and establish a direct association considering Odd's ratio and 95% confidence interval of the difference (Table 2). However, we couldn't comment on risk factor stratification from the corresponding Odd's ratio for each variable to establish association, as none of the corresponding 'P' value was statistically significant.

Table 2: Multivariate Logistic Regression Analysis

Variables	Odd's Ratio	95% Confidence Interval (CI)		P value
		Lower	Upper	
Serum Iron	1.724	0.621	4.783	0.295
Serum Ferritin	0.925	0.827	1.034	0.171
Serum TIBC	0.003	0.000	1.366	0.063
Serum Transferrin	2.710	0.719	1.021	0.060
Transferrin Saturation	0.050	0.001	5.013	0.203
Fasting Blood Sugar	0.293	0.000	8.850	0.994

Pearson Correlation was done between fasting blood sugar and serum iron concentration, serum ferritin, serum TIBC, serum transferrin & transferrin saturation only in cases (Table 3). There was only a very weak correlation between fasting blood sugar and serum ferritin concentration (Fig 7) with an 'R' value of 0.142; however the 'p' value obtained was 0.376 which was not of any statistical significance. There was no correlation between fasting blood sugar and the other parameters.

Table 3: Pearson Correlation between fasting blood sugar and other parameters

Variables	R value*	P value
Serum Iron	0.088	0.583
Serum Ferritin	0.142	0.376
Serum TIBC	0.027	0.869
Serum Transferrin	0.027	0.867
Transferrin Saturation	0.068	0.673

*R value between 0.8 to 1.0 was considered to be indicative of strong correlation

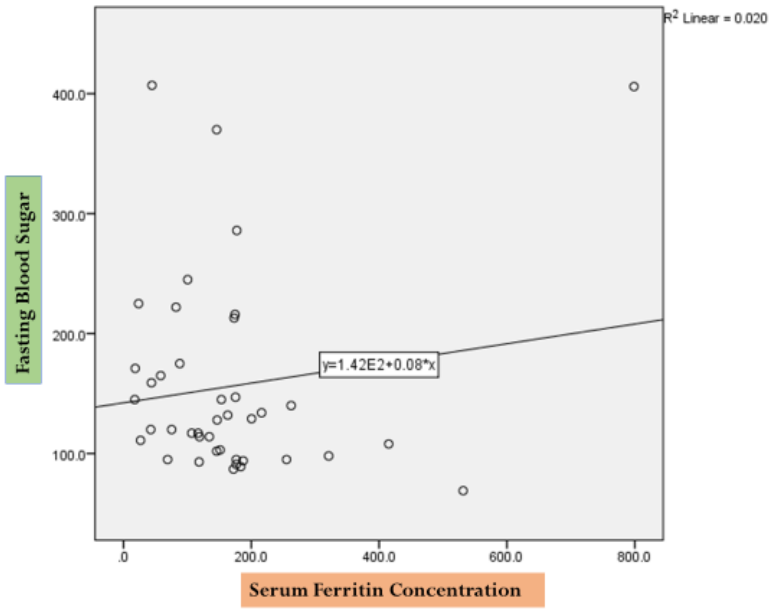

Fig 7: Correlation chart between FBS and serum Ferritin concentration

DISCUSSION

Most studies and latest literatures reveal that iron parameters viz. serum ferritin, serum transferrin, serum iron (Fe), serum TIBC (Total Iron Binding Capacity) and transferrin saturation are altered in T2DM patients. These parameters are now understood to be closely related to glucose metabolism and may also be considered as etiological factors causing T2DM [8,9].

In our study we found out that serum ferritin, serum iron and transferrin saturation were significantly higher in cases as compared to controls while serum transferrin & serum TIBC were slightly lower in the case group as compared to controls. Fasting blood sugar i.e. fasting plasma sugar was expectedly higher in cases in contrast to controls.

Serum iron concentration refers to the amount of circulating Fe^{3+} that is bound to serum transferrin i.e. transferrin bound iron only and does not include the iron contained in serum as free hemoglobin [10,11,12].

Fernández-Real et al in 2002 had revealed that there is a bi-directional relationship between iron and glucose metabolism and concluded that serum iron influences glucose metabolism even in absence of significant iron overload or even in a state of iron deficiency [9]. Nagarajrao R et al showed serum iron to be significantly increased in diabetic patients [13]. Kapoor S et al in a study found serum iron to be normal in T2DM patients [14]. Dulal et al in 2014 in a case control study revealed serum iron to be slightly high in T2DM patients [15]. Manikandan et al showed mean serum iron to be decreased in cases as compared to controls [7].

Free iron being a potent prooxidant increases the cell oxidative stress by participating in Haber Weiss & Fenton reaction, that generates highly toxic free radicals or reactive oxygen species viz. hydroxyl radical (OH•) and hydroperoxyl radical (HOO•) that are capable of inducing lipid peroxidation [16] and further causes oxidative stress and damage to tissues which alters the risk for T2DM [17].

Dandona et al & Rosen et al concluded that free iron is extremely toxic. Iron influences insulin action and thereby glucose metabolism. Iron interferes with insulin inhibited glucose production by the liver. The initial and most common abnormality associated with iron overload conditions is insulin resistance at liver. This finally causes inhibition of insulin internalization and insulin actions that finally results in peripheral hyperinsulinemia and subsequently insulin resistance [18,19].

Serum ferritin is the primary storage form of iron in the body that serves as an index of body iron stores. Ferritin is a positive acute phase reactant protein that is elevated in inflammatory diseases. It stores iron in such a form so that iron gets shielded from body fluids and unable cause oxidative damage [10,11,12].

Medalie et al concluded that in general population, body iron stores are directly associated with the development of glucose intolerance and pathogenesis of type II diabetes [20]. Shaw et al also confirmed that tissue iron excess increases the production of free radicals which in turn amplifies the steps involved in inflammatory lesion [21]. Duffy et al concluded that free iron is reduced from ferric state (Fe3+) to

ferrous state (Fe2+) state that plays a major role in lipid peroxidation process. As the concentration of free iron increases in body, it finally accumulates in the liver as ferritin. Thus, ferritin, an iron storage protein functions as a source of free iron for promotion of superoxide-dependent lipid peroxidation [22].

Dandona et al concluded that hepatic iron overload syndrome is characterized by increased prevalence of glucose tolerance and diabetes, with hyperferritinemia [18]. Moczulski et al concluded that serum ferritin, a reflector of body iron stores was significantly higher in diabetic patients when compared to controls and the ferritin level significantly increased as duration of diabetes increased [23]. Cantur KZ et al confirmed in their studies that poorly controlled diabetes patients had hyperferritinemia [24]. Ren et al in their study also showed that there is significant correlation between increased serum ferritin in diabetes compared to individuals with normal blood sugars and hyperferritinemia may be one of the causes for development of insulin resistance before overt diabetes [25]. Rahier et al proposed that increased ferritin levels in the β-cell can be possibly explained by the fact that ferritin exhibits antioxidant properties and the β-cell is particularly sensitive to oxygen radicals. This high amount of ferritin also explains why iron is preferentially retained in the β-cell itself causing an increase in β-cell mass initially in T2DM patients [26].

Tissue iron excess significantly contributes to production as well as amplification of injury that is caused by free radicals and also modulates various steps involved in the inflammatory lesion. Iron stores are now understood to be directly associated with insulin sensitivity, insulin secretion, and pathogenesis of type 2 diabetes. Free iron also stimulates ferritin synthesis by a positive feedback mechanism, while oxidative stress actually increases the release of free iron from ferritin. Therefore, increased oxidative stress and insulin resistance both together cause endothelial and tissue damage [9].

Bertelsen et al concluded oxidative stress induces insulin resistance thereby influences glucose metabolism by decreasing internalization of insulin and increases ferritin synthesis thereby influences iron metabolism [27].

Insulin has also been shown to be responsible for the increased ferritin synthesis by stimulating ferritin mRNA. Conversely, iron influences glucose metabolism by regulating insulin [28].

Transferrin or siderophilin is the primary transport form of iron in the plasma. The protein part that is responsible for transport of iron from one organ to another is apotransferrin. The apotransferrin-Fe3+ complex is known as transferrin. It contains twohigh-affinity binding sites for Fe3+.When both sites are occupied with Fe3+, it is called holotransferrin (Tf-Fe).Body's homeostatic mechanisms minimize the potential toxicity of free or unused highly toxic iron by forming transport proteins in form transferrin. Thus**,** plasma concentration of transferrin is regulated primarily by availability of iron. In iron deficiency, this level is increased and in iron excess there will be a decrease. Transferrin is a negative acute phase reactant protein and low concentrations are observed in inflammation or malignancy. There is also a reciprocal relationship between synthesis of ferritin and transferrin receptor (TfR1). The rates of

synthesis of TfR1 and ferritin are reciprocally related to intracellular iron levels. When iron is low, TfR1 synthesis is increased and synthesis of ferritin is decreased. The reverse is noticed when iron is abundant and there is no further requirement of iron [10,29].

Moreover, protein glycation as seen in diabetes, affects glycation of transferrin, which further amplifies its pathogenic potential by stimulating free iron release from transferrin, increasing the cell oxidative stress and directly causing endothelial and tissue damage [9].

Typically, only about 30% of the iron binding sites in transferrin are occupied that refers to transferrin saturation and may increase to more than 45% in iron overload conditions although the transferrin concentration is normal. Transferrin saturation (Tsat) may be estimated from the TIBC and serum iron by the following relationship: **Transferrin saturation (%) = 100 x serum iron/TIBC** [10,11,12,29]

Approximately 10% of T2DM patients with elevated ferritin levels have transferrin saturations more than normal [9].

Thomas MC et al found prevalence of elevated transferrin saturation (> 35%) to be 3–4 fold higher in patients with T2DM [8]. Ellervik C et al showed that transferrin saturation ≥50% was associated with a two to three-fold increased risk of developing T2DM [30]. Kapoor S et al showed that transferrin saturation was significantly higher and concluded that increased blood glucose in T2DM, changes the osmolality of blood which may cause hemolysis due to increased fragility of RBC and can interfere with hemoglobin and iron metabolism that increases serum iron and correspondingly increased transferrin saturation [14].

Serum TIBC refers to the maximum amount of iron that can be bound to iron binding proteins viz. transferrin. Transferrin accounts for most of the total iron-binding capacity (TIBC) of serum, However, in healthy individuals approximately only one-third (roughly 30%) of this capacity of transferrin i.e. only about one-third of the iron-binding sites of transferrin are saturated with iron keeping a large percentage as reserve known as unsaturated iron binding capacity (UIBC). TIBC is increased in persons with iron deficiency and is decreased in those with chronic inflammatory disorders viz. diabetes and in malignancies [10,11,12].

.Kapoor S et al in their study found TIBC and UIBC to be significantly lower in T2DM patients that indicates mild iron overload [14]. Senghor A et al & Dulal et al found that mean serum TIBC level was slightly lower in diabetic patients as compared to controls [6,15].

CONCLUSION

This study proves that estimation of iron and all iron related parameters will be useful in diabetes mellitus patients with regard to understanding the pathogenesis, to their evaluate the diabetic complications, evaluate the oxidative stress, associated inflammatory disorders etc., all of these which in turn will help in overall

management of diabetic patients. Iron profile may be considered as a routine & mandate investigation in the evaluation of diabetes mellitus patients henceforth.

REFERENCES

[1] Powers, A.C., 2015, "Diabetes Mellitus, Classification and Pathophysiology," In: Harrison's Principles of Internal Medicine, 19th Edition, Mc Graw Hill, pp. 2399 - 2406.

[2] Ramachandran, A., Snehalatha, C., 2012, "Epidemiology and Basic considerations of Diabetes," In: API Textbook of Medicine, 9th edition, Jaypee Brothers Medical Publishers (P) Ltd, pp. 321 – 323.

[3] Polonsky, K.S. and Burant, C.F., "Type II Diabetes Mellitus," In: William's textbook of Endocrinology, 13th edition, Elsevier, pp. 1386 – 1418.

[4] World Health Organization. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1. Geneva.

[5] Pramiladevi, R. et al, 2013, "Serum Ferritin Levels In Type II Diabetes Mellitus," SJAMS, 1(5), pp. 472 – 475.

[6] Senghor, A., et al, 2012, "Serum Ferritin, Iron, TIBC, Hb in male patients with dysglycemia," Int J Biol Med Res. 2012; 3(2) pp. 1609-1611.

[7] Manikandan, A., et al, 2015, "Study of Iron Status In Type 2 Diabetes Mellitus," IJCBR, 2(20) pp. 77-82.

[8] Thomas, M.C., et al., 2004, "Elevated iron indices in patients with diabetes," Diabet Med., 21(7), pp. 798-802.

[9] Fernández-Real, J.M., et al., 2002, "Cross-talk between iron metabolism and diabetes," Diabetes, 51(8), pp. 2348-54.

[10] Kennelly, P.J., et al, 2015, "Plasma Proteins & Immunoglobulins" In: Harper's Illustrated Biochemistry, 30th Edition, Mc Graw Hill, pp. 672- 677.

[11] Higgins, T., et al, 2015, "Iron, and Bilirubin" In: Tietz Fundamentals of Clinical Chemistry & Molecular Diagnostics, 7th Edition, Elsevier, pp. 508-513.

[12] Adamson, J.W., 2015, "Iron Deficiency and Other Hypoproliferative Anemias" In: Harrison's Principles of Internal Medicine, 19th Edition, Mc Graw Hill, pp. 627.

[13] Nagarajrao, R. and Alharbi, A.S. 2015, "Evaluation Of Serum Zinc, Copper, Magnesium And Iron Levels In Type 2 Diabetes Mellitus Patients", IJAR, 3 (2) pp. 960 – 965.

[14] Kapoor, S. and Sharma, K.A., 2015, "Study of serum parameters of iron metabolism in type 2 diabetes mellitus patients," J Chem Pharm Res, 7(3) pp. 1839-1844.

[15] Dulal, H.P., et al, 2014, "Status of iron, oxidant and antioxidants in chronic type 2 Diabetes mellitus patients," Nepal Med Coll J, 16 (1) pp. 54-57.

[16] Reif, D.W., 1992, "Ferritin as a source of iron for oxidative damage," Free Rad Biol Med, 12 pp. 417–427.

[17] Tuomainen, T-P, 1997, "Body iron stores are associated with serum insulin and blood glucose concentrations," Diabetes Care, 20 pp. 426–428.

[18] Dandona P, et al. "Insulin resistance and iron overload**.** Ann Clin Biochem," 1983; 20 pp. 77–79.

[19] Rosen P, et al. The role of oxidative stress in the onset and progression of diabetes and its complications. Diabetes Metab Res Rev. 2001; 17 pp. 189 –212.

[20] Medalie, J.H., et al, 1975, "Major factors in the development of diabetes mellitus in 10,000 men," Arch Intern Med, 135 pp. 811– 817.

[21] Shaw, J.E. and Chisholm, D.J. 2003, "Epidemiology and prevention of type 2 diabetes and the metabolic syndrome," Journal of Clinical Endocrinology and Metabolism, 179(7) pp. 379 - 383.

[22] Duffy, S.J., et al, 2001, "Iron chelation improves endothelial function in patients with coronary artery disease," Circulation, 103(23) pp. 2799–2804.

[23] Moczulski, D.K., et al., 2001, "Role of hemochromatosis C282Y and H63D mutations in HFE gene in development of type 2 diabetes and diabetic nephropathy," Diabetes Care, 24(7) pp. 1187- 1191.

[24] Cantur KZ, et al. 2003, Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res, 29 pp. 299-306.

[25] Ren, Y., et al. 2004, "Elevated serum ferritin concentration in a glucose impaired population and in normal glucose tolerance first degree relatives in familial type 2 diabetic pedigrees," Diabetes care. (2); 27 pp. 622-623.

[26] Rahier, J.R., et al, 1987, "The hemochromatotic human pancreas: a quantitative immunohistochemical and ultrastructural study," Diabetologia, 30 pp. 5–12.

[27] Bertelsen, M., et al., 2001, "Oxidative stress impairs insulin internalization in endothelial cells in vitro**,"** Diabetologia, 44:605–613.

[28] Yokomori, N., et al, 1991, "Transcriptional regulation of ferritin messenger ribonucleic acid levels by insulin in cultured rat glioma cells," Endocrinology. 128 pp. 1474–1480.

[29] Hortin, G.L., Amino Acids, Peptides, and Proteins. In: Tietz Fundamentals of Clinical Chemistry & Molecular Diagnostics, 7th Edition, Elsevier; 2015 pp. 305-306.

[30] Ellervik, C., et al., "Elevated transferrin saturation and risk of diabetes: three population-based studies," Diabetic care. 2011; 34 (10) pp. 2256-2258.