The association between inefficient repair of oxidative DNA lesions and common polymorphisms of the key base excision repair genes as well as their expression levels in patients with Rheumatoid Arthritis

Keywords
rheumatoid arthritis, polymorphisms, comet assay, BER, BER genes

Abstract

Introduction
RA is a common autoimmune heterogeneous joint disease of still unknown etiology. One of the characteristic features of RA is oxidative stress, most probably induced and stimulated by inappropriate B and T-cell activity. We have hypothesized, that oxidative stress together with impaired DNA damage response to oxidative DNA lesions could be responsible for increased incidence in RA patients of some diseases with genetic instability background such as lymphoma.

Material and methods
We determined the level of oxidative DNA lesions and the kinetics of removal of DNA damage induced by TBH in PBMCs of 30 RA patients and 30 healthy individuals. The metrics from DNA repair study were correlated with genotypes of common polymorphisms of key BER genes as well as their expression levels. DNA repair were evaluated by comet assay, the genotypes of the polymorphism were determined by Taqman Assay and PrimeTime qPCR Assay was used to analyze the expression profile of genes related to BER.

Results
We observed an association between RA occurrences and impaired DNA repair in PBMCs. After dividing the subjects by controls' quartiles of DNA repair efficiency, we found an association between an increased risk of RA and inefficient DNA Repair. We also identified interaction between inefficient DNA Repair and polymorphism of the UNG gene (rs246079), and lower expression of key BER genes – MUTH, NEIL3 and UNG.

Conclusions
Our result suggest that the genetic variations within BER genes as well as epigenetic factors may be linked with RA by the modulation of the cellular response to oxidative stress.

Rheumatoid arthritis (RA) is a common autoimmune heterogeneous joint disease of still unknown etiology. The pathology of RA leads to chronic inflammation of the joint tissues, which causes joint cartilage and bone destruction. One of the characteristic features of RA is oxidative stress, most probably induced and stimulated by inappropriate B and T-cell activity. This results in accumulations of oxidative DNA lesions in peripheral blood mononuclear cells (PBMCs) isolated from RA patients as we shown previously. We have hypothesized, that oxidative stress together with impaired DNA damage response (DDR) to oxidative DNA lesions (limited to base excision repair pathway - BER) could be responsible for increased incidence in RA patients of some diseases with genetic instability background such as lymphoma and lung cancer. Therefore we determined the level of oxidative DNA lesions and the kinetics of removal of DNA damage induced by Tert-butyl hydroperoxide (TBH) in PBMCs of 30 RA patients and 30 healthy individuals. The metrics from DNA damage and repair study were correlated with the genotypes of common polymorphisms of the key BER genes as well as their expression levels. DNA damage and repair were evaluated by alkaline single cell gel electrophoresis (comet assay), the genotypes of the polymorphism were determined by Taq man SNP Genotyping Assay and PrimeTime qPCR Assay was used to analyze the expression profile of genes related to BER. We observed an association between RA occurrences and impaired DNA repair in PBMCs. After dividing the subjects by controls' quartiles of DNA repair efficiency, we found an association between an increased risk of RA and inefficient DNA Repair (ORs and 95%CI: 2.4 and 0.34-17, 32 and 4.6-222.6, 104 and 8.5-1279.2, for the 2nd –4th quartiles, respectively, compared with the 1st quartile). We also identified interaction between inefficient DNA Repair and polymorphism of the UNG gene (rs246079), and lower expression of key BER genes – MUTH, NEIL3 and UNG. Therefore, our result suggest that the genetic variations within BER genes as well as epigenetic factors may be linked with RA by the modulation of the cellular response to oxidative stress and these polymorphisms may be a useful additional marker in this disease along with the genetic or/and environmental indicators of oxidative stress. However, these conclusion need to be validated in larger studies.

Keywords: rheumatoid arthritis; BER; comet assay, expression, polymorphisms

1. Introduction

Rheumatoid arthritis (RA) is a common autoimmune heterogeneous joint disease of still unknown etiology. In RA inflammation starts in the synovial membrane and spreads further to surrounding tissues leading finally to cartilage and bone destruction [1,2]. Various genetic [3,4] and epigenetic [5] factors were associated with RA, but also the environmental factors like cigarette smoke, viral and bacterial infections play a significant role in development of RA [6,7]. The pathogenesis of RA is regulated by T and B-cells. B-cells are involved in the production of autoantibodies such as rheumatoid factor (RF) [8] and anti-cyclic citrullinated peptide autoantibodies (ACPA) [9,10]. T-cells produce a variety of pro-inflammatory cytokines, especially tumor necrosis factor (TNF), interleukin 6 (IL-6) as well as vascular endothelial growth factor (VEGF), which are involved in degradation of bone and cartilage within the joint [11]. The pathogenesis of the disease may also be related with IL-33. Extracellular IL-33 is a critical enhancer of tumor necrosis factor (TNF)-induced RA synovial fibroblast activation [12]. IL-33 in experimental models of collagen-induced arthritis exhibits triggering properties and the administration of interleukin 33 intensifies the disease process [13, 14].

One of the characteristic features of RA is oxidative stress [15], most probably induced and stimulated by inappropriate B and T-cell activity. It is connected with higher level of reactive oxygen species (ROS) production in RA patients as compared with healthy subjects. The main factor that increases the release of ROS in RA patients is overproduction of TNF [16]. TNF controls cell proliferation or cell death. The altered level of nuclear factor kappa-B (NF-κB) increases the TNF-induced death, concurrent with sustained Jun N-terminal kinase (JNK) activation via the death response. JNK activation in NF-κB-deficient cells depends on the ROS, but ROS influence in stimulations of JNK is still unclear [17]. Moreover, TNF-alpha, by increasing in endothelial cells during ischemia/reperfusion (I/R) injury the expression of arginase, leads to a decrease in the availability of l-arginine for nitric oxidase synthase, thereby

increasing the production of O2−•. Overproduction of O2−• impairs the NO-mediated vasodilation and favors endothelial dysfunction. TNF-alpha inhibitors significantly reduce inflammation in patients with chronic autoimmune diseases, improving endothelial function and reducing the risk of acute cardiovascular complications [18,19]. ROS also contribute to the depolymerisation of hyaluronic acid, causing the increased bone resorption and reduction of joint viscosity. The elevated ROS level was correlated with various clinical parameters of RA like seropositive, seronegative status or DAS score [20].

The negative ROS effect in RA is not limited to joints. Excessive ROS production results in accumulations of oxidative DNA lesions in peripheral blood mononuclear cells (PBMCs) isolated from RA patients as we shown previously [21]. We have hypothesized, that oxidative stress together with impaired DNA damage response (DDR) to oxidative DNA lesions (limited to base excision repair (BER) pathway) could be responsible for increased incidence in RA patients of some diseases with genetic instability background such as lymphoma and lung cancer [22]. Therefore the aims of this work is to evaluate repair of oxidative DNA lesion and correlate it with common polymorphism of BER genes as well as their expression level as the results from our previous studies suggest that polymorphism of DNA repair genes may have a phenotypic effects in repair of oxidative DNA lesions [24-26]. Therefore we determined the level of oxidative DNA lesions and the kinetics of removal of DNA damage induced by Tert-butyl hydroperoxide (TBH) in PBMCs of 30 RA patients and 30 healthy individuals. The metrics from DNA damage and repair study were correlated with the genotypes of common polymorphisms of the key BER genes as well as their expression levels. DNA damage and repair were evaluated by alkaline single cell gel electrophoresis (comet assay), the genotypes of the polymorphism were determined by Taq man SNP Genotyping Assay and PrimeTime qPCR Assay was used to analyze the expression profile of genes related to BER.

2. Materials and Methods

Patients

The study group included 30 patients with RA (22 women and 8 men; mean age 59.3 ± 15 years) selected from patients of the Department of Rheumatology and outpatient clinic and 30 volunteers (24 women and 6 men; mean age 61.5 ± 11.3) recruited from patients without symptoms of chronic inflammatory conditions. Such a number of subjects provides sufficient statistical power (>0.80) for final conclusions. This cohort study has been approved by the Institutional Bioethics Committee of the Medical University of Lodz (Lodz, Poland) (no. RNN/07/18/KE, approved date: 16 January 2018). All RA patients fulfilled the EULAR/ACR 2010 diagnostic criteria of RA. Persons with past or presence malignancy history in first family degree as a potential reason for DNA instability were excluded from the study.

Methods

Sample collection and preparation

Blood samples were collected from both, patients and controls into anticoagulant up to 9 ml tubes. Next, PBMC were isolated as well as DNA and RNA. Total RNA was isolated from peripheral blood using the RiboPure ™ RNA Purification Kit, blood kit according to the manufacturer's protocol. Isolated RNA was transcribed into cDNA to a final concentration of 100 ng using GoScript ™ Reverse Transcriptase according to the manufacturer's protocol. Genomic DNA was prepared using GeneMatrix Blood DNA purification Kit (EURx, Gdansk, Poland) according to the manufacturer protocol.

Analysis of SNPs

Genotypes were determined by using Taq man SNP Genotyping Assay. We analyzed ten SNPs in XRCC1, OGG1, UNG, MBD4, MUTYH, TDG, SMUG1 genes (respectively rs25487, rs1052133, rs246079, rs151095402, rs2307293, rs3219472, rs3219489, rs3219493, rs4135054 and rs3087404). The PCR reaction (total volume 25 μl) including 5 μl 5x HOT FIREPol® Probe qPCR Mix (Solis),

1μl DNA (100ng), 1,25ul 20x Taq man SNP primers and 17,75μl RNA free water conditions were as follows: polymerase activation (10min, 95°C), 30 cycles of denaturation (15s, 95 °C) annealing/extension (60s, 60 °C). Genotype determination was made in Bio-Rad CFX96 system (BioRad).

We performed PrimeTime qPCR Assay (Integrated DNA Technologies) to analyze the expression profile of 15 genes associated with BER, respectively: OGG1 (Hs.PT.58.38536541 and Hs.PT.58.38797078) MUTYH (Hs.PT.58.4052997 and Hs.PT.58.38885205), NTHL1 (Hs.PT.58.45671999), NEIL1 (Hs.PT.58.38858353), NEIL2 (Hs.PT.58.39722884), UNG (Hs.PT.58.39999493), TDG (Hs.PT.58.1836292.g), LIG3 (Hs.PT.58.4393909), PARP1 (Hs.PT.56a.112995), PARP3 (Hs.PT.58.39766379 and Hs.PT.58.40275253) MPG (Hs.PT.58.19216181), APEX1 (Hs.PT.56a.3182919), APEX2 (Hs.PT.58.26859287), MBD4 (Hs.PT.58.39947182), NEIL3 (Hs.PT.58.1086590) and SMUG1 (Hs.PT.58.27762894). We used 2 reference genes: ACTB (Hs.PT.39a.22214847) and RPLP0 (Hs.PT.39a.22214824). The expression of chosen as reference genes did were not sensitive for inflammation [27,28]. The total reaction volume was 10 μl including: 1ul cDNA, 1μl Primers, 2μl 5x HOT FIREPol® Probe qPCR Mix (Solis) and 6 μl Nuclease free water. Condition for the reaction were suggested by the manufacturer of the HOT FIREPol® Probe qPCR Mix: 15 minute enzyme activation at 95 °C , 40 cycles of denaturation (10s, 95 °C), annealing/extension of 60s, 60 °C. qPCR reaction was made in Bio-Rad CFX96 system (BioRad). Gene expression was calculated in relation to that of the reference genes (ΔCt sample = Ct target gene—Ct reference gene). Following, the relative mRNA expression were calculated as fold = $2^{-\Delta Ct}$ sample. Expression status was determined from the data regarding the expression value of the analyzed gene in the control patient population. Based on these results, 3 groups were defined. Samples with results above or equal the median value (3rd and 4th quartiles) were classified as normal expression (Group 1), those

located below the median in quartile 2 as low expression (Group 2) and in quartile 1 as very low expression/no expression (Group 3).

Analysis of efficacy of DNA repair

The alkaline version of the comet assay with modifications [21] was used to evaluate the efficacy of DNA repair. Comet assay is a rapid, sensitive and selective technique for quantifying and analyzing DNA damage in individual cells. The DNA damaging agents selected for this study was TBH. TBH is well known agent that induces oxidative DNA lesions repaired mainly by BER pathway [29]. We used TBH at 7 μM for 15 min on ice – this concentrations did not affect the viability of PMBCs (viability of PMBCs was always >80%). The kinetics of DNA repair was calculated by estimation of the level of DNA damage induced by TBH during the 60 min of repair process. The DNA damage measured immediately after exposure to TBH was set as 100% of DNA damage to avoid any problems associated with individual and RA driven variation to TBH sensitivity. Next, we measured percentage of repaired DNA after 60 min. By subtracting the obtained value from 100 we obtained the individual DNA repair efficiency (DReff, the lower the value the better). Next, the DNA repair ranks of individuals was determined similarly to expression analysis. All subjects were divided into four groups regarding quartiles for individual DNA repair efficiency of control group. Quartile 1 means highly efficient DNA repair, quartile 2 efficient repair, quartile 3 low efficient DNA repair and quartile 4 no repair.

Global methylation analysis

Global methylation was assessed using the MethylFlash TM Global DNA Methylation (5-mC) ELISA Easy Kit. In this assay, DNA is applied to the wells of the plate, which have a high affinity for DNA after treatment. The methylated DNA fraction is recognized using capture and detection antibodies and then quantified colorimetrically by reading the absorbance on a microplate spectrophotometer. 100 ng of isolated DNA from patients with rheumatoid arthritis

and controls were applied to the well strips included in the kit. The analysis procedure was performed according to the manufacturer's protocol.

Statistical analysis

Descriptive data were expressed as mean and standard deviations (SD). The data from comet assay are presented as the median ± range. The normal distribution of continuous variables was confirmed by the Shapiro–Wilk test. Mann–Whitney rank sum test was used to compare DNA damage between patients with RA and healthy controls and it was decided based on the normality test. The relationships among the continuous variables were assessed using the Spearman's rank correlation coefficient. Multinomial logistic regression analyses were performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the effects of DNA repair status and other variables on RA. All variables included in the final multivariate models were determined to be independent by assessing their collinearity. Age, sex, genotypes and expression level of BER genes, were included as independent variables in univariate and multivariate multinomial logistic regression analyses. Only matching variables and factors that altered the ORs by 10 % were included in the final multivariate models. The quality of the models was determined by Hosmer–Lemeshow test. All statistical analyses were performed with TIBCO Statistica 13.3 (Palo Alto, CA 94304, USA). In all tests, p value <0.05 was used.

3. Results

Characteristics of the study population

There were no significant differences in the distributions of age, sex and smoking status between cases and controls. Mean time of disease duration was 12.27 ± 10.7 years (from 1 to 37 years). Eighteen patients were currently (for at least one month before blood collection) treated with methotrexate, three patients with sulfasalazine, and nine patients did not receive disease modifying anti-rheumatic drugs (DMARDs) within the last month. Glucocorticosteroids (GCS)

were used for treatment of sixteen patients. Five patients had not rheumatoid factor levels (positive in 25 cases). In addition, we determined the level of C-reactive protein, CRP (18.7 ± 22.60 g/dL). The disease activity has been also assessed based on Disease Activity Score 28-joint count C reactive protein (DAS28) -CRP score (DAS <1.7 was defined as remission – 2 patients, DAS >1.7 and <2.6 was defined as low disease activity – 13 patients and DAS28 above 5.1 as high disease activity – 9 patients). All controls had CRP (C-reactive protein) within normal limits and did not have any chronic disease with inflammatory background.

Differences in DReff between RA patients and controls

PBMCs isolated from RA patients were more sensitive for TBH as compared to healthy patients (Figure 1A; median RA=13.515; 25%=10.090; 75%=13.515 vs. 6.350; 25%=3.685; 75%=8.540; Mann–Whitney rank sum test $p < 0.001$). Moreover, RA group has more subjects in groups classified as low efficient and inefficient DNA repair as compared with control (Figure 1B and Table 1). Similarly, we observed more people with inefficient DNA repair in the RA group treated with DMARDS (Table 1).

Differences in BER mRNA expression levels between RA patients and controls

We also observed generally lowered expression level of BER genes in RA than control. Significant statistical difference in the level of gene expression was calculated for: OGG1, MUTYH, NTHL1, LIG3, PARP3, APEX1, APEX2, MBD4 and PARP1 genes (Table 2). Next, the relative expression levels were grouped into quartile values of the controls to use in multivariate logistic regression analysis. Please note that in some subjects' expression of UNG, PARP1 and PARP were practically undetectable, and median is 0.

The allele and genotypes of key BER genes in RA patients and controls

The observed differences in the expression of key genes encoding BER proteins were not due to a change in the profile of CpG island methylation (Supplementary Figure 1).

RA and control groups were similar in the allele and genotypes distribution of (Supplementary Table 1). No association with RA was found using univariate logistic regression. This is not surprising given the sample size and aims of this manuscript since we would like to focus on correlation of phenotype with genotype (SNP) and epigenetic factors (expression ratio).

Associations between DReff and ~~risk of~~ RA

To estimate RA risk, the relative DReff were grouped into quartile values of the controls (Table 1 and Table 3). The crude ORs for RA risk associated with relative DReff in the second quartile, third quartile and fourth quartile were 2.4 (95% CI, 0.34–17), 32 (95% CI, 4.6–222.6) and 104 (95% CI, 8.5–1279.2), compared with the first quartile (the highly efficient DNA repair). After adjusting for SNP rs246079 in UNG gene and expression level of UNG, MUTYH and NEIL3 genes in multivariate logistic regression analysis, the ORs of DReff increased significantly in third and fourth quartile corresponding for low efficient and no repair group respectively.
We tested also possible correlations between DReff and clinical parameters of RA like DAS, RF, aCCP and CRP, however no correlations was found.

4. Discussion

We observed an association between RA occurrence, impaired DNA repair of oxidative DNA lesions in PBMCs, polymorphism of the UNG gene and expression of key BER genes – UNG, MUTYH and NEIL3. Therefore, our result suggests that the genetic variations within BER genes as well as epigenetic factors may be linked with RA by the modulation of the cellular response to oxidative stress and this polymorphism may be a useful additional marker in this disease or/and environmental indicators of oxidative stress. Because of oxidative stress mainly 8-oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamido-pyrimidine were formed in DNA, which arise as a result of attaching to the guanizine ring at the C8 position of the hydroxyl radical and their further transformations in redox reactions [30]. These DNA

lesions are the main substrate for BER pathway and are removed with the help of MUTYH and NEIL3 DGs. MUTYH is responsible for the removal of incorrectly substituted adenine and 2-hydroxyadenine paired with 8-oxo-dG whereas NEIL3 exhibits a broad substrate recognition spectrum and can excise both oxidized pyrimidines and purines except 8-oxo-G [31]. A few reports have been published describing the effect of altered expression of BER genes in RA. Most of them, linked PARP1 gene with the progression of inflammation in RA (reviewed at [32]. The second concerns the MUTYH gene. Observed serum level of this gene was 8.8% higher in RA than in healthy people [33]. Our research clearly indicates that patients with rheumatoid arthritis have reduced expression level of genes encoding two important for oxidative DNA lesions DGs in PBMC compared to the control group. Decreased gene expression may result in ineffective or complete lack of repair and our study is in the line with this hypothesis. Unrepaired damage can form double-stranded DNA strand breaks (DSBs) as a result of further cellular metabolism [34]. DSB is one of the most serious DNA damages, resulting often in cell death, aging or mutations. The latter can lead to the development of cancer and explain in this manner the significant number of people with cancer after developing RA. The mutagenic effects of oxidative DNA damage are well recognized and connected with DSBs. For example, GC→TA or CC→TT are signature mutations for oxidative DNA lesions, and they have been observed in the p53 tumor suppressor or the ras oncogene gene in various cancers.

The finding suggesting the potential involvement of UNG at the genetic and epigenetic level in the inefficient repair of oxidative lesions in RA is exciting. UNG is the primary glycosylase involved in the removal of uracil (U) from DNA. The involvement of UNG in the removal of oxidative DNA lesions is minimal in contrast to other glycosidases; however, it plays a very important role in promoting lymphocyte development. During lymphocyte development two molecular processes on DNA occurred – VDJ and class switching recombination (CSR). Both are important for the formation of a wide range of lymphocyte B antigen receptors and heavily

depend on somatic hypermutation (SHM). SHM is conducted in cooperation with BER and MMR systems and UNG plays a key role in this process by recognizing and processing U. Imbalance of UNG level increased risk of developing B-cell lymphomas however, it is not only carcinogenesis that should be addressed as UNG also probably seems to be involved in autoimmune diseases development. The involvement of UNG in autoimmune diseases is complex and loss of UNG or changes of its activity via SNP can affect RA progression in two ways. Firstly, it can be responsible for severely impaired CSR and antibody affinity maturation via SHM [35]. Secondly, it can induced apoptosis in B cells. Apoptosis results in DNA fragments release outside cell. DNA fragments can stimulate innate inflammatory responses via the activation of the stimulator of interferon genes adaptor protein (reviewed at [36]). These can produce feedback loop as inflammations generates ROS that introduced oxidized DNA lesions, which unrepaired leads to apoptosis.

These findings confirm those of our earlier studies [37], and suggest also that SNPs in DNA repair genes may be another genetic factors linked with RA and contribute to the etiology, pathogenesis and outcome of RA however, the groups were relatively small to enable a final conclusion, and further studies are required to address the question.

Although our work has the character of basic research clinical aspects can be derived from it. The antioxidant potency of the host should be increased in order to avoid the effects of oxidative stress at the DNA level in people with reduced DNA repair efficiency. This can be done with an antioxidant-rich dietary intervention, because such diet ameliorates the state of oxidative stress and improves the antioxidant potency [38, 39]. In addition, we believe that a closer understanding of the genetic background of RA in association with the clinical phenotype will allow for future to personalized treatment of RA.

Our study has some limitations. First of all , the influence of the drugs taken by the patient cannot be neglected. MTX as well as GCS have plethora effects on RA patients. MTX but not

GCS was associated with the slightly increasing level of endogenous DNA damage compared with RA patients without treatment as we reported previously [21]. However, considering the research hypothesis of this article, it is irrelevant whether the observed alterations in the activity of DNA repair processes and their association with genotype or gene expression alterations are due to RA phenomena or therapy. Although we are more convinced of the former option, since MTX and GCS are drugs which are generally considered safe, and no association between treatment with these drugs and cancer development has been demonstrated. Anyway, our study had mainly preliminary character and these results need confirmation in larger cohorts outside the Central European populations, as the main limitations of the study are a single-center study, sample size and ethnicity.

5. Conclusions

In conclusion, impaired repair of oxidized DNA lesions in RA patients may accelerate PBMC aging and contribute to RA phenotype and linked with RA cancer development. Genetic and epigenetic factors contribute to this phenomenon, however epigenetic factor that led to lower expression to BER genes need to be identified. We exclude possibility of different methylation pattern in RA patients as epigenetic factor. Thus we must consider the two last options - histone code and miRNA expression. The last one is particularly interesting as some miRNAs are being considered as biomarkers for RA, however they need validation in a real-world clinical setting (reviewed at [40]). Connecting player between RA and ineffective repair of oxidative DNA damage may be miRNA-155, as it is associated with the occurrence of both phenomena [41,42].

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Bioethics Committee of the

Medical University of Lodz (Lodz, Poland) (no. RNN/07/18/KE, approved date: 16 January 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to law regulations in country of correspondence author.

Conflicts of Interest: The authors declare no conflict of interest.

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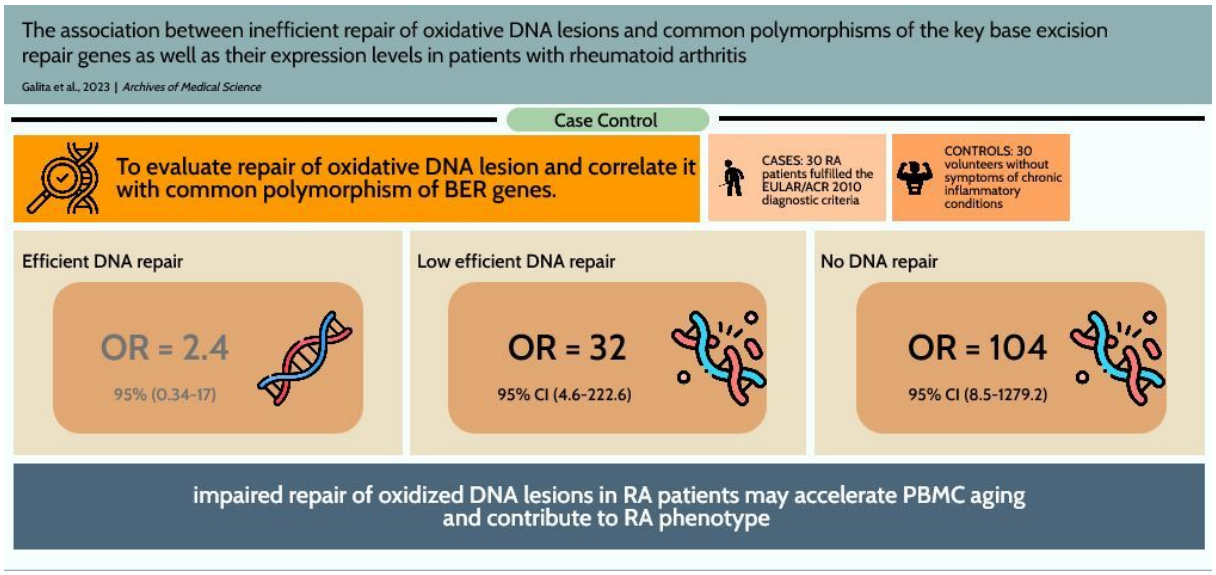
Figures

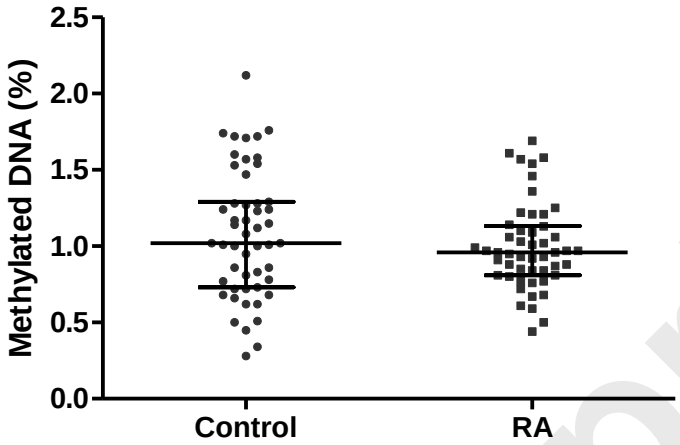
Figure 1. A) Peripheral blood mononuclear cells (PBMCs) show greater sensitivity for tert-butyl hydroperoxide (TBH) at 7 μM for 15 min as scored as DNA damage. PBMCs was isolated from 30 healthy controls (white box) and 30 rheumatoid arthritis (RA) patients (grey box). B) Distribution of individual DNA repair efficiency in 30 healthy controls (white box) and RA patients. Data are presented as median. Differences between groups were analyzed using the Mann–Whitney rank sum test Analysis, *** means $p < 0.001$


The association between inefficient repair of oxidative DNA lesions and common polymorphisms of the key base excision repair genes as well as their expression levels in patients with rheumatoid arthritis
Galita et al., 2023 | Archives of Medical Science
Case Control
To evaluate repair of oxidative DNA lesion and correlate it with common polymorphism of BER genes.
CASES: 30 RA patients fulfilled the EULAR/ACR 2010 diagnostic criteria
CONTROLS: 30 volunteers without symptoms of chronic inflammatory conditions
Efficient DNA repair
OR = 2.4
95% (0.34-17)
Low efficient DNA repair
OR = 32
95% CI (4.6-222.6)
No DNA repair
OR = 104
95% CI (8.5-1279.2)
impaired repair of oxidized DNA lesions in RA patients may accelerate PBMC aging and contribute to RA phenotype

Supporting Information

for

The association between inefficient repair of oxidative DNA lesions and common polymorphisms of the key base excision repair genes as well as their expression levels in patients with Rheumatoid Arthritis



SFigure 1. Patients with rheumatoid arthritis show similar levels of Global DNA methylation to healthy controls. Global methylation was assessed using the MethylFlash ™ Global DNA Methylation (5-mC) ELISA Easy Kit. Differences between groups were analyzed using the Student's t-test Analysis, *** means $p < 0.001$

STable 1. The allele and genotype frequency of the common polymorphisms in BER related genes in RA.

Genotype or allele	RA (n = 30)		Controls (n = 30)	
	Number	Frequency	Number	Frequency
XRCC1 (rs 25478)				
C/C	8	0.37	20	0.67
C/T	17	0.46	7	0.23
T/T	5	0.17	3	0.10
C	36	0.60	47	0.78
T	24	0.40	13	0.22
hOGG1 (rs 1052133)				
C/C	20	0.67	24	0.80
C/G	6	0.20	3	0.10
G/G	4	0.13	3	0.10
C	46	0.77	51	0.85
G	14	0.23	9	0.15

UNG (rs 246079)				
A/A	3	0.10	7	0.23
A/G	19	0.63	17	0.57
G/G	8	0.27	6	0.20
A	25	0.42	31	0.52
G	35	0.58	29	0.48
UNG (rs 151095402)				
C/C	29	0.97	30	1.00
C/T	0	-	0	-
T/T	1	0.03	0	0.00
C	58	0.97	60	1.00
T/T	2	0.03	0	0.00
MBD4 (rs 2307293)				
C/C	27	0.90	29	0.97
C/G	3	0.10	1	0.03
G/G	0	-	0	-
C	57	0.95	59	0.98
G	3	0.05	1	0.02
MUTYH (rs 3219472)				
C/C	22	0.73	24	0.80
C/T	6	0.20	6	0.20
T/T	2	0.07	0	0.00
C	50	0.83	54	0.90
T	10	0.17	6	0.10
MUTYH (rs 3219489)				
C/C	1	0.03	2	0.07
G/C	21	0.60	10	0.33
G/G	8	0.37	18	0.60
C	20	0.33	14	0.23
G	40	0.67	46	0.77
MUTYH (rs 3219493)				
C/C	30	1.00	28	0.93
C/G	0	-	0	-
G/G	0	0.00	2	0.07
C	60	1.00	56	0.93
G	0	0.00	4	0.07
TDG (rs 4135054)				
C/C	20	0.67	17	0.57
C/T	10	0.33	13	0.43
T/T	0	0.00	0	0.00
C	50	0.83	47	0.78
T	10	0.17	13	0.22
SMUG1 (rs 3087404)				
C/C	10	0.33	11	0.37
C/T	17	0.57	16	0.53

T/T	3	0.10	3	0.10
C	37	0.62	38	0.63
T	23	0.38	22	0.37

Table 1. The efficiency* of repair of DNA lesions induced by TBH (the lower the value, the better).

DReff	Range	Controls (n = 30)	RA (N = 30)	RA no DMARDS	RA DMARDS
Group 1 Highly efficient DNA repair	<16.04	16	2	2	0
Group 2 Efficient DNA repair	16.05-22.54	10	3	3	0
Group 3 Low efficient DNA repair	22.55-29.03	3	12	4	8
Group 4 No repair	>29.04	1	13	0	13

RA, Rheumatoid arthritis; *The efficiency was calculated as follow: The DNA damage measured immediately after exposure to TBH was set as 100% of DNA damage. Next, percentage of repaired DNA after 60 min. was measured. The individual DNA repair efficiency was obtained by subtracting this value from 100.

Table 2. Comparison of the mRNA expression levels of 16 core base excision repair genes between 30 rheumatoid arthritis (RA) patients and 30 controls. Analysis was performed using PrimeTime qPCR Assay. All expression values were multiple by 10^3 for better presentation of data.

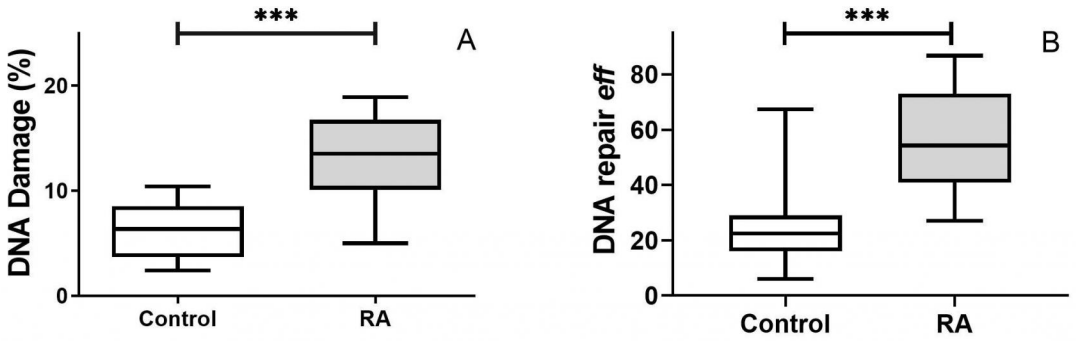
	Median/IQR		
Gene	RA (n=30)	Control (n=30)	p*
OGG1	3.102/5.719	6.302/10.883	0,006051
OGG1#	2.925/10.598	9.165/11.264	0,0003
MUTYH	0.000/0.730	0.796/1.055	0,008
MUTYH#	0.467/1.455	0.860/2.991	0,0611
NTHL1	1.982/4.996	3.19/5.824	0,0479
NEIL1	0.67/1.592	0.771/3.022	0,3208
NEIL2	3.631/12.007	7.124/0.139	0,1462
UNG	0.000/1.001	0.502/0.868	0,4404
TDG	1.484/9.673	1.696/4.013	0,7635
LIG3	1.919/5.64	4.231/5.954	0,0076
PARP3	0.000/0.001	0.226/0.907	0,0005
PARP3#	5.439/10.344	7.274/12.384	0,0964
MPG	5.439/10.100	7.274/12.384	0,0995
APEX1	12.082/29.179	22.290/15.943	0,0274
APEX2	20.980/29.314	29.038/26.569	0,0479
MBD4	0.853/1.736	2.167/4.465	0,0001
NEIL3	21.043/48.529	26.42/33.452	0,5324
PARP1	0,000000/0,003974	5.031/10.653	0,0047
SMUG1	7.724/34.175	20.019/27.396	0,6228

RA, Rheumatoid arthritis; * Mann–Whitney rank sum test; #different transcript

Table 3. Logistic regression analysis of DNA repair efficiency in RA cases and controls

DReff	Crude OR (95% CI)	Adjusted OR (95% CI)			
		rs246079 (UNG)	MUTYH	UNG	NEIL3
Group 1	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Group 2	2.4 (0.34-17)	2 (0.24-16.7)	2.5 (0.33-19.25)	2.66 (0.34-20.4)	2.32 (0.31-17.2)
Group 3	32 (4.6-222.6)	69.3 (6.5-852.9)	40.7 (4.7-349.1)	48.3 (5.35-436.4)	40.6 (4.9-338.9)
Group 4	104 (8.5-1279.2)	212.3 (11.1-4065.1)	209.2 (10.7-4106.3)	258.6 (13.8-4847)	303.4 (12.1-7580.6)

CI, confidence interval; RA, rheumatoid arthritis; OR, odds ratio. DReff by quartile based on the quartile values of control subjects.


***
A
DNA Damage (%)
20
10
0
Control
RA
***
B
DNA repair eff
80
60
40
20
0
Control
RA