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Association of Serum Iron Profile among Patients with Type 2 Diabetes Mellitus with or without Diabetic Nephropathy

Farheen Salim[1*], A. K. M Shahidur Rahman[2], Asif Ishtiaq[3], Gazi Arifur Rahman[4], Ferdous Jahan[5], Md. Delwar Hossain[6], Nazmun Haque[7], Hasina Akter[8]

[1]Assistant Professor, Department of Biochemistry and Molecular Biology, Green Life Medical College, Dhaka, Bangladesh
[2]Medical Officer, Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[3]Post-doctoral Researcher, Faculty of Medicine, University of Cyberjaya, Cyberjaya, Malaysia
[4]Medical Officer, Department of Urology, National Institute of Kidney Diseases and Urology (NIKDU), Dhaka, Bangladesh
[5]Assistant Professor, Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[6]Assistant Professor, Department of Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[7]Registrar, Department of Transfusion Medicine, M H Samorita Hospital & Medical College, Dhaka, Bangladesh
[8]Associate Professor (Current charge), Department of Biochemistry, Ashiyan Medical College, Dhaka, Bangladesh

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***Corresponding author:** Dr. Farheen Salim
Assistant Professor, Department of Biochemistry and Molecular Biology, Green Life Medical College, Dhaka, Bangladesh, Email: farheensalim50@gmail.com

Abstract **Original Research Article**

Background: Diabetes mellitus (DM) is the most significant public health challenges in both developed and developing countries. Diabetic nephropathy (DN) is one of the major causes of morbidity and mortality among patients with DM worldwide. Iron modify glucose metabolism and glucose metabolism impinges on iron. ***Objective*:** To evaluate serum iron profile in type 2 diabetic patients with or without diabetic nephropathy. ***Methods*:** This cross-sectional study was conducted at Department of Biochemistry and Molecular Biology, Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorder (BIRDEM), Dhaka, Bangladesh from January 2019 to December 2019. Total 150 participants were enrolled according to the selection criteria and were divided into three groups, 50 patients were with type 2 diabetes mellitus (T2DM), 50 patients were with DN and another 50 respondents were healthy controls. All relevant data were recorded and their serum iron profile was estimated accordingly. Data were analyzed and compared by statistical tests. ***Results:*** Male and female were 41.3% and 58.7% respectively and majority (31.3%) was in 51-60 years. The mean value of HbA_1C of DN patients was significantly higher among groups ($p<0.001$). It was found that serum iron and serum ferritin were significantly ($p<0.001$) higher and total iron binding capacity (TIBC) was significantly ($p<0.001$) lower in T2DM and DN patients compare with healthy controls. The mean serum creatinine level was significantly ($p<0.001$) higher and mean value of estimated glomerular filtration rate (eGFR) was significantly ($p<0.001$) lower in DN patients. There were significant negative correlation of serum iron and significant positive correlation of TIBC with eGFR in patients with DN; but a negative correlation of serum ferritin with eGFR in patients with DN. ***Conclusion*:** It was observed that, serum iron, serum ferritin levels were significantly increased and TIBC was significantly decreased in diabetic nephropathy. This study concluded that high serum iron, serum ferritin and low total iron binding capacity is associated with poor renal status among patients with DN.

Keywords: Type 2 Diabetes Mellitus (T2DM), Diabetic Nephropathy (DN), Iron Profile, Renal Status.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic, non-communicable, chronic disease with high morbidity and mortality. Worldwide DM is considered as the 7th leading cause of death and currently estimated 9.3% world populations are suffering from this disease [1]. DM is one of the most significant public health challenges of 21st century. Over the past three decades, globally the total number of diabetic patients has exceeded more than double [2]. Diabetes mellitus initiates a number of vascular events that affects most of the organs and the main consequences are renal failure, visual impairment, ischemic heart disease, strokes and peripheral vascular occlusive disease [3]. Diabetic nephropathy (DN) is one of the major micro-vascular complications of diabetes and the leading cause of end stage renal disease (ESRD) globally, causing high morbidity and mortality in patients with diabetes [4]. As compared with Caucasians,

South Asians have three-fold greater risk of developing DM and an almost 40-fold greater risk of developing DN, possibly due to a higher prevalence of insulin resistance in the later [5]. In DN there are structural and functional changes in kidneys. The pathophysiologic changes in diabetic nephropathy include hyperfiltration and microalbuminuria followed by worsening of renal function associated with cellular and extracellular derangement [6]. These changes result in a clinical presentation that is characterized by proteinuria, hypertension and progressive reductions in kidney function [4]. In DN various cells involved include glomerular podocytes, mesangial and endothelial cells, tubular epithelium and interstitial fibroblast and vascular endothelium [4].

Iron is a transition metal that acts as an oxidant and is one of the most valuable micronutrients for maintenance of optimum health [7]. Impaired iron metabolism leads to oxidation of lipid and protein which damages RBC (red blood cell) membrane [8]. Increased accumulation of iron affects insulin synthesis and secretion in the pancreas [9]. Iron deposition in the liver may cause insulin-resistance by interfering with the activity of insulin to suppress hepatic glucose production [9]. Catalytic iron converts poorly reactive free radicals like H_2O_2 into highly reactive one like hydroxyl radical and superoxide anion that can initiate and propagate the cascade leading to oxidative damage [8]. Several studies have shown the role of oxidative stress in diabetic patients with iron overload [10-13]. Ferritin is an index of body iron stores and is associated with development of glucose intolerance and type 2 diabetes mellitus (T2DM) [13]. On the other hand, there was no increase in serum iron among those with diabetes mellitus [14]. Also, a more recent prospective study found no association between ferritin levels and risk of T2DM [15]. Studies in recent years found serum iron and serum ferritin increase while TIBC (total iron binding capacity) and hemoglobin decrease in T2DM patients as compare to healthy control subjects [13, 16]. Iiron profile is made up of several blood tests that give information about iron in blood. An iron profile helps to find out different iron related markers like- serum iron, serum ferritin, total iron binding capacity (TIBC) and transferrin saturation [17]. The iron profile measures total amount of iron in blood. It also checks to see if the iron attaching to protein as it should. The iron profile may also tell how much iron is in the body; besides what is in the red blood cells. So, evaluation of iron profile could be a useful tool to predict different complications of diabetes mellitus. In DN, renal damage might be accelerated by iron overload, with significant pro-oxidant and pro-inflammatory consequences. Iron accumulation has been reported in the proximal renal tubules in diabetic nephropathy [18]. In this background, this study was aimed to evaluate serum iron profile among patients with T2DM with or without DN.

2. METHODOLOGY

2.1. Study Design

This cross-sectional study was conducted at Department of Biochemistry and Molecular Biology, Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorder (BIRDEM), Dhaka, Bangladesh from January 2019 to December 2019. The study protocol was approved by institutional review board (IRB), BIRDEM, Dhaka, Bangladesh.

2.2. Study population

A total of 150 subjects of both genders aged above 30 years were selected purposively for this study. They were grouped as-

Group I (a): 50 diabetic patients with nephropathy according to KDIGO guideline [4].

Group I (b): 50 diabetic patients without nephropathy as labeled by endocrinologist.

Group II: 50 normal healthy controls.

Patients undergoing dialysis or renal transplantation, patients having previous blood transfusion (in last 3 months), patients with hepatic disorders/malignancies/acute infection/fever, patients having haemoglobinopathies or genetic mutations which cause iron overload, patients taking vitamin or mineral supplements or drugs that alter serum magnesium levels (such as-diuretics, aminoglycosides, amphotericin B), patients having malabsorption or diarrhea, chronic alcoholic subjects and pregnant/lactating women were excluded from this study.

2.3. Study procedure

After selection of the study population, the purpose, objective and benefits of this study were disclosed to them. Informed written consent was obtained from each study subject. Their demographic profile, detailed clinical and medication history were recorded accordingly. Then different biochemical parameters of each study patient/subject like- serum iron, serum ferritin, total iron binding capacity (TIBC), glycated hemoglobin (HbA_1C), serum creatinine levels and estimated glomerular filtration rate (eGFR) were measured following standard procedure. All biochemical measurements were carried out at the clinical biochemistry section, Laboratory Department, BIRDEM General Hospital, Dhaka, Bangladesh.

2.4. Normal values of the study parameters

In this study normal values of different biochemical parameters were used according to the clinical biochemistry section, Laboratory Department, BIRDEM General Hospital, Dhaka, Bangladesh.

Parameters	Reference value
HbA_1C (%)	Diabetes: 6.5% or higher Prediabetes: 5.7% to 6.4% Normal: <5.7%
Serum Iron (µmol/L)	Male: 10.6-28.3 µmol/l Female: 6.6-26.0 µmol/l
Serum Ferritin (mg/ml)	Male: 30-400 mg/ml Female: 15-150 mg/ml
TIBC (µmol/L)	40.8-76.6 µmol/l
Serum creatinine (mg/dl)	0.8- 1.2 mg/dl
eGFR (ml/minute/1.73m^2)	>90 ml/minute/1.73m^2

2.5. Statistical Analysis

Statistical analysis was performed with the help of windows-based software statistical package for social sciences (SPSS), version- 22. All data were presented as mean with standard deviation (SD) and frequency with percentage for normally distributed data. Data were compared using ANOVA test and Chi square test. Pearson's correlation analysis was applied to assess the correlation. Statistical tests were considered significant when $p<0.05$.

3. RESULTS AND OBSERVATION

This cross-sectional study was carried out at BIRDEM, Dhaka, Bangladesh to evaluate serum iron profile among patients with T2DM with or without DN. In this study total 150 participants were enrolled. Among them 50 diagnosed diabetic nephropathy patients were selected as group I(a), 50 diagnosed diabetic patients without nephropathy were selected as group I(b) and 50 healthy individuals were selected as group II. Of the total participants male and female were 41.3% and 58.7% respectively. Most of the respondents (31.3%) were in 51-60 years age group followed by 26% was in 41-50 years age group, 23.3% in 61-70 years age group, 18% in 31-40 years age group and only 1.3% was in 71-80 years age group. Most of the respondents (60%) were from urban area, 26% was from suburban area and 14% from rural area. Among the participants 10% had no institutional education, 22% was completed primary level education, 18% completed secondary school certificate (SSC) level education, 18.7% completed higher secondary certificate (HSC) level education and 31.3% was completed their graduation. Among the participants 71.3% were sedentary worker, 23.3% were light worker, 2.7% were moderate worker and 2.7% were heavy worker (Table-1).

Table-1: Baseline characteristics of study population in 3 groups (N= 150)

Characteristics		Frequency (n)	Percentage (%)
Gender	Male	62	41.3
	Female	88	58.7
Age (years)	31- 40	27	18.0
	41-50	39	26.0
	51-60	47	31.3
	61-70	35	23.3
	71-80	2	1.4
Residence	Urban	90	60.0
	Suburban	39	26.0
	Rural	21	14.0
Education	No schooling	15	10.0
	Primary	33	22.0
	SSC	27	18.0
	HSC	28	18.7
	Graduation	47	31.3
Physical exercise	Sedentary	105	70.0
	Light work	37	24.6
	Moderate work	4	2.7
	Heavy work	4	2.7

In this study, 32% was male and 68% was female in diabetic nephropathy group, but male was predominant in diabetic patients without nephropathy group (72% versus 28%), although 20% was male and 80% was female participants were in control group (Figure-1). Most of the diabetic cases (33.3%) were treated by both insulin and oral medication, some cases were only on insulin (16%), some used only oral

medication (14%), and only 2% were on lifestyle modification (Figure- 2).

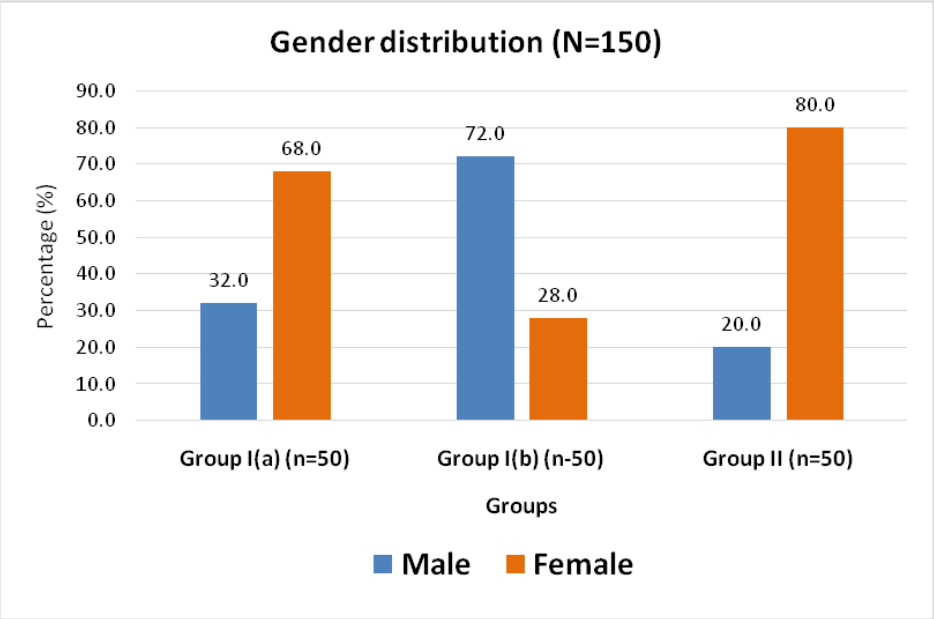

Figure-1: Gender distribution among 3 groups (N= 150)

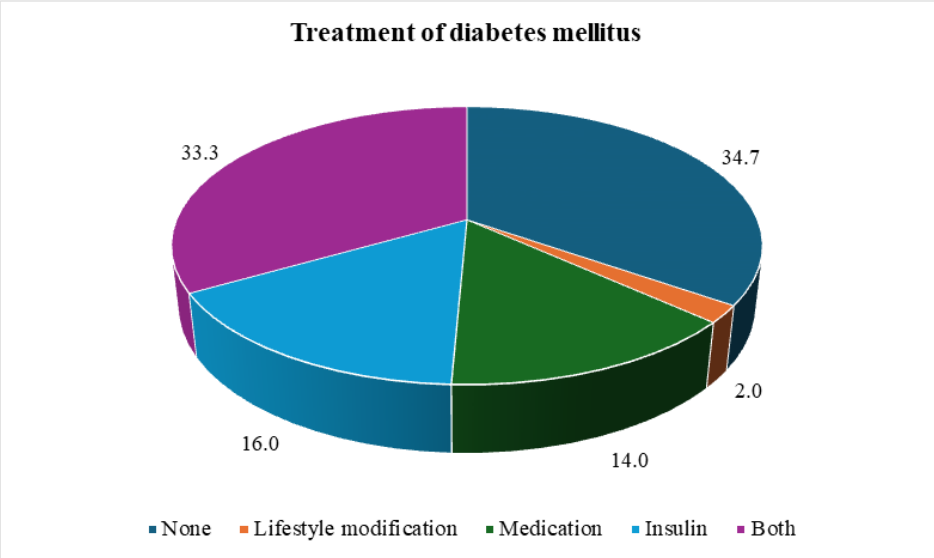

Figure- 2: Treatment of DM among diabetic patients with DN and without DN (N= 100)

It was observed that; duration of DM was 0-5 years, 6-10 years and >10 years among 50%, 30% and 20% patients respectively in case of T2DM without DN and 0.0%, 18%, 82% respectively in case of DM with DN (Table-2).

Table-2: Comparison of duration between T2DM without DN group (group Ib) and diabetic nephropathy group (group Ia) (N= 100)

Duration of diabetes (years)	Group I(b) (n= 50)		Group I(a) (n= 50)		*p* value*
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
0-5 years	25	50.0	0	0.0	<0.001[s]
6-10 years	15	30.0	9	18.0	
>10 years	10	20.0	41	82.0	

*Statistical analysis was done by chi square test, s= significant

In this study, the mean±SD level of HbA_1C (%) of diabetic patients with nephropathy was 10.33±2.07%, in case of DM without DN that was 8.33±2.16% and in healthy controls it was and 5.72±0.53%. It was observed that the mean value of HbA_1C among DN patients was significantly higher in comparison to other two groups (p<0.001) (Table-3).

Table-3: Comparison of HbA_1C level among the 3 study groups (N= 150)

Variable	Group I(a) (n=50) Mean±SD	Group I(b) (n=50) Mean±SD	Group II (n=50) Mean±SD	*p* value*
HbA_1C (%)	10.33±2.07	8.33±2.16	5.72±0.53	<0.001^s

Values are expressed as the mean± SD, *Statistical analysis was done by ANOVA test to compare among groups, s= significant

Comparison of functional status of kidney among the groups showed that, the mean(±SD) serum creatinine and mean(±SD) estimated glomerular filtration rate (eGFR) of DN group were 3.16±0.99 mg/dl and 18.32±6.19 ml/minute/m^2 respectively; in case of DM without DN group which was 0.92±0.13 mg/dl and 82.10±13.27 ml/minute/m^2 respectively and in healthy control group these were 0.75±0.09 mg/dl and 98.52±13.27 ml/minute/m^2 respectively. It was found that the mean serum creatinine of diabetic nephropathy patients was significantly higher in comparison with other two groups (p<0.001). Moreover, we observed that mean value of eGFR among diabetic nephropathy group was significantly lower in comparison with other two groups (p<0.001) (Table-4).

Table-4: Comparison of functional status of kidney among the groups (N= 150)

Variables	Group I(a) (n=50) Mean± SD	Group I(b) (n=50) Mean± SD	Group II (n=50) Mean± SD	*p* value*
Serum creatinine (mg/dl)	3.16±0.99	0.92±0.13	0.75±0.09	<0.001^s
eGFR (ml/minute/m^2)	18.32±6.19	82.10±13.27	98.52±13.27	<0.001^s

Values are expressed as mean±S,D *Statistical analysis was done by ANOVA test to compare among groups,. s= significant

Comparison of iron profile among three groups (T2DM with DN, T2DM without DN and healthy control group) showed that the mean±SD concentrations of serum iron, serum ferritin and total iron binding capacity (TIBC) in T2DM with DN group were 15.28±6.2 µmol/l, 177.6±156 mg/ml, 51.67±8.70 µmol/l respectively; which were 14.39±3.8 µmol/l, 114.82±111.84 mg/ml, 63.90±9.80 µmol/l respectively in case of T2DM without DN group and 11.28±3.64 µmol/l, 36.61± 28.96 mg/ml, 67.29±8.04 µmol/l respectively in case of healthy control group. It was observed that, serum iron and serum ferritin levels were significantly increased in T2DM with DN group in comparison with other two groups (p<0.001). On the other hand, TIBC was significantly decreased in T2DM with DN group in comparison with other two groups (p<0.001) (Table-5).

Table-5: Comparison of iron profile among the groups (N= 150)

Variables	Group I(a) (n=50) Mean± SD	Group I(b) (n=50) Mean± SD	Group II (n=50) Mean± SD	*p* value*
Serum iron (µmol/l)	15.28±6.2	14.39±3.8	11.28±3.64	<0.001^s
Serum ferritin (mg/ml)	177.6±156	114.82±111.84	36.61±28.96	<0.001^s
TIBC (µmol/l)	51.67±8.70	63.90±9.80	67.29±8.04	<0.001^s

Values are expressed as mean±SD, *Statistical analysis was done by ANOVA test to compare among groups, s= significant

Table-6 showed correlation between estimated glomerular filtration rate (eGFR) and other variables in three groups. There were significant negative correlation of serum iron and significant positive correlation of TIBC in T2DM patients with DN, but a negative correlation of serum ferritin with eGFR in T2DM patients with DN (Table-6).

Table-6: Correlation of eGFR with HbA_1C, serum iron, serum ferritin and total iron binding capacity (TIBC) among study subjects

Study groups			HbA_1C (%)	S. Iron (µmol/l)	S. Ferritin (mg/ml)	TIBC (µmol/l)
DM with DN	eGFR	r-value	+0.086	-0.290*	-0.031	+.423*
		p-value	0.552	0.041	0.831	0.002
DM without DN	eGFR	r-value	+0.123	-0.083	-0.035	+0.226
		p-value	0.393	0.566	0.809	0.114
Healthy Controls	eGFR	r-value	-0.125	+0.118	+0.062	+0.219
		p-value	0.386	0.416	0.667	0.127

Statistical analysis was done by Pearson correlation test. Values are expressed as the r: Pearson correlation coefficient. *Correlation is significant

4. DISCUSSION

This cross-sectional study was evaluated the serum iron profile among patients with type 2 diabetes mellitus (T2DM) with or without diabetic nephropathy (DN). The total study subjects were 150 included both sexes, they were divided into three groups and each group contains 50 subjects. Group I(a) was T2DM patients with DN, group I(b) was T2DM patients without DN and group II was healthy controls. The baseline characteristics of the study population were analyzed. The mean HbA_1C level in T2DM patients with DN was found significantly higher than other groups ($p< 0.001$) which reflect the finding of a related previous study [13].

Serum creatinine in group I(a), group I(b) and group II were 3.16±0.99 mg/dl, 0.92±0.13 mg/dl and 0.75±0.09 mg/dl respectively. It was significantly higher among DN group ($p< 0.001$). In accordance, Fan *et al.*, found serum creatinine was 2.65±0.21mg/dl, 0.72±0.16mg/dl and 0.79±0.18 mg/dl in type 2 diabetic patients with nephropathy, type 2 diabetic patients without nephropathy and healthy controls respectively; with a significant high in type 2 diabetic patients with nephropathy group ($p<0.025$) [19]. Another study also detected similar finding [20].

In this present study it was found that eGFR was significantly lower ($p<0.001$) in group I(a) than group I(b) and group II (18.32±6.19 ml/minute/m^2, 82.10±13.27 ml/minute/m^2 and 98.52±13.27 ml/minute/m^2 respectively). Fan *et al.*, also demonstrated that eGFR was significantly differed ($p<0.027$) among type 2 diabetic patients with nephropathy, type 2 diabetic patients without nephropathy and healthy controls (51.1±39.3, 113.4±26.3 and 101.0±39.4 ml/minute/1.73 m^2 respectively) [19]. Similar finding was shown in another study [20].

Mean serum iron in group I(a), group I(b) and group II were 15.28±6.2 µmol/l, 14.39±3.8 µmol/l and 11.28±3.64 µmol/l respectively. It was significantly different among 3 groups ($p<0.001$). In this context, Renuka and Vasanta found serum iron was 12.64±5.72 µmol/l, 9.7±6.45 µmol/l and 7.2±4.75 µmol/l in type 2 diabetic patients with nephropathy, type 2 diabetic patients without nephropathy and healthy controls respectively with a significant difference among the groups ($p< 0.001$) [13]. In our study population, there was a significant negative correlation of serum iron with eGFR in T2DM patients with DN. Glycation of hemoglobin contributes to substantial affinity for transitional metals and glycation of hemoglobin decreases ability of transferrin to bind ferrous iron. When concentrations of antioxidants are low, the reducing potential and anaerobiosis progressively increases, thereby facilitating a rapid release of iron from ferritin. Additionally, the ferroxidase activity of the heavy chain in apoferritin is also down regulated in this setting resulting in an increase in free iron as prooxidant agent. But one study documented that there was no increase in serum iron among the patients with DM [14].

Serum ferritin in group I(a), group I(b) and group II were 177.6±156 mg/ml,114.82±111.84 mg/ml and 36.61±28.96 mg/ml respectively. It was significantly different among 3 groups ($p<0.001$). Similarly, one previous study found that; serum ferritin was 357±28.5 mg/ml, 304±41.22 mg/ml and 192±60.5 mg/ml in type 2 diabetic patient with nephropathy, type 2 diabetic patient without nephropathy and healthy controls respectively with a significant difference between the groups ($p< 0.001$) [13]. In this study we found a negative correlation of serum ferritin with eGFR in T2DM patients with DN. However, these findings were inconsistence with a previous report [21]. Wolide *et al.*, found serum iron and serum ferritin levels were decreased in T2DM patients [21]. It was reported that, inadequate dietary intake, asymptomatic illness and poor absorption rate often associated with the decrease of serum iron and ferritin [21].

In this study, mean level of TIBC in group I(a), group I(b) and group II were 51.67±8.70 µmol/l, 63.90±9.80 µmol/l and 67.29±8.04 µmol/l respectively. It was significantly different among 3 groups ($p<0.001$). We observed a significant positive correlation of TIBC with eGFR in T2DM patients with DN. In a related study, Dhakad *et al.*, found TIBC was 53.72±24.6 µmol/l and 58.39±12.5 µmol/l in type 2 diabetic patient without

nephropathy and healthy control respectively with a significant difference between the groups ($p< 0.05$) [22].

There is a close relationship between iron profile and serum ferritin with T2DM because altered glucose metabolism can alter the iron profile and vice versa [13, 22, 23]. This bidirectional relationship occurs because of the alter iron profile or free iron induces oxidative stress and produces inflammatory cytokinases [22, 23]. Iron is a potent pro-oxidant and reactive oxygen species have been shown to interfere with insulin signaling at the cellular level [23]. Via Haber–Weiss and Fenton reactions, the free iron radicals initiate oxidation of biomolecules leading to generation of hydroxyl radical (OH^-). These radicals damage cellular membrane protein and nucleic acid. These events lead to insulin resistance and finally type 2 diabetes mellitus and thereby contributing to complications such as DN [24, 25]. The free OH^- causes non-enzymatic glycation of protein. The non-enzymatic glycation of proteins followed by a series of reactions and rearrangements resulting in the formation of advanced glycation end products (AGEs). These mechanisms, together with the interaction of the AGEs with their receptors (RAGE) induce reactive oxidative species (ROS) production. The Glycated transferrin has decreased ability to bind Fe^{3+} and thus induces the pool of free iron. The free iron and oxidative stress also promote the synthesis of ferritin [26]. This study demonstrated that serum iron and ferritin levels increase in diabetic nephropathy than T2DM without DN and healthy control group. Our results were supported by a couple of previous studies [13, 23, 27].

5. CONCLUSION

This study concluded that, serum iron and serum ferritin are significantly higher in patients with type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN) compare to healthy controls. On the other hand, TIBC is significantly lower in T2DM and DN compare with healthy controls. It was also observed that, there are strong negative correlation of serum iron with eGFR and significant positive correlation of TIBC with eGFR in patients with DN, but a negative correlation of serum ferritin with eGFR in T2DM patients with DN.

LIMITATIONS

This study was done only one tertiary care hospital in Bangladesh within a limited period of time. Moreover, detailed dietary habit of the study population was not considered. Effects of other antioxidants and trace materials on diabetic nephropathy were not measured.

RECOMMENDATIONS

Further multi-centered, prospective cohort study with large sample size with longer duration should be recommended.

Conflicts of Interest: All authors declared that there is no conflict of interest regarding this publication.

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