Research
ISSN 2413-0516

Myokines in alcoholic myopathy

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Objective Interleukin (IL)-15 is highly expressed in skeletal muscles, where it exerts anabolic effects, increase protein content in muscle fibres and promotes muscle growth. Alcoholics frequently suffer myopathy. Therefore, we analyse the level of IL-15 [and other myokines, such as tumor necrosis factor-α (TNF-α)] in alcoholics. Follow-up of skeletal muscle cytokines (myokines) such as IL-15 and TNF-α level in alcoholism, in an attempt to reveal if a certain level of myokines can be considered as a risk factor for short-term motility.
Methods IL-15 and TNF-α were determined by enzyme-linked immunoassay analytic techniques in blood samples of 70 chronic alcoholics and 70 age- and sex-matched controls, and then the levels of myokines were correlated with liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamate transferase (GGT), amount of ethanol consumed, duration and creatine kinase (CK) activity levels.
Results All the alcoholic patients were heavy drinkers (217.04 ± 149.93 g/day), who started at an early age (13.97 ± 8.96 years). IL-15, TNF-α levels and liver enzyme activity were significantly higher in these patients than in controls. Significant relationship was found between IL-15, quantity of ethanol consumption, TNF-α, CK, AST/ALT and between TNF-α and daily ethanol consumption (quantity) and GGT.
Conclusion A certain level of myokines such as IL-15 and TNF-α can be considered as a risk factor of alcoholics for short-term motility.
Keywords IL-15, TNF-α, alcoholic myopathy, liver impairment

Introduction

Interleukin (IL)-15 is a relatively newly described cytokine, highly expressed in skeletal muscle. IL-15 mRNA levels are highly up-regulated in response to strength training, and it seems to exert anabolic effects, increasing protein content in muscle fibres[1] and promoting myogenic differentiation and muscle growth. It would exert an opposite effect to that of tumor necrosis factor α (TNF-α), since it is able to antagonize muscle protein break-down in a cancer cachexia model.[2] Since its discovery in 1994,[3] it has become clear that it is secreted by many tissues, such as muscle, kidney, heart, lung, dendritic cells, monocytes and macrophages and also enterocytes,[4] but muscle is the major site of IL-15 mRNA transcription and probably secretion.[3] In addition to its actions on muscle, it also exerts many other functions on memory T cells, natural killer (NK) cells, eosinophils, neutrophils, monocytes and macrophages[5] and shares stimulatory actions on T-cells with IL-2, partly due to the similarity of their respective receptors. Indeed, the heterotrimeric IL-15 receptor is composed of beta and gamma chains identical to those of the IL-2 receptor, together with a specific alpha chain. It is, therefore, not surprising that it acts as a potent activator of T and B lymphocytes. It is also involved in the maintenance of T cell memory and in the activation of other immune cells, such as neutrophils and NK cells.[6] It may be also related to the pathogenesis of autoimmune diseases.[7] Regarding its metabolic function, it probably participates in the cross-talk between muscle and fat, leading to a reduction of the latter.

In chronic alcoholism, muscle wasting is a prominent feature. Chronic alcoholic myopathy has been found in nearly 50–60% alcoholics,[8–10] and it is defined by muscle atrophy, predominantly affecting type IIb fibres[11,12] and leading, sometimes, to incapacitating weakness. IL-15 increased after exercises and physical activity that occurs in alcoholics, due to encourage aggression or violence by disrupting normal brain function. According to the disinhibition hypothesis, for example, alcohol weakens brain mechanisms that normally restrain impulsive behaviours, including inappropriate aggression.[13] By impairing information processing alcohol can also lead a person to misjudge social cues.

TNF-α, a pro-inflammatory cytokine, also increases in acute or chronic alcoholics due to the impairment of liver. Studies have found that alcohol may increase the liver's sensitivity to inflammatory cytokines, such as TNF-α, in two ways. First, alcohol may directly or indirectly stimulate Kupffer cells to produce and release TNF-α into small channels (i.e. sinusoids) in which the blood flows through the liver. One indirect mechanism is that the alcohol induces an increase in the levels of a bacterial endotoxin in the blood. Second, alcohol may enhance the sensitivity of hepatocytes to TNF-α.[14]

The processes by which alcohol is broken down in the hepatocytes generate a variety of molecules that can be toxic to the liver, or it may interfere with normal physiological processes. For example, alcohol breakdown through the enzyme known as cytochrome P_{450} 2E1(CYP2E1) leads to the formation of small oxygen-containing molecules called reactive oxygen species (ROS). Unless they are rapidly eliminated or converted into harmless molecules by antioxidants, it can interact with and damage complex molecules in the cells (e.g. proteins and DNA).[15] Both increased and decreased levels of ROS can lead to apoptosis of hepatocytes.

Materials and Methods

Seventy alcoholic patients were included (patient group), who were admitted to Ibn-Rushd Teaching Hospital, Baghdad,

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(*Submitted: 17 April 2015 – Revised version received: 21 May 2015 – Accepted: 23 May 2015 – Published online: Summer 2015*)

Iraq. Their mean [± standard deviation (SD)] age was 40.35 ± 11.01 years. All of them were heavy drinkers of ethanol (>217 g/day) during a prolonged period (>13 years). In the control group (70 subjects), the mean (± SD) age was 33.0 ± 7.83 years, and they were nonalcoholic. Some clinical and biochemical parameters of patients and controls are shown in Table 1.

Cytokines and Biochemical Parameters

Blood samples were taken at 8.00 am in fasting conditions, and were immediately frozen at −80°C. The following parameters were determined: TNF-α by enzyme linked immune sorbent assay (ELISA; range of detection 32–2000 pg/ml); and IL-15 by ELISA (sensitivity <3 pg/ml; range of detection 15.6–1000 pg/ml). In addition, patients underwent liver enzyme assays, including aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase ALP, gamma-glutamyl transferase (GGT) and creatine kinase (CK).

Biostatistics

Spearman's correlation coefficient was used to compare quantitative parameters. When variables did show a normal distribution, Student's t-test and Pearson's test were employed.

Results

As shown in Table 1, all the cytokines were determined, including IL-15 and TNF-α levels; liver enzyme activity were significantly higher in alcoholic patients than in controls. All the patients were heavy drinkers (217.04 ± 149.93 g/day) with many years of consuption (13.97 ± 8.96 years). Significant relationship was found between IL-15, quantity of ethanol consumption, TNF-α, CK, AST/ALT and between TNF-α and daily ethanol consumption (quantity) and GGT (see Figs. 1–6), respectively.

Significant correlations were found between some studied parameters, as presented in Figs 1–6.

Discussion

The high value of IL-15 levels may be due to muscle contraction and violence associated with convulsion and cramps happened to the alcoholic patients, resulted from many alteration in electrolyte, calcium and sodium ions, caused

Table 1. **Mean ± SD of serum AST, ALT, ALP, CK, GGT activity levels, AST/ALT activity ratio, IL-15 and TNF-α level for studied groups**

Parameter	Patient Group N = 70 Mean ± SD	Control Group N = 70 Mean ± SD	*P value*
AST (U/L)	20.01 ± 9.80	8.68 ± 3.79	0.08
ALT (U/L)	13.84 ± 6.27	7.51 ± 2.57	0.030
ALP (U/L)	62.98 ± 22.90	50.87 ± 17.81	0.001
AST/ALT*	1.68 ± 0.89	0.83 ± 0.24	0.001
GGT (U/L)	97.45 ± 15.58	28.68 ± 13.55	0.061
CK (U/L)	133.68 ± 44.43	50.40 ± 24.49	0.001
IL-15 (pg/ml)	59.01 ± 11.09	34.92 ± 9.03	0.053
TNF-α (pg/ml)	83.42 ± 10.12	41.68 ± 15.74	0.072

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphate; CK: creatine kinase; GGT: gamma glutamate transferase; IL-15: interleukin-15; TNF-α: tumor necrosis factor-alpha.

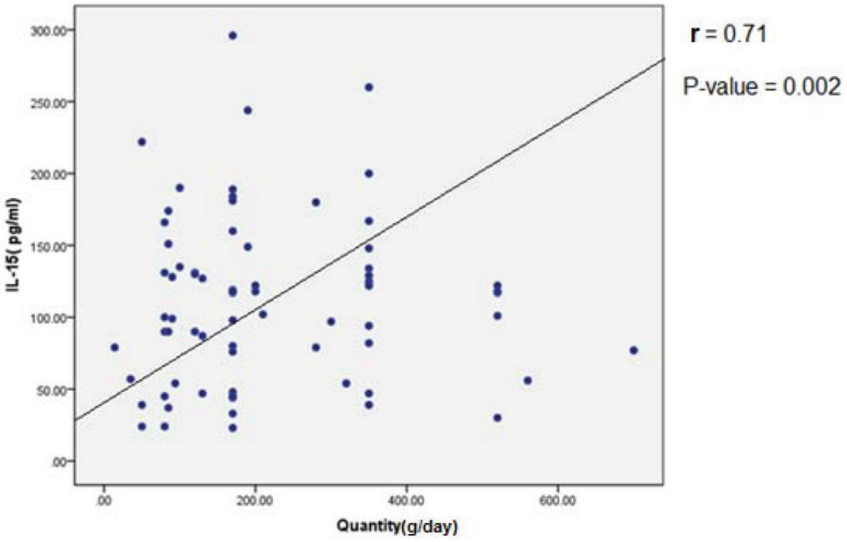

Fig. 1 **Correlation between IL-15 level and quantity of ethanol consumed per day in alcoholic patients group.**

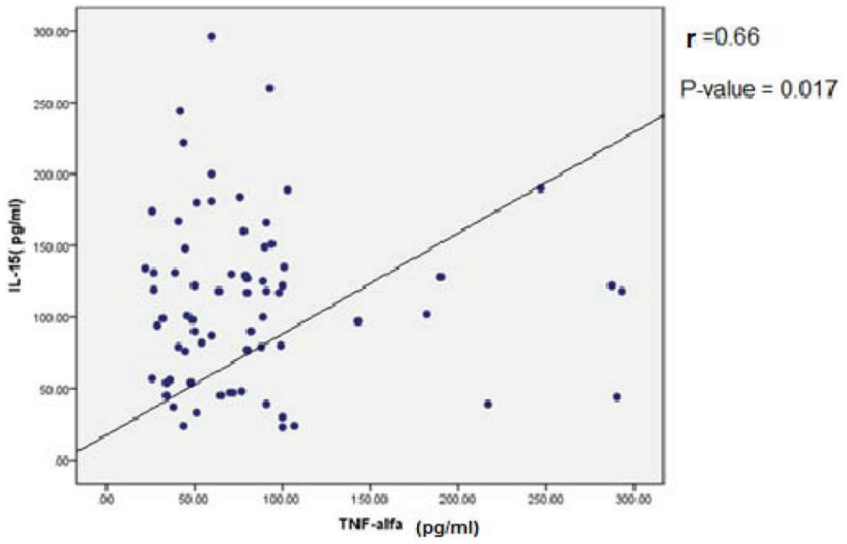

Fig. 2 **Correlation between IL-15 level and TNF-α in alcoholic patients group.**

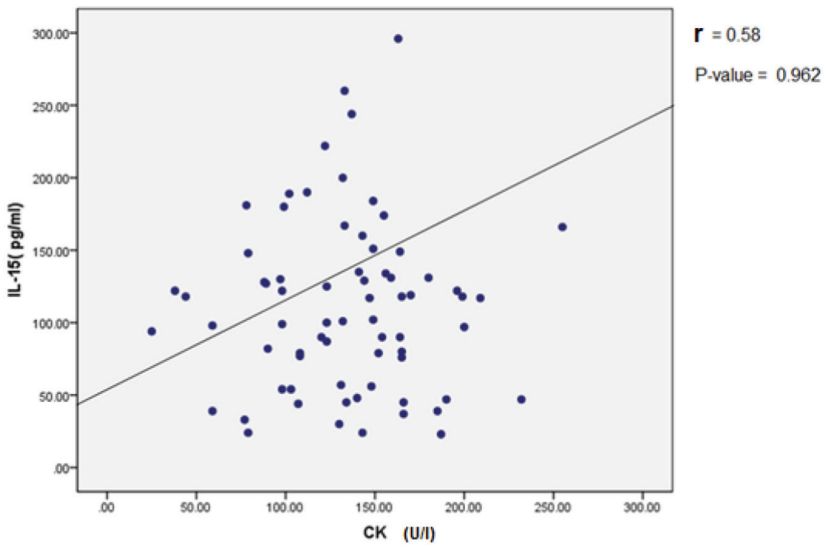

Fig. 3 **Correlation between IL-15 and CK activity level in alcoholic patient group.**

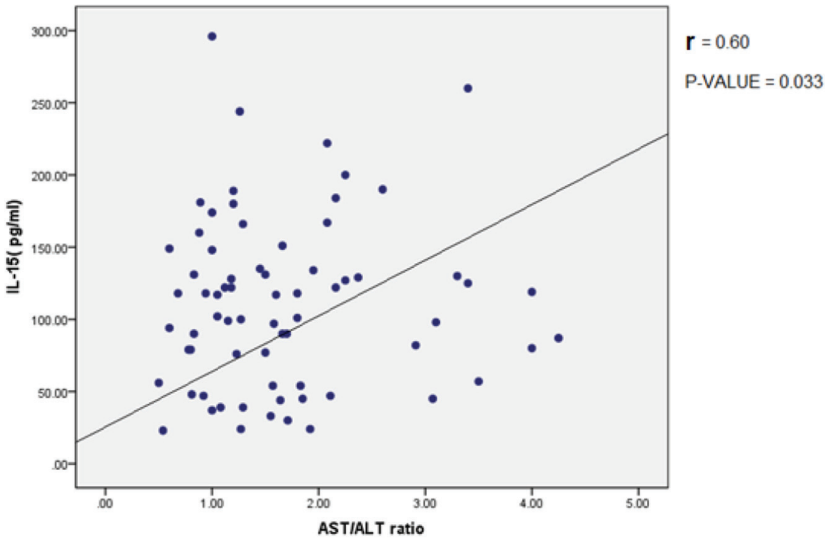

Fig. 4 **Correlation between IL-15 level and AST/ALT ratio in alcoholic patient group.**

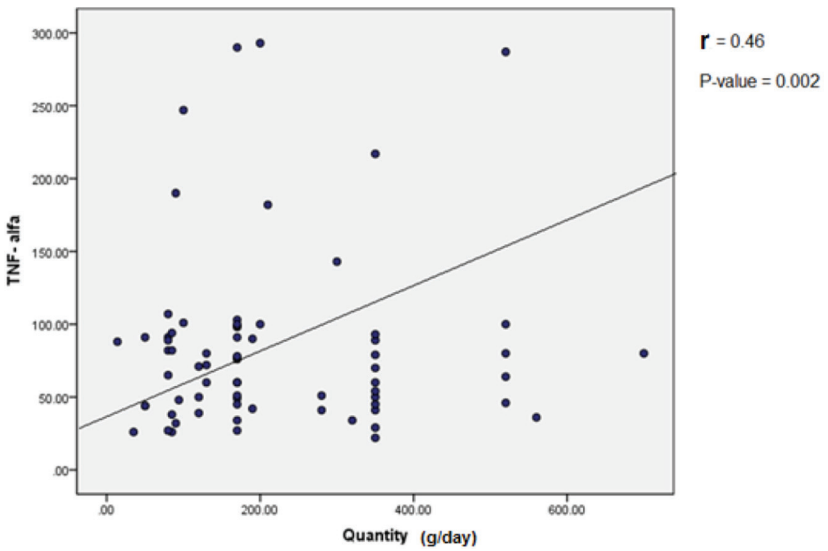

Fig. 5 **Correlation between TNF-α and quantity of ethanol consumed per day in alcoholic patient group.**

by changes in the membrane permeability, due to lipid peroxidation which occurred from reaction of acetaldehyde and phospholipids of muscle cells wall.[16]

IL-15 mRNA levels were up-regulated in human skeletal muscle following strength training, suggesting that IL-15 may accumulate within the muscle as a consequence of regular training or physical activity as happened to alcoholics.[17]

Oxidative stress produced from alcohol consumption is associated with numerous deleterious consequences for the cell (e.g. lipid peroxidation or even cell apoptosis or necrosis) and leads to different enzyme leakage to outside the cells, causing an increase in the level of these enzymes in the blood. So there were appositive correlation between IL-15, quantity of ethanol consumed, CK and AST/ALT ratio as shown in Figs. 1, 3 and 4, respectively.

The study found positive correlation between IL-15 and TNF-α (Fig. 2) due to liver impairment that happened in chronic alcohol due to damaging its cells (Kupffer cells) which converted to macrophages when activated by viruses or any foreign substances like alcohol, and endotoxins which were secreted from intestine bacteria due to alcohol effects travel to blood stream, then to Kupffer cells which activate them and secrete numerous cytokines including TNF-α.[18]

TNF-α was higher in alcoholics than in nonalcoholics (Table 1), this result agree with what was mentioned about the effects of alcohol consumption on different body organs.

One of the factors that can enhance the production of TNF-α is alcohol, endotoxin is released from the bacteria (gram-negative) normally living in the intestine when those bacteria die from alcohol and acetaldehyde (toxic substance). If endotoxin enters the blood stream and reaches the liver, it interacts with Kupffer cells, activating the cells to produce cytokines.

In a healthy person, endotoxin interacts primarily with Kupffer cells, and this interaction is considered crucial to secretion of TNF-α, which then interacts with receptors on both Kupffer cells and hepatocytes.

Endotoxins seem to be important in the development of early alcoholic liver disease. Thus TNF-α was correlated positively with quantity and GGT, Figs. 5 and 6, respectively. Alcohol

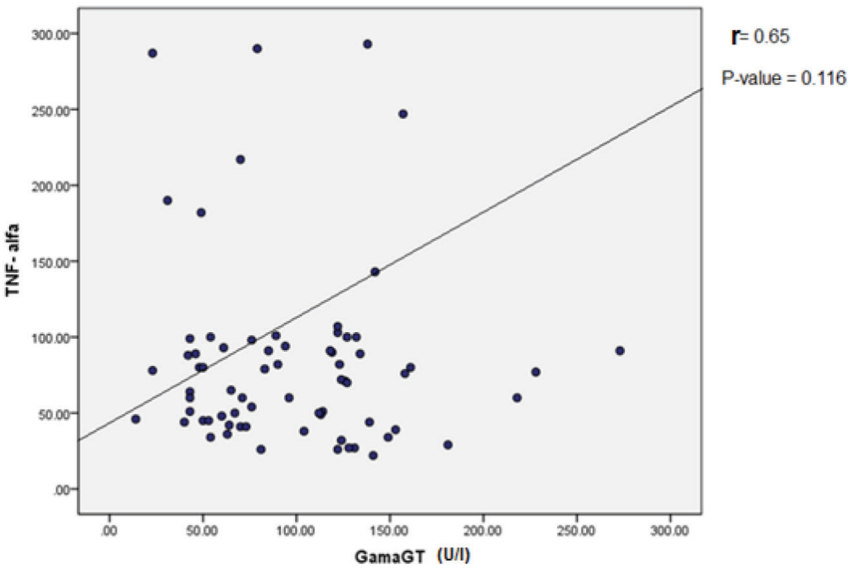

Fig. 6 **Correlation between TNF-α level and GGT activity levels in alcoholic patient group.**

also increased TNF-α by another way, it oxidises to acetaldehyde, which reacts with proteins and DNA and to form adducts. These adducts induce certain immune cells (recognize these adducts as foreign bodies) to produce many deafens cells like interleukins, interferon's and pro-inflammatory molecules like TNF-α.

Conclusion

IL-15 and TNF-α levels were higher in Iraqi alcoholics than in nonalcoholic. Both of them exert dangerous effects on the body and can consider as a risk factor for short-term motility.

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