

Original article

Role of cytokines in Granulomatous Colitis

Dr Keya Basu¹, Dr Sucharita Sarkar², Dr Subhrajyoti Karmakar³, Dr Dipan Das⁴, Dr Chhanda Das⁵,
Dr Rajib Sarkar⁶

Abstract:

Background: Intestinal tuberculosis (ITB) have significant immunological similarity with Crohn's disease (CD). T helper cells and various cytokines secreted by them play a very crucial role in the pathogenesis of both the diseases. It is of great clinical relevance in a country like India to perform a comparative study between CD and ITB with respect to pathogenesis. **Objectives:** To study the levels of four cytokines (IL-4, IL-17, IFN- γ & TGF- β 1) in serum of patients with CD & ITB which indirectly reflects the levels of CD4+ T cells. **Material and method:** An observational, cross-sectional study was done on patients, attending Gastroenterology clinic in a tertiary care hospital, with features of ileitis, colitis or ileo-colitis due to CD or ITB. 12 cases of Crohn's disease & 13 cases of intestinal tuberculosis were taken (control-20 cases). 5 biopsies were taken from the ulcerated mucosa & 3 biopsies were taken from normal mucosa. H & E study & serum levels of four cytokines (IL-4, IL-17, IFN- γ & TGF- β 1) were estimated. **Results & analysis:** In CD & ITB we found marginal increase of IFN- γ & TGF- β 1 compared to control. IL17 level was found decreased in ITB compared to control (p=0.001). CD4+ T cells study by ELISA also showed significant increase in the concentration of IFN- γ (p=0.007) in ITB. There was no change in IL4 levels. **Conclusion:** Cytokines have an important role in the pathogenesis of granulomatous inflammatory bowel diseases. The identification of cytokines & their role in the pathogenesis might be helpful for future therapy based on cytokines, and anti-cytokine antibodies.

Keywords: tuberculosis; Crohn's disease; cytokines; CD4+ T cells; granulomatous; inflammatory bowel diseases

Bangladesh Journal of Medical Science Vol. 20 No. 04 October'21. Page : 784-789
DOI: <https://doi.org/10.3329/bjms.v20i4.54135>

Introduction:

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. The primary site of infection is lung, other sites like intestine, meninges, bones, lymph nodes can also get affected. The gastrointestinal tract is, the most susceptible tissue to inflammatory responses even in normal conditions, because of its constant exposure to various bacterial antigens and antigens in food and toxic factors. In a

country like India, where tuberculosis is endemic, it is not very unlikely that the rising trend in inflammatory bowel disease might be a consequence of an immune response to bacterial (tubercular) antigens entering through the food.

The inflammatory bowel disease (IBD) entity includes predominantly two forms: Crohn's Disease (CD) & Ulcerative Colitis (UC). Though pathogenesis of this group of diseases is not fully

1. Dr Keya Basu, Associate Professor
2. Dr Sucharita Sarkar, Senior resident
3. Dr Subhrajyoti Karmakar, Senior resident
4. Dr Dipan Das, Senior resident
5. Dr Chhanda Das, Assistant Professor
Dept of Pathology, IPGMER and SSKM Hospital, Kolkata, India.
6. Dr Rajib Sarkar, Assistant Professor Dept of Gastroenterology, School of Digestive and Liver Diseases, IPGMER, Kolkata, India

Correspondence to: Dr Keya Basu, Associate Professor, Dept of Pathology, IPGMER and SSKM Hospital, Kolkata, India. Address- 233A/1 N S C Bose Road, Kolkata- 700092, E-mail address – kbasughosh@gmail.com

understood, an inflammatory response including granuloma formation (in case of Crohn's disease) is found. Interestingly, tuberculosis is also manifested by a granulomatous colitis, dominated by cells of the immune system from the intestinal mucosa represented by the neutrophils, macrophages and cytotoxic T-cells. These cells attack and destroy the cells in the vicinity, either by direct contact or indirectly by releasing soluble factors like reactive oxygen species, cytotoxic proteins, lytic enzymes or cytokines¹⁻⁴.

It is a known fact that in IBDs there is a loss of immune tolerance to intestinal flora that is mediated by various substances, including cytokines. Cytokines are small peptides secreted by activated dendritic cells and macrophages. They have an enormous role in transmitting intercellular signals, the stimulation of cell proliferation for the antigen specific effector cells and the autocrine, paracrine and endocrine mediation of local and systemic inflammation¹ as well as in wound healing⁵. Secretion of cytokines can be modulated by medicinal agents which can be used for therapeutic purpose⁶.

It is of great clinical relevance in a country like India to perform a comparative study between Crohn's disease and intestinal tuberculosis with respect to role of different T helper cells and various cytokines in the pathogenesis of both types of granulomatous colitis. The objective of this study was to see the levels of four cytokines (IL-4, IL-17, IFN- γ & TGF- β 1) in serum of patients with CD & ITB which indirectly reflects the levels of CD4⁺ T cells.

Material and method

The patients attending the Gastroenterology clinic and undergoing clinical, radiological and endoscopic evaluation with the evidence of colitis, ileitis or ileo-colitis due to Crohn's disease or intestinal tuberculosis were selected for the study. Patients with positive hepatitis markers; diabetes, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, or other autoimmune diseases; organ transplantation history; or history of treatment with steroids, immunosuppressant, or biological agents in the preceding 3 months before recruitment were excluded from the study population.

CD diagnosis was based on clinical presentations, endoscopic and radiological findings supported by histology and/or a non-response to, or a relapse after a trial of anti-tubercular therapy and exclusion of other compatible etiologies according to recent

guidelines. Disease activity was determined by the CD Activity Index (CAI)^{32/7}, and a CAI \geq 150 was defined as active CD. Intestinal tuberculosis was diagnosed based upon proper history, clinical examination, radiological findings, histological report and response to anti-tubercular drug. The study was carried for 18 months. A total of 45 cases were taken; Crohn's disease case-12, intestinal tuberculosis-13 and control-20 respectively.

Controls are defined as people suffering from any other disease other than CD or ITB and undergoing endoscopic-biopsy. Biopsies are taken from the ulcerated & normal mucosa. Haematoxylin and eosin stain was done for confirmation of histopathological diagnosis. Samples were collected from peripheral venous blood of these patients and serum levels of four cytokines (IL-4, IL-17, IFN- γ & TGF- β 1) were estimated by ELISA (Strictly following the guidelines in the user manual supplied with the ELISA kits for each and every cytokine – each sample is run in duplicate along with control and standard samples and the mean absorbance for each set is calculated then the average zero standard optical density is subtracted from it). Statistical forms were used to record the relevant demographic, clinical, laboratory data for each patient before uploading to a database maintained to track the clinico-pathological progress of the cohort. Microsoft access, excel 2013 and SPSS 20.0 (SPSS, Inc., Chicago, IL, USA), were used whichever appropriate for analysis. Data were presented as mean \pm standard deviation (SD) normally distributed data, or as median and inter-quartile range for non-normally distributed data. Normally distributed data were analysed using one-way ANOVA followed by Student–Newman–Keuls post-hoc testing. Comparisons between two groups were performed using an independent sample t-test. For non-normally distributed data, & analysis between groups were performed using the Kruskal–Wallis test. A P-value $<$ 0.05 was considered indicative of statistically significant differences.

Ethical clearance: Ethical clearance taken from Institutional Ethics Committee

Results & analysis:

A total of 12 CD & 13 ITB cases along with 20 healthy age-matched volunteers were included in the study. The study was done over a period of 18 months.

In our study group, there is male predominance (62.2%), 75% patient of CD & 76.9% of ITB patients.

Age distribution shows that these two diseases predominantly involve middle age group people.(CD- mean age 43 year & ITB-mean age 37.5 year). **Table 1** describes levels of various cytokines in serum obtained by ELISA in CD (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml (n=12). Mean values of various cytokines in CD were 1.529167,0,9.57,8.696 for IFN- γ , IL-4, IL-17 & TGF- β 1 respectively.

Table 1. Levels of various cytokines in serum obtained by ELISA in CD (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml)

	IFN- γ	IL4	IL17	TGF- β 1
1	1.84	0	NA	NA
2	2.04	0	NA	NA
3	1.2	0	8.62	NA
4	1.61	0	12.48	NA
5	1.4	0	NA	9.22
6	1.4	0	9.51	NA
7	1.3	0	NA	9.18
8	1.2	0	10.52	9.89
9	1.3	0	NA	8.07
10	1.1	0	NA	NA
11	1.36	0	NA	7.12
12	2.6	0	NA	NA
Mean	1.53	0	10.28	8.7

Table 2 describes levels of various cytokines in serum obtained by ELISA in ITB (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml.(n=13). Mean values of various cytokines in ITB were 1.623846, 0, 6.48, 8.637143 for IFN- γ , IL-4, IL-17 & TGF- β 1 respectively.

Table 2.Levels of various cytokines in serum obtained by ELISA in ITB (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml)

	IFN- γ	IL4	IL17	TGF- β 1
1	3.01	0	NA	NA
2	1.5	0	NA	NA
3	1.5	0	NA	5.73
4	2.11	0	NA	NA
5	1.1	0	NA	7.53
6	1.4	0	NA	13.45
7	1.5	0	14.52	NA
8	1.23	0	3.68	NA
9	1.31	0	NA	11.26
10	1.2	0	NA	6.35
11	1.41	0	9.28	NA
12	2	0	10.84	8.26
13	1.84	0	NA	7.88
Mean	1.62	0	9.58	8.64

Table 3 describes levels of various cytokines in serum obtained by ELISA in control group (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml (n=20). Mean values of various cytokines in ITB were 1.173333 ,0.804067, 148.254, 5.975333 for IFN- γ , IL-4, IL-17 & TGF- β 1 respectively.

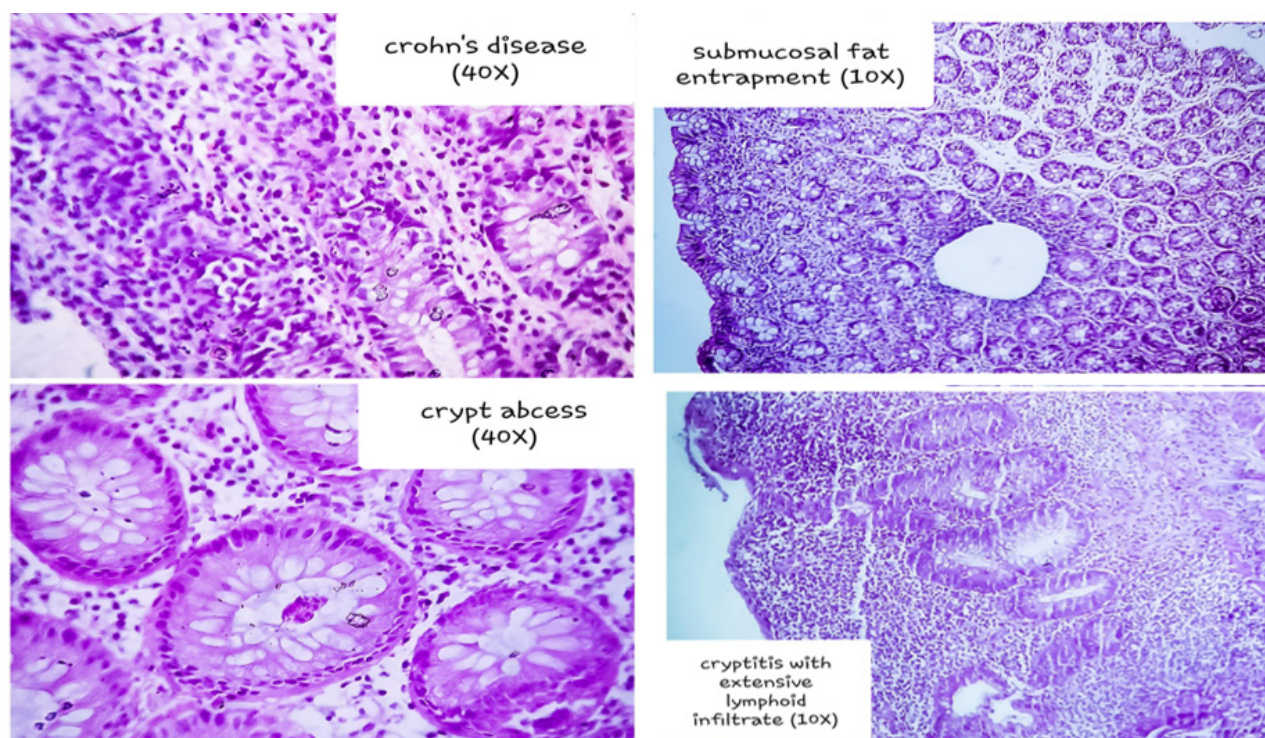
Table3.Levels of various cytokines in serum obtained by ELISA in control group (levels of cytokines are in pg/ml except IFN- γ & TGF- β 1 which is in ng/ml)

	IFN- γ	IL4	IL17	TGF- β 1
1	NA	1.86	NA	7
2	1.41	3.711	182.56	4.5
3	NA	6.49	NA	NA
4	1.26	0	136.13	5.08
5	1.03	0	146.32	5.68
6	1.38	0	150.24	4.3
7	1.2	0	162.88	NA
8	NA	0	NA	NA
9	NA	0	NA	NA
10	NA	0	NA	NA
11	1.15	0	121.43	5.04
12	1.12	0	113.34	6.21
13	1.02	0	163.92	5.26
14	1.06	0	157.24	7.24
15	1.31	0	152.32	6.68
16	0.96	0	129.56	5.97
17	1.26	0	128.54	7.64
18	1.11	0	182.5	4.89
19	1.08	0	118.6	5.58
20	1.25	0	178.23	8.56
Mean	1.17	0.80	148.25	5.97

Application of Levene’s test for Equality of variance & independent sample t test In CD & ITB cases revealed that there was marginal increase of IFN- γ & TGF- β 1 compared to control, whereas there was decrease in the level of IL17. There was no change in IL4 levels. In CD, increase of IFN- γ was statistically significant (p=0.013). Decrease of IL 17 was also achieved significance with p<0.001. In ITB, there was significant increase in the concentration of IFN- γ (p=0.007). IL17 level decrease in ITB compared to control achieved statistical significance (p=0.00).

Discussion:

Over the past decades, several works contributed significant progress in understanding CD immunogenesis, especially following the discovery of Tregulator and Th17 cells. It is postulating that



Upper left: Mononuclear inflammatory cells in lamina propria in Crohn's disease

Upper right: Submucosal fat entrapment in Crohn's disease

Lower left: Crypt abscess in Ulcerative colitis

Lower right: Cryptitis in Ulcerative colitis

cellular interactions can be modulated by the action of cytokines like Tumor Necrosis Factor α (TNF- α) Gamma Interferon (INF- γ) Interleukins (IL-1, IL-6, IL-4, IL-5, IL-10) Transforming Growth Factor β (TGF- β) or recently described cytokines like IL-13, IL-12, IL-18, IL-23, that can have a pro-inflammatory effect as well as an anti-inflammatory effect ^{5/8}. Moreover, CD is mediated by the Th1/ Th17 cytokines with increase in production of INF- γ ¹.

The Th17 subgroup contributes to CD pathogenesis by producing multiple pro-inflammatory factors, including TNF- α , IL-6 and IL-1 β ¹. Antibodies against IL-6, which drives Th17 pathway activation, negate the CD acute-phase response, suggesting promising applications in clinical research. ^{6/9} However, antibodies against the primary Th17 cytokine IL-17 failed to show benefits in CD patients ^{7/10}, leading to controversy regarding the role of Th17 cells, whether protective or destructive, in CD ^{8,9/11,12}, or about a possible competition between Th17 and Th1 subsets in CD ^{10/13}. Interleukin 4 (IL-4) produces the differentiation of naive T helper cells (Th0 cells) to

Th2 cells & a defective immunosuppressive effect ^{11/14}.

To explain the roles of CD4+ T-cell subgroups in CD patients, we included CD patients without any immunosuppressive therapy and any other immunological diseases. Importantly, the majority of patients included in this study were newly diagnosed and have not received any treatment. Therefore, we hope that the results thus obtained would illuminate correctly the real immunogenesis of CD, at least in our population.

During careful measurement of the various sub types of CD4+ T cells in systemic circulation by ELISA, IL17 level in ELISA achieved highest significance in co-relation with healthy control. However, there were several limitations to this study. First, the sample size of CD patients and healthy controls was small and might not have allowed discerning otherwise significant differences in observed T-cell subgroup population trends in CD versus control participants. The restricted cohort size might not reflect the whole population well. Secondly, we did not investigate

CD4+ T cells in mucosal specimens. To some extent, CD is a mucosal immunological disease. However, it is technically difficult to isolate enough mucosal lymphocytes for experiments, unless large surgical pieces were taken. So, we took peripheral blood samples which was simple, noninvasive & suitable for limited resource setups. However, we did not longitudinally explore possible changes in circulating CD4+ T-cell subgroups before and after treatment in the same individuals. This might be beneficial to elucidate the direct roles of CD4+ T cells as CD evolves.

The four cytokines we tested, among them IFN- γ was secreted by Th1 cells, IL4 was secreted by Th2 cells, IL17 was secreted by Th17 cells & TGF- β 1 was secreted by Treg cells. In the CD group we found that there was marginal increase of IFN- γ & TGF- β 1 compared to control, whereas there was decrease in the level of IL17. There was no change in IL4 levels as it was not detected in all our cases and most of our controls.

After statistical analysis we found that the increase of IFN- γ was statistically significant ($p=0.013$) and it correlated well with similar findings of Kang Chao et al in the Chinese population^{12/15}.

In our study the decrease of IL 17 also achieved significance with $p=0.001$, and this finding correlated well with the findings of Abdurrahman Sahin, Turan Calhan, Mustafa Cengiz, et al. in "Serum Interleukin 17 Levels in Patients with Crohn's Disease: Real Life Data."^{13/16}.

In conclusion, we found that a significant imbalance among Th1, and Th17 cells existed in CD patients. Further studies should be conducted in different populations to elucidate the precise mechanistic roles of diverse CD4+ T-cell subsets in CD development and progress.

Our study findings are similar to the findings of Kang Chao et al in the Chinese population and Abdurrahman Sahin, Turan Calhan, Mustafa Cengiz, et al in Turkey^{12,13/15,16}.

ITB is caused by *Mycobacterium tuberculosis* and morphologically resembles CD. Though this disease is an infectious disease there is significant immunological similarity with CD.

Being a granulomatous disease in the immunogenesis of ITB there is up-regulation of Th1 and Th17 cells and

down-regulation of Treg cells. IHC studies revealed that the local mucosa of ITB has many CD4+ Th1 cells and few Th17 cells. Th2 cell concentration in local mucosa was same as that of the control group. There is significant decrease in Treg cell population in ITB compared to the control group.

Detection of the levels of cytokines secreted by above mentioned CD4+ T cells by ELISA also showed significant increase in the concentration of IFN- γ ($p=0.007$). IL4 concentration secreted by Th2 cell was similar to the control group in our population & the difference is not statistically significant. The concentration of IL17 secreted by Th17 cells also showed significant decrease compared to the control group.

Our finding of upregulation of IFN- γ in ITB correlated with the study done by Pugazhendhi et al^{14/17}. TGF- β 1 concentration in serum in our ITB patient group did not achieve any significant change compared to the control group.

IL17 level decrease in ITB compared to control achieved statistical significance ($p=0.001$), but this finding do not correlate with the findings other scientists.^{12,14/15,17} As this study was conducted in a tertiary care hospital, getting newly diagnosed CD and ITB patient in the absence of other immunological disorder was very difficult.

Limitations of the study:

1. The sample size is relatively small (45 cases).
2. This study is done in a single institute over a limited period of time. So this could not be projected over the entire population.
3. Follow up of this persons could not be done as because of shorter duration.
4. Any immunological disorder or intake of any immunosuppressive drugs, though carefully excluded, but still can alter the test results to a great extent
5. Colonoscopic tissues were often found to be very small for doing both HPE & such an elaborate panel of IHC.

Conclusion

Cytokines have an important role in the pathogenesis of granulomatous inflammatory bowel diseases. The identification of new cytokines & identification

of their role in the pathogenesis might be helpful for future therapy based on cytokines, and anti-cytokine antibodies.

Conflict of interest: None

Funding disclosures: No disclosure

Authors's contribution:

Data gathering and idea owner of this study: Dr Keya Basu

Study design: Dr Keya Basu, Dr Sucharita Sarkar

Data gathering: Dr Sucharita Sarkar, Dr Subhrajyoti Karmakar,

Writing and submitting manuscript: Dr Keya Basu, Dr Sucharita Sarkar

Editing and approval of final draft: Dr Keya Basu, Dr Sucharita Sarkar, Dr Subhrajyoti Karmakar, Dr Dipan Das, Dr Chhanda Das, Dr Rajib Sarkar

References:

- Moldoveanu Al.C ,Diculescu M , Braticevici C. F.Cytokines in inflammatory bowel disease. *Rom. J. Intern. Med;* 2015; **53**(2):118-127 <https://doi.org/10.1515/rjim-2015-0016>
- Tuberculosis Fact sheet N°104". World Health Organization. November 2010.
- Global tuberculosis report 2015
- World Health Organization. Global tuberculosis control: epidemiology, strategy, financing. (2009). pp. 6-33
- Hoosen, M. The Immunomodulatory, Nitric Oxide and Cytokine activity of Septilin™. *Bangladesh Journal of Medical Science*, 2019;**18**(4), 675-688. <https://doi.org/10.3329/bjms.v18i4.42869>
- Omar, J., Shafii, N., Zainan, A. E., Sirajudeen, K., & Abdullah, M. Evaluation of wound healing biomarkers of Interleukin 6 (IL-6), Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinases 9 (MMP-9) in post Lower Segment Caesarean Section (LSCS) patients consuming Channa Striatus extract. *Bangladesh Journal of Medical Science*, 2020;**19**(3), 520-526. <https://doi.org/10.3329/bjms.v19i3.45870>
- Best WR. Predicting the Crohn's disease activity index from the Harvey-Bradshaw Index. *Inflamm Bowel Dis.* 2006 Apr;**12**(4):304-10 <https://doi.org/10.1097/01.MIB.0000215091.77492.2a>
- Sanchez-Munoz F, Dominguez-Lopez A, Yamamoto-Furusho JK. Role of cytokines in inflammatory bowel disease. *World journal of gastroenterology : WJG.* 2008; **14**(27):4280-8. <https://doi.org/10.3748/wjg.14.4280>
- Ito H, Takazoe M, Fukuda Y et al. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. *Gastroenterology* 2004; **126**: 989-96 <https://doi.org/10.1053/j.gastro.2004.01.012>
- Hueber W, Sands BE, Lewitzky S et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 2012; **61**: 1693-700. <https://doi.org/10.1136/gutjnl-2011-301668>
- Raza A, Shata MT. Letter: pathogenicity of Th17 cells may differ in ulcerative colitis compared with Crohn's disease. *Aliment. Pharmacol. Ther.* 2012; **36**: 204 <https://doi.org/10.1111/j.1365-2036.2012.05124.x>
- Monteleone I, Sarra M, Pallone F et al. Th17-related cytokines in inflammatory bowel diseases: friends or foes? *Curr. Mol. Med.* 2012; **12**: 592-7. <https://doi.org/10.2174/156652412800620066>
- Mikami Y, Kanai T, Sujino T et al. Competition between colitogenic Th1 and Th17 cells contributes to the amelioration of colitis. *Eur. J. Immunol.* 2010; **40**: 2409-22 <https://doi.org/10.1002/eji.201040379>
- Iijima H, Takahashi I, Kishi D, Kim JK, Kawano S, Hori M, et al. Alteration of interleukin 4 production results in the inhibition of T helper type 2 cell-dominated inflammatory bowel disease in T cell receptor alpha chain-deficient mice. *The Journal of experimental medicine.* 1999; **190**(5):607-15. <https://doi.org/10.1084/jem.190.5.607>
- Chao K, Zhang S, Yao J et al. Imbalances of CD4+ T-cell subgroups in Crohn's disease and their relationship with disease activity and prognosis. *J Gastroenterol Hepatol.* 2014 Oct; **29**(10):1808-14 <https://doi.org/10.1111/jgh.12592>
- Sahin A, Calhan T, Cengiz M, et al., "Serum Interleukin 17 Levels in Patients with Crohn's Disease: Real Life Data," *Disease Markers*, 2014. <https://doi.org/10.1155/2014/690853>
- Pugazhendhi, S.; Jayakanthan, K.; Pulimood et al. Cytokine gene expression in intestinal tuberculosis and Crohn's disease. *The International Journal of Tuberculosis and Lung Disease*, **17**, Number 5, 1 May 2013, pp. 662-668(7) <https://doi.org/10.5588/ijtld.12.0600>