Cytokines level and oxidative damages in some egyptian patients with alopecia areata

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Alopecia areata (AA) is a chronic, inflammatory and autoimmune disease. In human scalp areas affected by alopecia areata, an excessive expression of IL-1β and disequilibrium in pro-inflammatory and anti-inflammatory cytokines was detected. The aims of the present research was to determine the level of lipid peroxide (MDA), paraoxanase 1 (PON 1), interleukine 1β (IL1β) and IL6 in serum of AA male and female patients and find the possibility relation. A total of 100 participant from the Egyptian population, 50 were patients with AA of various types and 50 were healthy controls. Serum MDA, PON, IL1β and IL6 levels were measured in both males and females AA patients. AA patients comprised 30 male and 20 female patients (15–52 years). The healthy controls comprised 23 male and 27 female individuals (15–52 years). Serum MDA, IL1βlevels were significantly increased in AA patients compared to controls. While significant decrease in both PON and IL6 was detected. The female participants in patients showed significantly lower IL6, while significantly higher IL1β levels compared to male participants. AA is associated with low levels of IL6, PON, while high levels of IL1β and MDA in patients AA. Female participants show severe effect in both IL1β and IL6. More studies are required to prove its possible role in AA pathogenesis.

Keywords: adaptive immunity, alopecia areata, autoimmunity, innate immunity, antioxidant, PON, lipid peroxide, cytokines.

INTRODUCTION

Alopecia areata (AA) is non-scarring hair loss and considered one of chronic, inflammatory and autoimmune disease (Alkhalifah et al., 2010). AA is a disease with various clinical displays. It affects the scalp, although any hair bearing area of the skin can be affected (Alkhalifah et al., 2010). The loss of hair shows restricted patches, hair loss development and participation of the entire scalp (alopecia totalis), or the participation of the entire body (alopecia universalis) (Yang et al., 2013). Histologically, AA is linked with auto-aggressive T cells directed against the anagen hair follicles (Prie et al., 2015). The aethiopathogenesis of AA is unclear and various factors such as the genetic background of patients, emotional and environmental stress are implicated in its progress (Alkhalifah et al., 2010; Prie et al., 2015). The balance disturbance of antioxidant/oxidant is a prevalent character in the stress of autoimmune, emotion and environment.

IL-1β over expression is recorded in patients with AA at the early stages of disease, while disease susceptibility and severity are determined by IL-1α receptor antagonist and IL-1a polymorphisms. In severe AA patient's, a more expression of the disease is detected due to polymorphisms of gene, have inadequate levels
of IL-1 receptor antagonist, (Gregoriou et al., 2010), while an elevated frequency of the allele 2 of the IL-1 receptor antagonist gene was demonstrated in extensive AA hair loss patients (Tarlow et al., 1994).

Lipid peroxidation demonstrates the critical point of oxidative stress, which produce post exposure of cell membrane lipids to ROS (Koca et al., 2005; Yenin et al., 2014). Lipid peroxides and their malondialdehyde (MDA) products can influence the common function of most mammalian cells and their level linked with the lipid peroxidation degree (Briganti et al., 2003). Further, Paraoxonase 1 (PON 1) is a Ca-dependent esterase connected with HDL and participates in HDL anti atherogenic effects (Ramadan et al., 2013). Lower activity of paraoxonase has been found in serum and tissues of alopecia areata patients. So, the objective of the study was to estimate the serum levels of PON1, MDA, IL1β and IL6 in AA patients and explore a probable relation in both male and female.

MATERIALS AND METHODS

The present study included 100 individuals from the Egyptian population: 50 AA patients and 50 healthy controls. All patients of AA were recruited from the excellence Centre of National Research Centre, Egypt; during a period of 6 months (1 January 2017 to 30 June 2017). The study protocol was approved by the Medical ethical Committee of National Research Centre. Written informed consent was signed by patients and by one parent in the case of minors (below 18 years). The Patients with any scalp disorders such as irreversible alopecia, trichotillomania and scalp psoriasis were excluded from the study. The patients who had received any treatment within previous 3 months were excluded from the study, as well as patients with any diseases based on the immune patho mechanism, which could influence serum concentrations of IL-1β, IL6 deficiency or a disease that could affect their levels (e.g. renal diseases, cancers, Systemic LupusErythematosus or psoriasis) and those who were experiencing spontaneous re growth of terminal hair at the time of presentation. History of the patients, including age sex, duration, history of adequacy of sun exposure and family history of similar condition, was recorded carefully.

The extent of scalp hair loss was determined by dividing the scalp into four quadrants, followed by visually determining the percentage of scalp hair loss in each quadrant and adding the numbers together, with a maximum score of 100%. This was determined according to the severity of alopecia severity in alopecia tool (SALT) scores (Olsen et al., 2004). Scalp hair loss (S) was classified as follows:

- S0: no hair loss;
- S1: 25-49% hair loss;
- S2: 50-74% hair loss;
- S3: 75-99% hair loss;
- S4: 100% hair loss.

Patients were classified according to the pattern of AA into patchy AA or extensive AA (alopecia totalis/alopecia universalis (AT/AU)).

**PercentageChange:**

Mean Controls – Mean patients / Mean Controls x100

**Statistical analysis**

All statistical calculations were carried out using computer programs statistical package for the social science (SPSS; SPSS Inc., Chicago, Illinois, USA) version 15, for Microsoft Windows. Comparisons of numerical variables between the study groups were made using the Co-state, where unshared letters are significant at P≤0.05.

**Biochemical Determinations**

**Lipid peroxidation**

Lipid peroxidation was assayed in serum by measuring the thioisobarbituric-acid-reacting substances as previously described by Ruiz-Larrea et al. (1994) in which the thioisobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm (using UV-VIS Recording Spectrophotometer (Shimadzu Corporation, Australia).

**Interleukins-6 and IL-1β**

This High Sensitivity ELISA enables the analysis of Interleukin-1beta in low concentrations from serum. They measured by ELIZA, the microtiter plate provided in this kits has been pre-coated with an antibody specific to factor to be measured (IL-6). Standards or samples are then added to the appropriate microtiter plate wells with a biotin conjugated antibody specific to factor to be measured (IL-6). Next, Avid in conjugated to Horseradish Peroxidase (HRP) is added to each micro plate well and incubated. After TMB substrate solution is added, only those wells that contain parameter to be measured, biotin-conjugated antibody and enzyme conjugated Avid in will exhibit a change in color. The enzyme-
substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm.

**paraoxonase**

We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25°C. The basal assay mixture included 1.0mM paraoxon and 1.0 mM CaCl₂ in 0.05 M glycine buffer, pH 10.5. One unit (IU) of paraoxonase activity is defined as 1μmol of p-nitrophenol formed per min, and activity was expressed as U/l of serum (Bafikol et al., 2005).

**RESULTS**

AA patients comprised 30 male and 20 female patients (15–52 years). The healthy controls comprised 27 male and 23 female individuals (15–52 years). Serum PON, and IL6 levels were significantly decreased in AA patients, while MDA and IL1β levels were increased compared to controls (P≤0.05). No significant differences in PON, MDA level between male and female participants, while significant difference in IL1β and IL6 was detected between males and females patients comparing with their corresponding controls with percentages change 87.80% and 55.55% for IL1β and 33.89 and 45.39% for IL6, respectively (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>MDA(umole/L)</td>
<td>6.5.13±0.05</td>
<td>6.58±0.15</td>
</tr>
<tr>
<td>PON(U/l)</td>
<td>22.75±1.2</td>
<td>20.75±1.2</td>
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<tr>
<td>IL1β(pg/ml)</td>
<td>0.41±0.01</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td>IL6(pg/ml)</td>
<td>1.21±0.11</td>
<td>1.41±0.41</td>
</tr>
<tr>
<td>Patients (N)</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>MDA(umole/L) % change</td>
<td>3.00±0.03</td>
<td>2.90±0.00</td>
</tr>
<tr>
<td>PON(U/l) % change</td>
<td>14.13±1.0</td>
<td>13.00±1.20</td>
</tr>
<tr>
<td>IL1β(pg/ml) % change</td>
<td>0.77±0.02</td>
<td>0.70±0.03</td>
</tr>
</tbody>
</table>

Table 1: Comparison between the studied groups as regards serum PON, MDA, IL-1β and IL-6.

Data are expressed as Mean ±SD of 50 male and 50 female subjects either control or patients. Statistical analysis is carried out using SPSS computer program, where unshared letters are significant at P≤0.05.

**DISCUSSION**

Several works demonstrated high MDA levels in plasma, erythrocytes and biopsies from scalp in AA patients. The levels of tissues TBARS was two times higher in early phase than late AA phase compared to normal subjects (Naziroglu and Kokcam, 2000; Akar et al., 2005). Also Koca et al., supported previous works and detected serum MDA higher levels in AA patients (2005). Further, Abdel Fattah et al.,(2011); Shah and Sinha (2013) declared elevated MDA levels in plasma and tissue of AA patients. The authors also observed, more drastic effects for poly AA, alopecia totalis, alopecia universal which is directly proportional with the time (≥6 months). i.e. the longer the disease, the higher MDA levels. Additionally, Yenin et al. (2014) and Bakry et al., (2014) also recorded markedly higher plasma MDA levels in AA patients compared with healthy subjects.

In concomitant with the present results, Prie et al., (2015) found lower paraoxonase activity in serum and tissues of patients with alopecia areata.

In an immune operation, it was documented that T lymphocytes is implicated in preventing hair cycle and causing reversible loss of hair. Advanced development in the AA perception has found that the local and systemic cytokines regulation play the critical effect in its pathogenesis (Tanyasiri et al., 2005). Harman and Nevis(1993) detected IL-1α effects on growth of hair follicle and fiber production of hair in vitro, due to a marked decrease in the synthesis of DNA of hair, hair follicle anti-proliferative effect and suppression of fiber growth of hair. These results speculated that IL-1 may have a function in the AA pathogenesis through direct inhibitory actions on the follicle of hair, beside its role in stimulation of immune/ inflammatory system (pro inflammatory mediator). Additionally, Kasumagić-Hallilovic et al., (2012), observed that complete abrogation in hair growth by IL-1β in cultured human hair follicles. In *in vivo* experiments, hair follicle has been protected by IL-1α from the chemotherapeutic agents cytotoxic effects, suggesting inhibitory effect of IL-1α on cell division of hair follicle matrix (Husein et al., 1990; Jimenez et al., 1991). Moreover, *in vitro* studies have illustrated that IL-1α along with IL-1β and TNF-α, promotes matrix cells vacuolation inside the bulb of follicle and a reduction in the matrix size.
folicular melanocytes disorganization as well as changing in precortical cells differentiation and keratinization (Kasumagić-Halilovic et al., 2012). These changes in morphology of hair follicle are identical to those found in AA and indicate that IL-1α and IL-1β may play an effective role in the inflammatory hair disease pathophysiology. A restricted number of literatures have demonstrated serum IL-1β levels in AA patients. In contrast with the present findings, Kasumagić-Halilovic et al., (2012), showed that serum IL-1β levels in AA patients did not significantly differ from that detected in normal control.

Galbraith et al., (1999), detected an increase IL-1β 1,2 genotype frequency in severe alopecia areata patients, with allele 2 of the IL-1β +3953 polymorphism proposing a strong connection with IL-1β increased production (Gregoriou et al., 2010). The present study is considered the first study examined the level of IL6 in serum of AA patients. In this context, it was found that, the disequilibrium between cytokines production, and relative increase of pro-inflammatory cytokines, versus anti-inflammatory cytokines, such as IL-4 and IL-10 may implicated in the continuation lesions of alopecia areata, as detected in biopsies of human scalp (Gregoriou et al., 2010).

CONCLUSION
It could be concluded that, AA is strongly associated with increase production of MDA, IL1β, while inhibition in PON and IL6 cytokines and these abnormalities were more severe in female than in male subjects compared to control.

CONFLICT OF INTEREST
Authors declared no conflict of interest

ACKNOWLEDGEMENT
All authors acknowledge the excellence Centre of National Research Centre for helping in collecting blood sampling.

AUTHOR CONTRIBUTIONS
Sherief M. Hussein, Ragia H.Weshahy and Hany A.Shehata collecting blood sample, diagnosis, designed the experiment and reviewed the manuscript. While, Hanan F. Aly and Eman R.Youness performed the experiments, data analysis and also wrote the manuscript. All authors read and approved the final version.

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