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ORIGINAL ARTICLE

Evaluation of Relationship between Serum Visfatin and Ghrelin Levels with Serum Ferritin Concentration

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ABSTRACT

Iron deficiency is the most prevalent and worldwide form of malnutrition. Visfatin is an adipocytokine with insulin-like and inflammatory effects that increases in obesity and diabetes. Ghrelin as another adipocytokine is contributing to the regulation of appetite and obesity. Regarding the relation of adipocytokine and ferritin (an iron content indicator with adipose tissue) it is logical and suitable to investigate visfatin and ghrelin levels in iron deficient state. Generally sixty seven people participated in the case - control study. These subjects were referred to laboratory by physician during routine checkup. Subjects in the case and the control groups had serum ferritin below and above 10 µg/l, respectively. The hematological parameters were measured. Also serum concentrations of ferritin, visfatin, and ghrelin were measured by ELISA techniques and two groups were statistically analyzed. Hematologic parameters did not demonstrate significant differences between the case and the control groups. However, serum concentrations of visfatin and ghrelin were significantly reduced in the case subjects compared to the control groups. Our data revealed that serum concentrations of visfatin and ghrelin decreased in the case group, and this finding showed that measurements of these adipokines may be useful in the prediction of iron deficiency.

Keywords: *Iron deficiency, Ferritin, Visfatin, Ghrelin*

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INTRODUCTION

Iron deficiency is a condition with decreased total iron content in the body. In other words, the amount of iron is lower with respect to the formation of hemoglobin, iron containing compounds, and the related enzymes. The outcomes of reduction in iron stores differ from restricting the erythropoiesis to severe anemia with ineffective erythropoiesis and dysfunction of metalo-enzymes [1, 2].

Iron deficiency is usually a result of negative balance in iron turnover and is considered as a gradual process. Certain events take place when the total body iron is beginning to fall; first, iron storage in hepatocytes and macrophages of the liver, spleen and bone marrow is depleted. In this condition, plasma iron content is insufficient for the compensation of daily destructed hemoglobins in the bone marrow. Thus the amount of free erythrocyte protoporphyrins increases, which leads to the production of microcytic red blood cells and finally there is a drop in hemoglobin levels [3, 4].

Serum ferritin levels are associated with total body iron stores. Therefore, the measurement of serum ferritin is the easiest and most accurate laboratory test to estimate iron stores [5, 6]. Normal value of ferritin is dependent on age and sex. Serum ferritin reaches less than 15µg/l when iron stores are depleted. This amount of ferritin could reflect the iron deficiency [7-9]. Some studies have considered amount lower than 12 µg/l or less than 10 µg/l [10]. In some conditions, such as inflammatory disorders, these tests should be interpreted slightly differently since ferritin is an acute phase reactant [5, 11].

Adipose tissue as an important endocrine organ secreting a number of biologically active peptides is called adipokine. Since the last ten years, more than fifty different types of adipokines have been known. Adipokines have different physiological effects on various organs such as brains, bones, reproductive organs, livers, skeletal muscles, blood vessels and immune cells [12].

Ghrelin is one of the adipokines that are involved in iron metabolism [13]. This hormone is secreted mainly by gastric mucosal cells and has a key role in appetite regulation [14]. Direct intra cerebroventricular injection of ghrelin increases food intake and body weight [15]. This hormone stimulates hunger feeling and its secretion through the peripheral or central nerves increases appetite and food intake. It has been reported that loss of appetite in anemia may be due to a decrease in ghrelin levels. Several studies have shown that ghrelin levels increase after the treatment of iron deficiency anemia [13].

Visfatin is another adipokine known as colony-enhancing factor of ß cell. It is predominantly secreted by visceral adipose tissue [16, 17]. This hormone has been characterized in the cytoplasm and nucleus of many organs such as lungs, brains, spleens, kidneys and testes [17]. Visfatin increased in obesity, Insulin resistance, type 2 diabetes mellitus, and pro-inflammatory states [18]. It has been found that visfatin is an important mediator of inflammation that induces a dose-dependent induction of IL-1B, TNF-α and IL-6 [19]. Several studies have shown the increasing visfatin in diabetes and obesity [20]. Some studies have shown that the risk of iron deficiency in increases in obesity. The prevalence of iron deficiency anemia in obese children is more than normal-weight babies [21].

Given the above, it appears that serum concentrations of visfatin and ghrelin are associated with iron deficiency anemia; therefore, the present study was conducted to evaluate the serum values of ghrelin and visfatin in relation to amounts of serum ferritin as a biomarker for iron deficiency state.

MATERIALS AND METHODS

This study has been accepted by Bushehr University of Medical Sciences Review Board (Registration number 1808, Date 5.3.2013). Sixty-seven people participated in this case-control study. Thirty four women with a mean age of 27.79±7.29 year and ferritin ≤10 µg/l as the case group and thirty three subjects with a mean age of 27.6±10.79 year (10 men and 23 women) with ferritin >10 µg/l was considered as the control group [22]. These individuals were referred to laboratory by physician during the routine checkup. The study was approved by the ethics committee at the Bushehr University of Medical Sciences. All participants in the study were without any specific diseases such as cardiovascular, diabetes, high blood pressure, kidney disorders and had no history of drug use during the last six months. Blood samples were taken between 8 and 9 a.m. after 12h overnight fast. The collected samples were transferred into tubes with and without anticoagulant for further evaluation of Complete Blood Cell Count (CBC) and serum preparation respectively. CBC and RBC indices were measured by convenient cell counter (Cell Counter Full Diff, Switzerland). Serums were stored at - 80 ˚C until the time of the ferritin, visfatin and ghrelin measurements. Serum concentrations of ferritin (Monobind Kit), visfatin (Nampt visfatin / PBEF kit, Enzo, Germany), and ghrelin (DRG international,USA) were evaluated by Enzyme linked immunosorbant assay (ELISA). Weight and height were measured using standard techniques stadiometer. Body mass index (BMI) was calculated with the formula as body weight (kg)/Height (m^2) and considered for matching the subjects in the two groups.

Statistical analysis

SPSS Version 16 software was used for the data analysis to compare quantitative variables between the two groups (independent t-test) and regarding the relationship between quantitative variables, the Pearson correlation coefficient was used in both groups. P ≤0.05 was considered as the significance level. Covariance was also used in order to remove the confounding effects.

RESULTS AND DISCUSSION

Statistical analysis showed no differences in demographic variables such as age, weight, height, and BMI in the case group compared to the control group (Table 1).

Hematologic parameters such as WBC, RBC, PLT, Hb, HCT, MCH, MCHC, and MCV were not significantly different between the case and control groups (Table 2).

Statistical analysis revealed that serum concentrations of visfatin and ghrelin significantly more decreased in the case group than in the control group (Table 3).

There was negative relation between visfatin and Hb in the case group, so an increase in hemoglobin levels decreases visfatin value. However, this relationship was not significant in the control group. There was no significant relation between visfatin levels with WBC, RBC, HCT, MCV, MCH, MCHC, and PLT in both case and control groups.

As demonstrated in this study, our results showed that serum visfatin levels significantly decreased in the low level ferritin group than in the control group.

Visfatin as an adipokine is mainly secreted by visceral adipose tissue [17]. Inter relationship between adipogenesis and iron metabolism parameters was previously reported [16]. Serum levels of ferritin are related to body fat distribution, visceral fat, liver fat content, and insulin resistance [23]. The high visfatin expression has been found in the tissues such as liver, muscle, and bone marrow considered important in iron metabolism [24]. Fernandez-Real et al. have shown that serum visfatin is associated with parameters of iron metabolism as well as prohepcidin and sTfR (soluble transferrin receptor levels) [16]. Serum sTfR concentration was significantly associated with iron and ferritin requirements of cells and higher concentration of ferritin are accompanied with lower sTFR levels [10, 25]. Fernandez's study revealed that serum visfatin is correlated negatively with sTfR concentration and this could suggest that an increase in iron stores could lead to increased visfatin synthesis [16]. The Increased concentration of plasma and tissue iron stores induces hepcidin synthesis, which thus causes to lower the duodenal iron absorption. Serum prohepcidin represents the endogenous levels of hepcidine [26, 27].

There is a significant correlation between serum ferritin and prohepcidin in children with iron deficiency anemia [28]. Prohepcidin is also produced by liver [29].Visfatin and prohepcidin seem to have a significant or positive correlation with iron stores [16]. In the current study the decreased concentration of visfatin in the low ferritin subjects can be explained by these connections.

The results of this study indicated significantly reduced ghrelin the level in low ferritin group compared to the control subjects. These findings corresponded with Akarsu's report in 2007, which showed with a reduction the ferritin levels, ghrelin level will drop proportionally [30]. Ayse Dogen and colleagues in 2013 showed that after iron therapy, hepcidin and ghrelin levels significantly increased in the treated patients [31]. Furthermore, in another study Jarocka-Cyrta and coworkers (2010), also showed the increased number of duodenal ghrelin positive cells in patients with celiac disease [32]. Since there is a direct correlation between ferritin levels and hepcidin, our findings are consistent with the literature. Although Isguvenr and coworkers demonstrated that there was no a significant correlation between BMI and ghrelin levels [13], in the present study both groups were similar in terms of BMI to minimize the effect of BMI on the evaluated parameters. Thus, we can say that the reduction of ghrelin levels is independent on BMI in the low ferritin subjects.

Table (1): Frequency of demographic parameters in case and control groups.

Groups / ***Parameters***	**Control group(Ferritin>10µg/l)**			**Case group(Ferritin≤10µg/l)**			
	Mean± *SD*	**Min**	**Max**	**Mean± *SD***	**Min**	**Max**	***P(<0.05)***
Age(year)	27.6±10.79	5	54	27.79±7.29	9	41	0.934
Weight(kg)	62.9±17.18	19	87	59.82±9.42	45	81	0.364
Height(cm)	161.6±14.73	111	192	161.91±6.35	149	175	0.912
BMI(kg/m^2)	23.6±4.62	15.4	32.4	22.65±3.09	16.5	30.1	0.324

Table (2): Frequency of hematologic parameters in case and control groups

Groups / ***Parameters***	**Control group (Ferritin>10µg/l)**			**Case group (Ferritin≤10µg/l)**			
	Mean± *SD*	**Min**	**Max**	**Mean± *SD***	**Min**	**Max**	***P(<0.05)***
WBC ($\times10^3/\mu l$)	7.35±2.42	4.7	17.7	7.63±2.04	4.4	12.7	0.605
RBC ($\times10^6/\mu l$)	5.02±0.69	3.72	6.41	4.71±0.63	3.95	6.82	0.066
Hb (g/dl)	12.99±1.88	10.3	18.1	12.26±1.25	9.6	14.7	0.065
HCT (%)	39.26±5.36	31.2	54.7	37.16±3.95	22.5	43.6	0.073
MCV (fl)	79.13±11.8	59.7	95.7	81±9.76	59.1	98.2	0.482
MCH (pg)	26.16±4.41	18.2	31.7	26.11±3.56	19.5	32	0.959
MCHC(gr)	33.1±1.66	29.2	35.5	32.4±1.71	27.1	35.1	0.092
PLT ($\times10^3/\mu l$)	300.78±78.5	178	492	309.35±65.6	188	508	0.629

Table (3): Ferritin, Visfatin and ghrelin levels in the case and control groups

Groups / ***Parameters***	**Control group (Ferritin>10µg/l)**			**Case group (Ferritin≤10µg/l)**			
	Mean± *SD*	**Min**	**Max**	**Mean± *SD***	**Min**	**Max**	***P(<0.05)***
Ferritin (µg/l)	75.32±46.85	20	175.1	6.68±2.98	1	10	0.000
Visfatin(ng/ml)	3.17±1.82	0.34	7.49	2.23±1.43	0.45	5.87	0.022
Ghrelin(pg/ml)	1404.87±532.8	491	2720	820.64±337.06	211	1830	0.000

Our findings show that serum visfatin and ghrelin have direct the relation to ferritin level and potentially could predict the progress of the iron deficiency state. Furthermore, the current investigation indicated that serum visfatin and ghrelin levels directly related and BMI does not affect their variation. Meanwhile, we suggest investigating the relationship of both biomarkers with more indices of iron status and more number of subjects.

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