Evaluation of antioxidant and oxidant status, including levels of Malondialdehyde (MDA) and Superoxide Dismutase (SOD), in the Indian population with alopecia areata

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Abstract---Abstract: Hair loss is caused by alopecia areata (AA), which is a non-scarring inflammatory and autoimmune disorder. The aetio pathogenesis of alopecia areata is uncertain, however autoimmune, genetic predisposition, emotional, and environmental stress are all thought to have a role in its development. Antioxidant/oxidant balance disruption is a common sign of autoimmune, psychological, and environmental stress. As a result, our research looks at the effects of oxidative stress in alopecia areata. Materials and Methods: This was observational prospective study, The present study included 150 A.A patients(cases) and 150 controls attended to Department of Dermatology in collaboration with Department of Biochemistry, LN Medical College and Research center & J.K Hospital, Bhopal. The levels of Iron and Ferritin was estimated by colorimetric Method Results: A total of 300(150 cases of AA +150 controls were included SOD & MDA with mean+ SD are given in the Table no.1. The two groups were comparable (P<0.0001), (P<0.0001). The difference in the values SOD and MDA parameters in respect of these groups was highly statistically significant (P<0.0001*).
Introduction

Internally, such as enzyme activity or activated neutrophils, and externally, such as ultraviolet radiation, reactive oxygen species (ROS) are formed, creating oxidative stress on the skin (UV). The etiology of skin diseases such as vitiligo and alopecia areata is thought to be linked to an insufficient antioxidant system and rising levels of reactive oxygen species (ROS). Vitiligo is a common pigmented skin disorder in which melanocytes are selectively killed, according to the auto cytotoxic hypothesis. Oxidative stress has been considered as the major pathogenic event in melanocyte degeneration, with H2 O2 accumulation in the epidermis of those with active disease.

A change in the antioxidant pattern has been detected in the skin [8] erythrocytes, [9],[10] peripheral blood mononuclear cells [9],[12] and serum [13,14] of vitiligo patients, with significantly higher levels of Superoxide Dismutase (SOD). The activity of catalase (CAT) in the epidermis [15], peripheral blood mononuclear cells [9], and epidermal cells [16] has been found to be decreased. Alopecia areata (AA) is a common and recurrent chronic inflammatory disorder of the hair and nails. It can affect people of all ages [17] and both sexes. Many factors have been proposed as potential contributors to the aetiology and pathogenesis of AA, including the patient’s genetic constitution, family history, atopic state, nonspecific immune and organ-specific autoimmune reactions, possible emotional stress, infections agents, and neurological factors.

Method

Material And Methods

This was a hospital-based observational prospective study undertaken in the Department of Dermatology in cooperation with the Department of Biochemistry at LNMC and J.K. Hospital in Bhopal from November 2019 to November 2020, with 150 AA patients (cases) and 150 controls. All patients with AA who came in for routine screening at the Department of Dermatology had their blood samples taken and utilized to calculate serum Iron and serum Ferritin levels. The hospital’s specialized dermatologist had diagnosed all of the AA patients.

Prospective open label observational research was used as the study design.

The study will last 18 months.

300 patients were included in the study.

Calculation of sample size: The sample size was calculated using a single proportion design.
The population from which we randomly chose our sample was estimated to be around 20,000 people. We assumed a 10-percent confidence interval and a 95-percent confidence level. The exact sample size for this study was 150 Alopecia Areata sufferers and 150 healthy people as controls.

**Subjects and selection method:** Patients with Alopecia Areata who presented to the Department of Dermatology affiliated with LNMC & JK Hospital with Alopecia Areata formed the research population.

Group A (N=150) - was made up of 150 healthy people who acted as a control group (Non-Alopecia)

150 individuals with Alopecia Areata were in Group B (N=150).

**Inclusion criteria**

1. Be willing to engage in the study
2. Be of either sex
3. If you're under the age of eighteen,
4. Patients signed a written permission form and consented to participate in the trial.

**Exclusion criteria**

1. Pregnant women and breastfeeding mothers are excluded.
2. Females or those on hormonal contraceptives, and
3. patients with endocrine abnormalities.
4. Hereditary variables are not taken into account.
5. Those who are undergoing significant surgery
6. Participants taking iron, folic acid, or vitamin B12 supplements.

**Methodology for the procedure**

After obtaining written informed consent, the data of the selected patients was collected retrospectively using a well-designed questionnaire. The questionnaire asked about age, gender, nationality, height, weight, and consanguineous marriage, as well as physical activity and lifestyle behaviours including smoking and drinking.

Throughout the trial period, the same team of laboratory technicians performed all biochemical assays using the same approach.

- SOD
- MDA

The Beckman Coulter AU 480 Auto analyzer was used to measure these.

**Analytical statistics**
On a personal computer, data was created using MS Excel and analyzed using IBM's SPSS programmer version 20. The diagnostic accuracy of each biomarker was established. The data was statistically characterized using range, mean, standard deviation (SD), and frequencies (number of occurrences). The Two paired t-test (Independent) was used to do a comparison between two groups. A P value of less than 0.0001 is statistically significant. The significance level of P 0.0001 was used as the cutoff value.

**Ethical Approval**

The study was authorized by the institutes' ethical committee, and all patients gave their informed permission.

**Result**

A total of 300(150 cases of AA +150 controls were included T3, T4 & TSH with mean± SD are given in the Table no.1. The two groups were comparable (P<0.0001), (P<0.0001).

**MDA**

MDA levels in controls is $3.5393 \pm 0.7644$ nmol/ml

MDA levels in AA is $6.6452 \pm 1.4846$ nmol/ml

❖ The difference in the values Serum Iron parameters in respect of these groups was highly statistically significant (P<0.0001*)

**SOD**

SOD levels in controls is $191.72 \pm 28.571$ units/ml

SOD levels in controls is $55.747 \pm 22.41$ units/ml

❖ The difference in the values Serum SOD parameters in respect of these groups was highly statistically significant (P<0.0001*).

Table no 1 Shows metabolic parameters of patients of the 2 groups (Both Controls & AA).

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROLS</th>
<th>ALOPECIA AREATA PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>MEAN ± SD</td>
<td>MEAN ± SD</td>
</tr>
<tr>
<td></td>
<td>3.5393 ± 0.7644</td>
<td>6.6452 ± 1.4846</td>
</tr>
<tr>
<td>SOD (units/ml)</td>
<td>191.72 ± 28.571</td>
<td>55.747 ± 22.41</td>
</tr>
</tbody>
</table>
FIG:1 The Pie diagram showing No. of samples in ALOPECIA AREATA PATIENTS and controls

Table-2: This table shows the Critical Value, T-value and P-Value
### Discussion

In research by Koca R comparing blood antioxidant levels in patients with alopecia and healthy persons, the following conclusions were discovered: serum malondialdehyde levels were considerably higher in patients compared to healthy people. The activity of the superoxidase dismutase enzyme was significantly reduced when compared to that of healthy persons. This study shows that superoxidase enzyme activity is connected to an increase in fat peroxidation in individuals with areata alopecia, and that fat peroxidation is a key factor in the aetiology of areata alopecia (Koca et al., 2005).

Our findings show that AA patients had lower SOD and greater MDA levels in their blood than controls, with a statistically significant P value (p<0.0001).

SOD levels increased, according to Perihan ztürk1, zer Arcan’s study. This is almost certainly an anti-oxidant defence system. Our findings imply that lowered SOD activity may not be enough to limit superoxide radical generation since extra radicals may be produced and not easily broken down by SOD. In contrast to the previous studies (140), their SOD results differed from those of Akar et al (173). Different laboratory procedures, different materials (such as serum or tissue) analyzed, different inclusion/exclusion criteria for cohort selection, or a compensating mechanism in the body might all explain the discrepancies in the results.

With a statistically significant P value (p<0.0001), our findings reveal that AA patients had lower SOD and higher MDA levels in their blood than controls.

Alopecia Areata is an autoimmune disease that has been kept alive. Many studies have revealed that oxidative stress plays a role in alopecia Areata, with hydrogen peroxide (H2O2) accumulating in the affected skin's epidermal layer [161]. Oxidative stress is caused by reactive oxygen species (ROS) and other radicals. The formation of reactive oxygen species has been related to a decrease in antioxidant levels in the skin (ROS). [6] MDA levels are greater in Alopecia Areata, whereas SOD levels are lower, according to Naziroglu et al’s research.

As a result, we investigated MDA levels in alopecia patients to determine oxidative stress, SOD activity, and the antioxidant system.

SOD is a metalloenzyme family that protects cells against superoxide radical damage. The conversion of superoxide to H2O2 is accelerated by SOD. In our

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Critical Value</th>
<th>t-value</th>
<th>P-value</th>
<th>Statistically</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.971</td>
<td>22.7037</td>
<td>P&lt;0.0001</td>
<td>extremely statistically significant</td>
</tr>
<tr>
<td>SOD (units/ml)</td>
<td>1.969</td>
<td>45.7087</td>
<td>P&lt;0.0001</td>
<td>extremely statistically significant</td>
</tr>
</tbody>
</table>
study, however, we found a low quantity of SOD in the sera of AA patients compared to controls. SOD activity in AA patients has been studied in a variety of ways, with inconsistent results.

This finding of SOD in AA patients' sera is similar to that of Rafet et al. [32], who found a considerably lower amount of SOD in AA patients' sera than controls.

In the scalp tissue of individuals with active AA, however, Akar et al. [27] discovered a significant increase in SOD activity. They believe that antioxidant defences aren't a concern for AA. AA patients, however, exhibited lower SOD and higher MDA levels in their blood than controls, with a statistically significant P value (P<0.0001).

Dell'Anna et al. [118], on the other hand, discovered that leukocytes from Alopecia Areata patients showed higher SOD activity [28]. In one study, SOD levels were found to be normal, whereas in others, they were shown to be abnormally high. [43], [7], and [89] Maresca et al. Alopecia Areata sufferers, on the other hand, showed lower levels of SOD and greater levels of MDA than controls. Both SOD and MDA have extremely statistically significant P values (P<0.0001) in our data.

**Conclusion**

Plasma MDA levels were high in AA patients, which might harm erythrocytes exposed to high free radical concentrations. These findings indicate that the oxidant-antioxidant system is imbalanced, hinting that oxidative stress may play a role in Alzheimer's disease etiopathogenesis.

These data show that oxidative stress-related variables are greater in AA patient scalp scrapes than in controls, implying that they may play a role in disease etiology. Changes in antioxidant enzyme activity in AA patient scraping samples might be a local outcome of elevated levels of oxidative stress.

With this illness, oxidative stress may injure every layer of the skin, not just the hair follicle.

These findings support the hypothesis that free radical-mediated damage is the initial stage in the pathophysiology of AA because they reveal that the oxidant-antioxidant system is out of equilibrium.

**References**

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