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HFE gene changes in obese individuals in Mosul

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Abstract

Obesity represent one of major health problem worldwide in previous work in our laboratory significant change in iron status was notic. This study try to discover the relationship HFE gene copies number and obesity. This case control study included 100 subjects classified as 50 obese and 50 normal weight as the control group. Their ages ranged from 12 to 22 years old and carried out in Mosul during the period of April to June 2022. Complete blood picture was used to study the change in blood parameters. In addition serum ferritin was evaluated using ELISA kit.Gene copies number was assess using the GoTaq qPCR. The results of this work showed that in obese patients highest levels of hemoglobin found in compare to control ($p<0.05$) As observed in patients leukocyte level was which significantly higher than control $p<0.05$. Elevated levels of Platlate were observed in a significant higher proportion of patients than controls, Neutrophil and Lymphocyte also showed a significantly higher level in obese than control. High serum ferritin levels in obese individuals was observe in compare to controls. There was significant increase in the number of copies of the HFE gene in obese patients compare controls. In conclusion, HFE gene copies number increase in obese individuals as compensatory mechanism.

Keynote: HFEgene, obesity, CBC, Ferritin, total antioxidant capacity

1. Introduction

Hereditary hemochromatosis (HH), a disorder of iron metabolism characterized by increased intestinal iron absorption. Dietary quality, alcoholism and other life-style factors can increase the risk of iron overload, especially among at risk populations (Prabhu et al., 2020). The hemochromatosis gene HFE was discovered in 1996, more than a century after clinical and pathologic manifestations of hemochromatosis were reported. Linked to the major histocompatibility complex (MHC) on chromosome 6p, HFE encodes the MHC class I-like protein HFE that binds beta-2 microglobulin. HFE influences iron absorption by modulating the expression of hepcidin, the main controller of iron metabolism(Barton,2015).

Clinical HFE hemochromatosis is characterized by excessive storage of iron in the liver, skin, pancreas, heart, joints, and anterior pituitary gland. In untreated individuals, early symptoms include: abdominal pain, weakness, lethargy, weight loss, arthralgia, diabetes mellitus; and increased risk of cirrhosis when the serum ferritin is higher than 1,000 ng/mL. Clinical HFE hemochromatosis is more common in men than women (Barton,2015).

The HFE (high-iron) protein, a main limiting factor of duodenal iron absorption that interacts with the transferring receptor (TfR) and lowers its affinity for transferrin, is encoded by the HFE gene, which is located on chromosome 6. The classical HH, also known as type I hemochromatosis, is linked to mutations in this gene (Feder and et al., 1996).

Despite the fact that the disease's symptoms are frequently vague, if organ damage has already been occur, it is frequently irreversible. Compared to the normal population, hemochromatosis patients absorb more iron. But when people are between the ages of 20 and 40, and iron accumulation levels are at a concentration of roughly 20 to 40 g of iron, clinical indications brought on by excessive deposition of this metal start to appear. When the iron reserves are still insufficient, the clinical manifestations hardly ever show up in patients less than 20 years of age, and most of them present with symptoms between the ages of 40 and 50 (Hanson et al., 2001;Trinder et al., 2002; Whittington et al., 2002; Kowdley et al., 2013 ; Beutler ,2006).

Considering the lack of genetic studies in Iraq about iron storage in obese individuals, especially in Mosul, and the fact that early detection of the genetic predisposition to the disorder is important to reduce the overall burden of clinical disease, this article aimed at identifying a possible relationship between HFE gene expressions, hemoglobin level in young obese subjects.

2. Materials and methods

This case control study included 90 subjects classified as 50 obese and 50 normal weight as the control group. Their ages ranged from 12 to 22 years old and carried out in Mosul during the period of April to June 2022.

2.1. Inclusion and exclusion criteria

The main criterion for including such patients in the study was the increase in hemoglobin levels and a BMI greater than 29.9 kg/m2 in both males and females between the ages of 12 and 22, who were diagnosed to have metabolic syndrome. Individuals who had anemia, acute infectious diseases, pregnancy, renal diseases, liver diseases, thalassemia, or had received blood transfusions in the past were excluded from the study. For the control group, healthy patients of both sexes were selected with hemoglobin levels within normal limits and a BMI between 18.5 and 24.9 kg/m2.

The study was approved by the local ethical committee of the Ninavah Health Directorate. Informed consent was obtained from all participants after being informed of the aims of the study. Data collection: A self-administrative questionnaire was carried out using a standardized questionnaire. Samples were collected using dry plastic syringes. A tourniquet was used to make the veins more prominent. 2 mL of blood was collected in EDTA and kept at -20° until analysis. The other part was put in Jel, immediately processed and refrigerated, and the serum was frozen for ferritin testing purposes. BMI is obtained by calculation

according to the formula: weight(kg) ÷ height (m^2) (Who ,2000).

The data was analyzed using the Excel 2007 computer program. Comparisons were made between groups; results were expressed as percentages (%) and mean standard deviations (SD); to analyze differences between groups, we used the independent samples t-test with a significance difference of (P-value 0.05) considered. Hematological parameters such as hemoglobin, packed cell volume (PCV), WBC count, platelet count, and neutrophil lymphocyte ratio were analyzed on a hematology analyzer (Swelab Alfa Standard, Sweden) for 90 subjects within 1 hour of obtaining the samples. Ferritin is measured by an enzyme-linked immunosorbent assay (ELISA) kit from(Bioassay Technology Laboratory, China. The normal serum ferritin levels were 15–300 ng/mL in males and 15–200 ng/mL in females.

DNA extraction was performed on 20 selected samples in the obese group and 20 in the control group, using the AddPrep Genomic DNA Extraction Kit (AddBio, Korea).DNA concentration and purity were determined using the Nano Photometer N50 Touch (Implen Gmbh, Germany).The primers for amplification of the HFE gene were designed using the NCBI (primer blast) program. The forward primer (HFEF) was 5'-GGCAAGGGTAAACAGATCCC-3' (20 mer) and the reverse primer (HFER) was 5'-CACAATGAGGGGCTGATCCA-3' (20 mer), The forward housekeeping gene was 5'-CGGGTCTTTGCAGTCGTATG-3'(20 mer), and the reverse housekeeping gene was 5'-CTGTTTCTGGGGACTAGGGG-3'(20 mer).

Gene copies number was assessed using the GoTaq qPCR master mix from Promega (Promega Corporation, USA, A6000) by the PCR Max device (Stone, Staffordshire, ST15 0SA, UK). Then, using qPCR software MxPro3005P (Ecological Research Software), the thermal circulator was programmed . Repeat reactions for each gene of interest and for maintenance genes were performed for each sample. To compare gene expression differences between our samples, Cts were calculated. The ΔCt value calculated for each sample is the difference between the Ct of the gene of interest and the internal gene. ΔΔCt is measured as the difference between the ΔCt values of the test and control samples. The fold change in gene expression is measured by 2-(ΔΔCt).

3. Results

This study included 90 subjects: 50 patients and 40 controls. The information about age and body measurements of all subjects was determined as presented in Table 1.

Table 1. BMI and age of patient groups compared to the control group

	Obese N=50	Control N=40	P. value
Age	17.6±3.5	17.7±3,1	.874
BMI	35.9±3.99613	22.9±1.3	.000

In obese patients, the highest levels of hemoglobin found were with average of (14.2000±1.73570), and in control, levels of hemoglobin were (13.5525±1.05854). As observed in patients leukocyte level was (6.8720±2.27300) which significantly higher than control (5.8925±1.62613) with p. value of (0.024). Elevated levels of Platlate were observed in a significant higher proportion of patients than controls, Neutrophil and Lymphocyte also showed a significantly higher level in obese than control as represented in Table 2.

Table 2. CBC parameter and derivative values. These parameters were compared between the patient group and the control group.

Parameter	Obese	Control	p-value
Hemoglobin	14.2±1.70	13.5±1	**0. 041**
Hematocrit (%)	43.7±5.2	±3.1	**0 .039**
Leukocyte	6.8±2.2	1.6	**0.024**
Platelet	358.5±121.4	304.8±84.37	**0.020**
Neutrophil	57.1±8.7	60.5±6.8	**0.042**
Lymphocyte	38.1±8.5	34.9±6.8	**0.058**
Monocyte	2.8±1	3.1±1.6	**0.230**
Eosinophil	1.8±1.4	1.3±1.3	**0.063**
Basophil	0.26±0.40	0.15±0.36	**0.208**

The highest ferritin levels was significantly higher (119.1±34.3) in obese individuals, than in controls group (97.98±23.4) with a p< 0,001 Figure 1.

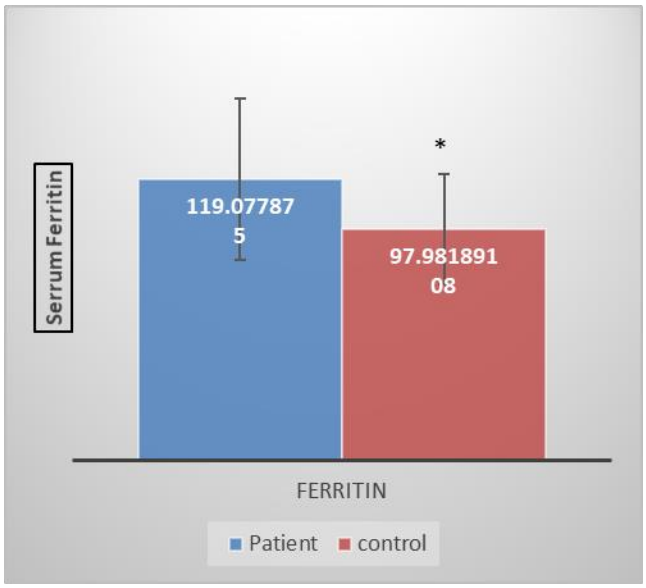
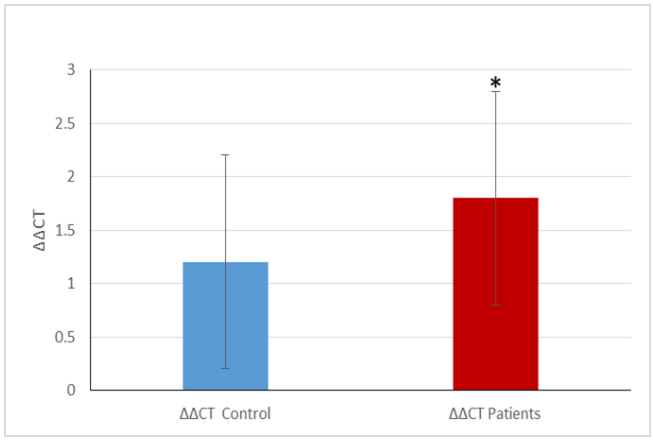


Figure 1. Serum Ferritin concentration in both studied groups

There was significant increase in the number of copies of the HFE gene in obese patients compare controls. HFE copies number was directly correlated with the BMI of the patients.



Figures 2. Changes in the number copies of a HFE gene between studied groups

4. Discussion

Elevated hemoglobin, ferritin, erythropoietin, and haptoglobin concentrations are observed in subjects with MetS. All components of MetS are associated with higher hemoglobin levels, elevated hyperglycemia, TG, abdominal obesity, low high density cholesterol, and higher ferritin levels are all linked (Hämäläinen et al., 2012). According to a study conducted on Korean adolescents and adults between the ages of 16 and 80, increasing serum ferritin concentrations were linked to a higher risk of MetS components such as elevated waist circumference, elevated glucose levels, reduced HDL-C levels, and elevated TG levels (Shim et al., 2017) and oxidative stress has been shown to be a risk factor for tissue damage, and therefore for developing type 2 diabetes (Leiva et al., 2013). This is because high levels of iron in cells and tissues will lead to the production of free hydroxyl radicals. (Datz et al, 2013), which are harmful to cells and can result in DNA damage, cell death, and fibrosis with subsequent tissue remodeling (Pietrangelo et al,2010; Kew,2014).

In our study, Hemoglobin level was significantly higher than control. In both women and men, hemoglobin may be a role in the diagnosis of newly developing MetS. Increased hemoglobin corresponded with an increased incidence of MetS and abdominal obesity in men. These findings have implications for the clinical availability of serum Hb as a predictor of MS and NAFLD (Chung et al., 2017; He et al., 2021; Hashimoto et al., 2015).

Ferritin also has a positive correlation with MetS, liver injury, and BMI. (Suárez-Ortegón et al., 2018). Iron status parameters in patients were significantly altered by metabolic syndrome. Females had significantly lower serum ferritin levels than males. (AL-Kataan,2013)

A normal iron storage level was linked to a risk of MetS components and reduced HDL-C in Chinese children aged 6 to 12 years old, particularly in hyperglycemia and reduced HDL-C.(Zhang et al., 2020). Obese people with high serum insulin levels had leukocyte correlations with MetS morbidity parameters. (Ryder et al., 2014). A genetic disorder called hereditary hemochromatosis (HH) is brought on by excessive iron absorption and organ iron accumulation. Numerous clinical consequences, including cirrhosis, arthritis, cardiopathies, diabetes, sexual problems, and skin darkening, are brought on by this accumulation. Several authors have suggested that pathological

processes such cellular oxidation, cardiovascular disease, and cancer are connected to iron levels that are close to the upper limit of normal. (Sullivan ,1981 ; Araujo et al., 1995 ; Huang ,2003 ; Robinson et al., 2005 ; Beutler ,2006; Yuan and Li , 2008).

In the early stages of the disease, approximately 75% of people with hemochromatosis are asymptomatic when they are diagnosed, and it is frequently discovered at random in connection with routine blood screening.(Pedersen and Milman, 2009) Patients experience a greater incidence of symptoms and an increased risk of organ involvement as a result of increasing iron accumulation in various tissues and organs. (Bassett et al., 1984). Thus, the clinical picture will differ depending on how early or how late the diagnosis is made and how early or how late iron-depletion treatment is started. Men begin to have symptoms after the age of 30, and women begin to experience them after menopause. Fatigue, arthralgias (Deugnier et al. 2019), and stomach discomfort are common starting symptoms, as well as decreased libido and erectile dysfunction in men. (Milman et al., 2019) Because of this, we chose research samples ranging in age from 12 to 22 in order to make an early diagnosis if possible. In addition there are no previous studies of HFE expression in obese people with metabolic syndrome.

Elevated serum transferrin-iron saturation (TS) and serum ferritin concentrations were the biochemical indicators of hemochromatosis. Men's normal serum ferritin values are around 300 ng/mL, while women's readings are around 200 ng/mL, according to the HEIRS Study (Adams et al., 2005). There is no defined "normal" range of serum ferritin levels in HFE hemochromatosis patients. There is a possibility for values between subnormal and several thousand.

Serum ferritin levels frequently increase with time in untreated clinical HFE hemochromatosis patients. An elevated serum ferritin concentration alone cannot rule out iron overload because serum ferritin is an acute-phase reactant and elevated serum ferritin levels might be caused by non-iron liver issues as well as inflammatory diseases including liver disease, alcohol use, and obesity. (Wong and Adams, 2006; Barisani et al.,2008; Adams et al., 2013) or neoplastic diseases (especially when the serum TS is normal) (Barton et al., 1993).

Determine HFE gene expression in obese patients with metabolic syndrome was the goal of this investigation. The identification of distinctive signatures in a variety of disorders using gene expression has proven beneficial .One of the main goals of the current study was to analyze gene expression from peripheral blood cells of obese people in order to identify novel molecular characteristics of the disease that may prove useful as biomarkers. When comparing patients and controls, we discovered a significant difference in the gene expression of HFE. These findings reported that there was an up-regulation of the HFE gene in obese patients compared to controls, suggesting that this increase in HFE expression modulates the expression of hepcidin. This idea was further confirmed by the finding that hepcidin was markedly downregulated as a result of the physiological reaction to iron overload. Hepatocytes have such a big effect on how much intestinal iron is absorbed under physiological circumstances. To promote iron absorption, hepcidin is expressed at low levels. The opposite happens, with increased hepcidin expression and decreased intestinal iron absorption, when iron stores are large. However, the control of duodenal iron absorption is disturbed as a result of decreased hepcidin synthesis in HFE-associated HH or in HFE knockout animals exhibiting paradoxical enterocyte iron insufficiency.

When iron is abundant, the body limits iron uptake from the duodenum by downregulating HFE expression, resulting in a reduction in iron uptake. It would also make sense to up-regulate HFE expression in the iron-overloaded liver in order to reduce the additional uptake of transferrin-bound iron into this organ. In iron homeostasis, as shown in human, in vitro, and animal models, an increase in serum and tissue iron raises hepcidin levels. (Pigeon et al., 2001; Lin et al., 2007).

The development of obesity-related problems has been linked to both inflammatory and oxidative conditions. Gathering the current knowledge on the molecular and cellular changes seen in these circumstances has become essential because pediatric obesity raises the likelihood of acquiring a number of comorbidities in both childhood and adulthood. (González-Domnguez et al., 2020). The defense response, known as the acute-phase response (APR), aims to minimize the damage on one side and eradicate or at the very least isolate the harmful substance on the other. Every substance that compromises the integrity of tissues causes a localized response known as inflammation, which affects the iron balance across the entire body. (Roy et al., 2004). Iron is transferred from the circulation to the storage sites of the reticuloendothelial system in order to decrease the availability of iron under inflammatory situations. (Weiss,2005).

The acute-phase protein hepcidin is largely produced by hepatocytes (Moris et al., 2021). Hepatic hepcidin production is modulated by different influences: circulating iron and iron stores, erythropoietic activity, hypoxia, and inflammation (Pigeon et al., 2001; Nicolas et al,2002; Nemeth et al,2003; Lee et al,2004; Nemeth et al,2004; Roy et al,2004; Datz et al, 2013). Obesity increases hepcidin levels (Bekri et al., 2006; Sanad et al., 2011), Rodrguez-Mortera et al. in their study showed that hepcidin increases in obese adolescents are associated more with inflammation and metabolic changes than with iron metabolism (Rodrguez-Mortera et al., 2021) , It is created more often when there is inflammation and an excess of iron (Aranda et al., 2018).

As is well known, obesity is a state of chronic low-grade inflammation that is strongly connected with increased mortality and severe chronic morbidity from a variety of reasons, including systemic arterial hypertension, diabetes, and liver pathology. As shown by elevated plasma concentrations of acute-phase proteins and cytokines like C-reactive protein (CRP), interleukin-6 (IL-6), Mohamed-Ali et al. (1997), tumor necrosis factor (TNF), Dandona et al. (2004), and plasminogen activator inhibitor-1, these conditions are linked to a state of chronic low-grade systemic inflammation. (Alessi et al., 2000) Adipose tissue is now understood to play a role in the generation of proinflammatory cytokines, which in turn play a role in the metabolic syndrome.

Barisani et al., in their study on patients with DHIO (dysmetabolic hepatic iron overload) to describe the expression of hepcidin and other iron-related molecules, showed that hepcidin mRNA significantly correlated with indices of lipid metabolism, namely, total cholesterol, LDL, and triglycerides, and that there was a significant inverse relationship between urinary hepcidin and biochemical and clinical parameters, i.e. serum iron, transferrin saturation, HOMA, and BMI (Barisani et al., 2008).

To sum up, the findings of the presented study, indicates that iron tended to decrease or be within the normal range in the massively obese. In spite of the high gene expression of HFE, this may be due to the elevated hepsidin concentration resulting from the inflammatory condition due to obesity. The possibility of a mutation in the gene excluded because the levels of ferritin are within normal limits. In addition, according to the information collected from patients by means of the questionnaire, patients did not complain of symptoms of hemochromatosis. According to the iron assays that were conduct on the patients' blood, hemoglobin was within the normal limits compared with the control, and from this, we conclude that there is no iron accumulation. Further studies will be necessary to identify the pathways that cause HFE upregulation, iron overload in obese cases, and hepcidin expression as well.

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