Systemic Redox Imbalance Along with Increased Serum Sialic Acid is Prevalent in Patients with Active Vitiligo: A Study from a Tertiary Care Teaching Hospital of Eastern India

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Abstract

Background: Vitiligo is one of the common depigmenting disorders causing disfigurement and affecting the quality of life. Redox imbalance is known to play a contributory role in melanocyte destruction. Serum sialic acid (SA) is an important marker of the acute-phase response and is associated with oxidative protein damage. Aim: The aim of this study was to analyze the status of oxidative stress markers and serum SA in vitiligo patients and to correlate the same with disease activity. Materials and Methods: The different oxidative stress parameters namely superoxide dismutase (SOD), malondialdehyde (MDA), and serum SA were measured spectrophotometrically using standard biochemical methodologies in all the study subjects. Results: Serum SOD and MDA values were higher in patients with active vitiligo (n = 23) as compared to stable vitiligo (n = 20) and healthy controls (n = 20). The MDA/SOD ratio was higher in patients with active vitiligo (P<0.0001). Serum SA was increased in active vitiligo as compared to stable vitiligo and healthy controls (P<0.0001). Conclusion: This study indicates that patients with active vitiligo demonstrate enhanced MDA/SOD ratio and increased serum SA. The studied parameters can serve as an important tool to monitor disease activity in vitiligo.

Key Words: Active vitiligo, malondialdehyde, redox imbalance, serum sialic acid, superoxide dismutase

Introduction

Vitiligo is an acquired manifestation of the skin characterized by depigmentation and occurs globally irrespective of sex, age, and race. The disease manifests into progressive melanocyte destruction, and the rate of depigmentation varies from person to person. Although vitiligo is nonfatal, it really affects the social status of the patients more badly than any other fatal diseases. Most of the patients throughout the world are affected by vitiligo irrespective of their present social status. Although the worldwide prevalence rate is 1%, in some parts of India, it is as high as 8.8%.[1] Several hypotheses have been made regarding the underlying pathomechanisms in vitiligo, but still the disease pathology is incompletely understood.

Growing amounts of evidence indicate a prominent role of oxidative stress in vitiligo-related melanocyte degeneration.[³] Majority of the serum proteins are glycoproteins in nature and in which the glycans have terminal sialic acid (SA) residues. SAs are a diverse family of sugar units with a nine-carbon backbone. It is well known that the changes in protein glycosylation play an important role in the pathogenesis and progression of diseases. Alterations of glycoprotein expression are known to induce oxidative stress and tissue damage.[⁴] As SA levels reflect the glycosylation changes and acute-phase reaction in diseases, investigating their profile will provide the earliest glimpse of the disease activity.[⁵]

To evaluate whether disease activity is associated with a systemic oxidative stress, this study was undertaken.
to investigate the oxidation/antioxidation parameters in active and stable vitiligo as compared to controls. Superoxide dismutase (SOD) level reflects the antioxidant status; malondialdehyde (MDA) level on the other hand is an indicator of lipid peroxidation; and MDA/SOD ratio, an index of redox imbalance, was estimated in patients and control population. Since SAs are regarded as acute-phase indicators, their estimation will give an insight into the disease activity.

Taken together, this study aimed to evaluate the redox status in the vitiligo patients in comparison with healthy controls along with elucidating the status of serum SA in those study subjects. Further, the utility of these biochemical/immunological markers to monitor the disease status of vitiligo patients had also been explored in this study.

Materials and Methods

Study population

This study was a cross-sectional, analytical study of patients with vitiligo. Peripheral blood was collected from 43 informed and written consented patients with vitiligo from the Dermatology Outpatient Department at School of Tropical Medicine, Kolkata. This study was approved by the Institutional Ethics Committee. The study duration was from December 2015 to August 2017. Vitiligo patients recruited in this study were classified into active and stable disease cases, with the criteria for active disease being extension of existing lesions and/or appearance of new lesions within the past 6 months. Percentage of body surface area (BSA) involved was measured by taking the patient’s palm as 1% of the total BSA. The inclusion criteria were all patients with vitiligo who were above the age of 18 years and who had no history of smoking or alcohol intake, and were not on any other medication. Clinically diagnosed vitiligo patients with any other disease symptoms were excluded from this study. The same number of age- and sex-matched healthy subjects without any clinical manifestation of depigmentation of the skin or any other autoimmune diseases from the teachers, students, and staff of the institute was also enrolled. Blood serum was isolated by centrifugation and stored at −20°C refrigerator until further use.

Evaluation of oxidative stress in all the study subjects

MDA was estimated in the serum of all study subjects by following Pasha and Sadasivudu’s method. SOD enzymatic activity with respect to inhibition of autooxidation of pyrogallol in the serum was determined by following the method of Marklund as described by Jyothi et al.

Estimation of serum sialic acid

Serum SA was measured by a modified method of Warren as described earlier. A total of 100 µL sample was hydrolyzed by 10 µL of 1.5 mol/L sulphuric acid and incubated at 80°C for 1 h. After cooling at room temperature, 50 µL of 0.2 mol/L sodium metaperiodate was dissolved in 10.5 mol/L, 85% orthophosphoric acid and was added to the cooled hydrolyzed samples and kept at room temperature for 20 min. Further, 500 µL of 0.77 mol/L sulphate sodium meta-arsenite was dissolved in 56 mmol/L sulfuric acid and was added to the sample containing mixture until the yellow-brown color disappeared. Again, 1.5 µL of 42 mmol/L thiobarbituric acid in 506 mmol/L sodium sulfate was added and the mixture was incubated at boiling water bath for 15 min and cooled to room temperature. Following this, 2 µL of cyclohexanone was mixed with the cooled solution and mixed vigorously. Further, the mixture was centrifuged for 3 min at 500 g (relative centrifugal force). The supernatant was taken and the absorbance was read at 549 and 513 nm. The difference between A549 and A513 was calculated, and the sample concentration was read from the calibration curve of N-acetyleneuraminic acid.

Statistical analysis

GraphPad Prism software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for all statistical analysis. P<0.05 was taken as significant in all cases. t-test was done to determine the statistical significance among two groups of data. All data were represented as mean ± standard deviation.

Results

The study population included 43 patients with vitiligo of which 23 were active and 20 were stable. Of the 43, 22 (51.6%) were female [Table 1]. The age and gender distribution between groups was comparable [Table 1]. The overall disease duration (years) and median with range in active vitiligo was 1.00 (0.25–11.00) and 3.0 (0.58–14.00) in stable vitiligo [Table 1]. Vitiligo disease activity score was calculated as described earlier. The average vitiligo disease activity score

| Table 1: Study population |
|---------------------------|
| **Study parameters**      | Healthy controls | Stable vitiligo | Active vitiligo |
| No. of study subjects     | 20               | 20              | 23             |
| Age (mean±SD)             | 32±10.32         | 33.50±12.67     | 31.22±16.66    |
| Sex ratio (female:male)   | 1:1              | 1:1.22          | 1:0.91         |
| Disease duration (years)  | NA               | 3 (0.58–14)     | 1 (0.25–11)    |
| Percentage of total body  | NA               | 7 (1–20)        | 2 (1–10)       |
| surface area involved     |                  |                 |                |
| VIDA score                | NA               | +4 (+1+4)       | +4 (+2+4)      |
| VIDA score: Vitiligo disease activity score, NA: Not available, SD: Standard deviation |
was +4 in active vitiligo patients and was +1 in the stable variant. We have seen no gender-based prevalence in patients studied. However, the occurrence of vitiligo is found to be more in younger individuals [Table 1].

MDA level was found to be 1.66-fold increased in active vitiligo patients [Figure 1a]. SOD level in patient serum was found to be 1.13-fold increased in active vitiligo [Figure 1b]. Whereas, the MDA/SOD ratio is 1.45-fold increased in active vitiligo patients than controls and this was found to be significant by t-test [Figure 1c and Table 2].

Serum SA was found to be 1.21-fold increased in active vitiligo patients than controls [Figure 2b], and the changes are statistically significant (P<0.0001) [Table 2]. Values of serum SA were extrapolated from generated SA standard curve [Figure 2a]. Further, serum SA bears good correlation (r=0.99) with MDA/SOD ratio in all the enrolled active vitiligo patients.

Discussion

Substantial depigmentation of skin due to vitiligo has not only clinical importance but also needs social awareness to improve the lifestyle of patients. The successful management of idiopathic skin patches requires proper understanding of the disease pathophysiology.

Redox imbalance is known in vitiligo. There were no data of MDA/SOD of the same patients. MDA/SOD is an important marker of progression of inflammation and reported in other diseases. In this study, MDA levels were found to be high in serum, whereas SOD activity of serum was slightly raised than healthy persons. Lipid peroxidation is measured by MDA assay. An increased MDA activity signifies more disruption of cell membrane due to the presence of free radicals. Free radical homeostasis is necessary for the organism. To counterbalance generation of free radicals, the living system has antioxidant enzymes such as SOD. MDA/SOD ratio thus gives an insight into the overall systemic redox status of the patient, which was found to be significantly increased in patients. Some other inflammatory diseases also reported high MDA/SOD ratio. As initiation of melanocyte cell destruction leads to skin depigmentation, a high amount of free radicals are generated which eventually destroys the cells, thus leading to a high MDA/SOD ratio.

| Study parameters | Healthy controls | Stable vitiligo | Active vitiligo |
|------------------|-----------------|-----------------|----------------|
| MDA (nmol/mL)    | 0.51±0.06       | 0.67±0.02       | 0.85±0.06      |
| SOD (U/mL)       | 4.88±1.29       | 6.19±1.28       | 5.52±1.34      |
| MDA/SOD          | 0.11±0.04       | 0.11±0.02       | 0.16±0.04      |
| Sialic acid (mg/L) | 618.90±11.01   | 632.20±16.02    | 750.30±55.75  |

SD: Standard deviation, MDA: Malondialdehyde, SOD: Superoxide dismutase

Figure 1: Biochemical oxidative stress parameters of the study subjects. Panels (a-c) describe malondialdehyde, superoxide dismutase, and malondialdehyde/superoxide dismutase in all the study groups. ***represents P<0.0001, **represents P<0.001

Figure 2: Serum sialic acid in all study subjects. Panel (a) describes sialic acid standard curve followed in this study and panel (b) describes serum sialic acid levels in different study groups. ***represents P<0.0001
We observed a significant (P<0.0001) increase in the serum SA levels in active vitiligo patients as compared to stable vitiligo and healthy controls. In our study, total serum SA levels were significantly increased parallel to oxidative stress.

Hence, it can be suggested from this study that increased levels of SA might be considered as a defense mechanism against the increased oxidative stress in active vitiligo. SA is known to have antioxidant property and can act as a free radical scavenger. In conclusion, it can be said that oxidative stress may be involved in the pathogenesis of active vitiligo. The results of our study suggest higher MDA and SOD levels along with SA thus supporting the higher oxidative stress hypothesis in vitiligo.

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**Conflicts of interest**

There are no conflicts of interest.

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