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PII: S0027-5107(15)30002-6
DOI: http://dx.doi.org/doi:10.1016/j.mrfmmm.2015.05.003
Reference: MUT 11470

To appear in: *Mutation Research*

Received date: 23-12-2014
Revised date: 1-4-2015
Accepted date: 9-5-2015

Please cite this article as: M. Cuchra, L. Markiewicz, B. Mucha, D. Pytel, K. Szymanek, J. Szemraj, J. Szaflik, J.P. Szaflik, I. Majsterek, The role of base excision repair in the development of primary open angle glaucoma in the Polish population, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* (2015), http://dx.doi.org/10.1016/j.mrfmmm.2015.05.003

The role of base excision repair in the development of primary open angle glaucoma in the Polish population

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POAG – primary open angle glaucoma
hTM – human trabecular meshwork
RGC – retinal ganglion cell
BER – base excision repair
ROS – reactive oxygen species
IOP – increase intraocular pressure
CNS – central nervous system
AP site – *the apurinic/apyrimidinic (AP) site*
OGG1 – *8-oxoguanine DNA glycosylase*
MUTYH (MYH) – A/G-specific adenine DNA glycosylase
APE1 – mammalian apurinic/apyrimidinic endonuclease Ape1
XRCC1 – *X-ray repair cross complementing group 1*
APDRT – gene (poly (ADP-ribose) polymerase 1
c/d – cup disk ratio
VF – visual field
RNFL – Retinal Nerve Fiber Layer
RA – Rim Area
NFL – Nerve fiver layer
SNP – single nucleotide polymorphism

Glaucoma is a leading cause of irreversible blindness in developing countries. Previous data have shown that progressive loss of human TM cells may be connected with chronic exposure to oxidative stress. This hypothesis may suggest a role of the base excision repair (BER) pathway of oxidative DNA damage in primary open angle glaucoma (POAG) patients. The aim of our study was to evaluate an association of BER gene polymorphism with a risk of POAG. Moreover, an association of clinical parameters was examined including cup disk ratio (c/d), rim area (RA) and retinal nerve fiber layer (RNFL) with glaucoma progression according to BER gene polymorphisms.

Our research included 412 patients with POAG and 454 healthy controls. Gene polymorphisms were analyzed by PCR-RFLP. Heidelberg Retinal Tomography (HRT) clinical parameters were also analyzed.

The 399Arg/Gln genotype of the *XRCC1* gene (OR 2.50; 95% CI 1.54-4.07, p=0.0002) was associated with an increased risk of POAG occurrence. It was indicated that the 399Gln/Gln *XRCC1* genotype might increase the risk of POAG progression according to the c/d ratio (OR 1.93; 95% CI 1-3.73, p=0.04) and RA factor (OR 3.88; 95% CI 1.01-14.82 p=0.04) clinical parameters. Moreover, the association of RNFL factor with 399Arg/Gln *XRCC1* genotype distribution and POAG progression (OR 2.46; 95% CI 1.06-5.68) was also found. Additionally, the analysis of the 324Gln/His *MUTYH* polymorphism gene distribution in the patient group according to RA factor showed that it might decrease the progression of POAG (OR 0.14; 95% CI 0.02-0.89 p=0.05).
We suggest that the 399Arg/Gln polymorphism of the *XRCC1* gene may serve as a predictive risk factor of POAG.
Keywords
primary open angle glaucoma, gene polymorphism, BER, HRT, oxidative DNA damage

Introduction

Glaucoma rates are second after cataracts among the proximate causes of blindness. The most recent epidemiological studies have shown that up to 60 million people may suffer from glaucomatous neuropathy [1,2]. Moreover, approximately 9 million people have gone blind as a result of the progressive degeneration of retinal ganglion cells (RGC) [1,2]. Additionally, Quigley et al. noted that the most frequent type of glaucoma is primary open angle glaucoma (POAG), which constitutes 75% of all diagnosed cases [3]. The genetic background of glaucoma development has yet to be completely understood. The main risk factors for POAG development and progression are intraocular pressure increases (IOP), aging (increased risk over 40), gender (men are more likely to develop glaucoma [4]), race [5], family history and diabetes mellitus type 2 [6]. Moreover, ocular parameters including myopic refractive error, optic disc shape, and corneal thickness are considered to be additional risk factors for glaucoma development [7,8]. Furthermore, oxidative stress is also perceived as an important risk factor in glaucoma pathogenesis. The occurrence of oxidative stress may play an important role in RGC death [9]. Reactive oxygen species (ROS) are generated during normal cellular metabolism, during exposure to ionizing radiation and resulting from other environmental factors [10]. Gilgun-Sherki et al. found that glial cells and neurons, which are post-mitotic cells, are very sensitive to free radical impacts [11]. Additionally, in the brain, a low level of antioxidant enzymes is observed; thus, neuronal cells are especially susceptible to giving rise to oxidative DNA lesions [12]. Izzotti et al. noted that the level of oxidative DNA damage in the human trabecular meshwork (hTM) as well as retinal cells is significantly higher among POAG patients compared to the control group. Additionally, they postulated that a progressive loss of hTM cells may be associated with long-term ROS exposure [13,14]. Moreover, an increased level of IOP and loss of the visual field correlates with elevated levels of oxidative DNA damage in hTM. Sacca et al. indicated an increased level of oxidative DNA damage in circulating lymphocytes of POAG patients [15].
The human genome possesses several mechanisms that prevent cells from accumulating DNA damage and from passing the DNA lesions to offspring cells. Recent data demonstrate that DNA repair mechanisms may play a significant role in protecting brain cell viability and nervous system function [12,16]. A strong relationship between altered DNA repair pathways and neurodegenerative disease development was observed [17]. Base excision repair (BER) is the first DNA repair mechanism that protects cells from small base modifications including alkylation, deamination and oxidation. In addition, it is expected that BER is active during all stages of the cell cycle; therefore, it may be essential for both dividing and non-dividing cells [16]. There are some results suggesting that BER may play an essential role in the development and maintenance of the central nervous system (CNS) [18].

BER follows a pathway that consists of recognition and excision of the modified base, incision of the DNA backbone, formation of AP sites (apurinic/apyrimidinic sites), creation of 3'OH groups and 5'P ends and repair synthesis and ligation [12]. In the first step, the specialized DNA glycosylase recognizes and removes the modified base. 8-Oxoguanine DNA glycosylase (OGG1) is a major human glycosylase, which causes cleavage of the glycosylic bond between the oxidase base and the sugar. This results in formation of the apurinic/apyrimidinic (AP) site [19]. It is extensively emphasized that polymorphism in the *OGG1* gene may alter glycosylase function, which in turn decreases the ability to repair DNA lesions [20]. Khono et al. indicated that individuals carrying the hOGG1-Cys326 protein have a significantly reduced ability to repair 8-OHdG compared to ones carrying the hOGG1-Ser326 protein [21]. Another important DNA glycosylase is MUTYH (MYH) (A/G-specific adenine DNA glycosylase) with the crucial role of preventing oxidative DNA damage [22]. It prevents adenine from forming mismatched bonds with 8-oxoG. Changes in the MUTYH gene may compromise its function, leading to a decrease in the DNA repair capacity [23]. AP sites that are formed in this manner may be repaired by endonuclease APE1 (*mammalian apurinic/apyrimidinic endonuclease Ape1).* APE1 incises the phosphodiester backbone of the DNA - 5' to the lesion, leaving behind the strand break with the normal 3'-hydroxyl group and a non-conventional 5'-abasic terminus [24]. It was shown that individuals carrying the 148Glu allele may have changes in DNA binding efficiency resulting from the lower ability of APE1 to interact with other BER proteins [25]. Thus, the presence of the Glu allele may lead to increased vulnerability to ionizing radiation [26]. The X-ray repair cross complementing group 1 (XRCC1) gene encoding XRCC1 - scaffold protein has no known enzymatic activity. It interacts with DNA ligase III, DNA polymerase β, APE1 and ADPRT [27]. Cappelli et al. suggested that absence of *XRCC1* may lead to a decreased level of DNA ligase III [28]. Moreover, multiple studies have shown that the presence of 399Arg/Gln and 399Gln/Gln genotypes of the *XRCC1* gene is associated with lower DNA repair capacity and increased genomic instability [29,30]. The main function of ADPRT is binding to the DNA strand breaks and recruiting the XRCC1-Lig3α complex. Deficiency in its function may be linked to the 762Val/Ala polymorphism. Moreover, the presence of the Ala allele leads to decreased poly ADP-ribosylation activity [26]. Additionally, altered activity of ADPRT is symptomatic of ailments including inflammation diseases, diabetes and neurodegeneration [31].

It is worthwhile to note that the presence of Single Nucleotide Polymorphisms (SNPs) in DNA repair genes may change the function of the proteins. It may cause genetic instability and increase the risk of developing certain diseases [32]. To the best of our knowledge, the genetic background of the BER mechanism has not yet been widely studied in relation to POAG development. Therefore, the aim of this study is to confirm the hypothesis that oxidative DNA damage and lower efficiency of its repair play an essential role in the pathogenesis of POAG.

Materials and methods

Characteristics of patients

A total of 412 patients with confirmed POAG (275 females and 148 males, mean age 73±9 years) who were hospitalized in the Department of Ophthalmology, Medical University of Warsaw, and 454 (260 females and 194 males) age-matched controls (mean age 71±12 years), who were selected from subjects without glaucoma symptoms, were enrolled in the present study. All patients and control subjects were Caucasian. The characteristics of the patients are presented in Table 1. The correct volume of IOP is indicated from the applied therapy. At the time of the study, POAG patients were treated topically with one or a combination of typical anti-glaucoma medications including beta blockers (e.g., Timolol), prostaglandin analogs

(e.g., Latanoprost), carbonic anhydrase inhibitors (e.g., Dorzolamide) and alpha2 agonists (e.g., Brimonidine). The aim of the therapy is to reduce the IOP to a level that will not lead to further damage of the optic nerve. All patients with POAG were tested by Heidelberg Retinal Tomography (HRT). In this study, we focused on the following clinical parameters: disk ratio (c/d), Rim Area (RA), Retinal Nerve Fiber Layer (RNFL) and visual field (VF) and nerve fiber layer (NFL). To analyze the progression of POAG according to the above-mentioned clinical parameters, all POAG patients were divided into appropriate groups. RA parameter patients were divided into the following groups: early POAG changes (1.26-1.39 mm^2), middle-advanced glaucomatous loss (0.87-1.26 mm^2) and advanced glaucomatous loss (<0.81 mm^2). Each group was compared to a normal RA parameter value (1.39-1.78 mm^2). To assess the relationship between progression of POAG and the RNFL parameter, the patients were also categorized into appropriate groups: early glaucomatous loss (0.181-0.210 mm), middle-advanced glaucomatous loss (0.130-0.180 mm) and advanced glaucomatous loss (<0.13 mm). Each group was compared with the normal range of the RNFL (>0.20 mm). We also combined middle-advanced glaucomatous loss and advanced glaucomatous loss compared to early glaucomatous loss. In relation to changes in the optic nerve disc, all POAG patients were divided into two groups. In the first group, patients had a c/d volume between 0.3-0.7 (early POAG changes), and in the second group, the volume of the c/d ratio was between 0.7-1.0 (advanced POAG changes). For the VF parameter, the patients were compared in three groups: early (MD>-6 dB), moderate (-6>MD>-12 dB) and advanced (MD<-12 dB) glaucomatous loss. Each clinical parameter volume range was in agreement with the guidelines of the European Glaucoma Society (Terminology and Guidelines for Glaucoma IInd Edition, Dogma, Savona 2003, Italy). The diagnosis of glaucoma is determined on the basis of intraocular pressure, optic disc appearance and loss of visual field. Additionally, the medical histories included the following information about the patient: age and family history including present or former cancer or any genetic disease.

Patients were excluded from the study if they were subject to any of the following conditions that could possibly interfere with the results of the study: use of eye drops other than anti-glaucoma medication preparations, any ocular surgeries or laser treatments performed in the past in the eye from which the specimens were to be collected, present or prior treatment with glucocorticosteroids or immunosuppressive therapy (if these treatments had not been stopped at least 1 year before the surgery and collection of specimens), use of non-steroidal anti-inflammatory drugs (with the exception of low-dose aspirin, which had to be stopped 7 days before the surgery and collection of specimens), or prior and concurrent systemic antibiotic treatment during the last 7 days before the start of the study.

The study protocol was reviewed and approved by the Local Ethics Committee of the Medical University of Warsaw and Medical University of Lodz. Written consent was obtained from each participant before enrollment in the study.

Genotype determination

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions. The genotypes of 399Arg/Gln and 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1*, 324Gln/His *MUTYH* and 762Val/Ala *ADPRT* gene polymorphisms were determined by the PCR-based restriction fragment length polymorphism (PCR-RFLP) method. For analysis of the 148Asp/Glu *APE1* gene polymorphism, PCR-CTPP was used. The PCR reactions were carried out in a 20 μl volume. Each sample contained 10 ng of genomic DNA and 1.25 U of Taq polymerase (Qiagen, Chatsworth, CA, USA) in 1x PCR buffer, 1.5 mM of $MgCl_2$, 50 µM of dNTPs, and 250 nM of each primer. The sequences, cycling conditions, reaction enzyme and product size are presented in table 2.

DNA Repair Analysis

Sample collection and lymphocyte isolation

Ten peripheral blood samples from 4 males and 6 females who were 65 years of age and diagnosed with POAG, as well as 10 peripheral blood samples from healthy controls (4 males, 6 females) who were 60 years of age, were collected into EDTA-coated vials. Lymphocytes were isolated under sterile conditions by centrifugation on a Histopaque 1077 (Sigma, Saint Louis, MO, USA). Viability of lymphocytes was determined by use of trypan blue exclusion and was >97%. The suspension, at a density of 1×10^6 cells/ml of PBS, was prepared for the comet assay.

DNA repair and comet assay

To assess the DNA repair, isolated lymphocytes were exposed to 10 μM and 20 μM H_2O_2 for 10 min at 4 °C. The control lymphocytes were incubated in H_2O_2-free RPMI 1640 medium (Sigma, Saint Louis, MO, USA). After exposure to H_2O_2, the cells were centrifuged, washed and incubated in fresh RPMI medium at 37 °C for 30, 60 and 120 min. DNA damage was assessed by alkaline single-cell gel electrophoresis (comet assay) as described by Singh et al. with modifications [33]. Each slide was analyzed with a Delta Optical fluorescence microscope connected to a camera (Jenoptik) that was equipped with an image-analysis system. The tail DNA was used as an indicator of DNA damage. To prevent additional DNA damage, all steps were conducted under dimmed light. DNA oxidation level was assessed by use of the enzyme endonuclease III (EndoIII/Nth) and Fpg. After cell lysis, the slides were washed three times with enzyme buffer, covered with 40 μl of either *Endo*III and Fpg (1 unit/gel) or buffer alone and incubated at 37 °C for 45 min. Further steps of the comet assay were performed as described above.

2.4. BER functional assay

The base excision repair functional assay was originally designed by Y. Matsumoto [34]. For the purpose of this study, the only substrate preparation step was a modified, repair reaction that was carried out according to Matsumoto et al. [34]. Following the substrate preparation procedure, pBSII plasmid was multiplied in E. coli DH5α and isolated by a Qiagen maxiprep kit (*QIAGEN Plasmid Maxi Kit*) according to the manufacturer's instructions. The vector was double digested with 2.5 U of *Xba*I and 2.5 U *Xho*II fast digest enzymes for 1 hour (*Thermo scientific* - FastDigest). SAP enzyme (*Thermo scientific*) was applied to avoid self-ligation. 5'-TCGAGAATUCGATATCA-3' and 3'-CTTAAGCTATAGTAGATC-5' were labeled in a kinase reaction of 2 U T4 kinase polynucleotide (*Thermo scientific*) with 2 μl of ATP (6000 μCi), whereas the second oligonucleotide was incubated in the same condition but with unlabeled ATP. Equal amounts of two oligonucleotides were mixed and annealed by heating samples to 95 °C and gradually cooling the samples down to room temperature. The experimentally established 1:5 (vector:insert - molar ratio) was applied to perform overnight ligation with 1 U of T4 ligase (*Thermo scientific*). The construct was purified by elution from 1% agarose gel *(GeneJET Gel Extraction Kit - Thermo scientific).* The *Minute*^{TM} Total Protein Extraction Kit (*Invent Biotechnologies*) was utilized to isolate proteins from peripheral blood lymphocytes. All protein samples were adjusted to 2 μg/ml. After the repair reaction, DNA samples were separated in polyacrylamide gel. The bands were detected by autoradiography exposition for 2 h, 4 h and overnight at -20˚C. Ethidium bromide *(BioShop)* staining was utilized to visualize load control. Optical density quantification of bands was performed by In Genius Bio Image software (Syngene Cambridge, UK).

2.4. Data analysis

Student's t-test was used to compare the differences between age and sex in the control group and treatment. The distribution of genotypes and alleles compared to the groups was tested using a χ^2 test. The odds ratios (ORs) and 95% (95% CI) confidence intervals for estimating

the associations between genotypes of *XRCC1*, *OGG1, MUTYH, ADPRT* as well as *APE1* and the risk of POAG were calculated using a logistic regression model. Hardy-Weinberg equilibrium was tested using a Chi-square test to compare the observed genotype frequency with the expected frequency among the control group. P values of less than or equal to 0.05 were considered to represent statistical significance. If all expected cell frequencies were less than 5, a one-tailed Fisher Exact Probability Test was used. Values from the comet assay in this study are expressed as the mean ± SEM of 20 separate experiments from each analyzed patient and control. If no significant difference between variations was found as assessed by the Snedecor–Fisher test, the difference between means was evaluated by applying Student's t-test. Otherwise, the Cochran–Cox test was used. All analyses were performed using STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA).

3. Results

There were no differences in average age or sex of the patients and controls (P>0.05). The observed genotype frequencies of the 399Arg/Gln *XRCC1*, 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1* and 324Gln/His *MUTYH* genes in the control group were in agreement with HWE (P=0.007, χ^2=3.17; P=0.18, χ^2=1.94; P=0.22, χ^2=1.51). The observed genotype frequency of *762Val/Ala ADPRT* as well as *148 Asp/Glu APE1* among the controls was not in accordance with HWE (P=0.001, χ^2=5.93, P=0,00001; χ^2 = 93.3).

3.1. Distribution of genotypes in the following genes: 399Arg/Gln *XRCC1*, 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1,* 324Gln/His *MUTYH*, 762Ala/Val *ADPRT* and 148Asp/Glu *APE1* among POAG patients and healthy controls

The genotype and allele distributions of 399Arg/Gln *XRCC1*, 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1*, 324Gln/His *MUTYH*, 762Ala/Val *ADPRT* and 148Asp/Glu *APE1* gene polymorphisms are summarized in table 3.

We did not observe a statistically significant difference in the frequency of the genotypes and alleles of 194Arg/Trp *XRCC1,* 326Ser/Cys *OGG1,* 324Gln/His *MUTYH,* and 762Val/Ala *ADPRT* as well as 148Asp/Glu *APE1* gene polymorphisms between POAG patients and controls.

However, we showed that the presence of the 399Arg/Gln genotype (OR 1.38 95%; 1.02-1.89, P=0.03) as well as the 399Gln allele (OR 1.23 95% 1.02-1.50, P=0.03) of *XRCC1* may be associated with an increased risk of POAG development.

3.2. Gene-Gene interaction between 399Arg/Gln *XRCC1*, 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1,* 324Gln/His *MUTYH*, 762Ala/Val *ADPRT* and 148Asp/Glu *APE1*

In the analysis of the combined effect of the studied polymorphisms, we indicated the relationship between the presence of the Arg/Gln-Arg/Arg genotype (OR 1.44; 95% 1.03-2.02, P=0.02) of the 399Arg/Gln and 194Arg/Trp *XRCC1* genes with elevated risk of POAG development. Additionally, the presence of the Arg/Gln-Ser/Cys genotype (OR 1.76; 95% 1.07-2.89, P=0.02) of the 399Arg/Gln *XRCC1* – 326Ser/Cys *OGG1* may increase the risk of POAG development. Further analysis of the combined genotype Arg/Gln-Gln/His (OR 1.60; 95% 1.00-2.58 P=0.05) of the 399Arg/Gln *XRCC1* – 324Gln/His *MUTYH* genes has indicated that the presence of these genotypes may also be associated with an increased risk of POAG occurrence. *Moreover, we have shown that the presence of the Arg/Arg-Val/Ala genotype of the 399Arg/Gln XRCC1 and 762 Val/Ala ADPRT genes may decrease the risk of POAG development (OR 0.54; 95% 0.30-0.87 P=0.04).* All data are displayed in table 4.

3.3. Distribution of genotypes of the following genes: 399Arg/Gln *XRCC1*, 194Arg/Trp *XRCC1*, 326Ser/Cys *OGG1,* 324Gln/His *MUTYH*, 762Ala/Val *ADPRT* and 148Asp/Glu *APE1* in relation to clinical parameters in the POAG patient group

The relationship between the following c/d, RA, RNFL, NFI and VF clinical parameters and the risk of POAG progression was assessed. The distributions of genotypes and allele frequencies of all studied genes in relation to the above-mentioned clinical parameters are summarized in tables 5-7. Each parameter was analyzed for the right eye and left eye separately, and then the mean value of the clinical parameters for both eyes together was calculated in relation to the studied genes.
Analysis of the relationship between the c/d ratio and POAG progression indicated that the presence of the 399Gln/Gln genotype (OR 1.67; 95% 1.07-2.61, P=0.02) as well as the 399Gln allele (OR 1.29; 95% 1.04-2.62, P=0.02) of the *XRCC1* gene may be associated with an increased risk of POAG progression. Additionally, we suggested that the presence of the 148Glu/Glu (OR 0.07; 95% 0.01-0.55, P=0.001) genotype of the *APE1* gene may be associated with a decreased risk of POAG progression. To analyze this parameter, the group of POAG patients was divided into the following subgroups: early stage and advanced stage. c/d parameters in advanced stages of POAG were compared to early glaucomatous loss. The results of the data are-presented in table 5.
The correlation of the VF parameter with the studied gene polymorphisms has shown that attendance of the 762Val/Ala genotype (OR 1.63; 95% 1.03-2.50, P=0.02) of the *ADPRT* gene is associated with increased risk of POAG progression. However, the presence of the 148 Asp/Glu genotype (OR 0.60; 95% 0.40-0.90, P=0.01, OR 0.71; 95% 0.50-0.99, P=0.04) of the *APE1* gene may be associated with a decreased risk of POAG progression. For VF parameters, POAG patients were compared using three subgroups: early, moderate and advanced glaucomatous loss. In this study, the moderate and advanced glaucomatous loss group was compared with the early glaucomatous loss group. Additionally, moderate and advanced glaucomatous loss were combined and then compared with the early glaucomatous loss group. The results of this study are displayed in table 6.
For the RNFL factor, our study involved patients with early stage glaucoma, middle-advanced glaucoma and patients with advanced glaucoma. The results of this study have shown that the presence of the 324Gln/His genotype (OR 0.47; 95% 0.30-0.82, P=0.005) of *MUTYH* as well as the 762Val/Ala genotype (OR 0.14; 95% 0.07-0.29, P=<0.0001) of the *ADPRT* gene polymorphisms may be associated with decreased risk of POAG progression. Moreover, the presence of the 148Asp/Glu (OR 2.25; 95% 1.30-3.89, P=0.003, OR 1.51; 95% 1.01-2.25, P=0.04) genotype and the 148Glu allele (OR 1.45; 95% 1.02-2.06, P=0.04) of the APE1 gene may be associated with an elevated risk of POAG progression. The results of these data are summarized in table 7.
We did not observe any relationship between the RA and NFI clinical parameters and the risk of POAG development.

3.3. Comet assay
The aim of this study was also to assess the level of basal and oxidative DNA damage as well as the efficiency of their repair in lymphocytes of POAG patients in relation to healthy controls.

3.3.1. Basal DNA damage and DNA damage induced by hydrogen peroxide
An additional purpose of this study was to evaluate the level of basal and induced DNA damage in lymphocytes of POAG patients as well as healthy controls. We found a significantly higher level of basal DNA damage, expressed as a percentage of tail DNA when

the comet assay was carried out under standard conditions among a group of POAG patients in relation to healthy controls (P=<0.001). Additionally, the levels of oxidative DNA damage induced by 10 μM as well as 20 μM H_20_2 were significantly higher in POAG patients (P=<0.001 and P=<0.001) than in healthy controls. In each probe, 50 cells were appraised. The results of our data are presented in Figure 1.

3.3.2. Oxidative DNA damage

Moreover, to detect DNA oxidation, we used the modified alkaline comet assay with endonuclease III (*Endo*III/Nth) and Fpg that recognizes a wide range of oxidized purines and pyrimidines [35]. We found that the level of basal oxidative DNA damage that is recognized by *Endo*III as well as Fpg is significantly higher among POAG patients compared to healthy controls (P=<0.001). Furthermore, we evaluated the level of oxidative DNA damage induced by 10 μM and 20 μM H_2O_2 that is recognized by the *Endo*III and Fpg enzymes. The results of our study have shown the significantly higher level of oxidative DNA damage recognized by *Endo*III (P<0.001) for 10 μM H_2O_2 and (P<0.001) 20 μM H_2O_2 in POAG patients in relation to healthy controls. Additionally, we have also indicated a significantly higher level of oxidative DNA damage recognized by the Fpg enzyme (P=<0.001) in POAG patients. In each probe, 50 cells were appraised. The results are shown in Figure 2.

3.3. Kinetics of DNA repair

Additionally, we investigated the effectiveness of DNA repair after treatment with H_2O_2 at concentrations of 10 μM and 20 μM for 10 min. After exposure to H_2O_2, the cells were washed and incubated in fresh RPMI 1640 medium for 30, 60 and 120 min. The results have indicated that the normal lymphocytes recovered within a repair – incubation time of 60 min, while in lymphocytes isolated form POAG patients, the repair capacity was slightly decreased after 120 min of incubation. Moreover, a comparison of the level of DNA damage recognized by Nth and Fpg in lymphocytes incubated with or without H_2O_2 revealed an increase in DNA damage in POAG lymphocytes and controls. In each probe, 50 cells were appraised. The results are presented in Figure 3.

3.4. Evaluation of BER activity

Finally, we estimated the efficiency of the BER mechanism, measured as the removal of uracil bases from DNA[QCE1], in lymphocytes of patients with POAG in comparison with healthy controls. In this assay, a substrate carrying a single lesion at a defined position enables analysis of fully repaired intermediate products at the molecular level.

The figure shows repaired and unrepaired DNA fragments detected by autoradiography. As shown in figure 4a, BER activity is expressed as a percentage of repaired DNA. The results of our studies have indicated that in the cellular extract isolated from POAG patient lymphocytes, BER activity was at a level of 68.270% in relation to healthy controls (80.767%). The percentage of DNA repair is presented in Figure 4b.

Discussion

It is widely known that oxidative stress may lead to DNA strand breaks in differentiated neurons [36]. Moreover, the proper functioning of DNA repair mechanisms is crucial for maintaining genome stability. It is widely noted that altered DNA repair may result in cancer development [37] as well as neurodegeneration [38]. Moreover, it is suggested that the presence of polymorphisms in the genes encoding the main DNA repair enzymes may be associated with reduced DNA repair capacity.

It is suggested that oxidative stress may be one of the main risk factors for the development of Alzheimer's disease, Parkinson's disease, ALS and Huntington's disease. Additionally, it is important that the presence of oxidative stress in the anterior segment of the eye may be combined with glaucoma as well as lens cataracts. However, the presence of oxidative stress in the posterior segment of the eye is linked with molecular degeneration [39-41]. Because of the high rate of ROS production, oxidative DNA damage is the most common type of DNA lesion. Data have shown that oxidative DNA damage may play a valid role in degradation of the hTM, optic nerve and retinal ganglion cells [13]. Nizankowska and Kaczmarek showed that characteristics for glaucoma development and progressive neuronal death may be associated with the presence of oxidative stress [42]. Additionally, Sarkhabi et al. indicated that the level of 8-OHdG, the main marker of DNA oxidation, was increased in aqueous as well as in serum in a group of glaucomatous patients in comparison with cataract patients [43]. Our previous data supported the hypothesis regarding the role of oxidative stress in POAG development. We evaluated increased levels of oxidative DNA damage among POAG patients compared to healthy individuals [44].

There are several defense mechanisms that protect mammalian cells against accumulation of oxidative DNA damage. The major DNA repair pathway that is responsible for removing oxidative bases, deaminated adenines and cytidines is the BER mechanism. It is suggested that increased levels of oxidative DNA lesions in hTM cells among POAG patients may be associated with BER deficiency.

Several enzymes are required for proper function of this pathway. Therefore, we selected the following BER genes: *XRCC1, OGG1, MUTYH, ADPER* and *APE*1. The presence of polymorphic variants of the above-mentioned genes may result in changing the DNA repair capacity [26]. The role of the 399Arg/Gln *XRCC1* gene polymorphism was widely studied in relation to head and neck cancer [45]. There are only a few studies that were focused on neurodegenerative or ocular disease. The same data found the relationship between the presence of the 399Arg/Gln *XRCC1* gene polymorphism with ocular disorders including age-related cataracts [46] as well as neurodegenerative diseases such as Parkinson's [47] and Alzheimer's diseases [24].

Our preliminary study included 190 patients with POAG and 190 healthy controls. In the present paper, the study groups were significantly larger, including 412 patients with POAG and 454 healthy controls. This is a newly selected group of patients who were fully characterized with all relevant glaucoma clinical parameters (Gdx, c/d ratio, RA, RNFL, and VF), thus providing a wider view of the relationship between these parameters and particular genotypes as contributors to POAG development.

The results of this study confirmed our previous preliminary data [48] that the presence of the 399Arg/Gln genotype as well as the 399Gln allele of the *XRCC1* gene is associated with an increased risk of POAG development. On the contrary, the results from our investigations did not confirm the results from the Güven et al. study [49]. The difference between our study and that of Güven et al. may be a result of the population group difference. Moreover, it is postulated that the presence of the 399Arg/Gln genotype of the *XRCC1* gene may be associated with a higher level of DNA adduct as well as greater sensitivity to ionizing radiation and tobacco-related DNA damage [50,51]. Furthermore, we also analyzed the 194Arg/Trp polymorphism of the *XRCC1* gene in relation to POAG development. Some data correlated the role of this polymorphic variant of the *XRCC1* gene with the risk of late-onset Alzheimer's disease [27]. However, some reports excluded the role of this polymorphism in glioma development [52]. In this study, we suggested that 194Arg/Trp *XRCC1* has no influence on the risk of POAG in the Polish population. The results of these data enhanced the results of our preliminary study [48]. However, no previous studies concerning the role of the

194Arg/Trp *XRCC1* gene polymorphism and the risk of POAG development have been published.

Another analyzed genetic polymorphism was 326Ser/Cys *OGG1*. The results of many studies on the role of the above-mentioned polymorphic variant are inconsistent. Others have postulated that the presence of this polymorphism may play important roles in the development of sporadic amyotrophic lateral sclerosis (ALS) [53] as well as diabetes mellitus type 2 [54]. It is postulated that OGG1 participates in the repair of oxidative damage in neurons. Additionally, OGG1 activity was found in the mitochondria and nuclei of neuronal cells. Because guanine is the most susceptible to ROS attack, this polymorphic variant was selected as a risk factor for the development of neurodegenerative disease [55]. However, Coppedè et al. showed a lack of association between the 326Ser/Cys *OGG1* gene polymorphism and the risk of Alzheimer's and Parkinson's diseases [55,56]. The results from the presented data confirmed our preliminary study regarding the lack of association between the 326Ser/Cys *OGG1* gene polymorphism and the risk of POAG development in the Polish population [48].

Moreover, the 324Gln/His *MUTYH* gene polymorphism was also analyzed. To our knowledge, there is no other previous research on the influence of this polymorphism on the risk of neurodegenerative disease development. However, a previous study postulated a relationship between the presence of this polymorphism and the risk of lung cancer [57] and colorectal cancer development [37]. However, Ashton et al. did not indicate the importance of the presence of this polymorphism in the development of endometrial cancer [22]. The results of the present study do not indicate a relationship between the 324Gln/His of *MUTYH* gene polymorphism with POAG in the Polish population. To the best of our knowledge, there is no additional data that confirms the role of this polymorphism in development of neurodegenerative diseases. The results of this study confirmed the results of our preliminary study [48].

Some foregoing studies have suggested the role of the 762Val/Ala polymorphism of the *ADPRT* gene in disease development, including diabetic polyneuropathy type 1 [58] and breast cancer [59]. However, Wen et al. suggested that there is no significant association between the 762Val/Ala *ADPRT* gene polymorphism and gastric cancer [60]. Our findings show a lack of association between the 762Val/Ala of the *ADPRT* gene polymorphism with the risk of POAG development in the Polish population. The final analyzed genetic polymorphism was 148Asp/Glu of the *APE1* gene. Reports have suggested that the presence of 148Asp/Glu of the *APE1* gene polymorphism is associated with decreased activity of this enzyme, which may lead to the development of various diseases. Previous research has provided evidence that 148Asp/Glu of the *APE1* gene polymorphism plays an important role in the development of different types of diseases, e.g., breast cancer [61] and lung cancer [62]. Parıldar-Karpuzoğlu et al. showed no association between this polymorphism and the risk of Alzheimer's disease development [24].

The results of our data confirmed our preliminary study's results that presented a lack of association between this gene and the risk of POAG development [63].

Additionally, we expand our research on the role of the interaction between the 399Arg/Gln and the 194Arg/Trp polymorphism of the *XRCC1* gene, 399Arg/Gln of *XRCC1* and 326Ser/Cys of the *OGG1* gene as well as 399Arg/Gln of the *XRCC1* and 324Gln/His of the *MUTYH* gene [48].

We suggested that the presence of the above combinations of polymorphic variants may predispose people to POAG development. On the contrary, the presence of the combination of the 399Arg/Gln *XRCC1* gene and the 762 Val/Ala ADPRT gene may play a protective role in POAG development. To the best of our knowledge, there is no other data that presented the interaction between the studied genes.

Furthermore, we continued our research to establish the role of the association between RNFL, c/d and VF clinical parameters and the studied genes [48,63].
Heidelberg Retina Tomography was used to assess the following clinical parameters: RNFL, c/d, and VF. To date, information about the relationship between the previously mentioned clinical parameters and BER genes has been limited. In patients with glaucoma, the loss of ganglion cell axons leads to an increase in optic cup size and a reduction in neuroretinal rim area. We analyzed the genotypes and allele frequencies of the studied genes in relation to clinical parameters in patients with different stages of POAG progression. Damage of the optic nerve is estimated via the cup/disk parameter. The results of our data suggested that disturbance in the cup disk ratio may be associated with the presence of the 399Gln/Gln mutant genotype as well as the 399Gln allele of the *XRCC1* gene. It has been reported that RNFL thinning is a glaucoma symptom [64]. Therefore, we concentrated on the analysis of the correlation between RNFL with genetic polymorphisms of selected BER genes. The results of our studies have shown that the presence of the 324Gln/His genotype of the *MUTYH* gene as well as the 762Val/Ala genotype of the *ADPRT* gene play protective roles in POAG progression. The presence of the 148Asp/Glu genotype and the 148Glu allele of the *APE1* gene increases the risk of POAG progression. The visual field is the main parameter used to assess the progression of POAG. We have postulated that the presence of the 762Val/Ala genotype of the *ADPRT* gene is correlated with an elevated risk of POAG progression. However, the presence of the 148Asp/Glu of the *APE1* gene is associated with a decreased risk of POAG progression.
Notwithstanding the numerous studies on the etiology of POAG, there are no other data that combine the genetic polymorphisms of BER genes with clinical parameters among a group of patients with POAG. Moreover, in the present article, we also analyzed the level of basal DNA damage, oxidative lesions induced by H_2O_2 as well as the efficiency of their repair in lymphocytes of POAG patients in comparison with lymphocytes of healthy controls. Single-strand breaks of DNA may be created by the following environmental factors: UV light, X-rays, ionizing radiation, toxic chemicals as well as endogenous agents such as ROS. It is postulated that an elevated level of DNA strand breaks may arise from increased oxidative stress [65]. To assess the level of oxidative DNA damage, a modified comet assay with *Endo*III and Fpg enzymes was used. Both enzymes may recognize different types of oxidative damage. It is worth noting that Fpg is specific for oxidized purines and that *Endo*III preferentially incises DNA at oxidized pyrimidine sites [35]. The results of our study indicated an elevated level of basal DNA lesions as well as DNA damage induced by H_20_2 in POAG patients in comparison with healthy controls. After H_2O_2 treatment, the levels of DNA damage recognized by *Endo*III and Fpg were significantly higher in lymphocytes isolated from POAG patients than healthy controls ($P<0.001$). Additionally, we presented a slightly decreased DNA repair capacity in lymphocytes isolated from POAG patients in relation to healthy control lymphocytes. We also analyzed the level of DNA damage recognized by *Endo*III and Fpg enzymes. The results of our studies are similar to those presented by Mozaffarieh et al. They showed that POAG patients with PVD *(primary vascular dysregulation)* have a significantly higher rate of DNA lesions than both POAG patients without PVD and healthy controls with and without PVD [65].
DNA repair systems play crucial roles in genome stability. It is widely known that genetic instability may be associated with an increased risk of malignant transformation [66] and neurodegeneration [12]. BER is the crucial pathway that repairs DNA damage resulting from oxidation, alkylation and deamination of bases. Alternate actions of the BER pathway may lead to a variety of biological consequences including mutations, transcription blocking, replication and chromosomal aberration [67]. To explain whether reduction of BER efficiency is associated with POAG development, the activity of this pathway was measured in cellular

extracts that were isolated from whole blood POAG patients as well as healthy controls. In the current studies, we focused only on the BER pathway because this is the crucial mechanism that protects cells against oxidation. The results of our studies indicated a difference in the percentage of DNA repair levels in POAG cellular extracts in relation to cellular extracts that were isolated from healthy controls. We indicated that in POAG patients, the efficiency of DNA repair was approximately 68.270 % in comparison with 80.767 % in healthy controls. To the best of our knowledge, there are no data that present the role of the BER pathway in POAG development. The results of our data suggested that the presence of polymorphic variants of genes that encode crucial proteins of the BER mechanism may not be considered as the only risk factor for POAG development.

Conclusion

The present study was designed to investigate alterations in the BER pathway. Therefore, we traced the course of BER not only holistically but also in a detailed way, which was provided by the analysis of the kinetics of DNA repair by a comet assay. Regarding the fact that genotyping analysis concerned the genes involved in the recognition and incision of lesion sites, we intentionally applied a BER assay to a narrow observation area of events in addition to glycosylase action. Such a composition of research allows us to assess which stage of BER might play a crucial role in pathogenesis and whether the screened SNPs may potentially disturb BER. Our results herein enhanced our preliminary study regarding the role of SNPs in the pathogenesis of POAG [48,63]. Thus, it can be noted that POAG patients have decreased BER repair capacity. Through in-depth analysis of the BER function, we showed that this reduced activity is not only caused by glycosylase polymorphisms but also crucial genes responsible for the subsequent stages of BER. We postulated that the 399Arg/Gln genotype and the 399Gln allele of the *XRCC1* gene may have as important of a role in POAG development as glycosylases. Additionally, our data exhibited that patients with POAG have significantly increased levels of oxidative DNA damage in relation to healthy controls. Moreover, we observed a slight decrease in DNA repair efficiency in a group of POAG patients. Thus, we postulated that disturbance of DNA repair in POAG patients may be involved in the pathogenesis of POAG. In the context of POAG, it seems to be important to notice the connection between the polymorphism of BER genes, increasing both the level of endogenous oxidative DNA damage and efficiency of their repair.

Conflict of interest statement

None.

Acknowledgements:

This work was supported by grants N N402 375838 from the Polish Ministry of Science and Higher Education and 2012/05/B/NZ7/02502 from the National Science Center.

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Table I. The clinical parameters characteristic for primary open angle glaucoma (POAG) patients and control group.

	Parameters	Patient groups	Control groups
Number	Gender male/female	148/275	194/260
	Hypertension*	242	288
	Low blood pressure**	148	87
	Vascular disease	139	176
	Diabetes mellitus type 2	76	150
	POAG in family relatives	135	21
Mean ± SD	Age (years)	73±9	71±12
	Intraocular pressure, IOP (mmHg)	13.2±2.9	11.9±1.9
	Cup disk ratio (c/d) right eye/left eye	0.73±0.14/0.74±0.14	PNM
	Rim area (RA) right eye/left eye	1.41±0.42/1.28±0.38	PNM
	Retinal Nerve Fiber Layer (RNFL) right eye/left eye	0.35±0.10/0.21±0.10	PNM
	Visual field right eye/left eye	-7.26±7.01/-7.72±8.10	PNM
	NFI right eye/left eye	32.98±20.35/26.80±19.45	PNM

* Systolic pressure > 140; Diastolic pressure > 90 mmHg;
**Systolic pressure □ 90; Diastolic pressure □ 60 mmHg;
^ PNM - Parameter not measured;

Table 2: Oligonucleotide sequences and restriction endonucleases used in the 399 Arg/Gln *XRCC1,* the 194Arg/Trp *XRCC1*, the 326Ser/Cys OGG1, the 324 Gln/His *MUTYH*, the 762Val/Ala *ADPRT* genes polymorphisms analysis by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the 148 Asp/Glu *APE1* gene polymorphism analysis by PCR- CTPP

Gene polymorphism	Oligonucleotide sequence	Annealing	Product size	Enzyme	Source
399Arg/Gln *XRCC1*	5'-TTGTGCTTTCTCTGTGTCCA-3'; 5'-TCCTCCAGCCTTTTCTGATA-3'	61°C	615bp	*Msp*I	[38]
194Arg/Trp *XRCC1*	5'-GCCCCGTCCCAGGTA-3' 5- AGCCCCAAGACCCTTTCATC-3'	61°C	491bp	*Msp*I	[38]
326Ser/Cys *OGG1*	5'-GGAAGGTGCTTGGGGAAT-3' 5'-ACTGTCACTAGTCTCACCAG-3	57°C	200bp	*Fnu4H*I	[39]
324Gln/His *MUTYH*	5'-TGCCGATTCCCTCCATTCTCTCTTG-3' 5'-TCTTGGCTTGAGTAGGGTTCGGG-3'	64°C	292bp	*HpyCH4*III	[40]
762Val/Ala *ADPRT*	5'-TTTGCTCCTCCAGGCCAAC-3' 5'-TGGAAGTTTGGGACCGCTGC-3'	58°C	210bp	*BstU*1	[41]
148Asp/Glu *APE1*	5'-GATACGGCATAGGTGAGACC-3', 5'- TCTGTTTCATTTCTATAGGCGAT-3' 5'-TCCTGTCATGCTCCTCC -3' 5'-GTCAATTTCCTTCATGTGTGCCA 3'.	58°C	G allele (167 bp) T allele (236 bp)	-	**[42]**

10% of the samples were repeated and the results were 100% concordant.

Table 3: The genotypes and alleles frequency and odds ratios (OR) of the 399Arg/Gln and the 194Arg/Trp of *XRCC1*, the 326Ser/Cys of *OGG1*, the 324Gln/His of *MUTYH,* the 762 Val/Ala of *ADPRT* and the 148 Asp/Glu of *APE1* genes polymorphisms in patients with in primary open angle glaucoma (POAG) and the healthy controls

	Patients with POAG		Controls			
Genotype /Allele 399Arg/Gln *XRCC1*	**Number n=393**	**Frequency**	**Number n=436**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/Arg	118	0.30	164	0.37	1	
Arg/Gln	**192**	**0.49**	**192**	**0.44**	**1.38 (1.02- 1.89)**	**0.03**
Gln/Gln	83	0.21	80	0.18	1.44 (0.98 - 2.12)	0.06
Arg	428	0.55	520	0.60	1	
Gln	**358**	**0.45**	**352**	**0.40**	**1.23 (1.02 - 1.50)**	**0.03**
Genotype /Allele 194Arg/Trp *XRCC1*	**Number n=410**	**Frequency**	**Number n=454**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/Arg	363	0.89	398	0.84	1	
Arg/Trp+Trp/Trp	47	0.11	53	0.12	0.95 (0.63 - 1.44)	0.82
Arg	773	0.94	849	0.94	1	
Trp	47	0.06	53	0.06	0.95 (0.64 - 1.43)	0.89
Genotype /Allele 326Ser/Cys *OGG1*	**Number n=412**	**Frequency**	**Number n=454**	**Frequency**	**OR (95% CI)**	**P – value**
Ser/Ser	278	0.67	326	0.72	1	
Ser/Cys	117	0.28	112	0.24	1.24 (0.91 - 1.68)	0.17
Cys/Cys	17	0.04	16	0.03	1.25 (0.62 - 2.52)	0.53
Ser	673	0.82	765	0.84	1	
Cys	151	0.18	143	0.15	1.20 (0.93 - 1.54)	0.15
Genotype /Allele 324Gln/His *MUTYH*	**Number n=412**	**Frequency**	**Number n=445**	**Frequency**	**OR (95% CI)**	**P – value**
Gln/Gln	263	0.64	297	0.66	1	
Gln/His	136	0.33	132	0.30	1.16 (0.87 - 1.56)	0.31
His/His	13	0.03	16	0.04	0.92 (0.43 - 1.94)	0.82
Gln	662	0.80	726	0.82	1	
His	162	0.20	164	0.18	1.08 (0.85 - 1.38)	0.52
Genotype /Allele 762 Val/Ala *ADPRT*	**Number n=407**	**Frequency**	**Number n=452**	**Frequency**	**OR (95% CI)**	**P – value**
Val/Val	289	0.71	324	0.72	1	
Val/Ala	99	0.24	109	0.24	1.02 (0.74-1.39)	0.92
Ala/Ala	19	0.05	19	0.04	1.12 (0.58-2.15)	0.73
Val	677	0.83	757	0.83	1	
Ala	137	0.17	147	0.16	1.22 (0.95-1.58)	0.12
Genotype /Allele 148 Asp/Glu *APE1*	**Number n=400**	**Frequency**	**Number n=454**	**Frequency**	**OR (95% CI)**	**P – value**
Asp/Asp	148	0.37	168	0.37	1	
Asp/Glu	246	0.61	285	0.63	0.98 (0.74- 1.29)	0.89
Glu/Glu	6	0.01	1	0.002	6.81 (0.81 - 57.23)	0.04 ^
Asp	542	0.68	621	0.68	1	
Glu	258	0.32	287	0.32	1.03 (0.84- 1.26)	0.78

^ P values – if all expected cell frequencies are less than 5 was used one-tailed Fisher Exact Probability Test.

Table 4 Distribution of double-combined genotypes of the 399Arg/Gln–194Arg/Trp of *XRCC1, the 399Arg/Gln XRCC1 – the 326Ser/Cys OGG1*, the 399Arg/Gln *XRCC1* – the 324Gln/His *MUTYH* and the 399Arg/Gln *XRCC1* - 762Val/Ala ADPRT genes polymorphisms in primary open angle glaucoma (POAG) patients and healthy controls

	Patients with POAG		**Controls**			
Genotype 399Arg/Gln-194Arg/Trp	**Number n=393**	**Frequency**	**Number n=436**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/Arg-Arg/Arg	94	0.24	131	0.30	1	
Arg/Arg-Arg/Trp	23	0.06	29	0.07	1.10 (0.60 -2.03)	0.63
Arg/Arg-Trp/Trp	-	-	-	-	-	-
Arg/Gln-Arg/Arg	**175**	**0.46**	**169**	**0.40**	**1.44 (1.03 - 2.02)**	**0.02**
Arg/Gln-Arg/Trp	14	0.03	19	0.04	1.03 (0.49 - 2.15)	1
Arg/Gln-Trp/Trp	-	-	-	-	-	-
Gln/Gln-Arg/Arg	76	0.19	74	0.18	1.43 (0.94 - 2.17)	0.13
Gln/Gln-Arg/Trp	6	0.02	5	0.01	1.67 (0.48 – 5.64)	0.18 ^
Gln/Gln-Trp/Trp	-	-	-	-	-	-
Genotype 399Arg/Gln-326Ser/Cys	**Number n=391**	**Frequency**	**Number n=460**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/ArgSer/Ser	82	0.21	104	0.24	1	
Arg/ArgSer/Cys	32	0.08	49	0.11	0.83 (0.47 - 1.41)	0.49
Arg/ArgCys/Cys	4	0.01	7	0.02	0.72 (0.21 - 2.56)	0.43 ^
Arg/GlnSer/Ser	123	0.32	143	0.33	1.09 (0.75- 1.59)	0.65
Arg/GlnSer/Cys	**57**	**0.14**	**41**	**0.09**	**1.76 (1.07- 2.89)**	**0.02**
Arg/GlnCys/Cys	10	0.03	6	0.01	2.11 (0.74- 6.06)	0.16
Gln/GlnSer/Ser	55	0.14	60	0.13	1.16 (0.73 -1.85)	**0.53**
Gln/GlnSer/Cys	26	0.06	17	**0.04**	1.94 (0.99-3.81)	0.03
Gln/GlnCys/Cys	2	0.01	3	0.007	0.84 (0.14- 5.18)	0.62^
Genotype 399Arg/Gln-324Gln/His	**Number n=412**	**Frequency**	**Number n=454**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/ArgGln/Gln	77	0.20	97	0.24	1	
Arg/ArgGln/His	35	0.09	53	0.13	0.83 (0.49-1.40)	0.49
Arg/ArgHis/His	5	0.01	6	0.01	1.05 (0.31-3.57)	0.59
Arg/GlnGln/Gln	120	0.31	135	0.34	1.12 (0.76-1.65)	0.56
Arg/GlnGln/His	**65**	**0.17**	**51**	**0.13**	**1.60 (1.00-2.58)**	**0.05**
Arg/GlnHis/His	5	0.01	5	0.01	1.26 (0.35-5.51)	0.48^
Gln/GlnGln/Gln	54	0.14	46	0.12	1.48 (0.90-2.42)	0.12
Gln/GlnGln/His	27	0.07	0	0	-	-
Gln/GlnHis/His	2	0.01	3	0.01	0.84 (0.14-5.15)	0.61^
Genotype 399Arg/Gln-762Val/Ala	**Number n=412**	**Frequency**	**Number n=445**	**Frequency**	**OR (95% CI)**	**P – value**
Arg/ArgVal/Val	86	0.22	106	0.24	1	
Arg/ArgVal/Ala	**22**	**0.06**	**48**	**0.11**	**0.54 (0.30-0.87)**	**0.04**
Arg/ArgAla/Ala	7	0.02	8	0.02	1.04 (0.36-2.98)	0.58
Arg/GlnVal/Val	131	0.34	143	0.33	1.09 (0.75-1.58)	0.66
Arg/GlnVal/Ala	49	0.13	39	0.10	1.49 (0.89-2.48)	0.12
Arg/GlnAla/Ala	9	0.02	5	0.01	2.13 (0.69-6.61)	0.19
Gln/GlnVal/Val	55	0.14	58	0.13	1.12 (0.70-1.79)	0.62
Gln/GlnVal/Ala	25	0.06	16	0.04	1.85 (0.93-3.69)	0.08
Gln/GlnAla/Ala	1	0.002	5	0.01	0.24 (0.03-2.07)	0.16^

^ P values – if all expected cell frequencies are less than 5 was used one-tailed Fisher Exact Probability Test

Table 5: The genotypes and alleles frequency and odds ratios (OR) of the 399Arg/Gln and the 194Arg/Trp of *XRCC1*, the 326Ser/Cys of *OGG1* and the 324Gln/His of *MUTYH*, the 762 Val/Ala of *ADPRT* and the 148 Asp/Glu of *APE1* genes polymorphisms among group of POAG in relation to c/d ratio parameter

Genotype /Allele	**Advanced POAG changes 0.7-1.0**		**Early POAG changes 0.3-0.7**		**OR (95% CI)**	P – value
399Arg/Gln *XRCC1*	**Number n=337**	**Frequency**	**Number n=297**	**Frequency**		
Arg/Arg	90	(0.27)	99	(0.33)	1	
Arg/Gln	165	(0.49)	144	(0.48)	1.26 (0.88-1.81)	0.21
Gln/Gln	**82**	**(**0.24)	54	(0.18)	**1.67 (1.07-2.61)**	**0.02**
Arg	345	(0.51)	342	(0.58)		
Gln	**329**	**(0.49)**	**252**	**(0.42)**	**1.29 (1.04-1.62)**	**0.02**
194Arg/Trp *XRCC1*	**Number n=359**	**Frequency**	**Number n=313**	**Frequency**		
Arg/Arg	316	(0.88)	284	(0.91)	1	
Arg/Trp+Trp/Trp	43	(0.12)	29	(0.09)	1.16 (0.71-1.92)	0.55
Arg	675	(0.94)	597	(0.95)	1	
Trp	43	(0.06)	29	(0.05)	1.31 (0.81-2.13)	0.27
326Ser/Cys of *OGG1*	**Number** n=313	**Frequency**	**Number** n=363	**Frequency**		
Ser/Ser	219	(0.70)	233	(0.64)	1	
Ser/Cys	83	(0.27)	115	(0.32)	0.77 (0.55-1.08)	0.12
Cys/Cys	11	(0.04)	15	(0.04)	0.78 (0.35-1.74)	0.54
Ser	521	(0.83)	581	(0.80)	1	
Cys	105	(0.17)	145	(0.20)	0.81 (0.61-1.06)	0.13
324Gln/His *MUTYH*	**Number n=362**	**Frequency**	**Number n=316**	**Frequency**		
Gln/Gln	222	(0.61)	200	(0.63)	1	
Gln/His	131	(0.36)	108	(0.34)	1.09 (0.79-1.50)	0.58
His/His	9	(0.02)	8	(0.03)	1.01 (0.38-2.68)	0.59
Gln	575	(0.79)	508	(0.80)	1	
His	149	(0.21)	124	(0.20)	1.06 (0.81-1.38)	0.66
762 Val/Ala *ADPRT*	**Number** n=359	**Frequency**	**Number** n=315	**Frequency**		
Val/Val	249	(0.69)	223	(0.71)	1	
Val/Ala	94	(0.26)	78	(0.25)	1.08 (0.76-1.53)	0.67
Ala/Ala	16	(0.04)	14	(0.04)	1.02 (0.49-2.14)	0.55
Val	592	(0.82)	524	(0.83)	1	
Ala	126	(0.18)	106	(0.17)	1.05 (0.79-1.40)	0.73
148 Asp/Glu *APE1*	**Number** n=359	**Frequency**	**Number** n=315	**Frequency**		
Asp/Asp	131	(0.37)	100)	(0.32	1	
Asp/Glu	226	(0.63)	201	(0.64)	0.852 (0.62-1.18)	0.35
Glu/Glu	**1**	**(0.001)**	**11**	**(0.04)**	**0.07 (0.01-0.55)**	**0.001^**
Asp	488	(0.68)	401	(0.64)	1	
Glu	228	(0.32)	223	(0.36)	0.84 (0.67-1.05)	0.132

^ P values – if all expected cell frequencies are less than 5 was used one-tailed Fisher Exact Probability Test.

Table 6: The genotypes and allele frequency and odds ratios (OR) the 399Arg/Gln and the 194Arg/Trp of *XRCC1*, the 326Ser/Cys of *OGG1*, the 324Gln/His of *MUTYH*, the 762 Val/Ala of *ADPRT* and the 148 Asp/Glu of *APE1* genes polymorphisms in primary open-angle glaucoma (POAG) in relation to visual field (VF) parameter

Genotype /Allele	-6-12dB	>-12dB	>-6dB	<-6dB	**OR (95% CI)***	P – value	**OR (95% CI)****	P – value	**OR (95% CI)*****	P – value
399Arg/Gln *XRCC1*	**Number n=136 (Frequency)**	**Number n=135 (Frequency)**	**Number n=271 (Frequency)**	**Number n=286 (Frequency)**						
Arg/Arg	47 (0.35)	38 (0.28)	85 (0.31)	84 (0.29)	1		1		1	
Arg/Gln	61 (0.45)	68 (0.50)	129 (0.48)	143 (0.50)	0.76 (0.48-1.21)	0.25	1.05 (0.65-1.70)	0.84	0.89 (0.61-1.31)	0.55
Gln/Gln	28 (0.21)	29 (0.21)	57 (0.21)	59 (0.21)	0.85 (0.48-1.51)	0.57	1.08 (0.60-1.95)	0.78	0.95 (0.59-1.53)	0.84
Arg	155 (0.57)	144 (0.53)	299 (0.55)	311 (0.54)	1		1		1	
Gln	117 (0.43)	126 (0.47)	243 (0.45)	261 (0.46)	0.90 (0.67-1.20)	0.47	1.04 (0.78-1.39)	0.08	1.03 (0.82-1.31)	0.79
194Arg/Trp *XRCC1*	**Number n=131 (Frequency)**	**Number n=141 (Frequency)**	**Number n=272 (Frequency)**	**Number n=178 (Frequency)**						
Arg/Arg	111 (0.85)	129 (0.91)	240 (0.88)	252 (0.91)	1		**1**		1	
Arg/Trp+Trp/Trp	20 (0.15)	12 (0.09)	32 (0.12)	26 (0.09)	1.74 (0.93-3.26)	0.07	0.90 (0.44-1.84)	0.08	1.29 (0.75-2.23)	0.22
Arg	242 (0.92)	270 (0.96)	512 (0.94)	530 (0.95)	1		1		1	
Trp	20 (0.08)	12 (0.04)	32 (0.06)	26 (0.05)	1.68 (0.92-3.08)	0.09	0.91 (0.45-1.82)	0.78	1.23 (0.72-2.09)	0.44
326Ser/Cys *OGG1*	**Number n=121 (Frequency)**	**Number n=136 (Frequency)**	**Number n=257 (Frequency**	**Number n=239 (Frequency)**						
Ser/Ser	78 (0.64)	97 (0.71)	175 (0.68)	156 (0.65)	1		1		1	
Ser/Cys	37 (0.31)	33 (0.24)	70 (0.27)	73 (0.31)	1.17 (0.76-1.80)	0.53	0.73 (0.45-1.18)	0.19	0.85 (0.58-1.27)	0.43
Cys/Cys	6 (0.05)	6 (0.04)	12 (0.05)	10 (0.04)	1.20 (0.42-3.42)	0.73	0.96 (0.34-2.74)	0.58	1.07 (0.45-2.55)	0.88
Ser	193 (0.80)	227 (0.83)	420 (0.81)	385 (0.81)	1		1		1	
Cys	49 (0.20)	45 (0.17)	94 (0.19)	93 (0.19)	1.05 (0.72-1.55)	0.81	0.82 (0.55-1.21)	0.32	0.95 (0.67-1.27)	0.63
324Gln/His *MUTYH*	**Number n=164 (Frequency)**	**Number n=93 (Frequency)**	**Number n=257 (Frequency)**	**Number n=201 (Frequency)**						
Gln/Gln	102 (0.62)	65 (0.70)	167 (0.65)	122 (0.61)	1		1		1	
Gln/His	56 (0.34)	27 (0.29)	83 (0.32)	73 (0.36)	0.92 (0.59-1.42)	0.70	0.69 (0.41-1.18)	0.18	0.83 (0.56-1.23)	0.35
His/His	6 (0.04)	1 (0.01)	7 (0.03)	6 (0.03)	1.20 (0.37-3.82)	0.76^	0.31 (0.04-2.65)	0.25^	0.85 (0.28-2.60)	0.77^
Gln	260 (0.79)	157 (0.84)	417 (0.82)	317 (0.79)	1		1		1	
His	68 (0.21)	29 (0.16)	97 (0.18)	85 (0.21)	0.97 (0.68-1.40)	0.88	0.67 (0.43-1.09)	0.11	0.87 (0.62-1.20)	0.39
762Val/Ala *ADPRT*	**Number n=141 (Frequency)**	**Number n=143 (Frequency)**	**Number n=284 (Frequency)**	**Number n=58 (Frequency)**						
Val/Val	94 (0.67)	101 (071)	195 (0.65)	242 (0.73)	1		1		1	
Val/Ala	**45 (0.32)**	**32 (0.22)**	**77 (0.27)**	**72 (0.22)**	**1.61 (1.03-2.50)**	**0.03**	1.06 (0.66-1.71)	0.79	1.33 (0.91-1.92)	0.14

Ala/Ala	2 (0.01)	10 (0.07)	12 (0.04)	19 (0.06)	0.027 (0.06-1.19)	0.06	1.26 (0.57-2.81)	0.57	0.78 (0.37-1.65)	0.52
Val	233 (0.83)	234 (0.82)	467 (0.82)	556 (0.83)	1		1		1	
Ala	49 (0.17)	52 (0.18)	101 (0.18)	110 (0.17)	1.06 (0.73-1.54)	0.75	1.12 (0.78-1.62)	0.53	1.09 (0.81-1.48)	0.55
148 Asp/Glu *APE1*	**Number n=142 (Frequency)**	**Number n=137 (Frequency)**	**Number n=297 (Frequency)**	**Number n=58 (Frequency)**						
Asp/Asp	61 (0.43)	47 (034)	108 (0.36)	100(0.31)	1		1		1	
Asp/Glu	80(0.56)	87 (0.64)	167 (0.56)	219 (0.67)	**0.60 (0.40-0.90)**	**0.01**	0.86 (0.55-1.29)	0.43	**0.71 (0.50-0.99)**	0.04
Glu/Glu	1 (0.01)	3 (0.02)	4 (0.01)	6 (0.02)	0.27 (0.03-2.32) ^	0.20	1.06 (0.25-4.44)^	0.59	0.62 (0.17-2.25)^	0.34
Asp	202 (0.71)	181 (0.66)	383 (0.69)	419 (0.64)	1		1		1	
Glu	82 (0.29)	93 (0.34)	175 (0.31)	231 (0.36)	0.74 (0.54-0.99)	0.04	0.93 (0.69-1.25)	0.35	0.83 (0.65-1.05)	0.12

Table 7. The genotypes and alleles frequency and odds ratios (OR) of the 399Arg/Gln and the 194Arg/Trp of *XRCC1*, the 326Ser/Cys of *OGG1*, the 324Gln/His of *MUTYH* , the 762 Val/Ala of *ADPRT* and the 148Asp/Glu of *APE1* genes polymorphisms among POAG patients in relation to RNFL parameter

Genotype /Allele	0.20-0.187	0.187-0.130	<0.130	<0.187	0.31-0.20	**OR (95% CI)***	P – value	**OR (95% CI)****	P – value	**OR (95% CI)*****	P – value	**OR (95% CI)******	P – value
The 399Arg/Gln *XRCC1*	**Number n=99 (Frequency)**	**Number n=110 (Frequency)**	**Number n=134 (Frequency)**	**Number n=343 (Frequency)**	**Number n=153 (Frequency)**								
Arg/Arg	26(0.26)	34 (0.31)	34 (0.25)	94 (0.27)	48 (0.31)	1		1		1		1	
Arg/Gln	54 (0.55)	57 (0.52)	65 (0.49)	176 (0.51)	77 (0.50)	1.29 (0.72-2.34)	0.39	1.04 (0.60-1.82)	0.89	1.19 (0.69-2.06)	0.53	1.17 (0.75-1.81)	0.49
Gln/Gln	19 (0.19)	19 (0.17)	35 (0.26)	73 (0.22)	28 (0.18)	1.25 (0.59-2.66)	0.56	0.96 (0.46-1.99)	0.93	1.76 (0.91-3.42)	0.09	1.33 (0.76-2.32)	0.31
Arg	106 (0.54)	125 (0.57)	133 (0.50)	364 (0.53)	173 (0.57)	1		1		1		1	
Gln	92 (0.46)	95 (0.43)	135 (0.50)	322 (0.47)	133 (0.43)	1.12 (0.79-1.62)	0.44	0.99 (0.70-1.40)	0.51	1.28 (0.92-1.78)	0.14	1.15 (0.88-1.51)	0.31
The 194Arg/Trp *XRCC1*	**Number n=115 (Frequency)**	**Number n=116 (Frequency)**	**Number n=141 (Frequency)**	**Number n=372 (Frequency)**	**Number n=159 (Frequency)**								
Arg/Arg	107 (0.93)	103 (0.89)	125 (0.89)	335 (0.90)	149 (0.94)	1		**1**		1		1	
Arg/Trp+Trp/Trp	8 (0.07)	13 (0.11)	16 (0.11)	37 (0.10)	10 (0.06)	1.11 (0.43 -2.92)	0.82	1.88 (0.79-4.45)	0.14	1.91 (0.84-4.35)	0.12	1.65 (0.80-3.40)	0.17
Arg	222 (0.97)	219 (0.94)	266 (0.94)	707 (0.95)	308 (0.97)	1		1		1		1	
Trp	8 (0.03)	13 (0.06)	16 (0.06)	37 (0.05)	10 (0.03)	1.11 (0.43-2.86)	0.82	1.83 (0.79-4.24)	0.15	1.85 (0.83-4.15)	0.13	1.61 (0.79-3.28)	0.18
The 326Ser/Cys *OGG1*	**Number n=108 (Frequency)**	**Number n=115 (Frequency)**	**Number n=141 (Frequency)**	**Number n=364 (Frequency)**	**Number n=67 (Frequency)**								
Ser/Ser	70 (0.65)	78 (0.68)	97 (0.69)	245 (0.67)	108 (0.67)	1		1		1		1	
Ser/Cys	32 (0.30)	35 (0.30)	39 (0.28)	106 (0.29)	43 (0.27)	1.15 (0.66-1.98)	0.62	1.13 (0.66-1.92)	0.66	1.01 (0.60-1.69)	0.54	1.08 (0.71-1.65)	0.70
Cys/Cys	6 (0.06)	2 (0.02)	5 (0.04)	13 (0.04)	11 (0.07)	0.84 (0.30-2.38)	0.74	(0.05-1.17)	0.06	0.51 (0.17-1.51)	0.21	0.52 (0.23-1.20)	0.12
Ser	172 (0.80)	191 (0.83)	233 (0.83)	596 (0.82)	259 (0.80)	1		1		1		1	
Cys	44 (0.20)	39 (0.17)	49 (0.17)	132 (0.18)	65 (0.20)	1.02 (0.66-1.56)	0.92	0.81 (0.52-1.26)	0.36	0.84 (0.55-1.26)	0.40	0.88 (0.63-1.23)	0.46
The 324Gln/His *MUTYH*	**Number n=116 (Frequency)**	**Number n=116 (Frequency)**	**Number n=143 (Frequency)**	**Number n=375 (Frequency)**	**Number n=67 (Frequency)**								
Gln/Gln	65 (0.56)	69 (0.59)	105 (0.73)	239 (0.64)	97 (0.60)	1		1		1		1	
Gln/His	50 (0.43)	40 (0.34)	35 (0.24)	125 (0.33)	65 (0.40)	1.15 (0.71-1.86)	0.57	0.86 (0.52-1.43)	0.57	**0.47 (0.30-0.82)**	**0.005**	0.078 (0.53-1.14)	0.20
His/His	1 (0.01)	7 (0.06)	3 (0.02)	11 (0.03)	-	-		-		-			
Gln	180 (0.78)	178 (0.77)	245 (0.86)	603 (0.80)	259(0.80)	1		1		1		1	
His	52 (0.22)	54 (0.23)	41 (0.14)	147 (0.20)	65 (0.20)	1.15 (0.76-1.74)	0.50	1.21 (0.80-1.82)	0.36	0.67 (0.43-1)	0.06	0.97 (0.70-1.35)	0.86

The 762Val/Ala *ADPRT*	Number n=112 (Frequency)	Number n=121 (Frequency)	Number n=141 (Frequency)	Number n=346 (Frequency)	Number n=190 (Frequency)								
Val/Val	84 (0.75)	83 (0.69)	88 (0.62)	255 (0.74)	115 (0.72)	1		1		1		1	
Val/Ala	22 (0.20)	33 (0.27)	46 (0.33)	79 (0.23)	38 (0.24)	0.79 (0.44-1.44)	0.44	1.20 (0.70-2.07)	0.51	1.58 (0.95-2.64)	0.07	0.98 (0.60-1.46)	0.78
Ala/Ala	6 (0.05)	5 (0.04)	7 (0.05)	12 (0.03)	37 (0.04)	1.17 (0.38-3.62)	0.78	0.99 (0.30-3.23)	0.61	1.31 (0.44-3.86)	0.62	**0.14 (0.07-0.29)**	**<.0001**
Val	190 (0.85)	199 (0.82)	222 (0.79)	589 (0.85)	268 (0.84)	1		1		1		1	
Ala	34 (0.15)	43 (0.18)	60 (0.21)	103 (0.15)	52 (0.16)	0.92 (0.58-1.48)	0.74	1.11 (0.71-1.73)	0.63	1.39 (0.92-2.10)	0.11	0.90 (0.63-1.30)	0.58
The 148Asp/Glu of *APE1*	**Number n=116 (Frequency)**	**Number n=115 (Frequency)**	**Number n=118 (Frequency)**	**Number n=349 (Frequency)**	**Number n=159 (Frequency)**								
Asp/Asp	38 (0.33)	38 (0.33)	25 (0.21)	101 (0.29)	60 (0.38)	1		1		1		1	
Asp/Glu	77 (0.66)	77 (0.67)	90 (0.76)	244 (0.70)	96 (0.60)	1.16 (0.76-2.10)	0.36	1.16 (0.76-2.10)	0.36	**2.25 (1.30-3.89)**	**0.003**	**1.51 (1.01-2.25)**	**0.04**
Glu/Glu	1 (0.01)	0	3 (0.023)	4 (0.01)	3 (0.02)	0.52 (0.05-5.25)	0.50	-		2.40 (0.45-12.71)	0.26	0.79 (0.17-3.66)	0.53
Asp	153 (0.66)	153 (0.67)	140 (0.59)	446 (0.64)	216 (0.68)	1		1		1		1	
Glu	79 (0.34)	77 (0.33)	96 (0.41)	252 (0.36)	102 (0.32)	1.09 (0.76-1.56)	0.62	1.06 (0.74-1.53)	0.73	**1.45 (1.02-2.06)**	0.04	1.19 (0.90-1.58)	0.21

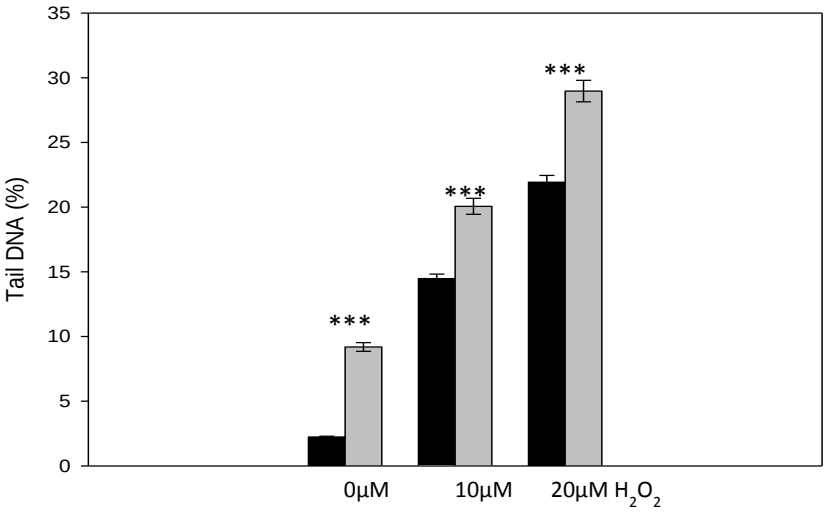

Fig 1. The level of basal and induced DNA damage in lymphocytes of patients with POAG (grey) in relation to healthy controls (black), measured by the alkaline comet assay. (*** p < 0001)

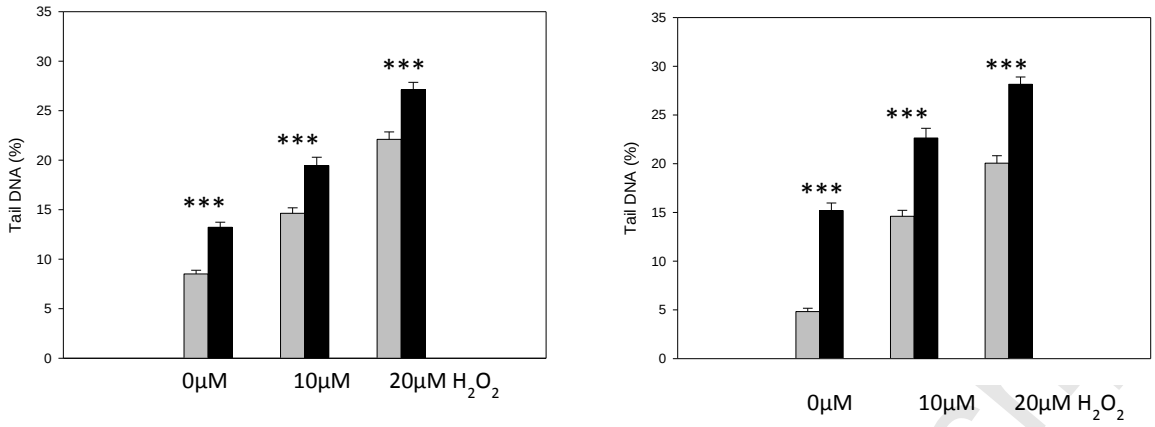

Fig 2. The level of basal oxidation and induced DNA damage by 10µM and 20µM H_2O_2 in lymphocytes of patients with POAG (black) in relation to healthy controls (grey), recognize by Nth (left panel) and Fpg (right panel) measured by the alkaline comet assay**.** (*** $p < 0.001$)

Fig 2. The level of basal oxidation and induced DNA damage by 10µM and 20µM H_2O_2 in lymphocytes of patients with POAG (black) and healthy controls (grey), recognize by Nth (left panel) and Fpg (right panel) measured by the alkaline comet assay**.**

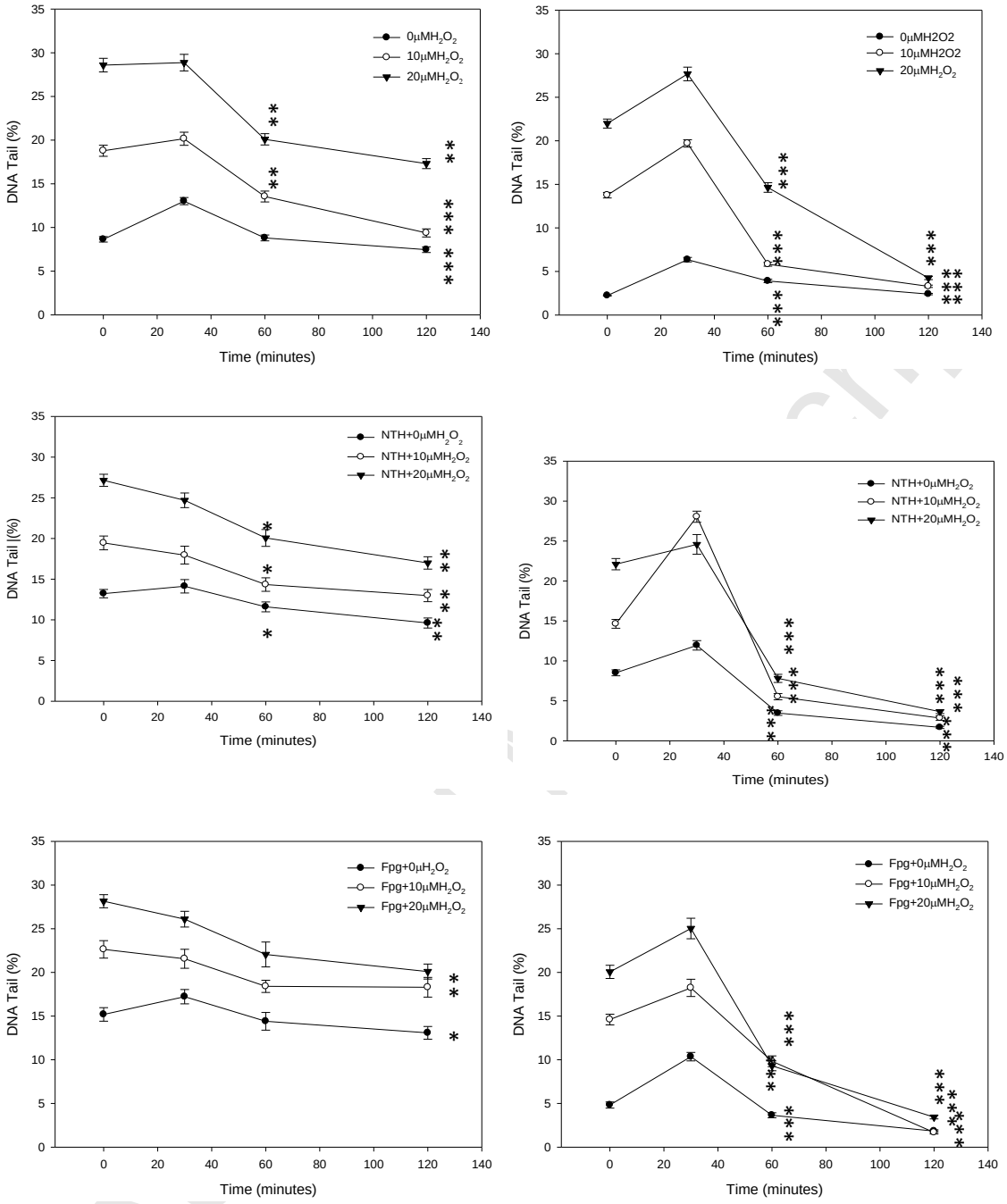

Figure 3. The kinetic of DNA repair in lymphocytes of POAG patients (left panel) and healthy controls (right panel) exposed to H_2O_2 at 10μM (⊸) and 20μM (⊸) for 10 minutes. We compare the percentage of DNA tail after 30, 60 and 120 minutes of incubation repair to time 0 in POAG patients and healthy control lymphocytes. Results obtained for Nth (middle panel), Fpg (lower panel) and without enzymes (upper panel). The values are displayed as mean ±SEM (n=10). (*p < 0.05, **p<0.01, *** p < 0.001

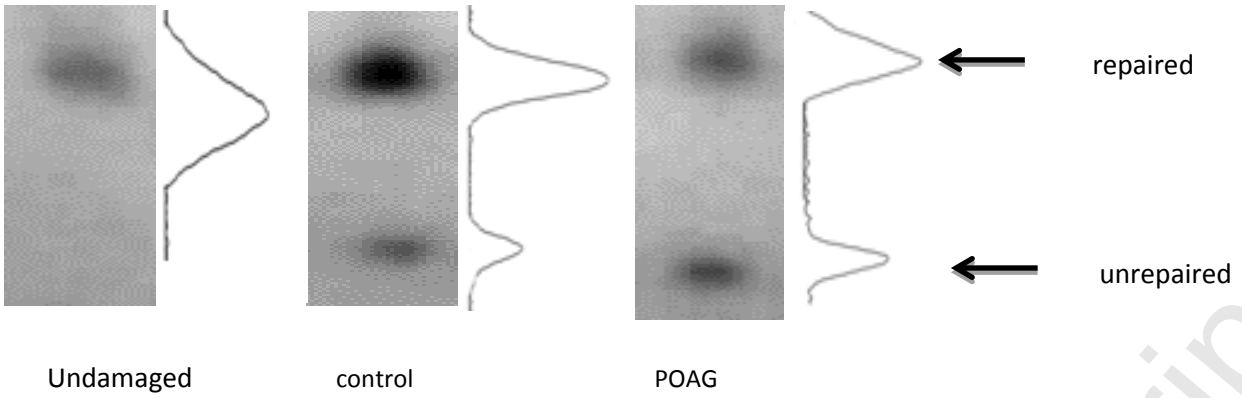

Figure 4a. BER activity in lymphocytes of POAG patients in comparison with healthy controls. Repaired and unrepaired DNA fragments after 1h incubations of substrate containing AP sites with cellular extracts obtained from lymphocytes POAG patients and healthy controls detected by autoradiography.

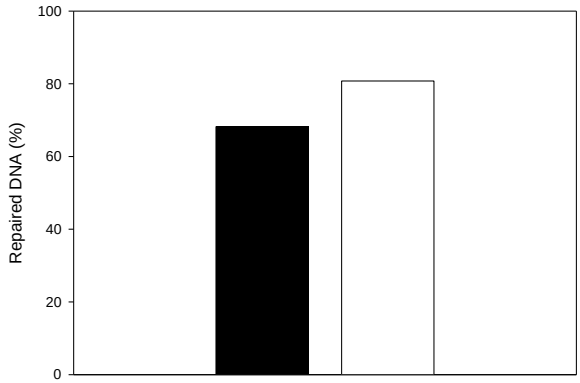

Figure 4b. Quantification on the BER activity expressed as a percentage of repaired DNA in cellular extracts is shown. The percentage of repaired DNA- the black bar represents POAG patients (68.20%) and the white bar represents healthy controls (80.767%)