

## Status of iron, oxidant and antioxidants in chronic type 2 Diabetes mellitus patients

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### ABSTRACT

Diabetes mellitus is a common health problem of the world. Iron may be a part of the cause of the disease and its complications. Iron is a trace element which produces reactive oxygen species (ROS) participating through Fenton reaction and that ROS may be a cause to produce oxidative stress and further diabetic complications. The study aims to access the iron and its effect in producing oxidative stress in type 2 diabetic patients. Serum iron, total iron binding capacity (TIBC) and percentage transferrin saturation are calculated as the index of iron. Malondialdehyde (MDA) is estimated as index of oxidant and vitamin C, vitamin E are measured as index of antioxidants. This is a case control study conducted in the department of Biochemistry in collaboration with department of Medicine at B P Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. 52 chronic type 2 diabetes mellitus patients and 52 age and sex matched normal healthy controls were included in the study. Plasma iron, TIBC, percentage transferrin saturation were found ( $89.14 \pm 30.50 \mu\text{g/dL}$ ), ( $266.78 \pm 48.80 \mu\text{g/dL}$ ), ( $36.61 \pm 14.31 \%$ ) in diabetic cases as compared to ( $83.98 \pm 24.19 \mu\text{g/dL}$ ), ( $279.08 \pm 40.23 \mu\text{g/dL}$ ), ( $31.05 \pm 10.98 \%$ ) of healthy controls. A significant increase in MDA level ( $6.35 \pm 1.52 \text{ nmol/ml}$  in cases and  $4.18 \pm 1.12 \text{ nmol/ml}$  in controls,  $p < 0.001$ ) and significant decrease in vitamin C ( $0.85 \pm 0.19 \text{ mg/dL}$  in cases and  $1.28 \pm 0.21 \text{ mg/dL}$  in controls,  $p < 0.001$ ) and vitamin E ( $0.85 \pm 0.25 \text{ mg/dL}$  in cases and  $1.34 \pm 0.38 \text{ mg/dL}$  in controls,  $p < 0.001$ ) were observed.

**Keywords:** Antioxidant, Iron, MDA, Oxidant, Oxidative stress, Type 2 DM, TIBC, Vitamin C, Vitamin E.

### INTRODUCTION

Type 2 diabetes mellitus is a clinical condition characterized by hyperglycemia due to the absolute or relative deficiency of insulin. It is also followed by pathological abnormalities like impaired insulin secretion, peripheral insulin resistance, and excessive hepatic glucose production. Although type 2 diabetes mellitus is a multiple etiological disease, emerging scientific evidences show there is somewhat relationship of the disease with iron metabolism. In recent years development of diabetes has been predicted with increased iron stores which is protective with iron depletion.<sup>1</sup> Although plasma concentration of iron is low total body iron content is approximately 4 gm in which a significant amount of iron is stored as ferritin and hemosiderin.<sup>2</sup> Iron is a transition element capable of reduction and oxidation activity and a potential harm is circumvented to body by binding iron with transport or storage proteins.<sup>3</sup> In recent years the role of iron has been investigated as a prooxidant which contributes to lipid peroxidation<sup>4</sup> causing oxidative stress. Ferric form of iron released from binding proteins can participate in production of free radicals by Haber-Weiss or Fenton reaction and cause oxidative damage. Role of iron is positively associated with the development of glucose intolerance and type 2 diabetes<sup>5</sup> as well as gestational diabetes mellitus. In fact iron level in serum is manifestation of storage iron, ferritin and there is increasing evidence

that glucose metabolism is influenced by high ferritin level in the body. It has been observed frequent blood donation improves insulin sensitivity<sup>6</sup> and constitute protective factor for the development of diabetes mellitus<sup>7</sup>. Serum ferritin, the storage form of iron is well correlated with baseline serum glucose<sup>8</sup> and beta cell function<sup>9</sup>. Iron in serum in ferrous form is the culprit for generation of free radicals. In our body iron is present usually in ferritin as well as in transferrin. For iron to act as a prooxidant agent it must be in free form and released by ferritin by the action of prooxidant that convert  $\text{Fe}^{++}$  to  $\text{Fe}^{+}$ . Glycation of transferrin decreases its ability to bind ferrous iron, hence increases free iron pool which in turn facilitates ferritin synthesis. A continuous production of free radicals causes increased lipid peroxidation and decreased antioxidant status. As a result Malondialdehyde (MDA), the end product and marker of lipid peroxidation is released to the plasma. Under physiological conditions damage due to free radicals is countered by antioxidants. When the excessive free radical formation takes place in the body antioxidant system can't cope up with the situation i.e. prooxidants overwhelm antioxidants. This improper balance between free radical production and antioxidant defense system tends to produce oxidative stress. This study aims to find out the level of iron and TIBC as iron status, MDA as oxidant status and Vitamin C and Vitamin E as antioxidant status.

## MATERIALS AND METHODS

This study was hospital based and case control study which was carried out in department of Biochemistry in collaboration with department of Medicine at B P Koirala Institute of Health Sciences, Dharan, Nepal. 52 chronic patients of type 2 diabetes mellitus and 52 age and sex matched healthy subjects were enrolled for the study (Fig. 1 & 2). Informed consent was taken from all the participants. Those diabetic patients who were also suffering from rheumatic heart disease, arthritis, infectious diseases, pulmonary tuberculosis, were excluded for the study.

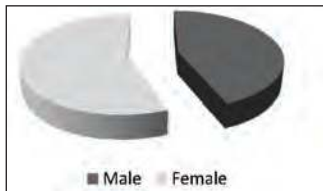


Fig. 1. Gender wise distribution of Case

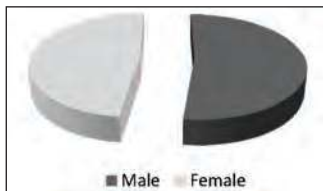


Fig. 2. Gender wise distribution of Control

Blood samples were collected in the fasting stage through vein puncture in two different vials, one EDTA and the other plain vial. Plasma and serum were separated by centrifugation. Plasma was utilized to estimate vitamin C and vitamin E as antioxidant parameters and serum was utilized for the estimation of MDA, Iron and TIBC. MDA was estimated by method of Yagi. This method is based on the formation of red pigment as a result of condensation lipid peroxidation breakdown products like MDA with thiobarbituric acid.<sup>10</sup>  $\alpha$ -tocopherol was estimated by Biery *et al* method in which  $\alpha$ -tocopherol is oxidized to tocopheryl quinone by ferric chloride and resultant ferrous ion forms complex with ethanolic  $\alpha$ - $\alpha'$  dipyridyl complex in aqueous medium.<sup>11</sup> Plasma Ascorbic acid was estimated by Sullivan *et al* method which depends on the reduction of ferric ion to ferrous ion by ascorbic acid forming a red-orange  $\alpha$ - $\alpha'$  dipyridyl

complex.<sup>12</sup> Iron and TIBC were estimated by using commercial kit produced by Ranbaxy Company. In which ferric ions are first reduced to ferrous ions by releasing in acid pH which ultimately react with ferrocene to form a violet colored complex. UIBC was estimated by the kit method. A known amount of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferrin at unsaturated iron binding sites. The additional unbound ferrous ions are measured using the ferrocene reactions. The difference in amount of ferrous ions added and the unbound ions are measured in the unsaturated iron binding capacity. TIBC is calculated by adding iron level and UIBC.

## RESULTS

In our study we found increased level of serum iron and percentage transferrin saturation, and decreased level of TIBC in diabetic patients (Table-1). Serum iron shared  $89.14 \pm 30.50$   $\mu\text{g/dL}$  in diabetic patients as compared to  $83.98 \pm 24.19$   $\mu\text{g/dL}$  in control ( $p=0.34$ ). TIBC was found  $266.78 \pm 48.80$   $\mu\text{g/dL}$  in diabetic cases as compared to  $279.08 \pm 40.23$   $\mu\text{g/dL}$  of healthy controls ( $p<0.16$ ). Transferrin saturation was found  $36.61 \pm 14.31$  % in diabetic cases as compared to  $31.05 \pm 10.98$  % of healthy controls ( $p<0.15$ ). When serum iron was stratified as male and female value shared  $94.72 \pm 36.92$   $\mu\text{g/dL}$  and  $92.35 \pm 24.16$   $\mu\text{g/dL}$  in males in cases and controls respectively ( $p=0.78$ ). Among females serum Iron level was found  $84.72 \pm 24.78$   $\mu\text{g/dL}$  and  $72.93 \pm 21.14$   $\mu\text{g/dL}$  in cases and controls respectively ( $p=0.13$ ). Serum TIBC and percentage transferrin saturation were also stratified as male and female. Serum iron was found increased, TIBC decreased, and percentage transferrin saturation increased in diabetes patients in both groups of males and females (Table-2). Likewise, MDA level was found to be  $6.35 \pm 1.52$  nmol/ml in cases and  $4.18 \pm 1.12$  nmol/ml in controls,  $p<0.001$  (Table-3). Vitamin C was found to be  $0.85 \pm 0.19$  mg/dL in cases and  $1.28 \pm 0.21$  mg/dL in controls,  $p<0.001$  and vitamin E was found to be  $0.85 \pm 0.25$  mg/dL in cases and  $1.34 \pm 0.38$  mg/dL in controls,  $p<0.001$  which were significantly decreased in diabetes patients as compared to healthy controls (Table-3).

**Table-1:** Distribution of serum iron, TIBC, and transferrin saturation in case and control

Groups	Iron ( $\mu\text{g/dL}$ ) (mean $\pm$ SD)	TIBC( $\mu\text{g/dL}$ ) (mean $\pm$ SD)	Transferrin saturation (%) (mean $\pm$ SD)
Case (n=52)	89.14 $\pm$ 30.50	266.78 $\pm$ 48.80	36.61 $\pm$ 14.31
Control (n=52)	83.98 $\pm$ 24.19	279.08 $\pm$ 40.23	31.05 $\pm$ 10.98
p-value	0.34	0.16	0.15

**Table-2:** Distribution of serum iron, TIBC and transferrin saturation in males and females

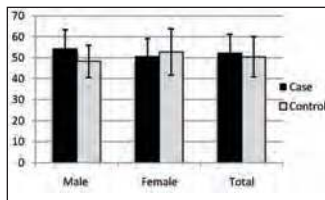
Groups	Iron ( $\mu\text{g/dL}$ ) (mean $\pm$ SD)		TIBC( $\mu\text{g/dL}$ ) (mean $\pm$ SD)		Transferrin saturation (%) (mean $\pm$ SD)	
	Male	Female	Male	Female	Male	Female
Case	94.72 $\pm$ 36.92	84.72 $\pm$ 24.78	256.26 $\pm$ 40.51	275.12 $\pm$ 53.69	38.23 $\pm$ 16.61	31.74 $\pm$ 11.69
Control	92.35 $\pm$ 24.16	74.93 $\pm$ 21.14	276.42 $\pm$ 42.03	281.95 $\pm$ 38.84	34.50 $\pm$ 11.37	27.33 $\pm$ 9.41
p-value	0.78	0.13	0.09	0.60	0.35	0.13

## DISCUSSION

Serum iron was found slightly high, TIBC slightly low and percentage transferrin saturation slightly high in diabetes patients (Table 1) but there was no significant difference in iron, TIBC and percentage transferrin saturation. MDA level was found significantly increased and vitamin C and Vitamin E were found significantly decreased in diabetes patients as compared to healthy controls (Table 3). There was high serum iron level in diabetic females as compared to control females (Table 2). The low level of iron in females may be due to the loss of iron through menstruation since age group ranges from premenopausal cases. Sheu *et al*<sup>13</sup> have also found the relationship between serum ferritin levels and insulin resistance, which existed only in diabetic females but not in males. Serum ferritin has been studied in detail and very few studies have conducted to see the association between serum iron and diabetes mellitus. Toumainen *et al*<sup>8</sup> have found that approximately 10% of type 2 diabetes patients with high ferritin levels have transferrin saturation greater than normal. Thomas *et al*<sup>14</sup> stated that the prevalence of elevated transferrin saturation was 3-4 folds higher in patients with diabetes, compared with historical prevalence described in the general population.

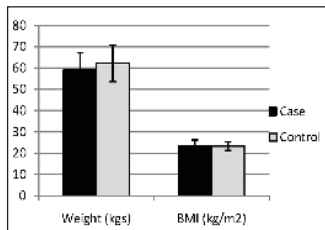
**Table-3:** Distribution of MDA and vitamin C and vitamin E in case and control

Groups	MDA (nmol/ml) (mean $\pm$ SD)	Vitamin C (mg/dL) (mean $\pm$ SD)	Vitamin E (mg/dL) (mean $\pm$ SD)
Case (n=52)	6.35 $\pm$ 1.52	0.85 $\pm$ 0.19	0.85 $\pm$ 0.25
Control (n=52)	4.18 $\pm$ 1.12	1.28 $\pm$ 0.21	1.34 $\pm$ 0.38
p-value	0.0001	0.0001	0.0001

**Fig. 3:** Age wise distribution of Case and Control

The different kind of result has been observed by Dinneen *et al*<sup>15</sup> who found no role of iron in diabetes mellitus. They determined distribution of iron histochemically by evaluating hepatic iron stores in autopsy specimens. No significant difference was observed. Similar type of result was also obtained by the study of Elis *et al*<sup>16</sup> whose study population comprised three subject groups with severe diabetic retinopathy, without retinopathy and non diabetic non retinopathy subjects. Serum iron and ferritin levels did not differ significantly between the three groups and there was no correlation between HbA<sub>1c</sub> level and serum iron and ferritin levels between the diabetic patients groups.

Malondialdehyde (MDA), the end product of lipid peroxidation was also studied in cases and controls. Significant increase in MDA was observed in cases as compared to controls (6.35 $\pm$ 1.52 Vs 4.18 $\pm$ 1.12 nmol/ml). MDA is produced as a result of lipid peroxidation which act as a marker of balance between prooxidant and antioxidant. In diabetes mellitus there is imbalance between prooxidant and antioxidant and prooxidant is actively predominant. As a result there is a high level of MDA present in sera of diabetic patients. This result is favored by other studies.<sup>17,18</sup> MDA level may be increased due to the action of iron by producing free radicals through Fenton reaction ultimately initiating chain reaction to cause lipid peroxidation.<sup>19</sup> Many studies have found increased level of lipid peroxidation even with small increment of iron concentration.

**Fig. 4:** Distribution of weight and BMI in Case and Control

There was significant decrease of vitamin C and vitamin E in cases as compared to controls ( $0.85 \pm 0.19$  Vs  $1.28 \pm 0.21$  mg/dL of Vitamin C and  $0.85 \pm 0.25$  Vs  $1.34 \pm 0.38$  of Vitamin E). Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins.<sup>20</sup> The reduction in vitamin C and vitamin E could be because of action of these vitamins as antioxidant in which both of them are used up to neutralize reactive oxygen species. Different studies have supported the above findings<sup>21,22</sup> and have shown significance decrease in antioxidant vitamins. In some studies it is also found the positive effects of vitamin C and vitamin E therapies in diabetic patients.<sup>23</sup>

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