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RESEARCH ARTICLE

STUDY OF SOME CYTOKINES IN SOME EGYPTIANS ADDICTED TO SOME COMMON STUFFS

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ABSTRACT

Background: Addiction is a complex disease, with physical, mental, social, and environmental factors. To be successful, treatment plans must address all these components. In some cases, hospitalization may be required.
Aim: The present study aimed to evaluate some cytokines in subject with addiction history to evaluate the effect of addiction on immune system.
Methods: This study was performed on 73 adult subjects and classified into 4 main groups, control, benzodiazepine addicted patients group (ABT group), opiate addicted patients before treatment group (AO before group) and opiate addicted patients after treatment with quietapine group (AO after group), that not suffer from diabetes, renal failure and liver disorder to exclude any inflammation. The four groups are within the age of (20-35) years and are all male to exclude female hormonal disturbance. In the study nine parameters were measured in the serum of both patients and healthy control group. These parameters were, Platelet derived growth factor (PDGF).Tumor necrosis factor (TNF), C-reactive protein (CRP), protein C, (Alanine amino transaminase)(ALT), (Aspartate amino transaminase) (AST), Creatinine, Urea and Glucose.
Results: For serum PDGF in the addiction dependent period, the results showed that the mean ± SEM of serum TNF was significantly lower in AO before treatment in the addiction dependent period (191.4 ± 12.2) and ABT in the addiction dependent period (268.6 ± 30.5) (pg/ml) as compared to normal (539.9 ± 72.1) and AO after treatment (403.8 ± 60.1) (pg/ml) groups, for serum PDGF in withdrawal period The results showed that the χ^{-} ± SEM of serum PDGF was significantly higher in AO before treatment in withdrawal period (865.0 ± 37.9) and ABT in withdrawal period (1243 ± 139.1) (pg/ml) groups as compared to normal (539.9 ± 72.11) and AO after treatment (403.8 ± 60.10) (pg/ml) groups Also, for serum TNF in the addiction dependent period the results showed that the mean ± SEM of serum TNF was significantly lower in AO before treatment in the addiction dependent period (191.4 ± 12.2) and ABT in the addiction dependent period (268.6 ± 30.5) (pg/ml) as compared to normal (539.9 ± 72.1) and AO after treatment (403.8 ± 60.1) (pg/ml) groups, for serum TNF in withdrawal period The results showed that the mean ± SEM of serum TNF was significantly higher in AO before treatment in withdrawal period (75.15 ± 11.4) and ABT in withdrawal period (268.6 ± 44.2) (pg/ml) groups as compared to normal (24.4 ± 3.7) and AO after treatment (30.8 ± 5.9) (pg/ml) groups. And, C-reactive protein, protein C, ALT, AST, creatinine, urea and glucose were within normal range in both groups.
Conclusion: The present study suggested that serum PDGF may be a useful marker in aiding diagnosis of immunodeficiency and serum TNF α may be a good marker for immunodeficiency disease. There is correlation between benzodiazepine level, opiate level and cytokine level. So we can use cytokine level as supportive parameter in patient who has no clear history about drug dose and addiction duration. Obtaining high cytokine level in the beginning of treatment allow physicians to put patient in the correct protocol of treatment. More studies should be performed to discover new markers for potential diagnosis of immunodeficiency in early stages, more studies on serum PDGF, TNFα and it's relation with benzodiazepine or opiate level.

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INTRODUCTION

Addiction is a state characterized by compulsive engagement in rewarding stimuli, despite adverse consequences (Mokdad *et al*., 2004). Benzodiazepine addiction causes anxiety, panic attacks, odd sensations, feelings of being outside the body and feelings of unreality, or confusion.

Sometimes the withdrawal symptoms are similar to the original anxiety symptoms (Malek and Bayer, 2004; Haddad *et al*., 2004; Jump up *et al*., 2006; Jump up *et al*., 2008). Opiate addiction is one of the most physically difficult addictions to treat, which means that an opiate rehab program equipped to provide you with both medical car and psychological support is essential. Opiate painkiller addiction, heroin addiction and methadone addiction are the three most common types of

opiate addiction and all three require opiate detox and addiction treat (Marx *et al*., 2014). Immunity is the ability to resist infection by an invading pathogen. The body quickly launches an immune response and prevents the symptoms of disease occurring. This can happen in two ways, naturally or artificially (Carapetis Jonathan *et al*., 2009). Cytokines can be used as markers of immunity in cross-section studies. Laboratory markers may be found to be more abnormal in advanced disease than in less advanced and asymptomatic infection (Najib *et al*., 1998). Cytokines play key roles in all immune responses, and offer important new avenues to explore, both in terms of mechanistic understanding of immunotoxicity and of developing new assays to test the immunotoxic potential of the novel compounds (Corsini and House, 2010). Effects on cytokines can be analyzed at the protein level, including direct detection of the relative concentration of various cytokines in the circulation following experimental treatment, and in activated peripheral blood mononuclear cells or diluted whole blood (Galbiati *et al*., 2010). Cytokines generally act primarily at a local level and that they are rapidly cleared from the circulation. Second, cytokines are extremely potent mediators that are active at very low concentrations and expressed only following cellular activation. Finally, for the detection of cytokines in activated peripheral blood mononuclear cells or diluted whole blood, the isolation and culture procedures may wash out important immune molecules and xenobiotics, and disrupt molecular networks between immune cells (Foster, 2001). Thus, the practical advantages of detection of cytokine levels in stimulated whole blood make it attractive for immunotoxicity screening purposes (House, 1999). The effects of different lymphocyte stimulants on cytokine production had been evaluated in clinical research, there may be differences between species (Schuerwegh *et al*., 2003).

MATERIALS AND METHODS

This study was performed on 73 adult subjects and classified into 4 main groups:

Group 1 (healthy control)**:** This group contains fifteen normal healthy adults from the community through announcing (20-34) years. The mean age of these patients was 27 years.
Group 2 (ABT patients)**:** This group contains twenty four patients. The mean age of these patients was 27.5 years with a range of (20- 35) years.
Group 3 (AO patients before treatment)**:** This group contains twenty four patients. The mean age of these patients was 27.5 years with a range of (20-35) years and
Group 4(AO patients after treatment with quetiapine)**:** This group contain ten patients, the mean age of these patients was 27.5 years with a range of (20- 35) years. Patients were selected from Ain shmas hospital, psychiatric department (Ain shamas University Hospital). Patients in group2 and group 3 are diagnosed as addicted immunodeficient patients with opiate or benzodiazepine level assay and complete blood picture. Full history was taken for all patients including personal history, smoking and working media. All addicted patients were newly diagnosed cases, did not receive any medication.

Sampling: The study was performed on control, AO before, ABT and AO after groups that not suffer from diabetes, renal failure and liver disorder to exclude any inflammation. The four groups are within the age of (20-35) and are all male to exclude female hormonal disturbance. Five ml of venous blood samples were withdrawn by BD vaccutainer system from patients and control. Blood samples were allowed to clot for 30 minutes and centrifuged at 4000 r.p.m. for 15 minutes and sera were separated into aliquots and stored at- 80̊ C till the time of analysis.

Laboratory investigations: In the study nine parameters were measured in the serum or plasma of both patients in both the addiction dependent period and after 28 days in withdrawal period and healthy control group. These parameters were, Platelet derived growth factor (PDGF), Tumor necrosis factor (TNF), C-reactive protein, Protein C, (Alanine amino transaminase) (ALT), (Aspartate amino transaminase) (AST), Creatinine, Urea and Glucose. Determination of (PDGF), (TNF) and Protein C was meassured using Enzyme linked immunesorbent assay method (ELISA) by Statfax 2100 analyzer (Warness technology, USA). Determination of ALT, AST, C-reactive protein, Creatinine, Urea and Glucose was measured using spectrophotometer evo100 (thermo-scientific, England).

Statistical analysis: Data of each patient was collected in a special file then it was coded and fed to the computer on windows7 worksheet version 5 for statistical analysis using graphpad prism version 6.01. Descriptive statistics were done including mean and standard error of mean.

RESULTS

Table (1): biochemical characteristics of ABT and AO before groups vs. AO after and control groups revealed that ABT and AO before groups were not statistically significant in age from AO after and control groups ($P > 0.05$ for each).

Table (1): Significant Correlations in serum PDGF concentration in the addiction dependent period between ABT group and each of AO after, control groups revealed that there were significant difference in serum PDGF concentration between ABT group and AO after, control groups ($P < 0.05$).

Table (1): Significant Correlations in serum PDGF concentration in the addiction dependent period between AO before group and each of AO after, control groups revealed that there were significant difference in serum PDGF concentration between AO before group and AO after, control groups ($P < 0.05$).

Table (1): Significant Correlations in serum PDGF concentration in withdrawal period between ABT group and each of AO after, control groups revealed that there were significant difference in serum PDGF concentration between ABT group and AO after, control groups ($P < 0.05$).

Table (1): Significant Correlations in serum PDGF concentration in withdrawal period between AO before group and each of AO after, control groups revealed that there were significant difference in serum PDGF concentration between AO before group and AO after, control groups ($P < 0.05$).

Table (1): Significant Correlations in serum PDGF concentration in withdrawal period between ABT group and AO before group revealed that there were significant difference in serum PDGF concentration between AO before group and AO after, control groups ($P < 0.05$). Table (1):

Significant Correlations in serum TNF concentration in the addiction dependent period between ABT group and each of AO after, control groups revealed that there were significant difference in serum TNF concentration between ABT group and AO after, control groups (P <0.05).

significant difference in serum TNF concentration between ABT group and AO before group (P <0.05). Table (1): Significant Correlations in serum C-reactive protein, protein C, ALT, AST, creatinine, urea and glucose in ABT and AO before groups vs.

Table 1. Mean ± SEM of age, PDGF, TNF, glucose, C-reactive protein, protein C, ALT, AST, creatinine and urea in the four groups

	Control group	ABT group	AO group before treatment	AO group after treatment
Age (years)	27.07±1.132	28.00±0.9	26.79±0.9286	26.79±0.9286
PDGF(pg/ml)	539.9[a] ± 72.1	268.2 ± 30.5	191.4 ± 12.2	403.8 [a] ± 60.1
Addiction period				
PDGF(pg/ml)	539.9±72.11	1234[ab] ±139.1	865.00[a] ±37.9	403.8±60.10
Withdrawal period				
TNF(pg/ml)	24.4 [a] ± 3.7	10.2 ± 0.66	7.3 ± 0.55	30.8 [a]± 5.9
Addiction period				
TNF(pg/ml)	24.4 ± 3.7	268.6[ab]± 44.2	75.1[a] ± 11.4	30.8 ± 5.9
Withdrawal period				
C-reactive protein(mg/dl)	4.076±0.228	4.25±0.150	4.00±0.199	3.00±0.26
Protein C(mg/dl)	105.6±3.62	106.9±3.58	105.0±3.52	91.70±2.608
ALT(mg/dl)	11.13±0.336	12.04±0.34	11.50±0.248	12.5±0.61
AST(mg/dl)	11.73±0.3003	11.92±0.329	11.54±0.225	12.30±0.78
Creatinine(mg/dl)	1.507±0.088	1.46±0.066	1,392±0.067	1.18±0.064
Urea(mg/dl)	22.33±1.57	27.38±1.30	28.88±1.40	21.80±1.61
Glucose(mg/dl)	91.13 ±2.52	102.5±2.86	101.6±2.102	92.80±2.901

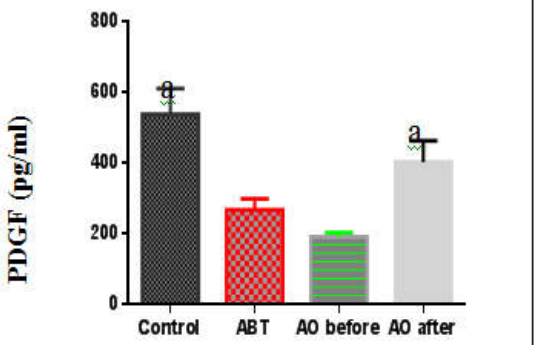

Figure 1. Serum PDGF in control, ABT in addiction period, AO before in addiction period and AO after groups, a: significant from normal groups at p < 0.05. b:significant from AO before group at p<0.05. Serum results presented as mean± SEM for all studied groups

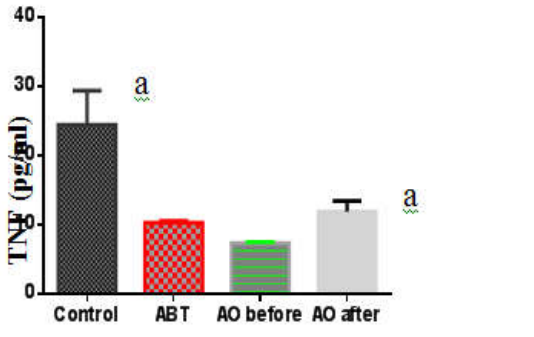

Figure 2. Serum TNF in control, ABT in addiction period, AO before in addiction period and AO after groups, a: significant from normal groups at p < 0.05. b:significant from AO before group at p<0.05. Serum results presented as mean± SEM for all studied groups

Table (1): Significant Correlations in serum TNF concentration in the addiction dependent period between AO before group and each of AO after, control groups revealed that there were significant difference in serum TNF concentration between AO before group and AO after, control groups (P <0.05). Table (1): Significant Correlations in serum TNF concentration in withdrawal period between ABT group and each of AO after, control groups revealed that there were significant difference in serum TNF concentration between ABT group and AO after, control groups (P <0.05). Table (1): Significant Correlations in serum TNF concentration in withdrawal period between AO before group and each of AO after, control groups revealed that there were significant difference in serum TNF concentration between AO before group and AO after, control groups (P <0.05). Table (1): Significant Correlations in serum TNF concentration in withdrawal period between ABT group and AO before group revealed that there were

AO after and control groups revealed that ABT and AO before groups were not statistically significant in serum C-reactive protein, protein C, ALT, AST, creatinine, urea and glucose from AO after and control groups (P >0.05 for each).

Figure (1): Showed that the mean ± SEM of serum PDGF was significantly lower in AO before treatment in the addiction dependent period (191.4 ± 12.2) and ABT in the addiction dependent period (268.6 ± 30.5) (pg/ml) as compared to normal (539.9 ± 72.1) and AO after treatment (403.8 ± 60.1) (pg/ml) groups, Figure (2): Showed that the mean ± SEM of serum TNF was significantly lower in AO before treatment in the addiction dependent period (7.3± 0.55) and ABT in the addiction dependent period (10.2 ± 0.66) (pg/ml) as compared to normal (24.4± 3.7) and AO after treatment (30.8 ± 5.9) (pg/ml) groups.

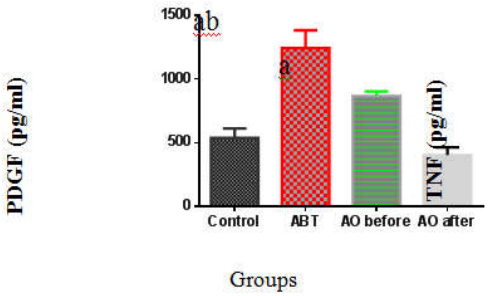

Figure 3. Serum PDGF in control, ABT in withdrawal period, AO before ABT in withdrawal period, and AO after groups, a: significant from normal groups at p < 0.05. b:significant from AO before group at p<0.05. Serum results presented as mean± SEM for all studied groups

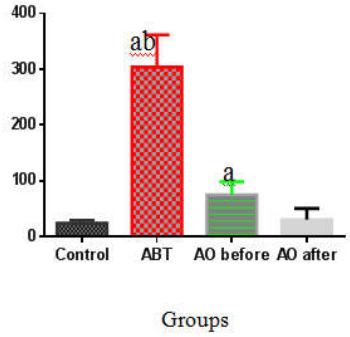

Figure 4. Serum TNF in control, ABT in withdrawal period, AO before in withdrawal period, and AO after groups, a: significant from normal groups at p < 0.05 b: significant from AO before group at p<0.05. Serum results presented as mean± SEM for all studied groups

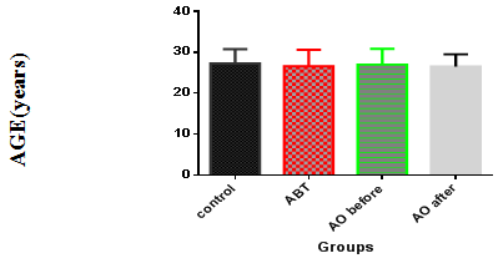

Figure 5. AGE in control, ABT, AO before and AO after groups, Serum results presented as mean± SEM for all studied groups

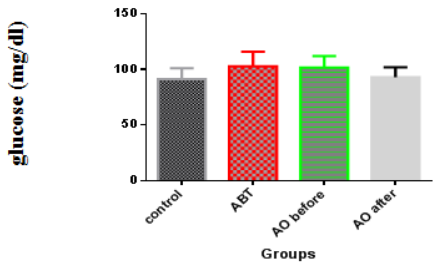

Figure 6. Serum Glucose in control, ABT, AO before and AO after groups, Serum results presented as mean± SEM for all studied groups

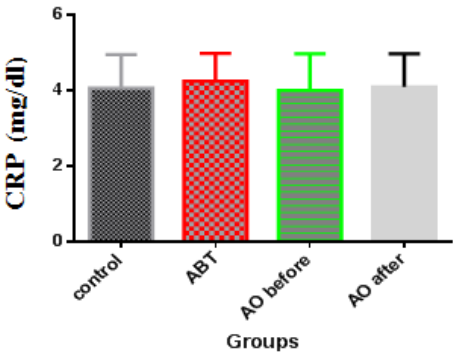

Figure 7. Serum C reactive protein in control, ABT, AO before and AO after groups, Serum results presented as mean± SEM for all studied groups

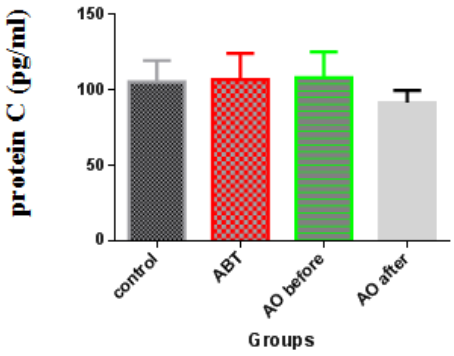

Figure 8. Plasma protein C in control, ABT, AO before and AO after groups, Plasma results presented as mean± SEM for all studied groups

Figure (3): Showed that the mean ± SEM of serum PDGF was significantly higher in AO before treatment in withdrawal period (865.0 ± 37.9) and ABT in withdrawal period (1243 ± 139.1) (pg/ml) groups as compared to normal (539.9 ± 72.11) and AO after treatment (403.8 ± 60.10) (pg/ml) groups. Figure (4): Showed that the mean ± SEM of serum TNF was significantly higher in AO before treatment in withdrawal period (75.15 ± 11.4) and ABT in withdrawal period (268.6 ± 44.2) (pg/ml) groups as compared to normal (24.4 ± 3.7) and AO after treatment (30.8 ± 5.9) (pg/ml) groups. Figure (5), figure (6), figure (7), figure (8), figure (9), figure (10), figure (11) and figure (12): showed that serum C-reactive protein, plasma protein C, serum ALT, AST, creatinine, urea and glucose were within normal range in both groups.

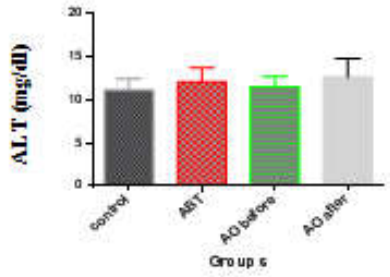

Figure (9): Serum ALT in control, ABT, AO before and AO after groups. Serum results presented as mean± SEM for all studied groups.

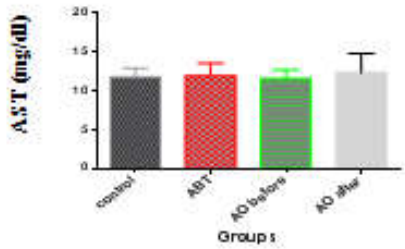

Figure (10): Serum AST in control, ABT, AO before and AO after groups. Serum results presented as mean± SEM for all studied groups.

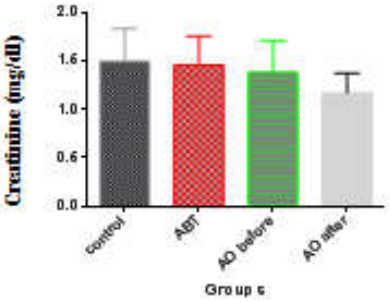

Figure 11. Serum Creatinine in control, ABT, AO before and AO after groups. Serum results presented as mean± SEM for all studied groups.

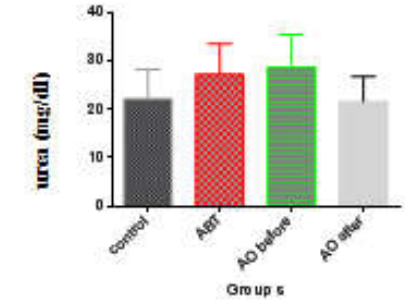

Figure 12. Serum Urea in control, ABT, AO before and AO after groups. Serum results presented as mean± SEM for all studied groups.

DISCUSSION

The use of drugs of abuse has generated several and serious health problem. There is a long recognized relationship between addictive drugs and increased levels of infections. The effects of opiates or benzodiazepines on immune system are receptor mediated, occurring both directly via specific receptors on immune cells and indirectly through similar receptors on cells of the nervous system. There have been numerous clinical reports on the association between infectious diseases and the use illegal drugs. Experimental studies using drugs of abuse support the clinical observations that these substances are associated with immune modulation (Herman F *et al*., 2003). Firstly, the present study found that PDGF level in the addiction dependent period was significantly lower in AO before groups and ABT group than normal and AO after groups, and TNF level in the addiction dependent was significantly lower in AO before group and ABT group than AO after and normal groups (Table 1).

In our study we found that chronic administration of opiate accompanied by suppression of lymphatic proliferation and impairment of immune response during addiction dependent period. This illustrated by (Josphine *et al*., 2010). who established that chronic morphine treatment significantly inhibits wound healing, similar to what is reported in the opioid use and abuse populations and therefore is a useful model to investigate the mechanisms underlying the inhibitory role of morphine in wound healing events.

In our study we found also that chronic administration of benzodiazepine was accompanied by suppression of lymphatic proliferation and impairment of immune response during addiction dependent period. This was illustrated by **(**Huemer *et al*., 2010). Who showed that many benzodiazepine induce prolonged impairment of cellular immune function in experimental animals after chronic low dosage administration. Secondly, the present study found that PDGF level in withdrawal period was significantly higher in AO before groups and ABT group than normal and AO after groups, and TNF level in withdrawal period was significantly higher in AO before group and ABT group than AO after and normal groups (*Table1*). The result of our study indicated that the mean serum level of cytokines in opiate addicted patient increased compared to control group in withdrawal period, and drugs used in treatment of opiate addiction such as methadone and buprenorphine cause hyper activation of the immune response through mu-opoid receptor activation. This was showed by (S Neri *et al*., 2005). who studied that long term treatment with methadone restore immune response and increase concentration compared with heroin abuser who present significantly lower lymphatic activity. However, this was showed by (Azarang *et al*., 2007). Who studied plasma profile of pro-inflammatory cytokines and chemokines in cocaine users under outpatient treatment, influence of cocaine symptom severity and psychiatric co-morbidity. In conclusion, cocaine exposure modified the circulating levels of pro-inflammatory mediators. Plasma cytokine/chemokine monitoring improved the stratification of cocaine consumers

seeking treatment and thus facilitated the application of appropriate interventions, including management of heightened risk of psychiatric co-morbidity. Also, (Bawa *et al*., 2013). Studied role of tumor necrosis factor alpha in opioid withdrawal syndrome and found that in opioid addiction, Hence suppression of proinflammatory cytokines (TNF) can significantly reduce opioid withdrawal syndrome. So, they conclude that opioid withdrawal induces glial activation and cytokines expression in different sites of the brain and, (Hutchinson *et al*., 2009). Found that morphine dependence was characterized by somatic and motivational signs of withdrawal that likely contribute to the maintenance of opioid addictive behavior. Morphine withdrawal induced glial activation and pro inflammatory mediator expression in the differ.

The results of this study showed that morphine withdrawal induces astrocytic activation to release TNF. Also, the result of that study indicated that the mean serum level of cytokines in benzodiazepine addicted patient increased compared to control group in withdrawal period. This was in agreement with (Hanan, 2013). Who showed that sub-chronic doses of clonazepam followed by a withdrawal period in adult male albino rats increased the production of cytokines in both treated groups. Finally, we found that when opiate addicted patients treated with quetiapine this resulted in improvement of patient immunity and reduction in cytokines levels to normal levels also, this was found with (Daniela *et al*., 2013). who studied impact of different antipsychotic on cytokines and tryptophan metabolites in stimulated cultures from patient with schizophrenia and found that quetiapine was identified to reverse the imbalanced cytokine levels in schizophrenia. In conclusion: This mean that patient addicted to opiate and benzodiazepine suffer from immunodeficiency and that lead to decrease in the level of serum PDGF and TNF-α. So we can conclude that serum PDGF may be a useful marker in aiding diagnosis of immunodeficiency, and serum TNF-α may be a good marker for immunodeficiency diseases. Also, during the withdrawal period for both opiate and benzodiazepine addicted patient the level of serum PDGF and TNF-α increase in correlation with the dose and duration of abused drug. So we can conclude that we can use cytokine level as supportive parameter in patient who has no clear history about drug dose and addiction duration. Obtaining high cytokine level in the beginning of treatment allow physicians to put patient in the correct protocol of treatment.

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