Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1):434-438

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Oxidative stress and cytokine role of patients with sickle cell disease

Eman R. Youness[a], Amina Abdel-Salam[b],Hanan F. Aly[c],WalaaA. AbulHamd[b], Marwa M. El-Sonbaty[d,e] and Sherien M. El-Daly[a]

[a] *Department of Medical Biochemistry, Medical Division, National Research Centre(NRC), 33 EL Bohouthst. (Former EL Tahrirst.), Dokki, Giza, Egypt*
[b]*Department of Pediatrics, New Children's Hospital, Cairo University*
,[c]*Department of Therapeutic Chemistry, Pharmaceutical and Drug Industries Division, National Research Centre(NRC), 33 EL Bohouthst. (Former EL Tahrirst.), Dokki, Giza, Egypt*
[d]*Department of Pediatrics, College of Medicine, Taibah University, Al- Madinah Al- Munawwarah, Kingdom of Saudi Arabia*
[e]*Department of Child Health, Medical Research Division, National Research Centre(NRC), 33 EL Bohouthst. (former EL Tahrirst.), Dokki, Giza, Egypt, Cairo, Egypt.(Affiliation ID:60014618)*

ABSTRACT

The present study is undertaken to demonstrate the oxidant-antioxidant status including serum lipid peroxidation product malondialdehyde (MDA), serum total antioxidant capacity (TAC), paraoxonase (PON), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) in Egyptian children with sickle cell anemia. Thus, the levels of MDA, TAC, PON, and TNF-α and VEGF were measured in 52 children with homozygous sickle cell anemia (30 males and 22 females) and 25 healthy age- and gender matched controls. The present results markedly declared significant elevated serum MDA level, while, serum AC and paraoxonase levels were significantly reduced in children with SCA as compared to control subject. On the other hand, cytokines levels, TNF-—α and VEGF levels were elevated significantly (P≤0.01) in SCA children as compared to normal control group. .There were significant change in the levels of MDA, TAC, PON, and TNF-α and VEGF were detected in sickle cell patients treated with hydroxyurea (HU) as related to untreated one. It was found that MDA level was found to be independent on PON and TAC. Also, an insignificant relationship were detected between serum MDA, TAC, PON and TNF-α and VEGF levels. Thus, it could be concluded that, the steady state between oxidant and antioxidant declaring the chronic oxidative stress, increased VOC frequency, decrease in PON level as well as increase cytokine levels in these patient.

Key words: Total antioxidant, Tumor necrosis factor-α, paraoxonase, vasoendothelia growth factor

---

INTRODUCTION

Sickle cell disease (SCD) is associated with vaso-occlusion, severe anemia, vasculopathy, and organs disorders. SCD leading to sickle hemoglobin (HbS) which arises from one mutation in the synthesis of the adult hemoglobin[1]. Sickled Hb can cause an overproduction of reactive oxygen species (ROS)[1]. These ROS cause oxidative stress which often leads to damage of cellular macromolecules; such as DNA, protein, and lipids, potentially leading to cellular apoptosis. A number of major cellular defense mechanisms exist to neutralize and combat the damaging effects of these ROS. One of these cellular defense mechanisms is the enzymatic system which includes superoxide dismutase, catalase, and glutathione peroxidase. Some previous studies stated that the activities of SOD, CAT and GSH-Px were reduced [2], while others reported that the activities of both SOD and GSH-Px were increased [3] in erythrocytes of SCD patients. A recent study has shown that the total antioxidant

capacity was reduced in SCA patients as compared to healthy controls[4].Various studies have shown that human sickle RBCs exhibit increased levels of thiobarbituric acid reactive substances (TBARS)[4].
SCD is causes 3.4% deaths in children under 5 years of age. Also, about eight to ten % SCA subjects develop childhood chronic anemia, fragility of erythrocytes as well as characteristics of vaso-occlusive crisis (i.e. thrombosis, fever, splenomegaly, joint pain, infections, lethargy, weakness, and events of stroke and heart failure)[3].Few studies indicated the role of immunologic association in SCA[3,5].It was found that the prevention of infection could correlated positively with vaso-occlusive crisis prevention in SCA patients[6]. Therefore, infectious complication could contribute to the severity of SCA by initiating inflammatory cytokines[3].Thus, this study aimed to evaluate the lipid peroxide, total antioxidant capacity, paraoxonase TNF-α and VEGF in Egyptian children with sickle cell anemia.

EXPERIMENTAL SECTION

This was a prospective case-control study conducted at the New Children's Hospital of Cairo University, Egypt, and at the Child Health and Medical Biochemistry Departments of the National Research Center, Cairo, Egypt. Fifty two children with established diagnosis of homozygous (HbSS) SCA (30 males and 22 females aged (11.10±2.10 years) and 25 healthy subjects (age- and gender-matched controls, 13 males and 12 females aged 10.70±3.00 years, were enrolled in the study after their legal guardians signed the informed consents. All recruited patients were in a steady state attending routine follow-up during the study period (from June2, 2014 to May, 2015). The study protocol was approved by the Ethics Committee of the Cairo University and by the Ethics Committee of the National Research Center, Cairo, Egypt. At enrollment, the number of severe painful episodes in the preceding 12 months was recorded (frequency of VOC per year), with a working definition of a VOC as pain in the extremities, back, abdomen, chest, or head that led to an unscheduled clinic or emergency room visit and required hospitalization, and that could only be explained by SCD, with exclusion of hand-foot syndrome, chest syndrome, osteomyelitis, and any episode of pain that was treated entirely at home [7]. Thirty patients (20 male and 10 female) were on hydroxyurea (HU) therapy with a mean dose of 20.00±3.50 (range 15-30 mg/kg/day, given orally once a day). Dose escalation was guided by clinical and hematological response with no attempt to reach the maximum tolerated dose (MTD). Blood samples for determination of MDA, PON, TAC, TNF-α and VEGF levels were collected as follows: 5mL of blood were collected into plain tubes and allowed to clot for 30 min at 25°C; it was then centrifuged at 3,000 rpm for 15 min at 4 ◦C, and the serum was separated into clean, properly labeled tubes for analysis.

Determination of lipid peroxidation*: Lipid* peroxidation was assayed by measuring the level of MDA. It was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al. [8] (in which the thiobarbituric acid-reactive substances react with thiobarbituric acid to produce a red colored complex with peak absorbance at 532 nm.

Determination of PON activity: Arylesterase activity of PON was measured spectrophotometrically in supernatants using phenylacetate as a substrate [9].

Measurement of serum TAC levels*:* Serum TAC levels were determined using an automated measurement method, which is based on the bleaching of the characteristic color of a more stable 2, 2-azino-bis (3-ethylbenz-thiazoline- 6-sulfonic acid, [ABTS]) radical cation by antioxidants(Beckman Coulter - Fullerton, CA, USA) [10]. The ABTS radical cation is decolorized by antioxidants according to their concentrations and antioxidant capacities. The results are expressed in mmol Trolox equivalents/L.

Determination of TNF-α and VEGF levels: Serum levels of both cytokines were estimated by Enzyme-linked immunosorbent assay (ELISA) method

Statistical analysis
Patients' data were analyzed using SPSS 17.0 for windows 7. Quantitative variables were expressed by mean and SD (Standard deviation), compared using unpaired t-student test and Mann-Whitney test. Spearman rank order test was used for correlating quantitative variables. Qualitative variables were expressed by numbers (Frequency) and percent compared between groups using Chi-square test. P-value was considered to be significant if < 0.05.

RESULTS

Table 1 declared that , the mean values of PON and TAC, were significantly decrease , while the MDA level was significantly increase in SCA patients as compared to normal one. Cytokines levels including TNF-α and VEGF

showed significantly elevated level in children with SCD as compared to normal control group. An insignificant change in oxidative stress biomarkers of SCA patients or cytokine levels in both genders (Table 1).

Marked significant changes in MDA,PON ,TAC, TNF-α and VEGF levels were observed in SCD patients treated with HU as compared to diseased untreated one (Table 2). An insignificant relation was detected between VOC frequency and MDA, PON, TAC, TNF-α or VEGF levels ($p \geq 0.05$).

No significant correlations were detected between PON level and frequency of VOC, MDA level, TAC,TNF-α or VEGF($p \geq 0.05$) (Table 3).

Table (1): Comparison of malonialdehyde, paraoxonase, total antioxidant capacity, VEGF and TNF-α in sickle cell patients as compared to control

Biomarkers	SCA patients (n=52)
	Mean ± SD
MDA (nmolk/mL)	3.00 ± 0.56[a]
PON (u/mL)	200.15±14.21[a]
TAC (mmol/L)	0.77±0.09[a]
VEGF(Pg/mL)	710 .00± 20.32 [a]
TNF-α(Pg/mL)	136.72 ±10.54 [a]

Males						
	Patients (30)	Control (13)			Patients (22)	Control (12)
Biomarkers	Mean ± SD	Mean ± SD			Mean ± SD	Mean ± SD
MDA (nmol/mL)	2.78±0.30[a]	0.91±0.03[b]			2.66±0.15[a]	0.93±0.03[b]
PON (u/mL)	200.80±15.0[b]	270.11±14.40[a]			204.2±24.1[b]	269.90±20.40[a]
TAC (mmol/L)	0.77±0.04[a]	1.00±0.10[b]			0.76±0.05[a]	1.02±0.08[b]
VEGF(Pg/mL)	709 .25± 20.32[a]	130.05±7.34[b]			710 .70±16.32[a]	131.00±6.45[b]
TNF-α(Pg/mL)	135.70 ±8.50[a]	27.00±5.43[b]			136.02 ±6.90[a]	26.90±4.11[b]

Biomarkers	Male Patients (n-30)	Female Patients (n=22)
	Mean ± SD	Mean ± SD
MDA (nmolk/mL)	2.78±0.30[a]	2.66±0.15[a]
PON (u/mL)	200.80±15.0[b]	204.20±24.10[b]
TAC (mmol/L)	0.77±0.04[c]	0.76±0.05[c]
VEGF(Pg/mL)	709 .25± 20.3[a]	710 .70± 16.3[a]
TNF-α(Pg/mL)	135.70 ±8.50[a]	136.02 ±6.90[a]

MDA, malondialdehyde; PON, paraoxonase; SCA, sickle cell anemia; TAC, total antioxidant capacity, Statistically significant value at p≤0.05.

Table (2): Levels of serum malonialdehyde, paraoxonase and total antioxidant capacity in sickle cell patients treated with HU and untreated HU

Biomarkers	SCA treated with HU (n=20)	SCA untreated with HU (n=10)
	Mean ± SD	Mean ± SD
MDA (nmolk/mL)	2.45±0.24[a]	3.00±0.56[b]
PON (u/mL)	230.10±12.2 [a]	200.15±14.2[b]
TAC (mmol/L)	0.99±0.13 [a]	0.77±0.09[b]
VEGF	345 .00± 11.3 [a]	710 .00± 0.3[b]
TNF-α	90.72 ±10.5 [a]	136.72 ±10.5[b]

HU, hydroxyurea; MDA, malondialdehyde; PON, paraoxonase; SCA, sickle cell anemia; SD, standard deviation; TAO, total antioxidant capacity. Unshared letter is significant at p≤0.05.

Table 3 : The relation between PON level, patients' and different biomarkers

	r-coefficient	p- value
VOC frequency (times/year)	0.24	**>0.05**
MDA(nmol/mL)	0.29	**>0.05**
PON(u/mL)	0.22	**>0.05**
TAC(mmol/L)	0.16	**>0.05**
VEGF	0.22	**>0.05**
TNF-α	**0.15**	**>0.05**

BMI, body mass index; MDA, malondialdehyde; PON, paraoxonase; TAC, total antioxidant capacity andVOC, vaso-occlusive crises. Statistical analysis reveal an insignificant correlations at p≥0.05

DISCUSSION

The role of oxidant damage to red cells in sickle cell anemia has been of interest in recent years. In a good connection with the present results, Alsultanet al [11], showed that the activities of SOD, CAT, and GSH-Px were significantly decreased in the SC subjects as compared with control normal subjects. The deficiency of the activities of these enzymes may be attributed to the high production of ROS in these patients which may destroy these antioxidant enzymes [12].Furthermore, the excess production of MDA, have additional toxic effects leading to alterations of the proteins, including antioxidant enzymes and protein receptors [13]. Alsultanetal [11] added that GSH is an essential cofactor for GSH-Px activity. It has been reported that GSH concentration was decreased in erythrocytes of sickle cell disease (SCD) individuals due to the excess production of ROS which consume GSH leading to the reduction in the activity of GSH-Px. Excess production of ROS may also have a serious adverse effect on cell membrane of RBCs resulting in protein and lipid peroxidation enhancing production of carbonyl and MDA concentrations which is the actual case [11].

In addition, SCA is associated with oxidative stress due to the imbalance in oxidant –antioxidant status in red blood cells leading to hemolysis [14]. Over production of MDA cause damage to the phospholipid of erythrocyte membrane which contributes to the formation of irreversible sickle cells (ISC) [15]. It was also suggested that the high amount of MDA can trigger erythrocyte phagocytosis [14]. Moreover, we also noted a significant ($p \leq 0.05$) reduction in the TAC level in SCA patients, comparing to control group, supporting the previous study of Foluke et al.[16], where TAC reflects the reducing property of non- protein individual antioxidant. Thus, high level of vaso-occlussive episodes is seen in patients with TAC lowest level [16].That suggested the reduction in low molecular weight antioxidants, may be triggered vaso-occlussive crisis. Various studies have shown the depleted levels of non-enzyme antioxidant molecules such as carotene, vitamin E, vitamin C, zinc and trace elements contributing antioxidant activity [13,17,18].

In concomitant with the El-Ghamrawy et al. [19], there were decreases in serum PON, TAC and an increase in MDA level. In contradictory with the present results Folukeet al. [16], declared that, HU therapy insignificantly affect the oxidative stress related to SCA children. While the present data are concomitant with the study of Folukeet al. [16] where they demonstrate that gender insignificantly affect the oxidative stress status of SCA children. Also, it was observed decrease in the activity levels of PON, and TAC [19] HU acts as an inducer of fetal hemoglobin expression, which reduces HbS polymerization in SCA patients, reducing mortality and VOC[19]. The present study declared the decrease in PON level in SCA patients. It was found that PON level was found to significantly increase and correlated positively with body weight and BMI, but it did not influenced VOC or hemolysis.

The present study reveals that children with sickle cell anemia demonstrated higher levels of serum inflammatory cytokines TNF-α and VEGF, these results are in coordinating with Veiga et al.[4] who found high level of TNF-α and VEGF despite of periodontal inflammation. Other studies have also reported similar findings to the present results, where elevated levels of inflammatory serum cytokines (TNF-α, VEGF), were detected in SCA patients [20-22]. Musa et al.[5] emphasized the role of an injured endothelium and activated monocytes resulting in an increase in VEGF level .The findings of the present study demonstrated that SCA children have high level of TNF- α which is an accordance with previous work [4]. Significant correlation was noticed in sickle cell patients between the level of inflammatory cytokines and leukocytes number, suggesting an important role of leukocytosis which is detected in SCA patients [4]. Sickle cell anemia patients have increased serum levels of circulating TNF-α and IL-8 at steady state and during crisis events [23]; these inflammatory molecules also possibly contribute to the complex mechanisms involved in vascular occlusion events. Moreover, inter-patient variations in cytokine levels could be attributed to gene polymorphisms [23]. Several studies reported on altered balance of inflammatory and anti-inflammatory cytokines in SCD patients during VOC, highlighted by elevation in pro-inflammatory cytokines [24] and reduction in anti-inflammatory cytokines levels in SCD patients compared to healthy individuals [25].
Cytokine imbalance was suggested to contribute to the pathogenesis of sickle cell pain crisis [26]. Reduced expression of anti-inflammatory cytokines, were reported in unselected SCD patients compared to healthy subjects [25] and in SCD patients with VOC compared to pain-free SCD patients [5]. The present study is also in agreement with recent study which reported elevated levels of TNF-α , in SCA Brazilian children of African descent origin [4]. IL-10 may also modulate the translation of TNF-α mRNA through interference with p38 MAPK activation, resulting in increased TNF-α production [23]. Furthermore, iron overload due to RBC hemolysis as a consequence of SCD or resulting from transfusion, leads to the generation of reactive oxygen species (ROS), and hence decreased IL-10 secretion and stimulation of TNF-α secretion [23]. These results suggest that the increased ROS generation in SCD, both in erythrocytes and from other sources, contributes to SCD pathology by activating endothelial expression of adhesion molecules and increasing endothelium–leukocyte and platelet adhesion as well as by reducing RBC deformability and making them more prone to hemolysis. Thus, ROS generation plays a part in vaso-occlusion and hemolysis, the twin pathophysiologic mechanisms of SCD.

In the present study we revealed that, children with SCD can have significant increase in the production of MDA, TNF-α, and VEGF, meanwhile positively correlate with low TAC and paraoxonase level as compared to normal control.

REFERENCES

[1]MSTonetti;NClaffey,*Journal of Clinical Periodontology*,**2005**,32(Suppl.6),210–213
[2]SDRoseff, Sickle cell disease: a review. *Immunohematology*,**2009**,25(2),67–74.
[3]LMusumadi;NWesterdale;HAppleby, Nursing Standard ,**2012**,26(26),35–40.
[4]PVeiga; C Schroth; RJ Guedes; R Freire ; SMGNogueira-Filho, *Archives of oral biology*,**2013**, 58 ,505-510.
[5]BOMusa; GCOnyemelukwe; JOHambolu; AIMamman; IH.Albarka, *Clinical and Vaccine Immunology*, **2010**,17(4),602–8.
[6]RReddeng-Lallinger; C, *Health Care*,**2006**, 36(10), 346–76.
[7]DSDarbari; OOnyekwere; MNouraie; CPMinniti; LLuchtman-Jones; SRana, *J Pediatric* ,**2012**,160,286---90.
[8]MBRuiz-Larrea; AMLeal; MLiza; MLacort; Hde Groot, *Steroids*, **1994**, 59,383---8.
[9]ADWatson;JABerliner; SYHama; BNLa Du; KFFaull; AMFogelman,*JClin Invest*, **1995**, 96, 2882---91.
[10]OErel, *ClinBiochem*,**2004**, 37, 277---85.
[11]AIAlsultan; MASeif; TTAmin; MNaboli; AMAlsuliman, *European Review for Medical and Pharmacological Sciences*, **2010**, 14, 527-538.
[12]KCWood;DNGrange, *ClinExper Pharm Physiol*, **2007**, 34, 926-932.
[13]SKJain; JDRoss; GJ Levy Duett J, *Biochem Med MetabBiol*, **1990**, 44: 37-41.
[14]ACChan; CKChow; DChiu, *ProcSocExpBiol Med*, **1999**; 222, 274-282.
[15]PHundekar; ASuryakar; AKarnik; RGhone; MVasaikar,*Biomedical Research*,**2010**, 21 (4), 461-464
[16]FFoluke ;AKayode; AJohan; KModupe,*J Natl Med Assoc*, **2008**, 100, 891---4.
[18]DAAdelekan; DIThurnham; ADAdekile, *Eur J ClinNutr*, **1989**, 43,
[19]RHasanato,*Ann Saudi Med*,**2006**,26 (1,17-21.
[20]MKEl-Ghamrawy; WMHanna; AAbdel-Salam; M MEl-Sonbaty; E RYounesse; AAdel ,*JPediatr (Rio J)*,**2014**, 90(3), 286---292.
[21]SCTaylor; SJShacks; SMVillican; JOlivares; GADinkins, *Lymphokine Research*,**1990**, 9(3,15–23.
[22]APathare; SAlKindi; AAAlnaqdy; SDaar; H Knox-Macaulay; DDennison, *American Journal of Hematology*,**2004**,77(4),323–8.
[23]NConran; STSaad; FFCosta; TIkuta, *AnnalsofHematology*,**2007**,86(4),255–61.
[24]SSarray;LRSaleh ;FLSaldanha;HHAl-Habboubi; NMahdi; WY Almawi,*Cytokine*,**2015**, 72 ,43–47.
24-Bandeira IC, Rocha LB, Barbosa MC, Elias DB, Querioz JA, Freitas MV, et al.Chronic inflammatory state in sickle cell anemia patients is associated withHBB*(/)*S haplotype. *Cytokine***2014**;65:217–21.
[25]BKeikhae; ARMohseni; RNorouzirad; M,Alinejadi; SGhanbari; FShiravi, Eur Cytokine Netw, **2013**, 24, 45–52.
[26]WBao; HZhong; DManwani; LVasovic; JUehlinger; MTLee,*Am J Hematol*, **2013**, 88, 736–40.