Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Hepcidin expression and iron parameters change in Type 2 diabetic patients

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ARTICLE INFO

Article history:
Received 26 January 2011
Received in revised form
21 March 2011
Accepted 22 March 2011
Published on line 21 April 2011

Keywords:
Hepcidin
Ferritin
IL-6
Type 2 DM

ABSTRACT

Aim: Iron may contribute to the pathogenesis of Type 2 diabetes mellitus (DM). The aim of this study was to determine iron regulator hepcidin and iron metabolic parameters in Type 2 DM patients, the relationships among them were evaluated in this specific sub-groups.

Materials and methods: The study included sixty-four people: 34 cases of diabetes and 30 age-matched controls. Serum hepcidin, IL-6, hsCRP, ferritin, sTfR, EPO as well as other clinical parameters were detected, and the associations between hepcidin levels and iron/inflammatory parameters were analyzed in diabetes and the controls.

Results: Serum ferritin and hepcidin levels in diabetic patients were significant higher than the controls ($p < 0.001$ respectively). A positive correlation between hepcidin and ferritin, as well as between ferritin and IL-6 levels was existed in diabetes and the control groups ($p < 0.001$ respectively).

Conclusion: All of these data demonstrated that the higher hepcidin levels in diabetic patients may be due to those higher ferritin and IL-6 levels, the elevated hepcidin might have adaptive value through down-regulated iron absorb and play an important role in pathogenesis of Type 2 DM.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action, or both. Diabetes and its complications have become a major public health problem in the world and its prevention has become a public health priority. Increasing evidence now suggests a potential role of iron in the pathogenesis of Type 2 DM [1]. Iron is a strong pro-oxidant that catalyses several cellular reactions leading to the formation of reactive oxygen species (ROS) and resulting in elevated oxidative stress, interfering with insulin secretion which is proposed to contribute to an increased risk of Type 2 DM [2]. In animal models, iron excess might result in β-cell oxidative stress and decrease insulin secretary capacity [3]. Apart from direct tissue damage, epidemiological studies have reported an association between iron overload and peripheral insulin resistance [4].

Ferritin is a widely used marker of iron status in epidemiological studies and accurately reflects body iron stores in healthy individuals [5]. Several cross-sectional and retrospective case–control studies have linked elevated ferritin levels with DM [6]. Furthermore, a few prospective studies have also reported that relatively high levels of ferritin are associated with an increased of risk of developing DM in apparently healthy individuals [7,8]. In diabetes, metabolic abnormalities may lead to increased ferritin levels through a variety of mechanisms, in certain insulin-sensitive cells such

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0168-8227/$ – see front matter
doi:10.1016/j.diabres.2011.03.028

as adipocytes, receptors for transferrin, glucose and insulin-like growth factor II co-localize in the cell membrane, and the presence of insulin resulted in the simultaneous translocation of all three proteins. Therefore, it has been hypothesized that insulin mediated glucose transportation may lead to increased transferring receptors on the cell surface, resulted in increased uptake of extracellular iron [9]. Ferritin is also an acute-phase reactant, its synthesis is up-regulated by infection or inflammation. Studies have demonstrated that pro-inflammatory cytokines such as tumor necrosisfactor (TNF)-α, interleukin-1 (IL-1) and interleukin-6 (IL-6) induce the expression of ferritin in cultured hepatic cell lines [10]. Type 2 DM is closely correlated with chronic inflammation, increased circulating concentrations of IL-6 and TNF-α were found in Type 2 DM [11]. So, the increased ferritin levels in type 2 DM probably also induced by their elevated inflammatory cytokines.

The hereditary hemochromatosis (HH) is a disorder of abnormal iron absorption resulted in the progressive accumulation of iron in the liver, heart, pancreas, and other organs. The most penetrate cases of HH presented with a classic triad of symptoms: hepatomegaly (associated with iron overload and cirrhosis), diabetes, and hyperpigmentation. Type 2 DM occurs in 25–75% of patients with hemochromatosis. HH is also a disease caused by a deficiency of hepcidin which associated with several iron-related disorder [12,13]. Hepcidin, a 25-amino-acid antimicrobial peptide, is the central regulator of iron homeostasis. Under normal circumstances, hepcidin expression and subsequent release into plasma prevents further absorption of iron from the duodenal enterocytes by preventing the efflux of iron by ferroportin channels, hence reduced amounts of iron delivery via transferrin to hepatocytes [14]. In response to iron loading in animal studies, hepcidin expression increased to prevent the further uptake of iron. Conversely, during iron deficiency, hepcidin expression decreased. Significant decreased hepcidin expression was found to contribute to HH, and the juvenile hemochromatosis, the most severe iron-overload disease, which associated with mutations in HJV or HAMP, is in this severe form of iron overload that the greatest deficiency of hepcidin has been documented [15]. Recently, research reported that the serum concentration of prohepcidin (a precursor of the mature hepcidin) was significantly higher in men with impaired glucose tolerance or Type 2 DM than in those with normal glucose tolerance, and that the serum prohepcidin level was negatively correlated with insulin sensitivity evaluated by the glucose clamp technique. Thus, they concluded that prohepcidin was associated with insulin resistance or impaired glucose metabolism [16].

Previous data allow us to presume that there may be existed relationship between hepcidin and diabetes. However, at present, there is spare information about the expression of hepcidin in Type 2 DM, whether the increased iron load in Type 2 DM due to a deficiency of hepcidin, or on the contrary, iron overload would up-regulate hepcidin expression? The aim of this study was to determine serum hepcidin levels and iron metabolic/inflammatory parameters in patients with Type 2 DM, and try to elucidate the relationships among them in this specific sub-population.

2. Subjects

This study was approved by the Ethics Committee of Shantou University Medical College and conducted from April to October, 2010. The study included sixty-four people: 34 cases of Type 2 DM and 30 age-matched controls. Incident cases of diabetes were defined by the appearance of any one of the following during follow-up: (1) a fasting ($\geq$8 h) glucose level $\geq$7.0 mmol/l, (2) a non-fasting glucose level $\geq$11.1 mmol/l; (3) use of diabetes medication, or (4) a self-reported physician diagnosis. All patients and control peoples gave written informed consent to the project's aims and to collection, analysis and use of the data for publication. Patients with malignancies and hepatic disease were excluded from our study, since a radiotherapy and/or chemotherapy might had altered the metabolism of the organism. None of our patients were under any treatment with iron or immunosuppressive drugs and/or received blood transfusion prior to the start of our study.

3. Materials and methods

Blood samples were obtained for hematological and biochemical tests after overnight fasting, using two types of containers, one of which contained ethylenediamine tetraacetic acid (EDTA), while the other without any anticoagulant. Blood in EDTA-containing vacutainers were analyzed by an automatic cell counter (COULTER LH 750 Hematology Analyzer) for the determination of the complete blood count including hemoglobin (Hb), platelet (PLT) and white blood cell (WBC). Non-anticoagulant blood was kept at room temperature for 2 h to ensure serum separation. Serum simples were analyzed for liver transaminases (AST, ALT, GGT), lipids (triglycerides, total cholesterol, HDL and LDL cholesterol) by an Automatic biochemical analyzer (Beckman LX20 Biochemical Analyzer). Serum ferritin, IL-6, hsCRP, EPO, hepcidin and sTfR were measured by ELISAs method (CUSABIO BIOTECH, Newark, New Jersey) according to manufacturers' instructions.

3.1. *Statistical analysis*

All data were given as mean ± standard [mean ± SD]. Statistical differences between two groups were carried out by non-parametric Kruskal–Wallis test or student's *t*-test. The associations of serum hepcidin with ferritin, sTfR, IL-6, hsCRP and other parameters were tested with Spearman rank correlation. Linear regression models were used to evaluate the effective of hematological variables, iron and inflammatory indicators on serum hepcidin production in each group. *p*-Value < 0.05 was considered as statistically significant.

4. Results

A summary of the results of the patients and control groups were presented in Table 1. The mean age of patients was 60.88 years. The levels of Glu and HbAc1 were significantly higher than the controls ($p < 0.001$ for both). The levels of