Effect of Ayurveda Treatment on Inflammatory Marker Levels

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Abstract

Background

Ayurveda has the potential to address chronic, lifestyle diseases, and its distinguishing feature is chronic inflammation. However, it is crucial to utilize modern advancements in cytokine markers to monitor the effectiveness of Ayurveda treatments.

Objective

The purpose of this study was to quantify the effectiveness of personalized Ayurveda-based treatment by examining the changes in cytokine levels for various non-communicable diseases such as diabetes, arthritis, GERD, etc.

Design, setting, participants, and interventions

The study was designed as an observational follow-up study, and it involved 60 patients with different disease conditions who underwent Ayurveda treatment. Pro-inflammatory cytokines (IFN-γ, IL-6, IL-17, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) were measured using a multiplex bead-based assay system.

Main outcome measures

The primary measure of outcome was the alteration in cytokine levels before and after Ayurveda treatment.

Results

The findings reveal a complex narrative where Ayurveda treatment consistently reduced pro-inflammatory cytokines across all diseases, although the impact varied depending on the specific condition. Notably, the treatment significantly decreased pro-inflammatory cytokines such as IFN-γ and IL-6, while increasing anti-inflammatory cytokines like IL-4 and IL-10. These responses were distinct in disease-specific contexts, highlighting the versatility of Ayurveda treatment. Diseases such as diabetes, GERD, and others exhibited unique changes in cytokine levels. Furthermore, the study assessed the balance between pro- and anti-inflammatory cytokines, demonstrating a shift towards equilibrium after treatment.

Conclusion

These results emphasize the potential of Ayurveda as an immunomodulatory approach to alleviate inflammation and enhance overall well-being.

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1. Introduction

Ayurveda, a venerable medical system with documented origins, places great emphasis on safeguarding one's health and preventing the onset of diseases. Nevertheless, in certain situations, diseases may emerge and manifest their symptoms. Ayurveda offers a comprehensive account of the premonitory signs and symptoms, manifested symptoms, and potential complications associated with various diseases. Despite advancements in technology, Ayurveda practitioners primarily rely on subjective symptoms for diagnosing diseases.

Disease is a multifaceted occurrence, involving a multitude of biomolecules and their pathways that contribute to the development and progression of various ailments. The quantification of these biomolecules is paramount for disease diagnosis, monitoring therapeutic responses, and tracking disease progression. Cytokines are a large, diverse family of small proteins or glycoproteins, function as chemical messengers in the transmission of intercellular signalling process. They are produced by leukocytes and other cells, playing pivotal roles in regulating the immune response [1]. Several reports have highlighted various factors that influence cytokine levels, underscoring the intricate nature of cytokine regulation [2].

One of the key hallmarks of chronic non-communicable diseases in inflammation in the body. Inflammation is a protective immune response that is triggered by harmful pathogens, dead cells, or irritants and is tightly controlled by the body's innate immune system. Inadequate inflammation can result in persistent infections, while excessive inflammation can lead to chronic or systemic inflammatory diseases [3]. Inflammageing, most commonly observed in the elderly, is characterized by elevated pro-inflammatory markers in the blood and is associated with a high risk of chronic disease, disability, weakness, and premature mortality [4].

Earlier research findings suggest that cytokines such as IL-6, IL-10 play a role in low-grade inflammation in metabolic diseases like diabetes, hypertension. The systemic chronic inflammation (SCI) characterized by increased pro-inflammatory cytokines levels, which progresses the disease further advances the disease and leads to the early development of complications and co-morbidities. Type 2 DM (T2DM) has been associated with an inflammatory host response controlled by the production of pro- and anti-inflammatory cytokines [5]. Studies have shown that increased IFN-γ levels and decreased IL-10 levels in

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patients with type 2 diabetes [6]. Polymorphisms in the IL-10 gene also increases the risk of diabetes. While, IFN-γ plays a crucial role in the development of T2DM as a pro-inflammatory factor [7], [8], [9].

Likewise, the pathogenic role of cytokines in diseases such as Gastroesophageal Reflux Disease, [10],[11] Osteoarthritis, [12],[13] Hypertension, [14] has been previously reported.

The primary objective of Ayurveda is to safeguard and uphold human well-being. Consequently, Ayurveda delineates the state of good health, variations in human health conditions, and methods to preserve and promotion of human well-being. Deviations from the fundamental state of good health indicate the development and manifestation of disease. Ayurveda then elucidates the causes of disease, its progression, manifestations, and corresponding treatments. Thus, Ayurveda places immense importance on maintaining the health of individuals in a healthy state and treating the ailments of patients, as crucial aspects of its practice. Additionally, Ayurveda emphasizes the significance of nutrition, lifestyle activities, environmental conditions, and daily routines, in maintaining good health and treating diseases, in conjunction with medications and treatment procedures.

Ayurveda therapeutic formulations typically consist of individual or combined herbal, mineral, and metal ingredients. These formulations contain synergistic multi-compounds that target multiple levels of drug action. The application of network pharmacology in Ayurveda drugs and formulations has revealed the molecular mechanism of immunomodulation in *Withania somnifera*, [15] the molecular targets of *Triphala* in cancer treatment, [16] and the effective inhibition and regulation of hub genes in the CKD and CVD pathways by *Chandanasava* [17].

Prakriti or human constitution, as defined by Ayurveda, is based on observable phenotype features that allow for individual categorization. Importantly, the personalized treatments are based on individual's *Prakriti* (unique phenotype) and other treatment related factors. Recent studies have demonstrated notable differences in gene expression,[18] biochemical parameters, [18] gut microbiome, [19] genetic variations, [20] DNA methylation, [21] and physiological responses to head-up-tilt among different *Prakriti* groups [22].

Ayurveda practitioners commonly assess the therapeutic effectiveness of a treatment course by comparing the reduction in symptoms and their severity, and their impact on

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disease-specific biochemical molecules. However, the significance of disease-related inflammatory markers, such as cytokines, has been overlooked in clinical practices. Consequently, this study aims to investigate the influence of Ayurveda treatment protocols on cytokine levels.

2. Methods

2.1. Study design

The present study is an observational follow-up study conducted within the Interdisciplinary Institute of Indian System of Medicine (IIISM) department at SRM Institute of Science and Technology, Kattankulathur, Chennai, India. The institutional ethical committee clearance was obtained and registered with the Clinical Trials Registry of India (CTRI) under registration number CTRI/2021/12/038909. Study participants were recruited from the Outpatient Department (OPD) of IIISM at SRM Hospital. All participants were informed about the study and provided informed consent.

2.2. Participants

The inclusion criteria for the study encompassed individuals aged 20 to 80 years, both male and female, seeking Ayurveda treatment at the OPD. Exclusion criteria included individuals under the age of 20 and pregnant or lactating women. Upon enrolment, Ayurveda physicians conducted examinations and diagnosed the participants' medical conditions, prescribing personalized medicines targeting the aetiology and symptomology of the patient. Symptoms of each patient were meticulously documented and assigned severity scores. During subsequent follow-up visits, symptoms were reassessed to evaluate therapeutic efficacy. In total, 497 patients were enrolled in the study. In a subset of participants (n = 60), the study assessed the levels of various cytokine markers to investigate the impact of Ayurveda treatment protocols on these markers. The pro-inflammatory and anti-inflammatory cytokines measured included IFN-γ, IL-6, IL-17, TNF-α, IL-1, IL-4, and IL-10.

2.3. Pro-inflammatory & Anti-inflammatory cytokine profiling

Blood samples were collected from the antecubital vein using a sterile needle and syringe and then transferred to vacutainers. Subsequently, the blood samples were subjected to centrifugation for 30 minutes at 800 g, leading to the separation of plasma, which was then carefully collected for further analysis. The circulatory levels of inflammatory cytokines including IFN-γ, IL-6, IL-17, TNF-α, IL-1, IL-4, and IL-10 were carried out using a Bio-Plex

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Pro™ Human Inflammation Panel in a multiplex bead-based assay system (Bio-Rad, Hercules, CA). Briefly, 50 µl of plasma samples were placed into a Bio-Plex Pro™ 96 flat-bottom well plate supplied by Bio-Rad, CA. The assay procedures were carried out in duplicate according to the manufacturer's instructions. Automated washing was performed using a Bio-Plex Pro™ wash station equipped with a magnet. Subsequently, the samples were acquired through cytometric imaging using a Luminex xMAP analyzer, specifically the Luminex 100 Milliplex Analyzer from Luminex Corp. The data obtained from these samples were analysed using the Bio-Plex Manager™ software 6.1, also provided by Bio-Rad. Cytokine concentrations were determined from standard curves that were prepared on each plate and expressed as picograms per millilitre (pg/mL).

2.4. Statistical analysis

Data are expressed as mean ± standard deviation values for biochemical parameters and geometric mean with 95% confidence interval values for cytokines. Paired samples t-test was performed to compare groups for continuous variables. All the analysis was carried out using JASP Statistical Package (Version 0.18.1) (https://jasp-stats.org/) and the value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participants enrolment

The study involved a cohort of 60 patients who were presented with various diseases and sought treatment at the OPD of the IIISM department at SRM Hospital in Chennai. Detailed demographic and disease-related information for these patients can be found in Tables 1 and 2.

3.2. Effect of Ayurveda treatment on cytokine levels

In the present study, an analysis was conducted on a panel of cytokines, which included four pro-inflammatory cytokines, namely IFN-γ, IL-6, IL-17, and TNF-α, as well as two anti-inflammatory cytokines, IL-4 and IL-10. Additionally, the cytokine IL-1, which can exhibit both pro-inflammatory and anti-inflammatory properties, was also measured.

In the initial phase of statistical analysis, the study assessed the overall impact of Ayurveda treatment on cytokine levels across all diseases that were part of the study. The statistical analysis revealed a significant reduction in pro-inflammatory cytokine levels following Ayurveda treatment. The pro-inflammatory cytokines IFN-γ (7.16 ± 3.37 vs 5.15 ± 2.94, p

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value < 0.001), IL-6 (8.66 ± 3.73 vs 6.47 ± 3.25, p value < 0.001), IL-17 (6.74 ± 3.48 vs 5.45 ± 3.34, p value < 0.001), TNF-α (7.38 ± 3.73 vs 5.15 ± 2.89, p value < 0.001) were significantly reduced after Ayurveda treatment. The anti-inflammatory markers IL-4 (5.65 ± 2.49 vs 6.29 ± 2.47, p value < 0.001) & IL-10 (5.36 ± 2.55 vs 5.81 ± 2.58, p value = 0.044) was significantly increased after Ayurveda treatment. However, IL-1 (5.68 ± 3.19 vs 5.67 ± 2.77, p value = 0.971) not found to be significantly varying after Ayurveda treatment (Table 3, Figure 1 (a-f)).

3.3. Effect of Ayurveda treatment on cytokine levels in diabetes mellitus

In the subsequent stage of statistical analysis, the patient group as a whole was subdivided into subgroups based on their specific diseases, and the impact of Ayurveda treatment on cytokine levels was examined within each subgroup. Notably, there were a total of 16 patients with diabetes mellitus in the study. The statistical analysis within this subgroup demonstrated a significant reduction in pro-inflammatory cytokine levels following Ayurveda treatment for diabetes mellitus. The pro-inflammatory cytokines IFN-γ (9.26 ± 3.67 vs 6.75 ± 3.45, p value < 0.001), IL-6 (10.43 ± 3.82 vs 7.55 ± 3.28, p value < 0.001), IL-17 (9.32 ± 3.70 vs 7.76 ± 3.64, p value < 0.001), TNF-α (10.32 ± 3.06 vs 7.05 ± 2.86, p value < 0.001) were significantly reduced after Ayurveda treatment. The anti-inflammatory markers IL-4 (7.87 ± 2.34 vs 8.61 ± 2.51, p value = 0.044) was significantly increased after Ayurveda treatment. However, IL-1 (8.12 ± 3.31 vs 7.79 ± 3.22, p value = 0.493) & IL- 10 (6.76 ± 2.54 vs 6.60 ± 3.31, p value = 0.806) not found to be significantly varying after Ayurveda treatment in diabetes mellitus (Table 4, Figure 2 (a – e)). Additionally, the impact of treatment duration was clarified through the comparison of follow-up visits in the 2nd and 3rd months. The follow-up visits in the 3rd month revealed a notable decrease in IL-6 and TNF-α levels when compared to the 2nd month follow-up (Figure 3).

3.4. Effect of Ayurveda treatment on cytokine levels in GERD

The total number of GERD patients recruited was 17. The statistical analysis showed that pro-inflammatory markers were significantly reduced after Ayurveda treatment. The pro-inflammatory cytokines IFN-γ (5.26 ± 2.55 vs 3.55 ± 1.21, p value < 0.001), IL-6 (7.06 ± 3.10 vs 5.55 ± 3.04, p value = 0.008), IL-17 (5.12 ± 2.45 vs 4.01 ± 2.10, p value = 0.005), TNF-α (5.30 ± 2.71 vs 3.71 ± 1.51, p value = 0.001) were significantly reduced after Ayurveda treatment. The anti-inflammatory markers IL-4 (4.47 ± 2.11 vs 5.28 ± 1.86, p value = 0.009) & IL-10 (4.64 ± 1.97 vs 5.48 ± 1.98, p value = 0.006) was significantly increased

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after Ayurveda treatment. However, IL-1 (4.33 ± 2.69 vs 4.33 ± 1.86, p value = 0.998) not found to be significantly varying after Ayurveda treatment (Table 5, Figure 4 (a -f)).

3.5. Effect of Ayurveda treatment on cytokine levels in Hypertension

The total number of Hypertension (HTN) patients recruited was 7. The statistical analysis showed that pro-inflammatory markers IFN-γ, IL-6, TNF-α was significantly reduced after Ayurveda treatment. The pro-inflammatory cytokines IFN-γ (7.93 ± 3.64 vs 5.67 ± 3.07, p value = 0.035), IL-6 (10.75 ± 2.98 vs 7.93 ± 2.79, p value = 0.028), TNF-α (7.69 ± 4.79 vs 5.08 ± 4.13, p value = 0.012) were significantly reduced after Ayurveda treatment. The other pro-inflammatory cytokine IL-17 not significantly varied after Ayurveda treatment in HTN patients. Similarly, anti-inflammatory cytokines IL-4 and IL-10 also not varied in HTN patients after Ayurveda treatment. Also, IL-1 not varied in HTN patients after Ayurveda treatment (Table 6, Figure 5 (a - c)).

3.6. Effect of Ayurveda treatment on cytokine levels in Osteoarthritis

The total number of Osteoarthritis (OA) patients recruited was 7. The statistical analysis showed that pro-inflammatory markers IFN-γ, IL-6, IL-17 & TNF-α were significantly reduced after Ayurveda treatment. The pro-inflammatory cytokines IFN-γ (*6.25 ± 1.30 vs 4.34 ± 1.73, p value = 0.023*), IL-6 (*8.09 ± 3.59 vs 6.43 ± 3.91, p value = 0.006*), IL-17 (*5.68 ± 1.33 vs 4.55 ± 1.48, p value = 0.004*), TNF-α (*6.42 ± 1.52 vs 4.41 ± 1.66, p value = 0.040*) were significantly reduced after Ayurveda treatment. The anti-inflammatory cytokines IL-4 and IL-10 also not varied in OA patients after Ayurveda treatment. Also, IL-1 not varied in OA patients after Ayurveda treatment (Table 7, Figure 6 (a – e)).

3.7. Effect of Ayurveda treatment on cytokine levels in other diseases

The remaining diseases mentioned in the Table 2 are categorised into a single group to understand the effect of Ayurveda treatment on cytokines level. The total number of other diseases (mentioned in Table 2) recruited was 13. The statistical analysis showed that pro-inflammatory markers were significantly reduced after Ayurveda treatment in these group. The pro-inflammatory cytokines IFN-γ (7.12 ± 3.36 vs 5.41 ± 3.43, p value < 0.001), IL-6 (7.76 ± 3.38 vs 5.58 ± 3.14, p value < 0.001), IL-17 (6.17 ± 3.44 vs 5.17 ± 3.14, p value = 0.012), TNF-α (6.85 ± 3.96 vs 5.14 ± 3.14, p value = 0.004) were significantly reduced after Ayurveda treatment. The anti-inflammatory cytokines IL-10, IL-1 & IL- 4 not found to be significantly varied after Ayurveda treatment in these group (Table 8, Figure 7 (a -d)).

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3.8. Effect of Ayurveda treatment on cytokine ratio levels in overall diseases

The ratio of pro-inflammatory to anti-inflammatory cytokines shows the treatment effect from pro-inflammatory to anti-inflammatory in diseases. The pro-inflammatory to anti-inflammatory cytokine ratios were significantly reduced in after treatment group. The cytokine ratio IFN-γ /IL-4 (1.32 ± 0.48 vs 0.80 ± 0.29, p value < 0.001), IFN-γ /IL-10 (1.35 ± 0.51 vs 0.90 ± 0.44, p value < 0.001), IL-6/IL-4 (1.69 ± 0.88 vs 1.08 ± 0.56, p value < 0.001), IL-6/IL-10 (1.83 ± 0.91 vs 1.22 ± 0.69, p value < 0.001), IL-17/IL-4 (1.25 ± 0.48 vs 0.84 ± 0.32, p value < 0.001), IL-17/IL-10 (1.35 ± 0.54 vs 0.94 ± 0.44, p value < 0.001), TNF-α /IL-4 (1.39 ± 0.62 vs 0.81 ± 0.31, p value < 0.001), TNF-α /IL-10 (1.57 ± 0.64 vs 0.92 ± 0.48, p value < 0.001) were significantly decreased after Ayurveda treatment (Table 9, Figure 8 (a – h)).

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Table 1. Demography details of the study subjects

Factor	Mean ± SD
Age (years)	38.25 ± 13.21
Gender (M/F)	32/28
Height (cm)	163.78 ± 6.29
Weight (kg)	73.46 ± 11.76
Systolic BP (mmHg)	125.75 ± 11.11
Diastolic BP (mmHg)	81 ± 8.57

Table 2. Disease list and number of patients in each disease category

Disease list	Patients number
Diabetes	16
Gastroesophageal reflux disease (GERD)	17
Osteoarthritis (OA)	7
Hypertension (HTN)	7
Hypothyroidism	1
Rheumatoid arthritis (RA)	1
Allergic rhinitis	2
Polycystic Ovarian Disease (PCOD)	2
Migraine	1
Vertigo	1
Bronchial asthma	1
Skin allergy	1
Hemiplegia	1
Anaemia	2

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Table 3. Cytokine levels before & after Ayurveda treatment in overall diseases

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	Mean ± (SD)	t value	p value
IFN- γ	7.16 ± 3.37	5.15 ± 2.94	8.867	< **0.001**
IL-6	8.66 ± 3.73	6.47 ± 3.25	8.961	< **0.001**
IL-17	6.74 ± 3.48	5.45 ± 3.34	7.384	< **0.001**
TNF- α	7.38 ± 3.73	5.15 ± 2.89	8.316	< **0.001**
IL-1	5.68 ± 3.19	5.67 ± 2.77	0.036	0.971
IL-4	5.65 ± 2.49	6.29 ± 2.47	-4.296	< **0.001**
IL-10	5.36 ± 2.55	5.81 ± 2.58	-2.058	**0.044**

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test.

Table 4. Cytokine levels in diabetes patients group before & after Ayurveda treatment

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	Mean ± (SD)	t value	p value
IFN- γ	9.26 ± 3.67	6.75 ± 3.45	4.677	< **0.001**
IL-6	10.43 ± 3.82	7.55 ± 3.28	5.902	< **0.001**
IL-17	9.32 ± 3.70	7.76 ± 3.64	4.649	< **0.001**
TNF- α	10.32 ± 3.06	7.05 ± 2.86	5.117	< **0.001**
IL-1	8.12 ± 3.31	7.79 ± 3.22	0.702	0.493
IL-4	7.87 ± 2.34	8.61 ± 2.51	-2.195	**0.044**
IL-10	6.76 ± 2.54	6.60 ± 3.31	0.250	0.806

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test.

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Table 5. Cytokine levels in GERD patients group before & after Ayurveda treatment

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	**Mean ± (SD)**	**t value**	**p value**
IFN- γ	5.26 ± 2.50	3.55 ± 1.21	4.476	**< 0.001**
IL-6	7.06 ± 3.10	5.55 ± 3.04	3.014	**0.008**
IL-17	5.12 ± 2.45	4.01 ± 2.10	3.300	**0.005**
TNF- α	5.30 ± 2.71	3.71 ± 1.51	3.990	**0.001**
IL-1	4.33 ± 2.69	4.33 ± 1.86	0.002	0.988
IL-4	4.47 ± 2.11	5.28 ± 1.86	-2.966	**0.009**
IL-10	4.64 ± 1.97	5.48 ± 1.98	-3.185	**0.006**

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test, GERD – Gastroesophageal reflux disease.

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Table 6. Cytokine levels in HTN patients group before & after Ayurveda treatment

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	**Mean ± (SD)**	**t value**	**p value**
IFN- γ	7.93 ± 3.64	5.67 ± 3.07	2.708	**0.035**
IL-6	10.75 ± 2.98	7.93 ± 2.79	2.892	**0.028**
IL-17	6.90 ± 4.08	5.09 ± 3.46	2.349	0.057
TNF- α	7.69 ± 4.79	5.08 ± 4.13	3.526	**0.012**
IL-1	5.94 ± 3.88	5.59 ± 3.16	0.373	0.722
IL-4	5.20 ± 2.13	6.08 ± 2.66	-1.692	0.142
IL-10	4.82 ± 2.55	5.56 ± 2.79	-1.512	0.181

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test, HTN – Hypertension.

Table 7. Cytokine levels in OA patients group before & after Ayurveda treatment

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	Mean ± (SD)	t value	p value
IFN- γ	6.25 ± 1.30	4.34 ± 1.73	3.018	**0.023**
IL-6	8.09 ± 3.59	6.43 ± 3.91	4.145	**0.006**
IL-17	5.68 ± 1.33	4.55 ± 1.48	4.586	**0.004**
TNF- α	6.42 ± 1.52	4.41 ± 1.66	2.607	**0.040**
IL-1	4.90 ± 1.68	5.08 ± 1.72	-0.514	0.626
IL-4	5.19 ± 1.80	5.48 ± 0.87	-0.597	0.573
IL-10	5.52 ± 3.06	5.92 ± 2.95	-0.849	0.429

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test, OA – Osteoarthritis.

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Table 8. Cytokine levels before & after Ayurveda treatment in other diseases

Cytokines	*Before treatment*	*After treatment*	Paired Samples t – Test	
	Mean ± (SD)	Mean ± (SD)	t value	p value
IFN- γ	7.12 ± 3.36	5.41 ± 3.43	4.775	**< 0 .001**
IL-6	7.64 ± 3.87	5.58 ± 3.14	6.205	**< 0 .001**
IL-17	6.17 ± 3.44	5.17 ± 3.85	2.947	**0.012**
TNF- α	6.85 ± 3.96	5.14 ± 3.14	3.516	**0.004**
IL-1	4.74 ± 2.43	5.18 ± 2.14	-1.781	0.100
IL-4	4.97 ± 2.11	5.33 ± 1.91	-1.672	0.120
IL-10	4.76 ± 2.64	5.32 ± 2.07	-1.394	0.189

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test.

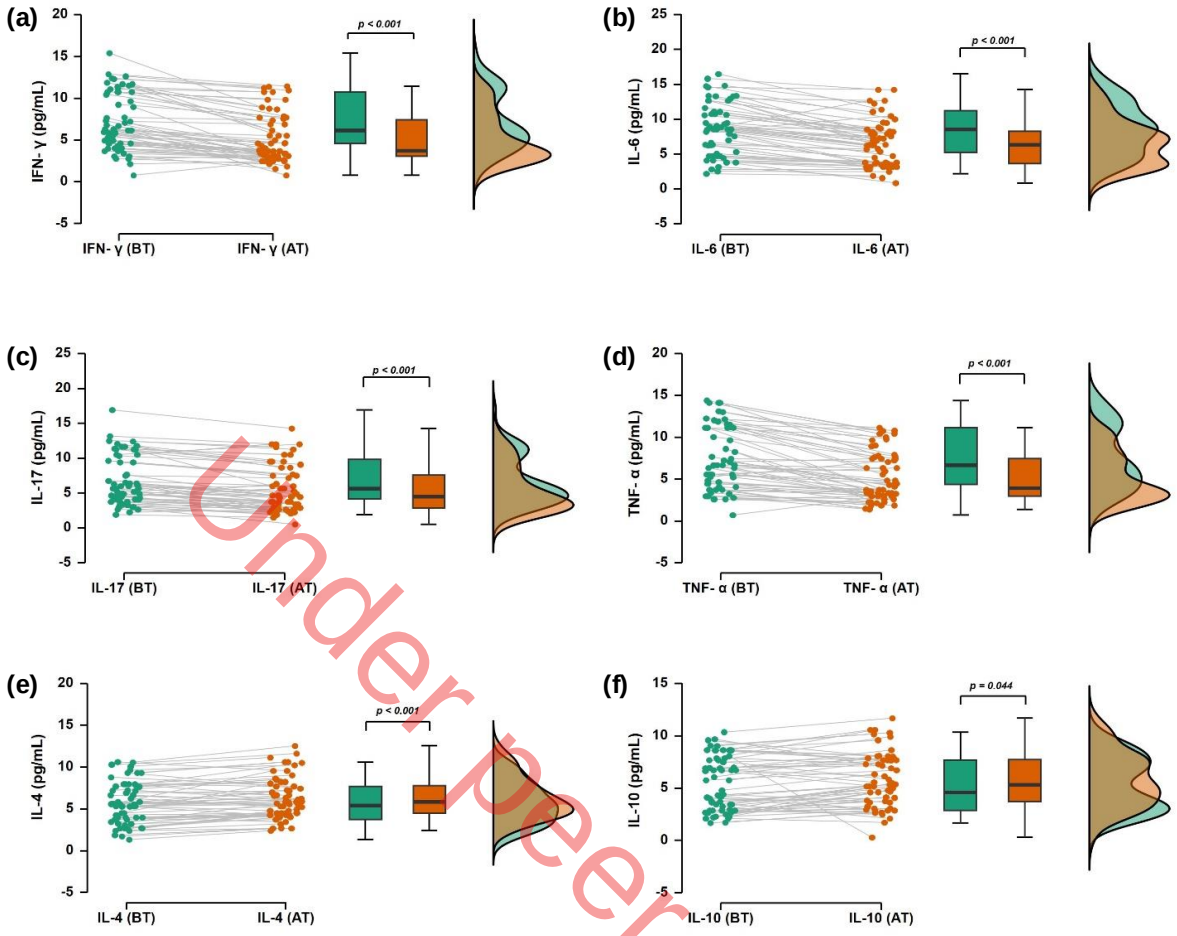

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Figure 1 (a – f) Effect of ayurveda treatments on cytokines levels in overall diseases

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in overall diseases. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: $p<0.001$, IL-6: $p<0.001$, IL-17: $p<0.001$, TNF- α: and anti-inflammatory markers IL-4: $p<0.001$, IL-10: $p<0.044$.

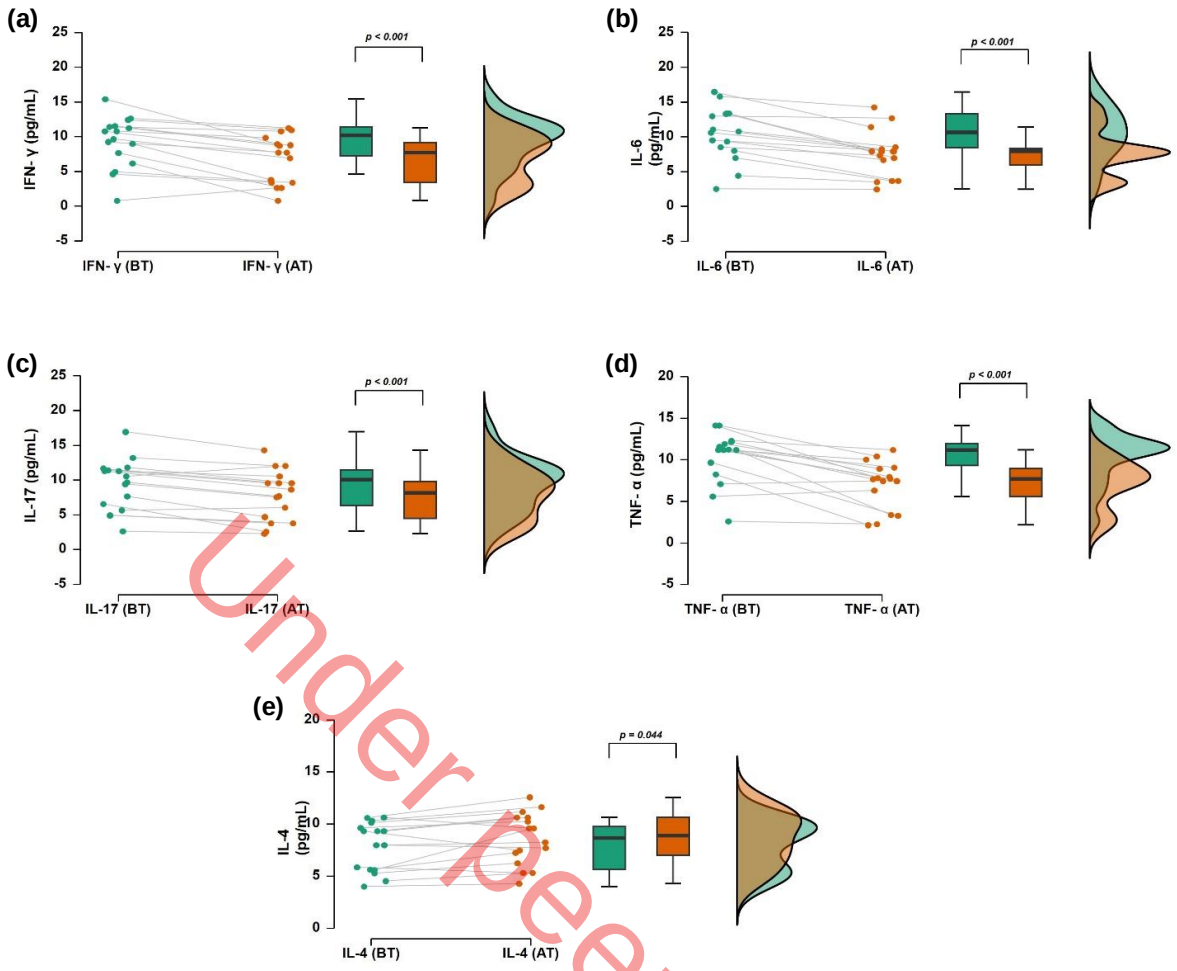

Figure 2 (a – e) Effect of ayurveda treatment on cytokine levels in diabetes mellitus

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in diabetes mellitus. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: $p<0.001$, IL-6: $p<0.001$, IL-17: $p<0.001$, TNF- α: and anti-inflammatory markers IL-4: $p<0.001$.

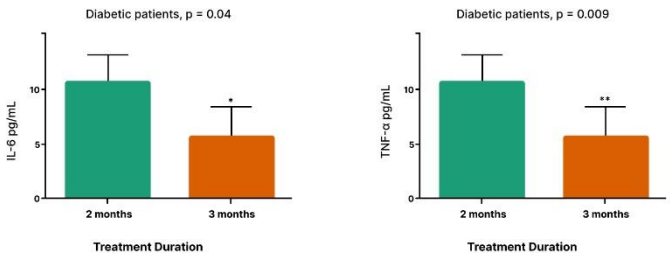

Figure 3 Effect of ayurveda treatment duration on cytokine IL-6 & TNF- α levels in diabetes mellitus

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The cytokines IL-6 & TNF- α showed significant difference between 2nd & 3rd month follow-up in diabetes mellitus.

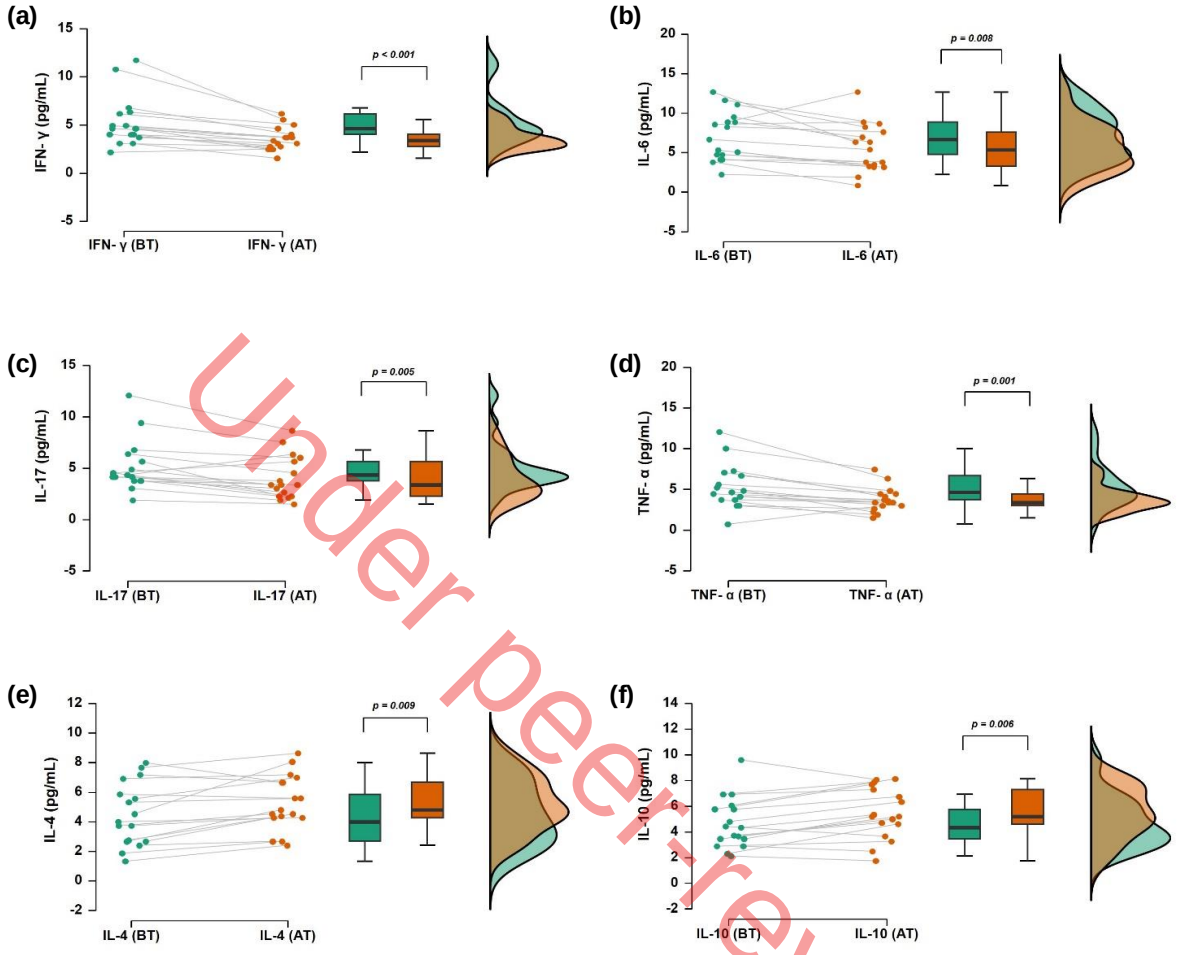

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Figure 4 (a - f) Effect of ayurveda treatment on cytokine levels in GERD

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in GERD. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: $p<0.001$, IL-6: $p = 0.008$, IL-17: $p = 0.005$, TNF- α: $p = 0.001$ and anti-inflammatory markers IL-4: $p = 0.009$, IL-10: $p = 0.006$.

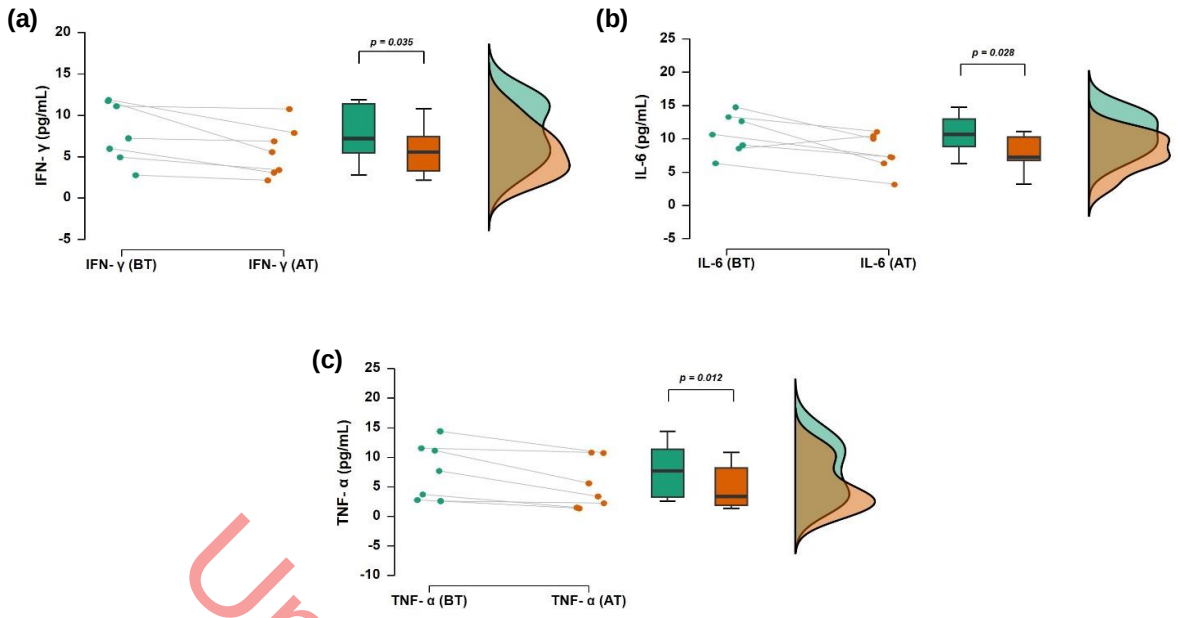

Figure 5 (a - c) Effect of ayurveda treatment on cytokine levels in hypertension

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in hypertension. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: p = 0.035, IL-6: p = 0.028, TNF- α: p = 0.012.

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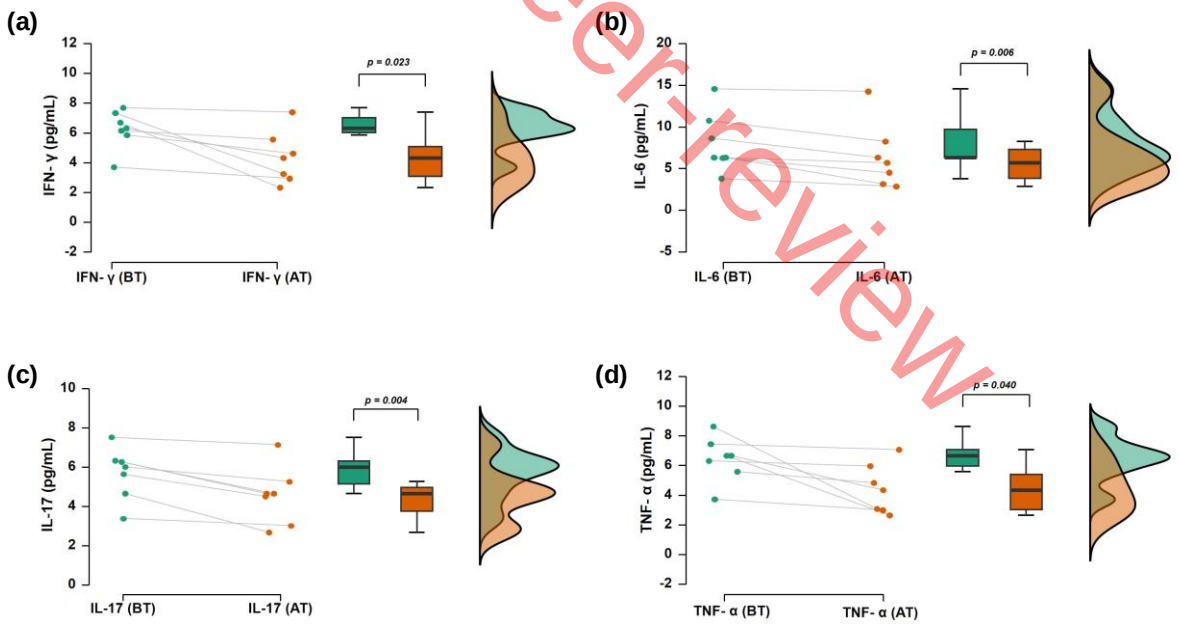

Figure 6 (a - d) Effect of ayurveda treatment on cytokine levels in osteoarthritis

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in osteoarthritis. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: p = 0.023, IL-6: p = 0.005, IL-17: p = 0.004, TNF- α: p = 0.040.

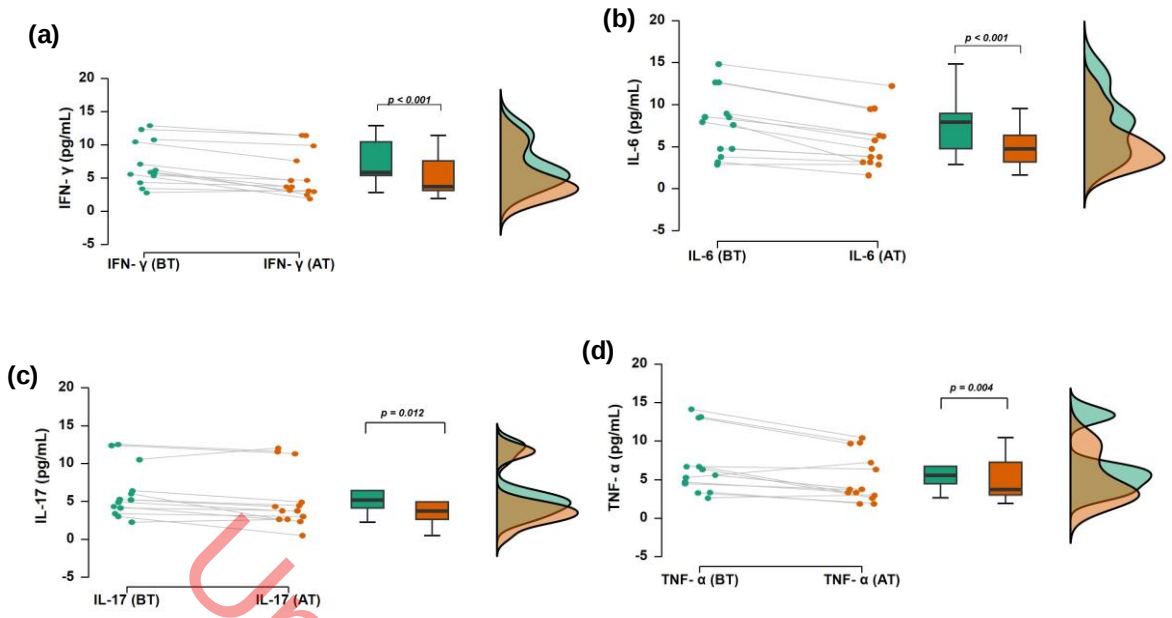

Figure 7 (a - d) Effect of ayurveda treatment on cytokine levels in other diseases

Serum levels of cytokines which showed significant difference before treatment (BT) & after treatment (AT) in other diseases. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated. For pro-inflammatory markers IFN-γ: p< 0.001, IL-6: p < 0.001, IL-17: p = 0.012, TNF- α: p = 0.004.

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Table 9. Cytokine ratio before & after Ayurveda treatment in overall diseases

Cytokine ratio	*Before treatment*	*After treatment*	Paired Samples T – Test	
	Mean ± (SD)	Mean ± (SD)	t value	p value
IFN-γ/IL-4	1.32 ± 0.48	0.80 ± 0.29	8.438	< **0.001**
IFN-γ /IL-10	1.35 ± 0.51	0.90 ± 0.44	6.210	< **0.001**
IL-6/IL-4	1.69 ± 0.88	1.08 ± 0.56	6.900	< **0.001**
IL-6/IL-10	1.83 ± 0.91	1.22 ± 0.69	7.391	< **0.001**
IL-17/IL-4	1.25 ± 0.48	0.84 ± 0.32	7.170	< **0.001**
IL-17/IL-10	1.35 ± 0.54	0.94 ± 0.44	5.958	< **0.001**
TNF-α/IL-4	1.39 ± 0.62	0.81 ± 0.31	7.727	< **0.001**
TNF-α/IL-10	1.57 ± 0.64	0.92 ± 0.48	5.311	< **0.001**

Values are expressed in mean ± SD, cytokine levels before and after treatment were compared using paired samples t – test.

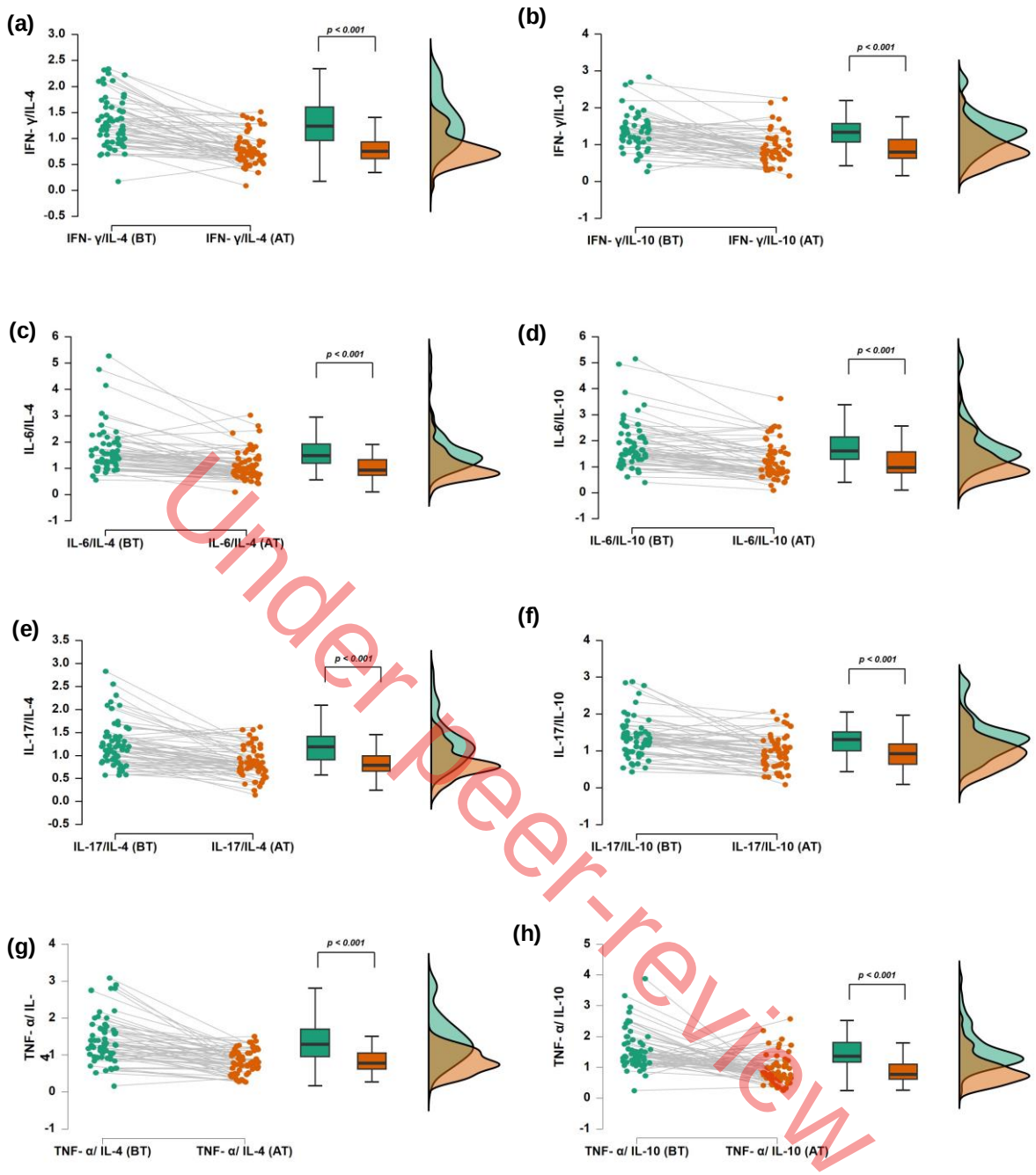

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Figure 8 (a - h) Effect of ayurveda treatment on cytokine ratio in overall diseases

Cytokines ratio which showed significant difference before treatment (BT) & after treatment (AT) in all diseases. The cytokine levels before and after treatment were compared using paired samples t – test and the p-value is calculated.

4. Discussion

In this study, we investigated the impact of Ayurveda treatment on pro-inflammatory and anti-inflammatory cytokine levels, revealing significant differences in these cytokine levels after treatment. The levels of pro-inflammatory cytokines exhibited a significant decrease following Ayurveda treatment. Conversely, anti-inflammatory cytokine levels, specifically IL-4 and IL-10, increased significantly after Ayurveda treatment. In addition, we also observed that among all diseases presented here, patients with diabetes showed higher levels of inflammatory markers. This is expected since progression of diabetes is associated with systemic inflammation in the body leading to various macro-vascular and micro-vascular complications.

Our findings indicate that Ayurveda treatment effectively reduces pro-inflammatory cytokines such as IFN-γ, IL-6, IL-17 & TNF-α in the circulation of patients with various diseases. Notably, the response to Ayurveda treatment varies depending on the specific disease. For instance, in diabetes patients, significant reductions were observed in all four pro-inflammatory cytokines mentioned. Similarly, in patients with GERD significant reductions in these pro-inflammatory cytokines were noted. However, for patients with HTN, only IFN-γ, and IL-6 exhibited a significant reduction after Ayurveda treatment and in OA patients, only IL-6 and IL-17 were significantly reduced after treatment.

In the context of anti-inflammatory cytokines, our overall disease analysis showed an increase in IL-4 and IL-10 levels following Ayurveda treatment. However, disease specific response was also observed. In diabetes patients, only IL-4 significantly increased after treatment. In GERD patients, both IL-4 & IL-10 observed to be increased after treatment. While in both HTN & OA, anti-inflammatory cytokine levels did not significantly vary after Ayurveda treatment.

The pro- inflammatory and anti-inflammatory cytokines play essential roles in the host immune response. The dynamic balance between pro- and anti- inflammatory cytokines is crucial for maintaining a stable, long term immune mechanism. The homeostasis of this dynamic balance is regulated through continuous feedback mechanisms at multiple levels, including levels of pro- and anti-inflammatory cytokines, molecular, organ, and entire system [23].

To assess the effect of Ayurveda treatment on the balance between pro- and anti-inflammatory cytokines, we have calculated the ratios between these types of cytokines.

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Interestingly, all pro- and anti-inflammatory cytokine ratios were found to be reduced after Ayurveda treatments, indicating a rebalancing effect on the immune response.

Previous studies have explored the role of Ayurveda treatment protocol in patients with Asthma measuring various cytokines, including like TNF α, IL-4, IL-6, IL-10, IL-13, IL-17A, GC-SF, IL-5, IL-7, IL-8, IL-1ß, IL12p70, GM-CSF, IFN-γ, MCP-1, MIP-1ß, and IL-2 before and after Ayurveda treatment. These studies demonstrated a decrease in IFN-γ, IL-6, TNF-α, IL-4 and increase in IL-10 after Ayurveda treatment in asthma patients. Our present study also showed a decrease in IFN-γ, IL-6, TNF-α cytokines after treatment along with increase in IL-10. However, our study showed an increase in anti-inflammatory cytokine IL-4 after treatment, whereas a previous study by Joshi et al., showed a decrease of IL-4 after treatment. This discrepancies may be attributed to disease specificity and the distinct treatment approaches in both studies [24].

Another study investigated the effects of the Ayurveda herb *Ashwagandha* (*Withania somnifera)* on cytokines IFN-γ and IL-4 in healthy individuals compared to placebo group. The study revealed that the levels of IFN-γ and IL-4 increased after 30 days of *Ashwagandha* treatment, however increase in IFN-γ in the treatment group contradicts our study results. The study revealed that the levels of IFN-γ and IL-4 increased after 30 days of *Ashwagandha* treatment. The increase in IL-4 corroborates with our study's results; however, the increase in IFN-γ in the treatment group contradicts our study results. This discrepancy may be due to the inclusion of only healthy subjects in the study, while the decrease in IFN-γ observed in our study could be a treatment response to the specific disease condition [25].

A study examining the immunomodulatory effects of *Andrographis paniculata*, another Ayurveda herb, found that cytokines IFN-γ and IL-4 increased after administration of the herb for 30 days. Additionally, a significant decrease in IL-2 was observed. While this study also showed an increase in IFN-γ and IL-4 after treatment, the increase in IFN-γ differs from our study's results [26]. These variations in study outcomes may be attributed to disease-specific responses and the uniqueness of Ayurvedic treatments for different conditions.

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5. Conclusion

In conclusion, Ayurveda treatment demonstrates the ability to reduce pro-inflammatory cytokines while increasing anti-inflammatory cytokines. This indicates that Ayurveda treatment has the potential to reduce the inflammatory response of various disease conditions by reducing pro-inflammatory cytokines and simultaneously inhibiting inflammation through the augmentation of anti-inflammatory cytokines irrespective of the specific disease. Additionally, Ayurveda treatment reduces the ratio between pro- and anti-inflammatory cytokines helping to maintain the dynamic balance critical for a healthy immune response. These findings underscore the immunomodulatory potential of personalized Ayurveda treatments in managing inflammatory aspects of various health conditions.

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Declaration of competing interest

The authors declare that they have no competing financial or personal interests that could have appeared to influence the work reported in this paper.

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