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The role of adhesion molecules and cytokines in patients with diabetic nephropathy.

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Abstract

Diabetic Nephropathy (DN) is one of the major microvascular complications of diabetes mellitus. There are immune disorders observed in these patients realized with the changes on the cell adhesion molecules and the cytokine levels because of various reasons. Therefore, the simultaneous determination and evaluation of adhesion molecules (sVCAM-1, sICAM, sPCAM-I, E-selectin and P-selectin) and the levels of cytokines (IL-2 and TGF-B) in patients with DN undergoing dialysis therapy for chronic renal failure was aimed in this study. The levels of all parameters were determined by immune-enzyme assay method. Notwithstanding that the values of the parameters of sVCAM, sICAM, pCAM-I, E-selectin and P-selectin were significantly higher before and after dialysis as compared to those of control group. The values of IL-2 and TGF beta-I were found to be increased as compared to those of the control group. After the comparison of the IL-2 values obtained before and after-dialysis, the values obtained after-dialysis were determined higher as compared to those of the control group and the values obtained before-dialysis. It was determined that TGF beta-I values obtained after dialysis are decrease in the values obtained before-dialysis. In our study, it was determined that there is an increase in all values obtained after-dialysis as compared to those obtained before-dialysis. Our study reveals the relation between the dialysis and the increase obtained the parameters studied. Changes occurring in adhesion and cytokine levels of patients with diabetic nephropathy are important in terms predetermination of potential complications and role of dialysis treatment.

Keywords: Diabetic nephropathy, Chronic renal failure, Haemodialysis, Cell adhesion molecules, Cytokines.

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Introduction

The diabetic nephropathy, which is one of the microvascular complications of diabetes mellitus, is a disorder characterized by the continuous proteinuria, decrease on the glomerular hypertension function and cardiovascular morbidity [1]. Diabetic nephropathy is particularly the major cause of morbidity and mortality in the case of type 1 and type 2 diabetes, and it is one of the leading causes of end-stage renal failure disease [2,3].

Several mechanisms are put forward in the development of diabetic glomerulosclerosis. The most widely accepted one is the non-enzymatic effects (Maillard reaction) of glucose and amino groups and the glycation of glomerular matrix proteins (oxidation) [4-6]. After this binding, intracellular signalling pathways are stimulated and thus resulted in the ROS formation and the release of cytokine and growth factor with the NF-κB activation, at the end the abnormal cell proliferation and increase on matrix come into existence. It causes vascular damage by increasing the vascular permeability, procoagulant activity and the expression of adhesion molecules [7]. The increased adhesion molecules are released into the plasma. The increase on the adhesion molecules causes to the start of the microvascular events. The endothelial injury is the beginning of the microvascular events. To determine what the reason is for the start of the endothelial damage is important for the prevention of the microvascular complications [8].

The expression of adhesion molecules is increased markedly in many tissues during acute and chronic inflammatory diseases. And also, the increase of the soluble adhesion molecules in the circulation during type 2 diabetic disorders may be the indication of endothelial damage and the activation of leukocytes in the diabetic environment [9]. Adhesion molecules are considered in four categories: the integrins, the selectins, the adhesion molecules included in the immunoglobulin superfamily and the cadherins [10]. The ICAM-1, which is one of the intercellular adhesion molecules and a member of the immunoglobulin superfamily, is a molecule expressed structurally on the endothelial cells [3,11]. Vascular Cell Adhesion Molecule-1 (VCAM-1) is a molecule expressed in endothelial cells and stimulates the adhesion between the endothelial cells and the leukocytes. The Very Late Antigen Proteins (VLA) group existing in leukocytes

associated with integrins. They are participating in the activation and co-stimulation of lymphocytes through the migration of lymphocyte and leukocyte into the inflammation area [10,12]. PCAM-I is a cell-to-cell adhesion molecules particularly existing on the endothelial cells and allows to the transmigration of leukocyte from endothelial [13]. E-selectins are present in active endothelial cells and expressions of them are increased by the pro-inflammatory cytokines such as IL-1, TNF-alpha [14,15]. P-selectins, however, are found on thrombocytes and endothelial cells. The selections in this group can be stimulated by various mediators such as selectins, thrombin, histamine, protein kinase C and the complement fragments [16]. Therefore, P-selectins show the characteristic of being expressed very quickly with membrane fusion of these granules.

Cytokines are hormone-like proteins or glycoproteins that play such an important role in the communications between the cells [17]. Over the past 10 years, especially the major advances in molecular biology have enabled a better understanding of the role of cytokines in the pathogenesis of chronic renal failure. Recently, a number of studies have shown that the, cytokines particularly IL-2, IL-6 and TNF-α, plays a very important role especially in the immune-mediated glomerulonephritis and interstitial damage [18]. The growth factor TGF-β is an important polypeptide synthesized in almost all cells, and regulates the majority of cellular events [19]. The most important effects of TGF- β in the context of wound healing is the stimulation of chemotaxis of inflammatory cells and the making the extracellular matrix synthesis increased [20,21]. IL-2 enhances the proliferation of T, B lymphocytes and Natural Killer (NK) cells and the formation of cytokine. The formed IL-2 causes the proliferation of the cell by acting as autocrine [22].

The simultaneous determination and evaluation of adhesion molecules (sVCAM-1, sICAM, sPCAM-I, E-selectin and P-selectin) and the levels of cytokines (IL-2 and TGF-B) in patients with diabetic nephropathy undergoing dialysis therapy for chronic renal failure was aimed in this study. Also it aimed to demonstrate the effectiveness of haemodialysis.

Material and Methods

Material samples

The patients treated with oral antidiabetic and insulin with old and new diagnosis and followed-up in the some private dialysis centers in Corum (Turkey) were included in the investigation. The patients to whom dialysis has been applied were divided into two groups, the patients before and after dialysis applied. Total of 127 samples were included in the study. The patients and healthy individuals included in the study were grouped as follows.

Group I: Healthy control group, serum samples of 27 individuals

Group II: The serum samples taken before dialysis of 50 patients with diabetic nephropathy under the treatment of dialysis

Group III: The serum samples taken after dialysis of 50 patients with diabetic nephropathy under the treatment of dialysis

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ondokuz Mayis University Hospital Ethics Committee (OMU-KAEK 2012/107). Serum samples were taken by protecting from the light and has stored at -86ºC until the study was started [23].

The determination of the adhesion molecules levels

The Enzyme Linked Immuno Sorbent Assay (ELISA) method was utilized for the determination of the levels of ICAM-1, VCAM-1, PCAM-I, E-selectin and P-selectin molecules. The ELISA kits (Bendermed System Diagnostics, eBioscience, Austria, the number of kits in order of; BMS241 (Human sICAM-1 Platinum ELISA extra sensitive), BMS232 (Human sVCAM-1 Platinum ELISA), BMS205 (Human sE-selectin Platinum ELISA) and BMS219/4 (Human sP-selectin Platinum ELISA) with high sensitivity were used for the determination of the molecule levels were used. (Measuring wavelength: 450 nm; reference wavelength: 620 nm).

Determination of the cytokine levels

The ELISA kits (Bendermed System Diagnostics, eBioscience, Austria, the number of kits in order of; BMS221 HS (Human IL-2 High Sensitivity ELISA) and BMS249/4 (Human TGF-beta1 Platinum ELISA)) were used for the determination of the levels of IL-2 and TGF-Beta-I. (Measuring wavelength: 450 nm; reference wavelength: 620 nm).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistic 22.0. Statistical significance threshold was considered 0.05. Values are expressed as mean ± standard distribution. The Mann-Whitney U test was used for comparison of control and pre/post-dialysis. For comparison of pre and post-dialysis T test was used. Values of statistical significant are expressed in the tables.

Results

The three experimental groups containing a total of 127 serum samples were formed; Group I has consisted of serums obtained from 27 healthy individuals; Group II and III have consisted of serums obtained from 50 patients with diabetic nephropathy, who were applied haemodialysis treatment, before and after dialysis respectively. The data obtained from all patients in terms of the gender, age and the dialysis duration were given in Table 1.

The determination of the adhesion molecules level

The results of sICAM-1 of the patients with diabetic nephropathy were compared with the results obtained from the control group of healthy individuals (244.56 ± 39.27), and it was found that it was significantly higher. However, the serum levels of sICAM-1 before haemodialysis were increased to relatively higher values as compared to that of the control group, and there was no significant and meaningful decrease observed in the serum levels of sICAM-1 after haemodialysis. The values obtained after dialysis were observed higher as compared to those of obtained before dialysis and those of the control group, moreover, there was a statistically significant difference found among these three groups (p=0.001) as shown in Table 2.

Table 1. *Demographic data of patients with diabetic nephropathy undergoing dialysis treatment.*

Dialysis period (year)	DN (n:50)	Control (n=27)
1-3	17	-
4-6	16	-
7-9	11	-
10 and above	6	-
The number of patients using oral anti-diabetic drug	21	-
The number of patients using insulin	29	-
Mean Age	56-65	50-62
Sex (male/female)	28/22	17/10

The sVCAM-1 results of the patients with diabetic nephropathy were compared with those of the control group of healthy individuals (4.22 ± 0.94), and there was an increase observed. In addition, it was observed that there was a certain amount of increase on the serum levels of sVCAM-1 before haemodialysis, whereas there was no significant and meaningful decrease observed in the serum levels of sVCAM-1 after haemodialysis. However, it was observed that the values obtained after dialysis were higher as compared to those of obtained before dialysis and those of the control group. In terms of statistical evaluation, there was a statistically significant difference found among these three groups (p=0.018) as shown in Table 3.

The result of PCAM-1 of the patients with diabetic nephropathy was found higher as compared to that of control group (0.32 ± 0.06). However, the serum levels of PCAM-1 before haemodialysis (0.59 ± 0.09) were increased to relatively higher values as compared to that of the control group, and there was no significant and meaningful decrease observed in the serum levels of PCAM-1 (0.67 ± 0.11) after haemodialysis. The values obtained after dialysis were observed higher as compared to those of obtained before dialysis and those of the control group, moreover, there was a statistically significant difference found (p=0.022 as shown in Table 2.

The result of E-selectin of the patients with diabetic nephropathy was found meaningful and higher as compared to that of control group (0.69 ± 0.11). However, the serum levels of E-selectin before haemodialysis was increased to relatively higher values as compared to that of the control group, and there was no significant and meaningful decrease observed in the serum levels of E-Selectin after haemodialysis. The values obtained after dialysis were observed higher as compared to those of obtained before dialysis and those of the control group, moreover, there was a statistically meaningful result found (p=0.001) as shown in Table 2.

The P-selectin results of the patients with diabetic nephropathy were compared with those of the control group of healthy individuals (3.46 ± 1.11), and there was a meaningful and a relatively higher increase observed. In addition, it was observed that there was a certain amount of increase on the serum levels of P-selectin before haemodialysis as compared to that of control group, whereas there was no significant and meaningful decrease observed in the serum levels of P-selectin after haemodialysis. It was observed that the values obtained after dialysis were higher as compared to those of obtained before dialysis and those of the control group (p=0.001) as shown in Table 2.

Table 2. *Adhesion molecules of all groups.*

Cell Adhesion Molecules	Control (n=27)	Diabetic Nephropathy (n=50)		P values*
		Pre-	Post-	
sICAM-1	244.56 ± 39.27	399.93 ± 77.81	485.67 ± 85.77	0.001
sVCAM-1	4.22 ± 0.94	13.56 ± 6.35	17.50 ± 5.01	0.018
sPCAM-1	0.32 ± 0.06	0.59 ± 0.09	0.67 ± 0.11	0.022
E-selectin	0.69 ± 0.11	2.12 ± 0.89	2.64 ± 1.05	0.001
P-selectin	3.46 ± 1.11	8.79 ± 2.13	11.02 ± 2.30	0.001

*The mean is difference is significant at the .05 level. All parameters were measured as ng/ml.

Determination of cytokine levels

The levels of IL-2 of the patients with diabetic nephropathy were compared with those of the control group of healthy individuals (0.36 ± 0.08), and there was a meaningful increase observed. Therefore, it was observed that the values obtained after dialysis were higher as compared to those of obtained before dialysis and those of the control group (P=0.353) as shown in Table 3.

The levels of TGF-Beta I of the patients with diabetic nephropathy were compared with those of the control group of healthy individuals (3.40 ± 0.31), and there was a meaningful increase observed as compared to that of the control group. Therefore, it was observed that there was a decrease obtained after dialysis as compared to those of obtained before dialysis,

but still higher as compared to those of the control group (p=0.024) as shown in Table 3.

Table 3. Cytokines levels of all groups.

Cytokines	Control (n=27)	Diabetic Nephropathy (n=50)		P-values*
		Pre-(n:50)	Post-(n:50)	
IL-2	0.36 ± 0.08	0.66 ± 0.09	0.71 ± 0.19	0.356
TGF-Beta I	3.40 ± 0.31	8.93 ± 2.89	6.93 ± 2.12	0.024

*The mean is difference is significant at the 0.05 level. All the parameters were measured as pg/ml.

Discussion

The Diabetic Nephropathy (DN) has been a leading cause of the end-stage renal disease in developed countries [3,24]. It is also one of the major microvascular complications of diabetes mellitus and is the most important cause of kidney failure [25]. There are many cases adversely affecting the metabolism with kidney failure. One of conditions that affect the metabolism negatively in patients with diabetic nephropathy is the changes observed in the levels of some adhesion molecules and cytokine [26]. There is a pretty strong relationship observed especially between the adhesion molecules and diabetic nephropathy. There is a plenty of study reporting the increase on adhesion molecules in patients with diabetes [26,27]. There are many negative effects observed because of these molecules, levels of which changes during diabetic nephropathy, especially in the cardiovascular system [3]. The determination and evaluation of adhesion molecules (sVCAM-1, sICAM, sPCAM-I, E-selectin and P-selectin) and the levels of cytokines (IL-2 and TGF-B) in patients with diabetic nephropathy undergoing dialysis therapy for chronic renal failure was performed in this study. In this study, it was determined that the levels of sVCAM-1, sICAM-1, PCAM-I, E-selectin and P-selectin in the patient groups with diabetic nephropathy was significantly higher than those of patients in the control group. Wu et al., has determined the increase in the levels of VCAM-1 and ICAM-1 released from endothelial cells in the study performed to detect the cardiovascular risk in patients with nephropathy [26]. Filiopoulus et al., has found in a study conducted that the plasma levels of VCAM-1 was higher in the patient group before the start of dialysis session in the case of comparing that of the observed SDBY patient group with that of healthy individuals in parallel with our data [28]. The circulation of VCAM-1 was reported the observation of high concentrations in DN patient group in the study performed by Desiree Luis Rodriquez [29]. Guler et al. has thought in the study on the investigation of the relationship between the level sICAM-1 in patients with Type 2 diabetes and nephropathy that the high level of sICAM-1 values may play role in DN development [30]. Kampoli et al., has reported the relationship between the increased level of CRP, IL-6, TNF-A, VCAM-1, E-selectin and P-selection molecules and the Type I and Type II diabetic nephropathy, retinopathy and cardiovascular diseases [31]. The level of P-selectin was found as statistically meaningful in the case of comparison between healthy control group and patient group in the study performed by Wang et al., The results of which are similar with those of our study [32]. It has been stated that the progressive increase in the level of P-selectin causes the renal failure in DN patients and the increase observed in the patients with Type 2 is correlated with the DN. The 106 patients with the haemodialysis treatment and diagnosed as peripheral artery disease were incorporated in the study during which the high levels of sICAM-1 and sVCAM-1 was used as the prediction for the peripheral artery diseases in the patients with haemodialysis treatment and as a conclusion, the correlation between high levels of sVCAM-1 and sICAM-1 and these patients with haemodialysis treatment was observed [33].

In our study, the levels of IL-2 and TGF beta were investigated in order to determine the changes in the cytokine levels in consequence of haemodialysis treatment. The increase in the level of IL-2 and TGF beta was observed in the group with the haemodialysis treatment group in the case of comparison between the control group and the group with diabetic nephropathy having haemodialysis treatment. However, it was determined that the levels of IL-2 were increased, whereas the value of TGF-beta was decreased in the case of the comparison the results obtained before and after haemodialysis in patients with diabetic nephropathy. The inflammatory cytokines contribute the development and the progression of diabetic nephropathy especially IL-1, IL-6 and IL-18 and TNF-α. The concentrations of these cytokines were increased in models with diabetic nephropathy and it was observed that they have affected the disease with multiple mechanisms. Furthermore, the increased levels of these cytokines in serum and urinary are in parallel with the progression of increased albuminuria and nephropathy [34,35]. Senatorski et al., has reported that the level of TGF beta and IL-6 has been increased significantly with the development of diabetic nephropathy in patients with diabetic nephropathy and is a good prognostic factor for the determination of nephropathy [17]. The high level of all immunity markers studied as compared to that of healthy individuals is the indication of a persistent inflammation in these patients.

The indication of the potential cases to be encountered in the early diagnosis, stages of disease and treatment status of diabetic nephropathy is important by using the detected levels of the parameters investigated in this study. In addition, there are studies with the simultaneous evaluation of adhesion molecules and cytokine levels. Therefore, the study we have performed is important in terms of the simultaneous evaluation of adhesion and cytokine levels. Additionally, our study is important for the evaluation of dialysis efficiency and identification of complications that may occur in patients with diabetic nephropathy.

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