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Original Article

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Can Antioxidative Status Be Involved in Type 1 Diabetes?

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease with beta-cell destruction, resulting in insulin deficiency. It is now clear that environmental factors also play a role in disease development. The prevalence of type 1 diabetes in children and young people in Portugal is 0.16% between 0 and 19 years of age. The main cause of death in T1DM is cardiovascular disease, and early endothelial dysfunction is its pathophysiologycal precursor. Hyperglycemia is associated with increased production of free radicals and increased oxidative stress. The aim of this study was to analyze the antioxidant status in a pediatric portuguese diabetic population.

Methods: The study was conducted to characterize and compare the antioxidant status in children aged 2 - 10 years old, with type 1 diabetes and healthy children. Plasmatic profile of total phenolic content (TPC), ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC) in children with diabetes and controls, pre-pubescent, and with BMI < 85th centile were evaluated.

Results: FRAP values were significantly lower in diabetic children compared with healthy controls ($P < 0.001$). There was not any statistical significant difference in the TPC and the TEAC determinations.

Conclusions: Young Portuguese diabetic children have a lower antioxidant status than healthy children.

Keywords: Antioxidant; Type 1 diabetes

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease with beta-cell destruction, resulting in insulin deficiency. The incidence of pediatric diabetes is increasing for T1DM [1]. A genetic predisposition seems to be necessary for developing the disease and is most often linked to genes in the HLA-complex [2]. Islet autoantibodies are detected in the majority of individuals at the time of diagnosis of T1DM, and these are directed against pancreatic proteins like insulin, glutamic acid decarboxylase (GAD), islet antigen 2 (IA-2), or zinc transporter 8 [3]. It is now clear that environmental factors also play a role in disease development [4].

Factors like maternal age at delivery, infections in early life, deficiency of specific nutrients during pregnancy, and/or early childhood have been associated with risk of T1DM [4]. Other suggested environmental risk factors for T1DM are alterations in gut microbiota and lack of general exposure to microbial factors [5].

The prevalence of T1DM in children and young people in Portugal is 0.16% between 0 and 19 years of age. In 2014, there were 17.5 new cases per 100,000 young people between the ages of 0 and 14 [6]. Portuguese people should be consuming a mediterranean diet, which is a traditional dietary pattern of the inhabitants of the Mediterranean countries, and considered to be one of the healthiest diets, rich in many nutrients, namely antioxidants [7, 8]. Despite of that, there are increasingly reports demonstrating a low adherence to this diet, and mainly between the youngest [9].

The main cause of death in T1DM is cardiovascular disease, and early endothelial dysfunction is its pathophysiologycal precursor, since very early [10]. Hyperglycemia is associated with increased production of free radicals and increased oxidative stress [11]. This is due to the increased production of reactive oxygen species (ROS) and decreased antioxidant state [12]. It was demonstrated that some non-nutrient compounds, such as the abundant plant phenolic substances, may contribute significantly to preventing oxidant damage [13].

Polyphenols show antioxidant activity *in vitro* and *in vivo* and therefore might protect from oxidative stress-related diseases such as cardiovascular disease [14, 15].

Although other authors analyzed the antioxidant status in diabetic children, different results have been obtained, and to our knowledge, this is the first study to analyze a pediatric portuguese diabetic population.

This work will focus on the antioxidant status profile in

pre-pubertal children, aiming to a possible impact of antioxidant on T1DM evolution and onset of complications. The authors analyzed the antioxidant status in children with established diabetes (> 6 months after diagnosis) and compared with healthy controls of the same age. In order to analyze the antioxidant status, different assays were used, namely ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC), both of which use organic radical producers. These methods determine the delay of radical generation and the ability to scavenge the radicals [16]. Total phenolic content (TPC) evaluates the phenolic compounds, that are metabolites with significant antioxidant potential [17].

Materials and Methods

Participants

Type 1 diabetic patients attending the Pediatric Endocrinology Clinic in Hospital de S. Joao, Porto, were selected for the study. The study group consisted of 23 diabetic children, with diagnosis for more than 6 months (12 males and 11 females) with a median age of 7.15 years. Exclusion criteria were BMI > 85th centile, the presence of puberty, complications such as retinopathy, nephropathy or neuropathy, other diseases or medications. Healthy aged matched individuals (N = 12) served as controls. All the diabetic children were treated with glargin and rapid analogues (lispro, aspart or glulisin) according to a multiple daily injections system.

The project was approved by the Institutional Ethics Commitee and all the patients parents/tutors signed an informed consent prior to commencement of the study.

Reagents and materials

Sample preparation

The blood was collected in tubes with EDTA. Samples were centrifuged at 2,000 g, 15 min at room temperature. Plasma was separated and stored at 20 °C until analysis.

Chemicals

Gallic acid, ascorbic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble analogue of vitamin E) standards were from Sigma-Aldrich or Fluka. Folin-Ciocalteu reagent, sodium fluorescein, 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) and $FeCl_3.6H_2O$ were from Sigma-Aldrich and 2,4,6-tripyridyl-s-triazine (TPTZ) was from Fluka. All remaining reagents of analytical grade were obtained from Merck. Standard antioxidant solutions were daily prepared and stored in the dark at 4 °C when not in use. Solutions were prepared with ultra-pure water (Millipore, Simplicity 185). All the spectrophotometric assays were performed in a Synergy HT W/TRF Multi Mode Microplate Reader with Gen5 2.0 software (BioTek Instruments, Winooski, VT, USA).

TPC

TPC values of plasma samples were determined by a colorimetric assay based on procedures described by Singleton and Rossi [18]. TPC was expressed as gallic acid equivalents (GAE; mg gallic acid/100 g of sample) through the calibration curves of gallic acid. Final results were in µg of GAE mL-1. Calibration curve ranged from 10 to 200 µg of GAE mL-1.

FRAP

FRAP values of plasma samples were determined by a colorimetric assay based on the method described by Benzie and Strain [19]. FRAP was expressed as ascorbic acid equivalents (AAEs; mg ascorbic acid/100 g of sample) through the calibration curve of ascorbic acid. Final results were in µg of AAE mL-1. Calibration curve ranged from 5 to 100 µg of AAE mL-1.

TEAC

TEAC assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS•+ radical in 6 min. TEAC values of plasma samples were determined by a colorimetric assay based on the method described by Re et al [20]. The total antioxidant capacity was expressed as percentage of inhibition (PI), according to the equation: where AbsABTS•+ corresponds to the initial absorbance of diluted ABTS•+ and Abssample corresponds to the absorbance of the sample after 6 min of reaction. TEAC values were expressed as Trolox equivalents (TEs; mg Trolox/100g of sample) using the standard curve of Trolox. Final results were in µg of TE mL-1. Calibration curve ranged from 5 to 100 µg of TE mL-1.

Calibration standards were daily prepared, and all samples were determined in triplicate. For all the analysis, we have $r \geq 0.99$.

HbA1c was determined with DCA 2000+ Analyzer (Bayer Inc., Tarrytown, NY, USA) immunoassay system (normal range: 3-6%).

Statistical analysis

Age comparision between diabetic children and controls groups was done with independente sample *t*-test and gender comparision between diabetic children and controls groups was done with Chi-square test.

Median values of TPC, FRAP and TEAC concentrations were reported because of the skewed distribution of data.

TPC, FRAP and TEAC values comparisions between

Table 1. Comparision of Mean Age and % of Female Gender in Diabetic Children (T1DM) and Controls

	T1DM (n = 23)	Controls (n = 12)	P
Age*, mean (SD)	7.5 (2.7)	6.6 (2.2)	0.349
Female gender, n (%)	11 (48)	5 (42)	0.728

*Age is missing in two cases from control group. SD: standard deviation.

diabetic children and controls groups were done with Mann-Whitney tests.

A linear regression was performed with logarithm of the logarithm of FRAP values as dependent variable and childrens age and group (diabetic children and controls) as independent variables.

A significance level of 5% was used in all the analyses.

Results

Significant differences in age and gender among diabetic children (T1DM) and controls were not found (Table 1).

The mean duration of disease was 3.29 years in diabetic children. The mean HbA1c was 8.17% in diabetic children.

As obesity was considered an exclusion factor, due to possible interference with metabolic oxidative systems quantification, both groups had a homogeneous BMI.

HbA1c in the diabetic group was higher than recomended by endocrine societies in general.

TPC, FRAP and TEAC values comparision between diabetic children and controls were presented in Table 2.

FRAP values were significantly lower in diabetic children compared with healthy controls ($P < 0.001$). There was not any significant difference in the TPC and the TEAC determinations between the groups.

Linear regression showed that the group of diabetic children had less log FRAP concentration than the controls ($\beta = -0.349$, $P < 0.001$) even adjusted for age ($\beta = -0.007$, $P = 0.616$).

Discussion

Oxidative stress is determined by a balance between ROS generation and antioxidant defense mechanisms that eliminate the superoxide radicals and similar compounds [21]. The antioxidant defense system consists of a series of specific enzymes, binding proteins and various low molecular weight antioxidants such as ascorbic acid, cysteine, glutathione and urate [21]. Some elements of this system are measured in the blood as markers [21]. Diabetes is considered a state of increased oxidative stress, but the data published to date are contradictory [22, 23].

Hyperglycemia generates free radicals through several biochemical pathways (nonenzymatic glycation, the polyol pathway and glucose oxidation) [24]. Free radicals can result in consumption of antioxidant defenses and enhanced susceptibility to lipid peroxidation [24].

In this study, markers of antioxidant defense in pediatric patients with T1DM were evaluated.

The study included a homogeneous group of patients with T1DM, and compared results with healthy controls. The results obtained in this study showed that healthy children had significantly higher antioxidant defense activity reflected by FRAP. This occurred despite of the fact that diabetic children are stimulated to follow a particularly healthy diet, with a higher consumption of fruit and vegetables.

Varvarokska et al demonstrated a lower level of antioxidant capacity among diabetic children, but with the same tendency exhibited by their healthy siblings [25]. In our study, there was a clear tendency for a lower antioxidant level in diabetic children, in relation to the control group.

Other authors showed that in young diabetic patients, systemic oxidative stress is present upon early onset of T1DM [26]. The fact of the diabetic children having a lower antioxidant status and considering their young age (all the children were pre-pubertal) leads to the need of a very careful follow-up, not only of cardiovascular risk, but also other pathological situations related to a higher oxidant level.

Conclusions

Young Portuguese diabetic children have a lower antioxidant status than healthy children. Therefore, a very careful follow-up is necessary and it is of particular importance promoting healthy habits that enhance the antioxidative defenses of the diabetic children since the very early years.

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Table 2. TPC, FRAP and TEAC Values Comparision Between Diabetic Children (T1DM) and Controls

	T1DM, median (min-max)	Controls, median (min-max)	P
Concentration TPC	1,814 (1,555 - 2,119)	1,804 (1,677 - 2,156)	0.862
Concentration FRAP	39.3 (30.3 - 48.3)	52.1 (35.9 - 111.8)	< 0.001
Concentration TEAC	838 (685 - 1,113)	885 (816 - 1,138)	0.058

min: minimum; max: maximum.

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