Cytokines plasma Levels in Active Tuberculosis and Post Treatment in Sudanese Tuberculosis Patients

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Abbreviations used in this paper: IL-12, Interleukin 12; IL-5, Interleukin 5; IL-10, Interleukin-10 ; ELISA, Enzyme linked Immune Sorbents Assay

Abstract - Background: The production of protective and anti-inflammatory cytokines such as Interleukin 12 (IL-12) , IFN-γ , Interleukin 5 (IL-5) and Interleukin 10 (IL-10) in response to Mycobacterial tuberculosis (M tuberculosis) play important roles to prevent progressive lung disease from developing and eventually death, but failure in immunity to resolve infection depends mainly on the balance and combinations between these cytokines. Therefore, the aims of this study are to determine the plasma level of IL-12 , IFN-γ, IL-10 and IL-5 in Sudanese tuberculosis patients and healthy controls and to compare these values with those obtained from patients on treatment .

Methodology: A case-control study which included 160 tuberculosis patients and 220 healthy matched controls from Sudan. The level of IL-12 , IFN-γ, IL-10 and IL-5 were measured using commercially Enzyme linked Immune Sorbents Assay (ELISA) kits

Result: The mean of INF -γ plasma level in patients with active TB was 103 pg/ml which was significantly lower than the mean of healthy controls (275 pg/ml) (p=0.001), also there were significant differences between untreated patients (mean=105 pg/ml) (p=0.0004), patients on two months of treatment (mean=102 pg/ml) (p= 0.042) compared with healthy controls. There was no significant differences between the mean of the patients on six months treatment (77.6 pg/ml) (p value =0.885) and healthy controls . For IL-12 concentration in patients with active TB was 135.7pg/ml which was significantly higher than healthy controls (mean 65.8 pg/ml) (p value =0.030) . There was no significant differences between the means concentration of untreated (125.21 pg/ml (p value = 0.745) and patients on two months of treatment(154.1 pg/ml) (p value =0.344) and patients on six months of treatment (152pg/ml) (p value =0.360) . The mean of IL-10 concentration in patients with active TB was 32pg/ml which was significantly higher than the mean (6.7 pg/ml) of healthy controls (p= 0.001) , also there were significant differences between the means of untreated patients and patients on two months of treatment compared with healthy controls (mean=39.55 pg/ml) (p=0.0004), (mean=20.00 pg/ml) (p=0.042) respectively and no significant differences on six months of treatment (mean=8.9 pg/ml) (p= 0.5962) . For the mean of IL-5 concentration in patients with active TB was 610 pg/ml which was significantly higher than the mean (563pg/ml) of healthy controls (p=0.0147) . There were no significant differences between untreated patients (mean=608.2pg/ml) (p=0.3984) and patients on two (mean=613 pg/ml) (p=0.0673), months and six months of treatment(mean=617pg/ml) (p= 0.4538) compared with healthy controls

In conclusion: We believe that these cytokines have important roles in the immune response to M tuberculosis and could be used in follow-up as indicators and monitoring the clinical effect of anti-tuberculous treatment.

Key words: Tuberculosis, cytokine, IL-12 , IFN-γ, IL-10, IL-5 and ELISA.

I. INTRODUCTION

Tuberculosis (TB), caused by intracellular organism *Mycobacterium tuberculosis*, remains one of the leading infectious diseases with a high morbidity and mortality in humans [1]. In 2014, according to the world health organization report estimated that 9 million people developed tuberculosis and 1.5 million people died as a consequence of the disease [2].

Most of these cases were found in Africa (4 million new cases), and Sudan one of the African country with high prevalence of tuberculosis [2, 3], the disease is responsible from 19.226 million new cases, equivalent to 151 cases per 100,000 inhabitants and nearly 1.5 million deaths annually [2-4].

Immunity of human to *M. tuberculosis* infection is mediated predominately by Th1 cytokines during the early stage and Th2 cytokines in the later stages of the infection,[5-9]. These cytokines play important roles to prevent progressive lung disease from developing and eventually death [9].The explanation of why immunity fails to resolve infection is depend mainly on the balance and combinations between Th1/ Th2 cytokines [9]. An understanding of the basis of these associations and correlation during TB could be useful in controlling protection/pathogenesis.

IL-12 and IFN-γ are central cytokines in the regulatory and effector phases of the immune response to *M tuberculosis* [8]. INF-y which produced by CD4+ T- lymphocytes and natural killer (NK) cells play an essential role of immunity against *M. tuberculosis* .It used as a marker of potentially protective immunity against *M. tuberculosis* by activating

macrophages to eliminate these intracellular pathogens [8]. It is the most potent cytokine to induce release of toxic nitric oxide from human monocytes/macrophages [10,11]. Orme IM *et al* observed that individuals with IFN-y and IFN-y receptor deficiency were more susceptible to disseminated mycobacterial disease [12].

Further studies have shown that *M. tuberculosis* inhibits the effects of IFN-y by affecting the signaling transcription of IFN-y responsive genes [13].

IL-12 has a central role in T cell mediated responses in inflammation and essential to generation of a protective immune response to *M. tuberculosis* [14,15], with main functions of enhance production of INF-γ by NK cells, dendritic cells and responsible of differentiation of CD4+ T-cell into Th1 capable of creating a protective granuloma.

On the other hand , the production of anti-inflammatory cytokines such as IL-4, IL-5 and IL-10 in response to *M tuberculosis* may down-regulate the immune response and limit tissue injury, but excessive production of these cytokines may result in failure to control the infection [9,16,17].

In the literature most reports on cytokines during TB are from studies on *in vitro* stimulated lymphoid cells or based on animal models with few reports on *in vivo* plasma levels. In the present study we therefore examined the levels of INF-γ, IL-12, IL-5, and IL-10 in the plasma of pulmonary TB patients and healthy controls.

II. MATERIALS AND METHODS

Study population

A prospective, cross sectional, case–control study was carried out during the period between 2015 and 2016 at Abu-Angah Hospital , Khartoum, Sudan. 160 patients with active pulmonary TB and 220 healthy controls were included. Heparinized blood samples were taken from all patients and healthy controls. All tuberculosis patients had microbiological (by culture and/or smear) or radiological evidence of *M. tuberculosis* disease (table 1).

Patients were divided into two groups; the untreated group (n-102) and on treatment group (n-58).Fifty eight patients received daily oral Isoniazid with pyrazinamide (30mg/kg) once a day in combination with rifampicin (10mg/Kg) in initial two months of regimen followed by isoniazid with rifampicin for four months in continuation phase during the study duration.

The healthy controls had no evidence of tuberculosis disease by clinical examination, and were matched on age, gender and BCG status (table 1). The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan. Written informed consents were obtained from all participants in the study. The collected blood samples were tested for other infectious diseases and that included hepatitis B (HBsAg, InTec products, INC, China), hepatitis C (Rapid Anti-HCV Test, InTec products, INC, China), syphilis (RAPIDAN TESTER, product code: RTTP01, Turkey), and HIV (HIV1, 2 Cassete test, Clinotech Diagnostics & Pharmaceuticals, Canada). Plasma samples stored at -20° C until use.

INF-γ, IL-12, IL-5, and IL-10 plasma level:

The plasma level of INF-γ, IL-12, IL-5, and IL-10 were measured using commercially Enzyme linked Immune Sorbents Assay (ELISA) kits according to manufacturer's protocol (Human INF-γ, IL-12, IL-5, and IL-10 ELISA Max ™ Deluxes Set Catalog Number: 430104 for INF-γ, IL-12 and 430704 for IL-5, and IL-10).

Statistical analysis

The significance of differences in INF-γ, IL-12, IL-5, and IL-10 concentrations in plasma were calculated with the Mann-Whitney Test (GraphPad Instat software Inc;La Jolla , CA , USA). P-value of <0.05 was deemed statistically significant. All statistical analyses were performed using SPSS for Windows v11.0 statistical analysis software.

III. RESULTS

Characteristics of tuberculosis patients and healthy control subjects

One hundred and sixty Sudanese tuberculosis patients were included into the study. The diagnosis of tuberculosis was based on the presence of *MTB* in a positive Ziehl-Nielson (ZN) smear of a sputum specimen and/or by positive culture with tuberculosis and radiological evidence (chest X-ray) (table 1).The control population comprised 220 healthy unrelated people from the same endemic area in Sudan, they were matched on gender and BCG-status (table 1) and showed no signs of any lung disease. Unfortunately the occupation of the control population differed from that of the patient population

INF-γ, IL-12, IL-5, and IL-10 plasma level:

Statistical analysis for our data showed that the mean of INF -γ plasma level in patients with active TB was 103 pg/ml which was significantly lower than the mean of healthy controls (275 pg/ml) (p=0.001) (figure 1), also there were significant differences between untreated patients (mean=105 pg/ml) (p=0.0004), patients on two months of treatment (mean=102 pg/ml) (p= 0.042) compared with healthy controls. On the other hand there was no significant differences between the mean of the patients on six months treatment (77.6 pg/ml) (p value =0.885) and healthy controls (figure.2).

IL-12 concentration in patients with active TB was 135.7pg/ml which was significantly higher than healthy controls (mean 65.8 pg/ml) (p value =0.030) (figure 3) . There was no significant differences between the means concentration of untreated (125.21 pg/ml (p value = 0.745) and patients on two months of treatment(154.1 pg/ml) (p value =0.344) and patients on six months of treatment (152pg/ml) (p value =0.360) (figure 4) .

The mean of IL-10 concentration in patients with active TB was 32pg/ml which was significantly higher than the mean (6.7 pg/ml) of healthy controls (p= 0.001) (figure 5), also there were significant differences between the means of untreated patients and patients on two months of treatment compared with healthy controls (mean=39.55 pg/ml) (p=0.0004), (mean=20.00 pg/ml) (p=0.042) respectively and no significant differences between patients on six months of treatment and healthy controls (mean=8.9 pg/ml) (p= 0.5962) (figure 6).

On the other hand the mean of IL-5 concentration in patients with active TB was 610 pg/ml which was significantly higher than the mean (563pg/ml) of healthy controls (p=0.0147) (figure7). There were no significant differences between untreated patients (mean=608.2pg/ml) (p=0.3984) and patients on two (mean=613 pg/ml) (p=0.0673), months and six months of treatment(mean=617pg/ml) (p= 0.4538) compared with healthy controls (figure 8)

IV. DISCUSSION

Immunity of human to *M. tuberculosis* infection is mediated predominately by Th1 cytokines during the early stage and Th2 cytokines in the later stages of the infection [5-9]. The protective immunity to resolve TB infection is dependent mainly on the balance and combinations between Th1/ Th2 cytokines [9]. An understanding of the basis of these associations and correlation during TB could be useful in controlling protection/pathogenesis.

To define the specific profile of cytokines produced in response to tuberculosis, IFN-γ and IL-12 production levels were examined as representative of Th1 responses and those of IL-5 and IL-10 were examined as representative of Th2 responses [18, 19].

In our results we found significantly raised plasma levels of IL-12, IL-5 and IL-10 and low level of IFN-γ in patients with tuberculosis compared with healthy normal controls.

IFN-γ , is a protective cytokine in *M. tuberculosis* infection by activation of macrophages to kill intracellular mycobacteria [21]. Reduced IFN-γ production in *M. tuberculosis* infection may be due to sequestration of IFN-γ producing cells at the site of disease, combined with systemic immunosuppression [22].

In contrast to our results, several studies demonstrated found that high levels of IFN-γ in TB patients compared with healthy controls [23-25]. An explanation for these differences in results may be due to difference in clinical status of the patients. Patients with active localized pleural TB produce high level of IFN-γ. It was supposed that IFN-γ provide protection in this clinical situation [20] .

Studies have reported that mononuclear cells from early-active TB patients can up-regulate pro-inflammatory cytokines, such as IL-12, with main functions to enhance production of INF-γ by NK cells, dendritic cells and responsible of differentiation of CD4+ T-cell into Th1 capable of creating a protective granuloma.

In our study, we have found high plasma levels of IL- 12 in TB patients compared to healthy controls. The increased IL-12 concentration in TB patients when compared with controls may be due to release of IL-12 into circulation during early stages of infection causing systemic symptoms and the levels depend on the clinical status of the patients.

Similar results were obtained in serum from pulmonary tuberculosis patients compared to healthy controls [26, 27]. One study showed no difference in IL-12 concentrations in TB patients and healthy controls [28]. This confliction may be due to the variation in genes distribution among populations. Many global studies done to detect polymorphisms in the genes of IL-12 and INF-γ and their receptors, they had reported the association between TB and gene polymorphisms [29]. We need more future studies to prove this association in our Sudanese population. In our study we found that the completion of the treatment does not affect the cytokines levels .

IL-10 which is a T regulatory cytokine plays a central role during chronic stage of pulmonary TB [30]. The IL-10 production is high during the infection promoting reactivation of TB. The excessive production of this cytokine results in failure to control the infection.

Our results showed that the levels of IL-10 and IL-5 were significantly higher in TB patients compared with control. IL-5 was previously reported to elevate in TB patients compared with control [31-33]. On the other hand many studies have reported the increased production of IL-10 in patients with active disease [34- 37]. There are few reports of elevating levels of IL-10 in plasma of the contacts compared to patients [38].The association of the high level of IL-10 and tuberculosis infection was also found in study done in Sudanese TB patients compared with control after *in vitro* stimulation of whole blood [3]. A Taiwan study described the high IL-10 production in patients compared to controls [39]. The high IL-10 and IL-5 levels in patients , suppresses immune response leading

to inadequate balance of pro and anti- inflammatory cytokines.

In our study, the possible effect of treatment on plasma IL-10 and IL-5 levels in TB patients were evaluated. We observed a statistically decreasing of IL-10 level during treatment. The IL-10 level in untreated TB patients and 2 months on treatment show significant differences when compared with healthy controls .This finding indicates that the IL-10 cytokine reduction is due to decreased bacterial load by treatment, and pervious study noted high *Mycobacterium tuberculosis* load associated with high IL-10 levels [36]. Similar studies showed decreased level of IL-10 in TB patient on treatment compared with healthy control [34,35, 37]. However, another study noted that IL-10 levels remain high at 2 months post therapy then decrease after 4 and 6 months post therapy [36].

IL-5 levels in our result showed no significant differences in 2 and 6 months of treatment compared with healthy controls.

In conclusion, measurement the plasma levels of several cytokines may be useful for evaluating the activity of TB disease and monitoring the clinical effect of antituberculous treatment. Further studies are needed to address the role of cytokines in immunity to TB under natural conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

V. ACKNOWLEDGMENT

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Table 1: Characteristics of the Study population

		Patients	Controls	p-value
Total number		160	220	
Mean age /yrs (range)		26 (11-70)	30 (11-70)	
Gender (male/female)		111/49	65/155	0.0041
Occupation	Governmental employee	22 (13.8)	21 (9.55%)	
	Workers	63 (39.4%)	44 (20%)	
	Other job	40 (25%)	40 (18.3%)	
	Jobless	3 (1.8%)	13 (5.9%)	
	Housewife	13 (8.1%)	17 (7.7%)	
	Student	19 (11.9%)	85 (38.6%)	
BCG vaccination		112 (70.6%)	212 (96.8%)	0.923
Definite tuberculosis	Presence of MTB in sputum based on both smear and culture	92(57.5%)	0 (0%)	
	Presence of MTB in sputum specimen only by smear	109(68.1%)	0 (0%)	
	Presence of MTB in sputum specimen only by culture	47 (29.4%)	0 (0%)	
Hepatitis C test		Negative	Negative	
Hepatitis B Ag test		Negative	Negative	
HIV1, 2 test		Negative	Negative	

Legends to figures:

Figure 1:

Shows the mean concentration of the Plasma INF-γ level pg/ml in TB patients (n=160, mean = 103 pg/ml) and healthy normal controls (n=220 , mean= 275 pg/ml) (p value =0.001)

Figure 2:

Shows the mean concentration of plasma INF-γ level in controls (275 pg/ml) , untreated (105 pg/ml) (p=0.0004), and on treatment(2 months=102 pg/ml (p= 0.042),6 months=77.6 pg/ml) (p value 0.885)

Figure 3:

Shows Plasma levels of IL-12 in TB patients (n=160 ,mean= 135.7pg/ml) and normal healthy controls(n=220 ,mean =65.8 pg/ml) p value 0.030.

Figure 4:

Shows the mean concentration of plasma IL-12 level in controls (65.8 pg/ml) ,untreated(125 pg/ml) (p value = 0.745) and on treatment(2 months=154.1 pg/ml (p value =0.344) ,6 months=152 pg/ml) (p value 0. 360).

Figure 5:

Shows the mean concentrations of plasma IL-10 levels (pg/ml) determined in patients (n-160) (32pg/ml) and healthy normal controls (n -220) (6.7 pg/ml) (p=0.001).

Figure 6 :

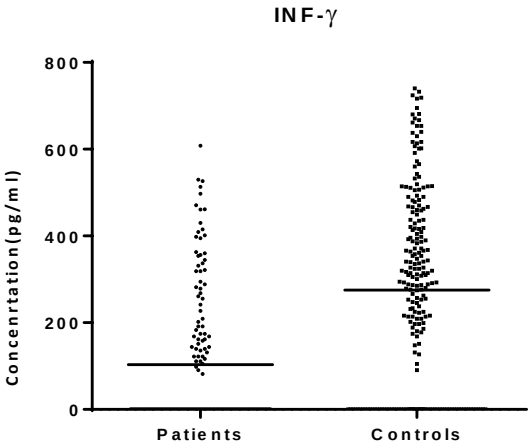
Shows the mean concentrations of plasma IL-10 levels (pg/ml) determined in healthy controls (n-220), and untreated patients(mean=39.55 pg/ml) (p=0.0004) and patients on 2 months(mean=20.00 pg/ml) (p=0.042) and 6 months of treatment(mean=8.9 pg/ml) (p= 0.5962) (n-160)

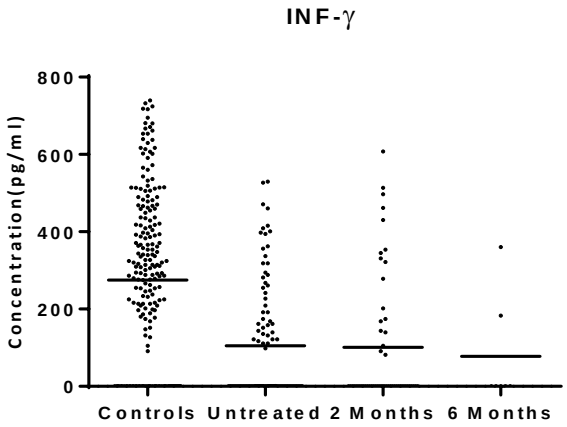
Figure 7:

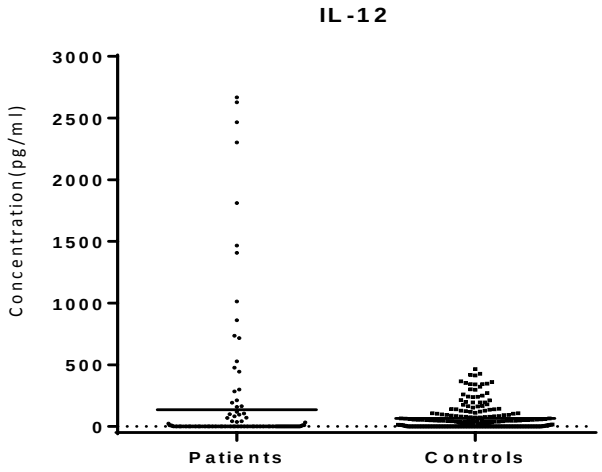
Shows the mean concentrations of plasma L-5 levels (pg/ml) determined in patients (610 pg/ml)(n-160) and healthy normal controls (n -220) (563pg/ml) (p=0.0147).

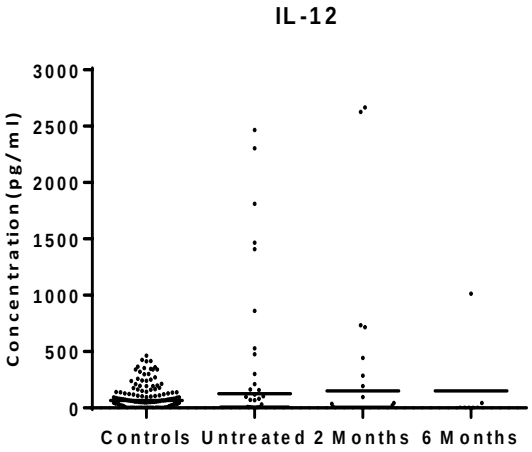
Figure 8:

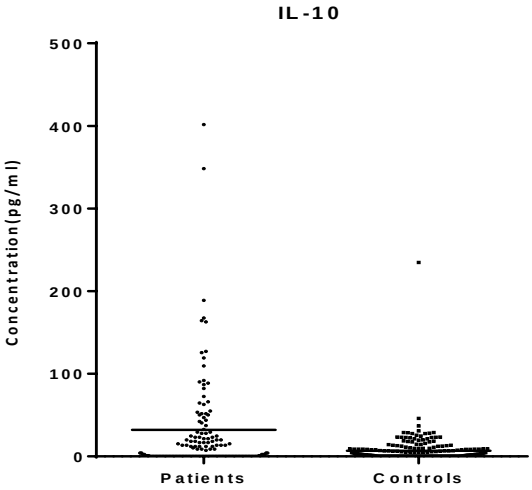
Shows the mean concentrations of plasma IL-5 levels (pg /ml) determined in healthy normal controls (220), untreated patients (mean=608.2pg/ml) (p=0.3984) and patients on treatment (two months (mean=613 pg/ml) (p=0.0673)and 6 months(mean=617pg/ml) (p= 0.4538)) *(n*-160).

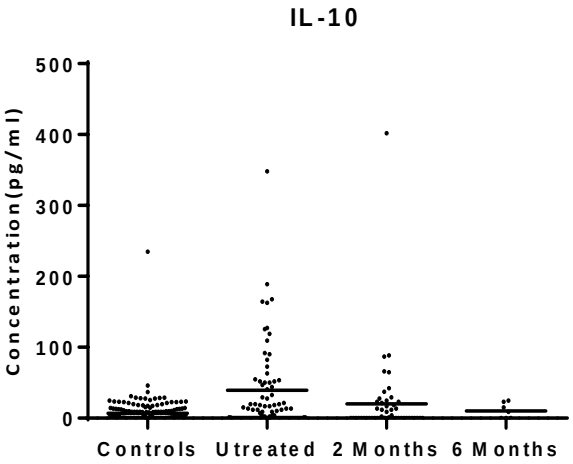


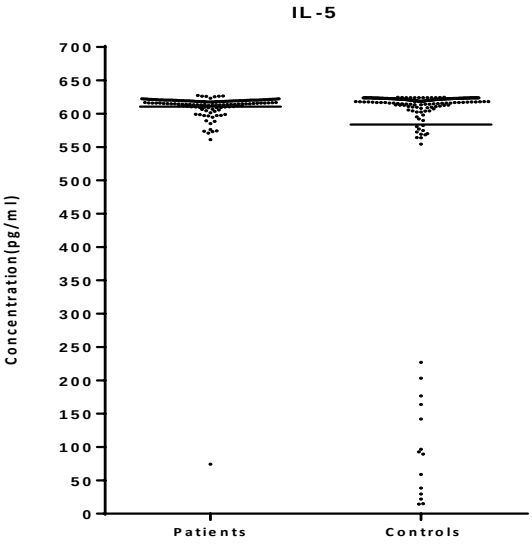


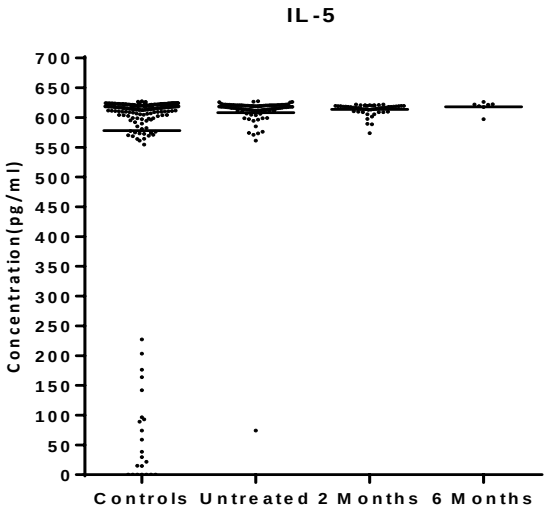





IL-10
Concentration(pg/ml)
500
400
300
200
100
0
Patients
Controls


IL-10
Concentration(pg/ml)
500
400
300
200
100
0
Controls
Utreated
2 Months
6 Months


IL-5
Concentration(pg/ml)
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Patients
Controls


IL-5
Concentration(pg/ml)
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Controls
Untreated
2 Months
6 Months