ISSN: 2578-7365

Research Article

Journal of Chemistry: Education Research and Practice

Effect of Narrowband-UVB Phototherapy Treatment on Total Ferroxidase Activity and Ceruloplasmin Oxidase Activity in Sera of Iraqi Vitiligo Patients

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Submitted: 05 Apr 2020; **Accepted**: 21 Apr 2020; **Published**: 15 May 2020

Abstract

Vitiligo is an acquired pigmentary disorder in which white depigmented macules and patches of skin appear on different parts of the body, affecting all ages and both sexes equally. Focal, segmen-tal, generalized and universal are considered the most characteristic patterns of vitiligo. One of the most effective treatment of this disorder is using light therapy, and in particular ultraviolet (UV) light. Among them narrowband ultraviolet B phototherapy (NB-UVB) is the most widely used. Oxidative stress is considered to be one of the possible pathogenic events in melanocyte loss. Imbalance in the oxidant/antioxidant system have been demonstrated in blood of vitiligo patients. To demonstrate the safety of using NB-UVB phototherapy treatment in vitiligo Iraqi patients. Patients with vitiligo group were treated with NB-UVB regimen as three times weekly on non- consecutive days for a total of 20 sessions (about 2 months). Total ferroxidase (activity and specific activity), ceruloplasmin concentration, ceruloplasmin oxidase (activity and specific activity) and serum copper were determined in all participants before and after NB-UVB phototherapy. The above biochemical parameters were measured in serum samples of 30 vitiligo patients before and after treatment with the narrow band UVB phototherapy. NB-UVB treatment was found to be associated with a significant reduction in copper ion concentration, but no significant differences in total ferroxidase (activity and specific activity), ceruloplasmin oxidase (activity and specific activity), and ceruloplasmin concentration. The present results provide evidence which support using narrow band UVB phototherapy as a safe method for vitiligo treatment.

Keywords: Total Ferroxidase, Ceruloplasmin Oxidase, Copper, Vitiligo, Narrowband-Uvb, Phototherapy.

Introduction

Vitiligo (an acquired pigmentary skin disorder) is considered as a multifactorial polygenic disorder with a complex pathogenesis [1]. Generally it is accepted that melanocyte destruction is the key event in pathogenesis of this disease [2]. Such destruction is explained by many theories which include: an intrinsic defect in melanocyte, autoimmune mechanism, and neuro mechanism [3].Moreover, many scientists reported oxidative stress as being the initial event in melanocyte death [3, 4].

Among the method that are used for treatment of this disease is the phototherapy which its first use was in 1997 [5]. Narrow band-UVB has been reported to be the most effective phototherapy of this disease, which aims to restore the functional integrity of epidermis & melanocytes by possible activating residual melanocyte, as well as to suppress the immune reaction [6]. Even though UV light was reported to increase the oxidative stress in blood by forming many types of cytotoxic free radicals, but the exact mechanism of the effect of using NB-UVB in the treatment of many inflammatory dermatory is not known [7, 8].

Ceruloplasmin (Cp) is a blue enzyme contains 6-7 copper (II) per molecules and binds with 90-95 % of copper ion in the blood. The first isolation of this glycoprotein enzyme was in 1944 by Holmber [9]. Multiple functions of ceruloplasmin have been described, including copper transport, iron homeostasis, oxidation of various amines (oxidase activity), oxidation of Fe(II) to Fe(III) (ferroxidase activity) for subsequent uptake by transferrin and ferritin, antioxidant activity, and endogenous modulation of the inflammatory response [10]. In other word, this protein resembles albumin & transferrin in that all of them are regarded primarily as transport proteins with multiple physiological & biochemical activities [11].One of its known function is preventing free radical production by oxidizing the highly toxic ferrous to ferric ion through its ferroxidase activity, thus it reduces the amount of free radicals (hydroxyl and superoxide) by inhibiting Fenton and Haber-Weiss reactions. Therefore, it is considered as an important antioxidant in serum [12]. In other word, this blood soluble protein, among other multi-copper ferroxidases in human body serves a basis for the precise control of iron efflux in different tissues and through its oxidase activity plays an important role in copper detoxification [13, 14].

This study is a part of a series studies carried out in our laboratory that aimed to look up the mechanism, at the molecular basis, of

using NB-UVB phototherapy to treat Iraqi vitiligo patients, as well as to justify the safety of its use as a method of treatment [15, 16]. In order to achieve this and since no clear indication has been found in the literature devoted to clarify the effect of this treatment on ceruloplasmin oxidase activity, as well as on total ferroxidase activity, the present study focus on these points.

Materials and Methods

The present study was done on 30 vitiligo patients, their mean ages ± SD was 28.5 ± 11.2 years. These cases of vitiligo were selected from Dermatology Department in Baghdad Teaching Hospital / Baghdad /Iraq. A careful history and examination ruled out any other associated disorder and use of any drug(s), which could interfere with the studied parameters. Blood samples of the patients were drawn twice before and after 20 session of phototherapy with NB-UVB. Treatment with a NB-UVB was usually administered 3 days weekly but never on 2 consecutive days, for an average of 7 weeks. Usually a standard starting dose of 200 (mJ/cm2) was used with increments 20% per treatment depending on skin phototype. For each samples total ferroxidase (activity and specific activity), ceruloplasmin concentration, ceruloplasmin oxidase (activity and specific activity) and concentration of serum copper were determined in all participants before and after NB-UVB phototherapy treatment & as illustrated below.

Determination of Total Ferroxidase Activity

Erel 1998 method [10] was used to measure this activity, using 3-(2-pyridyl)-5,6 bis(2-[5- furylsulfonic acid]-1,2,4 triazine as chromogen. The activity was calculated using the following equation: --

Enzyme Activity (*U/L*)=(*C1-C2*)×38.166

C1 = concentration of the substrate at the beginning of the enzymatic reaction (60 µmol / L).

C2 = concentration (µmol / L) of the substrate at the end of the enzymatic reaction.

Determination of Ceruloplasmin Oxidase Activity

The method that was used for determination of this activity in serum is based on Rice method [17] where p-phenylenediene was used as substrate. And the unit of activity was calculated as follows: -

International Unit=ΔA×Final multiplication

Where: ΔA = the differences in the absorbance at 540 nm.

And the final multiplication factor equal to 349 as calculated from the following relationship:

$$Final\ multiplication\ factor = \frac{10000}{\varepsilon \times incubation\ time \times b}$$

Where: A=Absorbance, the ε=Molar absorptivity of the base which equal to 1.91 L/ mol. cm, Incubation time= 15 minutes, b = 1 cm. While the concentration of ceruloplasmin (Cp) was calculated according to Holmberg, et al. and Ravin [18, 19] .

Determination of Copper Concentration

Flame atomic absorption spectrophotometry was used to determine the concentration of copper according to [20].

Statistical Analysis

Statistical program IBM SPSS version 20 was used in the statistical calculations. The paired samples t-test was used for the difference's analysis between patient before and after treatment with NB-UVB. A value of the $p<0.05$ (two-tailed) was considered statistically significant.

Results

A statistical analysis of total ferroxidase (activity and specific activity) and Cp oxidase (activity and specific activity) in the studied patients before and after NB-UVB phototherapy treatment is shown in Table 1. No significant differences were noticed in either total ferroxidase (activity and specific activity), or Cp oxidase (activity and specific activity).

Table 1: Comparison of the activity and specific activity of the total ferroxidase and Cp oxidase using paired samples t-test in vitiligo patients before and after NB-UVB phototherapy treatment

		N	Mean	Std. Deviation	P value
P value	Total ferroxidase activity (U/L) before treatment	30	51.202	3.493	0.454
	Total ferroxidase activity(U/L) after treatment	30	52.044	4.488	
Pair 2	Total ferroxidase specific activity (U/g) before treatment	30	0.638	0.094	0.093
	Total ferroxidase specific activity (U/g) after treatment	30	0.603	0.067	
Pair 3	Cp oxidase activity (U/L) before treatment	30	74.289	26.988	0.158
	Cp oxidase activity(U/L) after treatment	30	85.349	42.968	
Pair 4	Cp oxidases specific activity (U/g) before treatment	30	0.916	0.3061	0.465
	Cp oxidase specific activity (U/g) after treatment	30	0.982	0.495	

* P value is significant at the 0.05 level (2-tailed).

The [Cp], [Cu] and the ratio of copper concentrations to Cp concentration [Cu] / [Cp] are presented in Table 2. Significant differences are found in both copper ion concentration and the ratio of [Cu]/ [Cp] ratio (pairs 2 and 3), with no significant differences in Cp concentration.

Table 2: Comparison of [Cp], [Cu] and the ratio of [Cu]/ [Cp] using paired Samples t-test in vitiligo patients before and after NB-UVB phototherapy

		N	Mean	Std. Deviation	P value
P value	Cp concentration (mg/dl) before treatment	30	135.01	47.255	0.269
	Cp concentration (mg/dl) after treatment	30	149.70	77.304	
Pair 2	Cu concentration (mg/L) before treatment	30	0.097	0.0108	0.048*
	Cu concentration (mg/L) after treatment	30	0.058	0.0186	
Pair 3	[Cu]/[Cp] ratio before treatment	30	0.0067	0.00477	0.049*
	[Cu]/[Cp] ratio after treatment	30	0.0045	0.00213	

* P value is significant at the 0.05 level (2-tailed).

Discussion

It has been reported that patients with vitiligo suffer from disturbance in the oxidants/antioxidants balance, which results in increased oxidative stress in their bodies. Even though NB-UVB phototherapy has been considered as one of the most effective treatment of this disease, controversy still exist as exact mechanism of its action at the molecular basis, as well as the safety of its uses in the treatment. One of the proposed mechanisms of its action is through relieve of the oxidative stress by reversing the oxidant / antioxidant imbalance [21].

Among the important antioxidants molecule in the body is Cp which is an abundant glycoprotein that through its cuprous oxidase activity have been suggested to play an important role in copper detoxification [22]. Cp acts as antioxidant also through its ability to bind copper and thus prevent this ion from involvement in generation of free radicals in the body. Meanwhile the release of iron from liver relies on Ferroprotein (Ferroportin is the only known iron exporter [23]) and the ferroxidase activity of many multi-copper ferroxidase proteins among these proteins is Cp [24].

No studies have been reported so far on the effect of the phototherapy treatment on Cp, neither as a protein nor as an enzyme performing its oxidase activity. The observed non-significant alteration in this protein concentration as well as in its oxidase activity indicated that Cp may play a minor role in the mechanism of this type of therapy. On the other hand, Cp among other multi-copper proteins (zyklopen & hephaestin) through their ferroxidase activities serves as a basis for control of iron efflux in different tissues [24]. This later metal in its ferrous state with copper are known to generate free radicals through their involvement in Fenton reaction. As results from Table 1, the total ferroxidase activity seems to have no involvement in this type of treatment. Meanwhile the observed reduction in the measured copper concentration, as well as in the ratio of copper ion concentration to the concentration of ceruloplasmin [Cu]/ [Cp], which refers to the amount of available free copper ion in the serum, seems to be one of the factors that contributes in the reduction of free radical generation upon the phototherapy treatment As it is clear from Table 2. the copper concentration decreases significantly after the treatment. Such decrease suggests that a disturbance in the oxidant / antioxidant ratio is induced by phototherapy which may be explained as follows. On one side such reduction in copper concentration means a reduction in the free radical generation via Fenton and Haber-Weiss reactions in which copper is involved. On the other side copper is structural component of the one of the major antioxidants mechanism in human bodies i.e. superoxide dismutase, which catalyses the scavenging of superoxide ion. Therefore, the observed decline in the concentration of copper causes a reduction in SOD activity. Such reduction in this enzyme activity may be one of the mechanisms that lead to accumulation of free radicals in the vitiligo patients.

Based on the above results, it seems that copper is one of the key player in the disturbance of the oxidant / antioxidant reported to be induced by this type of phototherapy.

In order to further justify using this type of treatment, as a safe method with certainly, further work is recommended to investigate the changes in other biochemical parameters with using a larger number of participants as well as longer period of phototherapy treatment.

Conclusions

NB-UVB may be considered as a viable and safe therapeutic option in the treatment of vitiligo.

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