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The impact of oxidative stress and some endogenous antioxidants on type 1 diabetes mellitus

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ABSTRACT

Type 1 diabetes is one of the autoimmune diseases and is characterized by the destruction of beta cells in the pancreas, which leads to a complete loss or deficiency of the insulin hormone. This breakdown in beta cells is the result of several environmental, genetic, and oxidative factors are considered among the factors accused of destroying beta cells, so this study is designed to evaluate the effect of some oxidative factors in insulin dependent diabetes patients. The study includes 80 patients with type 1 diabetes compared with 40 healthy individuals as control group. The results showed a significant increase in the levels of total oxidant status (TOS) and malondialdehyde (MDA) and Transferrin in diabetic patients and a significant decrease in the levels of superoxide dismutase (SOD) and glutathione reductase (GR) comparing with healthy control. The study also noted over expression (folding change) for COX 7B in T1DM patients. The study concludes that oxidative status and enzymatic and non-enzymatic endogenous antioxidants play an important roles in the breakdown of beta cells, and thus it is one of the suggested factors responsible for developing type 1 diabetes mellitus.

KEYWORDS: Type 1 diabetes mellitus; TOS; MDA; SOD; GR; COX 7B gene

INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) is one of the most common chronic autoimmune diseases in children. The disease is characterized by the destruction of β cells, leading to hyperglycemia, and to a lifelong insulin-dependent state. Diabetes mellitus is found in every population in the world and in all regions, including rural parts of low- and middle-income countries. The number of people with diabetes is steadily rising (World Health Organization; 2016). Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. It occurs in human cells when the obstetrics of reactive oxygen species (ROS) increases and crushes the body's antioxidant ability and defenses. Damage in lipids, carbohydrates, proteins and nucleic acids will occur if the free radicals are not removed by the antioxidants forces (Phaniendra, A. *et al*, 2015). There is rising clue which have linked oxidative stress to a diversity of pathological cases, included cancer, cardiovascular diseases, chronic inflammatory disease and diabetes mellitus (DM) (El Faramawy, S. M., & Rizk, R. A. 2011). It has been announce of oxidative stress participation in the development and pathogenesis of diabetic complications. This involves weakness of insulin action and increased in the complication appearances (Ceriello, A., 2006). Therefore, the total oxidant status (TOS) and malondialdehyde (MDA) is usually used to estimate the overall oxidation

state of the body (Erel, O., 2005; Nair, V., *et al,* 2008). Malondialdehyde (MDA) is the steady, terminal product of lipid peroxidation, and this product produce through the decay of polyunsaturated fatty acids. It is the better indication for tissues damage by free radical and oxidative stress (Lavie, C. J., & Milani, J. N., 2011). Antioxidants are chemical or biological factors that capable to depart the potentially harmful action of free radicals such as reactive nitrogen species (RNS) and reactive oxygen species (ROS). The oxidation process of other macromolecules is avoided or retarded, by antioxidants. The damaging impact of free radicals in cells is decreased or finished by antioxidants (Mariyam, S., *et al,* 2020). The first group of these antioxidants is intracellular enzymes such as superoxide dismutase (SOD) and glutathione reductase (GR) (Fang, Y. Z. *et al,* 2002). SOD is considered a first-line defense against ROS. This enzyme is present in nearly all cells, and converts $\bullet O_2^-$ into H_2O_2 (Ighodaro O. M. and Akinloye O. A., 2018). Glutathione reductase (GR) which else called glutathione-disulfide reductase (GSR) is an enzyme that stimulates the lowering of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a susceptible molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Forman, H. J. *et al,* 2009). The second group is intracellular non-enzymatic antioxidant which known as made-up antioxidants or dietary complements and the body complexes antioxidant system is influenced by dietary absorption of antioxidant vitamin and mineral such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione (Pierce, J. D., *et al.,* 2004). Glutathione's (GSH) and bilirubin are prominent endogenous antioxidant cytoprotectant (Sedlak, T. W., *et al.,* 2009). Iron-binding proteins, transferrin and ferritin also play a role in antioxidant defense by preventing the catalyzation of free radicals through chelation (Shkolnik, K., *et al.,* 2011). Cytochrome c oxidase (COX), the terminal component of the respiratory chain complex of most aerobic organisms, is composed of 13 subunits in mammals. Three COX subunits are encoded by mitochondrial DNA, and 10, including COX 7B, are encoded by nuclear DNA (Sedlak, T. W., *et al.,* 1993), that functions as an electron-driven proton pump and plays fundamental roles in eukaryotic cell respiration and aerobic energy production (Soto, I. C. *et al.,* 2012). The aims of this study is investigate the effects of oxidative stress and antioxidants factors on T1DM.

MATERIALS AND METHODS

Study groups: The current study was conducted on 80 patients (48 males and 32 females). The patients were diagnosed clinically by physicians as having type 1 diabetes mellitus (T1DM). Diagnosis of T1DM cases were carried out according to the treating physician and symptoms (i.e. ketoacidosis, polyuria, polyphagia, polydipsia, or rapid weight loss with marked hyperglycemia) supported by evaluation of HbA1C level which must be greater than 6.5% percent on two separate tests; the presence of ketones in the urine and/or autoantibodies in the blood can distinguish type 1 from type 2 diabetes (Kahanovitz, L. *et al,* 2017). The control group consist of 40 apparently healthy individuals (25 male and 15 female) who had no pathological state at time of this study.

Blood sample collection: We collected five ml of blood from both groups. Each sample was divided into two portions:

1- Two ml of blood in anticoagulant (EDTA) tube for molecular study and immediately store at –20°C until use, 250 µl of blood tube was added in Trizol tube for RNA extraction and kept in deep freeze until used.
2- Three ml of blood in the gel tube and allow the sample to clot for a few minutes at room temperature, followed by serum separation from the clot by centrifugation for 15 minutes at 1000 g. Then the serum was divided into several eppendroff tubes, labeled and stored at -40 ˚C one for, TOS, MDA, SOD and GR ELISA assay (SinoGeneClon, China) procedure and for bilirubin and transferrin.

Oxidative and antioxidants factors study: The concentration of TOS, MDA, SOD or GR in the samples were estimated by using ELISA assay. Transferrin was performed by percentage Iron /Total Iron Binding Capacity (TIBC) depend on Human (Germany) kit assay at (595) nm. Bilirubin was performed depend on kit assay procedure Biolabo laboratories (France) and the absorbance was measured at (550) nm.

Molecular study: Total RNA of all samples was extracted using the AccuZol kit and the protocol provided by the manufacturer. Total RNA was reversely transcribed to complementary DNA (CDNA) using (CDNA kit, CMHTOJI Company / Russia). The procedure was carried out in a reaction volume of 25 ul according to the manufacturer's instructions. The expression levels of COX 7B gene estimated by Quantitative Real Time PCR (RT-qPCR). To confirm the expression of target gene, RT-PCR EVA Green assay was used. The mRNA levels of endogenous control gene β Actin were amplified and used to normalize the mRNA levels of COX 7B gene. Primer that used for RT-qPCR is listed in table (1).

Table 1. Primers Sequences Used in RT-qPCR

Gene	**Primer**	Sequence (5′→3′ direction)	References
COX 7B	**Forward**	**5′- CGT CTC CAA GTT CGA AGC ATT -3′**	**(Reynier F., *et al,* 2010)**
	Reverse	**5′- ACT TGT GTT GCT ACA TAT GTC C -3′**	
β-actin (Referenc e gene)	**Forward**	**5′ -CTG GAA CGG TGA AGG TGA CA -3′**	**(Panarina, M. *et al.*, 2014)**
	Reverse	**5′ -CGG CCA CAT TGT GAA CTT TG -3′**	

Statistical Analysis: Statistical analyses were done using GraphPad Prism software version 8.4.3. (Statistical Package for Social Sciences) in association with Microsoft Excel 2013 and social science statistics. An estimate was considered statistically significant Kits P value was less than the level of significance of 0.05. Gene expression was calculated using $2^{-\Delta\Delta ct}$ method.

RESULTS

In present study, the mean serum concentrations of TOS and MDA were significantly higher ($P<0.05$) among T1DM patients (26.10±2.44 pg/ml and 2.14±0.14 mmol/L respectively)

compared to healthy individuals (16.62±2.46 pg/ml and 1.29±0.13 mmol/L respectively), (table 2). According to gender, there wasn't any significance differences between mean serum concentration in male and female patients for each of TOS and MDA (28.10±3.00 pg/ml and 2.03±0.16 mmol/L respectively) and (34.20±4.40 pg/ml and 2.38±0.26 mmol/L respectively). According to family history of patients, the mean serum concentration of TOS and MDA had no significant differences (P>0.05) between patients with positive family history (40.00±18.00 pg/ml and 2.86± 0.88 mmol/L respectively) and negative family history patients (22.40± 2.90 pg/ml and 2.19± 0.17 mmol/L respectively), also there was no significant differences in the level of serum concentration in TOS and MDA among T1DM patients according to duration of injury groups, ≤10 years group (30.90±6.60 pg/ml and 2.38±0.30 mmol/L respectively) compare with ≥11 year group (30.30± 11.00 pg/ml and 2.43± 0.79 mmol/L respectively).

Table 2: The serum concentrations of oxidative parameter (TOS and MDA) in T1DM patients

Subject	TOS (pg/ml)				MDA (mmol/L)			
	T1DM patients		Healthy control		T1DM patients		Healthy control	
Mean ± SE	26.10±2.44		16.62±2.46		2.14±0.14		1.29±0.13	
SD	46.30		11.30		2.22		0.69	
P- value	0.0316*				0.0001*			
According to gender								
	Male patients		Female patients		Male patients		Female patients	
Mean ± SE	28.10±3.00		34.20±4.40		2.03±0.16		2.38±0.26	
SD	15.6		18.2		1.01		1.23	
P- value	0.266**				0.263**			
According to age groups								
Age range (Year)	1-10	11-20	21-30	31-40	1-10	11-20	21-30	31-40
Mean ± SE	32.52± 2.20	33.20± 8.60	22.90± 4.30	42.20± 7.40	2.718± 0.36	2.60± 0.42	2.021± 0.18	2.628± 0.26
SD	3.76	54.50	14.30	12.8	0.721	2.68	0.641	0.456
P- value	0.407**	0.011*	0.010*	0.042*	0.110**	0.001*	0.001*	0.030*
According to family history								
	Positive family history		Negative family history		Positive family history		Negative family history	
Mean ± SE	40.00±18.00		22.40±2.90		2.86±0.88		2.19±0.17	
SD	78.3		19.3		3.75		1.13	
P- value	0.359**				0.464**			
According to duration of injury								
	≤10 years		≥11 years		≤10 years		≥11 years	
Mean ± SE	30.90±6.60		30.30±11.00		2.38±0.30		2.43±0.79	
SD	47.7		22.6		2.27		1.57	
P- value	0.964**				0.954**			

*= Significant (P<0.05); **=Non Significant (P>0.05); SD= Standard Deviation; SE= Standard Error

In the current research the mean serum concentrations of SOD and GR were significantly lower (P<0.05) among patients with T1DM (15.91± 1.10 U/mL and 296.90± 13.00 pg/ml respectively) compared to healthy individuals (23.20± 3.10 U/mL and 433.10± 18.00 pg/ml respectively) table (3). In addition, there wasn't any significance difference between male and female patients mean serum concentration for SOD (20.50± 2.70 and 20.10± 3.80 U/mL respectively). The same results showed no significant difference between first age group and last group of T1DM patients (14.62 ±1.90 and 15.55±2.60 U/mL respectively) in compare with control group. In contrast the (11-20 year and 21-30 year) showed significant differences when compared with healthy control. Statistical analyses showed that GR serum concentration in all age groups of patients with T1DM were significantly different when compared with healthy control of same age range (P<0.05). The mean serum concentration of SOD and GR for T1DM patients had no significant differences (P>0.05) between positive family history (22.30 ± 5.60 U/ml and 352.00 ±76.00 pg/ml respectively) and negative family history patients (18.50 ± 2.00 U/ml 296.00± 16.00 pg/ml respectively), also there was no significant differences in the mean serum concentration of GR among T1DM patients have duration of injury less than ≤10 years (293.60 ± 13.00 pg/ml) than patients have duration of injury more than ≥11 years (342.1± 42.00 pg/ml). However, there was significant differences for SOD serum concentration between ≤10 years (21.00 ± 2.40 U/mL) and ≥11 year (13.06± 1.00 U/mL) among duration of injury in T1DM patients.

Table (3): The serum concentrations levels of enzymatic antioxidants parameters (SOD and GR) in T1DM patients

Subject	SOD (U/mL)				GR (pg/ml)			
	T1DM patients		Healthy control		T1DM patients		Healthy control	
Mean ± SE	15.91± 1.10		23.20± 3.10		296.90± 13.00		433.10± 18.00	
SD	7.91		14.50		97.90		88.10	
P- value	0.034*				0.0001*			
According to gender								
	Male patients		Female patients		Male patients		Female patients	
Mean ± SE	20.50± 2.70		20.10± 3.80		272.40± 14.00		336.00 ± 22.00	
SD	16.80		17.80		89.00		102.00	
P- value	0.927**				0.019*			
According to age groups								
Age range (Year)	1-10	11-20	21-30	31-40	1-10	11-20	21-30	31-40
Mean ± SE	14.62 ±1.90	14.90 ± 1.3	13.90± 0.79	15.55 ±2.60	306.0± 24	305.00 ±17	274.60 ±25	241.70 ± 49
SD	4.58	7.35	2.22	4.57	58.1	106	91.7	84.4
P- value	0.56**	0.04*	0.04*	0.70**	0.008*	0.028*	0.001*	0.04*
According to family history								

	Positive family history	Negative family history	Positive family history	Negative family history
Mean ± SE	22.30 ± 5.60	18.50 ± 2.00	352.00 ±76.00	296.00± 16.00
SD	20.9	13.0	312	107.00
P- value	0.526**		0.479 **	
According to duration of injury				
	≤10 years	≥11 years	≤10 years	≥11 years
Mean ± SE	21.00 ± 2.40	13.06± 1.00	293.60 ± 13.00	342.1± 42.00
SD	17.60	2.09	98.70	84.30
P- value	0.004*		0.353**	

*= Significant (P<0.05); **=Non Significant (P>0.05); SD= Standard Deviation; SE= Standard Error

The mean serum concentrations of transferrin was significantly higher (P<0.05) among patients with T1DM (0.17± 0.005 mg/dl) compared to healthy individuals (0.14± 0.011 mg/dl), opposite to bilirubin was significantly lower (P<0.05) among patients with T1DM (0.357 ± 0.028 mg/dl) compared to healthy individuals (0.452 ± 0.035 mg/dl). According to gender, there wasn't any significance differences between male and female patients mean serum concentration for bilirubin. Table (4) showed that, there were a significant differences (p<0.05) in males and females for transferrin in T1DM patients, (0.389 ± 0.042 and 0.322 ± 0.028 mg/dl respectively) when compare with each other.

The mean serum concentration of transferrin and bilirubin according to family history for T1DM patients had no significant differences (P>0.05) between positive family history (0.1754± 0.0092 and 0.376 ± 0.033 mg/dl respectively) compared to negative family history patients (0.1725± 0.0060 and 0.359± 0.037 mg/dl respectively), also there was no significant differences in the mean serum concentration (P>0.05) of transferrin and bilirubin among T1DM patients according to duration of injury groups, ≤10 years group (0.1738± 0.0054 and 0.359± 0.030 mg/dl respectively) compare with ≥11 year group (0.1676± 0.0061 and 0.420± 0.086 mg/dl respectively).

Table (4): The serum concentrations levels of non-enzymatic antioxidant parameter (transferrin and bilirubin mg/dl) in T1DM patients

Subject	Transferrin (mg/dl)		Bilirubin (mg/dl)	
	T1DM patients	Healthy control	T1DM patients	Healthy control
Mean ± SE	0.17± 0.005	0.14± 0.011	0.357 ± 0.028	0.452 ± 0.035
SD	0.04	0.05	0.214	0.176
P- value	0.009*		0.038*	
According to gender				
	Male patients	Female patients	Male patients	Female patients
Mean ± SE	0.17± 0.006	0.17± 0.008	0.389 ± 0.042	0.322 ± 0.028
SD	0.0386	0.0416	0.257	0.135
P- value	0.622**		0.183**	
According to age groups				

Age range (Year)	1-10	11-20	21-30	31-40	1-10	11-20	21-30	31-40
Mean ± SE	0.17 ± 0.014	0.17 ± 0.0059	0.17± 0.014	0.20± 0.021	0.233± 0.042	0.322± 0.025	0.462± 0.055	0.767± 0.35
SD	0.033	0.04	0.05	0.04	0.103	0.157	0.198	0.603
P- value	0.01*	0.006*	0.878**	0.169**	0.125**	0.110**	0.980**	0.529**
According to family history								
	Positive family history		Negative family history		Positive family history		Negative family history	
Mean ± SE	0.1754± 0.0092		0.1725± 0.0060		0.376 ± 0.033		0.359 ± 0.037	
SD	0.0391		0.0401		0.135		0.246	
P- value	0.797**				0.727**			
According to duration of injury								
	≤10 years		≥11 years		≤10 years		≥11 years	
Mean ± SE	0.1738± 0.0054		0.1676± 0.0061		0.359± 0.030		0.420± 0.086	
SD	0.0410		0.0137		0.223		0.192	
P- value	0.458**				0.532**			

*= Significant (P<0.05); **=Non Significant (P>0.05); SD= Standard Deviation; SE= Standard Error

The results showed an over expression (folding change) for COX 7B in T1DM patients (4.80) when compared to the healthy control group (1.00) (table 5). On the other hand, the levels of COX 7B gene expression reported a high significant increase in females (4.05) when compared with males T1DM patients (2.46). The gene expression of COX 7B in age groups (1-10 year, 11-20 year, 21-30 year and 31-40 year) for T1DM patients (1.80, 3.63, 6.57 and 14.20 respectively) reported a significant increase when compared with healthy control (1.00).

Moreover, the overexpression of COX 7B are appeared in patients with familial T1DM (2.11) and with patients without family history (2.30) when compare with healthy control (1.00), moreover, the patients with negative family history showed a slight increase in gene expression than the patients with positive family history. The gene expression of COX 7B was significantly higher mRNA expression in patients who have duration of injury (**≤10 years** and **≥11 year)** (**4.50** and **6.27** respectively) compared to control group (1.00).

Table (5): mRNA expression of COX 7B in T1DM patients and control subjects.

subjects	Mean CT(gene)	Mean CT(actin B)	Mean ΔCT(test)	Mean ΔCT(control)	Mean ΔΔCT	Mean fold change ($2^{-\Delta\Delta CT}$)
COX 7B						
Patient	18.83	21.62	-2.80	-0.53	-2.26	4.80
Control	21.09	21.62	-0.53	-0.53	0.00	1.00
According to gender						
Male	18.77	21.62	-2.85	-1.55	-1.30	2.46
Female	20.99	21.62	-0.63	1.39	-2.02	4.05

According to age groups (years)						
1-10	19.31	21.62	-2.31	-1.46	-0.85	1.80
11-20	18.91	21.62	-2.71	-0.86	-1.86	3.63
21-30	18.81	21.62	-2.81	-0.09	-2.72	6.57
31-40	18.57	21.62	-3.05	0.78	-3.83	14.20
According to family history						
Positive	20.01	21.62	-1.61	-0.53	-1.08	2.11
Negative	19.89	21.62	-1.73	-0.53	-1.20	2.30
According to duration of injury						
≤10 years	18.92	21.62	-2.70	-0.53	-2.17	4.50
≥11 year	18.44	21.62	-3.18	-0.53	-2.65	6.27

DISCUSSION

The present study conducted to determine the role of oxidative stress in T1DM patients (TOS, MDA and COX 7B gene). The results in table (2) showed that TOS is increased in T1DM patients compared to healthy control and this findings in consistent with several previous studies (Beyazyıldız, E. *et al,* 2013; Baser, H. *et al,* 2016; Tabur, S. *et al,* 2016).

Several research have showed that oxidative stress leads to damage of pancreatic islets, by necrosis or apoptosis of ß cells (Bonnefont-Rousselot, D., 2002). In fact, diabetic patients are exposed to elevated oxidative stress because of some mechanisms, involving glucose autoxidation and non-enzymatic protein glycation (Amina, B. S. G. *et al,* 2015). Non-enzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Shiff bases and more stable products are formed. Advanced glycation end products (AGEs) are then formed through oxidative reactions and cause irreversible chemical modifications of proteins (Amina, B. S. G. *et al,* 2015).

Moreover, another oxidative stress parameter was studied. Malondialdehyde (MDA) is the steady, terminal product of lipid peroxidation, and this product is produced through the decay of polyunsaturated fatty acids. It is the better indication for tissues damage by free radical and oxidative stress (Lavie, C. J., and Milani, J. N., 2011). High lipid peroxidation production in diabetic patients are of private benefit due to their hyperlipidemia is believed to be a high risk factor for the development of vascular complications. (Martín-Gallán, P., *et al,* 2003; Karam, I., *et al,* 2015).

In table (2), our data showed significant increase in level of MDA in T1DM patients in compare with healthy control, These results are in line with past studies (Varvarovská, J., *et al.,* 2003; Amina, B. S. G. *et al*, 2015; Mishra, A., *et al*, 2016; Kiran, B. S., *et al*, 2016).

The cleared increase in Malondialdehyde release might be referred to the increase in peroxidative damage to lipids from oxidative stress developed through diabetes (Kiran, B. S., *et al,* 2016). In general, increased of reactive oxygen species molecules led to the cellular macromolecules spoilage such as lipids, proteins and nucleic acids (Cabiscol, E., *et al,* 2000). Our results have found that TOS and MDA serum concentrations which are the most important marker of oxidative stress, were elevated significantly in male and female diabetic patients when compared with

healthy control. This obviously show that regardless of the sex, diabetic patients were exposed to elevated oxidative stress through lipid peroxidation which were in parallel with previous results (Waggiallah, H. and Alzohairy, M., 2011; Taher, M. A. and Nassir, E. S., 2011; Kumawat, M., *et al,* 2013).

Although that there is slight increase in the mean serum concentration of both TOS and MDA in female, but there is no significant differences ($p>0.05$) between male and female when compared with each other. This current results were in line with Brunelli, E. *et al,* (2014) which found that females present a medium state of oxidative stress that is significantly higher compared to males. In contrast, other study reported that in vivo biomarkers of oxidative stress were elevated in young male than in female of matched age (Ide, T., *et al*, 2002). In similar, it was noted that reactive oxygen species production was increased in the vascular cells from mens than in the cells from womens (Matarrese, P., *et al*, 2011). In addition, clinical and experimental data suggested a greater antioxidant potential in females over males (Bhatia, K., *et al,* 2012). These studies indicate that there is an apparent association between gender and oxidative stress, where women seem to be less susceptible to oxidative stress. Also Kander, M., *et al.,* (2017), found that gender is connected with differences in oxidative stress. Females appear to be less susceptible to oxidative stress. This may be because of the antioxidant properties of estrogen.

In the present study, there are a significant differences in the mean of serum concentration of TOS and MDA according to age groups ($p<0.05$) when compare with healthy control of matched ages, except that in (1-10 years) group and these results agree with several past studies (Domínguez, C. *et al*, 1998; Martín-Gallán, P., *et al*, 2007; Amina, B. S. G. *et al*, 2015; Pinchuk, I. *et al,* 2019).

Domínguez C. *et al*, (1998), in his study on children and adolescent found that Malondialdehyde and protein carbonyl group levels in plasma were progressively higher in diabetic children and adolescents than in control subjects ($P < 0.0001$). Pilar Martín-Gallán, P., *et al*, (2007), found that T1DM patients had higher oxidative damage to lipids, proteins and DNA showed by analysis of plasma and erythrocyte Malondialdehyde, carbonyl proteins and leukocyte 8-hydroxy-deoxyguanosine, all of which were significantly elevated at beginning, decreased through the first 1.5 years of development, and rose gradually after that. The level of lipid in plasma were highly joined with lipid and protein oxidation products. Furthermore, other study elucidated the increased concentrations of plasma MDA, eight days after clinical beginning of diabetes when metabolic control had returned to normal; this proposes that oxygen free radicals may previously have exerted their cytotoxic effects in the early clinical stage of the disease (Dominguez, C., *et al*., 1998). Moreover, in children and adolescents with T1DM, MDA levels continued to increase over the course of the disease, marked high production of free radicals and leading to lipid peroxidation and cell oxidative injury, which is considered by some authors to be related to the development of diabetic complications (Wolff, 1994; Amina, B. S. G. *et al*, 2015).

In current research, there is no significant differences in levels of TOS and MDA in patients with familial T1DM in compared with patients who not have family history ($p>0.05$). For duration of exposure to diabetes type 1, the TOS and MDA showed no significant differences when compared groups ($\leq$10 years and $\geq$11 year) with each other's. These results for family history and duration

of exposure, may give us view that there are no relationship between oxidative parameters with family history and duration of injury.

The research results reported that mRNA gene expression for COX 7B gene in T1DM patients were up regulated in compare with healthy control table (5).These results agree with Basu, S., *et al,* (2005) who found that both COX- and cytokine-mediated inflammatory pathways are significantly associated to type 1 diabetes. Also, these results in line with Huang, C., *et al,* (2006) and Reynier, F., *et al,* (2010). Intracellular reactive oxygen species are generated over the oxidative phosphorylation pathway by the mitochondrial electron transport chain in response to hyperglycemia (Brownlee M., 2001). The increase in glucose levels may result in high production of electron donors in the citrate cycle, which in turn creates a high potential in the mitochondrial membrane (Brownlee, M., 2005). It has been suggested that once a certain threshold is overtaken, high membrane potential suppresses electron transport at mitochondrial respiratory chain complex III and elevates the half-life of superoxide-generating intermediates such as ubisemiquinone (Du, X. L. *et al,* 2001). Subsequently, as a result of total high gene expression in the oxidative phosphorylation pathway, it is probable that mitochondrial membrane potential may exceed this threshold, resulting in decreased electron transport at respiratory chain complex III and high production of superoxide and reactive oxygen species (Huang, C., *et al,* 2006).

The results of this study showed high gene expression of male and female of T1DM patients in compare with healthy control. But mRNA gene expression of female patients was higher than male patients. In fact, no studies have deal with this subject. Because of an elevated in mitochondrial area seen with transmission electron microscopy was reported in endothelial cells of women with T1DM (Cester, N., *et al,* 1996; Sivitz, W. I., and Yorek, M. A., 2010). This give the evidence for overexpression in COX 7B gene of T1DM female patients in compare with T1DM male patients. Table (5), showed the significant differences between age groups of T1DM patients when compare with healthy control. Also, there are clear differences between age groups when compare with each other. The gene expression rises with progression of age in T1DM patients for unknown reasons. This may be back to the hyperglycemia that accumulate the oxidative stress and lead to mutation in COX 7B gene and cause overexpression, also for inappropriate treatments.

COX 7B gene showed no significant differences between T1DM patients with family history and T1DM patients without family history.

According to duration of injury, there is a clear differences between the two groups of our study, ≥11 year group indicated higher gene expression in COX 7B than other group. These result disagree with result of Basu, S., *et al,* (2005), who was studied the relationship between the COX and cytokine mediated inflammatory responses in T1DM and found that no relation was appear between the duration of DM and the inflammatory biomarkers or metabolic measurements. The expression of COX 7B gene according to duration of injury increases with the progression of disease and cause accumulation of ROS and gradually the complication is appear.

Our results showed no significant difference according duration of injury of two groups of T1DM patients in mean of serum concentration of SOD when compared with two groups, in opposite to GR had a significant differences.

This study shows a significant correlation between serum level of transferrin and bilirubin with T1DM. Higher serum concentration of transferrin in T1DM agree with the results of Ellervik, C. *et al,* (2011) who indicated that elevated transferrin saturation confers a two- to threefold increased risk of developing any form of diabetes, as well as type 1 and type 2 diabetes separately. But these results disagree with van Campenhout, A., *et al,* (2003), who suggested that lower transferrin concentration and its glycation can, by enhancing the pro-oxidant effects of iron, contribute to the increased lipid peroxidation observed in diabetes. Bilirubin, a posterior lowering product of biliverdin, also has useful impacts in terms of the prevention of diabetes mellitus and its complications. Additionally, bilirubin was likewise reported to supply preservation against metabolic syndrome, and to be negatively related with overweight and obesity. Bilirubin serum concentration negatively significant in T1DM patients with healthy control. In addition, age groups for bilirubin level have no significant differences between age groups and healthy control groups from same age ranges.

CONCLUSION

The study concludes that oxidative status and enzymatic and non-enzymatic endogenous antioxidants play an important roles in the breakdown of beta cells, and thus it is one of the suggested factors responsible for developing type 1 diabetes mellitus.

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