IJBC 2020; 12(3): 84-88

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Glutathione Peroxidase as an Oxidative Stress and Insulin Resistance Marker in a group of Egyptian Patients with Normoglycemic Sickle Cell Disease

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ARTICLE INFO

Article History:
Received: 27.05.2020
Accepted: 06.07.2020

Keywords:
Anemia
Sickle cell disease
Insulin resistance
Oxidative stress
Glutathione peroxidase
HOMA-IR

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ABSTRACT

Background: Sickle cell disease (SCD) which is characterized by abnormal hemoglobin structure has a carrier rate of 9-20% in Egypt. Reactive oxygen species (ROC) in SCD cause multiple organ injuries, endocrine complications and metabolic alteration such as insulin resistance (IR) in children and young adults. We aimed to determine the presence of IR in normoglycemic SCD patients and also assessing the concentration of glutathione peroxidase (GPX) and its correlation with insulin resistance in patients with SCD.
Methods: Sixty patients were enlisted from pediatric hematology outpatient clinic, Cairo university hospital together with 31 age and sex-matched healthy children not suffering SCD or other hematological disorders. Oral glucose tolerance test (OGTT) was checked at the beginning. Serum fasting insulin levels and glutathione peroxidase measurement was done using enzyme-linked immunosorbent assay (ELISA). Beta cell function and insulin resistance was calculated using the "Homeostasis Model of Assessment" according to the following equation (fasting insulin x fasting glucose)/22.5. Calculations are based on mmol/l for glucose and mU/ml for insulin considering values more than 1.4 as insulin resistance (IR)
Results: There were no impaired glucose tolerance in both patients and control group. On calculating HOMA-IR, 34 (56.7%) had IR compared to the absence of IR in 26 (43.3%) and all the subjects in the control group (P<0.001). Patients with SCD had lower GPX concentration than the control group with negative correlation with serum insulin and insulin resistance.
Conclusion: The correlation between oxidative stress markers represented by GPX and the development of insulin resistance despite normal serum glucose levels.

Please cite this article as: Abdou DM, Hamdy MM, Salama NM, Elgammal, Amrousy YM. Glutathione Peroxidase as an Oxidative Stress and Insulin Resistance Marker in a group of Egyptian Patients with Normoglycemic Sickle Cell Disease. IJBC 2020; 12(3): 84-88.

Introduction

Sickle cell anemia is a hematological disorder with high prevalence in Africa and the Middle East.[1] Egypt has a high frequency of Hb S carriers which can be explained by the high rate of consanguinity marriage.[2] SCD is a monogenic disorder causing progressive destruction in many organ systems.[3] It is caused by the substitution of the amino acid valine instead of glutamic acid in the beta globulin at position number 6, rendering sickle cell trait or SCD in heterozygotes or homozygotes, respectively.[1] This substitution will produce abnormal hemoglobin (Hb S) instead of the normal adult hemoglobin A. Hb S has different characteristics, deoxygenated Hb S polymerizes and results in sticky crescent-shaped RBCs when exposed

to low oxygen conditions. Sickled RBCs occlude the microvascular circulation triggering microvascular vaso-occlusion, tissue ischemia and infarction.[4]

Reactive oxygen species (ROS) are byproducts of cellular metabolism. RBCs are more susceptible to oxidative stress than any other tissue. In SCD, there is over production of hydrogen peroxide, hydroxyl radical and lipid oxidation at a higher rate than normal RBCs,[5, 6] which accentuates hemoglobin denaturation with further auto-oxidation, damage to the DNA, proteins and lipids.[7]

Insulin resistance (IR) is the impaired response of the cells to insulin, leading to the compensatory increase of insulin secretion along with increased serum glucose.[3] ROS-induced insulin resistance could be due to its interference with insulin signaling and uptake or direct effect of ROS on insulin receptors.[8, 9] Moreover, ROS can inhibit the translocation of Glucose transporter type 4 (GLUT-4) causing higher glucose levels with more increase in insulin secretion.[10]

The current study was aimed to detect IR in normoglycemic SCD patients, and to assess the association of glutathione peroxidase (GPX) as an antioxidant with IR in normoglycemic patients with SCD.

Materials and Methods

A comparative case-control study was investigated on 60 patients with SCD together and 31 age and sex-matched subjects as a control group. Patients were recruited from hematology Outpatient Clinic of the Cairo University Children hospital from July 2018 to January 2019, according to the fulfilled inclusion criteria. Patients having diabetes mellitus or any other endocrinopathy and those on any drug that affected glucose metabolism were excluded from the study. Oral glucose tolerance test (OGTT) was performed and interpreted according to World Health Organization criteria (http://www.who.int/diabetes/publications/Definition) to diagnose impaired glucose tolerance. Participants were selected after the study protocol was approved by the institutional ethical committee, at the Faculty of Medicine, Cairo University according to Helsinki Declaration II. Consent forms were obtained from the patients after they were informed about the study.

Biochemical analysis: Serum glucose was measured by oxidase method. For oral glucose tolerance test (OGTT); after overnight fasting, an anhydrous maximum 75 gm of glucose was given and the specimens were collected at fasting, 1 and 2 hours after the glucose load.

Immunoassay analysis: Serum insulin was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) technique, according to the manufacturer's instructions. (Insulin Human ELISA Kit, Invitrogen, Catalog # KAQ1251, Thermo Fisher Scientific, https://www.thermofisher.com). Serum Human Glutathione Peroxidase was also measured by ELISA technique (Kit ab193767) according to the manufacturer's instructions provided by Abcam.

HOMA-IR: The Homeostasis Model Assessment of beta cell function and Insulin resistance was calculated using the following equation (fasting insulin x fasting glucose)/22.5. Calculations are based on mmol/L for glucose and mU/mL for insulin considering values more than 1.4 as insulin resistance (IR).[11]

Statistical analysis: Regular data were statistically described in terms of mean ± standard deviation (± SD), while irregular data as median and range, or frequencies (number of cases) and percentages when appropriate. Numerical data were tested for the normal assumption using the Kolmogorov Smirnov test. The Student t-test for independent samples was used for numerical values

Table 1: Demographic and biochemical characteristics of the participants

Variable	Case (N=60)	Control (N=31)	P value
Age (year), Mean±SD	18.4±7.6	20.7±8.7	0.21
Sex	32 (53.3%)	16 (51.6%)	0.87
Male (%)	28 (46.7%)	15 (48.4%)	
Female (%)			
Weight percentile Mean±SD	32.5±29.6	36±29.8	0.27
Height percentile Mean±SD	27.7±27.2	40.5±30.5	0.01
Insulin (µIU/ml) Mean±SD	13±6.6	7.2±2.7	< 0.001
HOMA-IR Mean±SD	2.5±1.5	1.1±0.3	<0.001
Glucose	80.6±16.3	73.5±15.8	0.88
FBS (mg/dl) Mean±SD			
Glucose	149.9±27.7	163.9±21.3	0.023
1-hour PP (mg/dl) Mean±SD			
Glucose	113.6±13.8	107.4±13	0.95
2-hour pp (mg/dl) Mean±SD			
Ferritin (ng/ml) Mean±SD	1417.9±839.7	126±46.9	<0.001
Hb (g/dl) Mean±SD	8.2±1.1	12±1	<0.001
GPX (ng/ml) median (range)	0.33 (0-33)	14.90 (1-30)	<0.001
Insulin resistance	34 (56.7%)	0 (0%)	<0.001
Yes	26 (43.3%)	31 (100)	
No			

µIU/ml= micro international unit per milliliter, SD= standard deviation, HOMA-IR= Homeostatic model assessment of beta cell function-insulin resistance, FBS= Fasting blood sugar, mg/dl= milligram per deciliter, PP= postprandial, ng/ml= nanogram per milliliter, Hb= hemoglobin, g/dl= gram per deciliter, GPX; Glutathione Peroxidase

for normally distributed data, while for not normally distributed data, Mann Whitney U test was used. Chi-square (x^2) test was used for categorical data and when the expected frequency was less than 5, the exact test was used. For correlation spearman rank correlation, value less than 0.05 was considered statistically significant. All statistical tests were done by Statistical Package for the Social Science (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The study evaluated serum Glutathione Peroxidase (GPX) concentration in 60 SCD patients and 31 healthy participants. The demographic and biochemical data are summarized in Table 1. Serum Glutathione Peroxidase levels were significantly lower in patients with SCD than control participants (P<0.001). Fasting insulin and HOMA-IR levels were lower in control when compared to SCD patients (fasting insulin 7.2±2.7 *vs* 13±6.6 µIU/ml (P<0.001), and HOMA-IR 1.1±0.3 *vs* 2.5±1.5, (P<0.001). Ferritin level was higher in the SCD patients compared to the control group (1417.9±839.7 vs 126±46.9 ng/ml, P<0.001). After calculation of the HOMA-IR; patients were classified into "insulin resistant" IR and non-IR as is demonstrated in Table 2. The correlation of serum GPX with clinical and laboratory data of the SCD patients are shown in Table 3. A negative correlation was found between GPX and HOM-IR (Figure 1).

Discussion

In the last decade, survival rate of the patients with SCD is increased owing to the taking advantage of supportive care and using disease-modifying agents such as hydroxyurea;[12, 13] however, giving rise to more complications related to the disease, among them are metabolic and endocrine in 2% of the patients.[14] The association of SCD with oxidative stress has been well

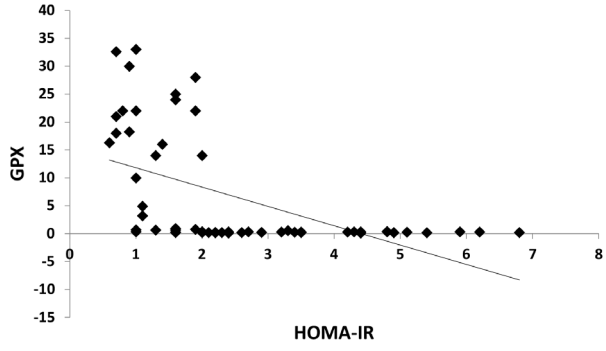

Figure 1: Correlation between HOMA-IR and serum GPX among patients with SCD.

Table 2: Demographic and clinical data of IR and non-IR group in patients with SCD

Variable	Insulin resistant (N=34)	Non-insulin resistant (N=26)	P value
Age (year): median (range)	15.5 (10-38)	18.5 (10-40)	0.97
Sex			0.94
Male	18 (52.9%)	14 (53.8%)	
Female	16 (47.1%)	12 (46.2%)	
Genotype			0.94
Hb SS	29 (85.3%)	22 (84.6%)	
Hb Sβ	5 (14.7%)	4 (15.4%)	
VOC frequency /year mean±SD	9.8±10.8	5.6±4.4	0.006
Age (month) at 1st diagnosis Mean±SD	16.5±12	26.4±30.1	0.002
Age (month) at 1st transfusion mean±SD	16.3±12.1	26.8±32.8	0.004
GPX (ng/ml) median (range)	0.29 (0-28)	16.30 (0-33)	<0.001

VOC; Vasso-occlusive Crisis, GPX; Glutathione Peroxidase

Table 3: Serum GPX correlation with clinical and laboratory data in the SCD patients

Variable	Glutathione Peroxidase	
	R	P value
Insulin	-0.643	<0.001
HOMA-IR	-0.696	<0.001
Insulin resistance	-0.625	<0.001
FBG	-0.382	0.003
Glucose, 1-hour PP	-0.237	0.068
Glucose, 2- hour PP	-0.284	0.028
Serum Ferritin	0.031	0.816
Hydroxyurea intake	-0.101	0.442
VOC severity	0.194	0.137
VOC frequency	-0.013	0.929
Hb	-0.041	0.754
hemoglobin genotypes: SS and Sβ	0.043	0.743

studied.[14, 15] It has been proved that vaso-occlusive crisis is precipitated by the accumulation of the free oxygen radicals and insufficiency of the antioxidants causing defect in vascular relaxation and endothelial adherence.[16] Glutathione peroxidase together with other enzymes including superoxide dismutase and catalase has shown efficacy as a defense mechanism against reactive oxygen species.[17]

In this study, the mean height percentile of the patients and control group was 27.7 and 40.5 cm, respectively with a P value 0.01, which was similar to another study that verified that children with SCD have a high incidence of short stature.[18]

Fasting insulin concentration was significantly higher in the patients with SCD than the control (13±6.6 µIU/ml *vs* 7.2±2.7 µIU/ml, P<0.001). Again our results were similar to the previous study who found higher levels of fasting insulin in patients with SCD.[19]

Thirty-four patients (56.7%) were identified as insulin resistant having HOMA-IR levels ≥1.4; while none of the subjects in control group demonstrated insulin resistance, which was consistent with a study showing that subjects with SCD had significantly higher HOMA-IR index versus the control group.[19]

Serum ferritin level was also different between SCD patients and the control which was explained by receiving blood transfusion.[3, 20] In the current study, there was no statically significant correlation between serum ferritin and GPX levels in SCD patients.

Patients with SCD had significantly lower values of GPX than the control group. The lower values of this enzyme with antioxidant activity could be due to overproduction of ROS in SCD patients.[5, 15, 19, 21]

According to the results of HOMA-IR, patients with SCD were divided into 2 groups: IR (n=34) and non-IR (n=26). The age at the diagnosis of patients of the IR group was significantly lower than that of the non-IR group; also, the age at the first transfusion of patients of the IR group was significantly lower than that of the non-IR group (P value: 0.002 and 0.004, respectively). It has reported that patients with SCD are more likely (2.5 times) to develop diabetes mellitus as a result of longer duration of the disease and more frequent blood transfusion.[22] Patients within the IR group demonstrate a significant frequency of vaso-occlusive crisis (VOC) in comparison to the non-IR group (mean: 9.8±10.8 crisis/year *vs* 5.6±4.4 crisis/year, P value: 0.006), although there was no significant difference in the severity of VOC between the two groups. VOC is an important indicator of both SCD severity and early death.[23]

Considering GPX concentration as an antioxidant in the SCD patients; there was a negative correlation with insulin level, HOMA-IR status and insulin resistance with r: -0.643, -0.696 and -0.625, respectively, (P<0.001 for all), suggesting an association between low GPX level and insulin resistance. Nur E et al. showed that HOMA-IR had a negative correlation with superoxide dismutase and catalase in non-obese, non-diabetic Korean children, which asserts the role of antioxidants as proactive agents against cell damage and emphasizes on the role of oxidative stress in chronic dysfunction and destruction of the different organs of the body.[24]

In our study, we could not show any correlation between GPX concentration and VOC severity or frequency and also among different hemoglobin variants.

Conclusion

The present study showed that there were high serum insulin levels and HOMA-IR in SCD patients despite normal serum glucose levels, indicating the presence of insulin resistance. The lower age at diagnosis and receiving blood transfusion, and lower concentration of antioxidant enzymes such as glutathione peroxidase appear to play key roles in developing this altered metabolism of glucose. Lower values of glutathione peroxidase in SCD patients had a negative correlation with insulin level, HOMA-IR status and Insulin resistance.

Funding: The study was funded by authors with no other funding agents or institutes.

Ethical approval: All procedures performed in the study were under the ethical standards of the Kasr Al-Ainy School of Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patient consent: All the procedures were approved by the ethical committee of the Kasr Al-Ainy School of Medicine, and written informed consent was obtained from each participant after a full explanation of the study protocol.

Conflict of Interest: None declared.

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