Relation between Body Iron Store and Insulin Resistance in Type 2 Diabetes

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Abstract:

The clinical impact of interaction between body iron status (serum iron and ferritin) and type 2 diabetes has been investigated in this study . Thirty six females were enrolled, eighteen type 2 diabetes and eighteen apparently healthy. These two groups were matched for age and body mass index BMI. The eighteen diabetes females were matched for age, BMI, pharmacological treatment (oral hypoglycemic agent), and chronic diabetes complications .

The biochemical parameters measured for both groups (control and diabetes patient) were fasting insulin(I_o),fasting blood glucose (G_o) , serum iron and ferritin .

A significant increase in all parameters in patients compared to healthy control was noticed . The insulin resistance (IR) which was calculated by the equation:-

IR= (I_o µIU/ ml × G_o mmol/ L)/22.5 was clearly demonstrated to be significantly higher in female diabetes patient(275.9 ± 22.7) compared to control (41.5±11.3) .

Key words: Type 2 diabetes., Body iron store .

Introduction:

Insulin is an anabolic hormone that stimulated the cellular uptake of many nutrients hexoses , amino acids , cations and anions.[1]

Insulin resistance(IR) defined as a reduced glucose response to a given amount of insulin[2].As the body attempts to overcompensate for poor insulin action by pumping out more insulin from the pancreas ,insulin level rise, so this development of insulin resistance result in compensatory hyperinsulinemia characterizes the transition from IR to type2 diabetes[3,4].

The central role of iron in biology is illustrated by the fact that this is the fourth most abundant element in living organisms .Iron has additionally proven to be fundamental in the selection imposed by evolution, given its close relationship with oxygen.[5]

Iron is absorbed from different parts of the small intestine ,transported bound to transferrin and is taken up from the blood by a high affinity specific transferrin receptors ,and stored in reticuloendothelial cells of the liver, spleen ,and bone marrow bounded to ferritin and haemosidrin.[6]

Iron stores, expressed as serum ferritin concentrations ,have been proposed to be a component of the insulin-resistance syndrome.[7]

Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells, redistributing transferrin receptors from an intracellular membrane compartment to the cell surface , since transferrin receptors have been shown to colocalize with insulin- responsive glucose transports in the microsomal membranes of cultured adiposytes ,suggesting that

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regulation of iron uptake by insulin occurs in parallel with its effect on glucose transport [8]. Reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver.Hepatic extraction and metabolism of insulin is reduced with increasing iron stores , leading to peripheral hyperinsulinemia. [9]

The aim of this work is to clarify , at least in part , the interaction of iron and body iron store (ferritin) with insulin resistance (IR) in healthy females , age and body mass index (BMI) matched patients with type 2 diabetes , and to anticipate the possible complication of such interaction.

Materials and Methods:

Subjects: The samples were taken from eighteen females type 2 diabetes mellitus DM age 49 – 62 years with BMI > 30 kg/m^2. They were selected from(Al- Kadhemyia Teaching Hospital /Diabetes Care Unit) , during the year 2007, All patients were diagnosed by physicians . Eighteen healthy control females age 50 - 59 years with BMI over 30 kg/m^2 were enrolled in this study.

Collection of Blood Samples:

Blood samples were obtained from an overnight fasting patients and control. Using disposable syringes venous blood samples were aspirated at 8 - 10 am , collected into two plane plastic tubes , one of them was centrifuged at 3000 rpm within 30 minutes to get serum. Fasting blood-glucose was measured using enzyme calorimetric method directly , the rest of the serum was stored at - 4 ^{o}C till used.

Body Mass Index BMI:

BMI was obtained by measuring weight in kilograms and height in meters then the following equation was used [10]:

$$BMI = weight / (height)^2$$

Fasting blood glucose was determined in the sera of patients and control using a ready Kit from Randox Laboratories, England ,where the glucose is oxidized to D-gluconate by glucose-oxidase with the formation of H_2O_2 in the presence of peroxidase to form a red dye mixture of phenol and 4-aminoantipyrine.The absorbance was measured at (505 nm) against a blank.[11]

Fasting insulin was determined using ELISA which is an enzymatic one/step sandwich type immunoassay (DSL-10-600 ACTIVE)® ' UK. The absorbance was measured at wavelength 450 and 620 nm after stopping the reaction. The values of each test was obtained using insulin standard curve which was constructed from the plot of the absorbance versus insulin concentrations. The insulin concentration and the unknown can be calculated.[12]

Insulin resistance was calculated by the equation :[13]

Insulin Resistance IR= $(I_o \times G_o) / 22.5$

Where :

Fasting Insulin (I_o) measured in µIU/ml

Fasting blood Glucose (G_o) measured in mmol/L

Free serum iron was measured according to the method of Henry 1984[14] where the dissociation of iron as Fe^{+3} from the transferrin complex was achieved by the addition of an acidic buffer containing hydroxyl amine which reduces Fe^{+3} to Fe^{+2}.The chromogenic agent, Ferene, forms a highly colored Fe^{+2} complex that was measured photo-metrically at 560 nm.

Ferritin quantitative test was performed using a solid phase enzyme linked immunosorbent assay. The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse

monoclonal anti-ferritin antibody-enzyme (horseradish peroxidase) conjugate solution.

The samples which tested, were allowed to react with the antibody resulte in the ferritin molecules that being sandwiched between the solid phase and enzyme linked antibodies, incubation was carried out at room temperature for 45 min. The wells were washed with distilled water and a solution of tetramethylborate reagent was added and incubated at room temperature resulting in the development of a blue color which was stopped by the addition of the stopping solution which change the color to yellow that was measured spectophotometrically at 450 nm. The concentration of ferritin was directly proportional to the color intensity from a standard curve which was constructed by plotting the absorbance of a set of standards against the concentration on a linear graph paper .

Statistical significant difference between the two groups was assessed by student-t test considering P values less than 0.05 to be significant. The results are expressed as mean $\pm$S.D.

Results and Discussion:

The biochemical parameters; Insuline, FBG , Iron ,Ferritin tested in this study are illustrated in table (1) for the female patients type (2) DM. A Significant elevation in fasting insulin and in fasting blood glucose in patients compared to that of matched age and BMI female are obvious , which result in the significant increase found in IR insulin resistant for the patient group compared to control.

Table 1: Insulin, G_o, IR, Iron, Ferritin of healthy and type 2 diabetes females.

Parameters	Healthy control	Patients	P value
Insulin (μIU/ ml)	11.5 ± 6.3	31 ± 8.2	P = <0.05
FBG mmol/ L	3.6 ± 2.3	8.9 ± 3.2	P = <0.05
IR	41.5 ± 11.3	275.9 ± 22.7	P = <0.05
Iron μmol/ L	18 ± 3.1	37 ± 4.2	P = <0.05
Ferritin mgm/ ml	120 ± 8.1	222 ± 28.3	P = <0.05

Total serum iron of DM patients showed a high significant increase compared with that of control , also a significant increase in body iron store(ferritin) for patients compared to control was found.

The insulin resistance is a state in which a given concentration of insulin produce a less than expected biologic effect so it means that does not work optimally at its target tissues to drive glucose into cells .This has numerous adverse consequences including glucose and insulin levels that are significantly higher than control[15].The entry of glucose into the target cells is by a fascillated diffusion. The specific protein involved in this process is the glucose transporter which is a transmembrane helical segments, which is insulin dependant in muscle and adipose tissues, so any defect in these transporters recrement from intracellular sites to the surface of the cells may help to explain the insulin resistance displayed by patients with type 2 DM (1). IR appear to be closely linked to total body iron stores in the healthy general population. [16]. Insulin resistance which is a complex cellular pathology affects multiple organ systems and predisposes patients to a hard metabolic defects [17] with high prevalence in the population associated with high death rate fundamentally through cardiovascular heart diseases, even in non diabetic subjects [18].

The impact of transition metals, in general and iron ,in particular has been a matter of study during the last decades, because iron is a potent pro-oxidant that increases the oxidative stress, results in hyper insulinism and insulin resistance . Free iron also exerts a positive feedback on ferritin synthesis , while oxidative stress increases the release of iron from ferritin. Thus, ferritin can act both as a

source of iron, which induces oxidative stress, and as a mechanism that protect against iron toxicity [19] .

Hyperferritinemia is present in high prevalence in patients with type 2 DM, particularly in poorly controlled patients, probably reflecting increased oxidative stress. Short term improvement in glycemic control is followed by variable decrease in serum ferritin concentration [16,20] .

During this century with increased life expectancy, a protective mechanism has become determinant, high carbohydrate diet and less heme Iron consumption is recommended due to both insulin resistance and increase oxidative stress [21].

The relation between IR and iron status in patients with type 2 DM should be tested on large scale clinical trials , searching for the usefulness and cost-effectiveness therapies which decrease iron and ferritin such as blood letting and iron-chelators to improve insulin resistant syndrome

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العلاقة بين خزين الحديد بالجسم ومقاومة الانسولين في مرضى السكري النوع 2

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الخلاصة:
تم التحري عن التداخلات السريرية لحالة الحديد في الجسم والتي تشمل (حديد مصل الدم والحديد المخزون في الفيريتين) عند مرضى داء السكري غير المعتمد على الانسولين(النوع 2) .
شملت الدراسة 36 انثى ، من الاناث 18 مصابة بداء السكري (النوع 2) و 18 انثى من الاصحاء .
كانت المجموعتين متقاربتين بالعمر وفي مؤشر كتلة الجسم (BMI).
اما مجموعة المريضات فقد كانت متماثلة فيما بينها من حيث العمر ومؤشر كتلة الجسم والعلاجات الفموية الخافضة للسكر(مثل الكلبنكلامايد والميتفورمين)ومضاعفات داء السكر.
تم قياس الدوال الكيموحيوية لمجموعتي الاصحاء والمرضى وقد شملت :- الانسولين، الكلوكوز بعد الصيام، الحديد في مصل الدم والفيريتين.
لوحظت زيادة معنوية عالية في جميع هذه الدوال عند المرضى مقارنة بالاصحاء. وقد كانت هذه الزيادة واضحة في مقاومة الانسولين (IR) والتي تم حسابها حسب المعادلة :-

IR = (الانسولين µIU/ ml × الكلوكوز mmol/ L) / 22.5

حيث وجدت (22.7 ± 275.9) مقارنة بالاصحاء (11.3 ± 41.5)