ORIGINAL ARTICLE

IL-17 Assay in Adult T-cell leukemia/lymphoma Patients with Dermatophytosis

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ABSTRACT

Key words: IL-17, T cell leukemia lymphoma patients, Dermatophytosis

This work aimed to: Assess IL-17 level as proinflammatory cytokine and predictor for the outcome of inflammatory process in ATLL patients with dermatophytosis. Isolation and identification of different types of dermatophytes infecting patients with ATLL.

Methodology: 58 subjects were included in this study (16 adult patients with adult T-cell leukemia / lymphoma clinically diagnosed to have dermatophytosis, 14 adult patients with adult T-cell leukemia / lymphoma clinically diagnosed to have no dermatophytosis, 12 age and sex matched patients clinically diagnosed to have dermatophytosis and 16 Age and sex matched apparently healthy Controls). Samples were examined microscopically using 20% KOH and cultured on into SDA containing chloramphenicol (0.5%) with/without cycloheximide (0.5%) and Dermatophyte test medium (DTM).

Results: in the non-ATLL patients with dermatophytosis, the serum IL-17 level was significantly increased compared with the healthy controls. In ATLL patients either with or without dermatophytosis, the IL-17 levels were significantly lower than those in the healthy controls. There was no significant difference in the IL-17 level between ATLL patients with dermatophytosis and those without dermatophytosis. Again, it is suggested that ATLL patients have low levels of IL-17, which cannot be enhanced by the presence of dermatophytosis. Among patients with ATLL with dermatophytosis (Group I) T. rubrum was the commonest dermatophyte causing infection; 64% of samples (tineacorporis 46%, tineaunguium 18%), whereas T. mentagrophytes was the 2nd commonest dermatophyte; 27 % (tineaunguium 27%), lastly T. tonsurans; 9% (tineacorporis 9%). In patients with Non-ATLL with dermatophytosis (Group III) T. rubrum was also the commonest dermatophyte causing infection; 64% of samples (tineacorporis 7%, tineaunguium 14%, tineapedis 43%), whereas T. mentagrophytes was the 2nd commonest dermatophyte; 29 % (tineaunguium 7%, tineapedis 22%), lastly T. tonsurans; 7% (tineacorporis 7%). Conclusion: Our data provides clinical evidence linking Th17 cells to immune deficiency in ATLL and opens a new avenue in the study of tumor immunotherapy based on promoting Th17 cell population.

INTRODUCTION

Interleukin-17 (IL-17) and IL-17-producing cells have been shown to play important roles in inflammation and the immune response. IL-17 is believed to be mainly produced by T helper 17 (Th17) cells, a unique helper T-cell subset different from Th1 and Th2 cells. Other subsets of T cells such as Gamma delta T cell (γδT) and natural killer T (NKT) cells have also been found to produce IL-17 in response to innate stimuli. IL-17 acts as a proinflammatory cytokine that can induce the release of certain chemokines, cytokines, matrix metalloproteinases (MMPs) and antimicrobial peptides from mesenchymal and myeloid cells. This leads to the expansion and accumulation of neutrophils in the innate immune system and links innate and adaptive immunity in vivo.

Furthermore, increasing evidence indicates that IL-17 and IL-17-producing cells are involved in the pathogenesis of various diseases such as allergies, autoimmune diseases, allograft transplantation and even malignancy. They may also play protective roles in host defense against infectious diseases and promote induction of cytotoxic T lymphocyte (CTL) responses against cancer. Targeting of the IL-17 axis is under investigation for the treatment of inflammatory disorders.
Adult T-cell leukemia/lymphoma (ATLL) is a malignancy of mature CD4+ T cells caused by human T-cell lymphotropic virus type 1. HTLV-1 infection is prevalent in southern Japan, Caribbean country, middle east and Africa.  

Superficial dermatophytosis, such as tinea and candidiasis, is quite common in ATLL patients, as approximately 50% of the patients develop cutaneous mycotic infections, and the incidence of dermatophytosis of indolent type of ATLL patients was higher than that of aggressive type. Dermatophytosis of ATLL patients tended to be intractable even with antifungal treatments, and inflammatory reactions to fungi are less prominent in ATLL patients than non-ATLL dermatophytosis patients.

These findings have suggested the defective immunity against dermatophytes in ATLL patients. Because superficially infected fungi in the stratum corneum of the epidermis cannot directly contact with T cells infiltrating in the upper dermis, some perturbation of epidermal innate immunity has been postulated. 

Interleukin (IL)-17–producing helper T (Th17) cells represent a lineage of effector T cells critical in host defense, and dysregulated Th17 cell responses mediate a wide variety of autoimmune and inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, and atopic dermatitis. 

IL-17 stimulates keratinocytes to produce various cytokines, such as granulocyte macrophage colony-stimulating factor, tumor necrosis factor α, CXC chemokine ligand 10, and vascular endothelial growth factor. IL-17 can also enhance the expression of antimicrobial peptides such as human β defensin (HBD)-2 and LL-37 in keratinocytes, which play an essential role in cutaneous innate immunity against fungi. These reports suggested that Th17 cells and IL-17 might play an important role in host defense against superficial dermatophytosis.

**METHODLOGY**

**Subjects and clinical evaluation:**

This study was conducted during the period from May-2013 to Dec-2014; The study included four groups: 

**Group I:** 16 adult patients with adult T-cell leukemia/lymphoma attending Mansoura Oncology Center and clinically diagnosed to have dermatophytosis. 

**Group II:** 14 adult patients with adult T-cell leukemia/lymphoma attending Mansoura Oncology and clinically diagnosed to have no dermatophytosis. 

**Group III:** 12 age and sex matched patients attending outpatient clinic of dermatology in Benha& Mansoura university hospitals and clinically diagnosed to have dermatophytosis. 

**Group IV:** 16 Age and sex matched apparently healthy Controls. 

An informed consent was taken from all subjects in the study.

**Inclusion criteria:**

The diagnosis of adult T-cell leukemia / lymphoma (ATLL) was based on the clinical features, histopathologically, cytologically proven mature T-cell malignancy and presence of anti-HTLV-1 antibody[7]. Dermatophytosis was diagnosed clinically and confirmed with microscopic examinations using KOH preparations. No dermatophytosis patients regardless of the presence of ATLL had taken any immunosuppressant drugs, systemic steroids or chemotherapy.

**Measurement of serum IL-17 level:**

The serum IL-17 was measured by ELISA using the Human IL-17A High Sensitivity ELISA Immunoassay Kit (Glory Science), according to the manufacturer's instructions: as following; Sample Preparation; Five ml of peripheral blood samples were taken from each of the patients & controls then transferred to serum separating tubes and left to coagulate at room temperature for 10-20 min, centrifuged at the speed of 2000-3000 rpm for 20-min. The supernatants were carefully harvested, if precipitation appeared, the samples centrifuged again. Aliquots of serum samples were frozen at ~80°C until analysis.

**Mycological evaluation:**

From patients of group I (ATLL with dermatophytosis) and patients of group III (Dermatophytosis); sample(s) from site(s) of suspected dermatophyte infection was (were) collected and subjected to mycological examination. 

**Sample collection:** The suspected skin lesion or nail (s) was (were) cleaned with 70% alcohol to remove contaminants. Scrapings were taken with a sterile scalpel blade and collected in a sterile clean paper. After collection of the specimen, the paper was folded to form a flat packet. It was closed by paper clip. The paper was labeled with the patient’s name, number and date.

**Mycological examination:** Each collected specimen was divided into two portions. The first portion of the specimen was examined microscopically using 20% KOH. The second portion was cultured on into two sets of media: SDA containing chloramphenicol (0.5%) with/without cycloheximide (0.5%) and Dermatophyte test medium (DTM). Negative test: yeast cells only (or with pseudohyphae) always two pieces.

**Statistical analysis:**

The serum concentrations of IL-17 were compared between the ATLL with dermatophytosis patients, ATLL without dermatophytosis patients, dermatophytosis patients without ATLL, and healthy controls. All statistical analyses were carried out using GraphPad Prism 6.01 statistical package. Shapiro–Wilk normality test was applied in order to find out if the values of the continuous variables were normally distributed. Student’s t-test, Mann–Whitney test or one-way ANOVA were used for comparison of continuous variables between groups while categorical data was...
compared between groups by using Chi-square $\chi^2$ test or Chi-square $\chi^2$ test with Yates correction. All $P$ values less than 0.05 were considered statistically significant.

RESULTS

Patients and Controls:
The patients and controls were categorized into 2 groups of age: 70 years or older and younger than 70 years. About 36 (62 %) subjects were < 70 years, while 22 (38%) subjects were ≥ 70 years. Of 58 patients, there were 30 (52%) males, 28 (48%) females. The Chi-square $\chi^2$ test and one-way ANOVA revealed that in the individual related factors including male/female ratio, age & age group, there were no statistical differences between the groups.

Table 1: Age and sex distribution of the study groups

<table>
<thead>
<tr>
<th>Patient-related factors</th>
<th>ATLL/dermatophytosis</th>
<th>ATLL</th>
<th>Dermatophytosis</th>
<th>Healthy control</th>
<th>Total</th>
<th>Percent</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>16</td>
<td>58</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>71.13 ± 10.7</td>
<td>66.71</td>
<td>62.50 ± 9.5</td>
<td>64.19 ± 5.08</td>
<td>66.36</td>
<td>0.0732</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>52-88</td>
<td>52-79</td>
<td>49-78</td>
<td>53-72</td>
<td>49-88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 70 year</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>36</td>
<td>62%</td>
<td>0.0523</td>
</tr>
<tr>
<td>≥ 70 year</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>22</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>10</td>
<td>52%</td>
<td>0.4658</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>28</td>
<td>48%</td>
<td></td>
</tr>
</tbody>
</table>

In the non-ATLL patients with dermatophytosis, the serum IL-17 level was significantly increased compared with the healthy control or ATLL patients with dermatophytosis. In ATLL patients either with or without dermatophytosis, the IL-17 levels were significantly lower than those in the healthy controls. There was no significant difference in the IL-17 level between ATLL patients with dermatophytosis and those without dermatophytosis.

Fig. 1: Statistical Analysis of Serum IL-17 level in study groups.
Mycological evaluation results:

Table 2: Type of isolated dermatophyte causing infection among patients with ATLL with dermatophytosis (Group I)

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Organism</th>
<th>Number of samples (22)</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea corporis</td>
<td>Trichophytonrubrum</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Trichophytontonsurans</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Tineaunguium</td>
<td>Trichophytonmentagrophytes</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Trichophytonrubrum</td>
<td>4</td>
<td>18</td>
</tr>
</tbody>
</table>

*T. rubrum* was the commonest dermatophyte; 64% of samples (tinea corporis 46%, tineaunguium 18%), whereas *T. mentagrophytes* was the 2nd commonest dermatophyte; 27% (tineaunguium 27%), lastly *T. tonsurans*; 9% (tinea corporis 9%).

Table 3: Types of isolated dermatophyte causing infection among patients Non-ATLL with dermatophytosis (Group III)

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Organism</th>
<th>Number of samples (14)</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea corporis</td>
<td>Trichophytonrubrum</td>
<td>1</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Trichophytontonsurans</td>
<td>1</td>
<td>7%</td>
</tr>
<tr>
<td>Tineaunguium</td>
<td>Trichophytonmentagrophytes</td>
<td>1</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Trichophytonrubrum</td>
<td>2</td>
<td>14%</td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>Trichophytonrubrum</td>
<td>6</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>Trichophytonmentagrophytes</td>
<td>3</td>
<td>22%</td>
</tr>
</tbody>
</table>

Table 4: Statistical analysis of serum IL-17 level in study groups

<table>
<thead>
<tr>
<th>Bonferroni's multiple comparisons test</th>
<th>Mean Diff.</th>
<th>95% CI of diff.</th>
<th>Significant</th>
<th>Summary</th>
<th>Adjusted P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophytosis vs. Control</td>
<td>31.03</td>
<td>29.42 to 32.64</td>
<td>Yes</td>
<td>****</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ATLL-Dermatophytosis vs. Control</td>
<td>-2.050</td>
<td>-3.539 to -0.5607</td>
<td>Yes</td>
<td>**</td>
<td>0.0042</td>
</tr>
<tr>
<td>ATLL vs. Control</td>
<td>-2.009</td>
<td>-3.550 to -0.4674</td>
<td>Yes</td>
<td>**</td>
<td>0.0069</td>
</tr>
<tr>
<td>ATLL-Dermatophytosis vs. ATLL</td>
<td>-0.04107</td>
<td>-1.583 to 1.500</td>
<td>No</td>
<td>ns</td>
<td>&gt; 0.9999</td>
</tr>
</tbody>
</table>

Among patients with Non-ATLL with dermatophytosis, *T. rubrum* was the commonest dermatophyte; 64% of samples (tinea corporis7%, tineaunguium14%, tinea pedis 43%), whereas *T. mentagrophytes* was the 2nd commonest dermatophyte; 29% (tineaunguium 7%, tinea pedis 22%), lastly *T. tonsurans*; 7% (tinea corporis7%).

![Fig. 2: Trichophytonrubrum on DTM](image1)

![Fig. 3: T. mentagrophytes on SDA](image2)
DISCUSSION

IL-17 is a glycoprotein (~20 kDa) of 155 amino acids and is the founding member of the IL-17 family of cytokines, which includes IL-17A (also termed IL-17), IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. A number of studies have shown that IL-17 induces the production of a set of key inflammatory cytokines and chemokines in various tissues to stimulate the inflammatory cascade further.

Adult T-cell leukemia/lymphoma (ATLL) is a malignancy of mature CD4+ T cells caused by human T-cell lymphotropic virus type I. HTLV-1 infection is prevalent in southern Japan, Caribbean country, middle east and Africa.

Superficial dermatophytosis, such as tinea and candidiasis, is quite common in ATLL patients, as approximately 50% of the patients develop cutaneous mycotic infections, and the incidence of dermatophytosis of indolent type of ATLL patients was higher than that of aggressive type. Dermatophytosis of ATLL patients tended to be intractable even with antifungal treatments, and inflammatory reactions to fungi are less prominent in ATLL patients than non-ATLL dermatophytosis patients.

Dermatophytosis is infections of keratinized tissues, such as skin, hair and nail, caused by a group of related filamentous fungi, which are also known as the ringworm fungi. The etiologic agents of dermatophytosis can be classified according to their natural habitats into three categories, geophilic, zoophilic and anthropophilic. Members of all three groups can cause human infection.

Dermatophytosis includes several distinct clinical manifestations. The severity of the disease depends on the strain or species of infecting fungus, the sensitivity of the host and the site of infection. There are three genera of dermatophytes, Trichophyton, Microsporum and Epidermophyton.

These findings have suggested the defective immunity against dermatophytes in ATLL patients. Because superficially infected fungi in the stratum corneum of the epidermis cannot directly contact with T cells infiltrating in the upper dermis, some perturbation of epidermal innate immunity has been postulated.

Patients with ATLL often suffer from various infections, such as pneumocystis carinii, pathogenic fungi, viruses, and parasites, and these infections occasionally result in death. In particular, superficial dermatophytosis is quite common in ATLL patients, as more than 60% of the ATLL patients have tinea pedis/unguium/corpusis, candidiasis and other cutaneous mycotic infection. The characteristics of tinea in ATLL include non-annular configuration and lack of inflammatory change.

Th17 cell–derived cytokines stimulate keratinocytes to produce antimicrobial peptides and ATLL malignant T cells are assumed to reduce the number and/or function of Th17 cells.

Interleukin (IL-17)–producing helper T (Th17) cells represent a lineage of effector T cells critical in host defense, and dysregulated Th17 cell responses mediate a wide variety of autoimmune and inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, and atopic dermatitis.

IL-17 stimulates keratinocytes to produce various cytokines, such as granulocyte macrophage colony–stimulating factor, tumor necrosis factor α, CXC chemokine ligand 10, and vascular endothelial growth factor. IL-17 can also enhance the expression of antimicrobial peptides such as human β defensin (HBD)-2 and LL-37 in keratinocytes, which play an essential role in cutaneous innate immunity against fungi. These reports suggested that Th17 cells and IL-17 might play an important role in host defense against superficial dermatophytosis.

ATLL cells produce various chemokines and cytokines, such as IL-10 and transforming growth factor (TGF)-β1, which cause immunosuppression in ATLL patients. In particular, IL-10 exerts an inhibitory effect on macrophages and suppresses the cytokine production by Th17 cells.

Peric et al. & Eyerich et al. reported that the production of antimicrobial peptides by epidermal keratinocytes was depressed in ATLL patients, which presumably attenuated the innate immunity of skin surface environment. IL-17 enhances various antimicrobial peptides such as HBD-2 and LL-37 in keratinocytes, but neither hBD-1 nor hBD-3.

The aim of this study was to assess serum IL-17 level as a proinflammatory cytokine and a predictor for the outcome of inflammatory process in ATLL patients with dermatophytosis. Also, Isolation and identification of different types of dermatophytes infecting patients with ATLL was done.

The current study was conducted from May 2013 to Dec 2014, and included 58 subjects. The study included four groups; Group I: 16 adult patients (8 males & 8 females) with adult T-cell leukemia/lymphoma clinically diagnosed to have dermatophytosis, Group II: 14 adult patients (8 males & 6 females) with adult T-cell leukemia/lymphoma clinically diagnosed to have no dermatophytosis, Group III: 12 (4 males & age and sex matched patients clinically diagnosed to have dermatophytosis and Group IV: 16 (10 males & 6 females). Age and sex matched apparently healthy Controls.

Of these groups, group I & group 2 included 30 (52%) patients who have ATLL. 16 (52 %) patients were female and the other 14 (48 %) cases were males. Their ages ranged from 52 to 88 years (mean age is 69 years ± 10.2 SD).

These obtained results are in harmony with that detected by Vose and E Matutes who demonstrated...
that the median age at diagnosis of ATLL is in the sixth decade and there is no gender prevalence.

In this study, all of the 36 samples (nail and skin) from 28 patients with dermatophytosis (Group I and Group III) were subjected to different mycological examinations, including direct microscopic examination (using 20 % KOH) and culture.

Among patients with ATLL with dermatophytosis (Group I), T. rubrum was the commonest dermatophyte causing infection; 64% of samples (tinea corporis 46%, tineaunguium 18%), whereas T. mentagrophytes was the 2nd commonest dermatophyte; 27% (tineaunguium 27%), lastly T. tonsurans; 9% (tinea corporis 9%).

In patients with Non-ATLL with dermatophytosis (Group III), T. rubrum was also the commonest dermatophyte causing infection; 64% of samples (tinea corporis 7%, tineaunguium 14%, tineapedis 43%), whereas T. mentagrophytes was the 2nd commonest dermatophyte; 29% (tineaunguium 7%, tineapedis 22%), lastly T. tonsurans; 7% (tinea corporis 7%).

These results were, to some extent, in agreement with the data obtained by C Mugge, UF Haustein and P Nenoff who demonstrated that the percentage of the anthropophilic fungus T. rubrum among the dermatophytes causing tineaunguium is 91% in Germany. T. rubrum and T. interdigitale (previously known as T. mentagrophytes) are also responsible for about 90% of all cases of dermatophytosis in Poland. The same figures have been reported in Great Britain and Sweden.

This could be attributed to that the epidemiology of dermatophytic infection is affected by migration pattern, increase in tourism, locality and changes in socioeconomic condition of the people 18.

In all 4 groups, the serum IL-17 was measured by ELISA using the Human IL-17A High Sensitivity ELISA Immunoassay Kit (Glory Science), according to the manufacturer's instructions. The serum concentrations of IL-17 were compared between the ATLL with dermatophytosis patients, ATLL without dermatophytosis patients, dermatophytosis patients without ATLL, and healthy controls.

As regards assessment of serum IL-17 level in the study groups, our results shows that in the non-ATLL patients with dermatophytosis, the serum IL-17 level was significantly increased compared with the healthy controls. In ATLL patients either with or without dermatophytosis, the IL-17 levels were significantly lower than those in the healthy controls. There was no significant difference in the IL-17 level between ATLL patients with dermatophytosis and those without dermatophytosis. Again, it is suggested that ATLL patients have low levels of IL-17, which cannot be enhanced by the presence of dermatophytosis.

These data were in parallel to that achieved by Y Sawada et al. who reported that the frequency of peripheral Th17 cells and the serum level of IL-17 was significantly decreased in ATLL patients, as compared with healthy controls. As well as, ATLL patients with dermatophytosis had lower IL-17 levels than did those without dermatophytosis.

On the other hand our findings were more or less similar to that detected by T Miyagaki, et al. who reported that IL-17A not significantly elevated in lesional skin of cutaneous T-cell lymphoma. Also a recent study carried out in Oncology Center, Mansoura University reported that serum levels of IL-17 and IL-21 in untreated acute leukemia patients were significantly higher than in controls and correlated positively with levels of circulating Th17 cells 20.

This discrepancy may be due to the different biological characteristics among different tumor types, and the possibility that the function of Th17 cells may vary according to different cancer cause, type, and location, as well as stage of cancer 21.

CONCLUSIONS

Our data provides clinical evidence linking Th17 cells to immune deficiency in ATLL and opens a new avenue in the study of tumor immunotherapy based on promoting Th17 cell population.

REFERENCES