IL-4, IL-12 and TNF-α Cytokines Productivity in Chronic Hepatitis C Infected Patients Treated with Bee Stings

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Abstract

Background: Bee venom (BV) has been traditionally used in oriental medicine to relieve pain and to treat chronic inflammatory diseases such as rheumatoid arthritis. In Egypt, viral hepatitis B and C are endemic with high prevalence rate.

Aim: To explore the effect of BV by stings on immunomodulation by describing the pattern of some cytokines productivity in chronic hepatitis C (HCV) infected patients.

Methods: Forty HCV patients were included in the study, they were classified into 3 groups; patients treated with bee stings (n=28), patients treated with interferon (n=5) and patients treated with conventional liver support drugs and bee stings (n=7). Bee stinging program was carried out for 9 months according to M. Nagib program. Interleukin-4 (IL-4), Interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF-α) levels were measured by enzyme linked immunosorbent assay (ELISA). The quantitative HCV RNA was conducted by reverse transcriptase polymerase chain reaction (RT-PCR). Liver function and hematological assessment were carried out. All measurements were done before, after 3 months, 6 months and 9 months of bee stinging program.

Results: There was gradual increase in IL4 and decrease in IL12 and TNF-α in all groups. There was significant difference in IL4 and TNF-α level through the sessions of treatment in patients treated with bee stings and liver support drugs. In patients treated with interferon, there was no significant difference in IL-4, IL-12 or TNF-α levels through the sessions of treatment. Liver enzymes decreased in patients treated with bee stings and liver support drugs.

Conclusion: Bee stings have associated with increased IL4 level, decreased level of TNF-α and improved some parameters in liver functions.

Key Words: Bee venom – Interleukin-4 (IL-4) – Interleukin-12 (IL-12) – Tumor necrosis factor alpha (TNF-α) – Venom immunotherapy (VIT) – Hepatitis C (HCV).

Introduction

APITHERAPY, the medicinal use of honey bee products, has been practiced since ancient times. It was described by Hippocrates (circa 400 B.C.) and Galen (circa 130-200 A.D.), who used honey, bee venom, pollen, propolis, and other substances in their medical practice. Today, honey bee products are widely used to treat arthritis and other inflammatory, autoimmune, and degenerative diseases. Also, pollen and bee bread may improve liver function and strengthen the heart, as well as provide amino acids to the nervous system [1]. BV is known to be a very complex mixture of active peptides, including melittin, phospholipase A2, apamin, adolapin, and mast cell degranulating peptide and several bioactive amines such as histamine, dopamine, norepinephrine and serotonin [2,3] suggested that the primary allergic components of BV, histamine and phospholipase A2 induced IL-10 production by T helper (Th)-2 cells and suppressed T-cell proliferation. The anti-inflammatory and analgesic properties of BV therapy are related to the modulation of adrenor-receptor activity and serotonergic neurotransmission [4].

IL-4 is a highly pleiotropic cytokine that is able to influence Th cell differentiation. Early secretion of IL-4 leads to polarization of Th cell differentiation toward Th2 like cells. IL-4 has marked inhibitory effects on the expression and release of the proinflammatory cytokines. It is able to block or suppress the monocyte derived cytokines, including IL-1, TNF-α, IL-6 and IL-8 [8].
IL-12 is a heterodimeric cytokine essential for cell-mediated immunity against microbial infection. With Interferon (IFN) α/β, they play critical roles in defense against viruses through antimicrobial as well as immunoregulatory effects [6]. IL-12 is a key factor in the induction of T cell-dependent and independent activation of macrophages, generation of Th1 and cytotoxic T cells. IL-12 is well-known for regulating Th1/Th2 differentiation and increasing survival of CD4+ T cells by preventing apoptosis [7].

TNF-α is a member of the growing TNF ligand family that is involved in immune regulation. Ma, et al. [8] revealed that TNF-α is a potent inhibitor of IL-12 p40 and p70 secretion from human monocytes. TNF usually has been considered as main component of antiviral activity [9]. In patients with chronic HCV infection serum TNF is upregulated and this corresponds to enhanced hepatocellular expression of TNF [10]. Increased levels of TNF are enhanced in chronic HCV infection and reflect histologic activity of the disease [11]. The liver contains a large compartment of cells of the innate immune system that have the potential for rapid release of proinflammatory cytokines with potent synergistic actions e.g., TNF, IL-12, IL-18, and IFN-γ. Dysregulated release of these cytokines can trigger extensive liver injury [12].

The aim of this study was to explore the effect of BV by stings on immunomodulation by describing the pattern of IL-4, IL-12 and TNF-α in chronic HCV infected patients.

Subjects and Methods

The present study was carried out in the period from January 2008 to June 2009. The bee stinging program was carried out in the bee venom therapy research center at the Faculty of Agriculture and Environmental Sciences, Suez Canal University, El-Arish, Egypt.

Forty HCV patients were included in the study; their mean age was 38 ± 7.8 years. They included thirty males (80%) with mean age of 40 ± 7.8 years and ten females (20%) with mean age of 39 ± 5.8 years old. The patients were divided into 3 groups. Group 1 included patients treated with bee stings (n=28), group 2 included patients treated with interferon (n=5) and group 3 included patients treated with conventional liver support drugs and bee stings (n=7). The included patients were randomly selected from patients attending the bee venom therapy research center searching for oriental treatment for HCV so the number of patients included in group 2 and group 3 is relatively smaller than the first group. All patients were negative for HBsAg and HCV-RNA positive. Patients having hepatocellular carcinoma were excluded from the study. Informed written consent was taken from patients before starting treatment. Blood samples were taken from the patients before, after 3 months, 6 months and 9 months of treatment except HCV-RNA which was tested before, after 3 months and 6 months of treatment.

Bee stinging program:

Bee stinging program was carried out according to M. Nagib program [13]. The duration of the program ranges between six months to one year and half. It is scheduled for five days weekly (Saturday till Wednesday). In the first week, on Saturday; patients were stung only one sting in the right hand as a test. From Sunday to Wednesday, they were stung one sting at the lower part of vertebral column (Fig. 1). In the second week, patients were stung one sting at the lower part of vertebral column and three stings at the anatomical site of the liver from the back (Fig. 2). This program continued every day except Thursday and Friday three months. Then, the dose was increased in the following three months to become one sting at the lower part of vertebral column and ten stings at the anatomical site of the liver from the back (Fig. 3). This program continued every day except Thursday and Friday till the end of the program.

Determination of IL-4, IL-12 and TNF-α:

Serum levels of IL-4 and IL-12 were measured by enzyme linked immunosorbent assay (ELISA) with a biosource international Immunoassay Kit (Belgium). TNF-α was measured by a sandwich enzyme immunoassay according to the manual, using kits of Immunotech Company (France).

Serological assessment:

The quantitative HCV RNA was conducted by a reverse transcriptase polymerase chain reaction (RT-PCR) Taqman technique using light cycler 1.5, Roche.

Hematological assessment:

Complete blood picture to assess total leukocytic count (TLC), red blood cells (RBCs), haemoglobin (Hb) and platelet count (PLT). Measurements were done by Heco cell counter, Radim diagnostics, Seac S.r.l.
Statistical analysis:

The SPSS Statistical Analysis Package version 15 (SPSS, Inc., Chicago, IL) was used for analysis of data. Data was presented as mean ± SD. Analysis of variance (ANOVA) test was used to find the difference between variables in the three groups and the difference between variables at the end of every session (every 3 months). F-value was measured and the mean difference is significant at 0.05 levels. Post hoc range tests was used to test the difference between each pair of means, we used T3 DUNNET Alpha.

Results

Liver functions:

Regarding liver functions tests in the three studied groups, there was gradual decrease in GGT level in groups 1 and 3 versus mild increased level in group 2. While ALT and AST were decreased in group 3 compared to group 1 and 2.

Hematological parameters:

There was no significant difference among the three groups regarding the TLC, HB, RBCs and platelets during the follow-up period except for group 2, platelet reduction was markedly observed.

Assessment of viral load:

Table (1) showed that, there was no significant difference between the three groups regarding viral load although there was decreased level of viral load in the three groups during follow-up period. No patient reached the sustained viral response (PCR results for example, negative or below the detectable level).

Immunological profile:

In the three groups of the study, there was gradual increase in IL-4 and decrease in IL12 and TNF-α. (Figs. 4-6) demonstrate the pattern of IL4, IL-12 and TNF-α respectively in the three studied groups.
Table (2) showed the statistical differences between variables after every session of treatment in the three groups. ANOVA test revealed that, there was significant difference in basal PLT level between the three groups. After 3 months of treatment, there was significant difference in TLC, RBCs and PLT between the three groups. After 6 months, there was significant difference in Albumin, TLC, RBCs and PLT. After 9 months, there was significant difference in GGT level, RBCs and PLT. Post hoc analysis revealed that, there was significant difference in basal INR between group 1 and 2 (p < 0.05), there was significant difference in basal RBCs between group 1 and 3 (p < 0.05), finally, there was significant difference in RBCs after 3 months of treatment between group 1 and 3 (p < 0.05).

Table (3) showed the statistical differences between variables after every sting in the three groups. ANOVA test revealed that, there was significant difference in IL4 level through the sessions of treatment and Post hoc analysis revealed that, there was significant difference between basal IL4 level and IL4 level after 3 months (p < 0.05), after 6 months (p < 0.001) and after 9 months of treatment (p < 0.001), also, there was significant difference between IL4 level after 3 months and after 6 months (p < 0.01) and after 9 months of treatment (p < 0.01) and finally, there was significant difference between IL4 level after 6 months and after 9 months of treatment (p < 0.01). There was significant difference in TNF-α level through the sessions of treatment but Post hoc analysis revealed no significant difference between different sessions of treatment.

Table (2): The statistical difference between variables after every session in every group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bee sting treated group</th>
<th>Interferon treated group</th>
<th>Bee sting and Liver support treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F Sig.</td>
<td>F Sig.</td>
<td>F Sig.</td>
</tr>
<tr>
<td>IL4</td>
<td>12.140 .000**</td>
<td>5.177 .310</td>
<td>30.066 .000**</td>
</tr>
<tr>
<td>IL12</td>
<td>1.889 .140</td>
<td>.139 .925</td>
<td>1.028 .404</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.407 .022*</td>
<td>1.755 .495</td>
<td>4.183 .021*</td>
</tr>
</tbody>
</table>

Table (3): The statistical difference between variables after every sting in the three groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>GGT</td>
<td>.006</td>
<td>.994</td>
<td>.302</td>
<td>.742</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.50</td>
<td>.238</td>
<td>1.50</td>
<td>.238</td>
</tr>
<tr>
<td>TLC</td>
<td>.970</td>
<td>.390</td>
<td>8.518</td>
<td>.002*</td>
</tr>
<tr>
<td>RBCs</td>
<td>3.243</td>
<td>.053</td>
<td>4.941</td>
<td>.016*</td>
</tr>
<tr>
<td>PLT</td>
<td>4.234</td>
<td>.024*</td>
<td>5.213</td>
<td>.013*</td>
</tr>
</tbody>
</table>
Discussion

Venom immunotherapy (VIT) is an efficient treatment of hymenoptera venom allergy. The mechanism of VIT is based on the induction of tolerance of allergen specific Th2 cells. The mechanisms of this T cell modulation could depend on cytokines produced by other cell types such as IL-12, TNF-α and IL-10 by monocytes [14]. Bee venom phospholipase A2 (PLA) is the major allergen in bee sting allergy. PLA-specific IgE responses are the result of imbalanced Th 2-cell differentiation. There are multiple mechanisms driving the differentiation of naive CD4+ T cells into Th1 or Th2 cell phenotypes. Most of them are linked to the conditions occurring during initial or repeated encounters with the allergen, in the context of an antigen presenting cell (APC). The different types of APC and their availability to display particular cytokine production profiles, pattern recognition receptors, costimulatory molecules and specific HLA haplotypes are key determinants for human Th1 and Th2 cell polarization [15].

Although Jutel, et al., [16] revealed that BV immunotherapy could lead to induction of the interleukin IL-10 and resulted in decreased IL-4 and IL-5 and increased IFN-γ secretion. In the current study, we found a gradual increase in IL-4 level in the three studied groups. In agreement with our results Fan, et al., [17] who revealed that, the levels of Th2 cytokines (IL-4 and IL-10) were significantly increased in chronic HCV infected patients compared with normal controls. Natural killer T cells produce predominantly IL-4 when stimulated with glycolipid-presenting hepatocyte [18]. Also, BV contains about 1% histamine and Packard and Khan [19] claimed that, histamine plays a significant role in up-regulating anti-inflammatory cytokines including IL-4, IL-5, IL-10 and IL-13. Finally, Meiler, et al., [20] revealed that, histamine receptor 2 up-regulated on specific Th2 cells displays a dual effect by directly suppressing allergen-stimulated T cells and increasing IL-10 production.

In the present study, we found a decrease in IL-12 and TNF-α level in the three studied groups. In agreement with our results Hodge-Dufour, et al., [21] revealed that TNF inhibits interferon-g priming for production of high levels of IL-12 by macrophages and Ma, et al., [8], reported that TNF-α is a potent inhibitor of IL-12 secretion from human monocytes. VIT induces a monocyte activation characterized by a delayed overproduction of IL-12 and TNF-α. These cytokines could be relevant to the inhibition of Th2 cells during VIT.

Therefore, VIT induced tolerance could depend not only on the specific action of venom antigens on T cells, but also on a secondary non-specific action of monocytes [14]. The balance between IL-12 and IL-4 during an infection may regulate not only the dichotomy between Th1 and Th2 responses, but also the type of Th1 responses and their association with the anti-inflammatory cytokine IL-10 [22]. IL-12 has, itself, been shown to be targeted by various viruses, including HCV [23]. Suppression of IL-12 production would be predicted to hinder HCV clearance, a prediction that has received circumstantial support by recent genetic studies Mueller, et al., [24]; Yin, et al., [25] and Houldsworth, et al., [26]. Lee, et al., [27] revealed that inflammatory cytokines such as IL-12 provide important signals for differentiation and survival of activated CD8 T cells.

Regarding results of TNF alpha in the present study. Mahdy, et al., [28] revealed that, TNF-α level was significantly increased in Egyptian patients with HCV-antibody seropositive and RT-PCR negative and in HCV-antibody seropositive and RT-PCR positive. This result is in contrary to our results of significant decrease in the level during treatment with bee stings. TNF-α have a direct antiviral effect by a noncytolytic mechanism [29]. Persisting infection is facilitated by diversity of mechanisms that allow the virus to escape host adaptive immunity [30]. In most patients with chronic HCV infection, there is an active inflammatory reaction in the liver with up-regulation of proinflammatory cytokines, notably TNF-α and interferon (IFN)-γ Dumoulin, et al., [31] which display both cytotoxic and antiviral effects. This process contributes to the hepatocellular injury seen in most HCV infected patients but does not prevent HCV replication in liver cells [32]. HCV core protein binds to the cytoplasmic domain of certain members of the TNF-α receptor superfAMILY, modulating the sensitivity of infected cells to the stimulus provided by their respective ligands [33]. Inhibitors of TNF production in macrophages, such as IL-4 and IL-10 have been described [21]. By blocking Th1 cytokine production, TNF might limit the extent and duration of inflammatory response in vivo. Chronic TNF exposure suppresses the response of both Th1 and Th2 subsets and attenuates T-cell receptor signaling. Thus chronic TNF stimulation suppresses T-cell function in vivo and might have important implications for our understanding of pathogenesis in chronic inflammatory diseases [34].

Although Verhoef, et al., [35] revealed that phospholipase A2 immunotherapy (PIT) had no
effect on secretion of TNF-α, some authors found that BV and its major component melittin inhibit inflammatory stimuli such as TNF-α by preventing p50 translocation via an interaction between melittin and sulfhydryl group of p50 and these inhibit inflammatory reaction in the development of rheumatoid arthritis. They exert anti-inflammatory effects by suppressing the transcription of cyclooxygenase (COX)-2 genes and pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α. Park, et al., [36] Finally, Park, et al., [37] concluded that melittin and BV prevent lipopolysaccharide, and sodium nitroprusside induced nitric oxide and prostaglandin E2 production via an interaction between melittin and sulfhydryl group of p50 and these inhibit COX-2 cytokine responses decrease in parallel with viral load during successful therapy [40]. These results may explain the high level of IL4 as all patients in this study donot reach the sustained viral response (SVR). Sustained SVR is the goal of therapy for patients with chronic hepatitis C Fried, et al., [41]. Unfortunately, patients with chronic hepatitis C do not represent a homogeneous population with uniform responses to therapy. Therefore, developing an optimized therapeutic regimen suited to all patients has proved difficult [42]. Although viral eradication is well documented in patients with SVR, changes in antibodies to HCV status remain unclear. The diagnosis of resolved hepatitis C infection is based on the detection of the HCV-specific antibody and the absence of detectable serum HCV RNA. Complete or partial seroconversion of the HCV antibody is characterized by a progressive non-synchronized loss of these antibodies [43,44].

Early studies of patients treated with venom or inhalant allergen immunotherapy reported a reduction in proliferative responses to allergen Eusebius, et al., [45] and Jutel, et al., [46] with an overall shift away from Th2 to Th1 response Ebner, et al., [47]. However, other studies based on different cohorts of patients with pollen immunotherapy have not reproduced these findings Wachholz, et al., [48] and Francis, et al., [49]. A possible explanation is that inhibition of peripheral T-cell proliferation and Th2 cytokine production is not the fundamental event in immunotherapy Till, et al., [50]. There is discrepancy in cytokine changes during specific immunotherapy (SIT) and this may be related to several factors such as inconsistency. BV allergies are usually monosensitized in comparison to polyallergic and atopic hay fever patients. The protocols of the immunotherapies differ in these studies; in ultra-rush immunotherapy a high dose is given in the first day which is much more than the amount received in classical immunotherapy. Finally, the methodological differences in restimulation of T cells by anti-CD3 instead of antigen-specific stimulation may be responsible for differences in cytokine production [16].

Regarding liver enzymes, chronic HCV is characterized by the fluctuation in aminotransferase values. Fluctuations seem to represent effects of mutations in the virus or episodic immune reactions. Also, HCV has the property to involve bile duct epithelial cells, causing a significant cholestatic component with changes of GGT levels. One of the striking features for this study is decreased level of GGT in group 1 through the follow-up period while increased in the other two groups. This result needs further assessment on large scale of patients.

To conclude, the dilemma of cytokine response in bee stings in patients with chronic HCV is not well characterized because other mechanisms should be studied like role of chemokine receptors expression and its role in subsets of immune cells, such as Th2 cells, might be subject to selective recruitment as a result of restricted expression of certain chemokine receptors.

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References


