Pro-Inflammatory Cytokines in Omani Type 2 Diabetic Patients Presenting Anxiety and Depression

Masoud Al-Maskari[1*], Ahmed Al-Shukaili[2], Ali Al-Mammari[1]

[1]Department of Medicine, [2]Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

ABSTRACT

Background: The relationship of inflammatory cytokines with anxiety and depression has been reported, but their role in diabetic patients has not been fully elucidated. **Objective:** We examined whether an association between prevalence of anxiety and depression in Omani type-2 diabetic patients (n=30) and the levels of inflammatory markers such as IL-1β, TNF-α, IFN-γ and C-reactive protein (CRP) exists. **Methods:** Symptoms of anxiety and depression were screened using the Hospital Anxiety and Depression Scale (HADS) through self-rated questionnaires. IL-1β, TNF-α, IFN-γ, CRP, anti-TPO and anti-GAD65 antibodies were measured in patients' sera using commercially available ELISA assays. **Results:** In Omani type 2 diabetic patients, high prevalence of anxiety and depression along with high levels of inflammatory markers were detected. However, no correlation was observed between inflammatory markers and anxiety or depression. **Conclusion:** These results indicate that Omani type 2 diabetic patients are at great risk for developing anxiety and depression. Therefore, these complications need more care and attention. There was no association between scores of anxiety and depression with the levels of inflammatory cytokines. This may need to be elucidated in a larger cohort of patients.

Keywords: Antibodies, Anxiety, Depression, Inflammatory Markers, Type-2 Diabetes

INTRODUCTION

The relationship between inflammatory cytokines and depression has recently been reported (1-3). Two major roles of cytokines that may lead to depression and are relevant to the present study are the sensitization of stress circuits and the potential direct impairment of positive network activity. Cytokines appear to trigger two behavioural processes involved in depression: the first occurs rapidly and includes sickness behaviour, malaise

*Corresponding author: Dr. Masoud Al-Maskari, Department of Medicine, College of Medicine and Health Sciences, Sultan Qaboos University Hospital, Muscat, Oman, Tel: (+) 968 99311312, Fax: (+) 968 24141198, e-mail: mmaskari@squ.edu.om

and fatigue, the second is delayed and involves depressed mood. For example, IL1β acts through activation of the vagus nerve, which then stimulates primary and secondary projection areas including the dorsal motor complex. It also acts by stimulating macrophage-like cells in the circumventricular organs and endothelial cells of the CNS resulting in local production of cytokines and prostaglandin E2 (PGE2) (4,5). The prevalence of anxiety and depression among patients with diabetes is higher than the average within the general population. The reasons for this are not fully understood. There are two hypotheses about possible relationships: the mood disturbance as a manifestation of direct physiological effects of diabetes**,** and the mental symptoms due to the stress and strain of having a chronic somatic condition (6). It has been suggested recently that depression may precede diabetes and increase the risk of developing type 2 diabetes and its complications.

Furthermore, it has been hypothesised that type 2 diabetes is a manifestation of an ongoing acute-phase response that is primarily characterised by an increase in the concentration of inflammatory mediators such as C-reactive protein (CRP), interleukin (IL)-6 and IL1ß. Elevated levels of IL-6 were shown to increase the risk of diabetes (7-9). Inflammatory cytokines have been shown to precede the onset of type 2 diabetes and may lead to depression. Therefore, the aim of this study is to examine the prevalence of anxiety and depression in Omani type-2 diabetic patients and the association of these mood dysfunctions with the concentration of inflammatory markers such as IL-1β, TNF-α, IFN-γ and CRP.

PATIENTS AND METHODS

Thirty omani patients (22 males and 8 females, mean age 44 ± 16) newly diagnosed as type 2 diabetes based on WHO criteria (10) and 30 healthy blood donors as normal control (25 male and 5 female, mean ages 35 ± 7) were enrolled in this study. Patients attended our diabetic outpatients Clinic at Sultan Qaboos University Hospital (SQUH), and informed consent was obtained from each subject. The Medical Research and Ethics Committee (MREC) at the College of Medicine and Health Sciences, Sultan Qaboos University (SQU), approved this study.

Symptoms of depression were screened using the Hospital Anxiety and Depression Scale (HADS) (11,12). This consisted of seven items for depression (HADS-D) and seven for anxiety. Only HADS-D scores were utilized in this study and scores of 0–7 on either subscale were considered 'normal'. Whereas scores higher than 7 for each subscale were operationalized as having a tendency towards anxiety and depression, which were generated using self-rated questionnaires by each patient at the time of attending the clinic. Metabolic control was determined by HbA1c patients with values reflect the average level of blood glucose in the past 3 months. The test is widely accepted as a reliable and valid index of metabolic control. The cytokine concentration and the antibody levels against TPO (Thyroid peroxidase antibody) and GAD65 (Glutamic Acid Decarboxylase 65 antibody) were measured in patients serum using commercially available ELISA assays (R & D Systems, USA, and Binding Site, UK, respectively) according to the manufacturer's instructions.

Statistical Analysis. Analysis of the data was performed using SPSS software. Descriptive statistics and Pearson correlation were generated and reported, considering p value less than 0.05 as significant.

RESULTS

Demographic data are listed in Table 1. The Majority (25/30) of patients were having poor control of HbA1c (>7). Only 6 out of 30 (20%) patients had a depression score >7 and 10 out of 30 (33%) had an anxiety score >7.
As shown in Table 1, creatinine value was (mean ± SD) 66.8 ± 20.5; CBC values (mean ± SD) results were WCC (6.5 ± 2.5), Lymphocytes (2.4 ± 0.7) and Monocytes (0.5 ± 0.18).

Table 1. Patients characteristics (mean ± SD; n=30).

Parameter	Mean ± SD (range)
Age (Years)	47 ± 8.6 (35-62)
Weight (Kg)	78.1 ± 15.4 (51.0-122.6)
Height (Cm)	162.1 ± 8.6 (141.0-174.0)
BMI (kg/m^2)	29.7 ± 5.5 (22.9-42.2)
HBA1c (%)	8.98 ± 1.70 (6.5-13.0)
Depression score (units)	4.23 ± 2.75 (0-11)
Anxiety score (units)	5.13 ± 3.21 (0-12)
Creatinine (μmol/L)	66.8 ± 20.5
CRP (mg/L)	14.2 ± 53.9
WCC	6.5 ± 2.5
Lymphocytes	2.4 ± 0.7
Monocytes	0.50 ± 0.18
IFN-γ (pg/ml)	16.7 ± 6.3
TNF-α (pg/ml)	46.1 ± 171.6
IL-1β (pg/ml)	82.4 ± 286.8
TPO (IU/ml)	42.1 ± 87.3
GAD65 (U/ml)	117.0 ± 410.7

CRP=C-reactive protein, WCC=White Cell Count, IFN-γ=Interferon-Gamma, TNF-α=Tumor Necrosis Factor-alpha, IL-1β=Interleukin-1beta, TPO=Thyroid Peroxidase Antibody, GAD65=Glutamic Acid Decarboxylase 65 antibody

The concentration of inflammatory markers (mean ± SD) were CRP (14.2 ± 53.9), IFN-γ (16.7 ± 6.2), IL-1β (82.4 ± 286.8) and TNF-α (46.1 ± 171.6). Furthermore, the autoantibodies value of TPO and GAD65 were 41.1 ± 87.3 and 117 ± 410.7 respectively, indicated that 26.7% of patients were positive for anti-TPO antibodies and 16.7% of patients were positive for anti-GAD65 antibodies (Table 1).

As shown in Table 2, the inflammatory markers (CRP, IFN-γ, TNF-α and IL-1β), in type 2 diabetes patients are elevated compared to normal controls, but were not statistically significant.

Table 2. Profile of inflammatory markers in patients (n=30) compared to controls (n=30).

Parameter	Patients (mean ± SD)	Control (mean ± SD)
CRP (mg/L)	14.2 ± 53.9	4.0 ± 4.0
IFN-γ (pg/ml)	16.7 ± 6.2	15.5 ± 5.2
TNF-α (pg/ml)	46.1± 171.6	64.5 ± 78.2
IL1-β (pg/ml)	82.4 ± 286.8	68.0 ± 78.3

CRP=C-reactive protein, IFN-γ=Interferon-Gamma, TNF-α=Tumor Necrosis Factor-alpha, IL-1β= Interleukin-1 beta.

As shown in Table 3, depression score is significantly correlated positively with anxiety (p=0.000) and CRP (p=0.000) levels and the concentration of IL-1β is correlated positively with GAD65 (p=0.000). The inflammatory cytokines were not correlated with depression or anxiety, and there were no associations between anxiety and depression with hyperglycaemia (HBA1c) or body mass index (BMI).

Table 3. Correlation (r) of depression (D) and anxiety (A) scores with HBA1c, BMI, CRP, WCC, cytokines (IFN-γ, TNF-α and IL-1β), thyroid and diabetes antibodies (TPO and GAD65).

	D	A	HBA1c	BMI	CRP	WCC	IFN–γ	TNF-α	IL1-β	TPO	GAD65
D	---	**0.683***	0.167	-0.017	**0.570***	-0.033	-0.152	-0.009	-0.218	-0.011	-0.222
A	---	---	0.134	0.118	-0.148	0.089	-0.089	-0.084	-0.265	0.070	-0.224
HBA1c	---	---	---	0.019	-0.142	-0.221	-0.312	-0.257	-0.050	-0.181	0.000
BMI	---	---	---	---	-0.073	0.200	0.023	-0.169	-0.262	-0.010	-0.032
CRP	---	---	---	---	---	0.117	0.040	-0.051	-0.048	-0.053	-0.069
WCC	---	---	---	---	---	---	0.018	0.002	-0.183	-0.164	-0.314
IFN-γ	---	---	---	---	---	---	---	0.048	0.031	-0.102	-0.069
TNF-α	---	---	---	---	---	---	---	---	0.126	-0.132	-0.261
IL1-β	---	---	---	---	---	---	---	---	---	-0.051	**0.671***
TPO	---	---	---	---	---	---	---	---	---	---	---
GAD65	---	---	---	---	---	---	---	---	---	---	---

*p<0.05 (significant); HBA1c (Glycated hemoglobin A1c), BMI (Body mass index), CRP= C-reactive protein, WCC,White Cell Count, IFN-γ=Interferon-Gamma, TNF-α=Tumor Necrosis Factor-alpha, IL-β=Interleukin-1 beta, TPO=Thyroid Peroxidase Antibody, GAD65=Glutamic Acid Decarboxylase 65 antibody.

DISCUSSION

In this study, we showed that Omani type 2 diabetic patients have high prevalence of anxiety and depression. However, anxiety and depression scores did not correlate with inflammatory markers probably due to small number of patients. Inflammatory markers were slightly higher in Omani type 2 diabetic patients than the healthy control group. Although this finding was not statically significant it is in agreement with a previous study wich showed that patients with an elevation of inflammatory markers had a roughly threefold increased risk of developing type 2 diabetes compared with the low-level reference group (13,14).

Interestingly, our study showed that auto antibodies such TPO are significantly correlated with depression score as well as some inflammatory cytokines. Our study also showed that IL-1β was significantly correlated with anti-GAD antibodies and IFN-γ was correlated significantly with anti-TPO antibodies. To the best of our knowledge, this is the first study to describe the correlation of these inflammatory markers with the development of anti-GAD antibody or TPO antibody. Several studies have shown that IL-1β inhibits GAD65 expression both *in-situ* and in animal models. Hao & Palmer (1995) found that IL-1β dramatically inhibits GAD-65 expression, and TNF-α and IFN-γ have no effect on GAD-65 expression (15). Moreover, limitations of this study such as the small size of the studied population which is not sufficient for comparison and correlation, should be taken into consideration.

Furthermore, our data suggest that the pattern of inflammatory cytokines is important in the pathogenesis of type 2 diabetes in term of the development of autoantibodies. These findings are in agreement with the fact that inflammatory reactions depend on a group of cytokines rather than on a single one. The concept that inflammation may be involved in the pathogenesis of type 2 diabetes has been elucidated in several studies showing that sensitization of insulin signalling by salicylates is induced via inhibition of the activity of IB kinase β. IL-1β is known to the IB kinase β and might thereby induce insulin resistance (16,17).

Depression has been associated with hyperglycaemia and diabetes-related complications (18). Our data failed to show a significant correlation between hyperglycemia and depression. In addition, we were not able to show an association between inflammatory markers and the level of anxiety and depression, most likely due to the small sample size. However, we have found an inverse relationship between HbA1C (hyperglycemia) and depression score, but the association was not significant. There are contradictory literature regarding the relationship of poor glyceamic control and depression (19). Several reports indicated that depression is associated with hyperglycaemia in patients with type 1 and type 2 diabetes. Our findings are in agreement with a study, which showed no associations between depression and hyperglycaemia (20). It has been reported that several factors were correlated strongly with depression in type 2 diabetes. These factors include low level of education, physical inactivity, subjective somatic complaints, and physical impairment (21). In this study, we did not investigate such factors.

In conclusion, these results indicate that Omani type 2 diabetic patients are at great risk for developing anxiety and depression. Therefore, these complications need more care and attention. This is the first study, to our knowledge, reporting an association of IL-1β and GAD65 antibodies in Omani Type 2 diabetic patients. Finally, the poor association between anxiety and depression with inflammatory markers may require a larger sample size.

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