Research Article

Evaluation the effects of insulin on oxidant/antioxidant status in type 1 diabetic patients

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Abstract

Earlier works have revealed increased generation of reactive oxygen species (ROS) and decreased antioxidant levels in type 1 diabetes mellitus (T1DM). The current study aimed to investigate the effect of mixed insulin therapy on oxidative stress and antioxidant status in patients with T1DM. This study involved 75 participants who were divided into three groups: 20 healthy subjects as a control, 25 newly diagnosed patients with T1DM (without treatment) and 30 patients with T1DM treated with insulin (regular and Human Neutral Protamine Hagedorn (NPH)) twice daily. Fasting serum glucose (FSG), serum concentrations of insulin, malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH), and vitamins (C and E) were measured in all participants. Compared with the healthy control, serum glucose and MDA concentrations were observed to be significantly higher, while significantly lower concentrations of CAT, GSH, and vitamins (C and E) were found in both the treated and untreated diabetic groups. Although insulin therapy caused a significant decrease in blood sugar with a marked elevation in the levels of insulin, CAT, GSH and vitamin E compared to the untreated patients, the changes in the levels of MDA and vitamin C between diabetic groups were not significant. Moreover, the level of insulin resistance was significantly increased in insulin-treated patients as compared to the control and untreated diabetic groups. In conclusion, twice daily treatment with regular and NPH insulin can ameliorate hyperglycemia and improve antioxidant levels in patients with T1DM. However, the insulin regimen used in this study did not reveal a beneficial effect on oxidative stress and insulin resistance. Hence, exogenous antioxidants (vitamins C and E) can be used in combination with insulin to control these parameters.

Keywords

Introduction

Type 1 diabetes mellitus (T1DM) is a long-term metabolic disease marked by hyperglycemia and lower levels of insulin due to pancreatic β-cell dysfunction (Simmons and Michels 2015). Although numerous risk factors are associated with the development of T1DM, oxidative stress seems to have an essential role in the development of the disease and its complications (Vahalkar and Haldankar 2008; Merkhan et al. 2021). Oxidative stress occurs in T1DM due to excessive formation of reactive oxygen species (ROS), mainly superoxide anion and hydroxyl radicals, and a simultaneous reduction in antioxidant capacity (Evans et al. 2002; Ceriello 2006). Persistent hyperglycemia contributes to a sharp rise in ROS production, which in turn results in β-cell destruction and eventually an impairment of insulin production and secretion (Thongnak et al. 2017).

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Moreover, ROS results in the activation of various pathways, including sorbitol generation, glucose autoxidation, and oxidative phosphorylation (Brownlee 2001; Robertson 2004). These mechanisms lead directly to a rise in oxidative stress, which is linked to a cascade of tissue damage during diabetes. Oxidative stress has actually been considered one of the primary pathogenic processes in the development of diabetes and can play an essential role in the emergence of many late consequences, including cardiovascular, renal, and nervous system diseases (Ceriello 2000; Osawa and Kato 2005; Daneman 2006; Maiese et al. 2007). The harmful effects of ROS are counteracted by endogenous enzymatic (superoxide dismutase (SOD) and catalase (CAT)) and non-enzymatic (GSH, vitamin C and vitamin E) antioxidants (Hanchang et al. 2019; Kostopoulou et al. 2020; Almulathanon et al. 2021). SOD combats ROS by converting superoxide anion to hydrogen peroxide and oxygen, while catalase converts hydrogen peroxide into water and oxygen. On the other hand, the antioxidant effect of vitamin C includes increasing nitric oxide synthesis in endothelial cells to stabilize the nitric oxide synthase cofactor, whereas vitamin E inhibits ROS, including peroxyl and superoxide radicals, and singlet oxygen by direct interaction. Moreover, GSH, an intracellular thiol, removes free radicals by a direct scavenging effect (Jayakumar et al. 2014; Chukwunonso Obi et al. 2016). However, the antioxidant mechanism is disrupted in hyperglycemia due to increased generation of ROS (Omodanisi et al. 2017).

Insulin was found about a century ago and is considered to act exclusively on peripheral tissues, particularly muscles, liver, and adipose tissue (Yarube et al. 2019). It is still an essential medication for type 1 diabetic patients (Aathira and Jain 2014). Several findings showed that insulin has antioxidant effects (Chen et al. 2014; Borah and Das 2017). However, conflicting results have been observed in other studies. Idris et al. observed a marked increase in MDA levels, an oxidative stress biomarker, in diabetic rats treated with insulin (Idris et al. 2020). Moreover, a study conducted by Yarube et al. showed that insulin therapy results in an elevation of the MDA level in the brains of mice (Yarube et al. 2019). On the other hand, Kocic et al. study revealed that twice-daily Human Neutral Protamine Hagedorn (NPH) insulin therapy neither reduces the MDA level nor improves the antioxidant capacity of type 1 diabetic patients (Kocic et al. 2007). Despite its widespread use in the treatment of T1DM, there is no published data on the effects of NPH and regular insulin combination on MDA, CAT, GSH, vitamin E and C concentrations in type 1 diabetic patients. Thus, the relationship between MDA, CAT, GSH, vitamins (E and C) and glucose levels in insulin-treated patients with T1DM was not evaluated at the same time. Therefore, the current study aimed to explore the effect of a mixed insulin regimen on oxidative stress biomarker (MDA), enzymatic and nonenzymatic antioxidant levels in type 1 diabetic patients.

Materials and methods

Patients

The current study was conducted at Al-Waffaa diabetic Centre, Mosul, Iraq between January and July, 2020. In this retrospective cross-sectional study, 55 patients with T1DM and 20 controls aged between 12 and 31 years were involved. Diabetic patients were divided into two groups: 25 newly diagnosed patients and 30 patients treated with an insulin regimen that included injections of regular insulin (HumulinR-Lilly) with NPH (HumulinN-Lilly) before breakfast and dinner for 3–18 months. The control group included healthy subjects with age-matched patient groups. The study has gained approval from the Research Ethics Committee of the College of Pharmacy, University of Mosul. In addition, the Declaration of Helsinki guidelines were followed and written informed consent was received from all subjects prior to their inclusion in the study. The inclusion criteria included type 1 diabetic patients without chronic diabetes complications. The exclusion criteria included alcoholics, smokers, pregnant or lactating women, patients using extra medicines or vitamins other than insulin, and patients with complications of diabetes or medical conditions other than T1DM. The body mass index (BMI) of all participants was measured based on height and weight.

Biochemical analysis

Blood samples were obtained from all participants after overnight fasting, followed by a water bath incubation (10 min, 37 °C), and then centrifugation at 4,000× g for 10 mins to separate the serum. The collected serum was stored at -20 °C until analysis.

A colorimetric technique was used to measure fasting serum glucose (FSG) absorbance at 505 nm, utilising a kit obtained from BIOLABO (France) and a UV-VIS Spectrophotometer (Japan). An enzyme-linked immunosorbent assay (ELISA) was used to assess serum insulin absorbance at 450 nm, using a Monobind kit (USA) and a microplate reader (ELx 800, BioTek, USA). Insulin resistance was estimated according to the following equation:

$$\text{Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)} = \text{Insulin (μU/ mL)} \times \text{Glucose (mmol/L)} / 22.5 \text{ (Matthews et al. 1985)}$$

The serum MDA level was measured using a UV-VIS Spectrophotometer (Japan) according to the modified technique, (Guidet and Shah 1989) where the absorbance of the colored product produced by the reaction between MDA and Thiobarbituric acid (TBA) was determined at 532 nm.

Serum CAT activity was evaluated by a spectrophotometric measurement of the absorbance at 240 nm wavelength (Aebi 1984). A modified Ellman method (Sedlak and Lindsay 1968) was used to measure serum GSH, with the

absorbance determined at 412 nm. Serum levels of vitamins C and E were estimated by a colorimetric method based on a reduction in 2,6-dichlorophenolinophenol absorption at 520 nm (Omaye et al. 1979), and the generation of ferrous ions at 460 and 600 nm, respectively (Martinek 1964).

Statistical analysis

All data are expressed as mean ± standard deviation. Kruskal-Wallis test followed by a Dunn test were used for comparisons between different groups. The correlations between FSG and MDA with other parameters were evaluated by Spearman's correlation test. Statistical analyses were performed using GraphPad Prism version 8.0 (San Diego, California, USA). The difference was considered statistically significant at $p < 0.05$.

Results

The basic clinical characteristics of the control group and diabetic patients are outlined in Table 1. There were no significant differences between the groups.

Table 1. Basic clinical characteristics of the participating groups.

Parameter (unit)	Control (n = 20)	Newly diagnosed T1DM (n = 25)	Insulin (n = 30)
Age (years)	19.12 ± 4.5	19.1 ± 3.5	20 ± 2.5
BMI (kg/m²)	22.4 ± 0.4	22.3 ± 0.8	22.1 ± 0.5

In Table 2, newly diagnosed and insulin-treated diabetic patients showed significantly higher FSG and lower insulin levels compared to the control group. However, treatment with insulin resulted in a significant decrease in FSG and a significant rise in insulin levels compared with the newly diagnosed diabetic group. Concerning HOMA-IR, the untreated diabetic group showed a comparable level to the control, while the administration of insulin resulted in a significant increase compared with the other groups.

Table 2. Assessment of FSG, serum insulin and HOMA-IR for the participating groups.

Parameter (unit)	Control	Newly diagnosed	Insulin
FSG (mmol/l)	4.7 ± 0.6	13.1 ± 0.85 [a***]	10.5 ± 0.75 [a*** b***]
Serum insulin (µIU/ml)	11 ± 0.65	3.6 ± 0.84 [a****]	7.1 ± 0.72 [a*** b****]
HOMA-IR	1.98 ± 0.53	2.11 ± 0.33	2.95 ± 0.65 [a**** b****]

The data are shown as mean ± SD. [a] reflects a comparison of insulin-treated and newly diagnosed diabetic patients against healthy controls; [b] reflects a comparison between insulin-treated and untreated diabetic groups. (***p < 0.001; ****p < 0.0001) denotes statistically significant differences, using Kruskal-Wallis test followed by a Dunn's multiple comparison test.

As shown in Fig. 1A, the untreated and insulin-treated diabetic groups had comparable serum MDA levels, but were significantly higher compared to the control group. Moreover, both diabetic groups showed significantly lower serum levels of CAT (Fig. 1B), GSH, Vitamins C and E (Fig. 1C) compared with the control group. However, the
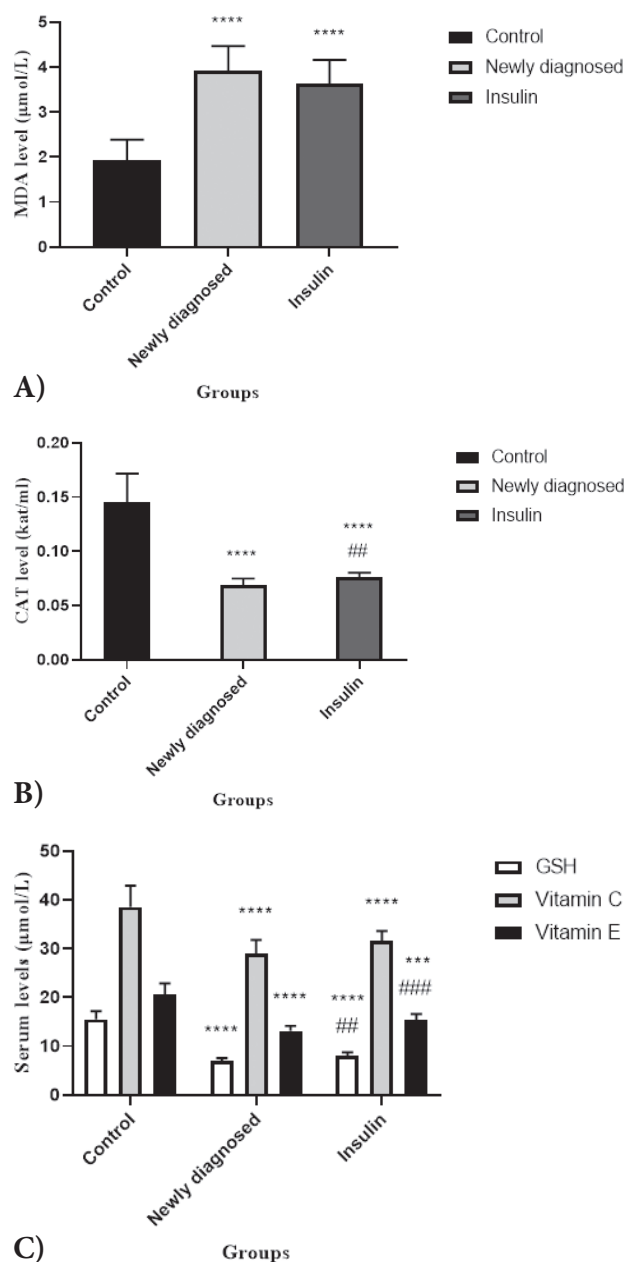

Figure 1. Effects of insulin on serum levels of **A)** MDA, **B)** CAT, **C)** GSH, vitamins C and E. The data are viewed as mean ± SD. (***p < 0.001; ****p < 0.0001) denotes statistically significant differences versus the control group; (##p < 0.01; ###p < 0.001) denotes statistically significant differences between insulin-treated and newly diagnosed patients, using the Kruskal-Wallis test followed by a Dunn's multiple comparison test.

insulin-treated group exhibited a significant elevation in the serum level of CAT, GSH and vitamin E in comparison to the untreated diabetic group.

Spearman's correlation analysis showed that FSG was significantly negatively associated with vitamins C and E in the newly diagnosed diabetic group. Meanwhile, a significant positive correlation between FSG and HOMA-IR was observed after insulin treatment. In addition, MDA was significantly inversely related to vitamins C and E in the insulin-treated group (Table 3).

Table 3. Correlations of FSG and MDA with variable parameters in insulin-treated and untreated diabetic groups.

Parameters	Newly diagnosed T1DM	Insulin
FSG / HOMA-IR	r = -0.05143	r = 0.7058[****]
FSG / Vitamin C	r = -0.5348[*]	r = -0.05238
FSG / Vitamin E	r = -0.4481[*]	r = 0.1773
MDA / Vitamin C	r = -0.07337	r = -0.5134[**]
MDA / Vitamin E	r = -0.1683	r = -0.4703[*]

(*p < 0.05; **p < 0.01; ****p < 0.0001) represent statistically significant correlations, were assessed by Spearman correlation analysis. r = correlation coefficient.

Discussion

Although insulin has been widely used in T1DM, contradictory findings have been found regarding its effect on oxidative stress. The current study was conducted to evaluate the potential effect of NPH plus regular insulin therapy on oxidative stress represented by lipid peroxidation and antioxidant levels in patients with T1DM. This study revealed a substantial increase in blood glucose levels accompanied by a significant decrease in insulin levels in untreated diabetic patients. Moreover, the oxidative stress biomarker, MDA, was significantly increased, whereas antioxidant levels represented by serum CAT, GSH and vitamins (C and E) were significantly decreased in the untreated diabetic group. Our results are in accordance with those of previous results (Firoozrai et al. 2007; Ramakrishna and Jailkhani 2007). It has been found that hyperglycaemia leads to the generation of various types of ROS, including hydroxyl radical, which attacks the unsaturated fatty acid chain, leading to lipid peroxidation and MDA formation (Giacco and Brownlee 2010; Bin-Jaliah et al. 2021). However, the present study did not show any correlation between blood glucose and the oxidative stress biomarker, MDA, in untreated diabetic patients. On the other hand, hyperglycaemia can lead to disruption of antioxidant systems by glycosylation (Zakaria et al. 2019). In accordance with a study by Ramakrishna and Jailkhani (2007), the link between hyperglycaemia and antioxidant reduction was confirmed in the current study by a negative association between FSG and serum concentrations of vitamins (C and E) in untreated diabetic patients.

A significant finding of our study was that patients treated with insulin exhibited high insulin resistance compared to untreated patients. In the current study, the FSG level of patients on insulin therapy was lower when compared to that of untreated diabetic patients, but was still higher than in the control subjects. Therefore, the rise in insulin resistance in insulin-treated patients may be due to poor glycaemic control, which in turn reduces insulin action by lowering the level of nitric oxide (Mohammad et al. 2021). Previous studies have revealed that nitric oxide contributes to the effect of insulin via intracellular mechanisms (Ding et al. 2000; Kocic et al. 2007; Ragy and Ahmed 2019). The correlation between FSG and HOMA-IR observed in this study further confirms the role of

hyperglycaemia in increasing insulin resistance. Another interesting finding in our study is that although insulin therapy resulted in an obvious increase in the serum levels of CAT, GSH, and Vitamin E compared to the untreated diabetic group, its effect on MDA was not significant. In line with our study, Kostopoulou et al. (2020) exhibited a significant increase in antioxidant capacity without any changes in MDA level following eight months of insulin treatment. In the case of our patients, hyperglycaemia may have resulted in elevated free radicals and lipid peroxidation through glucose autoxidation (Kocic et al. 2007). It has been found that marked variations in glucose levels in diabetic individuals lead to oxidative stress even in insulin-treated patients (Gao et al. 2012). However, the current study showed no significant association between FSG and MDA levels in the insulin-treated groups, which is in line with previous findings (Wentholt et al. 2008; Benhamou et al. 2014). Another possible explanation for decreased insulin activity against oxidative stress was due to insufficient antioxidant effects. In the present study, insulin therapy improved antioxidant status. However, no significant effect was found on the level of vitamin C. Vitamin C is a potent antioxidant that is water-soluble and found in the cytosol of the cell. In addition, it can prevent lipid peroxidation by scavenging ROS and regenerating the active form of vitamin E during oxidative stress (Pari and Saravanan 2007; Nair and Nair 2017). In this study, a significant inverse correlation was observed between serum MDA and vitamins (C and E), indicating the possible role of these antioxidants in counteracting oxidative stress in diabetic patients.

It's important to highlight that there are some limitations in this study. First, HbA1c has not been evaluated. Furthermore, this was a cross-sectional study and was conducted on a small number of patients. For further confirmation, follow-up studies with a larger sample size are needed.

Conclusion

Treatment with a twice-daily mixed insulin regimen (NPH and regular insulin) was effective for reducing FSG and improving serum insulin and antioxidants (CAT, GSH, and vitamin E) levels in patients with T1DM. However, insulin therapy did not attenuate the insulin resistance and oxidative stress represented by the MDA level. Accordingly, insulin therapy can be used in combination with exogenous antioxidants such as vitamins C and E to reduce oxidative stress.

Acknowledgements

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