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Research Article

CHANGES OF SOME BIOCHEMICAL BLOOD PARAMETERS IN WOMEN DUE TO DIABETES MELLITUS

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Abstract

This present study was conducted to evaluate thealteration occur in some biochemical blood parameters of 60 women due to diabetes mellitus. The tested biochemical blood parameters were Total Serum Bilirubin, Total Serum Protein, Total Blood Calcium and Serum Glutamic Pyruvic Transaminase (SGPT) or Alanine transaminase (ALT). The tested women were divided into three groups: 20 women with Insulin-Dependent Diabetes Mellitus (IDDM), 20 women that are Non-Insulin Dependent Diabetes Mellitus (NIDDM) and 20 women that have not been affected with (DM)which was conducted as a control group. The study showed a significant difference ($p<0.05$) between both IDDM and NIDDM compared with the control group in which the total serum bilirubin was decreased. However, no significant differences ($P>0.05$) were detected in both IDDM and NIDDM regarding total serum protein and total concentration of calcium compared to the control group. Moreover, there is a significant difference ($P<0.05$) in the level of SGPT in patients with IDDM and NIDDM compared to the patient who not suffered from Diabetic Mellitus.

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1. Introduction

Diabetes Mellitus (DM) is one of non-communicable disease, that has a major impact on health of human and the worldwide economy. It was found that the number of people affected by DM increased due to changes in lifestyle, ageing of population and urbanization (Chen Magliano and Zimmet, 2012). The International Diabetes Federation (IDF) reported that 463 million are currently living with diabetics, in which DM is considered one of the fastest-growing health challenges of the 21st century(Federation, 2019). The larger number of populations that will have DM according to the predict of IDF in 2035 including China, Japan, India, Indonesia, and four Asian countries in which the centre of DM epidemic will be in the Asia (Federation, 2019).

Bilirubin is acting as a powerful biological antioxidant and represents the end product of haem catabolism (Stocker *et al.*, 1987; Balla *et al.*, 2005). It was reported that bilirubin is negatively related to oxidative stress (Hasanein, Ghafari-Vahed and Khodadadi, 2017; Vitek and Schwertner, 2007). Many studies have showed that bilirubin at an acceptable elevated level is beneficial. Several Japanese studies mentioned that bilirubin is negatively associated with type two diabetes, glycated haemoglobin and peptide C

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in old and middle-aged people (Ohnaka *et al.*, 2010). Furthermore, the incidence of metabolic syndrome and DM were reduced by elevated bilirubin (Wu *et al.*, 2011). The protein was used as a biomarker to predict mortality of the long tear in patients who suffer from diabetes mellitus, coronary diseases and cancer. In addition to diagnosis and prognosis of acute and chronic type two diabetes mellitus (Zaidi *et al.*, 2019).

Changing the protein composition has been associated with an increase in serum viscosity which may respond to the microvascular sequelae. Albumin is the major protein that decreases in their level compared to the acute phase of proteins which increase in the level (McMillan, 1989).A study conducted in albino Wistar rats found that the synthesis of protein in all tissues was decreased due to deficiency of insulin and to the depressed synthesis of Hb (Pari and Amarnath, 2004). Others mentioned that insulin resistance in type two diabetes elevate the rate of blood proteins (creatinine and uric acid), which is characteristic of renal failure (Ferdi Abla and Chenchouni, 2018).

Calcium is one of the vital components that are needed in bone metabolism and other biological function such as muscle contraction, cell signalling, nerve impulse and blood clotting (Peacock, 2010). Serum calcium is Menten by parathyroid hormone (PTH), ionized calcium (Peacock, 2010) and 1,25-dihydroxy vitamin D [1,25(OH)2D] (Jorde *et al.*, 2013). The high concentration of serum calcium is associated with a wide range of health issues such as metabolic syndrome (Kim *et al*., 2010) and cardiovascular morbidity and mortality (Foley *et al*., 2008; Larsson *et al*., 2010). There is an association between diabetes incidence and serum total calcium. This association was independent of many risks factor of diabetes. The study confirmed there is an independent association between serum calcium and risk of diabetes (Sing *et al.*, 2016). The study concludes that both total calcium and albumin-corrected calcium was associated with an increased risk of diabetes in a Chinese population.

Serum glutamic pyruvic transaminase (SGPT) or Alanine transaminase (ALT) is used as a serum marker of liver disease, is considered as a good predictor of mortality from liver disease (Hyeon *et al.*, 2004). The level of Alanine transaminase in a patient with either type 1 or type 2 diabetes is higher three to four times compared to general populations (West *et al.*, 2006). The same study found the use of insulin in patientswith type two diabetes were reduce the elevation of Alanine transaminase.

2. Materials and Methods

The study group and clinical samples

The blood samples of 60 women were collected from women who Alasady Laboratory in Theqar which was divided into three groups. The first group (20 blood samples) of women was diagnosed with DM and dependent on insulin in their treatment (IDDM)these groups were taken insulin regularly. The second group (20 blood samples) of women are diagnosed with DM and do not depend on insulin in their treatment (NIDDM). The third group (20 blood samples) of women that have not been affected with DM according to their diagnosis and clinical history, this group was conducted as a control group. All the tested women in this study aged from 23-50 years. All the blood sample was collected using a disposable needle attached to a graduated hypodermic syringe in which about 4ml of venous blood was collected (Shenoy *et al.*, 2014).

Ethical Statement

Ethics agreement was obtained prior to the data collection in which agreement was obtained from all participant before their participation.

Total Bilirubin Concentration in the Blood Serum

The total bilirubin concentration in blood serum is estimated by using a bilirubin kit that has a measurement kit depending on two detectors that are:

A) The first detector is a total bilirubin reagent detector R1, which is sulphanilic acid at 45 mmol/L concentration, hydrochloric acid at 251 mmol/L concentration, caffeine 250 mmol/L concentration and sodium benzoate 450 mmol/L concentration.
B) The second detector is a nitrite reagent detector R2, which is sodium nitrite 5.5 mmol/L concentration.

Two tubes are taken for studying and were classified one with the blank and the other tube is the sample, in the tube with the blank 0.1 from R1 detector, and 1.0 from the sample serum that needs to be measured is added. Moreover, the other tube with the sample, 0.1 of R1, 0.2 of R2 and 1.0 from the serum sample that the total bilirubin concentration is needed to be measured, is added. The tubed are mixed well and kept at room temperature for 5 minutes. Then, the wavelengths of both tubes are measured by a spectrophotometer on the wavelength rate of 545 nm.

Total Serum Protein Determination

The determination of total serum protein is estimated by the Biuret method, as the peptide bonds will react with copper sulfate that gives a blue coloured complex which intensity matches with protein concentration in the blood serum (Henry *et al.*, 1974). Whereas, many solutions are used in this kit. Three test tubes are collected in each one of them 1ml of biuret solution is added, in the first tube 10 µl of blood serum is added, the second tube 10 µl of protein standard solution is added and the third tube 10 µl of a blank solution is added by a pipette, then mixed well and left in temperature 25 – 30 ˚C for 40 minutes. Then, the absorption of the standard solution and the blood serum is estimated by a Spectrophotometer with wavelength 545 nm, the determination of total serum protein is calculated by:

$$\text{Total Serum Protein} = \frac{\text{Sample Absorption}}{\text{Standard Solution Absorption}} \times \text{Standard Solution Concentration}$$

Total Serum Calcium Concentration

The total serum calcium is estimated by using a calcium kit (Stern *et al.*, 1975*)* which has many chemical detectors:

A) First detector R1 which is ethanolamine 600 mmol/L concentration.
B) Second detector R2 which is O-Cresolphtalein 0.52 mmol/L concentration and 8-Hydroxyquinoline 70 mmol/L concentration.
C) A standard solution is a Calcium solution 15 µg/dl.

Three plastic tubes were collected and divided into blank tubes, the sample tube and the standard solution tube. Furthermore, in the previous tubes, 1.0 ml from R1 detector was added then in addition 1.0 ml from R2 detector was also added then 2 ml from the standard solution and 2 ml from the serum sample that the total calcium concentration is needed to be measured. The tubes are mixed well and kept at room temperature for 5 minutes, then the wavelength is measured by a spectrophotometer on a wavelength 560 nm from the tube with the standard solution and the tube with the sample after measuring with the blank and comparing the results, then estimating the result of the total serum calcium concentration by this calculation:

$$\text{Total Serum Calcium} = \frac{\text{Sample Absorption}}{\text{Standard Solution Absorption}} \times \text{Standard Solution Concentration}$$

Serum Glutamic Pyruvic Transaminase (SGPT)

A commercially available kit (ALT Activity Assay by Sigma- Aldrich) was used to estimate the alkaline phosphate (ALP) or serum glutamic pyruvic transaminase (SGPT). The SPGT is determined by a coupled enzyme assay, the produced materials is appeared in a colourimetric (570 nm)/ fluorometric (λex = 535/λem = 587 nm) product, proportional to the pyruvate generated.

Statistical Analysis

The results are analyzed as the mean is determined and the Standard Error (SE) by using a T-test and an F-test.

3. Results

The impact of Diabetes Mellites on Total Bilirubin concentration

The results of this study showed that there is a significant decrease in the total bilirubin concentration in patients that depends on taking insulin about ($P < 0.05$), comparing with those control groups, as the concentration was (0.19 ± 0.90) were the control groups are (0.1 ± 0.08) respectively. Nevertheless, there is no significant difference in the bilirubin concentration of the patients with diabetes mellites who do not depend on insulin and the patients of the control group, which was about (0.08 ± 0.06). furthermore, no differences have been shown in bilirubin concentration between patients with diabetes mellites that depend on insulin and those that are not dependent on insulin (Table - 1).

The Impact of Diabetes Mellites on Total Protein Concentration

The results have showed no differences in the total protein concentration in patients with diabetes mellites that are dependent on insulin (55.6 ± 3.45) compared to the control groups which are (55.5 ± 3.60). Also, no differences have been shown in patients with diabetes mellites which are not dependent on insulin, which is about (55.7 ± 3.35) (Table 1). Hence, no differences have been shown when comparing the patients who are dependent and not dependent on insulin.

The Impact of Diabetes mellites on the Total Calcium Concentration

The results showed no differences are showed in calcium concentration in patients with diabetes mellites which are dependent on insulin that is about (7.35 ± 0.14) compared with the control group which is about (7.05 ± 0.35). Moreover, no differences have shown in the patients with diabetes mellites who are not dependent on insulin that is about (7.25 ± 0.13) (Table 1). Also, no differences are shown between patients that are dependent on insulin and those who are not dependent.

The Impact of Diabetes mellites on the Serum glutamic pyruvic transaminase (SGPT)

This present study showed a significant difference ($P < 0.05$) in the SGPT concentration of patients that depends on taking insulin, compared with control groups, as the concentration was (22.0 ± 0.10) were the control groups are (18.00 ± 1.11) respectably. Moreover, The SGPT was elevated significantly ($P < 0.05$) in the patients with diabetes mellites who are not dependent on insulin about (25.7 ± 5.13) compared with control groups (18.00 ± 1.11) (Table - 1). Moreover, there are no differences in the concentration of SGPT between the patients with diabetes mellites who take insulin and patients that are not dependent on insulin to control diabetes militance

Table - 1: Biochemical parameters among diabetes mellites participant and control participant (as mean ± SD) (N = 60)

Parameters	*IDDM participants (mean ± SD)*	*NIDDM participants (mean ± SD)*	*Control group (mean ± SD)*
Total Bilirubin Concentration	*0.19 ± 0.90*	*0.08 ± 0.06*	*0.1 ± 0.08*
Total Serum Protein	*55.6 ± 3.45*	*55.7 ± 3.35*	*55.5 ± 3.60*
Total Serum Calcium	*7.35 ± 0.14*	*7.25 ± 0.13*	*7.05 ± 0.35*
Serum glutamic pyruvic transaminase	*22.0 ± 0.10*	*18.00 ± 1.11*	*25.7 ± 5.13*

IDDM participants: Insulin dependent participants; NIDDM participants: Non Insulin dependent participants

4. Discussion

Diabetes mellitus is a defect occur in the metabolisms of the body, this metabolisms disorder occurs due to different causes such as environmental and hereditary causes. Leading to elevation in the blood sugar level (Hyperglycemia). Type two diabetes is the most common type of diabetes, which form 90 – 95% of all diabetes cases globally. Bilirubin is a natural

complies that have a toxic effect. However, unconjugated bilirubin possesses a strong antioxidant activity. That is way a little increase in bilirubin (hyperbilirubinemia) might have a beneficial effect on the health. A direct significant association was found between type two diabetes subject and Hba1C (Association, 2009). This study showed a significant decrease ($P < 0.05$) in the total bilirubin concentration in the patients with diabetes militances who depend on insulin, compared to the control groups. This result may due to the positive effect of insulin in reducing the level of serum glucose and this is in line with other studies that showed elevated bilirubin levels are negatively related to type 2 diabetes, and associated with a lower risk of diabetic complication (Stocker *et al.*, 1987; Fukui *et al.*, 2008; Vítek *et al.*, 2002). Another finding was no differences have showed in bilirubin concentration between patients with diabetes mellites that are not dependent on insulin and control groups. Moreover, no significant differences were been observed between patients who depend on insulin compared to patients who did not depend on insulin.

The total protein concentration was also estimated in this study. No differences in the total protein concentration in patients with diabetes mellites that are dependent on insulin compared to the control groups. Also, no differences have shown in patients with diabetes mellites who are not dependent on insulin compared to the control groups. Furthermore, no differences have shown when comparing the patients who are dependent and not dependent on insulin. A similar study had shown the same results, in which no significant differences in protein concentration between patients with diabetes mellites who are dependent on insulin and those who are not dependent on insulin especially during the month of Ramadan (Azizi and Siahkolah, 1998). Moreover, another study shows a significant decrease in albumin concentration, through the first years of patients with diabetes mellites (Kobbah *et al.*, 1998). Moreover, other studies have suggested that there are no significant differences in albumin concentration in patients with diabetes mellites during Ramadan month (Laajam, 1990; Mafauzy *et al.*,1990). Others mentioned that any increase in albumin concentration leads to an increase in total protein concentration (Uthman, 1994). The study shows no significant differences ($P<0.05$) in total calcium concentration in patients with diabetes mellites who are dependent on insulin and those who are not dependent on insulin compared to the control group.

A similar study found that serum calcium levels were not associated with type two diabetes (Kim and Hong, 2018; Kirii *et al.*, 2009). There are inconsistent reports about the relationship between serum calcium concentration and insulin sensitivity or insulin secretion. Lorenzo *et al.* (2014) mentioned that serum calcium, was weakly and negatively correlated with insulin sensitivity. This study showed that there was an increase in the level of SGPT in patients suffering from diabetes mellitus as compared to normal individuals. A similar study evaluates the level of SGPT in a patient with diabetes mellitus compared to a normal individual in which there was an increase in the liver enzymes (SGPT) recorded (Hanley *et al.*, 2004; Wannamethee *et al.*, 2005; Katiyar *et al.*, 2015). Moreover, a significant increase in the level of SGPT in patients who IDDM and NIDDM compared to the control group. Furthermore, no difference was recognized between the result of patients who IDDM and NIDDM. Another study mentioned that both controlled and uncontrolled type 2 diabetes individuals have a higher incidence of SGPT abnormalities than individuals who do not have diabetes especially individuals with fatty liver (Goyal *et al.*, 2014). Others conclude that hat current insulin use in patients with type 2 diabetes is associated with a reduced risk of elevated ALT (West *et al.*, 2006).

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