Oxygen-Dependent Bactericidal Activity of Leukocytes in Patients with Type 2 Diabetes Mellitus

Tip 2 Diyabetiklerde Lökositlerde Oksijen Bağımlı Antimikrobiyal Aktivitenin Tayini

Mustafa Kanat, Orkide Donma**, Cem Aygün*, Emine Sardoğan**, Özlem Ekmekçi**, Murat Hayri Sipahioğlu**, Reşat Özaras*

Abant İzzet Baysal University, İzzet Baysal Medical School, Department of Internal Medicine, Bolu, Turkey
*İstanbul University, Cerrahpaşa Medical School, Department of Internal Medicine, İstanbul, Turkey
**İstanbul University, Cerrahpaşa Medical School, Department of Biochemistry, İstanbul, Turkey

Abstract

Objective: In this study it is aimed to investigate the oxygen-dependent bactericidal activity of leukocytes in patients with diabetes mellitus type 2.

Materials and Methods: All the data obtained from 28 patients with diabetes mellitus were compared with 31 demographically matched control individuals. Leukocytes of patients with diabetes mellitus and healthy control group were separated from blood samples for determination of oxygen-dependent antimicrobial activity enzymes including superoxide dismutase (SOD), glutathione reductase (GSH-R), gluthatione peroxidase (GSH-Px) and catalase. As lipid peroxidation products, thiobarbituric acid reactive substances (TBARS) were measured.

Results: SOD, GSH-R, and catalase activities of patients with type 2 diabetes were not significantly different from control group ($p>0.05$). GSH-Px levels of patients with diabetes mellitus were significantly lower than control group (0.012±0.08 U/mg.pr versus 0.057±0.046 U/mg.pr, $p<0.05$). Measurements of TBARS were not different between patients with diabetes mellitus and control group (5.49±1.31 μmol/l versus 5.53±1.45 μmol/l, $p=0.905$).

Conclusions: It is difficult to suggest that in patients with diabetes mellitus the tendency to some known infections is due to alteration in oxygen-dependent bactericidal activity of leukocytes. *Turk Jem 2009; 13: 20-4*

Key words: Oxygen dependent bactericidal activity, tip 2 diabetes mellitus

Özet

Amaç: Bu çalışmada tip 2 diyabetik hastalarda lökositlerde oksijen bağımlı baktesididal aktivitenin etkilenip etkilenmediğini araştırdık.

Gereç ve Yöntemler: Çalışmaya 28 diyabetik hasta ve demografik açıdan benzer 31 normal birey alınmıştır. Çalışmaya alınan bireylerden alınan kan örneklerinden lökositler ayrıştırılmış ve oksijen bağımlı antimikrobiyal aktivitede rol oynayan enzimlerden superoxide dismutaz (SOD), glutathione reduktaz (GSH-R), gluthatione peroxidaz (GSH-Px), katalaz ile lipid peroxidation ürünü olarak, thiobarbiturik asid reaktive substans (TBARS) ölçülmüştür.

Bulgular: Tip 2 diyabetiklerde lökositlerde SOD, GSH-R, and katalaz aktiviteleri kontrol grubundan farksız bulunmuştur ($p>0.05$). GSH-Px düzeyleri ise diyabetiklerde kontrol grubuna göre daha düşük bulunmuştur (0.012±0.08 U/mg.pr'e karşı 0.057±0.046 U/mg.pr, $p<0.05$). TBARS düzeyleri ise diyabetik grupta kontrol grubundan farksız bulunmuştur (5.49±1.31 μmol/l karşı 5.53±1.45 μmol/l, $p=0.905$).

Sonuç: Diyabetik bireylerin bilinen bazı enfeksiyonlara karşı yatkın olmasının lökositlerdeki oksijen bağımlı bakterisidal aktivite ile ilişkili olduğunu söylemek zordur. *Türk Jem 2009; 13: 20-4*

Anahtar kelimeler: Oksije bağımlı bakterisidal aktivite, tip 2 diyabet

Introduction

Diabetes mellitus is a disorder characterized by altered glucose tolerance and impaired lipid and carbohydrate metabolisms. It is estimated that up to 8% of adult population has diabetes mellitus and the total number of people with diabetes mellitus is supposed to rise approximately from 191 million in 2005 to 330 million in 2025 (1,2).

Although the advent of new medical technologies and intensive medical management with insulin analogues or oral antidiabetic drugs significantly improved the outcome of complications caused

Address for Correspondence: Mustafa Kanat, MD, Abant Izzet Baysal University, Izzet Baysal Medical School, Department of Internal Medicine, Bolu, Turkey
Phone: +90 374 253 46 56 E-mail: mustafa.kanat@gmail.com **Recevied:** 07.07.2009 **Accepted:** 04.08.2009

by diabetes, it is still an important problem to handle the late onset diabetic complications which cause important morbidity and mortality. In earlier studies, it was suggested that infections played a role in increased morbidity and mortality related to diabetes mellitus (3,4). Unfortunately, most of these studies could not show any strong evidence for this idea (6-9). On the other hand, increased incidences of some infections were clearly shown among patients with diabetes mellitus (10-17).

Immunological studies conducted on individuals with diabetes mellitus revealed a substantial data that at least some of host defense mechanisms have weakened due to hyperglycemia (18,19). Among those, leukocyte chemotactic activity (20-22), leukocyte mobilization and adhesion defects (23-25), leukocyte phagocytosis and opsonization defects (26-28) and diminished bactericidal activity are frequently mentioned (29,30).

In addition to general defects of immunity, decreased immune response to infections due to microvascular or macrovascular dysfunction, urinary colonization due to retention of urine in bladder secondary to autonomous neuropathy, better growth of bacteria in urine with high glucose and some other non-immunologic or anatomic factors can also be suggested as major factors that sensitize patients with diabetes mellitus to infections (31-33).

In this study, it is aimed to investigate whether oxygen-dependent antimicrobial mechanisms are affected in individuals with diabetes mellitus. As indicators of oxygen-dependent bactericidal activity SOD, GSH-Px, GSH-R and CAT were studied, and thiobarbituric acid reactive substances (TBARS) were studied as indicators of free radical production. All the data obtained from patients with diabetes mellitus were compared with demographically matched control individuals.

Materials and Methods

The study protocol included 28 patients with diabetes mellitus and demographically matched 31 control individuals. Diabetic ketoacidosis, diabetic nephropathy, systemic infection, smoking, use of any drug that has a known effect on leukocytes (steroids, cancer drugs, immunosuppressors, interferons, etc.) or antioxidant drug use (melatonin, carnitine, vitamin drugs, Ginkgo biloba extract, allopurinol, etc.) were the criteria for exclusion from the study. For the measurement of biochemical parameters, from the individuals in patient and control groups venous blood samples were drawn after overnight fasting and were transferred into plastic tubes containing acid-citrate-dextrose (ACD) in order to separate leukocytes from other blood components e.g. erythrocytes, by dextran sedimentation method.

Method for preparation of leukocytes

Ten milliliters of anticoagulated blood collected in ACD, as the usual source of white blood cells, was placed into a 15-ml plastic centrifuge tube. Two ml of dextran solution in sodium chloride 5 g/dl, was added and mixed. The mixture was allowed to stand for 45 min. for sedimentation of cells. The supernatant was drawn off and discharged into another plastic centrifuge tube. At 4 °C, the tubes were centrifuged at 500xg for 10 min. The supernatant was drawn off and discarded (34).

Button of white blood cells was resuspended in 1 ml of cold sodium chloride, 0.9 g/dl. The cells were shock treated by adding 3 ml of ice-cold distilled water and mixed gently for 45 s, and by immediately adding 3 ml of cold sodium chloride, 1.8 g/dl and mixing, they were centrifuged at 500xg for 10 min. The supernatants were drawn off and discarded. The steps in stage 2 were repeated for second shock treatment. The specimens were stored at -80 °C until the enzyme assays were performed. The protein concentrations of each sample were determined (35).

Determination of protein, parameters of antioxidative system and lipid peroxidation.

Frozen specimens were allowed to thaw at room temperature and then the specimens were completely suspended by sonication, three bursts of 10 s each in a sonicator cup filled with ice water. Protein concentrations in specimens were determined by the method of Lowry (36). TBARS were measured using an established method after treatment with thiobarbituric acid (37). The samples, treated with an acid (TCA/TBA/HCl) solution to precipitate protein, were heated to produce a colored product, and were centrifuged. The absorbances were measured at 535 nm. The standard curve was constructed with malondialdehyde (MDA) that was generated by acid treatment of 1,1,3,3- tetrametoxypropane.

The specimens were incubated in 60 mM sodium-potassium phosphate buffer, pH 7.4, containing 65 μmol/ml H_2O_2 at 37 °C for 60 s for determination of CAT activities (38). The enzymatic reaction was stopped with 32.4 mM ammonium molybdate and the yellow complex of molybdate and H_2O_2 was measured at 405 nm, and the activities were calculated using the absorbances of the samples as well as the three different blank solutions.

Activities of GSH-R were calculated, without addition of FAD, by measuring the decrease in absorbance at 340 nm, due to oxidation of NADPH to NADP. GSH-R was determined in an assay in 0.1 M potassium phosphate buffer, pH 7.4, containing 80 mM EDTA, 7.5 mM GSSG, 2 mM NADPH (35).

Superoxide radicals generated by the employment of xanthine and xanthine oxidase react with 2-[4-iodophenyl]-3-[4-nitrophenol]-5-phenyltetrazolium chloride (INT) to form red formazan dye (39). The samples and the standards were treated with mixed substrate (0.05mM xanthine, 0.025 mM INT, and 80 U/L xanthine oxidase). The SOD activity was then measured by the degree of inhibition of this reaction.

GSH-Px catalyses the oxidation of reduced glutathione (4 mM) by cumene hydroperoxide (0.18 mM) (40). In the presence of GSH-R and NADPH (0.28 mM) the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decreases in absorbance at 340 nm were measured and GSH-Px activities were calculated.

Statistical analysis

Data collected in the study were analyzed using SPSS (Statistical Package for Social Sciences, version 12.0) for Windows. Results were expressed as mean±SE. Student t and Mann-Whitney U tests were used for evaluation of parametric and nonparametric data, respectively. A p value of 0.05 was considered significant.

Several parameters (oxidative and anti-oxidative) were studied together to eliminate any deviation in any one of the parameters. Since the studies may give variable results, we used TBARS which seems to have consistent and reproducible results for the calculation of power. The calculated power of the study was 80%.

Results

Demographic and biochemical characteristics of patients with diabetes mellitus and control group are summarized in Table 1. Patients in diabetes mellitus group included 13 female and 15 male patients with a mean age of 50.78±6.20 years. Control

group included 15 females and 16 males with a mean age of 50.35±5.56 years. The mean disease duration of patients with diabetes mellitus was 6.7±3.9 years. Body mass index (BMI) of patients with diabetes and control group was 28.21±3.37 kg/m^2 and 28.38±3.42 kg/m^2, respectively. Mean overnight fasting blood glucose of patients with diabetes was 178±62 mg/dl, while it was 88±7 mg/dl in control group. Glycosylated hemoglobin levels of patients with diabetes and control groups were 8.11±2.05% and 5.06±0.19%, respectively.

Mean SOD levels of patients with diabetes mellitus and control group were found to be 2.14±1.31 U/mg.pr and 2.48±1.19 U/mg.pr, respectively (p=0.289). Mean GSH-Px levels of patients with diabetes mellitus and control group were found to be 0.012±0.08 U/mg.pr and 0.057±0.046 U/mg.pr, respectively (p<0.01), (Figure 1-5) and mean GSH-R level of patients with diabetes mellitus was 1.92±1.84 U/mg.pr, while it was 2.33±1.41 U/mg.pr for control individuals (p=0.335). Catalase enzyme measurements of patients with diabetes mellitus revealed a mean value of 0.20±0.13 U/mg.pr, while it was 0.26±0.12 U/mg.pr for control group (p=0.096). Mean TBARS levels of patients with diabetes mellitus was 5.49±1.31 μmol/l and it was 5.53±1.45 μmol/l for control group (p=0.905).

Table 1. Comparison of demographic and biochemical characteristics of patients with diabetes mellitus and control group

	Patient group (n=28)	Control group: (n=31)	p value
Age (years)	50.78±6.20	50.35±5.56	NS
BMI (kg/m^2)	28.21±3.37	28.38±3.42	NS
FBG (mg/dl)	177.92±62.06	87.54±7.25	p≤0.001
HbA1c (%)	8.11±2.05	5.06±0.19	p≤0.001
SOD (U/mg.pr)	2.14±1.31	2.48±1.19	NS
GSH-Px (U/mg.pr)	0.012±0.08	0.057±0.046	p≤0.001
GSH-R (U/mg.pr)	1.92±1.84	2.33±1.41	NS
CAT (U/mg.pr)	0.20±0.13	0.26±0.12	NS
TBARS (μmol/l)	5.49±1.31	5.53±1.45	NS

NS: non-significant, BMI: Body mass index, FBG: Fasting blood glucose, SOD: Superoxide dismutase, SH-Px: Glutathion peroksidase, GSH-R: Glutathion reductase, CAT: Catalase,TBARS: Thiobarbituric acid reactive substances

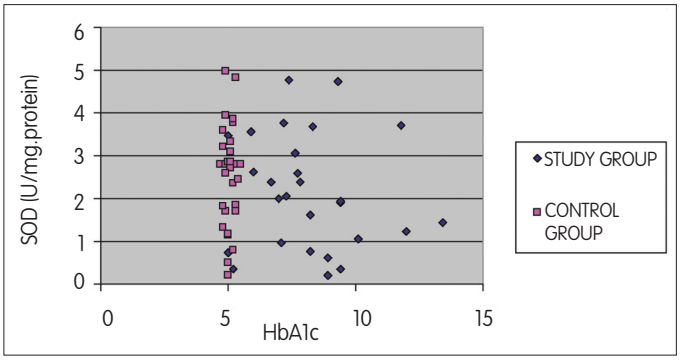

Figure 1. HbA1c levels in patients with diabetes mellitus and control group and its relationship with SOD levels

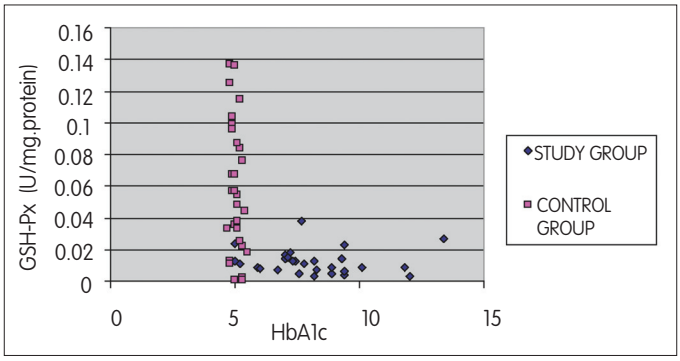

Figure 2. HbA1c levels in patients with diabetes mellitus and control group and its relationship with GSH-Px levels

Discussion

Intracellular killing process of leukocytes is conducted by oxidative or non-oxidative mechanisms. While some of the microorganism are killed with non-oxidative mechanisms, some other microor-

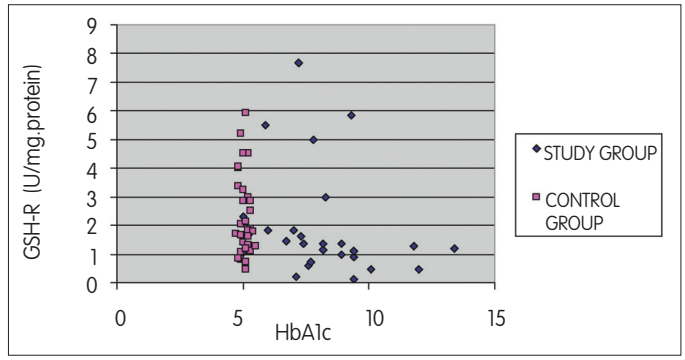

Figure 3. HbA1c levels in patients with diabetes mellitus and control group and its relationship with GSH-R levels

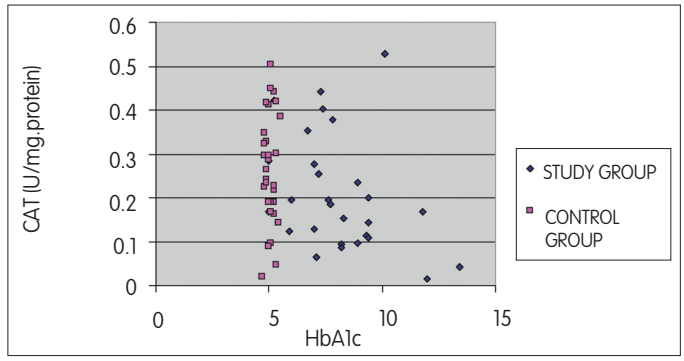

Figure 4. HbA1c levels in patients with diabetes mellitus and control group and its relationship with catalase levels

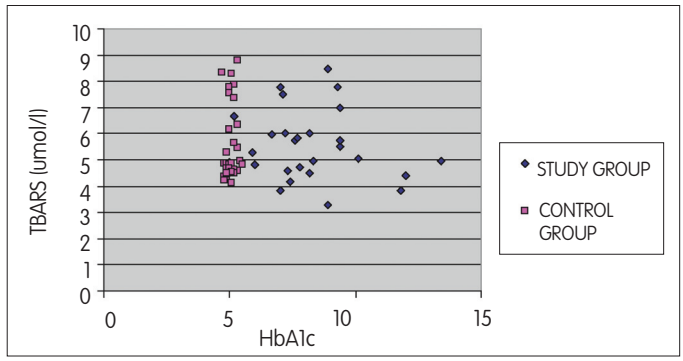

Figure 5. HbA1c levels in patients with diabetes mellitus and control group and its relationship with TBARS levels

ganism such as *Staphylococcus aureus, Escherichia coli, Serratia marcescens, Candida albicans, Aspergillus, Plasmodium, Leishmania* are killed by oxidative mechanisms which are also called "respiratory burst" (41-43). Respiratory burst is mainly characterized by a rapid increase in oxygen uptake and abrupt ROS production. ROS include reactive species such as superoxide anion, hydrogen peroxide, hydroxyl radicals and hypochlorous acid. Toxic oxygen products released after respiratory burst kill microorganisms shortly after phagocytosis (41). As an important point, in vivo biological activities of these free radicals are controlled by enzymes SOD, GSH-Px, GSH-R and CAT. Changes in the levels of these enzymes directly affect the levels of free radicals in leukocytes with the oxygen-dependent antimicrobial capacity of the cell. There is still little evidence concerning the scavenging potential of the circulating leukocytes of diabetic patients, especially regarding the well-documented impairment of immune system in diabetes mellitus and its proposed role in the development of diabetic complications. In order to be able to demonstrate the possible differences in terms of oxidative antimicrobial capacity of leukocytes between patients with diabetes mellitus and healthy individuals, the activities of those enzymes were measured in the present study. Except GSH-Px levels, we did not find a significant difference between patients with diabetes mellitus and healthy controls in terms of the levels of these enzymes that could directly affect the levels of free radicals in leukocytes with the oxygen-dependent antimicrobial capacity of the cell.

Some previous studies found SOD levels to be decreased among patients with diabetes mellitus (44,45). It was suggested that at high concentrations, glucose reacted with superoxide and as a result, there was a decline in the total amount of superoxide,,a natural substrate for SOD. As an adaptation mechanism, when the substrate levels were decreased, levels of SOD also decreased in the cell. However, it was not certainly reported in which conditions and at what concentrations glucose acted as an oxidant or an antioxidant. On the other hand, the activity of SOD was also suggested to be controlled by non-enzymatic mechanisms and therefore no change in SOD levels among patients with diabetes mellitus was also reported (46). In agreement with this hypothesis, in the present study we did not find any difference in terms of SOD levels between patients with diabetes mellitus and healthy individuals (2.14±1.31 U/mg.pr vs. 2.48±1.19 U/mg. pr, p=0.289).

For its activity, GSH-Px needs glutathione as a coenzyme and since its levels are known to decrease in patients with diabetes mellitus, levels of the enzyme may also be expected to decrease (44). GSH-Px is already shown to be susceptible to non-enzymatic glycation, and it may be inactivated under conditions of severe oxidative stress (47,48). Inactivation of GSH-Px by peroxynitrite and inactivation of GSH-Px in erythrocytes obtained from patients with type 2 diabetes mellitus when incubated with H_2O_2 were also shown previously (49,50). In our study, GSH-Px levels decreased accordingly, which could be considered as a sign of increased oxidative stress, although some other studies reported opposite findings (46).

GSH-R is responsible for the reduction of oxidized glutathione and needs NADPH as a cofactor. In patients with diabetes mellitus, due to increased NADPH poliol pathway and decreased regeneration of NADPH by pentose phosphate pathway the enzyme might be less effective. However, in our study we could not find any difference in terms of GSH-R levels between patients with diabetes mellitus and healthy individuals (1.92±1.84 U/mg.pr vs 2.33±1.41 U/mg.pr, p=0.335). In diabetes mellitus patients, CAT activities were previously shown to be unchanged (44). Similarly, in our study, catalase activity of patients with diabetes mellitus was found to be unaffected either. Additionally, we did not find any difference between patients with diabetes mellitus and healthy individuals in terms of TBARS levels, which are considered to be the important end products of the reaction between peroxi radicals and thiobarbituric acid (44).

In conclusion, our data suggest that enzyme levels which control the oxidative capacity of leukocytes, except GSH-Px, are not changed in patients with diabetes mellitus and it is difficult to suggest that the tendency to some known infections among patients with diabetes mellitus is mainly due to change in oxidative antimicrobial mechanisms.

Acknowledgements

This work was supported by the Research Fund of The University of Istanbul (Project number: T- 883/17072000).

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-53.
2. Sicree R, Shaw JE, Zimmet PZ: The global burden of diabetes. In: Gan D, ed. Diabetes atlas,2nd ed. International Diabetes Federation, Brussels 2003, pp15-71.
3. Robbins SL, Tucker AW Jr. The cause of death in diabetes: a report of 307 autopsied cases. N Engl J Med 1944; 231: 865-8.
4. Seymour A, Phear D. The causes of death in diabetes mellitus. A study of diabetic mortality in the Royal Adelaide Hospital from 1956 to 1960. Med J Aust 1963; 1: 890-4.
5. Thornton GF. Infections and diabetes. Med Cli North Am 1971;55:931-938.
6. Wheat LJ. Infection and diabetes mellitus. Diabetes Care 1980;3:187-197.
7. Joshi N, Caputo GM, Weitekamp MR, Karchmer AW. Infections in patients with diabetes mellitus. N Engl J Med 1999; 341: 1906-12.
8. Sasaki A, Horiuchi N, Hasegawa K, Uehara M. Mortality and causes of death in type 2 diabetic patients: a long-term follow-up study in Osaka District, Japan. Diabetes Res Clin Pract 1989; 7: 33-40.
9. Kessler II. Mortality experience of diabetic patients: a twenty-six year follow-up study. Am J Med 1971; 51: 715-24.
10. Farley MM, Harvey RC, Stull T, Smith JD, Schuchat A, Wenger JD, et al. A population-based assessment of invasive disease due to group B streptococcus in nonpregnant adults. N Engl J Med 1993; 328: 1807-11.
11. Breen JD, Karchmer AW. Staphylococcus aureus infections in diabetic patients. Infect Dis Clin North Am 1995; 9: 11-24.
12. Tierney MR, Baker AS. Infections of the head and neck in diabetes mellitus. Infect Dis Clin North Am 1995; 9: 195-216.
13. Vazquez JA, Sobel JD. Fungal infections in diabetes. Infect Dis Clin North Am 1995; 9: 97-116.
14. Leibovici L, Samra Z, Konisberger H, Kalter-Leibovici O, Pitlik SD, Drucker M. Bacteremia in adult diabetic patients. Diabetes Care 1991; 14: 89-94.
15. Zaky DA, Bentley DW, Lowy K, Betts RF, Douglas RG Jr. Malignant external otitis: a severe form of otitis in diabetic patients. Am J Med 1976; 61: 298-02.
16. Telzak EE, Greenberg MS, Budnick LD, Singh T, Blum S. Diabetes mellitus: a newly described risk factor for infection from Salmonella enteritidis. J Infect Dis 1991; 164: 538-41.
17. Sentochnik DE. Deep soft-tissue infections in diabetic patients. Infect Dis Clin North Am 1995; 9: 53-64.
18. McMahon MM, Bistrian BR. Host defenses and susceptibility to infection in patients with diabetes mellitus. Infect Dis Clin North Am 1995; 9: 1-9.
19. Gallacher SJ, Thomson G, Fraser WD, Fisher BM, Gemmell CG, MacCuish AC. Neutrophil bactericidal function in diabetes mellitus: evidence for association with blood glucose control. Diabet Med 1995; 12: 916-20.
20. Naghibi M, Smith RP, Baltch AL, Gates SA, Wu DH, Hammer MC, et al. The effect of diabetes mellitus on chemotactic and bactericidal activity of human polymorphonuclear leukocytes. Diabetes Res Clin Pract 1987; 4: 27-35.
21. Molenaar DM, Palumbo PJ, Wilson WR, Ritts RE Jr. Leukocyte chemotaxis in diabetic patients and their nondiabetic first-degree relatives. Diabetes 1976; 25: 880-3.

22. Mowat AG, Baum J. Chemotaxis of polymorphonuclear leukocytes from patient with diabetes mellitus. N Engl J Med 1971; 284: 621-7.
23. Sohnle PG. Neutrophil adherence in diabetes mellitus. J Lab Clin Med 1988; 111: 263-4.
24. Bagdade JD, Walters E. Impaired granulocyte adherence in mildly diabetic patients: effects of tolazamide treatment. Diabetes 1980; 29: 309-11.
25. Bagdade JD, Stewart M, Walters E. Impaired granulocyte adherence: a reversible defect in host defense in patients with poorly controlled diabetes. Diabetes 1978; 27: 677-81.
26. Bybee JD, Rogers DE. The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus. J Lab Clin Med 1964; 64: 1-13.
27. Davidson J, Sowden JM, Fletcher J. Defective phagocytosis in insulin controlled diabetics: evidence for a reaction between glucose and opsonizing proteins. J Clin Pathol 1984; 37: 783-5.
28. Alexiewicz JM, Kumar D, Smogorzewski M, Klin M, Massry SG. Polymorphonuclear leukocytes in non-insulin-dependent diabetes mellitus: abnormalities in metabolism and function. Ann Intern Med 1995; 123: 919-24.
29. Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. Diabet Med 1997; 14: 29-34.
30. Dziatkowiak H, Kowalska M, Denys A. Phagocytic and bactericidal activity of granulocytes in diabetic children. Diabetes 1982; 31: 1041-3.
31. Goodson WH, Hunt TK. Wound healing and the diabetic patient. Surg Gynecol Obstet 1979; 149: 600-8.
32. Hosking DJ, Bennett T, Hampton JR. Diabetic autonomic neuropathy. Diabetes 1978; 27: 1043-54.
33. Murphy DP, Tan JS, File TM. Infectious complications in diabetic patients. Primary Care 1981; 8: 695-714.
34. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; 27: 77-89.
35. Burtis CA, Ashwood ER, Tietz NW. Textbook of clinical chemistry. 3rd ed. W.B. Saunders Company, Philadelphia 1999, pp1784-86.
36. Lowry OH, Rosebrough NJ, Farr AJ, Randall RJ. Protein measurement with folin-phenol reagent. J Biol Chem 1951; 193: 263-75.
37. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-10.
38. Goth L, Meszaros I, Nemeth H. Clinical study of the determination of serum catalase enzyme activity. Hung Sci Instr 1984; 57: 7-12.
39. Ransod Superoxide Dismutase. Cat. No: SD 125.
40. Ransel Glutathione Peroxidase. Cat. No: RS 505.
41. Babior MB. Oxygen-dependent microbial killing by phagocytes. New Engl J Med 1978; 298: 659-68.
42. Kellogg EW, Fridovich I. Superoxide hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 250 (1975) 8812-17.
43. Nauseef WM, Clark RA. Granulocytic phagocytes. In: Mandell GL, Bennett JE, Dolin R, ed. Principles and practice of infectious diseases. Sixth ed. Elsevier Churchill Livingstone, Philadelphia, 2005, pp. 93-117.
44. Muchova J, Liptakova A, Orszaghova Z, Garaiova I, Tison P, Carsky J, et al. Antioxidant systems in polymorphonuclear leukocytes of type II diabetes mellitus. Diabet Med 1999; 16: 74-8.
45. Uchimura K, Nagasaka A, Hayashi R, Makino M, Nagata M, Kakizawa H, et al. Changes in superoxide dismutase activities and concentration and myeloperoxidase activities in leukocytes from patients with diabetes mellitus. J Diabetes Complications 1999; 13: 264-70.
46. Akkus I, Kalak S, Vural H, Caglayan O, Menekse E, Can G, et al. Leukocytes lipid peroxidation, SOD, GSH-Px, and serum and leukocytes vitamin C levels of patients with type II diabetes mellitus. Clin Chim Acta 1996; 244: 221-7.
47. Muruganandam A, Drouillard C, Thibert RJ, Cheung RM, Draisey TF, Mutus B. Glutathione metabolic enzyme activities in diabetic platelets as a function of glycemic control. Thromb Res 1992; 67: 385-97.
48. Condell RA, Tapell AL. Evidence for suitability of glutathione peroxidase as a protective enzyme: studies of oxidative damage. Arch Biochem Biophys 1983; 223: 407-16.
49. Grzelak A, Soszynski M, Bartosz G. Inactivation of antioxidant enzymes by peroxynitrite. Scand J Clin Lab Invest 2000; 6: 53-258.
50. Dincer Y, Alademir Z, Ilkova H, Akcay T. Susceptibility of glutathione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effects of glycemic control. Clin Biochem 2002; 35: 297-301.