



EVALUATION OF IL-6 AND TOLL LIKE RECEPTOR-2 IN SALIVA OF SOME IRAQI ADDICTED

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Abstract

Abuse substance and alcohol effect on immune function, so this study aimed to determine interleukin-6 and Toll like receptors concentrations in saliva of addicted. Seventy saliva samples were collected from addict patients and twenty samples from healthy (control) with age ranged (19-53) years. During the period from August (2019) to February (2020). Abuse substance were detected by using by Immunoassay (ABON™ Multi-Drug-urine) which is one step screen test panel used for the qualitative detection of multiple drugs in human urine. IL-6 and TLR2 concentrations were measured by ELISA method. The results appeared IL-6 concentrations were decreased in addicted (23.2196 ± 11.94204) compared with control (25.4063 ± 6.18878) pg/ml, while TLR2 concentrations were significantly increased in addicted (1.9967 ± 2.36047) pg/ml than control (0.8853 ± 0.43960) pg/ml. The conclusion of this study the abuse substance and alcohol effect on pro inflammatory cytokine and change the oral microbes by their role in increasing TLR2.

Key words: Iraqi addicted; receptor 2; environment, health life, immunology, interleukin 6, abuse substance, TLR2.

Introduction

Substance use disorders causing encompassing symptoms of depression and anxiety, the most common and costly disorders challenging healthcare systems worldwide. Psychological distress is highly percentage among people struggling with alcohol and illicit drug use disorders (Martinez *et al.*, 2018) Both substance abuse and stress have effects on immune system cytokines, that can be modulators of neuropsychiatric function. In addition to stress-induced effects on immune function (Loftis and Huckans, 2013).

Cytokines are important in Immune function, as measured by circulating cytokine levels. Cytokines are a heterogeneous group of signal-transducing proteins secreted by immune cells to regulate immune activity and communicate with the nervous and endocrine systems (Felger and Lotrich, 2013).

Methamphetamine (MA) is one of abuse substance that causes an increase in pro-inflammatory cytokines in animal models and in humans. Which activation of microglia and neuro-inflammation could, *via* effects on reward networks, mediate behavioral characteristics of addiction.

MA could induced inflammation contributes to abnormalities in dopaminergic brain regions and shows that IL-6 levels are associated with the functional connectivity of mesolimbic and corticostriatal pathways (Kohno *et al.*, 2018).

Toll like receptors (TLR) are germ line encoded receptors that recognize microbial patterns which shared by large groups of microorganisms. There are 13 mammalian TLRs and many of their ligands are known (Denny *et al.*, 2006). One study concluded The sTLR-2 in saliva could serve as a potential biomarker for caries activity (Zhao *et al.*, 2014). The aim of this study to know the effect of addicted on some TLR 2 and IL-6 in saliva.

Materials and Methods

This case-control study was conducted on (90) individuals divided into two groups: patients group and apparently health group with age ranged (19-53) years. The laboratory tests of this study were conducted in Laboratory of Microbiology, Department of Biology, College of Science, Babylon University and Central Public Health Laboratory, Baghdad. Urine and saliva were collected from both addicted and healthy control. Abuse substance were detected by using by Immunoassay (ABON™ Multi-Drug-urine) which is one step screen

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test panel used for the qualitative detection of multiple drugs in human urine. This product is used to obtain a visual, qualitative result and was prepared according to the instructions of the manufacturing company (AbonBiopharm - Hangzhou \ China).

Interleukin-6 and TLR2 concentrations

Seventy saliva samples were collected from addict patients by spitting in a calibrated sterile labeled screw capped test tube under resting conditions between 8.0-11.0 a.m.. Patients were asked to rinse or washing their mouths with pure water before sampling. In order to obtain a sample of total saliva as much as possible, the patients were instructed to collect saliva in their mouths at least for 5 min without swallowing and to spit into this tube. Saliva samples were kept in a cool place during the collection until transported on icebox to the laboratory and stored at (-20°C) in a deep freeze till the time of immunological analysis by ELISA. Also obtained twenty saliva samples were collected from healthy persons (control group) concentrations of Interlukin-6 (IL-6) and Toll-Like Receptor (TLR-2) were determinate in saliva of addict patients and control groups, using commercially available kits by the method of ELISA, according to the instruction provided by manufacturer Elabscience.

Statistical Analysis

The analysis of the current study was performed by using SPSS (Statistical Process for Social Sciences) version 23, in order to assess the results of this study. Results were expressed by descriptive data analysis such as tables (Frequencies, Percentages and Cumulative Percentages), mean \pm SD and contingency coefficients for the causes correlation ship of the contingency.

Results

The salivary IL-6 and TLR-2 levels were

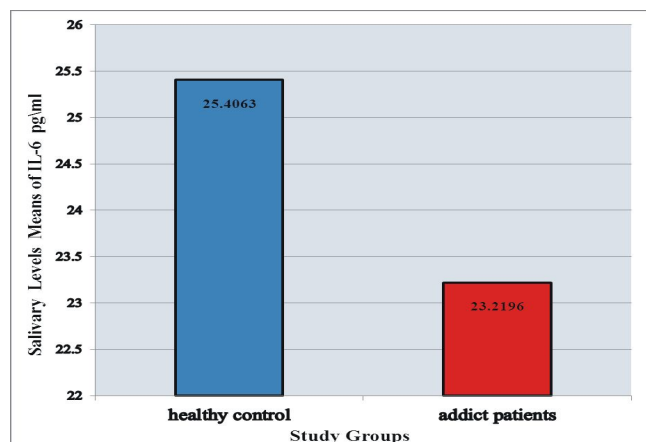


Fig. 1: The mean of saliva IL-6 in the addict patients and healthy control.

Table 1: IL-6 levels in the patterns of substance addiction of addict patients and healthy control.

Mean \pm SD.	Addicted
22.5675 \pm 5.01	Alcohol
21.91122 \pm 5.26311	Alcohol + Drug
27.6001 \pm 21.70	Single Drug
20.4063 \pm 5.28296	Mixed Drug
25.4063 \pm 6.1887	Control

determination in saliva of (70) addicts as well as, (20) healthy control. The results show in fig. 1, that mean level of IL-6 were a slight decrease in addict patients (23.2196 \pm 11.94204) pg/ml as compared with healthy control (25.4063 \pm 6.18878) pg/ml, with no statistical differences at ($P \leq 0.05$). In other word the present result revealed there are insignificantly variation in concentration of IL-6 in all saliva sample were taken from the patients subjects in comparing with healthy subjects.

The mean level of IL-6 (pg/ml) parameter in the addict patients with related to patterns of substance addiction.

In the current study was evaluate the concentration of IL-6 levels according to the patterns of substance addiction for addict patients, the single drug pattern recorded the highest mean of IL-6 level of these patients which was (27.6001 pg/ml) when compared with the other patterns of substance addiction, while the lowest mean of IL-6 level (20.4062 pg /ml) was shown in mixed drug pattern. The results showed no significant differences ($P \leq 0.05$) in the concentration of IL-6 levels between the patterns of substance addiction for addict patients, as shown in fig. 2. Also the present result revealed that the concentration of IL-6 levels in all the patterns of substance addiction were lower than those of control group (25.4063 pg/ml), except the concentration of IL-6 levels in the single drug pattern (27.6001pg/ml) was demonstrated a slight increase in comparing with healthy control fig. 2, table 1.

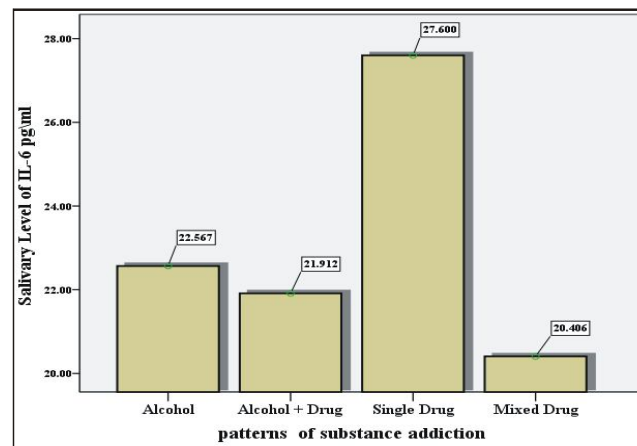


Fig. 2: The mean of saliva IL-6 in the patterns of substance addiction for addict patients.

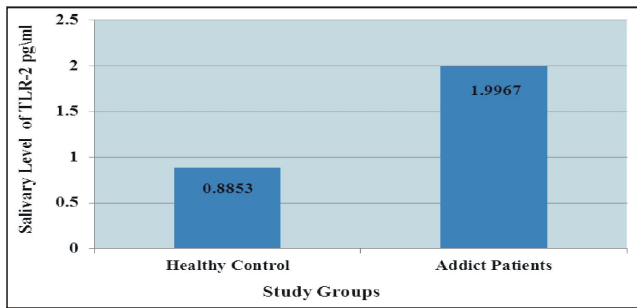


Fig. 3: The mean of saliva TLR-2 in the addict patients and healthy control .

Estimation of Salivary TLR-2

In the current study was evaluate the concentration of TLR-2 in all saliva samples were taken from the addict subjects and healthy subjects and the results show that mean level of TLR-2 was increased in addict patients (1.9967 ± 2.36047) pg/ml as compared with healthy control (0.8853 ± 0.43960) pg/ml, with high significant differences ($P \leq 0.05$). In other word the present result revealed there are statistical differences in concentration of IL-6 among the addict subjects and healthy subjects. This results were show in fig. 3.

In the current study was evaluate the concentration of TLR-2 levels according to the patterns of substance addiction for addict patients, the mixed drug pattern recorded the highest mean of TLR-2 level of these patients which was (3.1547 pg/ml) when compared with the other patterns of substance addiction, while the lowest mean of TLR-2 level (1.4090 pg /ml) was shown in alcohol + drug pattern. The results showed no significant differences ($P \leq 0.05$) in the concentration of TLR-2 levels between the patterns of substance addiction for addict patients, as shown fig. 4. Also the present result revealed that the concentration of TLR-2 levels in all the patterns of substance addiction were higher than those of control group (0.8853 pg/ml).

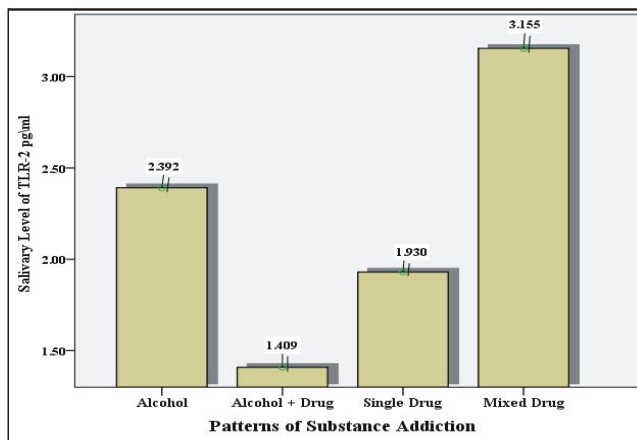


Fig. 4: The mean of saliva TLR-2 in the patterns of substance addiction for addict patients.

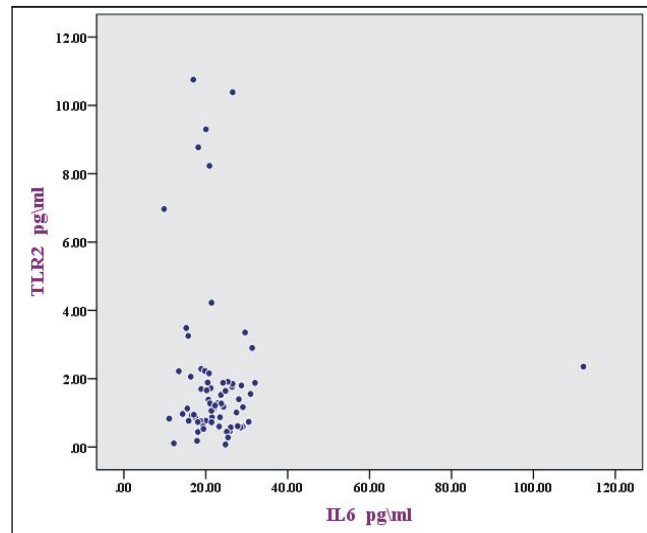


Fig. 5: The Correlation between IL-6 and TLR-2.

Relationships between IL-6 and TLR-2

To examine the relationship between the salivary immunological parameters that were determination in (70) addict patients, correlation test were carried out between these parameters (IL-6 and TLR-2). Pearson correlation coefficient revealed there are negative correlation between IL-6 and TLR-2 ($r = -0.044$). As shown in fig. 5.

Discussion

The current study found that concentration of proinflammatory cytokine, IL-6 were decreased compare with control this might be the alcohol and abuse substance act as immunomodulator, this agree with other study found human monocytes acutely exposed to alcohol show suppression of nuclear factor kappa B (NF- κ B) mediated production of pro-inflammatory cytokines (Mandrekar *et al.*, 2002) and acute alcohol treatment dose-dependently reduces IL-6, IL-12p40, IL-23 and IL-10 levels in bone-marrow-derived dendritic cells obtained from mice (Rendon *et al.*, 2012).

Single drugs was appeared increasing compare with others (alcohol, mixed and control) this similar to other studies, methamphetamine induced activation of microglia is dose-dependent and results in the production of pro-inflammatory cytokines including IL-6, IL-1 β and TNF- α (Beardsley and Hauser, 2012) in line with studies showing that MA exposure increases IL-6 RNA levels in an astrocytic cell line (Shah *et al.*, 2012) and that acute MA administration increases IL-6 mRNA levels in striatum (Goncalves, 2008; Sriram *et al.*, 2006),

Toll like receptors concentrations were increased in addicted compared with control, this may be the alcohol and abuse substance effect on dentil, many studies found that oral problem in addicted (Shekarchizadeh *et al.*, 2013;

Robinson *et al.*, 2005; Metsch *et al.*, 2002). These studies appeared the drugs include opiates, cannabis, hallucinogens, cocaine- and amphetamine-type stimulants effect oral health complications associated with drug abuse may result from direct exposure of oral tissues to drugs during ingestion and biologic interaction of drugs with normal physiology of oral cavity. A study on heroin injectors that regardless of their oral hygiene, these patients suffer from progressive dental caries (Madinier *et al.*, 2003). Toll like receptors (TLR) have been implicated as modulators of host-microbial interactions. Soluble TLR-2 and its co-receptor CD14 present in saliva can bind the cell wall components of cariogenic bacteria and modulate the disease process, these marker was increased with dental caries (Zhao *et al.*, 2014).

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