Study the Change of MDA, GSH and Hb 1 Ac and Glucose in Patients with Diabetes Mellitus Type I

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Abstract

A study was conducted for 6 months starting from November /2021 to April/2022 in Al-Hassan center for diabetes and endocrinology in Al-Husain medical city of Karbala. The total number of participants are (240) person include (150) patients with type I diabetes mellitus (TIDM), the males (54) (36 %), the females 96 (64%) and (90) healthy control group, males (36) (40%), females (54) (60%). In the recent study observed, the highest rate of age group in patients with T1DM in (12-17) (48%) and (18-23) (16%) confirms that high percentage of patients are children and adolescents. Also, all patients recorded in this study is a female and their percentage was (64%) while the males was (36%) confirms the most patients were diagnosed with diabetes are female this result demonstrated a relationship between gender and type I diabetes. also revealed a relationship between weight, height and body mass index (BMI) with gender. The considerable percentage of blood group for patients was showed in O (48%) and A (25.43%). Furthermore, observed increase in level of HbA1c and glucose concentrations in diabetic patients especially in females than males compared to the control group. Also detected in most patients an increase in concentrations of (MDA) enzyme and decreased in (GSH) enzyme concentrations and this can be considered as a diagnostic feature for type 1 diabetes.

Keywords: patients; MDA, GSH; Hb1Ac and glucose

1. Introduction

Diabetes mellitus is one of the most common endocrine disorders resulting from defects in insulin secretion or insulin action[1] . The primary sign of the diabetes is hyperglycemia in the blood, caused by insufficient pancreatic insulin secretion or low insulin-directed fostering of the glucose via target cells. DM could be classified to several the types, yet T1DM and T2DM are the two most common types. For T1DM, insulin renewal therapy is the backbone, while in T2DM, there should be lifestyle modification and a control diet [2] .Loss of functional β-cell mass is the key mechanism leading to diabetes mellitus as long as β-cells are able to compensate, for instance, for insulin resistance, normoglycaemia is preserved , The American Diabetes Association (ADA) defines type I diabetes mellitus (T1DM) as autoimmune β-cell destruction usually leading to absolute insulin deficiency and type II diabetes mellitus (T2DM) as progressive loss of β-cell insulin secretion frequently occurring on the background of insulin resistance . Novel ways of clustering patients with diabetes mellitus into subgroups that predict disease progression and risk of complications are being investigated [3] . T1DM is common worldwide and steadily increasing in frequency of incidence of about 3 % yearly. T1DM is responsible for about 5 % to 10% of total population of individuals who have diabetes, whereas T2DM has almost all cases. The number of individuals affected is predicted to rise to 642 million in 2040. (Mobasseri et al., 2020). In contrast to T2DM, in which both IR and decreased insulin secretion via the cells play a synergistic role, the pathogenesis regarding T1DM is caused by environmental, genetic, and immunologic factors which destroy the beta cells of endocrine pancreas and result in the insulin deficiency. It typically progresses over some period of several months to years throughout which period patients are asymptomatic, glycemic, and positive for the relevant autoantibodies. The autoimmune destruction process occurs in the persons who are genetically susceptible under triggering effects of at least one environmental factor [4]. A significant upstream event for increasing free radical generation is diabetes , Uncontrolled diabetes could cause the generation of free radicals , By interfering with the state of lipids, proteins, and DNA, oxidative stress, which is imbalance between antioxidants and pro-oxidants in cells, might cause cellular damage[5] . Antioxidants are substances which stop other compounds from oxidizing or neutralize free radicals, Antioxidants create a biological defense system against excessive ROS. Those bioactive compounds could scavenge free radicals or stop the production of ROS. Antioxidants could be exogenous, in other words, which occurs naturally in the human body, like uric

doi.org/10.31838/hiv22.02.536 Received: 25.06.22, Revised: 07.08.22, Accepted: 25.08.22

acid and glutathione; exogenous, in which case they're majorly derived from diet. Tea, coffee, red wine, as well as different kinds of vegetables and fruits (grapes, blueberries, and oranges) all significantly contribute to the diet's overall antioxidant capacity. Exogenous antioxidants, like carotenoids and vitamin E, also form an essential complementary component of a body's natural anti-oxidant defense system [6] .

2. Material and Methods

The current work was designed as case control study, which is also known as "prospective study" and "case-referent study [8], involved 240 individuals, 150 subjects of patients with TIDM, and 90 participants apparently healthy control group. Patients of different ages, ranging in age from one to fifty years, of both sexes, were diagnosed with T1DM. In the Al Naqaa laboratory of the Biochemistry Department, samples taken over a six-month period (November 2021 to April 2022) from the Al-Hassan center for diabetes and endocrinology in the Al-Husain medical city in the Kerbala Governorate were thoroughly processed.

Collection samples

With the use of a disposable syringe, five milliliters of venous blood were taken from each participant. Two portions of this blood were separated

The first portion (3 ml) was divided between two gel tubes and allowed to clot at the temperature of the room for about 30 min. The gel tubes were after that centrifuged at 4000 x g to get serum, and the first gel tube containing the serum was utilized to automatically evaluate blood glucose. The serum of the second gel tube was put into an Eppendorf tube and stored at a (-20oC) temperature until using it to estimate the enzymes (GSH and MDA).

The second portion of the blood sample (2ml) has been drawn into the EDTA tubes and utilized for the automatic and manual HbA1c and ABO group analysis.

3. Statistical Analysis

The version twelve of the computer program, SPSS, which has been utilized for data analysis. the data have been represented as mean standard deviation (± Sd). they were estimated differences among groups via using T test with the P value (i.e. the least significant difference) has been found for the comparison amongst the groups, and the results have been considered to have statistical significance at ($p \leq 0.05$).

4. Results and Discussion

Total sample (240) include 150 patient's cases of type I diabetes mellitus T1DM, 54 patients (36%) were males and 96 patients (64%) were females and 90 healthy persons, 36 persons (40%) were male and 54 persons (60%) female.

General parameters

Age

According to the results in table (1) showing no significant differences (P more than 0. 050) in age group less than 5 years and age group (42-50). There were high significant differences (P less than 0.001) in two age group (6-11) and (12-17) also a significant difference (P less than 0.05) in age group (18-23), (24-29), (30-35) and (36-41), as observed in table (4-1). Also results clarified that the more age group susceptible and affected by T1DM is (12-17) with a percentage of 48%. While the age groups less than 5 years and a group (42-50) it is the little patients recoded with T1DM at percentage 1.33% and 2.67% respectively.

Table 1: distribution of the study sample based on age.

Age (Years)	Patients		Control		Total	P value
	N	%	N	%		
>5	2	1.33	6	6.67	8	0.1573
6 – 11	20	13.33	---		20	0.00001 **
12 – 17	72	48	12	13.33	84	0.00001 **
18 – 23	24	16	6	6.67	30	0.00102 *
24 – 29	18	12	42	46.66	60	0.00195 *
30 – 35	6	4	18	20	24	0.01431 *
36 – 41	4	2.67	---		4	0.0455 *
42 – 50	4	2.67	6	6.67	10	0.52709
Total	150	100	90	100	240	0.00011 **

* means significance differences (p<0.05) ** means high significance differences (p<0.001)

Many studies have addressed and supported the findings of the present investigation, which shows that T1DM was strongly correlated with age, particularly in adolescence and infancy as shown in table 1. According to a recent analysis by the Number of Patients with T1DM in 2017, there are an estimated 1,106,500 million young people (under 20) living with T1DM globally, which is twice as many as there were in 2015 [8]. Incidence was lowest in youngest (1-4) and oldest (15-19) age groups, and it was highest in children aged 5 to 14 years. The incidence increased most significantly in children who were 10 to 14 years old [9]. Similar to this, a 60-year review of data from the UK Biobank indicates that up to half of all incidence cases of T1DM were identified as adults. Because of the difficulty in identifying T1DM from T2DM requiring insulin therapy and the possibility that more than 20% of individuals with T1DM are also receiving insulin, incidence rates in adult populations are rarely reported [10]. Based on one of the recent analyses, Iraq revealed a high frequency of T1DM as well, with over 8,000 adolescents and children suffering in 2019. When compared to other middle eastern nations, a moderate increase in prevalence and incidence of T1DM has been seen [11]. T1DM was reported to affect adults more frequently than previously thought in Western countries [12]. Diabetes prevalence and incidence are rising globally, The IDF projected those 425 million

persons who were aged between 20 and 79 had diabetes in 2017 (All types). Even though T1DM has generally been referred to as "juvenile diabetes" and is thought to affect children, new research indicates that it may really affect adults more frequently than previously thought. About a quarter of people with T1DM are adults, and adults ≥ 20 make up more than a million (or 85%) of all type diabetes cases in the US [13].

Gender

The results of table (2) revealed high significant differences (P less than 0.001) in the patient's group between male and female, also showing there were high significant differences (P less than 0.001) between patients and controls groups and between females in both groups. Also, a significant difference (P less than 0.05) between males in both groups and between males and females in control group. As indicated in Figure (4-1) the percentage of females (64%) is higher than the percentage of males (36%) in T1DM.

Table 2: distribution of the study sample based on gender.

Gender	Patients N (%)	Control N (%)	Total	P value
Male	54(36%)	36(40%)	90(%)	0.05778 *
Female	96(64%)	54(60%)	150(%)	0.00061 **
Total	150(100 %)	90(100 %)	240(100%)	0.00011 **
P value	0.00061 **	0.05778 *	0.00011 **	
* means significance differences (p<0.05) ** means high significance differences (p<0.001)				

In the recent study table (2) showing clearly females were the most suffer to T1DM and observed in the figure (1) as showing in previous studies which most agree with current study. In comparison to men, women were noticeably more possibly to report using an insulin pump [14]. In the case of comparing the rates in the females and males in 2002 - 2003 compared to 2012 - 2013, it seems that an increase in the incidence has been higher in the male patients compared to it in the female patients. APC, on the other hand, has been higher in the females. It is significant to note that incidence that was reported reflected one year, while APC reflected the trends of the incidence throughout the 11-year period. Males had a consistently higher T1DM prevalence compared to the females [9].The study on a number of 75 participants, the majority of the participants were females was, as the number of females was reached 39, while the number of males was 36 [15]. Another study of 214 adolescents (aged 13 to 18) with the T1DM in the central regions of the Saudi Arabia revealed that the female gender, longer T1DM durations and numerous daily injections were associated with females [8] .

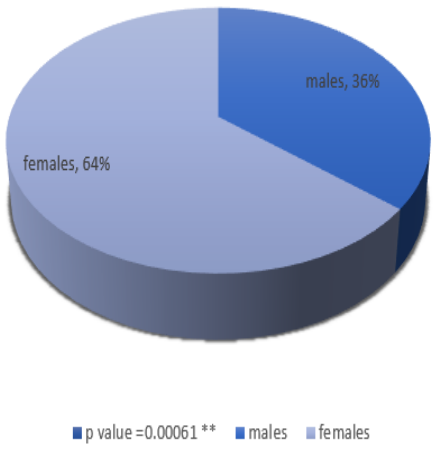

Figure 1. Comparison between gender of T1DM patients.

Length, Weight and Body Mass Index (BMI)

Based on the results of table (3), there have been insignificant decrease (P more than 0.05) in the length values in T1DM male's patients compared to controls, as the mean of length for T1DM male's patients and controls (1.495 and1.58) respectively. The results of the same table had revealed high significance decrease (P <0.001) for average of length between females in both groups (1.488 and 1.612) respectively. Also, high significance decrease (P less than 0.001) in mean of length between patients and control groups (1.491 and 1.599), respectively. Also showed no significant differences (p more than 0.05) between females and males in patient group control group.

The findings of the statistical analysis for weight, as showing in table (3), revealed a modest decrease (P more than 0.05) in the weight value in T1DM of male's patients compared to controls, as average of weight value for T1DM patients and controls (50.407 and 58.666), respectively. in the same table revealed significant decrease (P less than 0.05) of the mean of weight for T1DM of female's patients compared to controls (50.416 and 57.666), respectively. and decrease significantly (P less than 0.05) in groups of patients who have the T1DM compared to controls as the mean of weight value (50.413 and 58.066), respectively. Also showed no significant differences (p>0.05) between females and males in patient group control group.

The values of BMI for T1DM patients and the control (32.553 and 35.516, respectively) demonstrated a significant decrease (P less than 0.05) in mean BMI in comparison with control group. Additionally, there was a significant decline (P more than 0.05) in the mean BMI of female T1DM patients compared with the control group, with respect to both the BMI values for T1DM patients and the control group (33.451 and 35.69). Additionally, there were no significant differences (P more than 0.05) in either group's mean BMI for either gender or between group's mean BMI for men.

Table 3: data of body mass index with length and weight in control and T1DM patients.				
		Patients	Control	P value
		Mean ± SD(m)	Mean ± SD (m)	
Length	Male	1.495±0.221	1.58±0.335	0.1501
	Female	1.488±0.153	1.612±0.05	0.0001 **
	Total	1.491±0.181	1.599±0.214	0.0001 **
	P value	0.8198	0.4905	
Weight		Mean ± SD(Kg)	Mean ± SD (Kg)	
	Male	50.407±22.472	58.666±23.94	0.0997
	Female	50.416±16.492	57.666±6.419	0.0023 *
	Total	50.413±18.852	58.066±15.816	0.0014 *
	P value	0.9978	0.7707	
BMI		Mean ± SD (Kg/m)	Mean ± SD (Kg/m)	
	Male	32.553±11.467	35.516±9.677	0.2053
	Female	33.451±9.516	35.69±3.007	0.0950 *
	Total	33.128±10.267	35.62±6.498	0.0398 *
	P value	0.6076	0.9018	
* means significance differences (p<0.05) ** means high significance differences (p<0.001)				

According to a recent research, patients with T1DM have decreased weight, length, and BMI. DM is linked to numerous metabolic abnormalities within the body, such as indigestion of carbohydrates that results in weight loss and malnutrition [16]. Previous research also showed a reduction in BMI and weight. Women with T1DM had a markedly higher BMI than men did [17]. Female gender was connected with increased BMI, according to data from diabetes follow-up registry in Austria and Germany [14].

physiological parameters

Blood Group

Table (4) was showed there have been no significant differences (P more than 0.05) in blood group A and AB in both control and patient groups. Furthermore, high significance differences (P less than 0.001) were observed in blood groups B and O when compared between controls and patients with type I DM, and high significance differences (P less than 0.001) in all blood groups of controls and patients with type I DM, separately.

Table 4: The percentage of blood groups of the patients of TIDM and control group.				
Blood Groups	Patients	Control	Total	P value
A	47.5% 38 25.34%%	52.5% 42 46.66%	80	0.65472
B	81.25% 26 17.33%	18.75% 6 6.67%	32	0.00041 **
AB	70% 14 9.33%	30% 6 6.67%	20	0.07364
O	66.67% 72 48%	33.33% 36 40%	108	0.00053 **
Total	150	90	240	0.00011 **
P value	0.00001 **	0.00001 **	0.00001 **	
* Means significance differences (p<0.05) ** means high significance differences (p<0.001)				

According to table (4)'s findings, there were notable differences in the ABO group with T1DM, especially in groups O and B. Additionally, the results of a previous research revealed that blood group B was the most prevalent in Jodhpur City, succeeded by O, A, and AB in both females and males as well as general population. Additionally, the highest prevalence of diabetes has been discovered in blood group B, which was after that followed by O, A, and AB in both females and males as well as the general population [18]. According to the ABO blood type distributions, significantly more pancreatic cancer patients than control patients had been diagnosed with diabetes [19]. Results from the chi-square test indicated a negative or inverse relationship between the ABO blood groups and T1DM, and the O blood groups also revealed a negative relationship with DM, suggesting that those with the A and O blood types are less likely to develop T1DM. Yet, no connection between T1DM and blood types of AB and B was discovered. In 140 samples from healthy controls, blood group A has been the most common, followed by group A. Patients with T1DM had a high frequency of B blood group followed by the O blood group [20].

HbA1C

Table (5) showing there were high significant increase (P less than 0.001) in average of HbA1C in patients who have the T1DM compared with the control groups, HbA1C rate for the patients who have T1DM and the controls (10.345 & 5.206) respectively. As well as there was no significant differences (P more than 0.05) in both groups' patients and controls according to gender.

Table 5: percentage of HbA1c in Both patients with TIDM and control groups			
HbA1C	Patients	Control	P value
	Mean ± SD	Mean ± SD	
Male	10.1±2.062	5.283±0.466	0.0001 **
Female	10.483±2.649	5.155±0.247	0.0001 **
Total	10.345±2.462	5.206±0.354	0.0001 **
P value	0.3606	0.0935	
* means significance differences (p<0.05) ** means high significance differences (p<0.001)			

Results in table (5) revealed that diabetic patients' mean HbA1c values significantly increased compared to control group. In addition to that, when compared with the controls, patients who have T1DM had significantly higher levels of HbA1c, fasting glucose, and total cholesterol [21]; this finding is relevant to the current investigation. PPG, FPG, and HbA1c levels were greater in T1DM patients than in controls, as expected [22]. Women with T1DM exhibited higher HbA1c levels, BMI, HDL cholesterol, and a lower count of CD34+KDR+CD133+ as well as CD133+KDR+ than males did. Additionally, more women than men had obesity or overweight, smoking, thyroiditis, and sexual dysfunction [17].

Glucose

The concentration of glucose in T1DM patients was significantly very higher (P less than 0.001) than controls, according to the results of table (6) the mean of glucose for T1DM patients and controls is (222.464 and 101.4) mg/dl, respectively. In the same table also indicated insignificant reduction (P more than 0.05) in males' comparison to females in patients' group (217.74 and 225.12) mg/dl and in controls group (100.166 and 102.222) mg/dl, respectively.

Table 6: Concentration of glucose in both patients with TIDM and control groups

Glucose	Patients	Control	P value
	Mean ± SD (mg/dl)	Mean ± SD (mg/dl)	
Male	217.74±119.585	100.166±12.632	0.0001 **
Female	225.12±103.272	102.222±8.667	0.0001 **
Total	222.464±109.451	101.4±10.722	0.0001 **
P value	0.6922	0.3619	

* means significance differences (p<0.05) ** means high significance differences (p<0.001)

In current study the results have shown an increase in glucose levels in patients relative to controls subjects, which is consistent with many previous studies. In some study that supports current results the ratio of glucose concentrations in patients to healthy subjects was very high according to the statistical analysis (92.30± 3.28 control, 393.3±46.81 patients) [23]. Low levels of blood insulin and high levels of the blood glucose (i.e., hyperglycemia) are also linked to T1DM. Conversely, diabetes is characterized by high FBG (FPG ≥ 126 mg/dL). Even though the treatment with curcumin and chitosan-encapsulated curcumin left the diabetes state with persistent FBG issues, they were greatly reduced [24]. Hyperglycemia in the blood, which results from inadequate pancreatic insulin secretion or insufficient insulin-directed glucose uptake by target cells, is the primary sign of DM [2]. A lengthy latency period, which reflects the huge number of functional β cells which must be eliminated prior to the clinical manifestation of the disease, precedes the onset of symptomatic hyperglycemia [4]. According to data from diabetes follow-up registry in Austria and Germany, women were more likely to have poor glycemic control, an increased total cholesterol, BMIs, and LDL cholesterol [14].

(Malondialdehyde) MDA

Table (7) displays the results of the MDA levels in the control and patient. In the case when put to comparison with control group, there has been a significant increase (P less than 0.050) in concentration of MDA for patients with T1DM; the concentrations for male patients with T1DM and the control are (12.135 and 7.991) nmol/mL, respectively. shows a significant increase (P more than 0.05) in concentration of MDA for female T1DM patients as compared to the control groups, with respective concentrations of (11.51 and 9.343) nmol/ml for both groups. Moreover, there is a significant difference (p less than 0.05) between MDA concentrations of the T1DM patients and the control groups (11.735 and 8.802 nmol/ml, respectively). Lastly, both the control and patient groups did not significantly differ in the concentration of MDA between females and males.

Table 7: Concentration of MDA in both patients with TIDM and control groups

MDA	Patients	Control	P value
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	12.135±7.985	7.991±4.274	0.0054 *
Female	11.51±8.685	9.343±4.791	0.0926 *
Total	11.735±8.418	8.802±4.615	0.0026 *
P value	0.6640	0.1747	

* Means significance differences (p<0.05) ** means high significance differences (p<0.001)

In the case when put to comparison with control groups, the data in table (7) showed a substantial rise in MDA concentration in the patient groups. These findings are consistent with certain research that suggested a rise in MDA levels. A research that included 40 children who have the T1DM and 40 healthy controls found that the level of MDA (a measure of lipid peroxidation) that indicates an increase in the oxidative stress in the diabetics as it has been described in other studies, was significantly higher in the children with T1DM [25]. MDA concentrations have been considerably higher in the blood of T1DM individuals than the corresponding control group, according to research by Alghazeer et al. (2018). Additionally, diabetic males' levels of NO and MDA were marginally (but not substantially) higher than diabetic females' levels; these findings are consistent with recent findings. According to other research, the lipid peroxidation biomarker (MDA) has been considerably higher in both groups

(patients who have existing T1DM and patients with newly diagnosed T1DM) in comparison with the healthy group, which explains why serum MDA is likely connected with T1DM [26]. Even in people with well-controlled diabetes, dyslipidemia seen in the diabetes might have a significant effect on systemic inflammation through altered oxidative metabolism, resulting in increased lipid peroxidation (MDA) [27]. Throughout the initial observation period, MDA was positively correlated with poorer diabetes control as measured by a higher HbA1c, which is consistent with prior results [28].

Glutathione (GSH)

In table (8)'s GSH data, it was discovered that the concentration of GSH was significantly lower (P less than 0.05) in male T1DM patients as compared to the control group; concentrations were (7.745 and 10.961 nmol/mL, respectively). GSH concentrations in patients who have T1DM, and the control group are (7.045 and 9.243) nmol/ml, respectively, with a significant drop (P less than 0.05) in GSH concentration in female patients when compared to control groups. Additionally, there is a highly significant drop (p less than 0.001) in GSH concentration in T1DM patients in comparison to control group, with values of (7.325 and 9.861) nmol/ml, respectively. Finally, there are no discernible changes in the GSH concentration between the patient groups of male and female (p more than 0.05).

Table 8: Concentration of GSH in both patients with TIDM and control groups

GSH	Patients	Control	P value
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	7.745±1.627	10.961±6.047	0.0025 *
Female	7.045±4.433	9.243±3.94	0.0021 *
Total	7.325±3.586	9.861±4.857	0.0001 **
P value	0.3673	0.0371 *	
* Means significance differences (p<0.05) ** means high significance differences (p<0.001)			

The concentration of GSH in T1DM patients was significantly lower in this work; the findings have been consistent with previous investigations that also found a reduction in GSH concentration. Children with T1DM, particularly those with poorly controlled cases, showed low serum GSH that was statistically substantially lower in diabetic group than in the controls, which may have an impact on the erythrocyte GSHGPx system [29]. In a different study, Malik et al. (2018) described their findings in the following way: In T1DM patients with illness duration five years compared with disease duration, the levels of decreased GSH have been lower and the levels of catalase and superoxide dismutase have been higher. Children who have T1DM had considerably lower SOD and glutathione levels in comparison with their respective values in controls, whereas MDA levels have been considerably higher in the children who are T1DM in comparison to the control group, according to recent results that were also similar results for GSH observed in a study that has been conducted by El Amrousy et al (2020). The level of GSH has been observed to be significantly decreased in the diabetic men. GSH, on the other hand, was only elevated in diabetic females [30].

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