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SERUM FERRITIN LEVEL AND MYELOPEROXIDASE ACTIVITY IN TYPE 2 DIABETIC MALES ON ORAL HYPOGLYCEMIC AGENTS

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder, characterised by hyperglycemia caused by absolute or relative deficiency of insulin. Chronic hyperglycemia and enhanced oxidative stress have been implicated in the pathogenesis of diabetic vascular complications. The objective of the study was to find if there is an association between serum ferritin level and serum myeloperoxidase activity in type 2 diabetic males. Serum ferritin level was estimated by ELISA method and serum myeloperoxidase activity by spectrophotometric method using O-dianisidine dihydrochloride as substrate. A significant rise in serum ferritin level ($p < 0.001$) and myeloperoxidase activity ($p<0.001$) with associated hyperglycemia, is an indicator of inflammatory process in diabetics. Hemoglobin level was within physiological range. There was a significant positive correlation between serum myeloperoxidase activity and serum ferritin level ($p < 0.001$). The increased level of myeloperoxidase activity can further contribute to the accelerated progression of atherosclerosis in diabetic mellitus.

KEY WORDS: **Diabetes mellitus, ferritin, hyperglycemia, inflammation, myeloperoxidase.**

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INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterised by hyperglycemia caused by absolute or relative deficiency of insulin. The various factors which can contribute to hyperglycemia include reduced insulin level, decreased glucose utilization and increased glucose production [1]. Although the prevalence of both type 1 and type 2 diabetes mellitus is increasing worldwide, the predominance of type 2 diabetes is rising rapidly because of increase in obesity and reduced physical activity as countries are becoming more industrialized. Metabolic disturbances associated with diabetes mellitus include impaired glucose tolerance, insulin resistance, dyslipidemia, malnutrition and over nutrition [2]. Inflammation has been recognized as the underlying basis of type 2 diabetes mellitus, hypertension, chronic kidney disease and others. Inflammation has a fundamental role in mediating all the stages of atherosclerosis, from initiation through progression and ultimately leading to thrombotic complications. Cardiovascular complications are the leading cause of morbidity and mortality associated with diabetes. In type 2 diabetes, inflammation and activation of monocytes play a major role in reducing insulin sensitivity and loss of insulin secretion by islet cells. Inflammation and oxidative stress can accelerate the process of atherosclerosis in diabetes mellitus. Ferritin has been known as an index of body iron store. It is a ubiquitous and highly conserved iron-binding protein [3]. Ferritin is a positive acute phase protein and its intracellular and extracellular concentration in plasma is dependent on cellular secretion. Increased body iron stores are positively associated with the development of glucose intolerance, type 2 diabetes, and gestational diabetes. Serum ferritin has been considered as a component of insulin resistance syndrome. Serum ferritin concentration was also directly associated with uric acid, another component of the insulin resistance syndrome and inversely related with HDL-cholesterol [4]. Myeloperoxidase (MPO) is an oxidoreductase (EC 1.11.1.7), which is stored in the azurophilic granules of polymorphonuclear neutrophils [5]. It is strongly cationic hemoprotein with a molecular mass of 114 KDa consisting of two identical 72 KDa monomers linked by a disulphide bridge. MPO is involved in the intricate biochemical mechanisms involved in bacterial killing. MPO can generate hypochlorite ions by utilizing hydrogen peroxide which is produced in activated neutrophils and chloride ions. Hypochlorous acid further reacts with hydrogen peroxide and nitrates to form reactive oxygen and nitrogen species [6]. The acute inflammatory reaction also causes damage to membrane lipids, proteins and nucleic acids. MPO has been implicated in diseases associated with chronic non-microbial pathological processes, where oxidative stress and inflammation play dominant roles. Myeloperoxidase, a pro-oxidant enzyme released from the granules of leukocytes, monocytes and macrophages from the inflammatory sites can stimulate increased production of reactive oxygen species which can cause oxidative damage to the endothelium and vessel wall. Although MPO is crucial for the protection against invading pathogens, inappropriate activity of this enzyme could lead to host tissue damage. Increased activity of this enzyme has been implicated in pathological conditions such as cardiovascular disease, cancer, renal disease, lung injury, and Alzhiemer's disease [7]. Type 2 Diabetes mellitus is linked to augmented endothelial dysfunction and accelerated atherosclerosis. However, the association between serum ferritin level and serum myeloperoxidase activity in diabetes mellitus is not very well understood. The study was intended to determine the serum ferritin level and myeloperoxidase activity in type 2 diabetic males who were on oral hypoglycaemic agents and to find if there is any association between serum ferritin and myeloperoxidase activity in diabetics.

MATERIALS AND METHODS

The study population consisted of 45 healthy male controls subjects and 45 clinically diagnosed uncomplicated type 2 diabetic male patients between 25 to 60 years of age who are on oral hypoglycemic agents with duration of diabetes less than 10 years. An informed consent was taken from the patients before

the collection of sample. The ethical clearance was obtained from the Ethical Review Board of the institution. Patients on insulin therapy or with associated complications such as anemia, cardiovascular disease, nephropathy, retinopathy or any other macrovascular complications were excluded from the study. The relevant clinical history was taken and clinical examination of the patients was performed. Blood pressure measurement was done with a sphygmomanometer. The anthropometric measurements like weight, height, waist and hip circumference were recorded. Around 5 ml of venous blood sample was collected after a period of 12 hours overnight fasting. Fasting blood sugar, haemoglobin, lipid profile, serum ferritin level and serum myeloperoxidase activity were estimated in the sample. 1 ml of venous blood was collected in diabetic cases after 2 hours of post-prandial and blood sugar was measured. FBS, PPBS, total cholesterol, triglyceride, high density lipoprotein (HDL), hemoglobin were estimated by automated method and low density lipoprotein (LDL) was calculated using Friedwalds equation. The estimation of serum ferritin level was carried out by ELISA kit [8]. Serum myeloperoxidase activity was estimated by spectrophotometric method using – O-dianisidine dihydrochloride (3, 3'-dimethoxy benzidinedihydrochloride) as a substrate [9].

STATISTICAL ANALYSIS

The results are presented as mean ± SD and results of categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student 't' test (two tailed, independent) and Chi-square test, has been used to find the significance of study parameter. Pearson correlation has been established between MPO and other clinical variables. ROC curve analysis is performed to establish the cut off value of MPO activity.

RESULTS

The data of normoglycemic controls and type 2 diabetic males are compared with respect to fasting blood sugar (FBS), lipid profile, haemoglobin and inflammatory markers: S. ferritin and serum myeloperoxidase (MPO) activity.

Table 1
Distribution of Controls and Diabetic cases as per age group

Age in years	Cases		Controls	
	n	%	n	%
≤ 30	0	0.0	1	2.2
31-40	14	31.1	16	35.6
41-50	14	31.1	11	24.4
51-60	17	37.8	17	37.8
Total (n=)	45	100.0	45	100.0
Mean ± SD	47.13±8.34		44.62±9.74	

Table 2
BMI (kg/m^2) distribution in Controls and Diabetic cases

BMI (kg/m^2)	Cases		Controls	
	No	%	No	%
≤ 18.5	0	0.0	2	4.4
18.6-22.9	3	6.7	17	37.8
23-25	10	22.2	12	26.7
>25 - <30	29	64.4	14	31.1
≥ 30	3	6.7	0	0.0
Total	45	100.0	45	100.0

Table 3
Anthropometric measurements in Controls and Diabetic cases (mean ± S.D

Anthropometric parameters	Cases (n=45)	Controls (n=45)	P value
Waist circumference (cm)	95.68±7.67	88.00±8.23	**<0.001****
Hip circumference (cm)	92.40±6.06	91.86±6.16	0.672
Waist Hip Ratio	1.04±0.04	0.96±0.04	**<0.001****

The age distribution of control subjects and diabetic cases are shown in Table 1. The diabetic cases included in the study were above 30 years of age. There is no significant difference between the mean age of diabetic cases and controls. In the study, all the diabetic males have BMI greater than 18.5 kg/m^2. The mean BMI of cases is about 26.34±2.31 kg/m^2 and is found significantly higher than controls. Nearly 64.4% of the diabetics have BMI between 25 – 30 kg/m^2, indicating more number of cases are having higher BMI level (Table 2). A comparison of anthropometric measurements in diabetics and controls is shown in Table 3. There is a significant increase in waist circumference ($p<0.001$) and waist hip ratio in diabetics as compared to controls. The waist to hip ratio is significantly higher in diabetics as compared to controls ($p<0.001$), indicating diabetics are associated with central obesity.

Table 4
Fasting blood sugar level (mg/dl) in Diabetics and Controls (mean ± S.D)

FBS (mg/dl)	Cases		Controls	
	No	%	No	%
<100	0	0.0	45	100.0
100-125	0	0.0	0	0.0
≥126	**45**	**100.0**	**0**	**0.0**
Total	45	100.0	45	100.0
Mean ± SD	188.20±58.89		90.93±6.27	

Table 5
Comparison of Lipid profile between Diabetics and Controls (mean ± S.D)

Lipid Profile	Cases (n=45)	Controls (n=45)	P value
S.Total cholesterol (mg/dl)	154.07±8.93	152.87±6.45	0.467
S.Triglycerides (mg/dl)	123.53±11.85	121.53±13.33	0.454
S.HDL (mg/dl)	39.87±5.04	40.62±4.09	0.437
S.LDL (mg/dl)	90.04±5.80	87.96±6.03	0.098

Table 6
S. Ferritin, MPO activity and Hemoglobin levels in diabetics and controls (mean ± S.D)

Parameters	Cases (n=45)	Controls (n=45)	P value
Hemoglobin (g/dl)	14.41±0.98	14.34±1.47	0.794
S. Ferritin (ng/ml)	357.11±88.29	170.27±30.45	**<0.001****
MPO activity (U/L)	358.12±45.18	128.30±8.94	**<0.001****

All the diabetic cases in the study have fasting blood sugar (FBS) greater than 126 mg/dl. These are established cases of diabetes mellitus with a minimum duration of diabetes greater than 2 years after diagnosis and on oral hypoglycemic drugs. In the study, the controls have fasting blood sugar value less than 100 mg/dl. The FBS level in diabetics is about 188.20±58.89 mg/dl and the mean value of FBS in controls is about 90.93±6.27 mg/dl (Table 4). The serum total cholesterol, triglycerides, HDL and LDL levels are within normal range in both cases and controls (Table 5, 6). The hemoglobin level in cases and controls are within physiological range. The serum ferritin levels are significantly higher in male diabetics as compared to controls. A significant rise in serum ferritin level is observed, even though hemoglobin levels are within physiological limits. This indicates the rise in serum ferritin levels is independent of hemoglobin level. A significant rise in MPO activity in diabetics is observed as compared to controls. This indicates there is marked inflammation in diabetics, as rise in MPO activity is predominantly due to increased release of MPO from activated neutrophils.

Table 7
S. MPO activity (U/L) in diabetic cases and controls (mean ± S.D)

MPO activity (U/L)	Cases		Controls	
	No	%	No	%
<300	2	4.4	45	100.0
301-400	34	75.6	0	0.0
≥401	9	20.0	0	0.0
Total	45	100.0	45	100.0
Mean ± SD	358.12±45.18		128.30±8.94	

P<0.001*

Table 8
Distribution of S. Ferritin (ng/ml) in diabetic cases

S. Ferritin (ng/ml)	No. of patients (n)	%
<250 (Subgroup A)	9	20.0
≥250 (Subgroup B)	36	80.0
Total	45	100.0

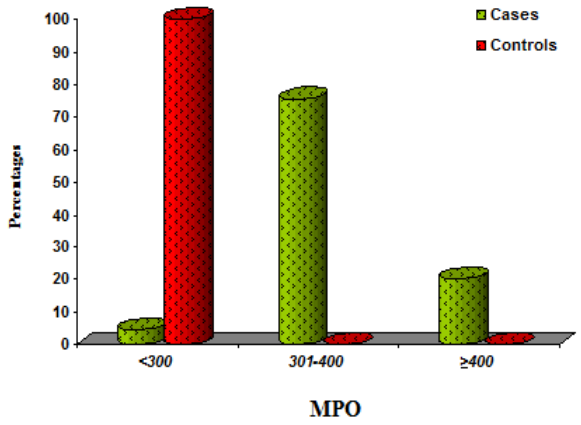

Figure 1
Percentage distribution of MPO activity (U/L) in diabetic cases and controls

Serum MPO activity in all the controls are less than 300 U/L whereas 75.6% of diabetic cases have serum MPO activity in the range of 301 – 400 U/L (Table 7). Nearly 20% have serum MPO activity greater than 400 U/L (Fig 1). MPO activity in diabetics is increased more than two folds in comparison to its activity in controls. The cut off level of serum ferritin was taken as 250 ng/ml. Out of 45 diabetic cases included in the study, 9 cases have serum ferritin level less than 250 ng/ml, whereas 36 diabetics have serum ferritin greater than equal to 250 ng/ml (Table 8). 80% of cases have serum ferritin levels greater than 250 ng/ml. Based on the cut off level of serum ferritin, the diabetic cases are again sub-grouped into: *Subgroup A:* Diabetic cases having serum ferritin < 250 ng/ml (normal serum ferritin level). *Subgroup B:* Diabetic cases having serum ferritin ≥ 250 ng/ml (high serum ferritin level).

Table 9
Comparison of demographic and anthropometric profiles in various subgroups of diabetics (mean ± S.D)

Demographic and anthropometric profiles	<250 (n=9) Subgroup A	≥250 (n=36) Subgroup B	P value
Age in years	50.44±7.54	46.31±8.42	0.186
Weight (kg)	76.89±5.71	74.11±9.19	0.394
Height (cm)	1.68±0.05	1.68±0.06	0.957
BMI (kg/m^2)	27.23±2.12	26.12±2.33	0.200
Waist circumference (cm)	97.37±7.18	95.25±7.83	0.466
Hip circumference (cm)	92.99±5.26	92.25±6.30	0.750
Waist hip ratio	1.05±0.03	1.03±0.04	0.376

There is no significant change in age (years), weight (kg), height (cm), BMI (kg/m^2), waist circumference (cm), hip circumference (cm) and waist to hip ratio between two subgroup (Table 9). The weight of diabetics in subgroup A is about 76.89± 5.71 kg and subgroup B is about 74.11 ± 9.19 kg, whereas height in both the subgroups are almost equal. BMI in subgroup A is 27.23 ± 2.12 kg/m^2 and in subgroup B is 26.12 ± 2.33 kg/m^2 (Table 9). BMI is slightly more in subgroup A than subgroup B but the difference is not significant (Table 9). The waist circumference in subgroup A is about 97.37 ± 7.18 cm and in subgroup B is about 95.25 ± 7.83 cm (Table 9). The hip circumference in both the subgroups is almost equal (Table 9). The waist to hip ratio in subgroup A is about 1.05 ± 0.03 which is slightly higher than in subgroup B which is about 1.03 ± 0.04 but is not significant (Table 9).

Table 10
Comparison of Lipid profile in two subgroups (mean ± S.D)

Lipid Profile	Subgroup A (n=9)	Subgroup B (n=36)	P values
S.Total cholesterol (mg/dl)	152.00±11.40	154.58±8.32	0.444
S.Triglycerides (mg/dl)	118.33±12.66	124.83±11.45	0.143
S. HDL (mg/dl)	41.22±5.02	39.53±5.06	0.373
S. LDL (mg/dl)	90.00±7.07	90.06±5.55	0.980

Table 11
Comparison of FBS, PPBS levels and S. MPO activity in two subgroups (mean ± S.D)

Parameters	Subgroup A (n=9)	Subgroup B (n=36)	P value
FBS (mg/dl)	153.33±23.28	196.92±62.01	0.046*
PPBS (mg/dl)	231.89±47.50	293.14±66.28	0.013*
S. MPO activity (U/L)	316.69±17.58	368.48±44.09	**0.001****
S. Ferritin (ng/ml)	230.48±9.63	388.76±68.01	**<0.001****

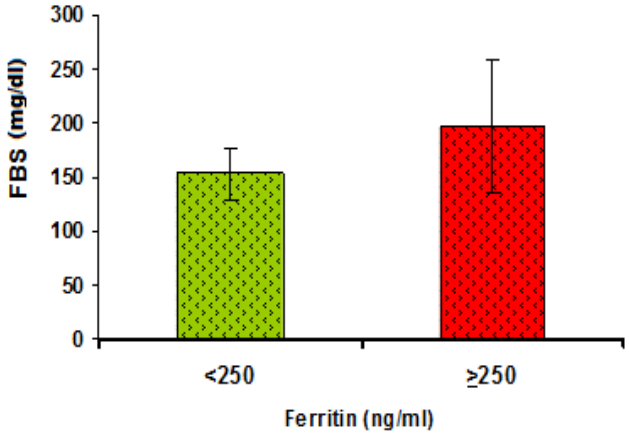

Figure 2
Fasting blood sugar (mg/dl) levels in subgroup A and B (mean ±S.D)

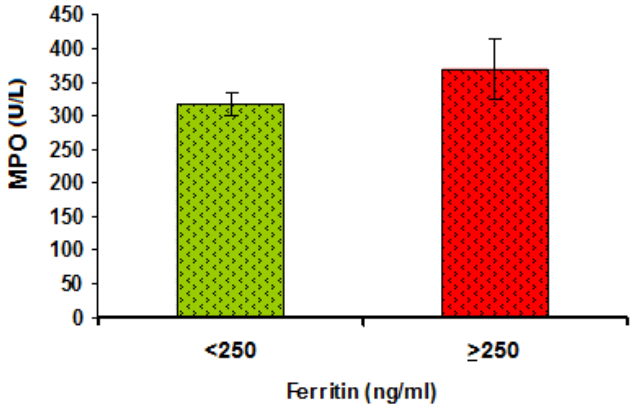

Figure 3
S. myeloperoxidase activity (U/L) in subgroup A and B (mean ±S.D)

There is no significant difference in serum lipid profile values between the two subgroups indicating change in ferritin levels are not associated with significant change in total cholesterol, triglycerides, HDL, and LDL levels (Table 10). There is no significant difference in lipid profile levels between diabetic cases and controls (Table 5). FBS is significantly higher in those cases having serum ferritin levels ≥ 250 ng/ml (subgroup B) as compared to diabetics having serum ferritin levels< 250 ng/ml (subgroup A) (Table 11), (Fig 2). This indicates that at higher blood sugar level, the level of serum ferritin, an inflammatory marker, is also high. There is significant increase in MPO activity when serum ferritin levels are ≥ 250 ng/ml (subgroup B) as compared to other subgroup (Fig 3).

Table 12
Pearson Correlation between S. MPO activity and S. Ferritin, FBS, and Lipid profile in cases:

MPO with other biochemical profiles	Cases (n=45)	
	r value	p value
MPO activity (U/L) vs S. Ferritin (ng/ml)	**0.612**	**<0.001****
MPO activity (U/L) vs FBS (mg/dl)	-0.097	0.526
MPO activity (U/L) vs S. T.Cholesterol (mg/dl)	0.136	0.373
MPO activity (U/L) vs S. Triglyceride (mg/dl)	0.173	0.256
MPO activity (U/L) vs S. HDL (mg/dl)	-0.195	0.199

Table 13
Pearson Correlation between S. MPO activity and FBS and Lipid profile in different levels of S. Ferritin in cases:

MPO with other biochemical profiles	Subgroup A (n=9)		Subgroup B (n=36)	
	r value	p value	r value	p value
MPO activity (U/L) vs FBS (mg/dl)	**-0.746**	**0.021***	-0.263	0.121
MPO activity (U/L) vs S. Total Cholesterol (mg/dl)	0.039	0.921	0.109	0.527
MPO activity (U/L) vs S. TGL (mg/dl)	0.316	0.407	0.062	0.719
MPO activity (U/L) vs S.HDL (mg/dl)	**-0.681**	**0.043***	-0.108	0.531

The Pearson correlation of MPO activity with fasting blood sugar, and serum ferritin, total cholesterol, triglyceride, HDL and LDL cholesterol is shown in Table 12. In the study, a significant positive correlation is observed between MPO activity and serum ferritin levels. The correlations of serum MPO activity with FBS and lipid profiles in diabetics is not very relevant (Table 12). The correlation of MPO activity with parameters other than ferritin was analyzed in subgroup A and B. In subgroup A with S. ferritin < 250 ng/ml, there is an inverse correlation between serum MPO activity and fasting blood sugar but in subgroup B with S. ferritin ≥ 250 ng/ml, there is no significant correlation of MPO activity with FBS. Statistically significant inverse correlation is also observed between serum MPO activity and HDL when serum ferritin level are within physiological range in diabetic males, however at elevated ferritin level, there is no significant correlation between serum MPO activity and HDL (Table 13).

Table 14
ROC curve to determinethe cut off level of S. MPO activity (U/L) in diabetics

Cut-off	Sensitivity	Specificity	LR+	LR-	AUC	P value
≤ 341.7(U/L)	**100.00**	69.44	3.27	0.00	0.860	**<0.001****

Table 15
S. MPO activity and S. Ferritin level as per duration of diabetes (mean±S.D)

MPO & Ferritin	Duration of diabetes in cases		
	≥2 – < 4 yrs (n=20)	≥ 4 - <6 yrs (n=9)	≥ 6 – < 8 yrs (n=6)
MPO activity (U/L)	**361.62±47.34**	357.25±49.25	341.91±26.30
S. Ferritin (ng/ml)	**358.58±97.67**	358.43±71.71	347.75±70.17

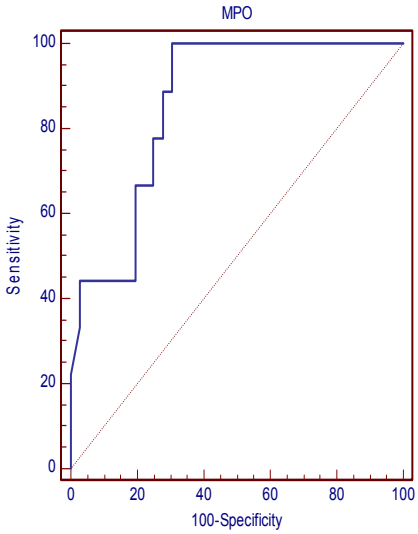

Figure 4
ROC curve to determine the cut off level of S. MPO activity (U/L) in diabetics

The ROC is used to establish the cut off range of serum myeloperoxidase activity in the diabetic cases in the study (Table 14). Further ROC curve has been used to find sensitivity and specificity of serum myeloperoxidase activity in diabetics. The cut

off level of serum MPO activity level was found to be 341.7 U/L. MPO activity below 341.7 U/L is more sensitive and is a better marker of inflammation and vice-versa. The area under the curve at cut off level of 341.7 U/L is about 0.86 (Fig 4). Further analysis was done to find if the duration of the disease after diagnosis will have any influence on serum ferritin levels and myeloperoxidase activity. There is an increase in both serum ferritin levels and in MPO activity irrespective of duration of diabetes (Table 15). However, in the period between 2-4 years after diagnosis of diabetes mellitus, the rise in S. ferritin and MPO activity is higher as compared to other subgroups (Table 15).

Table 16
Pearson Correlation between S. MPO activity and S. Ferritin as per duration of DM

Pair	Duration of diabetes in cases					
	≥2 –< 4 yrs (n=20)		≥ 4 - <6 yrs (n=9)		≥ 6 –< 8 yrs (n=6)	
	r value	p value	r value	p value	r value	p value
S. MPO activity (U/L) vs S. Ferritin (ng/ml)	**0.614**	**<0.001****	0.603	0.086	0.746	0.089

There is a significant positive correlation between MPO activity and S.ferritin levels in the early stages of diabetes ($p<0.001$) (Table 16). This correlation is not so significant when the duration of diabetes is increased. The rise in serum ferritin level is also associated with a significant rise in serum MPO activity in the early stages of DM. Although there is a rise in serum ferritin level and MPO activity as the duration of diabetes increases but no significant correlation is found (Table 16). In the study, there is an increase in serum ferritin level and myeloperoxidase activity associated with hyperglycemia.

DISCUSSION

Chronic hyperglycemia is a major etiological factor triggering both microvascular and macrovascular lesions in diabetes mellitus. Diabetes mellitus is associated with low-grade inflammation with increase in inflammatory markers. Persistent hyperglycemia in uncontrolled diabetics can cause inflammation and increased production of reactive oxygen species from glucose auto-oxidation can predispose to detrimental consequence. Serum ferritin generally considered as a marker of iron status in the body, is also a potent inflammatory marker [10]. Though serum ferritin acts as a storage form of iron in the body but under diverse stimulus can release free irons, which can take part in the generation of reactive radicals by Fenton's reactions [11]. Free radicals are formed in diabetics due to glucose oxidation, non-enzymatic glycation of proteins and oxidative degradation of glycated proteins. Elevated levels of reactive oxygen species and associated decline of antioxidant defense mechanisms can lead to damage of subcellular organelles, increased lipid peroxidation and development of insulin resistance. Inflammation and oxidative stress can accelerate the process of atherosclerosis in diabetes mellitus. In the study, all the diabetics had FBS greater than 126 mg/dl and they were on oral hypoglycemics drugs. The diabetics in the study group had increased waist circumference with associated increase in waist hip ratio (Table 3). BMI is increased in diabetics with nearly 64.4% having BMI greater than 25 kg/m^2. These results are consistent with some studies which have reported positive association of serum ferritin levels with higher BMI [12]. Increased body iron stores are associated with the development of glucose intolerance, type 2 DM and insulin resistance syndrome [13]. Various inflammatory factors can interfere with the synthesis and clearance of ferritin, consequently leading to the rise in serum ferritin level. The elevation in S. ferritin level can be due to causes other than derangement in iron metabolism [14]. In the study a significant increase in serum ferritin levels are found in diabetics as compared to controls (Table 6). Sharifi et al [15] have reported that serum ferritin levels are

increased in type 2 diabetes mellitus. Kaye et al [16] have reported elevated serum ferritin level as a risk factor for type 2 diabetes mellitus with iron overload. However, haemoglobin levels in the study were within physiological range, which excluded other haematological causes for iron overload. Males were only included in the study so that other confounding factors for increased iron turnover under physiological circumstances were ruled out. An important role for ferritin during the acute phase response is to restrict the availability of iron by sequestering into the cavity of the ferritin protein shell. Increased ferritin levels can cause pancreatic beta-cell dysfunction and increased insulin resistance, thereby leading to diabetes mellitus. Increase in serum ferritin concentration can also be due to leakage of tissue ferritin [14]. The level of serum ferritin represents the balance between its secretion and clearance, mainly in liver. Kalantar-Zadeh et al [17] have reported elevated levels of serum ferritin in diabetes mellitus due to inflammation, independent of iron stores. Ferritin is produced in macrophages, hepatocytes and adipocytes in conditions associated with inflammation and its production can be induced by the presence of inflammatory cytokines such as interleukin (IL)-β and tumour necrosis factor (TNF)-α [18]. Maiti et al [19] have reported inflammation in diabetes and atherosclerosis. There is increased predisposition towards atherosclerosis in diabetes mellitus as both diabetes mellitus and atherosclerosis are chronic inflammatory conditions. Under conditions of oxidative stress, ferritin can act as oxidant. ROS are found to be directly involved in the transcriptional activation of ferritin gene. Rogers et al [20] reported, IL-1β induces ferritin gene expression by translational control of its mRNA. The pathophysiologic mechanism for induction of ferritin synthesis in inflammation is different from iron-dependent ferritin gene expression. Qian et al [21] have reported that reactive oxygen species can interfere with insulin signalling at various levels in the insulin receptor function and can inhibit the translocation of glucose transporter, GLUT- 4 in the plasma membrane. This effect may be the main mechanism for insulin resistance resulting in hyperglycemia. Hyperglycemia associated with diabetes can lead to modification of macromolecules by forming advance glycation end products (AGE) and others, which can augment the production of pro-inflammatory markers like cytokines in vascular endothelium. The process of inflammation induces synthesis of various acute phase proteins such as hs-CRP and serum ferritin, predisposing to insulin resistance as well as atherosclerosis [22]. The rise in blood glucose level can bring about glycation of ferritin in hyperglycemic state. Glycated ferritin has longer life span and results in hyperferritinemia in type 2 diabetes as in the study. Iron deposition in the liver may further perpetuate insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production [4]. Elevated plasma ferritin concentration, obesity, metabolic syndrome, inflammation and lifestyle can predispose to development of diabetes. Serum ferritin is also considered as an important and independent predictor of DM [23]. Type 2 diabetes mellitus is linked to augmented endothelial dysfunction and accelerated atherosclerosis. Endothelial dysfunction is a major contributing factor to the pathogenesis of diabetic vascular complications. ROS such as superoxide ions and hydrogen peroxide (H_2O_2) have emerged as important molecules in the pathogenesis of diabetic vascular complications, including endothelial dysfunction. Vascular NADPH oxidases, the non-leukocyte oxidase, are found to be the major source of ROS in diabetic vasculature [24]. Increased levels of myeloperoxidase activity contribute to initiation and accelerated progression of atherosclerosis in diabetes [25]. MPO can use high-glucose stimulated, vascular non-leukocyte derived H_2O_2 to induce diabetic endothelial dysfunction by reducing nitric oxide bioavailability [26]. In the study, high serum MPO levels are found in diabetics along with high blood glucose levels (Table 7). Dougherty et al [27] have reported high levels of MPO, MPO-derived 3-chlorotyrosine, and HOCl -modified LDL in atherosclerotic lesions. In the study, high levels of myeloperoxidase activity were found in diabetics. Due to high blood glucose levels in subgroup B (serum ferritin ≥ 250 ng/ml), there is increased glucotoxicity which can contribute to oxidative stress and increased inflammation consequently resulting in higher

serum ferritin levels and MPO activity (Table 11). Increase in serum ferritin levels and MPO activity, markers of inflammation, can enhance insulin resistance and further contribute to hyperglycemia in uncontrolled diabetics. Sultan et al [28] have reported high ferritin levels which contribute to insulin resistance in diabetics. A negative correlation was found between myeloperoxidase activity and FBS in subgroup A but not in subgroup B (Table 13). ROC curve shows, MPO activity is a more sensitive marker of inflammation when the MPO activity is less than or equal to 341.7 U/L (Table 14).The fasting blood sugar levels is comparatively lower, when serum ferritin level are within normal range in diabetics as compared to diabetics who have ferritin greater than or equal to 250 ng/ml. Due to moderate hyperglycemia in the earlier group, a decrease in MPO activity was observed as compared to the later group. In subgroup A, the mean value of MPO activity was 316.69 ± 17.58 U/L and was less than the cut off range which was taken as 341.7 U/L (Table 11). High MPO activity in diabetics may be due to hyperinsulinemia [29]. Zhang et al [26] have reported vascular bound MPO could use high glucose stimulated hydrogen peroxide to amplify high glucose induced injury to the vascular wall. The severity of inflammation is of lesser degree in them as is evident by serum ferritin level within normal limits. A significant positive correlation was found between serum ferritin and MPO activity in the study ($p<0.001$) (Table 12). The study indicates there is associated increase in MPO activity and serum ferritin level in diabetics. In subgroup A, though serum ferritin levels are within normal limits, there was a significant rise in MPO activity as compared to controls. The rise in myeloperoxidase activity occurs even before obvious rise in serum ferritin level in the study indicating serum myeloperoxidase activity as a better sensitive indicator of inflammation as compared to serum ferritin. An inverse correlation was observed between serum MPO activity and HDL level when serum ferritin level was within physiological range in diabetic males however at elevated ferritin level, there was no significant correlation between serum MPO activity and HDL level (Table 12). When serum ferritin levels were within normal range, the increase in myeloperoxidase activity is less predominant even though FBS is raised. Subsequently, there is an increase in functional HDL which can effectively carry out its function of reverse cholesterol transport and anti-atherogenesis. In the study, there was no significant correlation of serum MPO activity with total cholesterol, triglycerides, and LDL level in either of the subgroups (Table 12). MPO was found to be linked with HDL, thereby inactivating the actions of HDL. The decrease in HDL level can also influence MPO activity. MPO has the capacity to convert anti-inflammatory HDL to pro-inflammatory HDL, thereby influencing endothelial dysfunction. MPO catalyzes reactions between hydrogen peroxides and chloride ions to generate potent oxidants including hypochlorous acid (HOCl) and reacts with nitric oxide and nitrite to produce reactive nitrogen species [30]. MPO and its oxidant byproducts are detected in lesions, and are especially abundant at sites of thrombosis. MPO can oxidize LDL to ox-LDL, which are taken up effectively by macrophage scavenger receptors and enhance foam cell formation. There was significant correlation between S. ferritin level and MPO activity in the initial years of diabetes ($p < 0.001$) (Table 16), but as duration of DM increases, there is elevation in inflammatory markers but no significant correlation was observed (Table 16). This may be attributable due to the influence of factors other than inflammation playing an important role in the progression of diabetes, thereby causing irreversible damages in the target tissues. Chronic systemic inflammation can induce insulin resistance and is a key mechanism relating both diabetes and metabolic syndrome. The prevalence of hyperferritinemia is about 55.6% in diabetics in the study. High serum ferritin level and increase in myeloperoxidase activity is a definitive indicator of inflammatory processes in diabetes mellitus. Due to increase in inflammatory markers, there is increase in insulin resistance, which in a cyclical process can further accelerate inflammation. As the disease progresses, even though oral hypoglycemics are supplemented, the end organs develop decreased sensitivity to insulin resulting in uncontrolled hyperglycemia.

CONCLUSION

There is increase in serum ferritin level and MPO activity, which are indicators of inflammation. Inflammatory response may have a dual role in DM, either it can have a causal relationship leading to insulin resistance or the response can be intensified by the hyperglycemic state ensuing in vascular complications. The elevation in MPO activity is observed even before there was an obvious rise in serum ferritin level. There was significant correlation between MPO activity and serum ferritin levels in the initial years of DM and as the disease progresses, other confounding factors come into action. The study needs to be carried out on a larger population size along with more sensitive markers of inflammation to decipher their exact role in the onset and progression of diabetes mellitus.

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