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Differential Expression of CXCL1, CXCL9 CXCL10 and CXCL12 Chemokines in Alopecia Areata

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Differential Expression of CXCL1, CXCL9 CXCL10 and CXCL12 Chemokines in Alopecia Areata

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ABSTRACT

Background: Alopecia Areata (AA) is a non-scarring, autoimmune disorder which causes hair loss. Inflammatory reactions are involved in hair loss of the scalp and/or body. The involvement of chemokine receptors in the pathogenesis of AA is well defined among which, CXCL1 acts on neutrophils and CXCL9, CXCL10 and CXCL12 and serve as T lymphocytes recruiters. **Objective:** To study the serum levels of ELR+ and ELR⁻ CXCL1, CXCL9, CXCL10 and CXCL12 in the patients suffering from AA and healthy controls. **Methods:** The study population of consisted of 30 patients suffering from AA and 30 healthy controls. Serum concentrations of CXCL1, CXCL9, CXCL10 and CXCL12 were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** Current results showed that AA patients had significantly elevated serum levels of CXCL9 and CXCL10 in comparison to controls ($p<0.001$). These results also demonstrated that serum levels of CXCL1 and CXCL12 were significantly decreased in AA patients compared to control ($p<0.001$). **Conclusion:** CXCL9 and CXCL10 are elevated in the AA patients and may be involved in the recruitment of T lymphocytes to the inflamed tissues. Moreover, due to the significant role played by these chemokines in angiogenesis/angiostatis phenomenon they could be considered as useful biomarkers in AA diagnosis and therapy.

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INTRODUCTION

Alopecia Areata (AA) is described as a non-scarring but autoimmune, inflammatory hair loss on the scalp and/or body. Several autoimmune diseases including thyroid disease, pernicious anaemia, diabetes mellitus and vitiligo are associated with AA but genetic aspects of the disorder are still poorly described (1-3). Recognized subgroups of AA include those patients with the complete absence of terminal scalp hair (Alopecia Totalis or AT) and loss of terminal scalp and body hair (Alopecia Universalis or AU) (2,4,5). Although, AA most often presents as patches of hair loss on the scalp but any other hair-bearing skin can also be involved. The AA diseased skin may slightly be blushed but otherwise appears normal (1,6). Hair follicles are preserved in AA and a potential for recovery of hair growth presents, even in longstanding disease states (2). Approximately 20% of patients suffering from AA show a positive familial history for the disorder. It is now well evidenced that T cells play a fundamental role in AA and the disease is a T cell dependent autoimmune disorder that specifically targets the skin (7). Because T cells are involved in the development of AA, it appears that factors which serve as recruiter/activator of these cells could potentially be considered as risk factors of autoimmune diseases including AA (8).

Chemokines are known as members of a subfamily of homologous (8-10 kDa) proteins belonging to the cytokine family. According to the presence, absence or position of the cysteine motifs in their N-terminus, chemokines are further categorized into four distinct sub-groups as CXC, CC, C, and CX3C (9-12). The CXC subgroup itself, based on the presence or absence of a conserved amino acid sequence called ELR motif (glutamate-leucine-arginine) in their N-terminus, are then subclassified in two following subclasses: The ELR^{+} and ELR^{-} CXC chemokines (13,14). The representative member of ELR^{+} of CXC chemokines is CXCL1 with angiogenic properties, whereas members of ELR^{-} CXC chemokines such as CXCL9 and CXCL10 inhibit angiogenesis. ELR^{+} chemokines act on neutrophils while ELR^{-} chemokines serve as T lymphocytes recruiters which their activation is a common event in autoimmune disorders (15). CXCL12 is an ELR^{-} CXC chemokine with angiogenic effects. CXCL12 (as $ELR^{-)}$ and CXCL1 (as ELR^{+}) are strongly chemotactic for lymphocytes and neutrophils, respectively. Both CXCL9 and CXCL10 as ELR^{-} are also T-cell chemoattractant, which are induced by IFN-γ (15,16). CXC chemokines bind to CXC chemokine receptors, the seven transmembrane- G protein coupled receptors and so far seven of CXC chemokine receptors have been discovered and designated as CXCR1 to CXCR7 (17). Due to the important roles of these chemokines (CXCL1, CXCL9, CXCL10 and CXCL12) in recruitment and activation of leukocytes, balancing angiogenesis/angiostasis and also based on the critical roles played by T lymphocytes in the pathogenesis of AA, we aimed to evaluate the serum levels of these chemokines in AA patients.

MATERIALS AND METHODS

Study Subjects. Blood samples were collected from 30 AA patients and 30 healthy controls. Patients were diagnosed as AA by expert specialists according to the medical history findings. The clinical diagnosis of AA was based on the presence of initially patchy alopecia with exclamation mark hairs. To avoid any disruptive factor, patients with a history of acute and chronic diseases were excluded from the study. An informed

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consent form was filled out by both patients and controls prior to enrolment in the study.
Cytokine Assays. Peripheral blood specimens were collected and serum specimens were isolated immediately and stored at -20$^{°}$C for further use. Serum concentrations of CXCL1, CXCL9, CXCL10 and CXCL12 were measured by ELISA (R&D systems, Minneapolis, UK). The sensitivity of assays was 2 pg/mL and inter and intra-assay assessments of reliability of the kits were conducted. Data were only used when inter and intra assays produced scores of CV<14% and CV <3%, respectively.

tatistical Analysis. Results are presented as mean ± standard errors (SE). All analysis were performed by SPSS (version 16; SPSS Inc.). One-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc tests were done to compare differences between different concentrations for each time point. For comparison of mean values of each concentration in different times ANOVA repeated measure with Bonferroni's post-hoc test was used. p<0.05 was considered as the statistically significant.

RESULTS

Our results demonstrated that the mean age of subjects was 18-30 years, including 16 males and 14 females in patient group. Analysis of our data demonstrated that the level of CXCL1, CXCL9, CXCL10 and CXCL12 did not correlate with gender in AA patients (Table 1).

Table 1.The association of sex and chemokine levels in AA patients.

| Chemokine	Male (pg/ml)	Female(pg/ml)	P Value
CXCL1	79.46 ± 6.35	79.06 ± 5.08	P=0.961
CXCL9	131.02 ± 8.68	130.66 ± 8.82	P=0.977
CXCL10	135.88 ± 11.71	187.11 ± 47.53	P=0.312
CXCL12	34.06 ± 3.53	36.56 ± 3.58	P=0.622

We also did not find any relationship between the age and chemokines levels in our study groups. Current results indicated that the mean serum levels of CXCL9 in AA patient and healthy group were 130.85 ± 6.09 pg/mL and 44.98 ± 0.37 pg/mL, respectively. The results also demonstrated that the mean serum levels of CXCL10 were 159.79 ± 23.09 pg/mL in patients and 33.94 ± 2.36 pg/mL in controls. The findings of study showed that the mean serum levels of CXCL1 in patients and control group were 79.28 ± 4.06 pg/mL and 125.64 ± 0.36 pg/mL, respectively. The mean serum levels of

CXCL12 were also significantly different in patients (35.21 ± 2.49 pg/mL) and control group (103.51 ± 13.02 pg/mL).

Table 2. Correlation between age and serum levels of CXC chemokines of AA patients.

Chemokine	18-25 year old patients (pg/ml)	25-35 year old patients (pg/ml)	P Value
Subjects (n)	15	15	
CXCL1	73.30 ± 5.91	84.509 ± 5.43	P=0.174
CXCL9	140.10 ± 9.33	122.76 ± 7.68	P=0.163
CXCL10	138.53 ± 14.63	165.89 ± 41.47	P=0.376
CXCL12	37.24 ± 3.98	33.43 ± 3.16	P=0.463

Our results revealed that CXCL9 and CXCL10 were significantly elevated and serum CXCL1 and CXCL12 levels were decreased in patients compared to controls (p<0.001) (Figure 1).
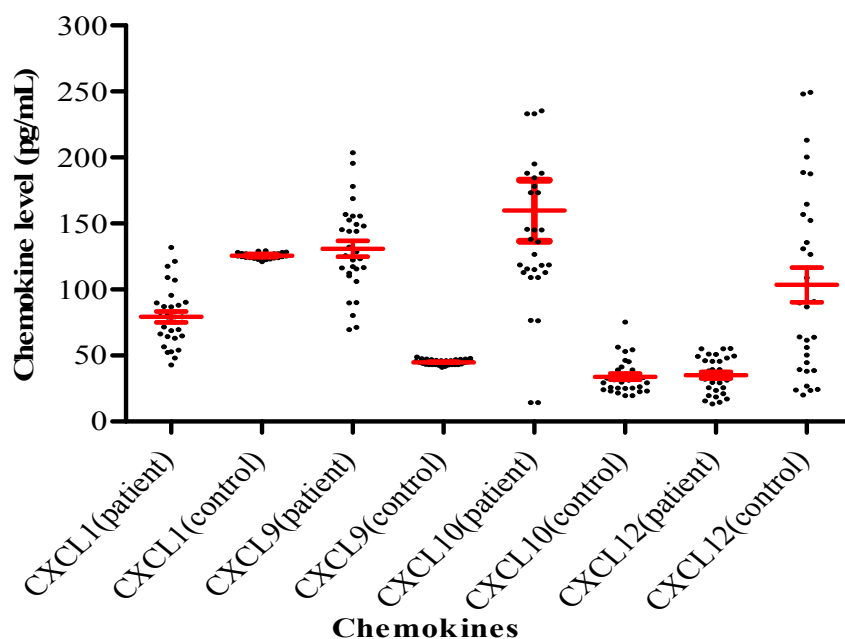
Figure 1. Serum levels of CXCL1, CXCL9, CXCL10 and CXCL12 in Alopecia Areata and healthy controls.

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DISCUSSION

In the present study, we investigated the serum levels of CXCL1, CXCL9, CXCL10 and CXCL12 in patients with AA and healthy controls. Notably, serum levels of CXCL9 and CXCL10 were significantly increased in patients with AA, while, CXCL1 and CXCL12 levels were decreased. Based on our results it may be concluded that CXCL9 and CXCL10, as the members of anti angiogenesis chemokines, are increased in the AA patients and are involved in the processes of infiltration of T lymphocytes into the inflamed tissues. These chemokines probably play a role in the pathogenesis of AA by balancing between angiogenetic and angiostatic chemokines (18,19). Previous studies revealed that T lymphocytes express CXCR3 receptor (20); hence, it seems that this chemokine receptor along with its ligands (CXCL9 and CXCL10) can induce T lymphocyte recruitment to the hair follicles. On the other hand, it has been established that T lymphocytes are recruited to the inflamed hair follicles and elevated expression of IFN-γ were reported in the AA patients (19,21,22). Therefore, increased serum levels of these two downstream targets of IFN-γ (CXCL9 and CXCL10) may probably be related to T lymphocytes recruitment as the source of CXCL9 and CXCL10. Gilhar et al. demonstrated that administration of IFN-γ induces AA with the development of para- and intrafollicular leukocytes infiltration in an animal model. Additionally, it is well identified that IFN-γ induces the expression of CXCL9 and CXCL10 (20,23); hence, a probable reason for up-regulation of the chemokines may be increased expression of IFN-γ in the AA patients. Our results also demonstrated that the serum levels of CXCL1 and CXCL12 decreased in the AA patients when compared to healthy controls.

Due to the fact that CXCL12 is a chemokine with dual effects on the immune system and the chemokine can shift its proinflammatory function to anti-inflammatory, thus, down regulation of this chemokine in the AA patients may be related to autoimmune conditions. Furthermore, it is plausible that failed angiogenesis in the AA patients are involved in hair loss. Because CXCL12 is an angiogenic chemokine, decreased CXCL12 levels may possibly in turn lead to decreased angiogenesis and cause hair loss in the patients. Previous studies revealed that vascular endothelial growth factor (VEGF), as an angiogenic factor, decreases in AA patients (25), hence, decreased expression of CXCL12 in the AA patients is in parallel with down-regulation of the VEGF (24,25). However, previous studies indicated that CXCL1 recruits antigen-specific T cells to the inflamed regions and this may partially explain its involvement in the pathogenesis of autoimmune diseases. The serum levels of CXCL1 were decreased in our studied AA patients. Thus, it appears that, CXCL1 may play important roles in the inflammatory conditions but it does not seem to play an important role in the pathogenesis of AA. Consistent with present findings, Mcphee et al. revealed that the serum levels of CXCL9, CXCL10 and their corresponding receptor (CXCR3) were increased during AA development in mouse models of AA (20). In line with our study, Kuwano et al. also reported that CXCL9, in parallel with other members of chemokine family such as CCL5, CXCL8 and CCL11 were increased in AA patients (19). Overall, our results suggest that CXCL chemokines may play important roles in the development of AA and thus could be considered either as useful biomarkers of disease activity or therapeutic targets for AA therapy.

ACKNOWLEDGEMENTS

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