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Relationship Between the Bee Venom Therapy and Tumor Necrosis Factor-308 Variation in the Management of Rheumatoid Arthritis, a Prospective Study

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Abstract: Bee venom (BV) was traditionally used to treat various inflammatory disorders including rheumatoid arthritis (RA). The current study aims to assess the anti-arthritic effect of BV and the relation between tumor necrosis factor (TNF)-308 polymorphism and BV treatment response in RA. Methods: 50 RA patients received BV injection for 6 months, with an evaluation of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), visual analog scale (VAS), disease activity score (DAS28-ESR), TNF- α , at baseline and after 6ms. Genotyping assay for TNF- α G308A rs1800629 gene polymorphism. Results: The mean age was 36.0 (29.0 -40.0) years; 90% were females and 10% were males with a mean disease duration 8 (5-10 years). Most of the studied patients (64%) had high disease activity and 37% had moderate disease activity with a mean 5.5 (4.7 -6.8) at baseline. Treatment with BV was associated with a significant improvement in ESR, CRP, VAS, and significant decline in the DAS28-ESR score with p-value <0.005. Most of cases achieved moderate and good EULAR response and a significant reduction of (TNF α) Level. TNF- α -308 genetic variant showed that the GG genotype (32 patients, 64 %) was more prevalent followed by AA genotypes (14 patients, 28 %). There was no difference between TNF- α G308 genotypes regarding the post-treatment response. Conclusion: Treatment with Bee venom can improve joint pain, disease activity, reduce ESR, CRP, and TNF α levels in RA patients. No difference between TNF- α G308 genotypes regarding treatment response.

Keywords: Bee venom, RA, TNF- α .

1 Introduction

Rheumatoid arthritis (RA) is recognized as a multisystem autoimmune disorder with a chronic course. The hallmark feature of RA is persistent symmetric polyarthritis (synovitis) that primarily involves small peripheral joints and may be associated with extra-articular involvement [1]. Autoimmune reaction to an external trigger like smoking, or an infectious agent is the initial step in RA pathogenesis. Followed by synovial proliferation of different immune cells which are organized by different sets of cytokines [1].

An important cytokine is TNF- α , which plays a crucial role in other cytokine activation and expression, promoting the expression of endothelial cell adhesion particles, angiogenesis and suppression of regulatory T cells [2]. The TNF- α encoding gene is placed on chromosome 6, within the class III region of the major histocompatibility complex. There are several single-nucleotide polymorphisms in the TNF- α promoter gene. Among these, a common polymorphism in the promoter, G to A substitution at position -308, has been studied in autoimmune diseases [3].

Disease activity control targeting remission or even low disease activity is an ultimate therapeutic goal in RA cases. Complementary and alternative medicine may be a choice for unsatisfied arthritis patients [4].

The use of natural products in conjunction with modern medication may provide a synergistic interaction, which could improve therapeutic potency while reducing side effects [5]. Bee venom (BV) was traditionally used to treat various chronic inflammatory disorders as RA. Anti-arthritic effect of BV was demonstrated in animal models and in humans [6,7] which potentiated by its immunomodulating effect. This effect come through suppression of the signaling pathways such as nuclear factor-kappa B and activator protein 1 that are involved in the activation of inflammatory cytokines including interleukin-6 (IL-6), IL-8, interferon- γ (IFN- γ), and TNF- α [8].

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The current study's objectives were to assess the anti-arthritic activity of BV and investigate the relationship between the TNF-308 polymorphism and treatment response.

2 Methodologies

The present research adopted the descriptive method due to its appropriateness for the research and its objectives. Fifty RA patients, who met the American College of Rheumatology (ACR)/European League Against Rheumatism 2010 categorization criteria for RA [9] were sequentially recruited from the VACERA research center in a prospective follow-up study. Exclusion criteria: Patients with hypersensitivity to bee venom, inflammatory arthritis other than rheumatoid arthritis or any systemic disease as diabetes hypertension, hyperlipidemia, coronary artery disease, renal or hepatic insults, and pregnant or lactating females were excluded from the study. Patients who receive steroids, DMARDs and biologic treatment were also excluded.

In the beginning, the histories of all enrolled RA patients were taken (about joint affection, disease duration, and medication). Musculoskeletal examination and clinical assessment of disease activity by calculating the disease activity score in 28 joints [DAS 28-ESR] [10].

The DAS 28-ESR score estimation requires, counting the number of tender and swollen joints, Erythrocyte Sedimentation Rate (ESR), and patient's visual analogue scale (VAS) rating. A score < 2.6 is defined as disease remission and a score $[>2.6 \text{ and } <3.2]$ is classified as low disease activity. A score $[>3.2 \text{ and } <5]$ indicates moderate disease activity whereas a score > 5.1 denotes high disease activity state. All the study participants had a DAS28-ESR value >3.2 (moderate and high disease activity) prior to commencing bee venom injection.

All these parameters [VAS, ESR, CRP, and DAS 28-ESR scores] were evaluated at baseline and after 6 months of bee venom therapy. Patients were classified according to improvement in DAS28 ESR from baseline and based on EULAR response criteria into good [DAS28-ESR is ≤ 3.2 and has decreased by >1.2], moderate [DAS28-ESR ≤ 3.2 plus a decrease >0.6 and ≤ 1.2] or [or a DAS28 ≤ 5.1 and >3.2 and a decrease >0.6] or [DAS28-ESR >5.1 and a decrease >1.2], and non-response [DAS28-ESR decreased ≤ 0.6 or DAS28-ESR >5.1 and a decrease ≤ 1.2] [11].

Laboratory measurements: were recorded at baseline and after 6 months, CBC was performed using the Cell_Dyn Ruby autoanalyzer (Abbot), liver and kidney function tests were done by using AU 4.0 autoanalyzer, the first hour's ESR was calculated using the Western technique, serum CRP was quantified by immunoturbidimetry (Beckman Coulter) on AU 680 instrument. CRP $> 6 \text{ mg/l}$ and RF $\geq 8 \text{ IU/ml}$ were regarded as abnormal results. The anti-cyclic citrullinated peptide (anti-CCP) level was measured by enzyme-linked immunosorbent technique (ELISA) using the QUANTA Lite® CCP3IgG ELISA, from INOVA Diagnostics. The manufacturer's methodology regarded a serum reading of more than 20 units as positive.

A 5ml of each sample obtained was left for one hour to coagulate at room temperature in a serum separator collection tube and centrifuged for 5 minutes at 3000rpm. The serum was collected and stored at -20°C until to be used to measure tumor necrosis alpha (TNF- α) serum level by human TNF- α enzyme linked immunosorbent assay (ELISA) using Human TNF- α ELISA kit, Glory Science Life Science Company, cat no: 15757, USA.

Five ml of peripheral venous blood sample were collected in EDTA tubes for DNA extraction from all included participants; using the QIAamp DNA blood kits, cat no: 51104 (Qiagen, Hilden, Germany). The procedure of extraction by silica-membrane-based technology. Genotyping assay for TNF- α G308A rs1800629 gene polymorphism was measured using Applied Biosystems™ TaqMan™ SNP Genotyping Assays “TNF- α G308A rs 1800629, cat no: PN4351379, assay ID: C_8848034879” from (ThermoFisher Scientific, Germany). The PCR TaqMan Genotyping Master Mix kit, cat no: 4371353 (ThermoFisher, Germany). The appropriate volume from PCR reaction mix is prepared with a total volume of $10\mu\text{L/well}$. The reaction mix of each sample is composed of $5\mu\text{L}$ of 2X TaqMan Genotyping Master Mix, $0.5\mu\text{L}$ of TaqMan assay (20X), and $4.5\mu\text{L}$ RNase free water. The thermal cycling protocol is optimized as follows” 950C for 10 minutes for AmpliTaq Gold, UP Enzyme Activation, followed by denaturation step at 950C for 15 seconds and annealing/extension at 600C for 1 minutes for 40 cycles. The qPCR was performed on Applied BioSystems PCR instrument (ThermoFisher Scientific, Germany).

The purified BV from the *Apis mellifera* species at a concentration of 1:1 was prepared at VACSERA, registered in the Egyptian Drug policy, and Planning center of Ministry of Health and Population No24206/2005. All the cases in the study received intradermal injections of BV (0.5 ml to 1 ml/day) for six months by following the injection schedule (table 1). Three sessions per week are advised, with many injections being given at each session, particularly at the painful places, and the dose is gradually raised based on the patient's ability to tolerate the venom up to 0.5ml/session. Skin sensitivity test by 0.05 ml of BV intradermally was done before the first injection at the flexor surface of the forearm, then evaluated for any local or systemic reactions (table 1).

Table 1: Schedule of bee venom injection.

Rush treatment	Day (1)	Day (3)	Day (5)	Day (7)	Day (9)
	0.05(ml)	0.1 (ml)	0.2 (ml)	0.3 (ml)	0.5 (ml)
Maintenance treatment	0.5 (ml) 2 - 3 times/week				

Proper orientation of the objectives of the study was followed by informed written consent from all subjects participating in this study.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20 (Armonk, NY: IBM Corp, 2018). Shapiro-Wilk test was used to verify the normality of distribution Quantitative data was described using range (minimum and maximum), mean, and standard deviation. Significance of the obtained results was judged at the 5% level. F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.

3 Results

Fifty RA patients were included. Their mean age was 36.0 (29.0 -40.0) years; of whom 90% were females and 10% were males with mean Disease duration 8 (5-10years). Assessment of disease activity at baseline using the DAS28-ESR score showed that most of the studied patients (64%) had high disease activity and 37% had moderate disease activity with a mean 5.5 (4.7 -6.8) (Table 2).

Table 2: Baseline characteristics and TNF 308 genotype of the studied subjects:

		RA patients N=50
Age in yrs	Median (IQR)	36.0 (29.0 -40.0)
Sex	Female (N&%)	45 (90%)
	Male (N& %)	5 (10%)
Disease duration in yrs		8(5-10)
Rheumatoid factor IU/ml Median(IQR)		55.0 (22.0 - 178.0)
ACPA^c IU^{**}/ml Median (IQR)		84.5 (44.0 - 200.0)
TNF 308 genotype	GG	32 (64%)
	AA	14 (28%)
	GA	4 (8%)
	G allele	68 (68%)
	A allele	32 (32%)

IQR, interquartile range, EULAR, European League Against Rheumatism; **ACPA**, anti-citrullinated protein antibodies; **TNF**; **Tumor necrosis factor**

Treatment with BV resulted in a significant reduction in ESR [baseline 67.0 (54.0 -98.0) vs after 6ms 31.5 (18.0 - 43.0)], CRP [baseline 46.0(23.0 -88.0) vs 12.0(5.0 -24.0)], and VAS [baseline 6.0(6.0-7.0) vs after 6ms 3.0 (2.0 - 4.0)], p value< 0.01. The data demonstrated a significant decline in the DAS28-ESR score from baseline 5.5 (4.7 -6.8) to that after 6 ms 3.9 (3.0-4.2) of Bee venom treatment with p-value <0.005. Assessment of serum TNF-α concentration pretreatment (68.7 ± 8.6) and after 6 ms of BV injection (54.1 ± 7.5) revealed a significant reduction in serum TNF-α concentration (p-value 0.00) (Table 3). Good EULAR response was achieved by 23 (46%) of patients, 23(46%) achieved moderate response, and no response in 8 (8%) of patients (Figure 1).

Table 3: Comparison between baseline parameters and after 6 months of treatment

	Baseline	At 6 ms	P value
WBC (x103 /mm3)	6.7(6.1 - 7.3)	6.7(5.9-7.2)	0.13
Hemoglobin (g/dl)*	11.9 ±1.01	12.02±0.9	0.720
Platelets (/mm3)*	322.7 ± 62.9	315.2 ±67.9	0.594
Creatinine ((mg/dl)	0.730.73(0.61 - 0.96)	0.675(0.56-0.89)	0.218
ALT U/L	36(22.0-60.0)	30(18.0 -55.0)	0.08
AST U/L	39 (23-55)	26.5 (17-44)	0.06
ESR mm/hr	67.0 (54.0 -98.0)	31.5 (18.0 - 43.0)	0.00
CRP (mg/dl)	46.0(23.0 -88.0)	12.0(5.0 -24.0)	0.00

DAS 28-ESR	5.5 (4.7 -6.8)	3.9 (3.0-4.2)	0.000
VAS	6.0(6.0-7.0)	3.0 (2.0 - 4.0)	0.00
TNF*(pg/mL)	68.7 ± 8.6	54.1 ± 7.5	0.000

*parametric, *WBC, white blood cell; ALT, alanine transaminase; AST, aspartate aminotransferase ESR, erythrocytic sedimentation rate; RF, rheumatoid factor; CRP, C-reactive protein; DAS, disease activity score; VAS, visual analogue scale, TNF, tumor necrosis factor.

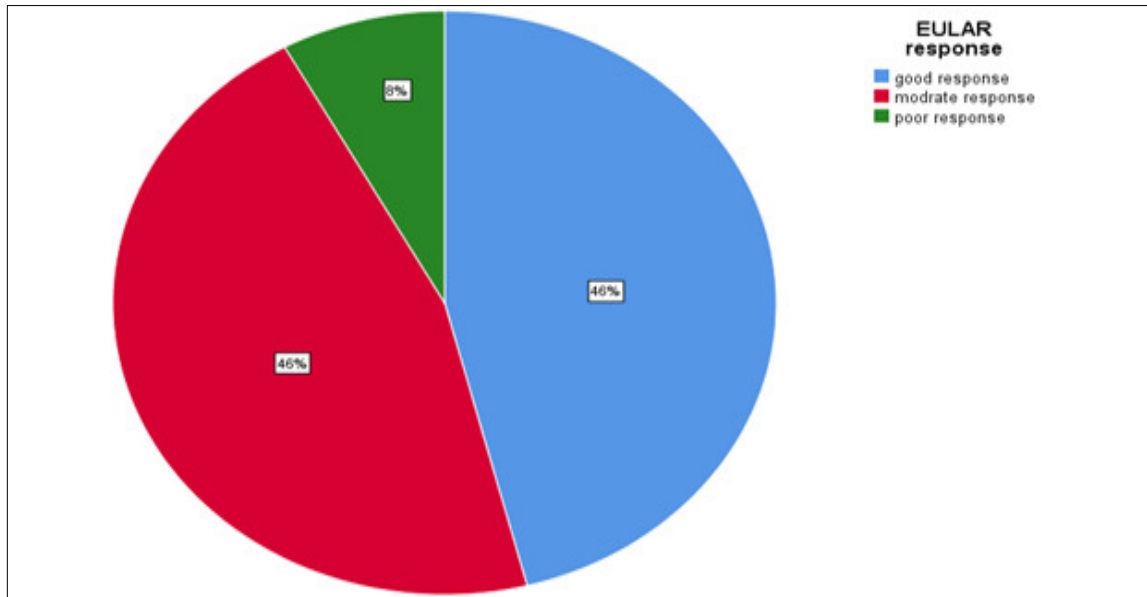


Fig. 1: EULAR response after bee venom therapy.

TNF-308 genetic variant in the studied subjects showed that GG genotype (32 patients, 64 %) was more prevalent in RA patients followed by AA genotypes (14 patients, 28 %) and GA genotype (4 patients, 8 %). In addition, G alleles are highly frequency 68 (68%) than A alleles 32 (32%) (**Table 2**).

There was no difference between TNF- α G308 genotypes Regarding the post treatment response, there was no significant difference between three genotypes with a mean DAS28 improvement nearly equal in each of them (-2), the same for the VAS (mean change -3), and CRP (mean change -30 to -20) in three genotypes. In addition, ESR was significant difference between three genotype (40.6 ± 19.6 , 26.9 ± 17.0 and 47.8 ± 11.9 , respectively) p value < 0.01 (**figure 2,3**). Based on the EULAR response criteria, TNF 308 genotypes were significantly associated with response to BV therapy, especially with AA and GG genotypes **table 4**.

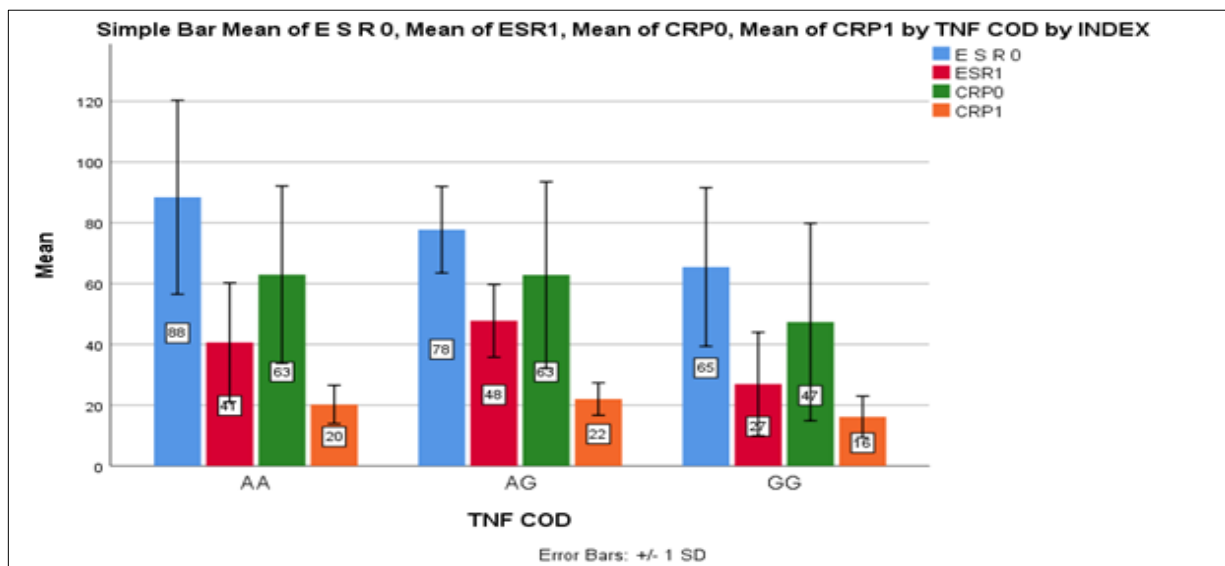


Fig. 2: ESR and CRP response to bee venom therapy in relation to TNF- α G308 genotypes

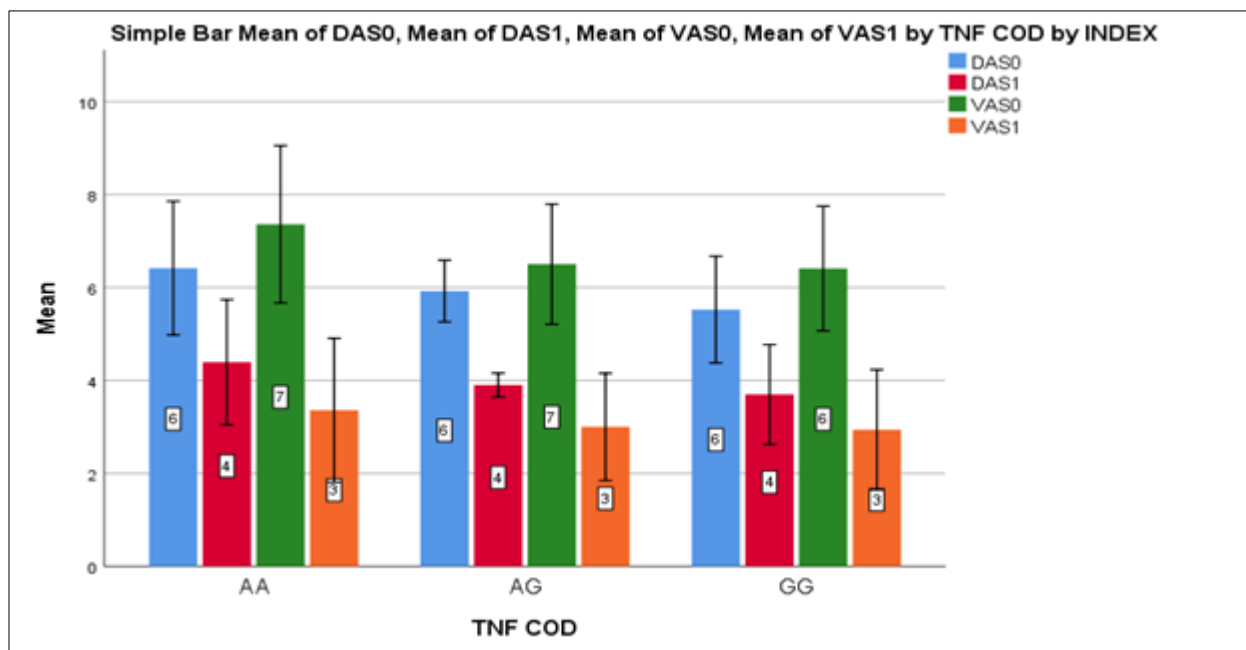


Fig. 3: DAS and VAS response to bee venom therapy in relation to TNF- α G308 genotypes DAS, disease activity score; VAS, visual analogue scale.

Table 4: Association between TNF 308 and bee venom response at 6 months:

EULAR response				
Genotype	Moderate	Good	Nonresponse	P value
AA	10(71.4)	3 (21.4)	1 (7.1)	0.000
GG	12(37.5)	19 (59.4)	1 (3.1)	
GA	1 (25)	1 (25)	2(50)	

4 Discussions

Bee venom has been investigated severally in vivo and vitro studies which give evidence of its multiple biological activities such as anti-mutagenic [11], anti-nociceptive [11], radioprotective [11], cytoprotective [12], anti-hepatotoxic [12], anti-oxidant [13,12], anti-microbial [13], anti-inflammatory [13,12], neuroprotective [13], anti-metastatic [14], and anti-tumor [14] effects.

Bee venom is formed of different effective substances. For thousands of years, people have used bee venom as a complementary or even alternative medicine. However, Scientists have just recently begun testing the safety and efficacy of bee venom for the treatment of inflammatory arthritis in clinical trials [15].

Since 1970 the disease-modifying antirheumatic drugs [DMARDs] were the most recommendable treatment for RA [16], a recent clinical guideline has been updated to recommend glucocorticoid, conventional synthetic DMARDs, and biological therapy, in various clinical situations [17]. Even so, there are still number of adverse reactions associated with this treatment, and not everyone will be able to reach remission, so alternative treatments may be sought by patients [18].

In the current study treatment with bee venom over 6 ms was associated by clinical improvement through significant reduction in DAS-ESR score, VAS, and significant reduction in inflammatory markers ESR and CRP. Moderate disease response was achieved in about have of the patients, while good disease response wase reported in 44%, non-response was only in 2 cases.

Experimental studies of BV's antiarthritic effects in artificially induced rheumatoid arthritis models were conducted severally. Recently, El-Tedawy et al, demonstrated that bee venom injection was associated with significant improvement in arthritic index score and knee joint swelling circumferences, with significant ESR reduction, in rat model with induced arthritis [19].

Furthermore, bee venom was found not only to reduce joint inflammation and swelling but also to preserve joint

structure through limiting the extent of the degenerative changes and enhancing the integrity, regeneration, and repair of joint tissue as proven by Elshafie et al, in the temporomandibular joint of albino rats with induce rheumatoid arthritis treated with bee venom for 12ws [20].

In human, Bee venom and methotrexate were equally effective in reducing RA symptoms as found by chen et al through assessment of (morning stiffness duration, swollen and tender joint count, VAS, ESR, and CRP level) without any associated significant adverse effects after bee-stinging treatment [21]. Moreover, liu et al, reported that bee venom therapy was superior to ordinary RA medication in reducing clinical signs of inflammation, lower relapse rate [22].

BV can suppress inflammatory cytokines, significant reduction in serum TNF- α level after 6ms of treatment compared to baseline level in the present study which revealed that systemic BV can display safe and anti-arthritis, anti-inflammatory and nociceptive therapeutic effect.

Parallel to that, a study was created to assess BV's impact on a mouse type-II collagen-induced arthritis (CIA). The findings demonstrated that among the serum proinflammatory cytokines, the BV group's production of TNF-alpha was decreased in comparison to that of the control group [23].

Similarly, 3 weeks treatment with BV in rat with Complete Freund's adjuvant induced arthritis revealed significant reduction in serum TNF- α concentration [5]. Moreover, combination of BV and anti-arthritis drug as methotrexate showed greater synergetic effect through reduction of TNF- α serum level concentration by 35%, potentiating methotrexate bioavailability, antiarthritis effects as well as reduce the associated hepatotoxicity when compared to methotrexate treated group. This is come in agreement with El-Tedawy et al, both TNF- α and IL-1 β levels were reduced significantly in BV group compared with the methotrexate group, reach those of normal control rats [19].

Investigating the genetic association of TNF- α 308 A/G in RA cases, the G allele and GG genotype were more frequently represented among studied RA patients. In agreement with our results, Mosaad et al and Zaghlool et al, revealed that the G allele and the GG genotype were more prevalent in RA patients, which is consistent with previous studies conducted in various parts of the world. [24,25,26,27, 28].

There was no significant difference between three genotypes, with a mean DAS28 improvement nearly equal in each of them (-2), the same for the VAS (mean change -3), and CRP (mean change -30 to -20), this is explained by low number of studied cases. Based on the DAS score changes, EULAR response criteria, genotype TNF 308 was significantly associated with response to BV therapy, especially with AA and GG genotypes.

To our knowledge, this is the first study for anti-cytokines effect of BV in human and association between genotype at TNF-308 and effect of BV therapy in RA has not previously been reported. Bee venom therapy is still an experimental therapeutic option for joint disease. The main defect in the current study is the small number of cases, no control group, and short duration of follow up. More will organize clinical trials over a large scale are necessary to confirm this effectiveness. Further studies should evaluate structural damage response on a large cohort, over long duration of treatment.

5 Conclusions

Bee venom therapy may be an effective option to control RA. Our results can support the potential anti-arthritis and anti-inflammatory effect of bee venom therapy in rheumatoid arthritis patients. Six months of treatment with Bee venom can control joints pain, disease activity, reduce ESR and CRP with improvement in TNF α levels. The G allele, and the GG genotype of TNF- α G308 genotypes were more frequently represented among RA patients. No difference between TNF- α G308 genotypes regarding treatment response. Bee venom therapy remains an experimental treatment option for arthritis.

6 Recommendations

We are still a long way from clinical application of BV therapy. Ongoing work on Bee venom and its component is required on a wide scale and over long duration to confirm its therapeutic anti-arthritis effects. Anti-arthritis effect of bee venom should be studied in relation to the usually used treatment in RA. Further steps towards the use of BV for pharmacological purposes are required.

Conflicts of Interest Statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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