RESEARCH ARTICLE

# Relationship between a pro- and anti-inflammatory cytokine imbalance and depression in haemodialysis patients

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**ABSTRACT.** ***Aim:*** **Depression represents the most frequent psychiatric disorder in patients on maintenance haemodialysis (HD), and it might be associated with secretion of inflammatory cytokines. In this study, we explore the possible correlations between depression and pro-inflammatory (IL-1β, IL-6, and TNF-α), anti-inflammatory (IL-10) cytokines, as well as high-sensitivity C-reactive protein (hs-CRP) serum levels.** ***Methods:*** **Eighty three HD patients were enrolled in this cross-sectional study. Depressive symptoms were measured with the Beck Depression Inventory (BDI), and 'elevated symptoms of depression' were defined as a BDI score of ≥16. Biochemical parameters (serum albumin, haemoglobin, ferritin, etc.) and dialysis dosage delivery (kt/v) were assessed. Serum IL-1β, IL-6, IL-10, TNF-α, and hs-CRP levels were measured using an ELISA method. Thirty two, healthy, age- and sex-matched individuals were included as the control group.** ***Results:*** **The prevalence of depression in HD patients was 61.4%. HD Patients with symptoms of depression had lower educational levels compared to non-depressed ones (P = 0.02), but did not differ with respect to age, gender, time on dialysis program, marital status, or smoking habits. Depressed patients also had significantly higher serum levels of IL-6, the IL-6-to-IL-10 ratio, as well as lower haemoglobin levels (P = 0.003, P = 0.002, and P = 0.02 respectively). No differences in mean serum IL-1β, IL-10, TNF-α, or hs-CRP concentrations were noted between the two groups. The BDI scores showed a significant, positive correlation with serum levels of IL-1β (P = 0.03), IL-6 (P = 0.001), TNF-α (P = 0.02), the IL-6-to-IL-10 ratio (P = 0.001), and a negative correlation with haemoglobin levels (P = 0.02).** ***Conclusions:*** **Maintenance HD patients with symptoms of depression may have higher serum levels of IL-6, a IL-6-to-IL-10 ratio, and lower haemoglobin concentrations. An imbalance between pro- and anti-inflammatory cytokines may play an important role in the pathogenesis of depression in HD patients.**

**Key words:** anti-inflammatory cytokines, depression, haemodialysis, haemoglobin, pro-inflammatory cytokines

Depression is the most common psychiatric disorder in patients on maintenance haemodialysis (HD), with prevalence rates of between 30-60%. Depression is associated with HD patient mortality [1-3]. Chronic, high plasma levels of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have been reported in chronic kidney disease (CKD) and maintenance HD patients [4, 5]. Inflammatory status in HD patients has been related to a high incidence of infection, uremic status, malnutrition, exposure to dialysis tubes and membranes, vascular accesses, and endotoxins [6]. High serum concentrations of proinflammatory cytokines may be associated with HD patient mortality [7]. Higher plasma concentrations of high sensitivity C-reactive protein (hs-CRP), IL-6, IL-1β, TNF-α, and ferritin (a positive, acute phase protein) compared to normal values have also been reported in depressed patients [8-10]; with a positive correlation between cytokine plasma concentrations and the severity of the depression [11-13]. Antidepressant treatment has been shown to lower serum IL-6 [14] and TNF-α [8] levels. Based on these findings, a possible correlation may exist between depression and proinflammatory cytokines. In this regard, many investigators have evaluated the association between depression and circulating cytokines such IL-6, TNF-α, IL-1, IL-10 (an anti-inflammatory cytokine), and hs-CRP in CKD and dialysis patients; however, conflicting results have been found and controversies still exist about this possible link. Some researchers found a significant, positive correlation between proinflammatory cytokines and depression severity in CKD and dialysis patients [12, 13, 15], conversely, others found no correlation between serum IL-1, IL-6, or TNF-α concentrations

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and depression in these patient populations [16-18]. Given the activated inflammatory state in many CKD patients, the relationship between depression and inflammation deserves further exploration.

Since both depression and inflammation are frequently seen in dialysis patients and are both associated with increased morbidity and mortality, we investigated the possible correlation between circulating cytokines, demographic characteristics, biochemical variables, and serum albumin, with depression in patients on maintenance HD in Iran, in a teaching HD center. Also, for first time, the balance between pro-and anti-inflammatory cytokines was evaluated in HD patients, with and without depression.

## DONORS AND METHODS

This cross-sectional study was performed in the HD center of the Imam Khomeini Hospital Complex, Tehran. Eligible subjects were adults between 18 and 90 years of age who were on maintenance HD for at least three months. All patients received HD for four hours, two to three times per week using a low flux, polysulphone dialyser and bicarbonate buffer. Thirty two, healthy, age- and gender-matched individuals were selected as the control group. Patients with autoimmune diseases, active infections, malignancy, severe mental illness, cognitive dysfunction, and current use of immunosuppressive, immunomodulator, corticosteroids, or antidepressant medications were excluded.

Demographic (including sex, weight, age, smoking, marital status, and educational level) and clinical data (including the cause of renal failure) were obtained from each patient's medical records.

The presence and/or severity of depression were assessed using the self-administered, Beck Depression Inventory (BDI), which was distributed by a healthcare assistant during an HD session. The BDI is a 21-item questionnaire that measures characteristic symptoms of depression. The possible total BDI score ranges from 0 to 63. The BDI questionnaire has been shown to have high sensitivity and specificity for the diagnosis of depression in dialysis patients [19]. A validation of the BDI in the Iranian population has been performed by Ghasemzadeh *et al.* [20]. A BDI score of $\geq$16 was adopted as the standard cut-off point to define depression in Iranian individuals, thus this value was used to classify patients as suffering from symptoms of depression.

After fasting overnight, morning, pre-dialysis blood samples were drawn. Samples were analyzed for determination of serum haemoglobin (Hgb), calcium, phosphorus, albumin, ferritin, iron, total iron binding capacity, intact parathyroid hormone (iPTH), uric acid, glucose, lipids, inflammatory mediators (hs-CRP, IL-1β, IL-6, TNF-α) and anti-inflammatory cytokine (IL-10) concentrations. Inflammatory and anti-inflammatory cytokines were assessed using the ELISA method with serum hs-CRP (Immulite, DPC Cirrus Inc., Los Angeles, CA, USA), IL-1β (Bender Med Systems, Austria), IL-6 (Amersham Bioscience, USA), IL-10 (Amersham Bioscience, USA), and TNF-α (Bender Med Systems, Austria) kits. Dialysis efficacy was calculated using the urea kinetics model and expressed as kt/v. The study protocol was approved by the local ethics Committee of the Tehran University of Medical Sciences. All patients provided written, informed consent form.

Data were analyzed using SPSS for windows (SPSS Inc., Chicago, IL, USA) version 18. All continuous variables are reported as the mean $\pm$ SD. Categorical variables were compared using the chi-square test. Student's t-test was used for normally distributed variables and the Mann-Whitney U test was used for parametric variables with non-normal distributions. Correlations between BDI scores and variables were investigated using the Pearson's correlation test and Spearman's correlation test for normally and non-normally distributed variables respectively. Multivariate logistic regression analysis was used to determine the independent risk factors for depression. Significance was defined as $P<0.05$.

## RESULTS

A total of 83 patients (52 males and 31 females) were enrolled in the study. Fifty-one out of these 83 patients (61.4%) had increased symptoms of depression (BDI $\geq$16). The mean BDI scores for patients with and without depressive symptoms were 27.8 $\pm$ 9.6 and 10.8 $\pm$ 4.7, respectively. The mean age of all participants was 57.4 $\pm$ 15.7 years (range 23-86 years). The mean body mass index (BMI) was 23.5 $\pm$ 3.7 kg/m$^2$ (range 14.2-35.6 kg/m$^2$). All the patients had been on maintenance HD for 5-312 months (mean 71.1 $\pm$ 57.3 months). The control group consisted of thirty two healthy individuals (16 males and 16 females), with a mean age of 52.9 $\pm$ 9.6 years old. Compared to the control group, HD patients had higher serum concentrations of IL-1β, IL-6, IL-10, TNF-α, triglyceride, and glucose, higher IL-1β-to-IL-10 and IL-6-to-IL-10 ratios, and lower serum HDL-cholesterol levels (*table 1*). BMI, serum total and LDL-cholesterol concentrations, and serum TNF-α-to-IL-10 ratio did not differ significantly between HD patients and control subjects.

Differences in demographic and clinical characteristics between HD patients with and without depression have been summarized in *table 2*. Patients with and without increased depressive symptoms were not significantly different with respect to age, BMI, gender, smoking, or marital status. However, educational level was significantly lower in patients with depression (P = 0.02). Etiologies of ESRD in these HD patients were hypertensive nephropathy (n = 21), diabetic nephropathy (n = 14), polycystic kidney disease (n = 9), reflux nephropathy (n = 6), nephrolithiasis (n = 4), chronic pyelonephritis (n = 3), and unknown (n = 26).

Biochemical findings for depressed and non-depressed HD patients are presented in *table 3*. Dialysis patients with symptoms of depression had a lower serum Hgb concentration (P = 0.02), higher serum IL-6 levels (P = 0.003) and a higher IL-6-to-IL-10 ratio (P = 0.002) compared to the patients without depression. Mean serum concentrations of IL-1β, IL-10, TNF-α, hs-CRP, albumin, calcium, phosphorus, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, glucose, ferritin, transferrin saturation, iPTH, and uric acid did not differ significantly between these two groups of patients.

With regard to the role of the effectiveness of the dialysis, we found no significant difference in kt/v values between the two groups of patient (P = 0.42). Also, there was no

**Table 1**
Comparison of biochemical parameters between haemodialysis patients and healthy control subjects

| | Control group (N = 32) | Haemodialysis group (N = 83) | P-value |
|---|---|---|---|
| **Age (years)** | 52.9 ± 9.6 | 55.9 ± 15.5 | 0.12 |
| **Gender (male/female)** | 16 /16 | 51/32 | 0.21 |
| **IL-1β (pg/mL)** | 1.2 ± 1.2 | 10.9 ± 5.2 | <0.001* |
| **IL-6 (pg/mL)** | 2.4 ± 1.1 | 6.5 ± 3.8 | <0.001* |
| **IL-10 (pg/mL)** | 1.4 ± 1.0 | 3.8 ± 1.7 | <0.001* |
| **TNF-α (pg/mL)** | 3.5 ± 2.6 | 10.4 ± 4.6 | <0.001* |
| **IL-1β / IL-10** | 1.0 ± 1.3 | 3.1 ± 2.3 | <0.001* |
| **IL-6 / IL-10** | 1.1 ± 0.9 | 1.9 ± 1.3 | 0.005* |
| **TNF-α / IL-10** | 2.7 ± 3.2 | 2.9 ± 1.5 | 0.17 |
| **Triglyceride (mg/dL)** | 131.9 ± 49.9 | 180.1 ± 84.9 | 0.002* |
| **Total cholesterol (mg/dL)** | 179.3 ± 29.7 | 175.4 ± 46.5 | 0.60 |
| **LDL-cholesterol (mg/dL)** | 103.9 ± 19.4 | 107.1 ± 31.31 | 0.53 |
| **HDL-cholesterol (mg/dL)** | 49.1 ± 17.1 | 41.6 ± 13.8 | 0.01* |
| **Glucose (mg/dL)** | 113.6 ± 49.7 | 137.4 ± 74.0 | 0.04* |
| **Body mass index (kg/m$^2$)** | 25.3 ± 4.1 | 22.4 ± 3.7 | 0.09 |

Data are presented as mean ± SD
* P< 0.05

**Table 2**
Demographic and clinical characteristics of haemodialysis patients classified according to BDI (N = 83)

| | | BDI<16 (N = 32) | BDI≥16 (N = 51) | Chi-square | P-value |
|---|---|---|---|---|---|
| **Gender** | Male | 22 | 29 | 1.17 | 0.28 |
| | Female | 10 | 22 | | |
| **Age** | ≤64 years | 22 | 30 | 0.85 | 0.65 |
| | 65-74 years | 6 | 12 | | |
| | ≥75 years | 4 | 9 | | |
| **Married** | Yes | 25 | 37 | 0.32 | 0.57 |
| | No | 7 | 14 | | |
| **Education** | Below high school | 13 | 36 | 7.66 | 0.02* |
| | High school | 15 | 13 | | |
| | Above high school | 4 | 2 | | |
| **Smoking** | Yes | 1 | 5 | 1.31 | 0.25 |
| | No | 31 | 46 | | |
| **Months on dialysis** | | 70.8 ± 55.4 | 68.3 ± 56.3 | | 0.20 |
| **Body mass index** | | 23.4 ± 3.6 | 23.8 ± 3.9 | | 0.69 |

Data are presented as number of patients in each group or mean ± SD
* P< 0.05

difference in mean time on dialysis between patients with and without depression.

Upon analysis of correlations between the BDI scores and biochemical variables, BDI scores showed a positive correlation with serum concentrations of IL-1β (P = 0.03), IL-6 (P = 0.001), TNF-α (P = 0.02), IL-6 to IL-10 ratio (P = 0.001) and a negative correlation with Hgb levels (P = 0.02) (*table 4*). There was a negative correlation between serum IL-6 levels and serum albumin and Hgb concentrations, and a positive correlation between serum IL-6 and hsCRP as well as IL-6 and ferritin levels, but these correlations did not reach statistical significance. *Figure 1* shows the correlation between BDI scores and the IL-6-to-IL-10 ratio.

Multivariate logistic regression analysis showed a direct correlation between the BDI score and serum IL-6 levels [P = 0.02, OR = 1.31 (95% CI = 1.04-1.49)], and between the BDI score and the serum concentration of TNF-α [P = 0.03, OR = 1.26 (95% CI = 1.08-1.89)].

## DISCUSSION

The findings of this study show that compared to the healthy control subjects, HD patients had higher serum IL-1β, IL-6, IL-10, TNF-α, triglyceride, and glucose concentrations, higher serum IL-1β-to-IL-10 and IL-6-to-IL-10 ratios, and lower serum HDL-cholesterol levels.

**Table 3**
Biochemical parameters of the HD patients stratified according to BDI (N = 83)

| | BDI<16 (N = 32) | BDI≥16 (N = 51) | P-value |
|---|---|---|---|
| **BDI** | 10.81 ± 4.73 | 27.88 ± 9.66 | < 0.001* |
| **IL-1β (pg/mL)** | 10.3 ± 4.4 | 11.3 ± 5.6 | 0.39 |
| **IL-6 (pg/mL)** | 5.3 ± 2.9 | 7.3 ± 2.7 | 0.003* |
| **IL-10 (pg/mL)** | 4.1 ± 2.0 | 3.7 ± 1.2 | 0.14 |
| **TNF-α (pg/mL)** | 9.6 ± 4.7 | 10.9 ± 4.6 | 0.17 |
| **hs-CRP (mg/dL)** | 4.78 ± 1.87 | 5.26 ± 2.52 | 0.31 |
| **IL-1β / IL-10** | 2.6 ± 1.4 | 3.5 ± 2.7 | 0.27 |
| **IL-6 / IL-10** | 1.3 ± 1.0 | 2.5 ± 1.6 | 0.002* |
| **TNF-α / IL-10** | 2.4 ± 1.3 | 3.2 ± 1.5 | 0.06 |
| **Haemoglobin (g/dL)** | 11.6 ± 1.6 | 10.9 ± 1.0 | 0.02* |
| **Albumin(g/dL)** | 4.5 ± 0.6 | 4.5 ± 0.7 | 0.97 |
| **Calcium (mg/dL)** | 10.1 ± 0.7 | 9.9 ± 0.8 | 0.29 |
| **Phosphorus (mg/dL)** | 7.1 ± 2.1 | 6.6 ± 1.6 | 0.28 |
| **Triglyceride (mg/dL)** | 217.3 ± 149.0 | 175.8 ± 100.1 | 0.17 |
| **Total cholesterol (mg/dL)** | 170.7 ± 52.5 | 182.3 ± 59.1 | 0.37 |
| **LDL-cholesterol (mg/dL)** | 101.3 ± 29.5 | 106.5 ± 33.9 | 0.48 |
| **HDL-cholesterol (mg/dL)** | 46.1 ± 17.1 | 40.5 ± 13.2 | 0.13 |
| **Glucose (mg/dL)** | 129.0 ± 86.7 | 127.1 ± 84.9 | 0.92 |
| **Ferritin (μg/L)** | 399.44 ± 162.26 | 484.84 ± 250.79 | 0.09 |
| **Transferrin (mg/dL)** | 291.1 ± 90.8 | 313.4 ± 9.7 | 0.28 |
| **iPTH (ng/L)** | 463.6 ± 366.1 | 532.3 ± 572.3 | 0.14 |
| **Uric acid (mg/dL)** | 7.9 ± 1.4 | 7.9 ± 2.1 | 0.94 |
| **Kt/v urea** | 1.5 ± 0.4 | 1.4 ± 0.5 | 0.42 |

Data are presented as mean ± SD
* P< 0.05

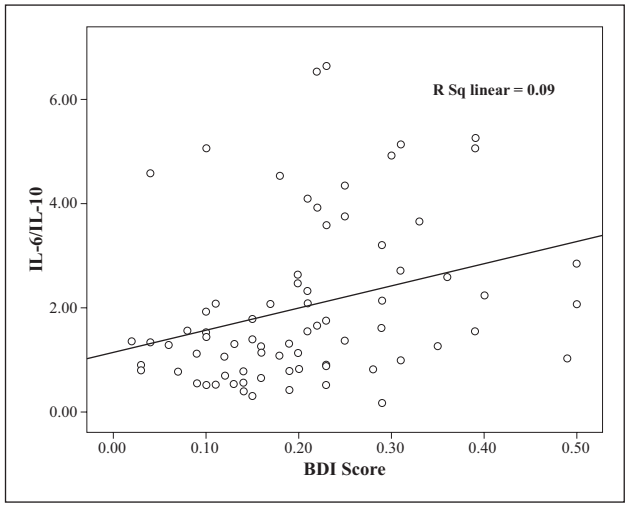


**Figure 1**
Correlation between global BDI and IL-6-to-IL-10 ratio in HD patients.

In addition, we found that serum levels of IL-1β, IL-6, TNF-α, and the IL-6-to-IL-10 ratio correlated positively and serum Hgb concentration correlated negatively with depression in HD patients. Although HD patients with depression had lower serum IL-10 levels and a BDI score that showed a negative correlation with serum IL-10 concentration, these results did not reach statistical significance. Multivariate logistic regression analysis showed direct correlations between depression and serum concentrations of IL-6 and TNF-α as independent factors in predicting depression in our HD patients.

Consistent with previous studies showing a 30-60% prevalence of depression in HD patients [1, 2], we found that 61.4% of our patients suffered from symptoms of depression. At the time of this study, none of the HD patients included were on antidepressant medications.

Advanced chronic kidney disease and dialysis are states that are known to be associated with chronic inflammation, as demonstrated by either increased levels of various pro-inflammatory cytokines or increased levels of positive acute-phase proteins [7, 21, 22]. Depression is independently associated with increased risks of morbidity and mortality [3, 4, 23, 24].

Proinflammatory cytokines have been shown to induce stress reactive neuroendocrine and central neurotransmitter changes reminiscent of those in depression [25], and it has been demonstrated that immunotherapy with IFN-α can precipitate depression [26].

Our results showed that serum IL-6 levels were significantly higher in HD patients than in the control group, and in HD patients with depression than in those without depression. The serum IL-6 concentration was positively associated with the severity of depression. In agreement

**Table 4**
Correlates of BDI and multiple variance in HD patients

| Variable | Correlation coefficient [a] | *P*-value |
|---|---|---|
| **Time on dialysis (months)** | -0.141 | 0.20 |
| **Haemoglobin (g/dL)** | -0.251 | **0.02*** |
| **Albumin (g/dL)** | -0.037 | 0.74 |
| **Total cholesterol (mg/dL)** | -0.206 | 0.07 |
| **LDL-cholesterol (mg/dL)** | -0.102 | 0.36 |
| **HDL-cholesterol (mg/dL)** | 0.085 | 0.45 |
| **Triglyceride(mg/dL)** | -0.134 | 0.08 |
| **Glucose (mg/dL)** | -0.025 | 0.82 |
| **Uric acid (mg/dL)** | 0.202 | 0.88 |
| **Ferritin (μg/L)** | 0.049 | 0.65 |
| **Transferrin (mg/dL)** | 0.054 | 0.77 |
| **iPTH (ng/L)** | -0.174 | 0.11 |
| **Calcium (mg/dL)** | -0.122 | 0.27 |
| **Phosphorus (mg/dL)** | -0.188 | 0.08 |
| **Kt/v urea** | -0.001 | 0.99 |
| **IL-1β (pg/mL)** | 0.236 | **0.03*** |
| **IL-6 (pg/mL)** | 0.370 | **0.001*** |
| **IL-10 (pg/mL)** | -0.067 | 0.07 |
| **TNF-α (pg/mL)** | 0.123 | **0.02*** |
| **hs-CRP (mg/dL)** | 0.096 | 0.38 |
| **IL-1β / IL-10** | 0.181 | 0.12 |
| **IL-6 / IL-10** | 0.375 | **0.001*** |
| **TNF-α / IL-10** | 0.203 | 0.06 |

[a]Pearson's correlation for IL-1β, IL-10, kt/v, phosphorus, LDL-cholestrol, total cholestrol, hs-CRP, haemoglobin, calcium, transferrin and albumin and Spearman's correlation for remains variables.
* P< 0.05

with our findings, Kalender *et al.* and Hung *et al.* reported the increase in IL-6 production in maintenance HD patients with depressive symptoms [12, 13]. Conversely, one study by Montinaro *et al.* and two studies by Dervisoglu *et al.* revealed that serum IL-6 levels were not significantly different among CKD and dialysis patients with and without depression [16-18]. Bossola *et al.* also found a correlation between IL-6 levels and fatigue, but not depression in HD patients [27]. The reason for the discrepancy between these studies is unclear and maybe due to a different sample size or the presence of different types of depression in HD patients. Nevertheless, our results suggest that IL-6 should be considered as an important mediator associated with depression in HD patients.

As previously mentioned, depression is associated with HD patient mortality [1-3]. Rao *et al.'s* study showed that plasma IL-6 levels are strongly associated with comorbidity in HD patients and are a powerful predictor of cardiovascular and all-cause mortality. [28]. Clinical studies have shown that increased circulating IL-6 is independently associated with progressive carotid atherosclerosis and coronary calcification in dialysis patients [29, 30]. Therefore, IL-6 may be the possible link between depression and morbidity and mortality in HD patients.

Sonikin *et al.* suggested that IL-6 is the only pro-inflammatory cytokines that positively and significantly correlated with BDI scores in patients undergoing chronic dialysis [15]. They assessed 27 HD patients of which about one third suffered depression. However, in the present study with larger number of HD patients, we have shown that TNF-α, another pro-inflammatory mediator, also correlates positively and significantly with BDI scores in maintenance HD patients. Thus, this is the first, albeit pilot study to our knowledge that establishes a relationship between the severity of depression and serum TNF-α levels in chronic, stable HD patients. Such a significant relationship between depression severity and serum concentrations of TNF-α has been shown in stable heart failure patients [31]. It has also been reported that in non-CKD and non-dialysis patients with major depression, serum TNF-α level are higher compared to healthy controls and that serum TNF-α concentrations decreased with antidepressant treatment [32].

Compatible with the findings of Montinaro *et al.* and Cilan *et al.*, our study revealed that HD patients had higher serum IL-1β than control subjects, and that all HD patients, with or without symptoms of depression, showed comparable serum concentrations of IL-1 [16, 33]. However in contrast to Montinaro *et al.*, our study, with a larger sample size, 83 *versus* 30 patients, showed a positive correlation between serum IL-1β concentrations and the BDI score (*table 3*).

We evaluated the association between one of the most important anti-inflammatory cytokines, IL-10, and depression in HD patients. Compared to the control group, HD patients had significantly higher serum IL-10 levels, and this finding was in agreement with the results of Montinaro *et al.*.[16] The IL-1β-to-IL-10 and IL-6-to-IL-10 ratios were also higher in HD patients than control subjects, whereas the TNF-α-to-0IL-10 ratio did not differ significantly between the two groups. Despite lower serum levels of IL-10 in HD patients with depression than those without depression, the difference did not reach statistical significance. In contrast to our result, Ko *et al.* showed that among patients undergoing peritoneal dialysis, subjects with depressive symptoms had lower IL-10 levels than patients without depressive symptoms [34]. This discrepancy may be due to the different dialysis methods which were used in the two studies. For the evaluation of the balance between pro- and anti-inflammatory cytokines, we calculated the individual ratios for serum concentrations of IL-1β, IL-6 and TNF-α to IL-10. Results revealed markedly increased IL-6-to-IL-10 ratios in HD patients with depression (P = 0.002, *table 3*). The of IL-6-to-IL-10 ratio was also associated positively with depression severity in our HD patients (P = 0.001, *table 4*)

IL-10 is a pleiotropic cytokine produced by Th2-type T cells, B cells, monocytes, and macrophages that inhibits a broad array of pro-inflammatory immune responses [35, 36]. Lower serum levels of IL-10 were shown in patients with unstable coronary heart disease [37, 38]. IL-10 inhibits IL-1β and TNF-α production [39]. A dysregulation of the cytokine balance could induce depressive symptoms due to higher levels of pro-inflammatory cytokines and lower levels of anti-inflammatory cytokines. It has been reported that antidepressants may attenuate the effects of proinflammatory cytokines by increasing the

production of anti-inflammatory cytokines such as IL-10 [40-42].

Uraemia, haemodialysis, and their consequent continuous complement activation induce the synthesis and release of IL-1 and IL-6, which have physiological roles in the immune response. However, in the absence of specific pathogens, this response results in immune failure. High levels of IL-1 and IL-6 are followed by secretion of IL-10, in an attempt to limit the inflammatory response [43]. An IL-10 gene polymorphism that detects high or low producers of IL-10 explains the differences in the individual immune system function in haemodialysed patients [44]. In 93 patients with ESRD, Holtzman *et al.* showed that a genetic predisposition to produce low levels of IL-10 is significantly related to depressive symptoms [45].

Therefore, we suggest that depression in HD patients may not only be affected by pro-inflammatory cytokines such as IL-6 and TNF-α, but may also be influenced by insufficient increase of anti-inflammatory cytokines such as IL-10. An imbalance between production of pro- and anti-inflammatory cytokines may be an important factor that influences the severity of depression in this patient population.

A bi-directional relationship between depression and inflammation seems to be present [32]. It is well-known that increased levels of inflammatory mediators can contribute to depressive symptoms [46, 47]. Possible mechanisms that correlate inflammatory cytokines and depression include the role of proinflammatory cytokines (IL-2, TNF-α, and INF-γ) on the activation of serotonin-degrading enzymes that decrease tryptophan and serotonin concentrations and result in the development of depression [48]. Another supposed mechanism involves IL-6-induced hypoactivity of the hypothalamic-pituitary–adrenal axis that changes the neurohormonal balance [49].

On the other hand, suppression of inflammatory cytokine activation alone, without antidepressant therapy can improve depressive symptoms. For example, in patients with psoriatic arthritis who were treated with etanercept, a significant reduction in depressive symptoms, independent of improvement in skin or joint problems, was observed [50].

Based on previous studies, positive as well as no correlations between inflammatory cytokines and depression have been reported in maintenance HD patients. Therefore, different subtypes of depression may exist, not all of them may be causally linked to the cytokine system.

Multivariate logistic regression analysis showed direct correlations between BDI scores and serum concentrations of IL-6 and TNF-α. These results suggest that IL-6 and TNF-α may be independently associated with the development of depression in HD patients.

In contrast to Hung *et al.* [13] , our results showed that serum Hgb concentrations were significantly lower in HD patients with depressive symptoms, and this laboratory marker was negatively associated with depression ($P = 0.02$, *table 4*). As seen (*table 3*), HD patients with and without depression had comparable serum transferrin saturation and ferritin. All patients in this study had access to erythropoietin and iron sucrose, free of charge as costs were covered by government insurance. Therefore, lower Hgb concentrations in HD patients with depression may be the result of resistant anemia due to the more pronounced inflammatory states in these patients. Animal and human studies have suggested a possible role of IL-6 and TNF-α in both erythropoiesis and the response to erythropoietin therapy in CKD patients [51, 52]. High circulating concentrations of TNF-α and IL-1 disturb iron distribution and induce resistance to parenteral iron with no increase in the serum Hgb level [53]. Although not statistically significant, we also saw a trend toward a negative correlation between serum levels of IL-6 and Hgb in our patients.

In inflammatory states, nuclear factor kappa B (NF-κB) decreased albumin gene expression and albumin synthesis. Thus depression may be closely associated with inflammation and malnutrition [54]. Hung *et al.* [13] found significant, negative correlations between BDI score and serum albumin levels. In another study on 30 maintenance HD patients, depressed HD patients showed a non-significant lower albumin concentration trend too [16]. We found no significant differences in serum albumin concentrations between HD patients with and without depression. Because serum albumin concentration could be influenced by other factors such as nutritional status, our result was not unexpected, as no significant difference was seen between our two groups of patients with respect to BMI.

Despite the positive, statistically non-significant correlation between serum concentrations of IL-6 and hsCRP or ferritin, confirming the results of Hung *et al.* [13], we too found no significant correlation between BDI scores and these two biomarkers.

In contrast to Hung *et al.* [13], our results did not show a significant correlation between kt/v, as dialysis dosage, and the BDI score. This discrepancy in results may be due to the smaller sample size in our study.

Although HD patients with depression symptoms have a lower educational level than those without depression, similar to the study by Ibrahim and co-workers, educational level was not found to significantly influence the BDI score [55]. Because our patients had not given reliable information about personal income, we could not translate the lower educational level to lower economic status. However, because no difference was seen with respect to albumin, transferring, and BMI as markers of nutritional status between our patients with and without depression, a lower educational level might not cause lower nutritional status.

The main limitations of this study were the relatively small sample size and inclusion of patients of a single, HD center.

In conclusion, patients on maintenance HD with symptoms of depression may have higher serum IL-6 levels, a higher IL-6-to-IL-10 ratio, and lower Hgb concentrations. When treating anemia in HD patient, it is important to consider the effect of depression and the consequent cytokine pattern in response to erythropoietin therapy. Depressive symptoms correlated positively with the serum concentrations of IL-1β, IL-6, the IL-6-to-IL-10 ratio, and TNF-α in these patient populations.

It seems that it is the imbalance between pro- and anti-inflammatory cytokines that is associated with depression in HD patients rather than the absolute levels of pro-inflammatory cytokines. The possibility that attenuation of inflammation might alleviate symptoms of depression in maintenance HD patients remains to be further explored in large, clinical trials.

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