

Original Article**Serum markers of Iron metabolism in individuals with diabetes mellitus from a population with high prevalence of anaemia**Sindu P.C.¹, Ramakrishnan V.R.²¹Department of Biochemistry, Amala Institute of Medical Science, Thrissur, Kerala..²Department of ENT, Jubilee Mission Medical College, Thrissur, Kerala, India.

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ABSTRACT

Introduction: Diabetes mellitus cause rise in the levels of cytokines which increases hepcidin secretion from liver. Hepcidin inhibits the release of iron from macrophages of reticuloendothelial cells and decreases the transport of iron from intestinal mucosa to blood. Anaemia of chronic inflammation cause rise in cytokines which leads to retention of iron at the stores as ferritin and fall in serum iron.

Aim: The present study was conducted to estimate serum markers of iron metabolism, serum iron, ferritin and haemoglobin and hsCRP from individuals with diabetes mellitus and in the control groups and to make comparison of these parameters.

Materials and methods: A total of 34 subjects were enrolled aged between 25 -50 years 17 subjects with diabetes mellitus and 17 age matched controls. Fasting blood samples were taken Hb, serum glucose; iron ferritin and hsCRP were estimated.

Results: We have observed that mean haemoglobin in both the groups were in the anemic level (<11g/dl) and there was no significant variation in haemoglobin levels in these groups. But there was a significant increase of serum ferritin in individuals with diabetes mellitus when compared with the control groups.

Conclusion: The alteration in iron metabolism in diabetes was found to be mediated by cytokines which increase the hepatic synthesis of hepcidin, which causes relative rise in ferritin in these cells the mild increase in CRP (hsCRP) is also explained by increase in cytokines.

Key words: Diabetes mellitus, Ferritin, Iron metabolism, hsCRP

INTRODUCTION

Ferritin is the storage form of iron in the reticuloendothelial cells and hepatocytes [1]. Increase in the iron stores as ferritin can result in higher level of plasma or serum ferritin. [2] .Serum ferritin is therefore used as a biochemical marker for assessment of body iron stores. Release of iron from reticuloendothelial cells and transport of iron across the basolateral membrane of intestinal mucosal cells into blood are mediated by transport protein ferroportin. [3, 1]

Ferroportin activity is inhibited by hepcidin which down regulates ferroportin and internalises it into intracellular vesicles [4]. This inhibition by hepcidin leads to fall in serum iron. Hepcidin is a peptide secreted by the liver. Cytokines, interleukin1 (IL 1) and interleukin 6(IL6) have been shown to increase secretion of hepcidin from liver [5]. Sustained increase in cytokines are seen in chronic infections and chronic inflammatory conditions such as

rheumatoid arthritis, resulting in increase in hepcidin levels and fall in serum iron leading to anaemia of chronic infection. Obesity, metabolic syndrome and diabetes also cause increase in cytokine levels. These conditions have been shown to increase the level of iron at the stores due to decrease in the iron released by macrophages of reticuloendothelial system. [6, 7, 8]. Highly increased store of iron as ferritin is considered to be toxic to cells that store iron such as hepatocytes, in iron overload condition [9]. Hereditary hemochromatosis, transfusion iron overload and dietary iron overload in African tribal populations are iron overload conditions that can lead to diabetes mellitus [6, 10]. In the presence of hydroperoxides or hydrogen peroxide free iron can generate hydroxyl radicals that are toxic to cells. Iron is sequestered in the ferric form during transport with transferrin or at the stores with ferritin. Oxidants can cause release of this bound form of iron. Reduction of ferric to ferrous may also release bound form of iron [6, 10]. Toxicity of

catalytic free iron leads to β cell damage, cirrhosis of liver, insulin resistance and diabetes mellitus in patients with iron overload conditions.

Surveys by National Nutrition Monitoring bureau (NNMB) and National Family Health Survey 3 (NFHS3) showed high prevalence of anemia in India [11].

NFHS3 has reported anemia prevalence 56.2% in women of 15-49 years 57.9% in pregnant women and 24.3% in men aged 15 -49. Iron stores may be depleted in a large number of individuals in the local population.

Type 2 diabetes mellitus is highly prevalent in the urban population of India, with a prevalence of 11.6% in the prevalence of diabetes mellitus in the urban population was 2.1% and in 2002 this has risen 12.1 % [13] There is a higher prevalence of impaired glucose tolerance than diabetes mellitus in subjects under 40 years The prevalence of diabetes mellitus reported from Kerala was 12.4% in urban population [14]

AIM AND OBJECTIVE

To estimate serum markers of iron metabolism, serum iron, serum ferritin and haemoglobin and hsCRP from individuals with diabetes mellitus and in the control groups, moreover to make comparison of these parameters in individuals with and without diabetes mellitus.

MATERIALS AND METHODS

Study design: Prospective case control study

Ethical consideration: Ethical approval was obtained and institutional guidelines were followed

Fasting blood samples were collected from local volunteers after obtaining their consent. Blood samples were taken by venous puncture, taken in plain glass tubes and allowed to clot. Serum was separated by centrifugation at room temperature for 4-5 minutes. For estimation of Hb, blood collected in plain glass tubes which contained a pinch of EDTA as the anticoagulant and hemoglobin was estimated by Cyanmethemoglobin method [15]. hsCRP was estimated by immunoturbidimetric method in fully automated biochemical analyzer Vitros5,1 FS . Glucose was estimated by glucose-oxidase method in fully automated biochemical analyzer Vitros5,1F S . Serum iron was estimated by liberating bound iron at low pH from serum transferrin and reacting with Ferrozine and ferritin by immunochemistry method.

Statistical analysis

Results were expressed as mean \pm standard deviation. Comparison of variables between the two groups was performed with student t test. The p values < 0.05 were considered statistically significant.

RESULTS

When serum parameters of iron metabolism were compared in normal and diabetic individuals we observed that ferritin was significantly elevated in diabetic samples. ($p < 0.01$) Serum iron was not significantly elevated in diabetic individuals. But mean haemoglobin in the control and diabetic groups were quite close to each other and lower than 11gm/dl. High sensitivity CRP was significantly elevated in individuals with diabetes mellitus. The results showed that there is significant increase in ferritin and hsCRP in diabetic individuals.

Table 1 Hb, S.Ferritin, S.iron and hsCRP levels in normal individuals and diabetic individuals

	Controls Mean \pm SD	Cases Mean \pm SD	t value P value
Hemoglobin	10.01 \pm 0.451	10.52 \pm 0.26	0.0352 P > 0.05
Serum Ferritin	14.96 \pm 2.8	42.26 \pm 11.86	2.98 P < 0.001
Serum iron	158.9 \pm 41.3	192.5 \pm 153.9	0.598 P > 0.05
hsCRP	0.44 \pm 0.3	2.25 \pm 2.1	4.95 P < 0.001

Fig. 1 Hb and S.Ferritin levels in normal individuals and diabetic individuals

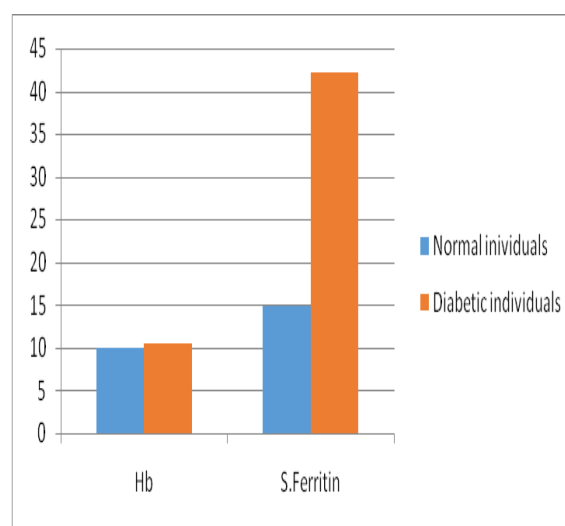
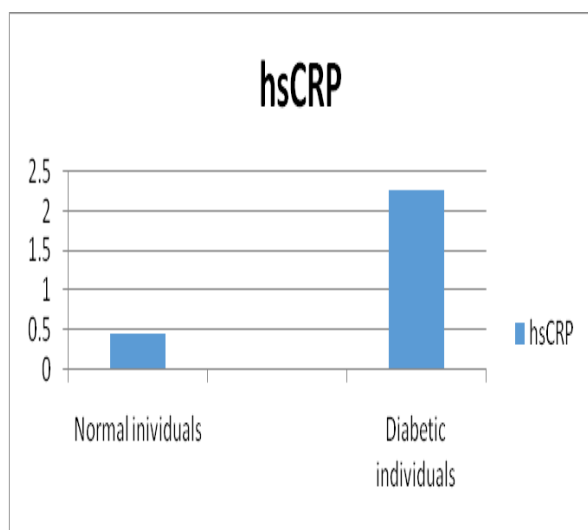


Fig. 2: hsCRP levels in normal individuals and diabetic individuals

DISCUSSION

The gradual transition from early metabolic abnormalities to impaired glucose tolerance, impaired fasting glucose and to diabetes takes several years. The earlier pre diabetic states are associated with insulin resistance which gradually progresses to β cell failure [16, 17, and 18]. Therefore in the initial stages of prediabetes insulin levels may be elevated. But β cell failure will impair insulin secretion and responsiveness of β cell to higher levels of glucose [16] Pathogenesis of insulin resistance is not well understood. But metabolic syndrome and obesity where insulin resistance is seen may lead to diabetes mellitus in later life. These conditions may also influence some of parameters of iron metabolism. The alteration in iron metabolism in these conditions are found to be mediated by cytokines IL-1, IL-6 and TNF α [5, 7]. The cytokines produced in diabetes mellitus and hypertension can increase the hepatic synthesis of acute phase protein CRP. The mild increase in CRP is measured as high sensitivity C reactive protein.

The cytokines also increase the hepatic synthesis of hepcidin. Hepcidin in turn inhibits release of iron from reticuloendothelial cells and decreases the transport of iron from intestinal mucosal cells across the basolateral membrane to blood [4].

This explains why serum iron is not elevated even though there is plenty of an iron store in individuals with diabetes. The retention of stored iron in the macrophages of reticuloendothelial cells, mediated by hepcidin causes a relative rise in ferritin in these

cells. This rise in ferritin at the cellular stores is reflected in the rise in serum ferritin, as serum ferritin is a marker of iron stores [2]

It was important to observe that the mean haemoglobin levels are in the anaemic range and serum ferritin was much lower in the local population than that reported in the western population [19]. There was no significant decrease in the level of haemoglobin in the control and diabetic group. Even in such anaemic conditions serum ferritin was significantly increased in diabetic individuals when compared to control group. But severe anaemia and higher serum ferritin levels are quite common in chronic infections and inflammatory conditions when compared with controls. It is also possible that there may be alternative sources of iron or alternate regulatory mechanism providing iron for haemoglobin synthesis in diabetes.

CONCLUSION

The alteration in iron metabolism in diabetes was found to be mediated by cytokines. The cytokines also increase the hepatic synthesis of hepcidin. Hepcidin in turn inhibits release of iron from reticuloendothelial cells a relative rise in ferritin in these cells. This rise in ferritin at the cellular stores is reflected in the rise in serum ferritin. The mild increase in CRP (hsCRP) is also explained by increase in cytokines.

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