Oxidative Stress and Antioxidant Status in Human Infected With Kala-azar

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الخلاصة

المالون ثنائي الالديهايد (MDA) هو الدليل الشائع الذي يستعمل للكشف عن وجود الاجهاد التأكسدي في النظام البايولوجي, كما تعتبر دورة الجلوتاثايون (GSH) أهم نظام دفاعي ضد الاكسده لأزالة السمية لصنف الاوكسجين المتفاعل (ROS) في كريات الدم الحمر. هدف هذه الدراسة هو قياس مستوى المالون ثنائي الالدهايد (MDA) كدليل لحالة الاكسدة ومستوى الجلوتاثايون المختزل (GSH) كدليل لحالة عدم التأكسد لدى المرضى المصابين بالحمى السوداء (Kala-azar). تضمنت الدراسة (٦٠) مصاب بمرض الحمى السوداء وتمت متابعتهم بعد أخذهم العلاج المتمثل بالعقار (Sodium stibogluconate) لمدة (٤) اسابيع . كما شملت الدراسة (٧٠) شخص طبيعي غير مصاب بالمرض من نفس العمر والجنس لغرض المقارنه (كمجموعة تحكم سوية). بعد معالجة النتائج احصائيا تم ملاحظة مايلي:

وجود زياده داله في مستويات المالون ثنائي الالدهايد (MDA) والجلوتاثايون (GSH) في كريات الدم الحمر عند المرضى المصابين بالحمى السوداء (Kala-azar) مقارنة مع الاصحاء. أما بعد العلاج فقد أنخفضت مستويات المالون ثنائي الالدهايد (MDA) والجلوتاثايون (GSH) أنخفاضاً دالاً مقارنة مع مستوياتها قبل العلاج. النتائج تقترح أن المرضى المصابين بالحمى السوداء تكون لديهم حالة اجهاد تأكسدي الذي في الغالب يحث مضادات التأكسد الداخلي مثل الجلوتاثايون(GSH).

Abstract

Malondialdehyde (MDA) is the most commonly marker that is used to investigate the presence of oxidative stress in biological system, and glutathione redox cycle is a major antioxidant defense system for the detoxification of reactive oxygen species (ROS) within erythrocyte.

In the present study, we aimed to evaluate erythrocyte malondialdehyde (MDA) as an indicator for the oxidative status and erythrocyte reduced

glutathione (GSH) level as indicators for the antioxidative status in Kala-azar patients.

The study included sixty patient with Kala-azar and they were followed-up after their complete chemotherapy with antileishmanial drug (sodium stibogluconate) for (4) weeks. Seventy normal healthy individuals of age and sex match who served as control. After data analysis the following observations were obtained, erythrocyte (MDA) and (GSH) level were significantly increased in Kala-azar patients as compared with normal healthy controls. After treatment, erythrocyte (MDA) and (GSH) level had decreased significantly as compared to patients before treatment groups. The results suggest that Kala-azar patients are in oxidative stress which most likely induces the endogenous antioxidant such as (GSH).

Introduction

Kala-azar (or visceral leishmaniasis) is the most sever form of leishmaniasis, it is caused by parasitic protozoan Leishmania donovani and transmitted to human by the bite of infected female sand fly Phlebotomus argentipes[1].

Once parasites inoculated in the skin are phagocytozed by macrophages which in turn produces reactive oxygen species (ROS) such us super oxide anion ($O^{\cdot-}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) as a host defense mechanism for killing the engulfed leishmania donovani, and are capable of damaging lipids and other biomolecules when produced in excess[2].

Erythrocyte (MDA) level is the most convenient marker used to detect oxidative stress and lipids peroxidation [3], although there are some factors that contribute to limit its utility [4,5]. The accumulation of (H_2O_2) decreases half life of erythrocytes by increasing oxidation of poly unsaturated fatty acids of membranes and also by oxidizing hemoglobin to methemoglobin [2]. Reduced (GSH) is ubiquitous cellular antioxidant that plays a central role in defense against a variety of diseases and both exogenous and endogenous insults [6]. Its functions include the detoxification [7] of these (ROS) by direct scavenging and also as a substrate for glutathione peroxidase which removes (H_2O_2) accumulated in the cells.

Erythrocyte is most vulnerable to (ROS). The aim of the present study is to investigate the oxidative stress in erythrocyte by estimating the level of (MDA) and antioxidant status in Kala-azar patients.

Materials and Methods

A total of (130) subjects were enrolled in the study. (60) Newly diagnosed Kala-azar patient's samples are collected from medical city hospital and they were followed-up after complete chemotherapy with sodium stibogluconate for (4) weeks. (70) Normal healthy control of comparable age and sex were

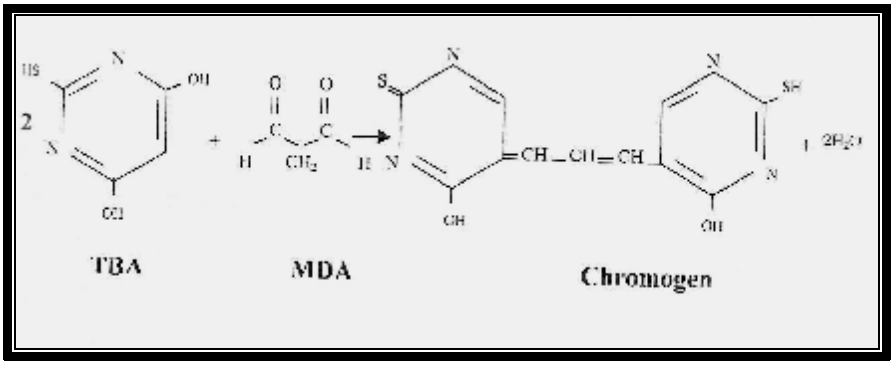
considered as normal controls. The patient before treatment group was also compared with their follow-up.

(5) ml of blood samples were taken in EDTA vials. The chemicals and reagents used in this study were of analar grade unless otherwise specified and were obtained from (BDH) chemicals Ltd., England; Hopkins and Williams, England; Sigma chemicals USA and Fluka, A.G., Germany.

Biochemical tests include erythrocyte (MDA) and (GSH) levels were determined in controls and Kala-azar patients (before and after treatment).

1 - MDA assay:

(MDA) was assayed according to the method of Ohkawa et al.[8]. With minor modification from Hirayama et al. [9]. The reaction to form thiobarbituric acid-reactive substances (TBA-RS) depends on the condensation of two molecules of (TBA) with one molecule of (MDA) to generate a reddish chromogen that absorbs light at (532) nm wave length.



2- Glutathione level: Determination of erythrocyte glutathione level was performed according to the method of Virgil [10] which is a modified version of that of Beulter [11]. Virtually, all of the non protein sulfhydryl groups of erythrocyte are in the form of reduced (GSH). 5,5-Dithiobis (2-nitrobenzoic acid) DTNB is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at (412) nm and is directly proportional to the (GSH) level [11].

3 - Hemoglobin concentration: Hb was followed using hemoglobin kit (Randox) procedure no. 540-UV 1996. In the presence of alkaline potassium ferricyanate hemoglobin is oxidized to methemoglobin. This then reacts with potassium cyanide to form cyanomethaemoglobin which absorbs at (540) nm. The intensity of this absorbance is directly related to total hemoglobin concentration.

The results were analyzed by student's "t" test to find out level of significance. P value ≤ 0.05 was considered significant. Analysis of data was performed using the software SPSS package.

Results

Table (1) demonstrates the mean ±SD of erythrocyte (MDA) level expressed (n mol/g Hb) of normal healthy controls and Kala-azar patients (before treatment group1 and after 4 week treatment group2).

Erythrocyte (MDA) level was significantly higher in group (1) as compared with controls ($p < 0.00001$). After treatment group (2) (MDA) level was decreased but it remained elevated significantly above that of controls ($p < 0.00001$).

Table (2) demonstrates the mean ± SD of erythrocyte (GSH) level expressed (m mol/gHb) of normal healthy controls and Kala-azar patients (before treatment group1 and after 4 week treatment group 2).

Erythrocyte (GSH) level was significantly higher in group (1) as compared with controls ($p < 0.0001$). After treatment group (2) (GSH) level was not significantly different from controls.

Table (3) demonstrates the mean ± SD of hemoglobin concentration expressed as (g/dl) of normal healthy controls and Kala-azar patients (group1 and group2).

Hemoglobin concentration was significantly lower in group (1) as compared with controls ($p < 0.0001$). After treatment group (2) Hb concentration was increased but it remained significantly below that of controls ($p < 0.001$)

Erythrocyte (MDA) level n mol/gHb	**normal healthy control**	**Kala-azar patients**	
		Group(1)	**Group(2)**
Sample size (n)	70	60	60
Mean ±SD	4.65 ± 1.57	7.78 ± 2.99	5.42 ± 1.97
Probability		P< 0.00001*	p< 0.00001**

*normal healthy controls versus group (1).

**normal healthy controls versus group (2).

Table 1: Biostatistical calculations and student (t-test) of erythrocyte (MDA) level for normal healthy control and Kala-azar patients (group1 and group2).

Erythrocyte (GSH) level m mol/gHb	normal healthy control	Kala-azar patients	
		Group(1)	Group(2)
Sample size (n)	70	60	60
Mean ±SD	6.78 ± 1.3	8.56 ± 1.2	7.08 ± 0.92
Probability		P< 0.0001*	Ns**

*normal healthy controls versus group (1).
**normal healthy controls versus group (2).

Table 2: Biostatistical calculations and student (t-test) of erythrocyte (GSH) level for normal healthy control and Kala-azar patients (group1 and group2).

Hemoglobin concentration g/dl	normal healthy control	Kala-azar patients	
		Group(1)	Group(2)
Sample size (n)	70	60	60
Mean ±SD	13.05 ± 1.56	7.56 ± 2.25	10.20 ± 1.78
Probability		P< 0.0001*	P< 0.001**

*normal healthy controls versus group (1).
**normal healthy controls versus group (2).

Table 3: Biostatistical calculations and student (t-test) of hemoglobin concentration for normal healthy control and Kala-azar patients (group1 and group2).

Discussion

In this study, we used erythrocyte (MDA) as an indicator for the prevalence of oxidative stress in Kala-azar patients. Our findings revealed an increased (MDA) level in erythrocyte of Kala-azar patients before treatment as compared with normal healthy controls (Table1). These findings are in agreement with Neupane et al. [2] and Seravslan et al. [12]. The elevated (MDA) level in erythrocyte of Kala-azar patients demonstrates the high incidence of these patients to oxidative stress. This evaluation could be described to an increase in the production of (ROS) as host defense mechanism against invaded parasites. Erythrocyte (MDA) level has witnessed a significant restoration after

the course of chemotherapy to the time of (4) weeks as compared with the level before treatment (Table1). The decreased (MDA) level after treatment may be causes decreased in the production of (ROS) due to the killing of microorganism.

A glutathione redox cycle is a major defense system for detoxification of (ROS) within the erythrocytes [13]. In this study, erythrocyte (GSH) level was significantly increased in Kala-azar patients before treatment as compared with healthy control (Table2). Neupane et al.[2] had reported a significant increased of erythrocyte glutathione level in visceral leishmaniasis and Huseyin V. et al.[14] in a study showed a significant higher in erythrocyte (GSH) level of cutaneous leishmaniasis. Thus, elevated (GSH) level may be attributed to the alteration in the activity of this enzyme in order to compensate with the increased (ROS) production in circulation. Erythrocyte (GSH) level dropped significantly after treatment (Table2) and all patients manifested, the (GSH) level become within the normal range of controls. The restoration of (GSH) level in Kala-azar patients may indicate a total improvement in erythrocyte metabolism as well as refreshed erythropoiesis.

The patients treated with (SSG) (sodium stibogluconate) for (4) weeks may be using (GSH) for their metabolism. This is in agreement with previous study done by carter et al. [15] who has reported that (GSH) is involved in (SSG) metabolism.

Furthermore, an inverse correlation was seen between (GSH) level and (MDA) level in erythrocyte with Kala-azar patients suggesting the ameliorative effect of higher red cells (GSH) in neutralizing (ROS) and decreasing the possibility of membrane lipid oxidation with consequence of producing (MDA).

The results in (Table3) showed decreased level of hemoglobin with Kala-azar patients before treatment, this suggest an important role of enhanced lipid peroxidation in pathogenesis of hemolytic anemia. Increased level of erythrocyte (MDA) and decreased level of hemoglobin have been described in experimental visceral leishmaniasis in hamsters by Sen et al. [16].

In conclusion, the human infected with Kala-azar are in oxidative stress. Increased ROS not only kill the parasites but also damage the cells and release MDA as a secondary marker of tissue damage. Increased erythrocyte GSH levels are induced endogenously or are not effectively utilized by the erythrocytes to counteract the ROS.

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