Tumor necrosis factor-α, Interleukin-10, and oxidant/ antioxidant status in patients with pulmonary tuberculosis: Effect therapy, a follow up study

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الخلاصة

لتقييم حالة الاكسدة / مضادات الاكسدة (ممثلا بالمالونديليهايد وحالة مضادات الاكسدة الكليه) عند التشخيص وخلال فترة اسبوعين على مدى الشهرين الاولين من العلاج مع قياس مستوى عامل النخر الورمي-الفا ، والانترليوكين-10قبل بدء العلاج ومع نهاية فترة العلاج المركز للشهرين الاوليين في مرضى التدرن الرئوي المشخصين حديثا بالمقارنة مع مجموعة الضبط من الاصحاء.

من بين 52 مريضا تمت مقابلتهم وفحصهم فقط 46 مريضا شاركوا في هذه الدراسة. في البداية تم سحب عينة دم ممن المرضى وتم قياس مستوى المالونديليهايد، مضادات الاكسدة الكلية، عامل النخرالورمي-الفا والانترليوكين -10، بعدها تم وضع المرضى على العلاج المركز لمدة شهرين مكون من 4 حبات من دواء رايمستار (حبة بجرعة ثابتةمن الايزونيزايد ،الريفامبسين، البيرازين امايد والايثانبيوتول) يوميا وخلال فترة العلاج وبفترات اسبوعين تم سحب عينات دم من المرضى لتحديد مستوى المالونديلهايد ومضادات الاكسدة الكلية وبنهاية الفترة المحددة والمقترحة للدراسة تم قياس مستوى المالونديلهايد ومضادات الاكسدة الكلية مع مستوى عامل النخر الورمي-الفا والانترليوكين-10 . ادخل الى هذه الدراسة ايضا 46 من الاشخاص الاصحاء، غير المدخنين من اعمار واجناس مقاربة لمجموعة المرضى اتخذوا كمجموعة ضبط بالنسبة للفحوص المختبرية الاولية للمالونديلهايد، مضادات الاكسدة الكلية، مستوى عامل النحرالورمي-الفا والانترليوكين-10 . بالنسبة لمجموعتي المرضى والضبط تم حساب معدل كتلة الجسم باستخدام معادلة خاصة.

كانت هناك زيادة معنوية في مستويات المالونديلهايد، مستوى عامل النخرالورمي-الفا والانترليوكين -10 مع انخفاض معنوي في مستوى مضادات الاكسدة الكلية في مرضى التدرن الرئوي في مرحلة ماقبل العلاج بالمقارنة مع مجموعة الضبط من الاصحاء. خلال العلاج كان هناك ارتفاعات تدريجية في مستوى مضادات الاكسدة الكلي وانخفاضا تدريجيا في مستوى المالونديلهايد كل اسبوعين خلال فترة العلاج الاولي المركز في الشهرين الاوليين. في نهاية الفترة المقترحة في هذه الدراسة كان هناك انخفاضا معنويا في مستويات المالونديلهايد عامل النخرالورمي -الفا والانترليوكين -10، مع زيادة معنوية في مستوى مضادات الاكسدة الكلية بالمقارنة مع النتائج قبل بدء العلاج.

التدرن الرئوي الحاد يكون مصحوبا بارتفاع في جهد الاكسدة مع تغيير في مستوى بعض السايتوكاينز وان العلاج الاولي المركز لفترة شهرين كان مصحوبا بتقليل جهد الاكسدة (منعكسا بانخفاض المالونديلهايد وارتفاع مستوى مضادات الاكسدة الكلية) وانعكاس التغيرات الحاصلة في مستويات عامل النخرالورمي -الفا والانترليوكين -10، من مجموعة السايتوكاينز الحادثة بسبب المرض.

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Abstract

To evaluate the oxidant / antioxidant status (represented by serum malondialdehyde"MDA"and total antioxidant status "TAS") at diagnosis and then at two-weeks intervals during the first two months intensive therapy, with the levels of tumors necrosis factor-α (TNF-α) and interleukin-10 (IL-10) before starting therapy and by the end of the 2 months therapy in newly diagnosed patients with pulmonary tuberculosis (PTB) in comparison to healthy controls.

Out of 52 patients interviewed and examined, only 46 patients with active PTB consented to participate in this study. Initially blood sample was taken and assay of serum MDA, TAS, TNF-α and IL-10 was done. Then patients were put on intensive 2 months therapy as 4 tablets of Rimstar ® (a fixed dose tablets containing isoniazid, rifampicin , pyrazinamide and ethambutol) daily. During therapy and at 2 weeks intervals, blood samples were taken and assay of serum MDA and TAS were done till the end of suggested period of the study, where assay of TNF-α and IL-10 also have been done. Included in this study also 46 healthy, non-smoker, age and sex-matched volunteers as a control group for the initial laboratory results of MDA, TAS, TNF-α and IL-10. For both patients and controls, calculation of the body mass index (BMI) was done using especial equation.

There was a significant increase in the serum levels of MDA, TNF-α and IL-10, with a significant decrease in the TAS in patients with PTB in the pre-therapy stage in comparison to healthy controls. During therapy, there was a gradual increase in TAS and a gradual decrease in MDA at the 2 weeks intervals till the end of the intensive 2-months therapy. By the end of suggested period of the study, there was a significant reduction in the serum level of MDA, TNF-α and IL-10 with a significant increase in TAS levels, in comparison to the pre-therapy stage.

Acute PTB was associated with an oxidative stress and changes in some cytokines levels and the initial 2 months intensive therapy was associated with a reduction in oxidative stress (as reflected by reduction in MDA and an increase in TAS levels) and reverse the changes in TNF-α and IL-10 levels induced by the disease.

Key words: Pulmonary TB, MDA, TAS, TNF-α, Il-10.

Introduction

Mycobacterium TB is one of the most widespread pathogens, it is estimated roughly that one third of the world's population is infected with it. It is responsible for 5-12 million cases of active TB each year, and about 3 million deaths [1,2].

The interaction of T-cells with infected macrophages is a critical factor of protective immunity against the bacteria, and the cytokines produced by these cells are important mediators of the immune and inflammatory responses [3]. When TB is in activity we can observe a decrease in the T helper 1 (Th1) response, together with an increase in the production and activity of T helper 2(Th2) cytokines [4]. These cytokines exert important roles to limit or exacerbate the disease depending on their balance and combination. An understanding of the basis of these associations and correlation during TB could be useful in elucidating protection and pathogenesis [5]. PTB is associated with increased oxidative stress as indicated by enhanced lipid peroxidation products. Mycobacteria are capable of inducing reactive oxygen species (ROS) production by activity both mono-nuclear and polymorphonuclear phagocytes that possess antimicrobial activity. The enhanced level of free radical production, although designated to combat the invader, has the potential to damage the host. However, host tissue damage is limited by the concurrent enhancement of the antioxidant defenses of the host. In TB patient there is also a suggestion of poor antioxidant defense that may expose to oxidative host tissue damage [6].The aim of this study was to evaluate the oxidant/ antioxidant status in a newly diagnosed patient with PTB, at diagnosis and at two weeks intervals during the initial intensive two months anti-TB therapy, with assessment of some cytokines levels (tumor necrosis factor -α (TNF-α) and interlukin-10 (IL-10))at diagnosis and by the end of the intensive therapy in comparison to healthy controls.

Subjects and methods

The study was conducted with the approval of the regional research of Mosul Health Administration, at the Advisory Clinic for chest and respiratory diseases at Al-Faisalyah, during the period from January 2009 to May 2011.

Out of 53 patients interviewed and examined, only 46 patients with active PTB consented to participate in this study and followed for the initial 2 months intensive therapy with anti-TB therapy. The inclusion criteria to this study include, typical symptoms of PTB, fibrocavitary lung inflitrate on chest radiograph and at last one sputum specimen staining positive with Ziehl Neelsen for acid-fast bacilli, being non-smoker, with no history of other illnesses (cardiovascular, renal, hepatic or diabetes mellitus) and on no drug therapy. Additional criteria for female patient being not-pregnant, not lactating.

Control subjects

Forty six apparently healthy volunteers, non-smoker with no previous history of PTB, recruited as controls to establish nominal values of serum MDA, TAS, TNF-α, Il-10.

For both the patient and the controls, BMI were calculated as follows:

BMI = Body weight (Kg) / Height (m^2) [7]

In patient group after diagnosis, blood samples were taken with assay serum MDA, TAS, TNF-α, IL-10. then they were put on 4 tablets / day of Rimstar ® (a fixed dose tablet containing isoniazid 75 mg, rifampicin 150 mg , pyrazinamide 400 mg and ethambutol) to be swallowed as a single dose in the moving before breakfast for the initial 2 months with vitamin B6 capsule 10 mg daily. Patient were seen at 2 weeks intervals,through which a blood samples were taken and assay of serum MDA, TAS were done. By the end of the suggested period of the study another blood samples were taken with assay of serum MDA, TAS, TNF-α, Il-10 were done.

Methods

1. Serum malondialdelyde (MDA) were estimated using thiobarbituric acid (TBA) assay (Buege and Aust, 1978) [8].
2. Serum total antioxidant status (TAS) were measured according to the method described by Miller et al (1993) [9]using a kit from random laboratories UK.
3. Serum TNF-α, IL-10 were measured by ELISA following the manufacturer's instructions on a commercial kit applied from (Metgenix, Biolource International, USA).

Statistical Analysis

Standard statistical methods were used to determine the Mean Standard Deviation (SD), minimum and maximum values. Unpaired t-test were used to compare the results of various biochemical parameters of patients with PTB and the controls. Paired t-test were used to compare the results of various biochemical parameters in patient with PTB in the pre-therapy and post-therapy stages. 95% confidence intervals (CI) were used with regard mean values of MDA, TAS, TNF-α, IL-10. P value ≤ 0.05 was considered to be significant[10].

Results

Table (1) shows the characteristics of the patients and the controls. By comparing mean values and 95% CI of the mean values of parameters under study, there was a significant differences between patients with PTB in the pre-therapy stage and the controls (Table 2).

Figure 1and 2 shows the 95% CI for the serum TAS and MDA respectively in patients with PTB in relation to the progress in therapy at 2 weeks intervals.

There was a significant reduction in the mean serum MDA, TNF-α and IL-10 with a significant increase in the serum levels of TAS in patients with PTB in the post-therapy stage in comparison to the pre-therapy stage (Table 3).

Table 1: The characteristic of patients with pulmonary tuberculosis and the controls.

Parameters	Controls	Patients	P-value
No.	46	46	NS
Age (years)	40.15 ± 10.35	38.83 ± 8.07	NS
BMI	23.10± 1.62	21.60±1.38	NS
ESR mm/hr	9± 5.37	60.46± 14.12	S
Male	38	38	NS
Female	8	8	NS

NS: non-significant differences at $p \leq 0.05$
S: significant differences using Unpaired t-test.

Table 2: Comparison of mean values and 95% CI of measured parameters between patients in the pre-therapy stage and the controls.

Parameters	Patients		Control		P-value
	Mean (SD)	95% CI of mean	Mean (SD)	95% CI of mean	
IL-10(pg/mL)	5.89 (0.89)	5.61-6.17	3.63 (0.87)	3.37-3.89	<0.0001
TNF-α(pg/mL)	57.52 (7.22)	55.24-59.80	40.57 (3.13)	39.64-41.50	<0.0001
TAS(mmol/L)	1.09 (0.17)	1.04-1.14	1.86 (0.18)	1.81-1.91	<0.0001
MDA(µmol/L)	2.05 (0.20)	1.99-2.11	1.10 (0.30)	1.01-1.19	<0.0001

Using Unpaired t-test.

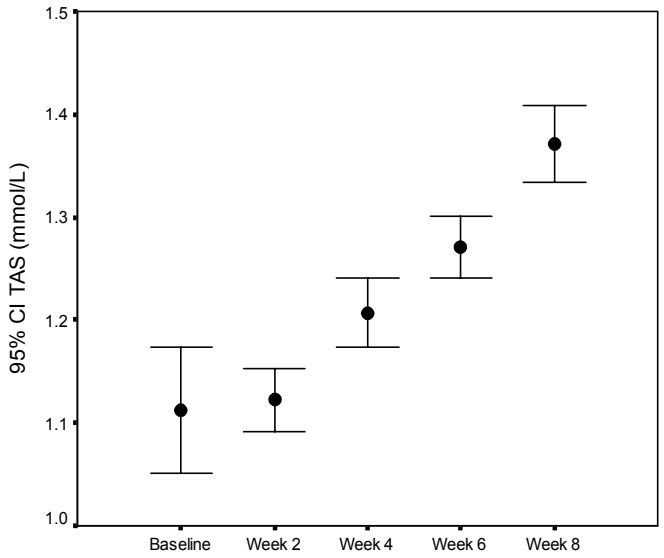

Figure 1: 95% CI of mean serum TAS levels at 2 weeks intervals during the first 2 months initial therapy.

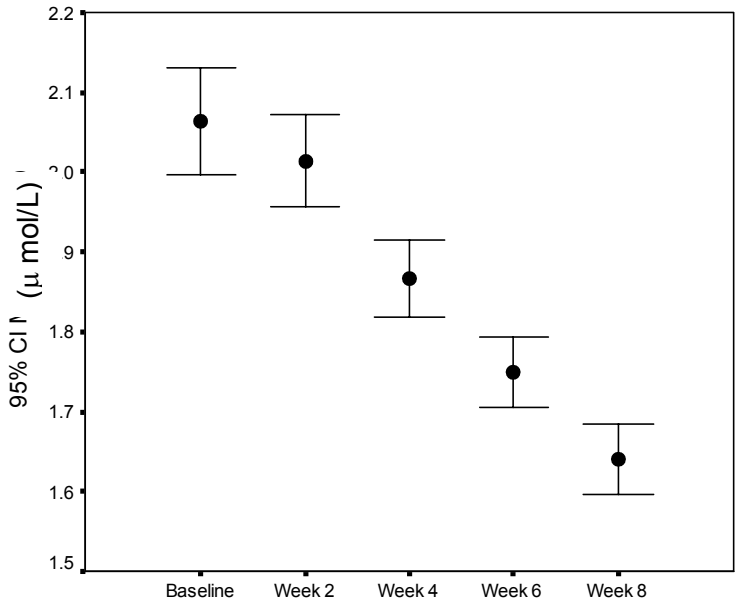

Figure 2: 95% CI of mean serum MDA levels at 2 weeks intervals during the first 2 months initial therapy.

Table 3: Comparison of mean values and 95% CI of measured parameters between patients in the pre-therapy and post-therapy stages.

Parameters	Before treatment Mean (SD)	After treatment Mean (SD)	Mean difference	P-value
IL-10(pg/mL)	5.89 (0.88)	3.79 (1.04)	2.1000	<0.0001
TNF-α(pg/mL)	57.42 (7.16)	49.83 (4.60)	7.5905	<0.0001
MDA(µmol/L)	2.06 (0.21)	1.64 (0.13)	0.4238	<0.0001
TAS(mmol/L)	1.11 (0.19	1.37 (0.12)	-0.2590	<0.0001

Using Paired t-test.

Discussion

1. Effects of PTB and anti-TB therapy on serum levels of TNF-α and IL-10.

While comparison of chest radiographs provides an accepted strategy for monitoring the efficiency of therapy, alternative immune–based assays would provide insights into the effectiveness of the antibacterial regimen and the host's immune response[11].

This study revealed a significant differences in the mean values of serum TNF-α and IL-10 levels in newly diagnosed patients with PTB in the pre-therapy stage in comparison to healthy controls, and significantly decreased by the end of the intensive two months therapy in comparison to pre-therapy values, but it dose not reach control values.

TNF-α is produced by macrophages, monocytes and dentritic cells in response to mycobacteria or its products. It has immuno-regulatory properties and brings about the activation of macrophages granuloma foramation, T-cell stimulation and regulation of chemokine induction, thus controlling infections[12]. There is evidence to suggest that TNF-α is necessary at the beginning of the inflammatory process in order to limit the multiplication of mycobacteria[5]. High levels of TNF-α were reported in chronic TB accompanied by the evaluated release of TNF-α receptors resulting in fever, necrosis and weight loss. This implies that TNF-α has both protective and immunopathologic effects[13].

This study results were in agreement with many research workers, who reported that TNF-α levels were high in the pleural fluid, plasma or monocyte culture of patients with TB before or after the onset of the treatment , when compared with controls [5,14]. Going with this study results Sahiratmadja et al [15] who reported that the high TNF-α initial levels in patients with PTB decreased significantly during treatment, with the inflammatory process decreasing at the same time.

Peresi et al [4], also in agreement with this study reported that TNF-α production by monocytes was lower in the controls than in patients with PTB, before therapy, after 3 months of therapy and at the end of therapy. Although cytokine production in the patients was always higher than in the controls, it decreased significantly during the treatment and the final levels were lower than the baseline levels.

With regard serum IL-10 levels, the production of such anti-inflammatory cytokines in response to mycobacterium TB may down-regulate the immune response and limit tissue injury, but excessive production of such cytokines may result in failure to control the infection[16]. Ellner [17]reported that the levels of the anti-inflammatory cytokine IL-10 are higher in cases with TB. This effect is useful, as it reduces the pro-inflammatory activity of TNF-γ and TNF-α as well as providing some protection against Th1 profile-induced tissue destruction, and although IL-10 levels are higher during the phase of great activity of the inflammatory process (pre-treatment) than during or after the end of the specific therapy, these levels always remain above normal during treatment[18].

In contrast to the results of this study, Moura et al [19], reported that high initial production of IL-10 in patients with PTB remained unchanged with the treatment, while Sahiratmadja et al [15] reported that cytokines levels were found to be equivalent to those of the control group at the end of therapy.

2. Oxidant / antioxidant status

This study revealed increased serum MDA and reduced serum TAS levels in newly diagnosed patients with PTB , with a gradual reduction in MDA levels and elevation in TAS levels every two weeks during the intensive 2 months therapy.

In agreement with this study results, Reddy et al., [20]. They concluded that in TB patients, free radical activity is quite high and antioxidant levels are low. Kaur et al., [21] also reported that there is oxidative stress and decreased antioxidant activity in patients with PTB (as reflected by increased MDA levels and reduced vitamin C, glutathione and superoxide dismutase). Going with this study result, the study conducted by Mohod et al [22]. They concluded that the levels of vitamin C as an antioxidant is significantly reduced with significant elevation in lipid peroxidation levels in patients with PTB.

With regard effect of therapy Plit et al., [23], reported that even after 6 months of successful chemotherapy, PTB is still associated with increased levels of circulating lipid peroxides and low plasma concentration of vitamin E.

In an attempt to kill mycobacteria, host cells (namely macropahes, neutrophiles and monocytes) generate huge amount of ROS. One of the manifestations of these ROS is lipid peroxidation. These high levels of ROS are often cytotoxic and may cause host tissue damage. Malnutrition is more common in patients with PTB than in the healthy subjects and could possibly explain the low levels of antioxidant potential. Furthermore, patients with TB are unable to produce sufficient of antioxidant to cope with their increased oxidative stress [20].

Since measurement of a single antioxidant parameter will not necessarily reflect the true free radical burden in patients with PTB [24,25] and as the levels of individual antioxidant might be affected to a different levels in a disease state for example Plit et al., [23] reported that on diagnosis of TB, plasma concentrations of vitamin C and B- carotene are low returning to normal values after chemotherapy, while vitamin E levels remain low throughout. This is why in this study measuring TAS was the choice , since it

represent the net result of the protective effect of antioxidant such as antioxidant enzymes, small molecule antioxidant (glutathione) and dietary antioxidant micronutrient.

In this study, serum TAS was observed to be low in patients with PTB, this could be due malnutrition or exhaustion in an attempt to neutralize the heavy load of free radical in these patients.

In support to this study results, Wiid et al., [26]. They reported that after one week of anti –TB therapy, the TAS levels of patients with PTB raised and they relate the increase in TAS levels to rifampicin therapy, since rifampicin has been shown to be a scavenger of free radicals [27]. But in contrast to this study results, they reported that the initial raise in TAS levels, was followed by a drop in its level by the second to the 4th week during therapy in a one month follow up study.

In agreement with this study, Lamsal et al., [28]. They reported elevated MDA and nitrite levels with concomitant depressed vitamin C and E levels as an indications of lipid peroxidation and oxidative stress, and a decease in MDA and nitrite levels with subsequent increase in vitamin C after 2 months of follow up indicate a good response to treatment with standard anti-TB therapy.

In this study, there was a gradual decrease in MDA levels and gradual increase in TAS levels every two weeks during the initial 2 months therapy. This might through a light about a possible supporting role of antioxidant therapy as an aid to the anti-TB therapy as suggested by Seyedrezazadeh et al., [29] who reported that a 2 month supplementation vitamin E and selenium therapy will reduce oxidative stress and enhances TAS levels in patients with TB treated with standard chemotherapy. This might be the first study with regard following newly diagnosed patients with pulmonary tuberculosis, for oxidant/antioxidant levels at two weeks intervals and TNF-α at the start and by the end of the intensive 2-months therapy.

In conclusion: PTB was associated with elevation in some cytokines levels (TNF-α and IL-10) and oxidative stress (as indicated by the increase in MDA levels and a reduction in TAS

levels) and the standard initial 2 months therapy was associated with reduction of TNF-α and Il-10 with a reverse of the oxidative stress.

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