The Role of Autoantibody and Antioxidant Enzymes in Patients with Type I Diabetes

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ABSTRACT

To determine autoantibodies and antioxidant enzymes as well as the correlation between them.

This study included 80 individuals, 40 patients with type 1 diabetes and 40 healthy individuals without diabetes (as a control group). The study was carried out during the period from December 2010 to the end of December 2012 at Al-Tahreer General Hospital, Al-Basra Maternity and Pediatric Hospital, and Al-Sader Teaching Hospital. Laboratory investigations were performed to estimate glutamic acid decarboxylase antibody (GADA) and islet cell antigen-2 antibody (IA-2A) by enzyme-linked immunosorbent assay (ELISA), antioxidant enzymes (glutathione peroxidase [GPX] and superoxide dismutase [SOD]), and glycosylated hemoglobin (HbA1c) (as a marker of glycemic control) for these patient and control groups.

The high prevalence of GADA and IA-2A had been demonstrated among patients with type 1 diabetes, which was significantly higher ($P < 0.001$) (72.5%) in comparison to 0% in the control group. These results are suggestive of the autoimmune characteristic of type 1 diabetes.

The age of onset of type 1 diabetes is found to affect the frequency of these autoantibodies. The frequency was significantly higher in patients who developed the disease in early childhood (91.7% for GADA and 58.3% for IA-2A) in comparison with those who developed the disease later on (40% for GADA and 20% for IA-2A); this probably occurred due to genetic and non-genetic factors.

Although the statistical analysis of the correlation between gender and autoantibodies showed no significant difference, female patients with type 1 diabetes were found to be more affected than male patients.

The frequency of these autoantibodies was found to decrease as the duration of type 1 diabetes increased. The prevalence of GADA and IA-2A in patients with duration of disease less than 5 years was 78.3% and 43.5%, respectively, and began to decrease to 0% for GADA and IA-2A in those with disease duration more than 12 years. These results are attributed to the depletion of islet cell autoantibodies with time.

Additionally, HbA1c levels were significantly higher in islet cell autoantibodies–positive patients than in islet cell autoantibodies–negative patients ($P < 0.001$). The difficulty in achieving glycemic control despite oral hypoglycemic drug and insulin therapy is attributed to the fact that the pathogenesis of disease in developing type 1 diabetes and latent autoimmune diabetes (LADA) in adults is due to β-cell destruction rather than insulin resistance as in classical type 2 diabetes.

The mean activity of both antioxidant enzymes (SOD and GPX) in red blood cells (RBCs) was significantly lower than the control ($P < 0.001$). Also the lower mean activity of both antioxidant enzymes (SOD and GPX) in RBCs showed a higher significant value in patients who had uncontrolled diabetes (HbA1c level > 8%) ($P < 0.001$).

Patients with LADA who were tested positive for GAD and IA-2A showed a significant decrease in the mean activity of SOD and GPX in comparison to patients with type 2 diabetes who were tested negative to autoantibodies; most of the patients with LADA also had a higher HbA1c level > 8% ($P< 0.001$).

There is a strong evidence of the role of autoimmunity in the pathogenesis of type 1 diabetes. The oxidative stress SOD and GPX are depleted as well. The correlation reflects the more oxidative stress with poor diabetic patients may progress the complications.

Key Words: Antioxidant, Autoantibody, Enzymes, Glycosylated hemoglobin, Type 1 diabetes mellitus.

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INTRODUCTION

It is now well recognized that diabetes is an epidemic disease in most countries that are undergoing socio-economic transitions. It is a major public health problem worldwide, with a high probability of developing type 1 and type 2 diabetes. Type 1 diabetes, formally known as insulin-dependent diabetes, accounts for approximately 15% of the diabetes population. It strikes to any age; however, it is generally seen in children and young adult (1). Type 1 diabetes is further classified as type 1a (autoimmune diabetes) and type 1b (idiopathic type) (2).

Prior to the clinical onset, type 1 diabetes is characterized by lymphocytic infiltration of islet cells and circulating autoantibodies against a variety of islet cell antigens, such as glutamic acid decarboxylase (GADA), islet cell antigen-2 from tyrosine phosphatase–like protein family (IA-2A), and insulin. At this stage, the measurement of GAD, IA-2A, and insulin autoantibodies can provide vital information and insight with regard to the autoimmune progression of diabetes (3,4).

The presence of disease markers that are measurable at this time may allow the opportunity to predict and prevent the clinical onset of disease (5,6).

While the majority of patients fall under the classical definition of either type 1 or type 2 diabetes, there are at least two subgroups of patients that bridge these classical barriers. Studies indicate that as many as 10%–15% of patients diagnosed with type 2 diabetes have circulating autoantibodies to either islet cell antigens, and these patients eventually become insulin dependent (7). The patients who are initially misclassified as type 2, in fact, have late-onset or slow-developing type 1 diabetes, which is sometimes referred to as latent autoimmune diabetes in adult (LADA)(8,9).

Many studies revealed that the presence of islet cell autoantibodies, which are regarded as immunological markers for the autoimmunity of the disease (5,6), will predict the development of diabetes in the relatives.

Current researches have shown that the measurement of GADA, IA-2A, and insulin can be of a significant value to the clinician in predicting, diagnosing, and managing patients suffering from diabetes (10,11).

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and the most common complications such as atherosclerosis, nerve damage, renal failure, male impotence and infection (12). Recently, some evidences suggest that oxidative stress may play an important role in the etiology of diabetes and diabetic complications (13). In healthy individuals, oxidative damage to tissues is prevented by a system of defenses that include antioxidant enzymes and small molecules, such as antioxidant vitamins, with scavenging ability (14). In patients with diabetes, an altered balance between the production of reactive oxygen species and antioxidant levels has been reported (15,16), but there is still a lack of data regarding the actual status of antioxidant enzymes in patients with diabetes. To gain more information about the activities of antioxidant enzymes, the study aims to determine autoantibodies (GADA and IA-2A) and antioxidant enzymes as well as the correlation between them.

MATERIALS AND METHODS

Subjects

During the period from November 2010 to December 2012, 80 individuals were included in the present study. They were divided into patient and control groups.

1. Patients group:

A total of 40 patients with type 1 diabetes from Centre of Researches and Treatment of Diabetes in AL-Tehreer Hospital, Al-sadder teaching hospital, and Basra Hospital for Maternity and Children were recruited for the case-control study.

2. Control group:

A total of 40 apparently healthy volunteers were involved in the study. Patients and controls were instructed and informed about the aim of the study and the investigation procedure, and their acceptances were documented. In addition, the work was approved by the ethical committee of the College of Medicine, University of Basrah, Iraq.

Laboratory analysis

After an overnight fasting, 5 mL of venous blood was collected from both patient and control subjects, and then divided into the following parts: 2.5 mL was transferred to EDTA (ethylenediaminetetraacetic acid)-containing tubes and used for hemoglobin (Hb) and HbA1c estimation within 48 hours. The remaining amount was separated by centrifugation at 3000 rpm for 10 minutes. A part of separated plasma was stored in separated plain tubes at –20°C before testing; all plasma tubes were allowed to thaw once (repeated thawing is avoided). Other 2.5 mL of whole blood was transferred to heparinized tubes, and these tubes were used for GPX and SOD enzyme estimation within 48 hours.

Diagnostic kits:

All the diagnostic kits were purchased from Human (Germany), EUROIMMUN (Germany), and Randox (United Kingdom). These include:

- Anti-GAD ELISA (IgG) Test (Kit No. EA 1022-9601 G) (EUROIMMUN).
- Anti-IA-2 ELISA (IgG) Test (Kit No. EA 1023-9601 G) (EUROIMMUN).
- Glycosylated hemoglobin HbA1c Test (Kit No.10657) (Human).
- Glutathione Peroxidase Test (Kit No .RS 504) (RANDOX).
- Superoxide dismutase Test (Kit No. SD 125) (RANDOX).

GADA and IA-2A and were measured according to the instructions of manufacturers. HbA1c was also measured (19). The antioxidants were measured based on the procedures given in the studies by Paglia and Valentine (17) and Arther and Boyne (18).

Statistical analysis

The statistical analysis was performed by SPSS version 15, chi-square test, Pearson chi-square, correlation coefficients (r), and crosstabs to determine the difference in the characteristics between patients and controls. The statistically significant differences were assessed with the chi-square test at two levels of probability ($P \leq 0.05$, $P \leq 0.001$).

RESULTS

A total of 40 patients of both sexes (22 females and 18 males) with type 1 diabetes were present. Their ages ranged between 2 and 31 years, with a mean of (11.88±6.8) years. The disease duration was between 6 months and 19 years.

Table 1 shows the prevalence of GADA and IA-2A among patients, while it is absent among the control group. The results of HbA1c were significantly higher among all patients than among the control group ($P > .001$). The results of SOD and GPX were significantly lower among patients than among the control group ($P > .001$) level of.The Hb (in g/dL) was lower among patients than among the control group.

The present study showed that the prevalence of islet cell autoantibodies is affected inversely by the duration of the disease (Table 2), as the percentages of autoantibodies in patients with the duration of disease less than 5 years were 78.3% and 43.5% for GADA and IA-2A, respectively, and the percentages for GADA and IA-2A begin to decline to 0% in those with the duration of disease more than 11 years.

TABLE 1: Biochemical characteristic among patient and control groups

Biochemical characteristics	Type 1 diabetes mellitus	Control of type1 diabetes mellitus
GAD antibody	27/40 67.5%	0/40
IA-2A	16/40 40%	0
%HbA1c	6.7–10.8	4.2–5.8
GPX U/g Hb	31.5 ± 3.4	48.0 ± 3.8
SOD U/g Hb	860 ± 60	1282 ± 60.4
Hb g/dL	9.5–12.7	10.5–14

$P < 0.01$

TABLE 2: Influence of duration of the disease in the prevalence of GADA and IA-2A in patients with type 1 diabetes.

Result duration	GADA positive		IA-2A positive	
	No.	%	No.	%
<5	18	78.3	10	4305
5–7	4	66.7	4	6607
8–10	5	71.4	2	28.6
>11	0	0	0	0
Total	27		16	
χ^2= 9.573	df= 3	P < 0.05	χ^2= 4.941	df = 3 P > 0.05

The relationship of GADA with the duration of disease showed a significant difference ($P < 0.05$), while it was not significant for IA-2A ($P > 0.05$) (Table 2).

According to HBA1c levels, the patients with diabetes were divided into three groups: patients with good diabetic control (GDC) with HBA1c level less than 7.0%, patients with fair diabetic control (FDC) with HBA1c level between 7.0% and 8.0%, and patients with poor diabetic control (PDC) with HBA1c level more than 8.0%.

As expected, there were significant differences in the mean level of HBA1c among the three groups of patients ($P < 0.01$ in all cases).

The activities of SOD and GPX in patients and controls were determined. As shown in Table 3, a significant reduction in the activities of both enzymes among patients was noticed ($P<0.001$).

Table 3: Mean activity of SOD and GPX in patients and controls.

Variables	Type 1 diabetes		T	P	T	P
	Patients	Control				
GPX (U/g Hb)	31.5	48.0	−20.4	.00	−24.6	.00
SOD (U/g Hb)	860	1282	−31.3	.00	−25.9	.00

Data are presented as mean ± SD. P < 0.001. T= −24.6 P < 0.001

Table 4: Correlation coefficients (R) between the activities of antioxidant enzymes (glutathione peroxidase [GPX] and superoxide dismutase [SOD]) and clinical characteristics of patients with Type 1 diabetes.

Clinical and biochemical characteristics	Type1 diabetes mellitus GPX (U/g Hb)		Type1 diabetes mellitus SOD (U/g Hb)	
	R	P	R	P
Age (year)	−.239	−.137	.254	.11
Duration of diabetes (year)	−306	>0.05	.301	>0.059
HbA1c (%)	−.762**	<0.001	−.697**	<0.001

**Correlation is significant at the 0.001 level.

Correlation between the activities of antioxidant enzymes (glutathione peroxidase and [GPX] superoxide dismutase [SOD]) and clinical and biochemical characteristics of patients with type 1 diabetes.

In patients with type 1 diabetes, a possible correlation between the activities of antioxidant enzymes (SOD and GPX) and age, sex, duration of diabetes, and levels of HBA1c were also studied (Table 4).

No significant correlations were observed between the activities of antioxidant enzymes (SOD and GPX) and each of the below-mentioned clinical characteristics of patients with type 1 diabetes; however, there were significant correlations between these enzymes and HBA1c (P <0.001).

DISCUSSION

Type 1 diabetes is a chronic inflammatory and multifactorial disease caused by a selective destruction of the insulin-producing β-cells in the islets of Langerhans. One theory regarding the etiology of type 1 diabetes is that it results from the destruction of pancreatic β-cells due to defective immune regulation by infectious or environmental agents that trigger the immune system in genetically susceptible individuals to develop an autoimmune response against altered pancreatic β-cell antigens. Currently, autoimmunity is considered a major factor in the pathophysiology of type 1 diabetes (20,21).

Type 1 diabetes is associated with the appearance of humoral and cellular islet autoimmunity, and a defective immune regulation appears to be involved (22).

The present study found that there are 27 (67.5%) GADA-positive and (33.3%) IA-2A-positive type 1 patients. This result is in agreement with 82.9%, 80.3%, 84%, and 82.8% estimated by Borg et al.(23), Pardoni et al. (24), Laadher et al. (25), and Suaad et al. (26), respectively. The above findings demonstrate the important role of islet cell autoimmunity in the pathogenesis of the disease. However, these results seem to be higher than that reported by Damanhouh et al. (27) in a study from Saudi Arabia on patients with type 1 diabetes, who showed 54% and 27% for GADA and IA-2A, respectively. Also, in a study from Taiwan, patients with type 1 diabetes showed 47% GADA and 23% IA-2A positive in their sera (28).

The reason for such differences in the prevalence of these autoantibodies may be attributed to difference in assays used, procedure sensitivity, and difference in patients' genetics and environmental characteristics.

These autoantibodies were found to be more prevalent in those who developed the disease during childhood and early puberty, and the prevalence began to decrease as the age of onset increased. These results are in agreement with a number of studies (26,29-31). This may be attributed to the genetic and non-genetic factors that influence the presence of disease-associated antibodies, the rate of progression to clinical onset of diabetes, and the severity of reduced insulin-secreting capacity (32).

Environmental factors have been implicated in the etiology of autoimmune diabetes (age-related non-genetic factors); these factors include early exposure to cow's milk, reduced rates or duration of breast feeding, vitamin D consumption, and the early introduction of cereals (33-36). So the striking post-pubertal decline in disease incidence could be caused by the loss of genetic or environmental effects in this age group (31).

Regarding gender, this study showed that females, in general, were more affected by diabetes mellitus than males, as there were 51.9% females and 48.1% males in the studied group. Although, the variation in gender distribution regarding islet cell autoantibodies is statistically non-significant, females also

showed a higher prevalence of islet cell autoantibodies (GADA and IA-2A) than males. This finding is in agreement with those demonstrated by other studies (36,37), which showed an increased frequency of islet cell reactivity in females than in males. However, a study by Lutale et al. demonstrated no correlation between gender and autoimmunity in patients with type 1 diabetes (38). The logical explanation for this difference may be attributed to sex hormones; females might respond more to conventional antigens due to sex hormones (39).

When the duration of the disease was taken in consideration as a factor affecting the frequency of these antibodies, it was found that the frequency of these antibodies decreased as the duration of the disease increased. These results were controversial. Although many studies showed the same results (24,36,40) demonstrating a decrease and disappearance of these autoantibodies with time, others showed an increase of antibodies with time (38). The studies explained this finding by seroconversion of patients from GADA and IA-2A negative at the onset of disease to GADA and IA-2A positive later on.

Although, there is no clear-cut explanation of the decrease of antibodies with increased duration as shown in the study by Lutale et al., the effect of immune tolerance could be the factor that plays a role. Alternatively, this could be attributed to the fact that there is nearly a complete destruction of islet cells with time (antigenic depletion), so the level of these antibodies decreases in association with the degree of disappearance or destruction of islet cell antigens. The disease progress of patients with islet cell antibodies may be worse, and the death may occur before reaching the late age of puberty.

In patients with diabetes, long-term damage, dysfunction, and failure of different organs are related to uncontrolled hyperglycemia (41,42). The genetic hypothesis suggests that complications from diabetes are genetically predetermined as part of the diabetic syndrome, whereas the metabolic hypothesis suggests that complications such as cellular and vascular damage are the effects of long-term hyperglycemia.

The mean activities of SOD and GPX depleted in patients with type 1 diabetes and in LADA (positive GAD autoantibody group). The depletion was more severe in patients with poor diabetic control HBA1c > 8%.

In the present study, the activity of antioxidant enzymes (SOD) in RBCs of patients suffering from type 1 diabetes mellitus was significantly lower than that in the control group. This finding is in agreement with a number of studies (43-49) and is not compatible with many others (50-56)

In patients with diabetes, the autoxidation of glucose results in the formation of hydrogen peroxide (H2O2), which inactivates SOD (57). Therefore, the accumulation of H2O2 may be one of the explanations for decreased SOD in these patients. Also, the characteristic feature of diabetes—hyperglycemia—enhances non-enzymatic binding of glucose to proteins. This phenomenon—glycation—causes structural and functional changes in proteins such as Hb, albumin, basal membranes of glomeruli, etc.

Antioxidant enzymes are endogenous proteins that work in combination to protect cells from reactive oxygen species (ROS) damage. Increased levels of products that cause oxidative damage to lipids and proteins have been detected in the serum of patients with diabetes, and their presence correlates with the development of complications (58).

Hyperglycemia, a hallmark of diabetic condition, depletes natural antioxidants and facilitates the production of ROS, which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, and DNA and exert cytotoxic effects on cellular components (59).

To control lipid peroxidation, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging ROS. The organisms' susceptibility to free-radical stress and peroxidative damage is related to the balance between the free-radical load and the adequacy of antioxidant defenses. Abnormally, high levels of lipid peroxidation and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and oxidative stress.

SOD exists in several forms. One form containing manganese is found in the mitochondrial matrix and the other containing copper and zinc is found in the cytoplasm. Cells are capable of increasing the synthesis of SOD in response to hyperoxidant stress. The extracellular fluid contains a unique high-molecular weight SOD. The enzyme binds to external endothelial cell surfaces and may be important in the pathogenesis of free-radical damage (60).

This study reveals a significant fall in SOD levels, which could be due to excessive oxidative stress. A decrease in SOD levels can result not only an increase in the superoxide-free radical but also an elevation of other ROS and intensification of lipid peroxidation processes in diabetes. In diabetes, the initial event resulting in the increase in ROS formation is the depletion of adenosine triphosphate due to its increased conversion to adenosine monophosphate, adenosine, inosine, and hypoxanthine. Xanthine

oxidase, in the presence of oxygen, converts hypoxanthine into xanthine and uric acid accompanied by superoxide formation. Hyperglycemia contributes to oxidative stress by virtue of the fact that monosaccharides and glycolytic intermediates can generate oxidative reactants. Glucose can enolize and thereby reduce molecular oxygen under physiological conditions in the presence of traces of transition metals yielding oxidizing agents like H2O2. The glycation reaction itself serves as a source of free radicals. The term autoxidative glycosylation is more appropriate to describe the glucose-dependent oxidative chemical modifications of proteins. Autoxidative glycosylation is initiated by the autoxidation of the aldose/ketose sugar to a more reactive dicarbonyl sugar (glucosone), which then reacts with the protein. Partially reduced oxygen intermediates like superoxide anion radical and H2O2 generated in the course of this autoxidation associated with glycation contribute to the oxidative stress. This is suggestive of the fact that increased autoxidative glycosylation of Hb may also have led to the enhanced generation of free radicals like the superoxide anion, thereby causing the depletion of SOD that quenches free radicals. It can, therefore, be concluded that hyperglycemia influences the etiopathogenesis of diabetes in more than one way (60).

The data in this study reveal that the GPX level was significantly low, indicating a decreased scavenging capacity of glutathione-dependent antioxidant-defensive system against elevated lipid peroxidation processes. GPX is one of the enzymes responsible for the removal of H2O2 produced as part of cellular metabolism. It is possible that the observed reduction in GPX in these diabetic samples may indirectly lead to increased lipid peroxidation, as lipid hydroperoxides are destroyed by GPX.

The low activity of GPX could be directly explained by the low content of glutathione found in patients with diabetes, as glutathione is a substrate and cofactor of GPX (61). Enzyme inactivation could also contribute to low GPX activity. GPX is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress (62). The inactivation of the enzyme may occur through glycation governed by the prevailing glucose concentration (63). Thus increased glycation in patients with diabetes and subsequent reactions of proteins may affect amino acids close to the active sites of the enzyme or disturb the stereochemical configuration and cause structural and functional changes in the molecule. The low activity of GPX causes the accumulation of H2O2 in patients with diabetes. This finding could also explain the progressive decrease in SOD in later stages of the diabetes. Similar findings were reported by various other studies (55,64-69).

Furthermore, there is a negative correlation between SOD and GPX depletion and poor diabetic control, which reflects that the oxidative stress with poor diabetic patients may lead to complications progress.

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