Level of Interferon - gamma, Interlukin – 6, frequency of Monocyte and Lymphocyte subsets among newly diagnosed Tuberculosis patients in northwest Ethiopia

Abstracts

Background

Tuberculosis is one of the fundamental health problems in the world. During TB infection immune cells and cytokines play the major role for the pathogenesis of the disease and also for host defence. Cells of immune system play a great role to decrease the progress of the disease with the coordination of cytokines. The aim of our study was to determine the level of immune cell and certain cytokine in TB patients and apparently health controls.

Methods

A comparative cross-sectional study was conducted among newly diagnosed TB patients in Northwest Ethiopia. Blood samples were collected from all study participants. Serum level of interferon-gamma and interlukin-6 were determined by using an Enzyme Linked Immunosorbent Assay (Ready-SET-Go! Kit), Frequency of Monocyte and T cell sub population were measured with Flow cytometry using BD Multi TEST^{TM} reagent. Mann-Whitney U test and spearman correlation were applied for statistical test by using graph pad prism.

Result

A total of 80 study participants comprising of 40 newly diagnosed smear positive TB patients and 40 healthy blood donors were included in the study. From our study participants 19 (23.75%) were females and 61(76.25%) were males with mean age of 30. Results from the study showed that the median level of IFN- ϒ (72.8) and IL-6 (13.45) in TB patients was higher when compared with controls IFN- ϒ (44.8) & IL-6 (4.08) ($P > 0.001$) while on the other hand, the median level of CD4 (565.5) and BMI (18.00) were lower compared to controls CD4 (857) & BMI (20.9) ($P > 0.001$)

Conclusion

Our study showed high level of IFN-Ɣ, IL-6 and low level of monocyte, CD4 and BMI in TB patients than the control groups.

Key words; Tuberculosis, CD4T cell, IFN-γ and IL-6, Gondar, Ethiopia

INTRODUCTION

Tuberculosis (TB) continues to be major public health problem, causing global morbidity and death yearly. Based on the recent WHO report, there were 9.6 million new TB cases per year and close to 2 million deaths (1). Factors related to the environment, host and pathogen could play significant roles for TB susceptibility (2). Immune status of the patient play a great role in the progression of TB and for the control of TB disease (3). The ability of the host to elicit potent cell mediated immune response determines disease development and outcomes associated with TB. Macrophage activation, cytokine production and other immune cells play roles in TB immunity and determine the outcome of the disease (4, 5).

T-helper 1(Th1) and T-helper 2 (Th2) immune responses are the major arms of immune responses for humoral and cell mediated immune system, respectively. The induction of the Th1 type immune response is very crucial to combat TB infection and characterized by secretion of interferon-gamma (IFN-γ). Cytokine such as IFN-γ, Tumor necrosis factor – alpha (TNF-α), IL-12 and Interleukin-6 (IL-6) are responsible chemicals to build the proactive immunity against TB pathogens during early stage of the infection. IFN- γ has versatile roles including facilitating the intracellular killing of the TB bacilli by macrophages (6, 7). It has a great role for the function and maturation of immune cells, and it stimulates production of other cytokines and activation factors to augment the function of innate immune system during infections (8, 9). In addition to IFN-γ, the inflammatory cytokines like IL-6 also play pivotal roles in TB immunity. The secretion of IFN-γ for the activation of macrophage by CD4 cells during TB infection is stimulated by the production of IL- 6 (10). IL-6 is a cytokine known as a kind of pleiotrophic nature and produced by many cells including T and B-lymphocytes. It plays a great role in the synthesis of C-reactive protein in

various stimuli (11, 12) and also has been revealed that it has a relevant role in the pathogenesis of tuberculosis like the secretion of IFN- γ during infection (10, 13).

Therefore, the present study aims to assess the levels of these two essential cytokines IL-6 and IFN-γ, which are greatly needed to combat TB infection and as well as assessing the levels of two immune cells which are T cells and monocyte. Thus, the level of these two cytokines and cells among active TB patients will be compared to the level from apparently healthy control.

MATERIAL AND METHODS

Study design and area

A cross-sectional study was conducted in newly diagnosed TB patients at the Gondar of University referral hospital from February to May 2014.Gondar University hospital is a tertiary level teaching and referral hospital with 450 beds for inpatients as well as rendering referral health services for over 5 million inhabitants in North-West Ethiopia. The hospital provides inpatient and outpatient services to the population in the surrounding area of Gondar town and the adjacent regions. The hospital has TB clinic where TB patients are getting their medication and further assessment during follow up period. For comparison purpose, apparently healthy controls were enrolled at the Gondar University Blood Bank.

Study participant

A total 80 study participants comprising of 40 newly diagnosed smear positive TB patients and 40 healthy blood donors were selected for the study; the mean age for the TB patients and the controls were 34 years (range 18 to 57) and 26 Years (range: 20 to 42), respectively. Number of females among TB patients is (11/40) 27.5% and (9/40) 22.5% among the control group. Among the TB cases, 70% (28/40) were diagnosed in the hospital as pulmonary TB and the remaining were extra pulmonary TB. The body mass index (BMI) of the study participants were, 17.98 for Tb patients and 20.64 for controls. From all study participants' informed consent were taken. Participants with known disease (HIV, diabetic, hypertensive and cancer) and pregnant women were excluded in the study and we include with the age group of 18 up to 65 years old. Socio-demographic data were collected using a structured and pretested questionnaire.

Blood collection

Six (6) ml of whole blood was collected from the study participant using serum separator (14) tubes and EDTA for measurement of cytokines using ELISA technique and the remaining one was used for blood cell count determination, respectively.

Serum level IFN-γ and IL-6 measurement

The levels of IL-6, and IFN-γ were measured using ELISA Ready-SET-Go! Kit. Anti-human cytokines (IL-6 and IFN-γ) capture antibodies (eBioscience) was coated in ELISA (Coat corning costar 9018) plates with 100 µl/per well in 1x coating buffer and the plates were sealed and incubated overnight at 2-8^{o}c. On the following day, the plates were aspirated and washed three times using 250µl wash buffer (1x PBS, 0.05% Tween 20). The standards (human cytokines lyophilized standard) were reconstituted and diluted according to the given instruction. A twofold serial dilution of the top standards with 100µl were performed in duplicates to make a standard curve for 8 points and also 100µl supernatant of samples were added to incubate at room temperature for 2 hours. After 3-5 washes, 100µl/well of detection antibody (anti human cytokine biotin) was added and incubated at room temperature for 1 hour. After 3-5 washes, 100µl/well of Avidin-HRP was added and incubated at room temperature for 30 minutes. Finally, 1M H3PO4 or 2N H2SO4 stop solution was added to stop the reaction, and the plate was read at 450nm length using ELISA reader. The analytical sensitivity and other technical protocols were followed as per the manufacturer guideline.

Cell counting using Flow cytometry

T-lymphocyte sub-populations were characterized using Multi TEST^{TM} fluorescent labelled monoclonal antibodies reagents against surface CD markers (CD_3-flurescein isothiocyanate (FITC), CD_8- phycoerythrin (PE) and CD_4-allophycocyanin (APC)) after erythrocyte has been lysed. FACS Calibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) was used for acquisition and analysis of T-lymphocyte sub-population by using Mulit TEST software (Becton Dickinson), and a minimum of 15, 000 events were captured during acquisition. Monocyte cell count done by coulter counter machine.

Statistical analysis

Data were analyzed using graph pad prism. Data were reported as frequency and percentage for categorical variables and mean and standard deviation (SD). Mann-Whitney U test was used to compare the cytokine levels between TB patients and healthy controls. Spearman correlation was used to correlate serum IFN-γ and IL-6 cytokines with peripheral CD4+, CD8+ and Monocyte cells. Statistical significance was considered at 95 % level of confidence and P value less than 0.05.

RESULTS

IFN-gamma, IL-6, and peripheral T-cell subpopulation and Monocyte

The level IFN-γ and IL-6 were significantly higher among active TB patients compared to healthy controls (median of 72.8 Pg/ml versus 44.0 pg/ml, $p < 0.0001$ for IFN-gamma; and 27.4 pg/ml versus 6.0pg/ml, $p < 0.0001$ for IL-6) (table 2). Similarly, significant difference in CD_4^+ T cell subsets ($p < 0.0001$) and monocyte ($p=0.018$) was obtained between active TB patients and healthy controls. (Table 1) & (Fig 1).

Correlation of serum IFN-γ and IL-6 cytokines with peripheral CD4+, CD8+ and Monocyte cells,

By using spearman correlation among cytokine and cells, IL-6 and $CD4^+$ showed weak negative correlation ($r = -0.4929$; $p = 0.001$ but monocyte and $CD8^+$ cell showed weak positive correlation ($r = 0.3962$; $p = 0.0167$). (Fig 2)

DISCUSSION

In our study, the level of CD4 cell count is significantly higher in the controls when compared with that of newly diagnosed TB-patients. This is in agreement with previously literature which showed that patients with TB case had low level of $CD4^{+}$T cell subsets when compared with that of the apparently health controls (14). Due to the protective effect of the immune response against this pathogen mediated by Th1 cells, lower levels of the cells leads to the progression of tuberculosis in the patients (3). Moreover, most of TB cases show low BMI in our study, which may play a great role for the decreasing of CD4 counts and their functionality by reducing the transportation of different co-factors and elements than controls with normal BMI value. This Low level of CD4 cells play a great role for the pathogenesis of TB disease, because it may be due to the low respond in delayed type immune response and due to low level of cell frequency may lead to decrease in granuloma formation, which is fundamental for proper immune response.

IFN-γ is one of the Th1 cytokine identified as the most important agents of anti-mycobacterial cytokine by activating macrophage to enhance intracellular killing (15)(16) as well as enhance the production of other cytokine (17). In our study the level of IFN-γ was significantly higher in TB patients compared with the control groups. The finding is consistent with other similar studies on TB (18-20) and the level of this cytokine is decrease through and after treatment (20) The probable reason why INF- γ is higher in TB patients while CD4 cell are low is perhaps due to the fact that IFN- γ comes out from both local production and spill over of it from the activated lymphocytes sequestered at the site of MTB infection. This condition can make the cytokine not being able to elicit downstream events involving ineffective activation of macrophages and intracellular killing of MTB. All these conditions play a great role for the pathogenesis of MTB infections.

The level of IL- 6 in TB patients is also higher than that of the controls, similar studies had the result which is in line with our findings (21, 22). On the other hand also study showed that there is increased IL- 6 in active TB disease condition when compared with that of the latent condition of TB infection (23). In addition study showed that the concentration of IL-6 is very high in TB patients with pulmonary cavities than without cavities. This is a great indication that IL-6 plays its own role for the TB disease outcome (24). High level of IL-6 during TB infection can inhibit the type 1 interferon signalling on macrophage and

consequently lead to the disease progression (25). Moreover , increased level of IL-6 is correlated with disease severity and decrease in the nutritional status of the patients (24), our findings showed this based on the level of BMI in TB patients in the study that were significantly lower than that of health controls.

The level of monocyte cell in the blood of TB patients is higher than that of the controls and show significant association, one of the probable reason that the increased frequency of the monocyte during TB infection is due to the production of pro-inflammatory cytokines and chemo attractant factors that direct the monocyte from the bone marrow to lymph node and other infected tissue where it act like macrophages. In this condition there may be an increase of monocyte in the vascular. On the other hand cytokines that is produced by infected macrophages may have an impact on monocyte by not changing it to macrophages thereby preventing it from going to the infected area. This high frequency of monocyte in TB patients may also contribute to the increased level of IL- 6 in TB patients since they have high level than the controls.

CONCLUSION

In our study patients with tuberculosis showed higher level of IFN-ϒ, IL-6 and monocyte with lower level of CD4 and BMI than the controls. This may indicate the progression of the disease. Future studies are required to describing the mechanisms of this value alteration during TB disease as well as for better clinical application of these parameters.

Abbreviations

BMI: body mass index

CD: cluster of differentiation

EDTA : Ethylenediaminetetraacetic acid

ELISA: Enzyme linked immunosorbant assay

IL: Interlukine

IFN -γ: Interferon gamma

TB: Tuberculosis

TNF-α: Tumor necrosis factor alpha

SD: Standard deviation

WHO: world health organization

Declarations

Aknowledgement

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

ML & EA: Conceived and designed the experiments; ML, MW &BM, carried out data collection and laboratory analysis; EA supervised the data collection and laboratory analysis; ML & SY ;analyzed the data and prepared the first manuscript draft EA , MW & ML; reviewed the draft. All authors read and approved the final manuscript.

Competing interests

The authors declare that there is no competing interest with regard to the present study.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the ethical committee of the School of Biomedical and Laboratory Sciences, University of Gondar. In addition, written informed consent was obtained from each participant.

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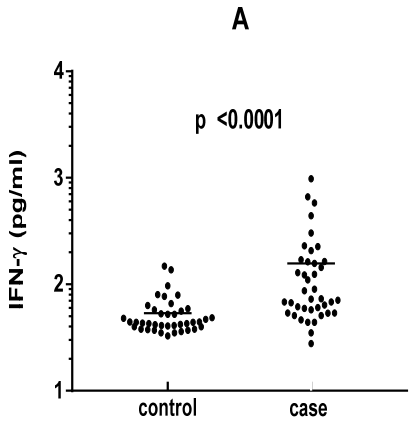
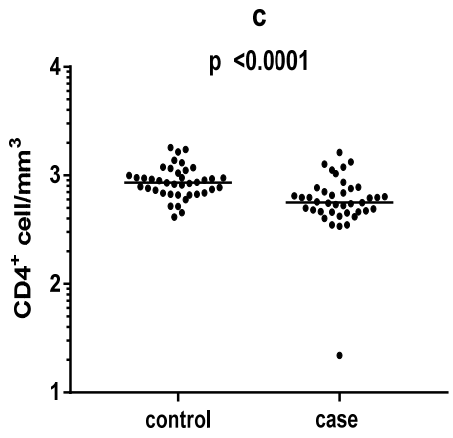
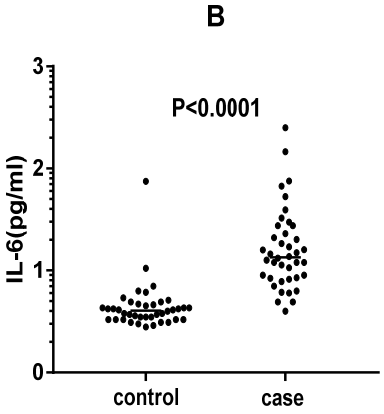
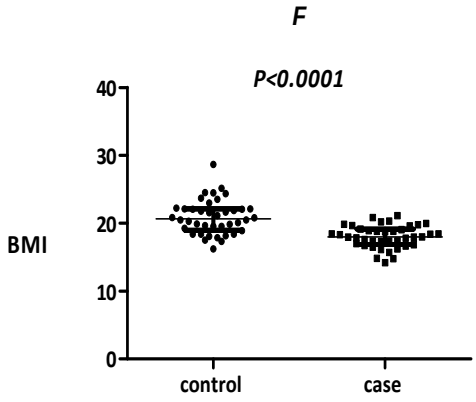
Table 1. Level of IFN-γ, IL- 6, CD4T cell, CD8 T cell and Monocyte cell in TB patients and controls

Parameters	TB patients	Control	p-value
IFN-γ(pg/µl) (median ±SD)	72.8	44.0	< 0.0001
IL-6 (pg/µl) (median ±SD)	13.45	4.05	< 0.0001
CD4 cells($x10^3$/µl)	565.5	857.0	< 0.0001
CD8 cells($x10^3$/µl)	539.5	551.5	< 0.4162
Monocyte ($x10^3$/µl)	400.0	300.0	< 0.0193


A
p <0.0001
IFN-γ (pg/ml)
4
3
2
1
control
case


c
p <0.0001
CD4+ cell/mm3
4
3
2
1
control
case


B
P<0.0001
IL-6(pg/ml)
3
2
1
0
control
case


F
P<0.0001
BMI
40
30
20
10
0
control
case

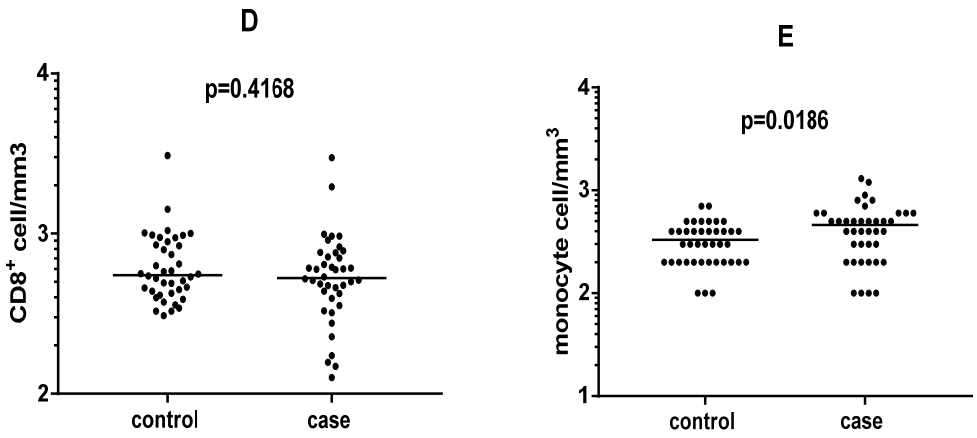
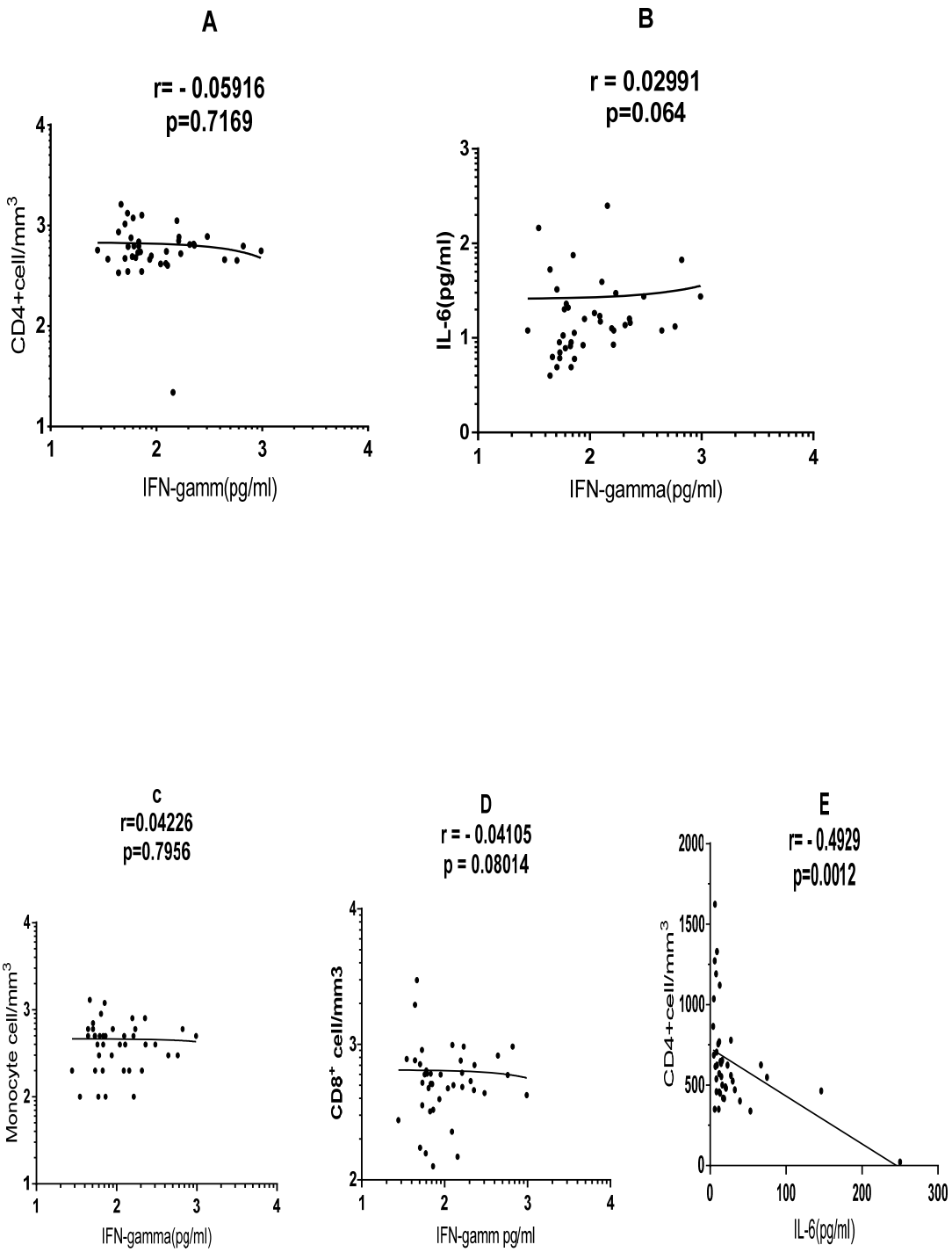


Fig. 1 **Serum level of IFN-gamma, IL-6, and measurement of peripheral blood CD4+ cells, CD8+ cells and Monocytes among newly diagnosed TB patients (n=40) and apparently healthy controls (n=40)**


A
r= - 0.05916
p=0.7169
CD4+cell/mm3
IFN-gamm(pg/ml)
B
r = 0.02991
p=0.064
IL-6(pg/ml)
IFN-gamma(pg/ml)
c
r=0.04226
p=0.7956
Monocyte cell/mm3
IFN-gamma(pg/ml)
D
r = - 0.04105
p = 0.08014
CD8+ cell/mm3
IFN-gamm pg/ml
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r= - 0.4929
p=0.0012
CD4+cell/mm3
IL-6(pg/ml)

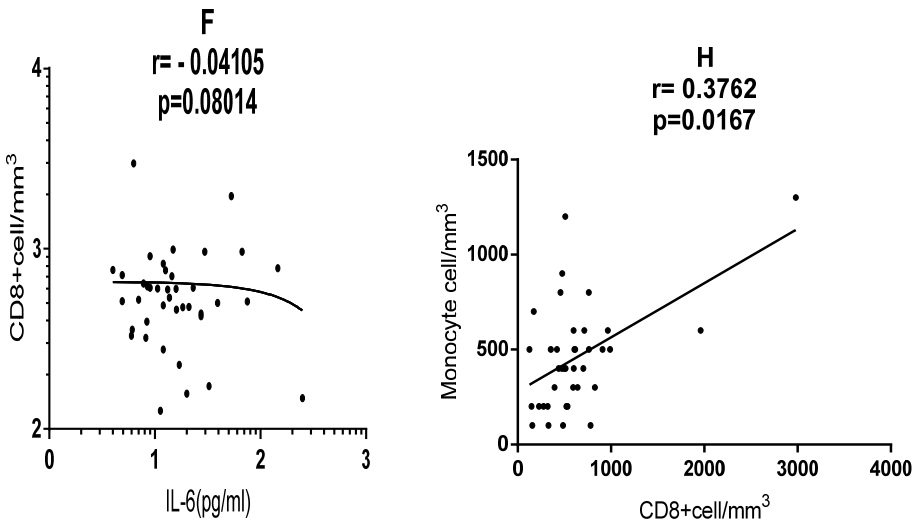

Fig.2 **Correlation of serum level cytokine with peripheral cell in newly diagnosed TB patients (A) IFN-γ with $CD4^+$ cells, (B) IFN-γ with IL-6, (C) IFN-γ with monocyte , (D) IFN-γ with $CD8^+$, (E) IL-6 with $CD4^+$ cells, (F) IL-6 with $CD8^+$ cells (G) IL-6 with monocyte cells and (H) CD8+ cell with monocyte cells.**