Clinical Biochemistry 49 (2016) 61–65

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Plasma antioxidant enzymes and lipoperoxidation status in children with Down syndrome

Jingyi He [a], Tingyu Li [a], Jie Chen [a], Youxue Liu [a], Feng Xiong [b], Jing Yang [b], Cui Song [a,*]

[a] Children Nutrition Research Center, Ministry of Education Key Laboratory of Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, CSTC2009 CA5002, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Children's Hospital of Chongqing Medical University, Chongqing 400014, PR China
[b] Children's Hospital of Chongqing Medical University, Central District, Chongqing, PR China

ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 15 August 2015
Accepted 28 August 2015
Available online 2 September 2015

Keywords:
Down syndrome
Oxidative stress
Biomarkers

ABSTRACT

Objectives: Oxidative stress (OS) may play a critical role in cell aging and neurologic disorders that are often seen in Down syndrome (DS) patients. The aim of this study was to determine the antioxidant enzyme level and lipoperoxidation status in blood from DS children.

Design and methods: In a cross-sectional study, we recruited a total of 36 DS children and 40 healthy controls (HCs). All subjects were free of infection according to the C reactive protein (CRP) value and routine peripheral blood profile. The activities of total superoxide dismutases (SODs), extracellular glutathione peroxidase (GPx3),malondialdehyde (MDA) and nitric oxide synthase (NOS) concentrations in peripheral blood were measured by spectrophotometric methods. The relationship of SOD and GPx3 was analyzed in the two groups.

Results: The two groups were similar with respect to age, gender and peripheral blood profiles. The total SOD activity was significantly increased, while the GPx3 activity was significantly reduced in the DS group compared to the HCs ($p = 0.000$, $p = 0.033$ respectively). The MDA level was higher in DS children ($p = 0.013$). There was no significant difference in NOS between DS and HCs ($p = 0.708$). A significant negative correlation between GPx3 and SOD activity was identified in DS ($r = -0.14$, $p = 0.018$) but not in the HC group.

Conclusions: Abnormal redox metabolism takes place in DS individuals. Reducing GPx3 may be a compensatory mechanism of protection against intracellular OS. Moreover, monitoring of decreases in GPx3 activity may be a useful biomarker for evaluating OS in DS patients.

1. Introduction

Down syndrome (DS) is a multiple malformation syndrome due to the trisomy of chromosome 21. DS patients are vulnerable to multiple diseases such as hematologic disorders, cardiovascular alterations and immunological impairment in addition to cognitive defects. Not only that, DS patients are also susceptible to age related neural degenerative diseases. Excessive oxidative stress (OS) is thought to play a critical role in accelerated cell aging and neurologic disorders that are often seen in individuals with DS [1,2] and can result from defects in reactive oxygen species (ROS) metabolism. ROS include several radicals and molecules such as the superoxide anion radical ($O_2-\bullet$), the hydroxyl radical ($\bullet OH$) and hydrogen peroxide (H_2O_2). ROS overproduction has deleterious effects on cell membrane structures, nucleic acids, lipid metabolism and protein function [3], and can lead to premature cell aging and cellular dysfunction.

There are several antioxidant defense pathways that involve proteins such as superoxide dismutases (SODs) and glutathione peroxidases (GPxs) to promote ROS scavenging to maintain redox homeostasis. SODs catalyze the dismutation of superoxide anion radicals ($O_2-\bullet$) to form hydrogen peroxide (H_2O_2). H_2O_2 is sequentially independently catalyzed to water by GPxs or/and catalase (CAT). Malondialdehyde (MDA) is the principal end peroxide product of polyunsaturated fatty acids (PUFAs) during lipid peroxidation induced by ROS (Fig. 1) [4]. Nitric oxide synthases (NOS) include three types in vivo, neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). These NOS isoforms have been identified for the catalysis of the oxidation of L-arginine to produce nitric oxide (NO).

While there are abundant studies that explored SOD1 and cytosolic or mitochondrial GPx activity in DS individuals, there are few studies concerning plasma GPx (GPx3) activity in DS children. In our study, we measured extracellular GPx3 level, plasma total SOD activity, MDA and NOS levels to assess the antioxidant defense system and the oxidative stress status of DS children.

Abbreviations: OS, oxidative stress; DS, Down syndrome; HCs, health controls; CRP, C reactive protein; SODs, superoxide dismutases; GPxs, glutathione peroxidases; MDA, malondialdehyde; NOS, nitric oxide synthase; ROS, reactive oxygen species; CAT, catalase; PUFA, polyunsaturated fatty acids; nNOS, neuronal NOS; eNOS, endothelial NOS; iNOS, inducible NOS; NO, nitric oxide; CHCMU, Children's Hospital of Chongqing Medical University; SD, standard deviation; IQI, interquartile interval; NEU, neutrophils; LYM, lymphocyte; RNS, reactive nitrogen species.

* Corresponding author.
E-mail address: songcui801106@163.com (C. Song).

http://dx.doi.org/10.1016/j.clinbiochem.2015.08.026
0009-9120/

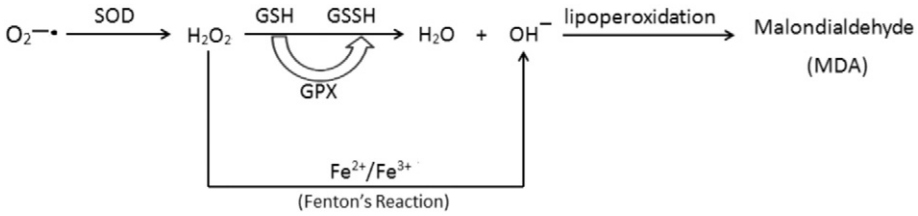

Fig. 1. Reactions of antioxidant enzymes and lipoperoxidation. Superoxide dismutases (SODs) catalyze the dismutation of superoxide anion radicals ($O_2-\bullet$) to form hydrogen peroxide (H_2O_2). H_2O_2 is sequentially catalyzed to water by glutathione peroxidases (GPxs). Malondialdehyde (MDA) is the principal end peroxide product of polyunsaturated fatty acids (PUFAs) during lipid peroxidation induced by reactive oxygen species (ROS).

2. Materials and methods

2.1. Participants

We recruited 36 DS children who were admitted to the Children's Hospital of Chongqing Medical University (CHCMU) from December 2013 to June 2014 and met the following inclusion criteria: clinical diagnosis of standard DS by karyotype analysis of peripheral blood and no systemic disease manifestations (including but not limited to congenital hypothyroidism, serious heart defects, hematological diseases, gastrointestinal malformations, acute respiratory diseases or any infectious diseases). A total of forty healthy controls (HCs) were recruited from the kindergartens and schools cooperating with CHCMU. None of the patients or control subjects had taken any medication or dietary supplements or other interventions in the 6 months preceding the study. This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the CHCMU. Signed informed consent forms were obtained from all participants.

2.2. Specimens

Blood samples were collected into drying tubes, heparin sodium-containing tubes and EDTA-K2 tubes for serum, plasma as well as for C reactive protein (CRP) and routine tests respectively. The serum and plasma were obtained by centrifuging blood samples at 2000 $\times g$ for 10 min within 30 min of collection. The serum and plasma were then separated from the cell layer and stored at −80 °C until analysis was performed. The CRP and routine tests were carried out within 30 min of collection by the Clinical Laboratory Department at CHCMU. Hemolysis samples were excluded.

2.3. Laboratory measurement

The total SOD activity, plasma GPx3, serum MDA and NOS were measured by the chemical colorimetric method. Samples for total SOD, GPx3, MDA and NOS were prepared following the instructions for the assay kit (Jiancheng Bio Co., Nanjing, China) and the final absorbance was determined at 550 nm, 412 nm, 532 nm and 530 nm respectively with BioTek's Epoch micro-volume spectrophotometer system.

2.4. Statistical analyses

Statistical analysis was performed with SPSS 22 software (IBM, NY, USA). A one sample Kolmogorov–Smirnov test was used to evaluate the normality of variable distribution. Values having normal distributions were expressed as mean ± standard deviation (SD), while skewed data were expressed as the median [interquartile interval (IQI)]. Comparison among the groups was performed using an independent t test for values with normal distribution, and a Mann–Whiney U test was done if the conditions of application were not fulfilled. Pearson's test was done for correlation analyses between certain quantitative variables. $P < 0.05$ was considered statistically significant.

3. Results

The basic information and peripheral blood routine profile for the DS and HC groups are shown in Table 1. A total of 36 patients and 40 HCs were included in the study. The mean levels of age and sex showed no statistically significant differences between the two groups ($p = 0.193$ and $p = 0.626$ respectively). The CRP value for every subject was lower than 1 mg/l. The peripheral blood routine profile and CRP value revealed no evidence for acute or chronic infections or inflammation for every subject, and there were no significant differences in absolute counts and the proportion of neutrophils (NEU) or lymphocyte (LYM) between the two groups ($p > 0.05$). Platelet and hemoglobin levels were similar between the two groups ($p = 0.321$, $p = 0.913$ respectively).

The total SOD catalytic activity in the DS children was significantly elevated compared to the controls ($p = 0.000$). Meanwhile, the DS group displayed a significantly decreased GPx3 activity compared to the control group ($p = 0.033$). The MDA level in DS subjects were significantly increased relative to the HCs ($p = 0.013$). There was no significant difference in NOS activity between DS and HC groups ($p = 0.708$) (Table 2, Fig. 2).

The significantly negative correlation of SOD and GPx3 was identified in the DS group ($r = -0.14$, $p = 0.018$) but not in the control group ($p = 0.225$) (Fig. 3).

4. Discussion

According to our data,there is no significant difference in age or gender between the two groups. No evidence of acute infection or inflammation was found in the CRP value and peripheral blood profiles of the participants enrolled in the study, which allows OS related to inflammation or infection to be ruled out.

There are many reports of excessive oxidative stress in DS subjects in the literature. SODs often represent the first line of defense against OS. SOD in peripheral circulation could probably be used as a biomarker for memory and cognitive decline in the DS population [5]. In humans three SOD isoforms have been identified: cytosolic Cu/Zn-SOD (SOD1)

Table 1
Basic information and peripheral blood routine profiles for the subjects[a].

Group	DS	HCs	P
Number	36	40	–
Age (years)[b]	1.0000 (0.4375, 5.9168)	2.2084 (1.1875, 4.5140)	0.193
Sex (M/F)	27/9	28/12	0.626
WBC ($\times 10^9$/l)	6.46 ± 2.16	7.51 ± 1.43	0.22
PLT ($\times 10^9$/l)	305.10 ± 76.76	274.70 ± 54.68	0.321
Hb (g/l)	134.30 ± 5.46	134.00 ± 6.63	0.913
NEU ($\times 10^9$/l)	3.36 ± 1.70	4.20 ± 1.01	0.201
LYM ($\times 10^9$/l)	2.66 ± 0.75	2.67 ± 0.55	0.992
NEU%	50.90 ± 10.17	55.70 ± 5.48	0.205
LYM%	42.20 ± 9.39	35.70 ± 5.25	0.072

WBC: white blood cells; PLT: platelet; Hb: hemoglobin; NEU: neutrophils; LYM: lymphocyte.
[a] Data are mean ± SD.
[b] Median and interquartile interval is presented for age.