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Role of Oxidative DNA Damage in Pathogenesis of Diabetic Neuropathy

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***Abstract*—**Oxidative stress is considered to be the cause for onset and the progression of type 2 diabetes mellitus (T2DM) and complications including neuropathy. It is a deleterious process that can be an important mediator of damage to cell structures: protein, lipids and DNA. Data suggest that in patients with diabetes and diabetic neuropathy DNA repair is impaired, which prevents effective removal of lesions. Objective: The aim of our study was to evaluate the association of the hOGG1 (326 Ser/Cys) and XRCC1 (194 Arg/Trp, 399 Arg/Gln) gene polymorphisms whose protein is involved in the BER pathway with DNA repair efficiency in patients with diabetes type 2 and diabetic neuropathy compared to the healthy subjects. Genotypes were determined by PCR-RFLP analysis in 385 subjects, including 117 with type 2 diabetes, 56 with diabetic neuropathy and 212 with normal glucose metabolism. The polymorphisms studied include codon 326 of hOGG1 and 194, 399 of XRCC1 in the base excision repair (BER) genes. Comet assay was carried out using peripheral blood lymphocytes from the patients and controls. This test enabled the evaluation of DNA damage in cells exposed to hydrogen peroxide alone and in the combination with the endonuclease III (Nth). The results of the analysis of polymorphism were statistically examination by calculating the odds ratio (OR) and their 95% confidence intervals (95% CI) using the χ2-tests. Our data indicate that patients with diabetes mellitus type 2 (including those with neuropathy) had higher frequencies of the XRCC1 399Arg/Gln polymorphism in homozygote (GG) (OR: 1.85 [95% CI: 1.07-3.22], P=0.3) and also increased frequency of 399Gln (G) allele (OR: 1.38 [95% CI: 1.03-1.83], P=0.3). No relation to other polymorphisms with increased risk of diabetes or diabetic neuropathy. In T2DM patients complicated by neuropathy, there was less efficient repair of oxidative DNA damage induced by hydrogen peroxide in both the presence and absence of the Nth enzyme. The results of our study suggest that the XRCC1 399 Arg/Gln polymorphism is a significant risk factor of T2DM in Polish population. Obtained data suggest a decreased efficiency of DNA repair in cells from patients with diabetes and neuropathy may be associated with oxidative stress. Additionally, patients with neuropathy are characterized by even greater sensitivity to oxidative damage than patients with diabetes, which suggests participation of free radicals in the pathogenesis of neuropathy.

***Keywords*—**Diabetic neuropathy, oxidative stress, gene polymorphisms, oxidative DNA damage.

I. Introduction

DIABETES is a chronic metabolic disease whose primary symptom is elevated blood glucose levels. Type 2 diabetes (T2DM), which accounts for about 90% of all cases of hyperglycemia, is due to abnormal action of insulin in the body. There is considerable evidence that hyperglycemia causes many of the major complication of diabetes including neuropathy [1].

Neuropathies are characterized by a progressive loss of nerve fiber function. The syndromes may be grouped under two general headings: diffuse and focal neuropathies. The diffuse neuropathies: distal symmetrical sensorimotor polyneuropathy (DPN) and diabetic autonomic neuropathy (DAN) are common, usually chronic, and often progressive. In DPN, typically, the most distal parts of the extremities are affected first and progress proximally with increasing duration or severity of diabetes. The signs and symptoms of this syndrome are decreased sensation and loss of reflexes. Diabetic autonomic neuropathy is the other form of diffuse diabetic neuropathy. The autonomic nervous system is composed of nerves serving the heart, gastrointestinal system and genitourinary system. DAN can affect any of these organ systems. Until now, we do not have any effective method to prevent the development of diabetic neuropathy and therapeutic modality to prevent the progression of symptoms [2].

It is generally assumed that hyperglycemia results in an increased production of reactive oxygen species (ROS) [3]. Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress playing a key mediatory role in the development and progression of diabetes and its complications due to increased production of free radicals and inappropriate antioxidant defenses [4].

Free radicals lead to macromolecular damage. Among oxidative DNA lesion are abasic sites, single strand DNA breaks, sugar moiety modifications, and deaminated and adducted bases [5]. Accumulation of DNA damage may promote mutagenesis, human pathogenesis and loss of homeostasis. These lesions are mainly repaired by base excision repair (BER) [6].

OGG1 and XRCC1 are proteins involved in the BER pathway. hOGG1 (human 8-oxoguanine glycosylase 1) is one of the key glycosylases that recognized and removes 8-hydroxy-2-deoxyguanine [7]. The XRCC1 (X-ray Repair Cross Complementing Group 1) protein act as a scaffold for other DNA repair proteins, such as DNA glycosylases, polymerase beta and ligase III [8]. In our research we seek the relationship between free radicals that contribute to increased levels of DNA damage and DNA repair pathway regularity responsible for their removal. In this pathway is involved several proteins fulfilling different functions. There is a possibility that polymorphism in the gene encoding a specific

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protein translates into its function, which can cause inefficient repair and increased levels of free radicals, is responsible for the emergence of diabetes and loss of nerve function. We tried to evaluate the association of the *hOGG1* (326 Ser/Cys), *XRCC1* (194 Arg/Trp and 399 Arg/Gln) gene polymorphisms with diabetes type 2 and diabetic neuropathy in a Polish population, to determine the changes in repair genes that are specific to neuropathy.

Single-cell gel electrophoresis (comet assay) is a simple method for detecting DNA damage. It can be used to estimate DNA damage at the individual cell level through strand breaks and alkali-labile sites generated from oxidative stress. We tried to determine the extent of DNA damage, the effectiveness of DNA repair and sensitivity to hydrogen peroxide. Hydrogen peroxide is a factor to induce DNA damage. Comet assay in combination with the enzyme endonuclease III which converts oxidized pirymidynes into strand breaks [9], have also reported a correlation between specific oxidative DNA damage.

II. Materials and Methods

A. Study Population

For the analysis of polymorphisms the study involved 173 patients (117 with diabetes mellitus type 2 and 56 with diabetic neuropathy). Age related subjects with normal glucose metabolism and no family history of diabetes (n = 212) served as the control group. Blood samples obtained from 5 patients and 2 control group subjects were used to comet assay. Participants were enrolled from Department of Internal Medicine, Diabetology and Clinical Pharmacology of the Medical University of Lodz, Poland. All subjects included into the study were unrelated Caucasians and inhibited Lodz district, Poland. This project was approved by the Ethic Committee of the Medical University of Lodz in accordance with the Declaration of Helsinki and each person was informed of the detailed study protocol, and signed informed consent. The diagnosis of T2DM was determined clinically according to the World Health Organization 1999 criteria [10].

B. PCR-RFLP Geontyping Assays

Genomic DNA was extracted from peripheral blood samples using Taq PCR Core Kit according to the manufacturer's protocol (Qiagen, Germany). The primers used in the amplification were: *hOGG1* 326 Ser/Cys 5'-ACTGTCACTAGTCTCACCAG-3' forward and 5'-TGAATTCGGAAGGTGCTTGGGGAAT-3' reverse which generate a fragment of 200 bp; *XRCC1* 194 Arg/Trp forward 5'-GCCCCGTCCCAGGTA-3' and reverse 5'-AGCCCCAAGACCCTTTCATC-3' which generate a fragment of 292 bp; *XRCC1* 399 Arg/Gln forward 5'-TTGTGCTTTCTCTGTGTCCA-3' and reverse 5'-TCCTCCAGCCTTTTCTGATA-3' which generate a fragment of 615 bp. About 50 ng genomic DNA in a total volume 20 µl was amplified using a MultiGene Thermal Cycler (Applied Biosystems, Foster City, California, USA). The reaction mixture consisted of 20 pmol each primer, 0.2 mM each dNTPs, 2.5 mM $MgCl_2$, 1x PCR buffer, 1 U Taq DNA polymerase (Qiagen, Germany) and nuclease-free water. PCR conditions were 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for *XRCC1* or 57 °C for *hOGG1* for 30 s, 72 ◦C for 30 s and finally 10 min at 72 °C. Amplified fragments were digested with appropriate restriction endonucleases (New England Biolabs, Beverly, MA) at 37 °C for 16 hour. The digested PCR products were stained with 0.5 µg/ml ethidium bromide (Sigma, Munich, Germany) and observed under UV image system (Bio-Rad Laboratories Inc., Richmond, California, USA). The *Fnu4*HI restricted product of *hOGG1* codon 324 Ser/Ser, Ser/Cys and Cys/Cys genotypes had band sizes of 200, 200/100 and 100 bp, respectively. The *Msp*I restricted product of *XRCC1* codon 194 Arg/Arg, Arg/Trp, Trp/Trp genotypes had band sizes of 292/174, 313/292/174 and 313/174, respectively. The *Msp*I restricted product of *XRCC1* codon 399 Arg/Arg, Arg/Gln, Gln/Gln genotypes had band sizes of 374/221, 615/374/221 and 615, respectively.

C. DNA Damage and Repair

Peripheral blood lymphocytes, isolated with the use of Histopaque and centrifugation, were used in the comet assay. To examine DNA damage cells were incubated with hydrogen peroxide to give a final concentration of 10 or 20 µM, for 10 min on ice. To assess the effectiveness of DNA repair in patients and healthy subjects, as well as lymphocytes exposed to hydrogen peroxide and untreated have been washed in fresh RPMI 1640 medium (Sigma, Munich, Germany) preheated to 37 °C. Some cells from suspension were used to test immediately, then after 15, 30 and 120 min later.

D. Comet Assay

The comet assay was carried out under alkaline conditions according to Singh et.al. [11], with modifications of Klaude et.al. [12]. Cells were suspended in Low Melting Point (LMP) agarose (0.75% in PBS) and spread on microscope slides precoated with 0.5% of normal agarose. Slides were then put in a tank filled with lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris and 1% Triton X-100, pH 10) for 1 h at 4 °C, and followed by incubation in a electrophoresis buffer (0.3 M NaOH and 1 mM EDTA, pH 13) for 20 min to allow unwinding of DNA. Electrophoresis was carried out for 20 min at 0.7 V/cm (30 mA). After electrophoresis, slides were washed in neutralization buffer (0.4 M Tris, pH 7.5), dried, stained with 2 µg/ml DAPI, and covered with a coverslip.

E. Endonuclease Assay

Nth recognize oxidized pyrimidines making an incision in the DNA strands than can be detected by the alkaline comet assay. To analyze the number of Nth-sensitive DNA lesions, slides were washed three times in an NthI buffer (40 mM Hepes-KOH, 0.1 mM KCl, 0.5 mM EDTA, 0.2 mg/ml bovine serum albumin, pH 8.0). Gels were then incubated for 30 min with 1 mg/ml of NthI in this buffer at 37 °C. Further steps were made as described previously.

F. Comet Analysis

Preparations were observed under 200 x magnifications. Images of comets for analysis were obtained using a COHU 4910 camera (Cohu, Inc., San Diego, CA, USA) equipped with UV-1 filter block consist an excitation filter (359 nm) and barrier filter (461 nm) connected to a fluorescent microscope (Nikon, Tokyo, Japan). Slides were scored using personal computer-based image analysis system, Lucia-Comet v. 4.51 (Laboratory Imaging, Praha, Czech Republic). Measurements were made for 50 cells per slide and the percent of DNA in comet tail was used as a quantitative measure of the DNA damage.

G. Analysis of Polymorphisms

Allelic frequencies were estimated by the allele counting method. $\chi 2$ test was used to examine the differences in the distribution of genetic polymorphisms between cases and controls. Odds ratio (OR) and its 95% confidence interval (CI) were used to estimate the association between genotype and the risk of diabetes mellitus and diabetes neuropathy with logistic regression models. In all cases, P value of less than 0.05 was considered statistically significant. Analysis was conducted with STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA).

H. Analysis of DNA Damage

The data analyzed were the percent of DNA in comet tail for 50 cells. All the values in this study were expressed as mean ± S.E.M. Differences between mean values were tested for using the *t*-test. P-value less than 0.05 were considered statistically significant. The data were analyzed using STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA).

III. Results

A. Analysis of Polymorphisms

The basic data (± standard error) of our patients and controls are shown in Table I. No statistically significant differences were found between the cases and controls in terms of age and sex distributions. Results are summarized in Tables II, III and IV. Table II shows the distributions of allele and genotype frequencies for *hOGG1* 326 Ser/Cys and *XRCC1* 194 Arg/Trp, 399 Arg/Gln gene polymorphisms in T2DM including diabetic neuropathy cases and controls. The variant allele (G) (46%, OR: 1.38 [95% CI: 1.03-1.83], P=0.3) and genotype (GG) (26%, OR: 1.85 [95% CI: 1.07-3.22], P=0.3) frequency of *XRCC1* 399 Arg/Gln polymorphism were significantly different between cases and controls but the *hOGG1* 326 Ser/Cys and *XRCC1* 194 Arg/Trp were not (P>0.05). Table II shows the distributions of allele and genotype frequencies for *hOGG1* 326 Ser/Cys and *XRCC1* 194 Arg/Trp, 399 Arg/Gln gene polymorphisms in diabetic neuropathy cases and controls. The statistic analysis indicated that there were no differences in genotype frequencies between cases and control groups (P>0.05). Table IV shows the distributions of allele and genotype frequencies for *hOGG1* 326 Ser/Cys and *XRCC1* 194 Arg/Trp, 399 Arg/Gln gene polymorphisms in T2DM cases and diabetic neuropathy patients. The statistic analysis indicated that there were no differences in genotype frequencies between both cases. The genotype distributions of both *hOGG1* and *XRCC1* in the cases and controls were consistent with Hardy-Weinberg equilibrium.

B. DNA Damage and Repair

In the course of the study, we decided to include comet assay. The extent of DNA damage can be expressed by measuring the percent of DNA present in the tail region by comet analysis. Figures 1 presented the initial level of DNA damage (time 0) and during the repair incubation (time of 15, 30 and 120 min) after exposure to hydrogen peroxide at 10 and 20 μM in healthy controls, patients with diabetes and diabetic neuropathy. Additionally, Figure 2 shows the oxidative DNA damage caused by the action of the Nth enzyme that recognizes oxidized pyrimidines and strand breakage caused by hydrogen peroxide at 10 and 20 μM at time 0 and during the repair incubation of the same groups of people. Peripheral blood lymphocytes exposed to hydrogen peroxide at 10 and 20 μM from healthy subjects exhibiting efficient repair with completion within 2 h (P>0.05), in both the presence and absence of the Nth enzyme. In cells of patients with diabetes complete repair can be observed only after the application of hydrogen peroxide concentration of 10 μM (P>0.05), in both the presence and absence of the Nth enzyme. In the case of incubation with the damaging agent at a concentration of 20 μM there is incomplete DNA repair, in both the presence (P<0.01) and absence of the Nth enzyme (P<0.05). In lymphocytes of patients with neuropathy has not been shown effective DNA repair in cells treated with hydrogen peroxide in both concentrations and also if applicable, or no Nth enzyme (P<0.0001). Efficacy of DNA repair, measured as relative decrease in DNA damage, in patients groups was significantly lower than in the controls. During 120 min of repair incubation lymphocytes of T2DM persons removed 21% and 20% the initial damage induced by hydrogen peroxide (10 and 20 μM), 64% and 9% by hydrogen peroxide (10 and 20 μM) and Nth, the cells in persons with diabetic neuropathy 5%, 0% and 38%, 13%, whereas the cells in control 56%, 81% and 58%, 86%, respectively.

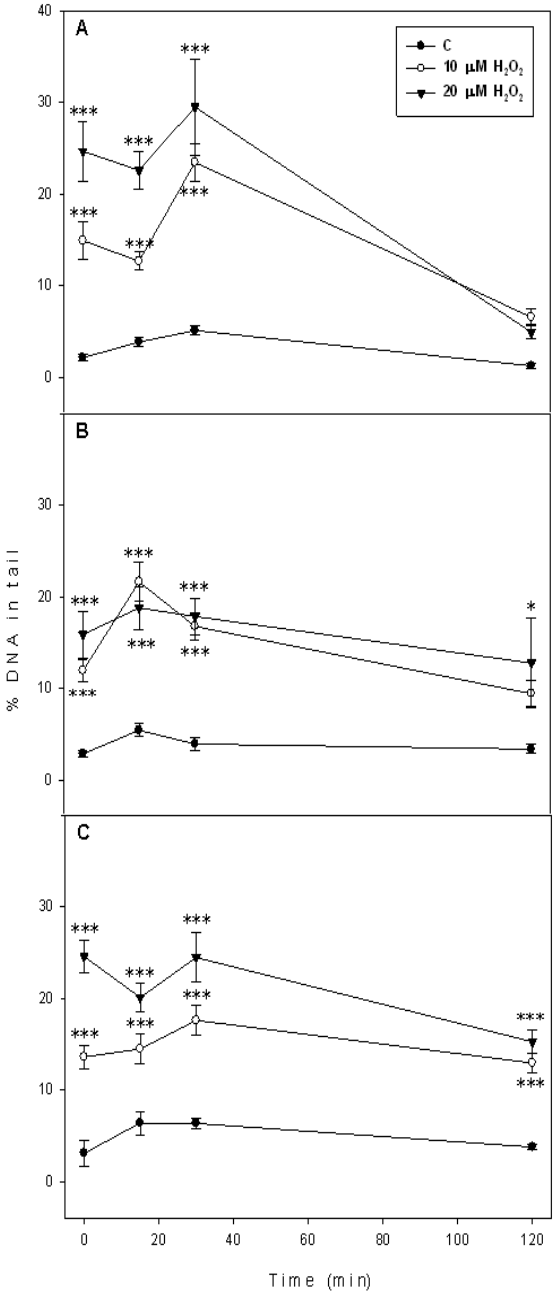

Fig. 1 DNA damage and repair in lymphocyte in the controls (A), type 2 diabetes (B) and neuropathy (C) patients. DNA damage (strand breakage) was induced by hydrogen peroxide at a concentration of 10 and 20 µM at 4 °C and measured as a percentage of the tail DNA in the alkaline comet assay. Data are expressed as means ± SEM. *P<0.05, **P<0.01, ***P<0.0001 as compared with control at the appropriate time of incubation

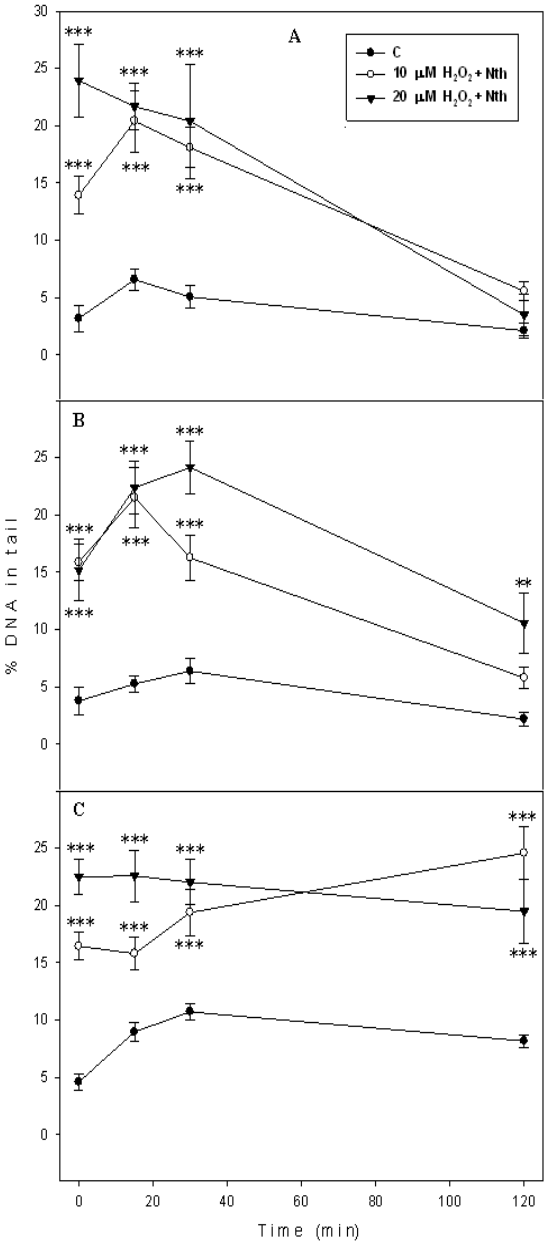

Fig. 2 DNA damage and repair in lymphocyte in the controls (A), type 2 diabetes (B) and neuropathy (C) patients. DNA damage (oxidative lesions and strand breakage) was induced by Nth enzyme and hydrogen peroxide at a concentration of 10 and 20 µM at 4 °C and measured as a percentage of the tail DNA in the alkaline comet assay. Data are expressed as means ± SEM. *P<0.05, **P<0.01, ***P<0.0001 as compared with control at the appropriate time of incubation

TABLE I
FEATURES CASES AND CONTROL SUBJECTS

	Cases			P value	
	T2DM patients (n=117)	Diabetic neuropathy patients (n=56)	Controls (n=212)	T2DM patients	Diabetic neuropathy patients
Mean age (years)	62,9±12,8	65,7 ±9,6	65,5 ± 15,9	0,912	0,995
Mean BMI	30,9 ±5,2	29,6 ± 7,9	28,9 ± 6,3	0,830	0,957
Female (%)	53 (45%)	33 (59%)	117 (55%)		

BMI – Body Mass Index; T2DM - Diabetes mellitus type 2.

TABLE II
DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES FOR GENETIC POLYMORPHISMS OF HOGG1 AND XRCC1 GENES IN DIABETES MELLITUS TYPE 2 (T2DM) INCLUDING NEUROPATHY PATIENTS AND THE CONTROLS

Polymorphism	T2DM diabetic neuropathy n=173 (%)	Controls n=212 (%)	OR (95% CI)	P
hOGG1 326 Ser/Cys				
Ser/Ser	113 (0.65)	145 (0.68)	Reference	
Ser/Cys	54 (0.31)	59 (0.28)	1.17 (0.75–1.83)	0.48
Cys/Cys	6 (0.03)	8 (0.04)	0.96 (0.32 – 2.85)	0.58
Ser	280 (0.81)	349 (0.82)	Reference	
Cys	66 (0.19)	75 (0.18)	1.10 (0.76 – 1.58)	0.62
XRCC1 194 Arg/Trp				
Arg/Arg	155 (0.90)	186 (0.88)	Reference	
Arg/Trp	18 (0.10)	26 (0.12)	0.83 (0.44–1.57)	0.57
Trp/Trp	0 (0.00)	0 (0.00)	-	
Arg	328 (0.95)	398 (0.94)	Reference	
Trp	18 (0.05)	26 (0.06)	0.84 (0.45–1.56)	0.58
XRCC1 399 Arg/Gln				
Arg/Arg	59 (0.34)	85 (0.40)	Reference	
Arg/Gln	69 (0.40)	92 (0.43)	1.08 (0.69–1.70)	0.74
Gln/Gln	45 (0.26)	35 (0.17)	1.85 (1.07-3.22)	0.03
Arg	187 (0.54)	262 (0.62)	Reference	
Gln	159 (0.46)	162 (0.38)	1.38 (1.03-1.83)	0.03

TABLE III
DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES FOR GENETIC POLYMORPHISMS OF HOGG1 AND XRCC1 GENES IN DIABETES NEUROPATHY PATIENTS AND THE CONTROLS

Polymorphism	Diabetic neuropathy n=56 (%)	Controls n=212 (%)	OR (95% CI)	P
hOGG1 326 Ser/Cys				
Ser/Ser	35 (0.63)	145 (0.68)	Reference	
Ser/Cys	19 (0.34)	59 (0.28)	1.33 (0.71-2.52)	0.37
Cys/Cys	2 (0.04)	8 (0.04)	1.04 (0.21-1.59)	0.61
Ser	89 (0.79)	349 (0.82)	Reference	
Cys	23 (0.21)	75 (0.18)	1.20 (0.71-2.03)	0.49
XRCC1 194 Arg/Trp				
Arg/Arg	52 (0.93)	186 (0.88)	Reference	
Arg/Trp	4 (0.07)	26 (0.12)	0.55 (0.18-1.64)	0.28
Trp/Trp	0 (0.00)	0 (0.00)	-	
Arg	108 (0.96)	398 (0.94)	Reference	
Trp	4 (0.04)	26 (0.06)	0.57 (0.19-1.66)	0.29
XRCC1 399 Arg/Gln				
Arg/Arg	22 (0.39)	85 (0.40)	Reference	
Arg/Gln	20 (0.36)	92 (0.43)	0.84 (0.43-1.65)	0.61
Gln/Gln	14 (0.25)	35 (0.17)	1.55 (0.71-3.36)	0.27
Arg	64 (0.57)	262 (0.62)	Reference	
Gln	48 (0.43)	162 (0.38)	1.21 (0.80-1.85)	0.37

TABLE IV
DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES FOR GENETIC POLYMORPHISMS OF HOGG1 AND XRCC1 GENES IN DIABETES NEUROPATHY PATIENTS AND THE DIABETES MELLITUS TYPE 2 (T2DM) PATIENTS

Polymorphism	Diabetic neuropathy n=56 (%)	T2DM n=117 (%)	OR (95% CI)	P
hOGG1 326 Ser/Cys				
Ser/Ser	35 (0.63)	78 (0.67)	Reference	
Ser/Cys	19 (0.34)	35 (0.30)	1.21 (0.61-2.40)	0.58
Cys/Cys	2 (0.04)	4 (0.03)	1.11 (0.19-6.37)	0.61
Ser	89 (0.79)	191 (0.82)	Reference	
Cys	23 (0.21)	43 (0.18)	1.15 (0.65-2.02)	0.37
XRCC1 194 Arg/Trp				
Arg/Arg	52 (0.93)	103 (0.88)	Reference	
Arg/Trp	4 (0.07)	14 (0.12)	0.57 (0.18-1.81)	0.33
Trp/Trp	0 (0.00)	0 (0.00)	-	
Arg	108 (0.96)	212 (0.94)	Reference	
Trp	4 (0.04)	14 (0.06)	0.56 (0.18-1.75)	0.31
XRCC1 399 Arg/Gln				
Arg/Arg	22 (0.39)	37 (0.32)	Reference	
Arg/Gln	20 (0.36)	49 (0.42)	0.69 (0.33-1.44)	0.31
Gln/Gln	14 (0.25)	31 (0.26)	0.80 (0.33-1.73)	0.43
Arg	64 (0.57)	123 (0.53)	Reference	
Gln	48 (0.43)	111 (0.47)	0.83 (0.53-1.31)	0.64

IV. DISCUSSION

Diabetes is a most common metabolic disorder and currently affecting more than 170 million individuals worldwide. Global estimates predict a further growth of almost 300 million within the next 20 years, with the greatest increases in the developing countries [13]. Type 2 diabetes the more prevalent form of hyperglycemia, results from insulin resistance and/or defective secretion by β cells, promote their damage. No active insulin contributes to imbalances in glucose metabolism and leads to the hyperglycemia. Prolonged exposure to elevated glucose induces both repeated acute changes in intracellular metabolism and cumulative long-term changes in the structure and function of macromolecules [14]. The main risk factors include genetic and environmental factors such as obesity and lack of physical activity. Hyperglycemia brings high risk of long-term, severe complications, for example neuropathy. Diabetic neuropathy causes peripheral somatic or autonomic nerve fibers dysfunction and affects most patients with diabetes [15]. A common phenomenon accompanying diabetes and its complications is oxidative stress and reduced antioxidant defense.

Elevated glucose causes oxidative stress due to increased production of mitochondrial ROS [16], nonenzymatic glycosylation reaction [17] and glucose autooxidation [18]. High levels of free fatty acids (FFA) observed under diabetic conditions contribute to production of ROS due to increased mitochondrial uncoupling [19] and β-oxidation [20]. In addition, hyperglycemia and FFA elevated oxidative stress leads to the activation of several other biochemical pathways that are associated with hyperglycemia-induced cell damage and play a significant role in the development of diabetic complications: the polyol pathway, the hexosamine pathway, protein kinase C (PKC) activation, and formation of advanced glycation end-products (AGE). Unused glucose enters the polyol pathway when aldose reductase reduces it to sorbitol. Hyperglycemia increases this enzymatic conversion. Sorbitol accumulates intracellulary, causing cell damage [21]. This process has been accompanied by decreases in NADPH and glutathione. The resulting loss of antioxidant reducing equivalents results in enhanced sensitivity to oxidative stress associated with intracellular ROS [22]. Hexosamine pathway is an additional pathway of glucose metabolism. At the beginning fructose-6-phosphate is converted to *N*-acetylglucosamine-6-phosphate by glutamine: fructose-6-phosphate amidotransferase [23]. The end product is UDP-N-acetylglucosamine. It is the substrate for the glycosylation of important intracellular factors including transcription factors. It has been suggested that the activation of this pathway leads to insulin resistance and the development of late complication of diabetes [24]. Additionally, activation of this pathway increase ROS formation [25]. Another pathway is associated with the protein kinase C. Under diabetic conditions the concentration of diacylglycerol is elevated. This is the activator of PKC [26]. Increased PKC activity arises from chronic hyperglycemia and is connected with many processes involved in the pathology of diabetic complication [27]. AGE/RAGE pathway is also associated with excessive production of reactive oxygen species. Non-enzymatic glycation process applies to proteins, DNA and phospholipids containing amino groups. This process involves the covalent binding of glucose to free amino groups. AGEs contribute to the formation of free radicals. AGE by combining with specific receptor for glycation end products (RAGE) on endothelial cells exacerbate oxidative stress [28]. AGEs are also mediators of late diabetic complications.

Oxidative stress causes damage to many cell components such as proteins, lipids and DNA. To DNA lesions include base modification, covalent binding of bases within DNA or DNA-protein cross-links, abasic sites and strand breaks. In human, oxidative DNA lesions are mainly repaired by the BER system. Proper repair of DNA is an extremely important phenomenon for cells. Any irregularity in this process may have serious consequences.

Data suggest the link between BER pathway disorder and higher risk of several diseases. Genetic variation in BER genes may alter repair function and contribute to diseases such as cancer [29] and some age-related diseases [30], [31]. Effective repair of DNA damage is based on the coordinated action of many proteins. Imbalance of the activities of those proteins will result accumulation of oxidative damage and mutation to DNA in the aging tissues [32]. In our study, we investigate the

association of *hOGG1* 326 Ser/Cys and *XRCC1* 194 Arg/Trp; 399 Arg/Gln gene polymorphisms with diabetes mellitus, diabetic neuropathy and controls with normal glucose metabolism. The protein products of these genes are involved in the BER pathway. Analysis of the *hOGG1* 326 Ser/Cys gene polymorphism in patients with T2DM has been described for the Chinese population. The study demonstrated that gene-gene interactions of the −23A>G and 326 Ser/Cys polymorphisms of the *hOGG1* gene was significantly more prevalent among Chinese patients with T2DM, whereas the relationship between 326 Ser/Cys polymorphism alone and risk of T2DM was not identified [33]. A study in the Polish population has been reported that *hOGG1* 326 Ser/Cys and *XRCC1* 399 Arg/Gln gene polymorphisms presented no risk of developing T2DM [34]. This is inconsistent with the results obtained by us, but it should be noted that the population analyzed in that study was much smaller. We observed that polymorphism in *XRCC1* 399 Arg/Gln gene may contribute to risk of T2DM in Polish population (OR: 1.07 for GG genotype). There is no connection of other polymorphisms with risk of diabetes. The relationship between genotypes analyzed by us and diabetic neuropathy susceptibility has not been investigated previously. Genotype GG obtained from diabetic neuropathy patients occurred more commonly (OR: 1.21) compared to controls, although the difference was not statistically significant. Further studies with larger populations are needed to confirm or refute this hypothesis.

Data suggest an increased cancer risk in diabetic patients, mainly in those with type 2 diabetes. The highest risk is for primary liver cancer, the average risk for pancreatic cancer and low for the colorectal, endometrial, breast, and renal cancers [35]. The cause of the association is not clear but may be connected with oxidative stress. This phenomenon may also contribute to apoptosis of nerves and loss of their functions. Increased oxidative DNA damage in T2DM patients has been demonstrated in experimental studies [36]. Alterations in mitochondrial DNA were also reported [37]. Polymorphisms in DNA repair genes, which may cause a decrease activity of proteins involved in this pathway can be the reason of the observed increase DNA damage. In addition, abnormal metabolic parameters and their correlation with DNA damage, suggest the risk of development of metabolic syndrome in diabetic group of rats but a possibility of repression by antioxidants because of their ability to counteract oxidative stress [38]. This is linked with a decrease in oxidative defense accompanying diabetes. In the present study we also tried to check susceptibility to DNA damage induced by hydrogen peroxide and the effectiveness of their repairs in freshly isolated peripheral blood lymphocytes from cases and healthy controls subjects. Complete DNA repair was observed in control exposure to hydrogen peroxide at a concentration of 10 and 20 μM after 120 minutes. Our results revealed that patients with type 2 diabetes have a decreased ability to DNA repair. Their cells subjected to incubation with the damaging agent at a concentration of 20 μM does not have complete repair after a certain time. In patients with neuropathy, it is even less than in people with T2DM because the cells of these patients after exposure to hydrogen peroxide at a concentration of both 10 μM and 20 μM do not have complete repair after a certain time. It is possible that patients with neuropathy are more sensitive to oxidative stress which is the cause of its development.

In conclusion, the present study demonstrates the association of *XRCC1* 399 Arg/Gln polymorphism with increased risk for diabetes. In addition, oxidative DNA damage in cells of diabetic patients and even more in neuropathy suggesting decreased efficacy of DNA repair mechanisms. It is closely associated with impaired oxidant-antioxidant balance. Oxidative stress may be related to the pathogenesis of diabetic neuropathy.

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