Immunological alterations in tuberculosis associated immune reconstitution inflammatory syndrome in HIV infected patients

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The tuberculosis associated immune reconstitution inflammatory syndrome (TB-IRIS) frequently complicates the course of HIV/AIDS and HIV-TB treatment and its immunological mechanisms are poorly understood. Here, we investigated T-cells frequencies, their secreted chemokines and cytokines. In this prospective case-control study, HIV/AIDS and HIV-TB patients during treatment with highly active antiretroviral treatment (HAART) and anti-TB treatment were followed for TB-IRIS development. Age, gender and BMI-matched patients without IRIS constituted as “Controls” (non-IRIS). Activation and proliferation were assessed in CD4 and CD8 cell compartments. CCR4, CCR6 and T-reg cells were also analysed in PBMCs. Cytokines (IL-2, IL-4, IL-10, IFN-γ and TGF-β1) and chemokines (IP-10, MCP-1, MIG and RANTES) were measured in culture supernatants. Of 560 enrolled HIV/AIDS patients, TB-IRIS developed in 50 (8.9%) patients (25 paradoxical and 25-unmasking) at a median interval of 35-days (IQR, 24-78). After ART therapy, CD8+ T-cell proportion decreased in both paradoxical and unmasking-TB-IRIS as compared to non-IRIS. Simultaneously, activation of CD4+ T-cells was observed in unmasking TB-IRIS only. Similarly, CD161+ T-cells, Th17-cells and inflammatory cytokines like IFN-γ, IP-10 and MIG elevated in both TB-IRIS subgroups as compared to non-IRIS. In conclusion, during HAART treatment the dominance of pro-inflammatory cells and cytokines in TB-IRIS patients favours the development of IRIS event. On the other hand, in non-IRIS patients relative increase of anti-inflammatory cells and cytokines prevents the development of IRIS event.

**Keyword:** AIDS, Cytokines, Immunophenotyping, T-cells, TB-IRIS

Introduction of highly active antiretroviral treatment (HAART) in the 1990s significantly reduced morbidity and mortality associated with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS)1. Recent WHO estimate of global Tuberculosis (TB) burden is 2.45 million (range 2.19-2.73) of which 0.62 million are HIV positive including 0.25 fatal cases2. In high TB burden countries like India, TB-associated immune reconstitution inflammatory syndrome (TB-IRIS) is a common complication among TB/HIV co-infected patients following HAART administration. The incidence of TB-IRIS in high TB burden countries ranges from 8 to 54%3; whereas in India, it is reported to be 8%4. This variation can be attributed to differences in case definitions and patient population studied.

Different case definitions were used for IRIS and TB-IRIS in the past until 2008 when a consensus case definition was given for use in resource-limited settings5. Laboratory parameters, such as plasma viral load and CD4+ cell counts are not considered as essential requirements for diagnosis of TB-IRIS. Almost all TB-IRIS cases occur within the first three months of HAART initiation with an exaggerated clinical presentation. Two main presentations of TB-IRIS have been described: (i) paradoxical TB-associated IRIS that occurs after starting HAART in patients receiving anti-TB treatment; and (ii) unmasking TB-IRIS which occurs as an exaggerated, unusually inflammatory initial presentation of TB in a patient receiving HAART for HIV/AIDS6. Increased antigen-specific interferon-gamma (IFN-γ) and vigorous helper T-cells (Th)-1 responses have been described in TB-IRIS7.

Exact immunopathological mechanisms for the development of TB-IRIS are poorly understood and complex interplay between the immune system and
TB-IRIS remains to be determined. Few clinical risk factors have been related to developing TB-IRIS, also suggested in many studies, such as patients with low CD4 counts at the time of ART initiation and further rapid increase in the CD4 count after ART are more likely to develop TB-IRIS. In the present study, we tried to assess the immunological profile of numerous T-cell subsets expression with their activation and proliferation status during TB-IRIS. We evaluated secreted cytokines and chemokines to decipher the T-cell functions during clinical events in HIV-infected patients.

Materials and Methods

Study participants

The patients were enrolled from the ART centre of the All India Institute of Medical Sciences (AIIMS), New Delhi. The study was conducted from July 2012 to May 2015 and was approved by the institutional ethics committee of the AIIMS, New Delhi (IESC/T-397/2012). A written informed consent was taken from every participant. Out of 692 HIV/AIDS patients screened, 560 were enrolled for this study. Following exclusion criteria were applied: age ≥18 years, presence of opportunistic infections other than TB, malignancy, patients already receiving TB drugs, seriously ill patients and pregnancy. The HIV infection was documented by a licensed third generation ELISA kit as described previously. HAART was administered to all enrolled patients after careful screening. Patients diagnosed with HIV-TB were first administered anti-TB drugs followed by antiretroviral (ARV) drugs after two weeks. The IRIS was diagnosed according to the criteria described in International Network for the Study of HIV-associated IRIS (INSHI) consensus case definition and classified into TB-IRIS and non-IRIS (with or without TB). The TB-IRIS patients were further divided into paradoxical and unmasking groups and the group of patients who had not developed IRIS represented as non-IRIS (Fig. 1). Blood samples were collected for flow cytometry at baseline and at the time of IRIS. In the non-IRIS group, due to undefined time duration for the occurrence of IRIS event, it was uncertain to predetermine definite end time point.

Immunophenotyping by Flow cytometry

Flow cytometric analysis was performed using cryopreserved peripheral blood mononuclear cells (PBMCs) using FACS Aria III flow cytometer (BD Biosciences, USA). The analysis was performed by BD FACSDiva version 6.1.3 software. Cryopreserved PBMCs were thawed in RPMI supplemented with 10% fetal calf serum and surface and intracellular staining were done according to anti antibodies manufacturer’s protocol. Following three different panels of anti antibodies (BD Biosciences, USA) were used for analysing T-cells and their subsets. Panel 1: CD3-APC-H7 (Clone SK7), CD4-Alexa Fluor 488 (Clone RPA-T4), CD8-PE-CF-594 (Clone RPA-T8), CD161-PE (Clone DX12), CD69-PerCP (Clone L78), and Ki67-Alexa Fluor-647 (Clone B56). Panel 2: CD3-APC-H7, CD4-Alexa Fluor 488, CD25-PE (Clone M-A251), and FoxP3-PE-CF-594; (Clone 259D/C7). Panel 3: CD3-APC-H7, CD4-Alexa Fluor488, CCR4-Alexa Fluor-647 (Clone 1G1), CCR6-PerPCy5.5 (Clone 11A9), and IL-17A-PE (Clone SCPL1362).

Gating strategy

For specific cell identification, PBMCs were primarily identified over SSC on Y-axis and FSC on X-axis. Further, signature T-cell surface marker CD3 were gated. For T-reg cells: CD3 gated cells were further subjected to identify CD4+CD25+ dual positive cells; these dual positive cells were gated to identify FoxP3 cells on the histogram. For IL17A: After analyzing PBMCs on FSC vs. SSC, gated lymphocytes were identified for CD3+CD4+ dual positive cells and further, mAbs to IL-17a cells were used on gated dual positive helper T-cells.

Cytokines and chemokines in culture supernatants

Cytokines and chemokines namely, interleukin (IL)-2, IL-4, IL-10, interferon gamma (IFN-γ), IFN-γ-induced protein (IP)-10, monocyte chemotactic protein (MCP)-1, monokine induced by IFN-γ (MIG), regulated upon activation normal T-cell expressed and presumably secreted (RANTES), and transforming growth factor (TGF)-β1 levels were estimated in unstimulated culture supernatants collected after 24 h incubation of PBMCs in complete RPMI-1640 media (supplemented with 10% FBS, 10mM L-glutamine), using enzyme-linked immunosorbent assay (ELISA) kits (IL-2, IP-10, MIG, and RANTES, Quantikine, USA; IL-4, IL-10, IFN-γ, MCP-1, and TGF-β1, e-Biosciences, USA). The assays were done according to the manufacturers’ protocol.

Statistical analysis

Baseline characteristics were compared between HIV-positive patients with and without TB using
Student’s t-test for normally distributed variables. Parameters without a normal distribution were compared through Wilcoxon’s rank-sum test and qualitative data. Categorical variables were evaluated by Chi-square test. Further, before and after HAART parameters of IRIS groups and in non-IRIS groups were compared by Wilcoxon’s sign rank test. The p-value of <0.01 and <0.05 were considered statistically significant. Entire analysis was done using statistical software Stata 12.1 (Texas, USA).

**Results**

**Patients’ characteristics**

Of 560 participants in the study, 184 (32.8%) were HIV-TB patients and 376 (67.2%) were HIV patients without TB. Among 560 patients, 50 (8.9%) participants were diagnosed with IRIS. The median time interval between initiation of HAART and development of unmasking and paradoxical TB-IRIS was 37 (IQR, 22–88) and 68 (IQR, 29-92) days.

![Table 1—Demographic and clinical characteristics of 560 HIV/AIDS patients](image)

[HIV, human immunodeficiency virus; TB, tuberculosis; BMI, body mass index; kg, kilogram; m, meter; PVL, plasma viral load. Age, weight, height and BMI data have been expressed as mean ± SD; gender as number (%) of male and female patients, CD4 cells and HIV-1 plasma viral load data have been expressed as median and interquartile range (IQR)]

respectively. Baseline details of enrolled patients are provided in Table 1. In Table 2, baseline data of
paradoxical and unmasking TB-IRIS with their age, gender and body mass index (BMI) matched comparative non-IRIS groups had been provided. After HAART initiation in both paradoxical and unmasking IRIS groups and in both non-IRIS groups (with and without TB), the baseline CD4 cell counts increased and plasma viral load (PVL) decreased (Suppl. Fig. 1). All supplementary figures are available only online along with the respective paper in CSIR-NISCAIR repository NOPR at http://nopr.res.in).

Frequency of CD4+ and CD8+ T-cells in TB-IRIS

In present study, it was found that in the overall IRIS group, the proportion of Th-cells decreased significantly at IRIS event. When the comparison was done between IRIS vs. non-IRIS patients, it was observed that the proportions of CD4+ T-cells were significantly decreased in paradoxical TB-IRIS after HAART, whereas in unmasking TB-IRIS patients the change was not significant. The analysis of activation was done through CD69+ and proliferation was done through Ki67+ expression on CD4+ T-cells. At IRIS event (IRIS vs. non-IRIS) in paradoxical TB-IRIS, CD69+CD4+ T-cells population increased significantly. Although, no significant change was observed in unmasking TB-IRIS patients. Simultaneously, after ART treatment the frequencies of Ki67+CD4+ T-cells were found significantly increased in both paradoxical and unmasking TB-IRIS (Fig. 2).

Further, the proportions of CD8+ T-cells (baseline vs. event) after HAART were found to be elevated in paradoxical TB-IRIS and decreased in unmasking TB-IRIS patients. Interestingly, the comparison between IRIS vs. non-IRIS showed significantly higher proportion of CD8+ T-cells after ART treatment in both IRIS subgroups. Simultaneously, CD69+CD8+ T-cells (baseline vs. event) at IRIS events were marginally decreased in paradoxical TB-IRIS and significantly decreased in unmasking TB-IRIS patients. When IRIS vs. non-IRIS comparison was done, this proportion was significantly higher in both types of IRIS group after ART treatment. On the other hand, between IRIS vs. non-IRIS, before and after ART treatment frequencies of Ki67+CD8+ T-cells at IRIS event were significantly decreased in paradoxical and unmasking TB-IRIS (Fig. 3). The CD4+/CD8+ ratio was also calculated. Before ART treatment (IRIS vs. non-IRIS) this ratio was higher in paradoxical and lower in unmasking TB-IRIS patients, however, the ratio after treatment was lower in the paradoxical group only (Suppl. Fig. 2).

Expression of CD161+ T-cells in TB-IRIS

After HAART initiation, baseline vs. IRIS event, expression of CD161+ on CD3+ T-cells was significantly increased in paradoxical TB-IRIS whereas no significant change was observed in unmasking TB-IRIS. Though, after treatment, Ki67+CD161+ T-cells were significantly elevated in paradoxical TB-IRIS group, while no change was found in unmasking TB-IRIS group. The frequency of CD69+CD161+ was higher during IRIS events (IRIS vs. non-IRIS) among both groups. (Suppl. Fig. 3). During IRIS (baseline vs. event), CD161+ expression was lower on CD4+ T-cell and higher on CD8+ T-cells in paradoxical TB-IRIS. No significant change
Expression of CCR4 and CCR6 on CD3+CD4+ T-cells in TB-IRIS and non-IRIS patients

Further, the expression of chemokine receptors CCR4+ and CCR6+ on CD3+CD4+ T cells were studied. In both paradoxical and unmasking TB-IRIS cases, in a comparison of baseline vs. event, CCR4+ and CCR6+ T-cell percentages were unchanged after ART treatment. Although, between IRIS vs. non-IRIS, CCR4+cells were higher and CCR6+cells were lower in both paradoxical and unmasking TB-IRIS patients after the HAART (Suppl. Fig. 5).

Expression of regulatory T-cells and Th17 cells

We evaluated the proportion of T-regulatory (T-reg) cells, based on the intracellular expression of FoxP3 (Fig. 4; panel-1). In both groups (IRIS vs. non-IRIS), before and after receiving therapy, the T-reg cell proportion was found to be decreased. Remarkably, among non-IRIS patients, baseline vs. event, T-regs cells were increased in paradoxical and decreased in unmasking controls after ART treatment, which was unchanged in both types of IRIS patients. The Th17 cell proportions were analyzed by intracellular IL-17A expression on CD3+CD4+ cells (Fig. 4; panel-2). The cells were seen higher at both time points in both paradoxical and unmasking TB-IRIS

was observed in unmasking TB-IRIS patients (Suppl. Fig. 4).

Fig. 2—Frequency of helper T cells with their activation and proliferation status in tuberculosis associated immune reconstitution inflammatory syndrome (TB-IRIS) and non-IRIS peripheral blood. (A) Representative fluorescence-activated cell sorter (FACS) plots showing frequency of CD3+CD4+ T cells, CD3+CD4+CD69+ T cells and CD3+CD4+Ki67+ T cells among gated T cell lymphocytes isolated from peripheral blood mononuclear cells (PBMCs); and (B) The bar diagram show the expression in percentage of helper T cells and their activation and proliferation status. [P values were observed between the groups by using Wilcoxon’s sign-rank test. The bars in grey with lines = before HAART initiation and black = after HAART initiation]
patients (IRIS vs. non-IRIS) in comparison to non-IRIS. While baseline vs. event, only unmasking IRIS group showed a significant increase in Th17A cells.

Measurement of cytokines and chemokines in the culture supernatant of PBMCs

The Th1 signature cytokines, IL-2 and IFN-γ during TB-IRIS in the culture supernatant of PBMCs were measured. Where, baseline vs. event no changes were found in IL-2 levels in both types of TB-IRIS patients, while the concentration of IFN-γ was found to be upregulated. Further, Th2 signature cytokines IL-4 and IL-10 were also analysed and significantly higher levels of IL-4 were observed in paradoxical TB-IRIS patients (baseline vs. IRIS event) and in unmasking TB-IRIS patients (IRIS vs. non-IRIS) after ART treatment. On the other hand, after ART (IRIS vs. non-IRIS) the concentration of IL-10 was higher in non-IRIS patients in comparison to the respective TB-IRIS groups. Interestingly, post ART treatment, anti-inflammatory cytokine IL-10 levels went higher in both types of non-IRIS patients (Suppl. Fig. 6). Another Th2 cytokine TGF-β1 level was found to be significantly increased after HAART in both sub-groups of IRIS. However, TGF-β1 was significantly higher at baseline and after ART treatment in the non-IRIS group in comparison to IRIS patients (Suppl. Fig. 7).

Ratios of cytokines and chemokines from Th1 and Th2 cells

After ART treatment, the ratio of IFN-γ/IL-4 (IRIS vs. non-IRIS) was increased in both groups of TB-IRIS patients. Simultaneously, the IFN-γ/IL-10 ratio was significantly higher (baseline vs. event as well as IRIS vs. non-IRIS) in both TB-IRIS patients (Suppl. Fig. 3—Frequency of cytotoxic T cells with their activation and proliferation status in tuberculosis associated immune reconstitution inflammatory syndrome (TB-IRIS) and non-IRIS peripheral blood. (A) Representative fluorescence-activated cell sorter (FACS) plots showing frequency of CD3+CD8+ T cells, CD3+CD8+CD69+ T cells and CD3+CD8+K67+ T cells among gated T cell lymphocytes isolated from peripheral blood mononuclear cells (PBMCs); and (B) The bar diagram show the expression in percentage of cytotoxic T cells and their activation and proliferation status. [P values were observed between the groups by using Wilcoxon's sign-rank test. The bars in grey with lines = before HAART initiation and black = after HAART initiation]
Fig. 6). The chemokine IP-10 and MIG levels were significantly increased after ART treatment (baseline vs. event) in both TB-IRIS patients. Interestingly, IRIS vs. non-IRIS comparison indicates IP-10 levels to be significantly higher during the event in both TB-IRIS group. However, after the treatment MIG levels were significantly higher in unmasking whereas lower in paradoxical TB-IRIS patients. The levels of MCP-1 and RANTES chemokines were also analyzed and found to be relatively unchanged after treatment in both IRIS and non-IRIS groups. However, RANTES (IRIS vs. non-IRIS) showed higher levels in paradoxical and lower in unmasking TB-IRIS patients after giving ART treatment. (Suppl. Fig. 7).

Discussion

In the present study, we specifically focused on IRIS development due to TB in HIV/AIDS patients during treatment. We analyzed various immunological parameters at baseline and during IRIS events in paradoxical and unmasking TB-IRIS with their respective comparators and non-IRIS groups. The findings of the present study suggest different immune responses in paradoxical and unmasking subgroups of TB-IRIS, and therefore analysis was done in paradoxical and unmasking TB-IRIS separately.

Following HAART administration, we observed a significant increase in absolute CD4 counts and a decrease in PVL in both subgroups of TB-IRIS as well as in non-IRIS patients and this is consistent with the treatment effect. During IRIS, an inflammatory response or dysregulation of the immune system occurs to both intact subclinical pathogens and residual antigens. It is possible that a combination of underlying antigenic burden, the degree of immune restoration following HAART, may lead to TB-IRIS.

During the TB-IRIS event, the proportion of CD4+ T-cells decreased as compared to the baseline. However, subgroup analysis showed that there was a significant decrease in percentages of CD4+ T-cells in paradoxical TB-IRIS only. A possible explanation for CD4+ T-cell population decline could be either a reduced thymic output and/or recruitment of these cells to additional new TB sites, occurring during TB-IRIS events. This decreased CD4+ T-cell expression in TB-IRIS patients affects the balanced IFN-γ, TNF-α and other cytokines that led to the declined immune response against any opportunistic infection such as M.tb which in turn could relapse of TB infection and facilitate to the development of TB-IRIS. While in unmasking TB-IRIS, it can be
postulated that due to latent TB infection, the CD4+ T-cell population remained relatively stable during the IRIS event in comparison to non-IRIS\textsuperscript{15}. Further, we explored the turnover of CD4+ T-cells in TB-IRIS development and studied activation (CD69+) and proliferation (Ki67+) profiles. Between baseline vs. IRIS event, decreased activation of CD4+ T-cells in paradoxical TB-IRIS and increased unmasking TB-IRIS during IRIS event suggest progression of existing TB disease or development of TB at a new site in paradoxical, could be the reason for observed differences in activation profiles of T-cells in two subgroups of TB-IRIS\textsuperscript{16}. On the other hand, among IRIS vs. non-IRIS, we observed an increased CD4+ T-cell proliferation in paradoxical TB-IRIS which can be attributed to variations in IL-2-dependent proliferation\textsuperscript{14} or compensatory proliferation due to loss of lymphocytes in the peripheral blood compartment during TB-IRIS event\textsuperscript{17}. However, no such changes were observed in unmasking TB-IRIS. The analysis here revealed that proportion of CD8+ T-cell and CD8+CD69+ T-cells was observed higher in paradoxical TB-IRIS, which could be attributed to a heightened immune response against prolonged TB disease\textsuperscript{18} or contributed partly by extended anti-TB drugs\textsuperscript{19}. This increase could also reflect a compensatory increase in the case of CD4+ T-cells losing their functional activity or proportion and vice versa\textsuperscript{20}. On the other hand, a lower percentage of CD8+ T-cells in unmasking TB-IRIS might be due to the absence of active TB and no anti-TB drugs were administered to this group. The ratio of CD4/CD8 was also estimated, the CD4/CD8 ratio is one of the clinical biomarker used to assess the patient’s immunity. Although many studies suggested that in the initial month of ART treatment, greater increase in CD4 count as well as a CD4/CD8 ratio and rapid decline in PVL are variables associated with TB-IRIS occurrence. However, in the present study, our data shows inverse protagonist role of the CD4/CD8 marker in the development of TB-IRIS.

We further deciphered dynamics of CD161+ expressing T-cells in TB-IRIS since these cells are known to play a key role in immune regulation against HIV and TB\textsuperscript{21}. Following HAART administration, CD161+ expressing CD4+ T-cells were decreased in paradoxical TB-IRIS group and this could be due to the recruitment of these cells in the previously established active TB disease. Additionally, it is known that M.tb cell wall lipids increase the expression of programmed death-1 (PD-1) receptors on CD161+ T-cells which eventually leads to apoptosis of these cells\textsuperscript{22} and contribute to the decreased expression of CD161+ on CD4+ T-cells, which could further attenuate protective responses thus contributing to TB-IRIS development. It is also possible that in paradoxical TB-IRIS elevated percentages of CD161+ on CD8+ T-cells might compensate for decreased CD4+ T-cells population\textsuperscript{20}.

Additionally, the Th homing receptors were also assessed, Th1 (pro-inflammatory; CCR6+) and Th2 (anti-inflammatory; CCR4+) cell populations. Baseline vs. IRIS event, no significant changes were observed in Th1 and Th2 populations in both subgroups during IRIS event. However, when we compared IRIS vs. non-IRIS, we found lower Th1 proportion after ART treatment in both TB-IRIS and this might be due to the migration of Th1-cell from peripheral blood to new disease sites resulting in protective inflammatory responses. The Th2-cell percentages were found to be higher in both TB-IRIS sub-groups once it compared between baseline vs. IRIS event. This could probably be due to the relatively inadequate migration of Th2-cell from peripheral compartment to disease sites, although additional experiments are required to ascertain this hypothesis, such as detailed chemokines and chemokine receptors profiling. Therefore, it appears that during the TB-IRIS event, a relative increase of Th1-cell and decrease of Th2-cell proportions at disease sites facilitated inflammation during TB-IRIS event.

Thus, the abundant proportion of pro-inflammatory cells (IL-17A) and their secreted cytokines and deficiency of T-reg cells during IRIS event among TB-IRIS group indicates the development of TB-IRIS. These findings are supported by previously reported studies as well\textsuperscript{23,24}. Also, in the non-IRIS group, elevated Th2 (anti-inflammatory) cytokines that help to prevent the development of TB-IRIS. This is further corroborated in the present study by the observation of elevated IL-10 and TGF-β1 cytokine levels in the non-IRIS group as these cytokines are secreted by T-reg cells\textsuperscript{25}. Observation of a marginally elevated IL-2 level in TB-IRIS cases in response to HAART administration could have promoted the proliferating ability of Th cells, helper CD161+ T-cells and increase in absolute CD4 counts\textsuperscript{14}. On the other hand, elevation in IFN-γ levels during TB-IRIS
event could reflect protective responses, however, unchanged IL-10 levels in IRIS sub-groups shifts the balance towards IFN-γ and ratio of IFN-γ to IL-10 increases significantly, promoting inflammation in TB-IRIS. Simultaneously, inadequate elevation in IL-4 levels in TB-IRIS aggravated the pro-inflammatory condition. In non-IRIS cases, both IFN-γ and IL-10 cytokines were increased; however, the rise in anti-inflammatory cytokine far exceeded this, preventing the development of TB-IRIS in this group. Further, in the present study, increased TGF-β1 levels after HAART in TB-IRIS were much less than non-IRIS which suggest an insufficient increase in TGF-β1 levels also led to uncontrolled inflammation and eventually TB-IRIS. Consistent with IFN-γ levels, IP-10 which is induced by IFN-γ also increased during TB-IRIS along with MIG and MCP-1; all three chemokines are positively regulated by IFN-γ. Put together, this could contribute towards increased inflammation and eventually TB-IRIS.

The main strength of the study lies in the assessment of immunological changes in both paradoxical and unmasking TB-IRIS. By contrast, most of the previous studies have focused either on combined TB-IRIS or the either form of TB-IRIS. Limitations of the present study were: (i) time point of blood collection in non-IRIS patients was chosen arbitrarily by consensus at 24 weeks, ideally it should have been first 6 weeks as TB-IRIS developed during this period; and (ii) we did not measure cells, cytokines and chemokines changes at disease site as it was technically challenging to obtain tissue from extra-pulmonary site.

**Conclusion**

It is concluded from this study, the dominance of pro-inflammatory cells, such as Th17 and cytokines (IFN-γ, IP-10 and MIG) in TB-IRIS patients favours the development of IRIS event. On the other hand, in non-IRIS patients relative increase of anti-inflammatory cells such as FoxP3 cells and cytokines (IL-10 & TGF-β) prevent the development of IRIS event.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

**Ethical statement**

This study was approved by Institutional ethical committee (IESC/T-397/2012), and written informed consent taken from all the study participants.

**References**