

Novel Therapeutic Modality Employing Apitherapy for Controlling of Multiple Sclerosis

Ahmad G Hegazi*, Khaled Al-Menabbawy, Eman H Abd El Rahman and Suzette I Helal
Department of Zoonotic Diseases, National Research Center, Dokki, Giza, Egypt

*Corresponding authors: Ahmed Hegazi, Department of Zoonotic Diseases, National Research Center, Egypt, Tel: +201001440063; Fax: +20237749222; E-mail: ahmedhegazi128@gmail.com

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Abstract

Objective: Study the effect of bee sting therapy (Apitherapy) in the treatment of Multiple Sclerosis.

Methods: Fifty patients with MS, their ages ranged between 26-71 years, were subjected to complete clinical and neurological history and examination to confirm the diagnosis. All cases were under their regular treatment they were divided into two main groups, Group I received honey, pollen, royal jelly and Propolis and were treated with acupuncture 3 times weekly, for 12 months, in addition to their medical treatment, while group II remains on their ordinary medical treatment only. Acupuncture was done by bee stings for regulating the immune system.

Results: Results revealed that 4 patients showed some improvement regarding their defects in gait, bowel control, constipation and urination, while 12 cases, showed some mild improvement in their movement in bed, and better improvement in bed sores, sensation, and better motor power, only two cases of them were able to stand for a few minutes with support. Interleukin (IL) 1 β , tumor necrosis factor alpha (TNF α), and IL-6 were detected. The level of TNF- α was significantly elevated in patients in Group II, while IL-1 β was reduced in Group II than Group I and no significant differences were found for IL-6 between the 2 groups. The mean values of IgE level in both groups of M.S. Patients were low, but with no statistical significance, while by the end of the study there were an elevation in the levels of IgE for both groups, which was statistically significant.

Conclusion: Although Apitherapy is not a curable therapy in MS, but it can be used to minimize the clinical symptoms of MS, and can be included among programs of MS therapy.

Keywords: Multiple sclerosis; Apitherapy; Bee venom; Cytokine; Food supplements

Introduction

Multiple sclerosis (MS) is a chronic and putatively immune mediated inflammatory disease of the central nervous system [1]. MS altered immune function in patients. It exhibits many of the hallmarks of an inflammatory autoimmune disorder including breakdown of the blood-brain barrier, the recruitment of lymphocytes, microglia, and macrophages to lesion sites, as well as the presence of multiple lesions [2]. It is characterized by the damage of fatty myelin sheaths around the axons of the brain and spinal cord, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms [3].

Apitherapy is the medical use of honey bee products. This includes the use of honey, pollen, bee bread, propolis, royal jelly, and apilarnil and bee venom. Most claims of apitherapy have not been proved to the scientific standards of evidence-based medicine and are anecdotal in nature. Bee venom therapy is an alternative form of healing. Bee venom therapy is the part of apitherapy which utilizes bee venom in the treatment of health conditions. However, bee venom is a complex mix of a variety of peptides and proteins, some of which have strong neurotoxic and immunogenic effects [4]. It has been used since ancient times to treat arthritis, rheumatism, back pain, skin diseases and in this modern age as an alternative therapy to treat autoimmune

diseases, Lyme disease and chronic fatigue syndrome [5-7]. Some reports have shown beneficial effects of bee venom in post herpetic neuralgia [8], fibromyalgia and multiple sclerosis [9]. Interleukin (IL) 1 β , tumor necrosis factor alpha (TNF α), and IL-6 are cytokines which mediate cellular responses during immune activation and inflammation. In Multiple Sclerosis (MS) they might be responsible for T-cell activation (IL-1 β), for demyelination (TNF α), and for immunoglobulin (Ig) synthesis (IL-6) within the central nervous system [10]. There is no standardized practice for the administration of bee venom. The aim of this investigation was to evaluate the effect of bee venom therapy (Bee Sting) and other bee products on the immunological status of cases with MS.

Materials and Methods

The aim of this study was to determine the immunological status of MS patients. Patients had to be free of immunomodulatory treatment during the study period and for at least the preceding 12 months. This study was carried out on fifty MS patients diagnosed and confirmed by clinical examination and radiological studies [11-13,7]. Twelve males and thirty eight females, their ages ranged between 26-71 years, with a mean of 38.7 ± 4.8 . 2, were selected from patients attending the outpatient clinic of Adult Neurology in National Research Centre, Dokki, Egypt," over a period of three years from September 2008 till April 2011. All cases were subjected to complete clinical and neurological history and examination to confirm the diagnosis. They

were signed consent according to medical ethic committee (14146). There were 32 cases with quadripareisis, (8 males and 24 females) and 18 cases with parapareisis (4 males and 14 females). All cases were under their regular treatment either by corticosteroids, or interferon. These cases were divided into two main groups, each group consists of 25 cases (6 males and 19 females), Group I received honey, pollen, royal jelly and Propolis and were treated with bee stings (from honeybee (*Apis mellifera* L.) workers of pure Carniolan race) 3 times weekly, for 12 months, started gradually by one sting, then gradually increase up to 25 stings per session-“after a sensitivity test was done”- in addition to their medical treatment, while group II remains on their ordinary medical treatment only. Informed consent was signed by the patients and their caregivers to participate in the study including the following:

Full general and neurological assessment, including full history and examination according to sheet prepared for the study, neurological examination on the beginning of the study to confirm the diagnosis, then the examination was repeated every two months to examine and detect the changes which may be happened for each case, and recorded in the form of scores starting from 0 for normal with no abnormal signs up to 5 for complete disability.

Apiacupuncture done by bee stings in the following points Du 13, 14, Li 11, S6, S9, points for cervical area and lumbar area and vision points GB2 and Li3 [14].

Supplementation

All patients of both groups were ordered to receive one tablet (Kirkland signature, USA) according to Bowling and Stewart [15]. Each Tablet Contains-% Daily Value: Vitamin A 2,500 I.U. 50% as Beta Carotene-40%, Vitamin C 90 mg-150%, Vitamin D3 500 I.U.-125%, Vitamin E 50 I.U.-167%, Vitamin K 30 mcg-38%, Thiamin (Vitamin B1) 1.5 mg-100%, Riboflavin (Vitamin B2) 1.7 mg-100%, Niacin 20 mg-100%, Vitamin B6 3 mg-150%, Folate (Folic Acid) 500 mcg-125%, Vitamin B12 25 mcg-417%, Biotin 30 mcg-10%, Pantothenic Acid 10 mg-100%, Calcium 220 mg-22%, Phosphorus 110 mg-11%, Iodine 150 mcg-100%, Magnesium 50 mg-13%, Zinc 11 mg-73%, Selenium 55 mcg-79%, Copper 0.9 mg-45%, Manganese 2.3 mg-115%, Chromium (as Chromium Picolinate) 45 mcg-38%, Molybdenum 45 mcg-60%, Chloride 72 mg-2%, Potassium 80 mg-2%, Boron 150 mcg, Nickel 5 mcg, Silicon 2 mg, Vanadium 10 mcg, Lutein (flower) 250 mcg, Lycopene 300 mcg.

Laboratory studies

Three milliliters of venous blood were drawn from fasting patients and controls with a sterile plastic syringe. A total of 2 ml. blood was gently placed in a dry clean plastic tube and left for 15 minutes to clot, then centrifuged at 3500 r.p.m for separation of serum, which then kept in deep freeze at -70°C until analysis. Serum samples were obtained from patients with clinically definite MS for estimation of serum levels of immunoglobulin E [16] using commercially available ELISA kits according to the manufacturers' directions. Serum cytokine levels (Interleukin 1 β , tumor necrosis factor alpha and IL-6) were assessed using enzyme linked-immunosorbent assays [17] using commercially available ELISA kits according to the manufacturer's directions (kits produced by the Bender MED System, Vienna, Austria). All these investigations were done at the beginning of the study and by the end of one year of supplementation and bee sting sessions.

Statistical analysis

All data obtained were statistically analyzed using Microsoft Excel and SPSS 11.5 for windows software package including, “to test, non-parametric Qui square, Mann Witney and anova test”. [18].

Results

In the current study, fifty patients diagnosed as MS, twelve males and thirty eight females, their ages ranged between 26-71 years, with a mean of 38.7 ± 4.8 . Thirty two cases with quadripareisis, (8 males and 24 female) and 18 cases with parapareisis (4 males and 14 females). During the period of the study there was a gradual assessment of signs and symptoms every 2 months for both groups of patients. These periodic assessments for both groups showed the improvement in the scores of signs recorded every two months as shown in Table 1 as well as in the scores of symptoms recorded during the same period among patients for both groups.

Results revealed that 4 patients out of 9 (44.4% of parapareisis cases), showed significant improvement regarding their defects in gait, bowel control, constipation and urination, while 12 cases out of 16 cases (75% of quadripareisis cases), showed mild improvement in their movement in bed, and better improvement in bed sores, sensation, and better motor power, only two cases of them (12.5%) were able to stand for a few minutes with support among patients of group I. As shown in Table 1 there was no statistically significant differences between group I and group II at the start of the study, regarding their general condition, depression, fatigue, sleeping heat tolerance, attention span, memory, rigidity, spasms and tremors, while by the end of the study there were significant improvement regarding general condition, depression sleeping, heat tolerance attention span, memory as well as rigidity and muscle spasms.

In this study, the mean values of IgE in both groups of M.S. Patients (Table 2) were low, but with no statistical significance, while by the end of the study there were an elevation in the levels of IgE for both groups, which was statistically significant among cases of group (I). The aims of the present study were to evaluate the pro-inflammatory tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , and IL-6. Results are shown as the mean \pm standard error in pictograms per milliliter (Table2). All patients with MS had significantly higher cytokine concentrations. The level of TNF- α was significantly elevated in Group II patients (6.1 ± 1.3 pg/mL) vs control Group I subjects (4.3 ± 0.1 pg/mL) at the end of experiment (12 months), as was the level of IL-1 β (123.0 ± 2.2 vs 224.1 ± 1.4 pg/mL; $P \leq .0001$) at the end of experiment respectively. In contrast, no significant differences were found for IL-6 between the group I and group II.

Discussion

MS affects the ability of nerve cells in the brain and spinal cord to communicate with each other effectively [19]. Theories include genetics or infections as causes for MS. Different environmental risk factors have also been found [20]. Life expectancy of people with MS is 5 to 10 years lower than that of the unaffected population [3]. Several subtypes, or patterns of progression, have been described. Subtypes use the past course of the disease in an attempt to predict the future course. They are important not only for prognosis, but also for therapeutic decisions [21]. Although there is no known cure for multiple sclerosis, several therapies have proven to be helpful.

	2 Months		4 Months		6 Months		8 Months		10 Months		12 Months	
	G 1	G 1I	G 1	G 1I	G 1	G 1I	G 1	G 1I	G 1	G 1I	G 1	G 1I
General Condition	4.87 ± 0.440	3.73 ± 0.5	4.57 ± 0.278	3.49 ± 0.26	3.87 ± 0.398	3.17 ± 0.23	3.71 ± 0.267	3.01 ± 0.22	2.85 ± 0.243	2.83 ± 0.13	2.37 ± 0.182	2.8 ± 0.12
Depression	3.50 ± 0.707	3.62 ± 0.24	3.62 ± 0.625	3.6 ± 0.35	3.37 ± 0.652	3.29 ± 0.12	3.00 ± 0.534	3.05 ± 0.72	2.62 ± 0.323	2.76 ± 0.45	1.75 ± 0.25	2.45 ± 0.33
No Energy Fatigue	3.75 ± 0.647	3.24 ± 0.6	3.37 ± 0.595	3.25 ± 0.35	3.62 ± 0.893	3.24 ± 0.38	4.25 ± 0.883	3.28 ± 0.28	2.87 ± 0.665	2.95 ± 0.85	2.75 ± 0.311	2.85 ± 0.35
Sleeping	3.75 ± 0.490	3.4 ± 0.58	3.37 ± 0.679	3.39 ± 0.46	2.50 ± 0.327	3.07 ± 0.12	2.37 ± 0.323	2.8 ± 0.62	1.75 ± 0.25	2.35 ± 0.25	1.37 ± 0.182	2.07 ± 0.12
Heat tolerance	3.75 ± 0.725	3.5 ± 0.75	3.37 ± 0.818	3.39 ± 0.16	2.50 ± 0.590	3.06 ± 0.590	2.37 ± 0.534	2.73 ± 0.63	1.75 ± 0.462	2.5 ± 0.42	1.37 ± 0.365	2.37 ± 0.365
Attention span	2.12 ± 0.479	2.1 ± 0.45	2.00 ± 0.534	2.06 ± 0.32	1.87 ± 0.398	1.93 ± 0.66	1.50 ± 0.267	1.67 ± 0.21	1.25 ± 0.163	1.53 ± 0.62	1.25 ± 0.163	1.38 ± 0.22
Memory	2.12 ± 0.350	2.3 ± 0.35	2.00 ± 0.462	2.13 ± 0.27	1.75 ± 0.365	2.05 ± 0.05	1.50 ± 0.188	1.92 ± 0.27	1.62 ± 0.182	1.82 ± 0.15	1.50 ± 0.188	1.72 ± 0.13
Rigidity	2.50 ± 0.50	2.48 ± 0.52	2.50 ± 0.654	2.39 ± 0.52	3.00 ± 0.731	2.17 ± 0.33	2.25 ± 0.411	2.06 ± 0.18	2.12 ± 0.479	1.92 ± 0.72	1.62 ± 0.323	1.84 ± 0.23
Spasms	2.37 ± 0.595	2.4 ± 0.58	1.87 ± 0.610	2.26 ± 0.19	2.50 ± 0.462	2.19 ± 0.25	1.75 ± 0.313	2.03 ± 0.18	2.50 ± 0.707	1.89 ± 0.09	1.87 ± 0.398	1.74 ± 0.398
Tremors	2.37 ± 0.32	2.36 ± 0.43	2.50 ± 0.654	2.3 ± 0.52	2.75 ± 0.674	2.28 ± 0.45	2.25 ± 0.453	2.2 ± 0.32	2.37 ± 0.679	2.17 ± 0.66	2.01 ± 0.15	2.12 ± 0.718
Headaches	1.87 ± 0.639	1.86 ± 0.19	2.62 ± 0.625	1.72 ± 0.23	2.12 ± 0.398	1.66 ± 0.32	1.62 ± 0.263	1.59 ± 0.28	1.75 ± 0.313	1.49 ± 0.19	1.37 ± 0.263	1.42 ± 0.23
Eye sight	2.50 ± 0.566	2.48 ± 0.36	2.50 ± 0.566	2.39 ± 0.34	2.25 ± 0.490	2.31 ± 0.09	1.87 ± 0.440	2.27 ± 0.62	1.87 ± 0.398	2.13 ± 0.18	1.75 ± 0.313	2.01 ± 0.13
Speech	1.62 ± 0.263	1.59 ± 0.63	1.62 ± 0.263	1.55 ± 0.17	1.87 ± 0.295	1.49 ± 0.24	1.50 ± 0.267	1.44 ± 0.15	1.50 ± 0.267	1.4 ± 0.28	1.37 ± 0.182	1.37 ± 0.08
Swallowing	1.37 ± 0.263	1.45 ± 0.21	1.37 ± 0.182	1.41 ± 0.22	1.37 ± 0.182	1.38 ± 0.42	1.12 ± 0.125	1.32 ± 0.19	1.25 ± 0.163	1.29 ± 0.33	1.12 ± 0.125	1.26 ± 0.25
Numbness	2.62 ± 0.375	2.5 ± 0.5	2.62 ± 0.375	2.42 ± 0.34	2.25 ± 0.453	2.23 ± 0.66	1.87 ± 0.295	2.07 ± 0.35	1.62 ± 0.375	1.91 ± 0.07	1.25 ± 0.163	1.72 ± 0.12
Balance	4.62 ± 0.800	3.49 ± 0.26	3.87 ± 0.685	3.37 ± 0.63	3.00 ± 0.843	3.02 ± 0.42	2.50 ± 0.707	2.71 ± 0.17	1.87 ± 0.692	2.47 ± 0.32	1.50 ± 0.834	2.05 ± 0.47
Walking	4.37 ± 0.595	3.3 ± 0.62	4.37 ± 0.595	3.27 ± 0.25	4.50 ± 0.707	3.14 ± 0.45	4.25 ± 0.75	3.07 ± 0.37	3.87 ± 0.742	2.96 ± 0.52	2.87 ± 0.639	2.87 ± 0.639
Hand coordination	2.62 ± 0.532	2.7 ± 0.28	2.62 ± 0.460	2.6 ± 0.34	1.87 ± 0.226	2.47 ± 0.56	1.75 ± 0.25	2.35 ± 0.77	1.50 ± 0.188	2.19 ± 0.28	1.37 ± 0.182	2.04 ± 0.12
Writing	2.62 ± 0.679	2.65 ± 0.25	2.87 ± 0.789	2.57 ± 0.09	2.12 ± 0.548	2.42 ± 0.52	1.87 ± 0.479	2.31 ± 0.08	1.75 ± 0.365	2.18 ± 0.33	1.62 ± 0.263	2.02 ± 0.26
Bladder & Bowel	4.12 ± 0.580	3.09 ± 0.35	4.12 ± 0.580	2.96 ± 0.65	3.50 ± 0.597	2.83 ± 0.42	3.25 ± 0.526	2.79 ± 0.67	2.37 ± 0.323	2.83 ± 0.13	2.12 ± 0.226	2.63 ± 0.16

Table 1: Scores of signs recorded every two months (1-12), for patients of both groups.

Bee sting therapy is increasingly used to treat patients with MS in the belief that it can stabilize or ameliorate the disease. However, there are no clinical studies to justify its use [22]. Found that there were no improvement of disability, fatigue, and quality of life. Bee sting therapy

was well tolerated, and there were no serious adverse events. They concluded that the treatment with bee venom in patients with relapsing multiple sclerosis did not reduce disease activity, disability, or fatigue and did not improve quality of life.

Groups	Mean at the start of the study				Mean at the end of the study			
	IgE	(IL) 1 β	(TNF) α ,	IL-6	IgE	(IL) 1 β	(TNF) α ,	IL-6
Group (I)	664 \pm 32	153 \pm 3.73	4.5 \pm 0.001	4.8 \pm 0.01	1031 \pm 14	123.0 \pm 2.2	6.1 \pm 1.3	14.8 \pm 0.05
Group (II)	491 \pm 42	222 \pm 4.13	2.6 \pm 0.002	4.6 \pm 0.04	1045 \pm 32	224.1 \pm 1.4	4.3 \pm 0.1	13.6 \pm 0.04

Table 2: Mean levels of IgE, Interleukin (IL) 1 β , tumor necrosis factor alpha (TNF) α , and IL-6 for both groups at the start of the study and by the end of the study

The therapeutic benefits of honeybee venom have been known for a long time to relieve pain and to treat inflammatory diseases, particularly for treatment of arthritic and rheumatic conditions in humans [23,24] and in animals [25-27]. Specific immunotherapy with bee venom can result in an almost complete protection against adverse (or allergic) reactions from stings in the great majority of cases [28].

In this study, there was a statistically significant improvement in patients regarding their immunity, their health and general conditions and this is in agreement to that explained by Park et al. and Prado et al. [29,30] that bee stings cause hemoconcentration which might be related to the marked edema induced by the venom. According to Janik et al. [8], course of treatment starts with testing the patient for allergy, which is known to occur in 1% of the general population. Bee venom is administered in the form of a direct bee sting or else by injection of a venom extract, and the treatment is usually given twice a week.

Bee Venom Acupuncture (BVA), as a kind of herbal acupuncture, exerts not only pharmacological actions from the bioactive compounds isolated from bee venom but also a mechanical function from acupuncture stimulation. BVA is growing in popularity, especially in Korea, and is used primarily for pain relief in many kinds of diseases [29-31]. Park et al. and Prado et al. stated that bee venom therapy is used by people with many different autoimmune disorders, including MS, rheumatoid arthritis, lupus and scleroderma. It is also used for a number of other diseases and conditions, including depression, skin conditions, menstrual cramps and varicose veins. It is claimed that bee venom therapy works with the patient's own body to reduce inflammation. The theory is that because the stings produce inflammation, the body mounts an anti-inflammatory response. Presumably, this would then work to reduce inflammation where the myelin is being attacked by the immune system in a person with MS [29,30].

The location of the sting is important, with the sting acting as a sort of acupuncture in combination with the effects of the venom, while others reported that the location is not important. The number of stings also varies widely from a few to hundreds and they may be administered either by live bees or by injection. This treatment can cause pain, and even result in death if the subject has an allergy to bee venom, which can produce anaphylactic shock [22]. For honeybee venom subcutaneous immunotherapy 100 or 200 μ g doses are considered effective. The median lethal dose (LD50) for honeybee venom has been reported in a number of reports [32,33] as 2.8 mg venom/kg body weight for intravenous and 3.8 mg venom/kg body weight for intraperitoneal delivery in mice. A 2004 randomized crossover study was conducted in the Netherlands among 24 people with either relapsing-remitting MS or secondary-progressive MS. While the treatment was well-tolerated, no beneficial effects were seen on the MRIs or clinically among these patients [21,19]. In our study

there was a statistically significant improvement regarding the clinical findings while the results of MRIs done to follow up for our patients showed that there is no more changes in the demyelinating lesions which means that demyelination shows no progression as shown in other previous investigation [7].

BV administration was reported to stimulate the function of immune system [34] and to affect the release of cortisol production which is known as natural anti-inflammatory agent [35]. Melittin which is the major component of BV was found to suppress inflammation by inhibiting Phospholipase (PLA) enzymatic activity [36]. This enzyme was abundantly released in severe inflammatory disorders and actively found to cause tissue and organ degradation which will lead to the loss of their functions [37]. Melittin was also found to block the production of neutrophil superoxide [38]. Bee venom (inflammation, allergy, cytotoxic, haemolysis), antibacterial, antiinflammatory, immunoactivating, immunosuppressive, analgesic, radioprotective, anticarcinogenic, accelerates heartbeat, increases blood circulation, lowers blood pressure, improves haemoglobin synthesis, anticoagulant, lowers cholesterol levels, membrane effects on blood cells, influences immuno-active blood cells and hormone levels, antiarrhythmic, heart stimulating therapeutic effects, improvement in hypertonia and arteriosclerosis [38-40].

The mechanism of action of bee venom was clarified as follows: bee venom blocks the building of proinflammatory substances and inhibits the proliferation of rheumatoid synovial cells. Today, bee venom is applied directly via sting or injection. This practice was initiated in 1964 in Russia [41] and has been further developed since then, mostly in the Far East [42].

Apamin accounts for less than 2% of venom dry weight, presents a neurotoxic action [42] and possesses unusual functional as well as structural properties [44]. It is remarkable among peptides in its ability to cross the blood-brain barrier and act on the central nervous system. Apamin is known to block calcium dependent potassium fluxes by binding to a Ca²⁺-dependent potassium channel [45]. Apamin is the smallest neurotoxic polypeptide known. It has been isolated from bee venom [46].

Nam et al. [47] who stated that bee venom is a mixture of many substances. In this study we found that there is a statistically significant improvement regarding their immunity and the improvement in their health and general conditions and this is explained by Park et al. and Prado et al. [29,30] bee stings cause hemoconcentration which might be related to the marked edema induced by the venom. Following bee stings there is an increase in various cytokines like interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , etc. In a mouse model using the subcutaneous route, rapid increases in serum alanine aminotransferase and aspartate aminotransferase transaminases, creatinine, urea nitrogen, uric acid, sodium and

chloride electrolytes, and creatine kinase were recorded [48,28,29]. The pain and swelling of the sting are caused by histamine, dopamine, serotonin, and norepinephrine. Several toxins are also present, including apamin, melittin, monamine, and mast-cell degranulating peptide. Lastly, the substances responsible for the allergic response include hyaluronidase and phospholipase-A2, enzymes that work to activate immune cells and produce Immunoglobulin E [47].

Despite a lack of scientific evidence, bee venom therapy has been reported by people with MS to increase stability, as well as reduce fatigue and spasticity. More than 1,300 people with MS have sent testimonials to the American Apitherapy Society in support of the therapy [19,7] this is agreed by our study as patients under bee venom therapy showed marked improvement in stature, stability, and spasticity, while our study is disagreed with Castro et al. [4] as they found that there were no definite conclusions regarding efficacy and therefore there was little evidence to support the use of honeybee venom in the treatment of MS.

The present study was conducted to evaluate the pro-inflammatory Tumor Necrosis Factor (TNF)- α and interleukin (IL)-1 β , and IL-6. Results are shown as the mean \pm standard error in pictograms per milliliter (Table2). All patients with MS had significantly higher cytokine concentrations. The level of TNF- α was significantly elevated in Group II patients (6.1 ± 1.3 pg/mL) vs control Group I subjects (4.3 ± 0.1 pg/mL), as was the level of IL-1 β (123.0 ± 2.2 vs 224.1 ± 1.4 pg/mL; $P \leq .0001$), respectively. In contrast, No significant differences were found for IL-6 between the 2 groups. These findings supported by the previous findings of different authors as Martins et al. [10] who found that 10 of the 13 cytokines or markers were significantly different between patients and age-matched control subjects. The increase in proinflammatory and down-regulatory cytokines in patients with MS is consistent with the progression of the disease because inflammatory and restorative processes seem to occur simultaneously. Following bee stings there is an increase in various cytokines like interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , etc. In a mouse model using the subcutaneous route, rapid increases in serum alanine aminotransferase and aspartate aminotransferase transaminases, creatinine, urea nitrogen, uric acid, sodium and chloride electrolytes, and creatine kinase were recorded Park et al. and Prado et al. Cytokines are significantly elevated in patients with MS are consistent with the pathologic features of this disease. Interleukin (IL) 1 β , Tumor Necrosis Factor alpha (TNF- α), and IL-6 are cytokines, which are increased during immune activation and inflammation [48,49]. IL-1 β is released from macrophages and endothelial cells and is involved in the synthesis and release of acute phase proteins. In patients with MS, IL-1 β is mainly expressed by microglial cells and infiltrating macrophages throughout the white matter and, especially, in acute lesions [50] and has been shown to promote oligodendrocyte death in tissue culture [51].

Several review articles have stressed the need for the identification of biomarkers in MS as a valuable tool in the diagnosis and identification of disease stages and subcategories of MS and in monitoring treatment of the disease [52-55]. Hagman et al [55] examined cytokine concentrations in 72 patients with clinically definite MS and 21 healthy control subjects. Of the 5 cytokines included in their study (IL-10, TNF- α , IL-6, IFN- γ , and IL-2) they found that TNF- α was significantly elevated in the MS patients compared with control subjects. Martins et al. observed [10] marked increases in the concentrations of proinflammatory IL-1 β and TNF- α , and anti-inflammatory (TH2)-type cytokines in patients compared

with control subjects, with 9 of the 13 cytokines or markers showing highly significant differences in these 2 groups. Serum TNF α was significantly higher in depressed and MS patients than in normal controls interferon (IFN)- γ ; interleukins (ILs)-1 β , 6 and tumor necrosis factor (TNF)- α . Significant increases between patients and control subjects were found for IL-1 β (mean, 26.0 vs 14.3 pg/mL; $P \leq .0001$), IL-6 (mean, 16.8 vs 7.5 pg/mL; $P=0.03$) and TNF- α (mean, 4.5 vs 1.6 pg/mL; $P=0.01$) [56].

Concerning to the levels of IgE which showed significant low levels among cases of both groups (I and II), while by the end of the study marked elevation of IgE levels. The previous study was assessed and they can be explained by Nam et al. [47] who stated that bee venom is a mixture of many substances. In this study, we found that there is a statistically significant improvement regarding their immunity and the improvement in their health and general conditions and this is explained by Park et al. and Prado et al. [29,30] bee stings cause hemoconcentration which might be related to the marked edema induced by the venom. Following bee stings there is an increase in various cytokines like interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , etc. The pain and swelling of the sting is caused by histamine, dopamine, serotonin, and norepinephrine. Several toxins are also present, including albumin, melting, Menominee, and mast-cell degranulation peptide. Lastly, the substances responsible for the allergic response include hyaluronidase and phospholipase-A2, enzymes that work to activate immune cells and produce immunoglobulin E [47].

Conclusions

From the results we conclude that bee venom injected intradermally could be a potential new therapeutic agent in the treatment of MS patients, with minimal tolerable side effects. Interleukin-1 β , 6 and TNF could be considered as an indicator in the treatment of MS with intradermal injection of bee venom. Larger randomized controlled complementary studies are needed to explore their efficacy. This work is a potential starting point for larger studies with wider scales of applications to confirm our findings.

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