Association between excess iron and type 2 diabetes

Diabetes is a lifelong and costly metabolic disease that could lead to several life threatening complications, such as diabetic nephropathy and cardiovascular disease (CVS). Therefore, a better understanding of its pathophysiology and identifying the possible mechanisms underlying this disease could be helpful in preventing its occurrence and complications. Obesity is found to be the major risk factor that derives type 2 diabetes (T2D). Recently several studies has demonstrated that there are other risk factors that participate in developing T2D such as iron accumulation in the liver. Studies showed that high levels of iron are associated with oxidative stress. Iron is considered a strong pro-oxidant through the production of hydroxyl radicals, powerful oxidant species. In addition, iron overload is found to be associated with lipid accumulation in the liver cells (steatosis) which leads to insulin resistance. Fatty liver, oxidative stress, obesity and other factors are considered triggering factors that increase the risk of developing T2D. Therefore, iron might be involved in diabetes pathophysiology. It is also anticipated to be a risk factor for developing insulin resistance and other diabetic complications. It is also important to investigate whether high iron levels could increase the risk of diabetes or having diabetes will elevate iron levels.

Background

Introduction

Diet and lifestyle play a major role in prevention of type 2 diabetes (T2D)[2]. Macronutrients, such as carbohydrates and fat should be restricted to decrease the chance of developing T2D. However, the impact of

micronutrients on the reduction of the incident of T2D is not well established [2, 3]. Several studies suggested that some micronutrients, such as calcium, magnesium, chromium and iron could cause insulin resistance and diabetes [2].

Iron is considered as an essential nutrient for humans as it is the major component of oxygen transporters in the body, and it is has a metabolic function as a cofactor for several enzymes. However, excess amount of iron is found to be harmful to the body by altering glucose metabolism and the production of reactive oxygen species that play a role in the generation of additional reactive oxidants, such as hydroxyl radicals in which iron salt plays a catalytic role in a reaction. This reaction is known as a metal catalysed Haber-Weiss reaction [4, 5]. Pancreatic islets are found to be more susceptible to oxidative stress as their defence against oxidants is weak. Mouse model with high levels of iron and oxidative stress mediates apoptosis of pancreatic islets with a subsequent decrease in insulin secretory capacity [5, 6]. Previous studies on animals showed an increase in diabetes incidence after parenteral administration of iron. Evidence on the role of iron in the pathophysiology of type 2 diabetes mellitus was first discovered in patients with classic hereditary hemochromatosis (HH). This hereditary disorder is characterised by a progressive accumulation of iron in the heart, liver, pancreas, and other parts of the body. The frequency of diabetes was found to be increased in those patients [5]. Other studies suggested that phlebotomy or iron chelation therapy will decrease iron level and thus improve insulin sensitivity in diabetic patients [7].

Other studies on thalassemic patients showed an increase in insulin resistance[5]. Although the exact mechanism on the participation of iron in the onset of diabetes is not exactly known, however, iron overload found to be associated with hepatic dysfunction, insulin deficiency and insulin resistance.[5]

Previous evidence confirms the association between iron overload and insulin resistance. However, further investigation is warranted to prove the relationship between the accumulation of iron and the progression to type 2 diabetes mellitus.

Recently, correlation between hepatic iron overload with chronic liver disease such as non-alcoholic fatty liver disease (NAFLD) have been considered. Like glucose, iron level is regulated by a mechanism similar to that for glucose. It is regulated by a hepatic peptide hormone called herpcidin. High levels of iron stimulate the synthesis of herpcidin, which in turn decreases the iron exporter ferroportin in macrophages and intestinal cells and thus reduce serum iron. High consumption of food that contains iron and glucose will also increase the risk of hyperglycaemia and iron overload. Males are at higher risk to develop iron overloading than females, because females lose iron from blood during menstrual cycle.[8]

Kowdley et al, investigated the role of serum ferretin levels and the accumulation of iron in NAFLD [9]. An increase in ferretin levels is associated with greater accumulation of iron in hepatic cells, but even in patients without iron overload, ferretin was associated with advance stages of NAFLD. This concludes that iron overload is unrelated to advanced stages of NAFLD

characterised by systemic inflammation. This study gives a contradictory role of iron overload in patients with NAFLD.[8]

Insulin-resistance is found to be related to hepatic iron-overload syndrome. Typically, in NAFLD, the iron accumulation is mild and involves hepatocytes and sinusoidal Kupffer cells. Nevertheless, iron reduction treatment was found to be beneficial in the treatment of NAFLD disease activity as well as increase in insulin sensitivity.[10]

Iron overload has also been found to be associated with diabetic complications, such as diabetic nephropathy and cardiovascular disease [5]. Animal studies showed an increase in the amount of iron in the kidneys. An increase in urinary iron was also found in patients with diabetic nephropathy [5]. In patients with CVD, high iron levels are associated with several complications such as myocardial infarction (MI) [5].

Diabetes is found to be associated with more than one risk factor including iron overload, fatty liver and obesity. More research is warranted to investigate the correlation between elevated iron levels and the incidence of T2D.

Obesity is another detrimental factor that is found to be prevalent in diabetic patients. It is considered a major cause of inflammation, which may be responsible for developing insulin resistance leading to diabetes. High levels of C-reactive protein (CRP) have been reported in obese patients [11]. Therefore, our study will also investigate the relationship between obesity, T2D and iron levels.

Aims and objectives

Because iron overload is found to affect major tissues involved in glucose and lipid metabolism (pancreatic B cells, liver, and adipose tissue)[12]. Iron overload is found to be related to several metabolic chronic disease, such as T2D, obesity, non-alcoholic fatty liver and other complications [12]. Our aim of the study is to investigate the association between excess iron in the body and T2D. This study also aims to elucidate how iron levels and T2D are related to obesity.

Plan of investigation

A cross-sectional observational study will be carried out. It will include a group of 180 participants. The study population will be categorized into four groups consisting of Group 1: Healthy individuals (controls); Group 2: Obese healthy individuals; Group 3: Normal weight diabetic patients; Group 4: Obese diabetic patients [13]. The inclusion criteria are patients aged between 40 and 70 years with a male to female ratio of 140: 40; only postmenopausal women will be included to reduce possible confounding by iron deficiency. The exclusion criteria are any history of uncontrolled hypertension, cardiac, pulmonary, and neurological complications. Participants having recent infectious, inflammatory or neoplastic conditions; or with laboratory evidence of inflammation (C-reactive protein > 0. 5 mg/dl or white blood cell count > 11, 000/uL, platelet count > 400, 000/uL), and individuals who is anaemic will be excluded [13]. Heavy smokers and alcoholics will also be refused enrolment.

Methods and materials

General measurements:

On the first day, every participant will be allocated to the appropriate group after conducting diferent measurements. Body mass index (BMI) will be measured for each patient by dividing their weight (in kilograms) by height (in meters) squared. Any participant with a BMI> 30 is considered to be obese. Then, the blood pressure will be taken by a standard sphygmomanometer for each patient while they are on a supine position. Patients will be asked to avoid drinking alcohol and caffeine 12 hours before the measurements.[14] Then each participant will be asked to fill in a 50-item food frequency questionnaire (FFQ) to assess their iron consumption.[2]

Oral glucose tolerance test (OGTT) will be used to measure blood glucose levels for participants then results will be recorded. Before OGTT they will be asked not to eat, or drink for up to 8-12 hours. Participants will have to ingest 75 g glucose in 250-300 ml water over five minutes. Then the blood samples will be taken after 2 hrs of glucose ingestion. (Normal value less than 140 mg/dL) [15].

Iron overload will be measured by ferritin blood test; overnight blood samples will be obtained. Ferritin can be measured in serum using serum separator tubes (SST), or plasma using lithium heparin or EDTA tubes. Centrifugation and separation within 24 h of sample collection is required and ferritin is stable for 7 days at 2-8°C. The samples must be centrifuged to remove precipitates and fibrin [16].

Statistical methods:

Statistical analyses will be performed utilising Minitab software. Descriptive statistics will be performed for each group. The mode of distribution for continuous variables will be detected according to Anderson darling test. Serum ferritin values for each group will be compared with the control group and the significant difference will be determined by Student t-test or Mann Whitney test according to the mode of distribution. A p-value lower than 0. 05 will be considered significant. By comparing ferritin levels of the control group with the obese nondiabetic group, we will determine if there is a significant relationship between obesity and iron levels. On the other hand the relationship between iron levels and diabetes will be determined by comparing the group of diabetic healthy weight with the control group. We could also determine the role of obesity in T2D. The association between the serum ferritin levels, BMI and blood glucose levels will be determined by Spearman’s rank correlation test. Higher correlation is demonstrated with Rho values close to 1.

The 50-item food frequency questionnaire (FFQ) filled in by each group of participants will be analysed trough Minitab software. This will help to evaluate the association between dietary intake of iron and the risk of T2D.

Fund should be allocated to purchase tools and materials to successfully conduct this project. Tools of measuring glucose level are essential during different phases of this project. These tools will estimate blood glucose levels and provide data that will enrich analysis. Serum separator is essential to estimate iron levels necessary to conduct analysis and find correlation with blood glucose level.

Estimation of iron and blood glucose and their correlation is advantageous for participants. The results of this project will reflect on their diet specifications by either increasing or decreasing iron content. This project will provide insights to how much iron should be minimally consumed to reduce the incidence of diabetes. Also, many recommendations for obesity will be flagged up in this project to reduce the incidence of diabetes and raise awareness toward diabetes and its complications.

This study also requires the participation of a part-time technical assistant in distributing the questionnaire, collection of data and feedback. An experienced researcher is required to guide me in the experimental part of the study as he has a broad expertise and knowledge in practical issues. Given the large sample size taken in this project, support needs to be offered by researchers and technical assistants to collect blood samples and conduct the required tests to meet the pre-determined objectives in a timely fashion

The use of participants in the study helps to make a difference in care for future diabetic patients by providing information about the risk factors that will derive the disease, with the possible interventions. It will also benefit the participant themselves by gaining knowledge about the different dimensions of diabetes and through helping them to improve their quality of life by changing their lifestyle and diet modification. Of note, this study will not cause any harm for participant.