

ASSOCIATION BETWEEN THE STAGE OF DIABETIC RETINOPATHY AND THIOL/DISULFIDE HOMEOSTASIS

Diyabetik Retinopati Evresi ile Tiyo/Disülfid Homeostazi Arasındaki İlişki

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ABSTRACT

Objective: This study investigate the association between the stage of retinopathy and thiol/disulfide homeostasis in patients with type 2 diabetes.

Material and Methods: Group 1, 100 diabetic patients without retinopathy; Group 2, 115 patients with non-proliferative diabetic retinopathy(NPDR); and Group 3, 72 patients with proliferative diabetic retinopathy(PDR). Disulfide, native thiol levels (NTLs) and total thiol levels(TTLs), disulfide/NT ratio (index 1), disulfide/TT ratio (index 2) and NT/TT ratio (index 3) of the patients were evaluated. Patients were excluded if they had cardiovascular or cerebrovascular diseases, or a diagnosis of inflammatory diseases or malignancy, if they were using any medications including lipid lowering agents, vitamins, or any antioxidants, or if they had a history of alcohol intake or smoking.

Results: There was no significant difference between the groups in terms of age and gender (p=0.098 and p=0.16, respectively). No significant difference was observed between the groups with respect to disulfide levels (p=0.22). NTLs and TTLs were lowest in Group 1 and highest in Group 3. Significant differences were found in NTLs and TTLs between all groups (for NTLs, p=0.01, p=0.001 and p=0.001, respectively; for TTLs, p=0.008, p=0.001 and p=0.006, respectively). Index 1, 2 and 3 values were not significantly different between Group 1 and 2 (p=0.83, p=0.43 and p=0.43, respectively), while significant differences were observed between Group 1 and 3 and between Group 2 and 3 (for all 3 indexes, p=0.001)

Conclusion: While there was no difference between the groups in terms of disulfide levels, NTL and TTL were found to be lower as the retinopathy stage increased.

Keywords: Diabetic Retinopathy; Disulfide; Native Thiol; Total Thiol

ÖZET

Amaç: Bu çalışmada tip 2 diyabetli hastalarda retinopati evresi ile tiyo/disülfid homeostazi arasındaki ilişki araştırıldı.

Gereç ve Yöntemler: Grup 1, retinopatisi olmayan 100 diyabetik hasta; Grup 2, proliferatif olmayan diyabetik retinopatisi (NPDR) olan 115 hasta; ve Grup 3, proliferatif diyabetik retinopatisi (PDR) olan 72 hasta. Hastaların disülfid, doğal tiyo seviyeleri (NTLs) ve toplam tiyo seviyeleri (TTLs), disülfid/NT oranı (indeks 1), disülfid/TT oranı (indeks 2) ve NT/TT oranı (indeks 3) değerlendirildi. Hastalarda kardiyovasküler veya serebrovasküler hastalıklar, inflamatuvar hastalıklar veya malignite tanısı varsa, lipid düşürücü ajanlar, vitaminler veya herhangi bir antioksidan dahil olmak üzere herhangi bir ilaç kullanıyorlarsa veya alkol alımı veya sigara içme öyküsü varsa hastalar çalışma dışı bırakıldı.

Bulgular: Gruplar arasında yaş ve cinsiyet açısından anlamlı bir fark yoktu (sırasıyla p=0,098 ve p=0,16). Gruplar arasında disülfid düzeyleri açısından anlamlı bir fark gözlenmedi (p=0,22). NTL'ler ve TTL'ler Grup 1'de en düşük, Grup 3'te en yüksekti. NTL'ler ve TTL'lerde tüm gruplar arasında anlamlı farklılıklar bulundu (NTL'ler için sırasıyla p=0,01, p=0,001 ve p=0,001; TTL'ler için sırasıyla p=0,008, p=0,001 ve p=0,006). İndeks 1, 2 ve 3 değerleri Grup 1 ve 2 arasında anlamlı farklılık göstermezken (sırasıyla p=0,83, p=0,43 ve p=0,43), Grup 1 ve 3 ile Grup 2 ve 3 arasında anlamlı farklılıklar gözlenmiştir (her 3 indeks için de p=0,001)

Sonuç: Gruplar arasında disülfid düzeyleri açısından fark bulunmazken, retinopati evresi arttıkça NTL ve TTL daha düşük bulundu.

Anahtar Kelimeler: Diyabetik Retinopati; Disülfid; Nativ Tiyo; Total Tiyo

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INTRODUCTION

Diabetes mellitus (DM) is a disease associated with serious mortality and morbidity. The prevalence of DM among all age groups is estimated to increase to 4.4% by 2030. Diabetic retinopathy (DR) and cataract are complications of DM that affect vision. DR is one of the microvascular complications of DM and a threatening disease causing irreversible loss of vision (1).

Thiol/disulfide homeostasis (TDH) plays critical roles in many processes including antioxidant protection and apoptosis. This homeostasis is believed to play a critical role in the etiopathogenesis of diabetes and an imbalance in TDH triggers oxidative stress or tissue inflammation (2,3). In our study, we investigated the association between retinopathy and TDH.

MATERIALS AND METHODS

A total of 287 patients followed with the diagnosis of diabetic retinopathy associated with Type 2 DM at the ophthalmology outpatient clinic Karabük University Hospital were included in the study after ethics approval and informed consent. (KBÜ Ethic committee, 11.1.2019 date, 77192459-050.99-E.1825, No:1/6) Patients were excluded if they had cerebrovascular disease, cardiovascular diseases such as hypertension and heart failure, infections, sepsis, rheumatic disease or a diagnosis of inflammatory diseases such as malignancy, if they were using any medications including lipid lowering agents, vitamins, or antioxidants or if they had a history of alcohol intake or smoking.

A total of 287 patients were divided into 3 groups according to the retinopathy status evaluated by fundus fluorescein angiography: Group 1, 100 diabetic patients without retinopathy; Group 2, 115 patients with NPDR; and Group 3, 72 patients with PDR. Disulfide, NTLs and TTLs, index 1 calculated as disulfide/NTx100, index 2 calculated as disulfide/TTx100 and index 3 calculated as NT/TTx100 of the patients were evaluated.

Blood samples were collected into biochemistry tubes for blood thiol/disulfide analysis after 8 hours of fasting. The amount of dynamic disulfide bonds was calculated by taking half the difference between TT and NT groups. After calculating NTLs, TTLs and disulfide levels, disulfide/TT percentages, NT/TT percentages and disulfide/NT percentages were determined.

Disulfide levels, disulfide/NT and disulfide/TT ratios and NTLs/TTLs were compared.

Statistical Analysis

SPSS Version 16.0 (SPSS, Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.), The chi-square test was used to compare the groups in terms of gender, the numerical variables were found to be normally distributed by the Kolmogorov Smirnov test and the ANOVA test was used to compare the groups.

RESULTS

Group 1 consisted of 100 patients (54 males and 46 females) with a mean age of 56.7 ± 4.26 years, Group 2 consisted of 115 patients (48 males and 67 females) with a mean age of 58.53 ± 4.1 years and Group 3 consisted of 72 patients (40 males and 32 females) with a mean age of 58.59 ± 3.54 years. No statistical difference was observed between the groups in terms of age and gender. ($p=0.098$ and $p=0.16$, respectively). The mean NTL was 235.94 ± 53.93 , 213.26 ± 54.25 and 178.72 ± 63.37 $\mu\text{mol/l}$, the mean TTL was 279.81 ± 54.16 , 253.57 ± 73.70 and 223.54 ± 59.91 $\mu\text{mol/l}$ and the mean disulfide level was 21.93 ± 4.96 , 20.15 ± 13.64 and 22.41 ± 5.86 $\mu\text{mol/l}$ in Group, 1, 2 and 3, respectively (Figure 1). Index 1 was 10.12 ± 5.22 , 9.51 ± 7.37 and 15.65 ± 11.02 , index 2 was 8.17 ± 2.85 , 7.49 ± 4.21 and 11.08 ± 5.14 and index 3 was 83.64 ± 5.71 , 85.0 ± 8.42 and 77.82 ± 10.28 in Group, 1, 2 and 3, respectively (Figure 2). No statistical difference was observed between the groups in terms of disulfide levels ($p=0.22$). NT and TT values were found to be gradually lower from Groups 1 to 3. Statistically significant differences were observed between the groups in terms of NTLs ($p=0.01$). There were statistically significant differences between the groups in terms of TTLs ($p<0.05$).

Index 1 value was not significantly different between Group 1 and 2 ($p=0.83$), while statistically significant differences were observed between Group 1 and 3, Group 2 and 3 (for both comparisons, $p=0.001$). There was no statistically significant difference between Group 1 and 2 in terms of index 2 ($p=0.43$), while statistically significant differences were observed between Group 1 and 3, Group 2 and 3 (for both comparisons, $p=0.001$). Index 3 value was not significantly different

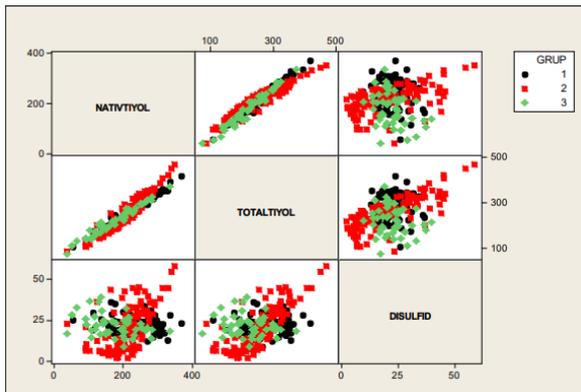


Figure 1. Mean Native Thiol Level (NNTIYOL), mean Total Thiol Level (TNTIYOL), mean Disulfide levels values

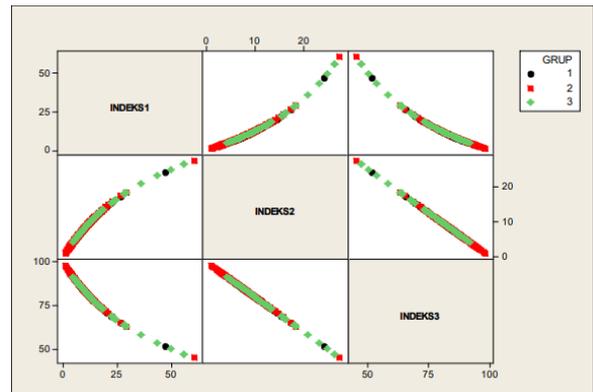


Figure 2. Index 1, Index 2, Index 3 values

between Group 1 and 2 ($p=0.43$), while statistically significant differences were found between Group 1 and 3, between Group 2 and 3 (for both comparisons, $p=0.001$). These results showed that the presence and stage of DR is inversely proportional with NTLs and TTLs. When compared to the disulfide levels, both NTLs and TTLs were lower in the PDR compared to the other two groups. In addition, NTL was lowest in the PDR group when compared to the TTLs.

DISCUSSION

A thiol plays a critical role in preventing oxidative stress. The thiol groups of sulfur-containing amino acids in proteins are oxidized by reactive oxygen radicals to form reversible disulfide bonds. Thiol/disulfide balance plays critical roles in antioxidant defense, apoptosis, enzyme activities, cellular signaling mechanisms (2). Diabetes is a chronic disorder as well as a state of increased oxidative stress. In diabetes, increased free radicals leading to structural and functional changes in proteins and genetic mutations. Moreover, in diabetes, the effects of free radicals can be overcome by the administration of exogenous antioxidants. Oxidative stress represents disruption in favor of free radicals. Many investigators emphasize that this imbalance leads to the vascular complications of diabetes (4-7). In 53 patients with cerebral venous thrombosis, thiol levels were lower, while disulfide levels were normal. A negative correlation was found between the number of thrombosed sinuses and NTLs and TTLs (8). In 31 patients with benign paroxysmal positional vertigo, index 1 and index 2 levels were significantly increased,

while NTLs and index 3 levels were decreased and these levels remained unchanged in the blood samples collected after successful treatment of vertigo (9). In 50 patients with rheumatoid arthritis, NTLs and TTLs were lower, while disulfide levels were higher, and the disease activity score correlated negatively with thiol levels and positively with disulfide levels (10). In another study conducted in 71 patients with hepatitis B-associated hepatitis, 50 patients with hepatitis B-associated cirrhosis and 45 healthy controls, NTLs and TTLs were found to be low in parallel with the degree of hepatic fibrosis (11).

In a study on 84 smoking patients, NT, TT and index 3 levels were significantly low, while index 1 and index 2 levels were significantly high. Carbon monoxide levels negatively correlated with NT, TT and index 3 levels, while positively correlated with disulfide, index 1 and index 2 levels (12). In 52 patients with Graves' ophthalmopathy (GO), NTLs were low, while disulfide and index 1 levels were high. NTLs were lower in active GO, while disulfide and index 1 levels were significantly higher in active GO. A negative correlation was found between clinical activity score and NT, whereas clinical activity score was positively correlated with index 1 (13).

In a study on diabetic patients, in all DM groups, NTLs and TTLs were low, whereas disulfide, index 1 and index 2 levels were high. The disulfide level was lower in the newly diagnosed DM group compared to the other DM groups (14). In a study 122 patients with type 2 DM without retinopathy, with NPDR and with PDR, index 1, index 2 and disulfide levels were higher

and NT, TT and index 3 levels were lower in the PDR group (15). In our study, disulfide levels were not different between the groups, while NTLs and TTLs were lower in the groups with retinopathy compared to the group without retinopathy and, again, lower in the PDR group compared to the NPDR group. TDH is severely disrupted in favor of oxidants. The limitation of our study was not evaluated by adding a healthy control group.

Our study evaluated the relationship between TDH and DR in a large series of patients in the literature. Our study is important in that it shows that in addition to the high level of oxidative stress in diabetic patients, oxidative stress increases with the increase in the stage of retinopathy. Although our results are very similar to the literature, we think that it will be useful for future studies since there are few studies on the subject.

CONCLUSION

TDH is affected to varying degrees in diabetic retinopathy patients, especially as the diabetic retinopathy stage progresses. TDH may have a critical role in the progression of diabetic retinopathy.

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