DNA damage and arterial hypertension. A systematic review and meta-analysis

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Oxidative DNA damage markers (8OHdG, comet assay, gammaH2AX) are becoming widely used in clinical cardiology research. To conduct this review of DNA damage in relation to hypertension in humans, we used databases (e.g. PubMed, Web of Science) to search for English-language publications up to June 30, 2022 and the terms: DNA damage, comet assay, gammaH2AX, 8OHdG, strand breaks, and arterial hypertension. Exclusion criteria were: children, absence of relevant controls, extra-arterial hypertensive issues, animal, cell lines. From a total of 79526, 15 human studies were selected. A total of 902 hypertensive patients (pts): (comet: N=418 pts; 8OHdG: N=484 pts) and 587 controls (comet: N=203; 8OHdG: N=384) were included. DNA damage was significantly higher in hypertensive pts than healthy controls (comet 26.6±11.0 vs 11.7±4.07 arbitrary units /A.U./; $P<0.05$ and 8OHdG 13.1±4.12 vs 6.97±2.67 ng/mg creatinine; $P<0.05$) confirmed with meta-analysis for both. Greater DNA damage was observed in more adverse cases (concentric cardiac hypertrophy 43.4±15.4 vs 15.6±5.5; sustained/untreated hypertension 31.4±12.1 vs 14.2±5/35.0±5.0 vs 25.0 ±5.0; non-dippers 39.2±15.5 vs 29.4±11.1 A.U.; elderly 14.9±4.5 vs 9.3±4.1 ng/mg creatinine; without carvedilol 9.1±4.2 vs 5.7±3.9; with coronary heart disease 0.5±0.1 vs 0.2±0.1 ng/mL) ($P<0.05$) confirmed with meta-analysis. DNA damage correlated strongly positively with serum glycosylated haemoglobin (r=0.670; $P<0.05$) and negatively with total antioxidant status (r=−0.670 to −0.933; $P<0.05$). This is the first systematic review with meta-analysis showing that oxidative DNA damage was increased in humans with arterial hypertension compared to controls.

Key words: DNA strand break damage, gammaH2AX, comet assay, 8OHdG, arterial hypertension, cardiovascular disease

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INTRODUCTION

The rapid accumulation of papers on deoxyribonucleic acid (DNA) damage in cardiovascular (CV) medicine requires the serious attention of clinical cardiologists[1-6]. DNA damage has recently begun to be investigated in a broad spectrum of CV medical contexts (risk factors, diseases, diagnostic and therapeutic procedures including pharmacological agents or intervention techniques) with the aim of stopping or preventing CV pathogenesis resulting predominantly from oxidative stress, as well as finding either a novel therapeutic target or an early and reliable prognostic or predictive factor. Research efforts on DNA damage in CV medicine are prolific, but need to be constructively managed. The authors believe that this review of oxidative DNA damage in arterial hypertension may be, at this point helpful[7].

METHODS

We searched multiple databases (PubMed/PubMedCentral, Web of Science) from inception until June 30, 2022 for studies on DNA damage and arterial hypertension (HT) using key words that are summarized along with inclusion and exclusion criteria and a flow diagram of article selection in Fig. 1 and Fig. 2.

Three main types of oxidative DNA damage were targeted. In ascending order according to the extent of DNA damage and biological harm for the organism and offspring, they are: oxidised guanine/guanosine, single strand breaks (SSBs), and double strand breaks (DSBs). These were assessed in terms of relevant biomarkers or analytical methods: 8-hydroxy-2'-deoxyguanosine (8OHdG), comet assay, and phosphorylated histone AX2 (gammaH2AX) (Fig. 3a).

The number of searched studies or other items is described using "N". Statistical significance was defined as *P*-value ($P<0.05$). Correlation was expressed as Pearson's correlation coefficient (r).

KEY WORDS	INCLUSION CRITERIA	EXCLUSION CRITERIA
• Arterial hypertension • DNA damage • DNA SSBs • DNA DSBs • DNA SBs • Comet assay • 8OHdG • γH2AX	• The most severe DNA damage – SBs • The most frequently studied DNA damage – 8OHdG • Arterial hypertension • Humans	• Failure of key words • Apoptosis of relevant analysis (TUNEL assay) • Telomerase changes • Epigenetic DNA modifications * • Cell - free DNA • Children, animal, cell - lines • Extra CV issues (neoplasma,...) • Other types of DNA damage markers • No relevant controls

Fig. 1. Selection criteria – DNA damage and arterial hypertension in humans.
Comet assay, an assay for DNA SSBs detection; CV, cardiovascular; DNA, deoxyribonucleic acid; DSBs, double strand breaks; gammaH2AX, phosphorylated histone 2AX (a marker of DNA DSBs); SBs, strand breaks; SSBs, single strand breaks; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; 8OHdG, 8-hydroxy-2'-deoxyguanosine; *DNA methylation, histone acetylation, micronucleic acid modification.

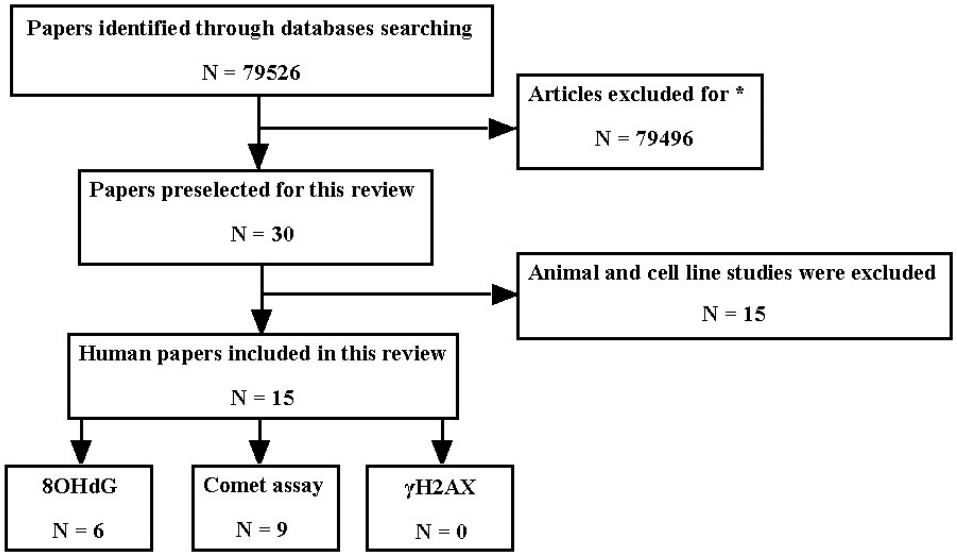

Fig. 2. Selection diagram – DNA damage and arterial hypertension in humans.
Comet assay, an assay for DNA single strand break detection; DNA, deoxyribonucleic acid; γH2AX, a marker for DNA double strand breaks; N, number; 8OHdG, 8-hydroxy-2'-deoxyguanosine; *not in English; not concerning 8OHdG/Comet assay/gamma-H2AX in arterial hypertension; not appropriate controls; duplicates; no free full text.

Meta-Analysis of Means were used – results are presented as mean difference with 95% confidence limit MD (95% CL). Forest plot was used for graphical presentation. χ^2 test was used to test the null hypothesis that all differencies are zero versus the alternative that all studies had the same non-zero differencies (heterogeneity tests). Level of significance was α=0.05. Statistical Software (NCSS 2021, LLC. Kaysville, Utah, USA, ncss.com/software/ncss) was used.

RESULTS

The 15 selected studies are summarised in Table 1. Only human studies were selected and only nuclear (not mitochondrial) DNA damage was investigated. Oxidative DNA damage markers were tested in blood and urine (not tissues). All selected studies are prospective, appropriately controlled (matched at least for age) using either a case control or a cohort control design. There were high standards with respect to publication ethics in all selected studies to the best of our knowledge. In toto 902 HT patients (pts) mean age 47 years and 587 controls were included (Table 1, Fig. 4B).

Human studies provide consistent data that HT/pressure overload increases oxidative DNA damage markers, when the blood pressure (BP) remained independently on treatment significantly higher compared to controls (Fig. 4B, Table 1, 2) (ref.[8-17,19-20,22]). Meta-analysis showed an average mean difference between hypertensive patients and healthy controls of 7.5 (6.6; 8.6) ng/mg creatinine for 8OHdG and 14.7 (6.4; 23.0) from the Comet assay (Fig. 3b.). Table 2 shows human studies categorised according to antihypertensive drugs into those with untreat-

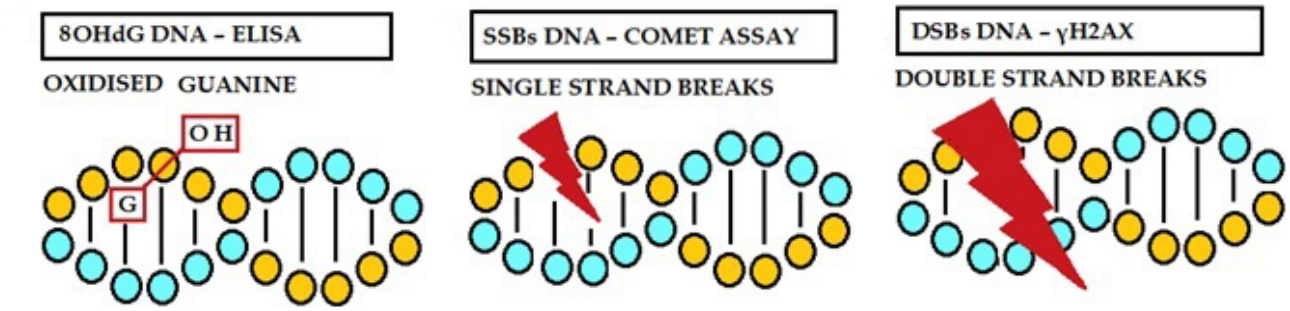

Fig. 3a. Oxidative DNA damage – the most important types and analytical methods.
Comet assay, an assay for SSBs; DNA, deoxyribonucleic acid; DSBs, double strand breaks; ELISA, enzyme – linked immunosorbent assay (an assay for 8OHdG); gammaH2AX, a marker for DSBs; SSBs, single strand breaks; 8OHdG, 8-hydroxy-2'-deoxyguanosine.

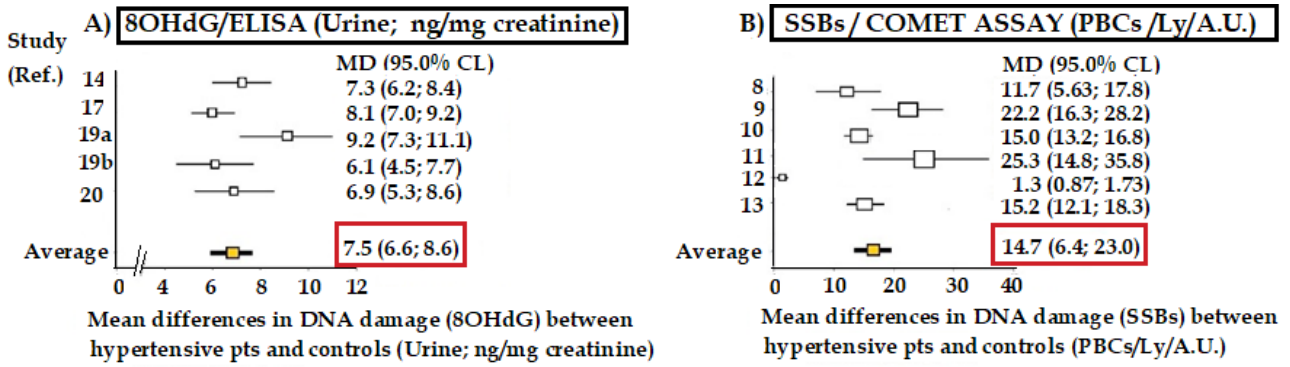

Fig. 3b. The DNA damage average mean difference (95% CL) between hypertensive patients and controls among the studies was **A)** 7.5 (6.6; 8.6) for 8OHdG/ELISA (Urine; ng/mg creatinine) with substantial heterogeneity (chi^2=601.7; *P*<0.0001) and **B)** 14.7 (6.4; 23.0) for SBs/COMET assay (PBCs /Ly/; A.U.) with (chi^2=140.8; *P*<0.0001). Fig. 3b is related to Table 1.
CL, confidence limit; ELISA, Enzyme-linked Immunosorbent assay; MD, mean differences; SBs, strand breaks; PBCs, peripheral blood cells; Pts, patients; Ly, Lymphocytes; A.U., arbitrary unit.

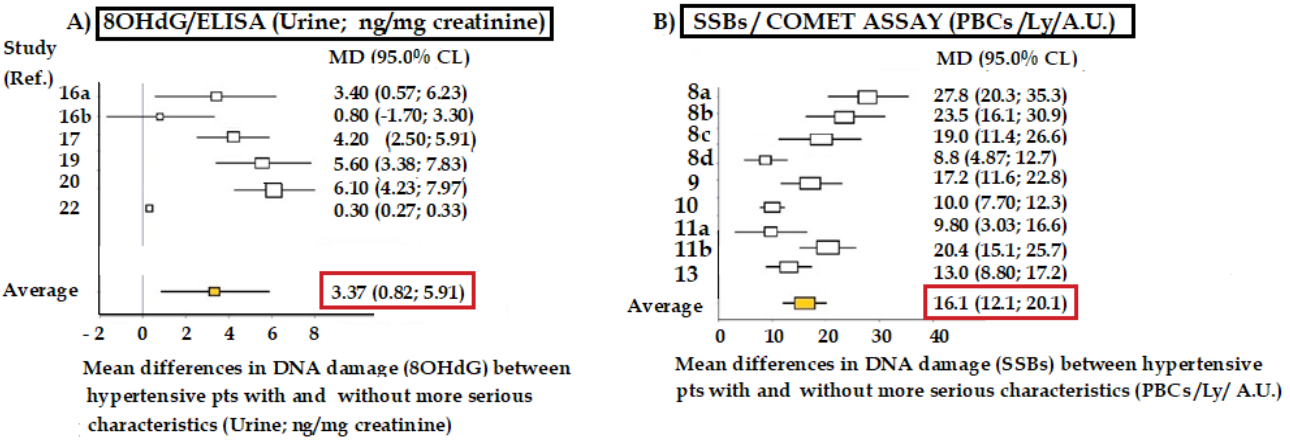

Fig. 3c. The DNA damage average mean difference (95% CL) between hypertensive patients with and without additive adverse effect among the studies was **A)** 3.37 (0.82; 5.91) for 8OHdG/ELISA (Urine; ng/mg creatinine) with substantial heterogeneity (chi^2=335.4; *P*<0.0001) and **B)** 16.1 (12.1; 20.1) for SBs/COMET assay (PBCs /Ly/; A.U.) with (chi^2=313.9; *P*<0.0001). Fig. 3c is related to Table 3.
CL, confidence limit; ELISA, Enzyme-linked Immunosorbent assay; MD, mean differences; SBs, strand breaks; PBCs, peripheral blood cells; Pts, patients; Ly, Lymphocytes; A.U., arbitrary unit.

Table 1. Humans – increased oxidative DNA damage in arterial hypertension vs healthy controls.

Author	Tested parameter (additive specif.)	Cohort N (Age [year/s])	Controls-Age mtch N (additive specif.)	DNA damage: type/ method Source, Units	Comparation C vs HT using DNA damage level $P < 0.05$	Ref.
SBs/ COMET ASSAY						
Gur M	HT	84 (50±6)	24	PBCs /Ly/, A.U.	C < HT 15.5±6.1 < 26.7±14.6	8
Yildiz A	HT	21 (45±7)	19	PBCs /Ly/, A.U.	C < HT 9.2±4.4 < 31.4±12.1	9
	"HT"	23 (46±6)	19	PBCs /Ly/, A.U.	C = *"HT"* *9.2 ±4.4 = 14.2±5.4*	
Subash P	HT	80 (50±9)	50	PBCs /Ly/, A.U.	C < HT 15.0±5.0 < 30.0±5.0	10
Gür M	HT	64 (48±7)	20	PBCs /Ly/, A.U.	C < HT 9.0±4.5 < 34.3±23.3	11
Saiedullah	M HT	46 (41±5)	40	PBCs /Ly/, A.U.	C < HT 5.4±1.0 < 6.7±1.0	12
Subash P	HT	100 (45±12)	50	PBCs /Ly/, A.U.	C < HT 14.5±4.2 < 29.7±10.6	13
8OHdG/ELISA						
Negishi H	HT	38 (52±1)	22	Urine, ng/mg creatinine	C < HT 10.1±1.1 < 17.4±2.4	14
Negishi H	HT	38 (52±1)	22	Urine, ng/mg creatinine	C < HT 10.1±1.1< 17.4±2.4	15
Lee J	HT	38 (54±12)	22	Plasma, ng/mL	C < HT 3.41±2.0 < 9.0±4.1	16
Subash P	HT	105 (45±9)	75	Urine, ng/mg creatinine	C < HT 5.7±2.4 < 13.8±4.4	17
Kotani K	HT (DM)	45 (62±9)	31 (DM)	Urine, ng/mg creatinine	C = HT 8.8 (6.9–10.5) = 7.9 (6.2–10.0)	18
Yavuzer S	HT (old)	30 (73±9)	30	Urine, ng/mg creatinine	C < HT 5.7±2.7 < 14.9±4.5	19
	HT (young)	30 (44±4)	30	Urine, ng/mg creatinine	C < HT 3.2±1.5 < 9.3±4.1	
Yıldırım E	HT	40 (44±4)	40	Urine, ng/mg creatinine	C < HT 3.8±1.9 < 10.7±4.9	20
	"HT"	36 (43±7)	40	Urine, ng/mg creatinine	C = *"HT"* 3.8±1.9 = 4.6±2.9	
Toljic M	GHT	21 (32; 23–38)	28 (PC)	Plasma, nM	C = HT 43.0±10.5 = 41.5±21.2	21
Zhao Y	HT (old, DM, CHD)	63 (72±8)	84 (old, DM, nonCHD)	Serum, ng/mL	C < HT 0.22±0.07 < 0.53±0.09	22

A.U., arbitrary unit; C, controls; CHD, coronary heart disease; DNA, deoxyribonucleic acid; DM, diabetes mellitus; ELISA, enzyme – linked immunosorbent assay; GHT, gestational HT; HT, arterial hypertension; *"HT" = WCH,* white coat hypertension; Ly, lymphocytes; mtch, matched; N, number; PBCs, peripheral blood cells; PC, pregnant healthy controls; SBs, strand breaks; specif, specification; 8OHdG, 8 – hydroxyl-2'-deoxyguanosine; Data are expressed as: means ±SD or (max – min) or median (interquartil range); Significant difference: P – value < 0.05.

ed HT, treated HT and HT without drug specification. All human studies selected consistently found significantly greater DNA damage level in HT pts with more adverse conditions compared to those without (Fig. 4C, Table 3) (ref.[8-11,13,16,17,19,20]). Meta-analysis showed in hypertensive patients higher average mean differences of DNA damage 7.5 (6.6; 8.6) for 8OHdG and 14.7 (6.4; 23.0) for Comet assay in those with an adverse factor compared to those without (Fig. 3b.).

No significant difference was seen in DNA damage compared to white coat hypertensive (WCH = "HT") pts with controls (Table 1, 2) (ref.[9,20]). No significant difference in DNA damage was found between treated hypertensive diabetics and normotensive diabetics[18] or between gestational hypertensive pts (GHT) on methyldopa and pregnant healthy controls (Table 1) (ref.[21]). Oxidative DNA damage expressed a stronger positive correlation with serum glycosylated haemoglobin (HbA1c), (r=0.670, P<0.0001) and a stronger negative correlation (r =−0.692 to −0.968, P<0.001) with total antioxidant status (TAS) (Table 4, Fig. 4D) (ref.[9-11,13,15,17]).

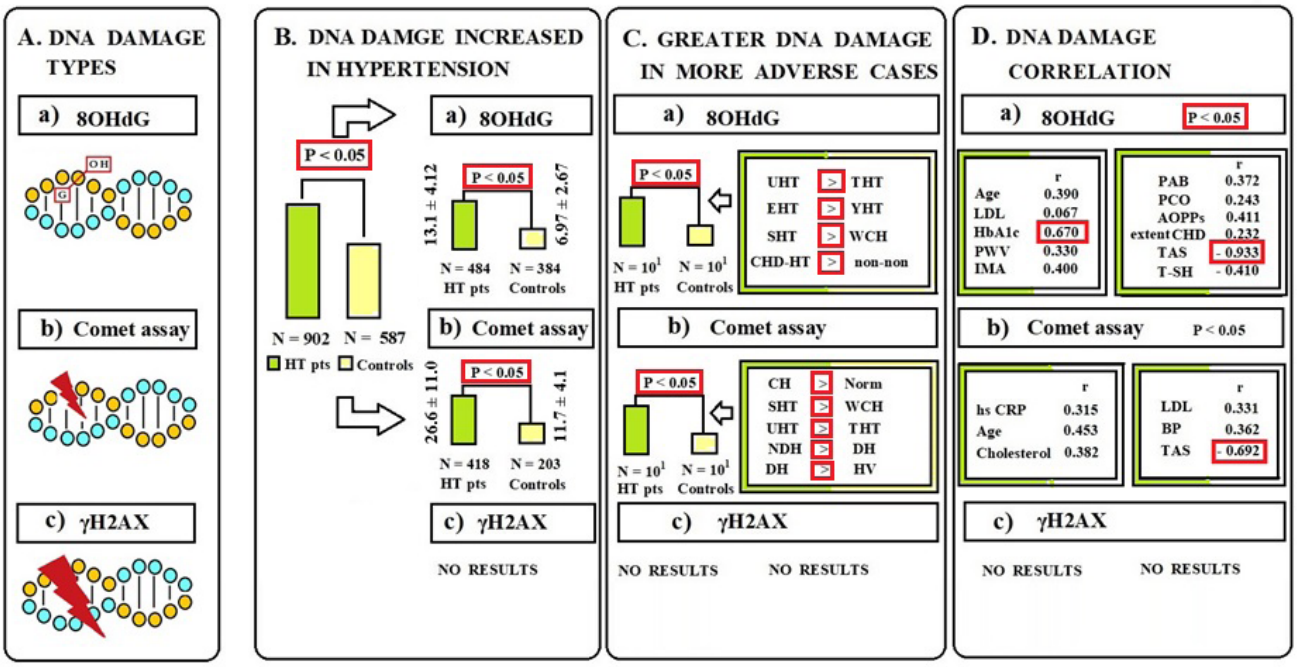

Fig. 4. Summary of review results – DNA damage and arterial hypertension in humans.

AOPPs, advanced oxidation protein products; BP, blood pressure; CH, concentric hypertrophic geometric cardiac pattern; CHD-HT, hypertension with coronary heart disease; comet assay (an assay for single strand breaks detection); DH, dipper hypertension; DNA, deoxyribonucleic acid; EHT, elderly hypertension; gammaH2AX (a marker of double strand breaks); HbA1c, serum glycosylated haemoglobin; hs CRP, high sensitive C - reactive protein; HT, arterial hypertension; HV, healthy volunteers; IMA, ischemia modified albumin; LDL, low density lipoprotein cholesterol; NDH, non-dipper hypertension; non-non, non-hypertension and non-coronary heart disease; Norm, normal; *P*<0.05, significant; PAB, pro-antioxidant balance; PCO, protein carbonyl; pts, patients; PWV, brachial-ankle pulse wave velocity; r: Pearson's correlation coefficient; SHT, sustained hypertension; TAS, total antioxidant status; THT, treated hypertension; T-SH, total-thiol; UHT, untreated hypertension; WCH, white coat hypertension; YHT, young hypertension; 8OHdG, 8-hydroxy-2'-deoxyguanosine (oxidised form of guanosine). For details related to: Fig 4A = see Fig. 3a.; Fig. 4B = see Table 1; Fig. 4C = see Table 3; Fig. 4D = see Table 4.

Table 2. Studies in humans according to antihypertensive therapy (oxidative DNA damage and arterial hypertension).

DNA DAMAGE	ANTIHYPERTENSIVE DRUGS			Ref.
marker/ assay	NO	YES	UNKNOWN	
	Author	Author	Author	
SBs/ Comet assay	Gur M			8
	Yildiz A			9
	Subash P	Subash P		10
	Gur M			11
	Saiedullah M			12
	Subash P	Subash P		13
8OHdG/ ELISA			Negishi H	14
			Negishi H	15
	Lee J	Lee J		16
	Subash P	Subash P		17
		Kotani K		18
		Yavuzer S		19
	Yildirim E			20
		Toljic M		21
			Zhao Y	22

DNA: deoxyribonucleic acid; SBs: Strand breaks; 8OHdG/ ELISA, 8-hydroxy-2'-deoxyguanosine; ELISA, enzyme – linked immunosorbent assay.

Table 3. Differences in oxidative DNA damage – according to additionally specified hypertensive patients.

Author / DNA DAMAGE	COMPARISON GROUP A	vs	GROUP B	DIFFERENCE ($P < 0.05$) in DNA DAMAGE (A.U.)	Ref.
SBs/ Comet assay (PBCs /Ly/, A.U.)					
Gur M	CH (N = 24 pts)	vs	Norm (N = 19 pts)	CH > Norm 43.4±15.4 > 15.6±5.5	8
	CH (N = 24 pts)	vs	CR (N = 22 pts)	CH > CR 43.4±15.4 > 19.9±8.0	
	CH (N = 24 pts)	vs	EH (N = 19 pts)	CH > EH 43.4±15.4 > 24.4±6.4	
	EH (N = 19 pts)	vs	Norm (N = 19 pts)	EH > Norm 24.4±6.4 > 15.6±5.5	
Yildiz A	SHT (N = 21 pts)	vs	WHC (N = 23 pts)	SHT > WCH 31.4±12.1 > 14.2±5.4	9
Subash P	UHT (N = 30 pts)	vs	THT (N = 50 pts)	UHT > THT 35.0±5.0 > 25.0 ±5.0	10
Gur M	NDH (N = 33 pts)	vs	DH (N = 31 pts)	NDH > DH 39.2±15.5 > 29.4±11.1	11
	DH (N = 31 pts)	vs	HV (N = 20 HV)	DH > HV 29.4±11.1 > 9.0±4.5	
Subash P	UHT (N = 50 pts)	vs	THT (N = 50 pts)	UHT > THT 36.2±11.4 > 23.2±9.7	13
8OHdG/ ELISA (Urine, ng/mg creatinine; Plasma, ng/mL; Serum, ng/mL⁑)					
Lee J	UHT (N = 17 pts)	vs	C - THT (N = 17 pts)	UHT > C – THT 9.1±4.2 > 5.7±3.9	16
	UHT (N = 21 pts)	vs	T - THT (N = 21 pts)	UHT = T – THT 9.0±3.9 = 8.2±4.1	
Subash P	UHT (N = 30 pts)	vs	THT (N = 75 pts)	UHT > THT 15.9±5.9 > 11.7±2.9	17
Yavuzer S	EHT (N = 30 pts)	vs	YHT (N = 30 pts)	EHT > YHT 14.9±4.5 > 9.3±4.1	19
Yildirim E	SHT (N = 40 pts)	vs	WHC (N = 36 pts)	SHT > WCH 10.7±4.9 > 4.6±2.9	20
Zhao Y	CHD - HT (N = 63 pts)	vs	nonCHDnonHT (N = 84 pts)	CHD-HT > nonCHDnonHT 0.5±0.1 > 0.2±0.1⁑	22

DNA, deoxyribonucleic acid; Ref, References; SBs, strand breaks; CH, concentric hypertrophic geometric cardiac pattern; N, Number; pts, patients; vs, versus; Norm, normal geometric cardiac pattern; CR, concentric remodelling geometric cardiac pattern; EH, eccentric hypertrophy geometric cardiac pattern; SHT, sustained hypertension; WHC, white coat hypertension; UHT, Untreated newly diagnosed hypertension; THT, treated hypertension; NDH, non-dipper hypertension; DH, dipper hypertension; HV, healthy volunteers; 8OHdG, 8 - hydroxy-2'-deoxyguanosine; ELISA, enzyme – linked immunosorbent assay; C–THT, carvedilol (2 months) – THT, T–THT, thiazide (2 months) – THT; EHT, elderly hypertension; YHT, young hypertension; CHD-HT, Hypertension with coronary heart disease (elderly cohort with type 2 diabetes mellitus); nonCHDnonHT, Normotensive pts without coronary heart disease (elderly cohort with type 2 diabetes mellitus); Significant difference: $P < 0.05$.

DISCUSSION

Until now, DNA damage had been tested in humans with HT only in blood and urine. In contrast to animal and cell-line studies (ref.[23-37]), no data are available for tissues in humans, most probably for practical reasons (clinical usefulness; patient-friendly sample taking; cost, time, and benefit effectiveness). Regarding the three types of oxidative DNA damage selected in this review, only comet assay (blood: N=418 pts) and 8OHdG (blood: N=122 pts; urine: N=362 pts) were tested in humans. In contrast to animal and cell-line studies (ref.[23-37]), there are no data available for gammaH2AX in humans, since this is the newest method (Fig. 3). Comet assay was tested in strictly untreated HT (4 studies; N=215 pts) (ref.[8,9,11,12]). These data are very important, to exclude artefacts in changes as DNA damage induced by medication. Unfortunately, comet assay was tested also in a mixed group with treated/ untreated HT (2 studies; N=180 pts) (ref.[10,13]). These studies have limited value for assessing the effect of HT *per se*. No study on comet assay was done in a pure group including only medicated HT pts. No differences in DNA damage should be expected in effectively – treated HT pts in whom BP became comparable to that of controls as a result of antihypertensive drugs. However, this remains an unresolved presumption.

8OHdG was also tested in strictly untreated HT (1 study; N=40 pts) (ref.[20]), or in a mixed group with/ without

Table 4. Correlation – oxidative DNA damage and sundry factors. Humans with arterial hypertension.

Author	DNA marker	r	*P*	Factor	Ref.
Gur M	SBs/ Comet assay	0.315	0.012	hs CRP	11
Gur M	SBs/ Comet assay	0.479	< 0.001	Age	11
Yildiz A	SBs/ Comet assay	0.453	0.039	Age	9
Subash P	SBs/ Comet assay	0.072	NS	Age	10
Negishi H	8OHdG/ ELISA	−0.012	NS	Age	15
Subash P	8OHdG/ ELISA	0.221	NS	Age	17
Yavuzer S	8OHdG/ ELISA	0.39	0.002	Age (elderly)	19
Gur M	SBs/ Comet assay	0.382	0.002	Cholesterol	11
Yildiz A	SBs/ Comet assay	0.550	0.010	Cholesterol	9
Subash P	SBs/ Comet assay	−0.045	NS	Cholesterol	10
Negishi H	8OHdG/ ELISA	−0.082	NS	Cholesterol	15
Subash P	8OHdG/ ELISA	0.044	NS	Cholesterol	17
Gur M	SBs/ Comet assay	0.331	0.008	LDL	11
Yildiz A	SBs/ Comet assay	0.539	0.012	LDL	9
Subash P	SBs/ Comet assay	−0.043	NS	LDL	10
Subash P	8OHdG/ ELISA	0.067	0.033	LDL	17
Negishi H	8OHdG/ ELISA	0.059	NS	HDL	15
Negishi H	8OHdG/ ELISA	0.018	NS	TAG	15
Saiedullah M	SBs/ Comet assay	0.362	< 0.05	BP	12
Negishi H	8OHdG/ ELISA	0.202	NS	BP	15
Subash P	8OHdG/ ELISA	0.027	NS	BP	17
Negishi H	8OHdG/ ELISA	−0.040	NS	HR	15
Negishi H	8OHdG/ ELISA	−0.235	NS	BMI	15
Negishi H	8OHdG/ ELISA	0.670	< 0.0001	HbA1c	15
Kotani K	8OHdG/ ELISA	0.330	< 0.05	PWV (HT - DM)	18
Yavuzer S	8OHdG/ ELISA	0.400	0.002	IMA	19
Yildirim E	8OHdG/ ELISA	0.396	< 0.001	IMA	20
Yildirim E	8OHdG/ ELISA	0.372	< 0.001	PAB	20
Yavuzer S	8OHdG/ ELISA	0.370	0.004	PCO	19
Yildirim E	8OHdG/ ELISA	0.243	< 0.05	PCO	20
Yildirim E	8OHdG/ ELISA	0.411	< 0.001	AOPPs	20
Zhao Y	8OHdG/ ELISA	0.232 to 0.424	< 0.05	extent of CHD	22
Gur M	SBs/ Comet assay	−0.692	< 0.001	TAS	11
Yildiz A	SBs/ Comet assay	−0.818	< 0.001	TAS	9
Subash P	SBs/ Comet assay	−0.792	< 0.0001	TAS	10
Subash P	SBs/ Comet assay	−0.914 to −0.968	< 0.0001	TAS	13
Subash P	8OHdG/ ELISA	−0.933	< 0.0001	TAS	17
Yavuzer S	8OHdG/ ELISA	−0.290	0.025	T – SH	19
Yildirim E	8OHdG/ ELISA	−0.410	< 0.001	T – SH	20
Subash P	SBs/ Comet assay	0.204	NS	Glc	10
Subash P	8OHdG/ ELISA	0.170	NS	Glc	17

AOPPs (uM), advanced oxidation protein products; BMI, body mass index; BP, blood pressure; CHD, coronary artery disease; DNA, deoxyribonucleic acid; ELISA, enzyme – linked immunosorbent assay; Glc, plasma fasting glucose; HbA1c, serum glycosylated haemoglobin;HDL, high density lipoprotein cholesterol; HR, heart rate; hs CRP, high sensitive C-reactive protein; HT – DM, hypertensive diabetics; IMA (ng/ml), ischemia modified albumin; LDL, low density lipoprotein cholesterol; NS, not significant; r, Pearson's Correlation coefficient, PAB (arbitrary units), prooxidant-antioxidant balance; PCO (nmol/mg/pr), protein carbonyl; PWV, brachial – ankle pulse wave velocity; Significance, *P*-value < 0.05; SBs, strand breaks; TAG, triacylglycerol; TAS, total antioxidant status; T-SH (uM), Total thiol; 8OHdG, 8-hydroxy-2'-deoxyguanosine.

antihypertensive drugs (2 studies; N=143 pts) (ref.[16,17]). In contrast to comet assay, 8OHdG was also tested in a group without any specification of treatment (3 studies; N=139 pts) (ref.[14,15,22]), and in a group with treated HT (3 studies; N=126 pts) (ref.[18,19,21]). Unfortunately, the absence of a treatment specification is limiting for data interpretation. Based on 8OHdG studies, the authors' view is that the most surprising study is that by Toljic et al., with no differences in plasma 8OHdG between GHT pts and healthy pregnant women. Unfortunately, BP description data are missing in this study[21]. Thus we can speculate that BP in the Toljic study was corrected by antihypertensive drugs (methyldopa in GHT) with no differences in BP compared to controls. This speculation may explain why there were no differences in 8OHdG between treated GHT and controls[21]. However, this hypothetical explanation is not confirmed in another study on treated HT pts by Kotani[18]. Kotani et al. found no significant differences in 8OHdG between type 2 diabetes mellitus (T2DM) pts with and without HT, despite the fact that there is a statistically significant difference in BP as well as between antihypertensive drugs[18]. At this point, the key question is how DM influences 8OHdG. The above hypothetical speculation is not excluded by Yavuzer et al.[19]. Yavuzer selected HT pts on therapy with calcium channel blockers and/ or diuretics. Despite the antihypertensive therapy in HT pts, Yavuzer found higher BP as well as 8OHdG in HT pts compared to controls[19]. Thus, elevated 8OHdG in HT pts in Yavuzer' s study may be due to ineffectively – treated HT (ref.[19]). On the other hand, it is important to mention that both calcium channel blockers and diuretics appear to have little effect on oxidative stress[16,19]. Medications against oxidative stress are angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor 1 blockers (ARBs), beta - blockers, statins, and fibrates[19]. Such medications should be avoided in studies testing the effect of different factors on oxidative DNA damage. The effects of different substances (especially vasodilators, vasoconstrictors, mechanical interventions) on different DNA damage types in HT animals were tested[24-33]. Regardless of antihypertensive pharmacotherapy, a majority of the selected studies demonstrated significantly higher DNA damage in HT 731 pts (349 pts; comet assay; blood) and (382 pts; 8OHdG; 122 in blood and 260 in urine) compared to controls[8-17,19,20,22].

DNA damage (at least using comet assay and 8OHdG) was higher in those with more adverse characteristics (concentric hypertrophy[8], sustained HT (ref.[9,20])), untreated HT (ref.[10,13,16,17]), non-dippers[11], elderly[19], CHD (ref.[22]) (Table 3). Unfortunately, data are based on small pts groups (10^1–10^2 of pts), and small numbers of studies (1^0) usually written by the same author team.

Among the parameters (age, cholesterol, low density lipoprotein cholesterol (LDL), BP, TAS, plasma fasting glucose (glc)) in which the correlation with both DNA damage types (comet assay, 8OHdG) were tested in humans, only TAS showed a consistently stronger negative significant correlation for both methods (comet assay; blood: r=−0.69 to −0.96, P<0.001, 4 studies, N=265 pts with untreated or mixed group) (ref.[9-11,13]) and (8OHdG; urine: r=−0.933, P<0.0001, 1 study, N=105 pts mixed group) (ref.[10]). However, these quite constant data on the TAS correlation are based on 4 studies written by 2 different author teams[9-11,13]. Surprisingly, regarding BP, the correlation was found for humans only for comet assay in blood (r=0.362), not for 8OHdG (ref.[12]), and more strongly in animals with comet assay in tissue[28].

Among the parameters in which only 8OHdG was tested (high-density lipoprotein cholesterol (HDL), triacylglycerol (TAG), heart rate (HR), body mass index (BMI), brachial-ankle pulse wave velocity (PWV), ischemia modified albumin (IMA), prooxidant-antioxidant balance (PAB), protein carbonyl (PCO), advanced oxidation protein products (AOPPs), extent of coronary heart disease (CHD), total thiol (T-SH)), only serum glycosylated haemoglobin (HbA1c) showed a stronger positive correlation (8OHdG; urine: r=0.670, P<0.0001) (ref.[15]). The relevance of this is uncertain, because the result is generated from a single study (N=38 pts with unspecified treatment) (ref.[15]).

Other results on DNA damage correlation gave weaker values (high sensitive C-reactive protein (hs CRP) (ref.[11]), PWV (ref.[18]), IMA, PAB, PCO, AOPPs, CHD extent, T-SH (ref.[19,20])), not constant (age[9-11,15,17,19], cholesterol[9-11], LDL (ref.[9-11,17])), or not significant results (HDL (ref.[15]), TAG (ref.[15]), BP (ref.[12,15,17]), HR (ref.[15]), BMI (ref.[15]), glc (ref.[10,17])). Such conflicting results may be due to discrepancies in therapeutic access to HT (treated, untreated, unspecified) or due to a limited number of probands.

CONCLUSION

This is the first systematic review with meta-analysis showing that oxidative DNA damage was increased in humans with arterial hypertension compared with healthy controls (Fig. 4B). Greater DNA damage (comet assay in blood; 8OHdG in blood/in urine) was observed in cases with the additional adverse characteristics (elderly, untreated, etc.) (Fig. 4C). A stronger correlation of DNA damage was observed for TAS and uncertainly (based on 1 study) for HbA1c (Table 4, Fig. 4D).

Search strategy and selection criteria

Multiple databases (PubMed, Web of Science) were searched to find English-language papers until June 30, 2022. The search terms were: DNA damage, comet assay, gammaH2AX, 8OHdG, strand breaks, arterial hypertension. Exclusion criteria were: children, absence of relevant controls, extra-arterial hypertensive issues, animal and cell line studies.

ABBREVIATION

ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin II receptor 1 blockers; AOPPs, advanced oxidation protein products; BMI, body mass index; BP, blood pressure; CH, concentric hypertrophic geometric cardiac pattern; CHD, coronary heart disease; CHD-HT, hypertension with coronary heart disease; 95% CL,

95% confidence limit; CV, cardiovascular; DH, dipper hypertension; DM, diabetes mellitus; DNA, deoxyribonucleic acid; DNA SSBs, DNA single strand breaks; DNA DSBs, DNA double strand breaks; DNA SBs: DNA strand breaks; EHT, elderly hypertension; ELISA, enzyme-linked immunosorbent assay; GHT, gestational hypertension; glc, plasma fasting glucose; gammaH2AX, phosphorylated histone 2AX; HbA1c, serum glycosylated haemoglobin; HDL, high density lipoprotein cholesterol; HR, heart rate; hs CRP, high sensitive C-reactive protein; HV, healthy volunteers; HT arterial hypertension; IMA, ischemia modified albumin; LDL, low density lipoprotein cholesterol; MD, mean difference; N, number; NDH, non-dipper hypertension; non-non, non-hypertension and non-coronary heart disease; Norm, normal; PAB, prooxidant-antioxidant balance; PCO, protein carbonyl; *P*–value, $P<0.05$; r, Pearson's correlation coefficient; pts, patients; PWV, brachial-ankle pulse wave velocity; ref, references; SHT, sustained hypertension; TAG, triacylglycerol; TAS, total antioxidant status; T-SH, total-thiol; THT, treated hypertension; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; T2DM, type 2 diabetes mellitus; UHT, untreated hypertension; WHC, white coat hypertension; YHT, young hypertension; 8OHdG, 8-hydroxy-2'-deoxyguanosine.

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REFERENCES

1. Hazuková R, Řezáčová M, Kočí J, Čermáková E, Pleskot M. Severe deoxyribonucleic acid damage after out-of-hospital cardiac arrest in successfully resuscitated humans. Int J Cardiol 2016;207:33-5. doi: 10.1016/j.ijcard.2016.01.046
2. Hazuková R, Řezáčová M, Köhlerová R, Tomek T, Cermakova E, Koci J. Comet assay in evaluating deoxyribonucleic acid damage after out-of-hospital cardiac arrest. Anatol J Cardiol 2017;18(1):31-8. doi: 10.14744/AnatolJCardiol.2017.7578
3. Hu L, Wang Z, Carmone C, Keijer J, Zhang D. Role of Oxidative DNA Damage and Repair in Atrial Fibrillation and Ischemic Heart Disease. Int J Mol Sci 2021;22(8):3838. doi: 10.3390/ijms22083838
4. Ramos KS, Brundel BJJM. DNA Damage, an Innocent Bystander in Atrial Fibrillation and Other Cardiovascular Diseases? Front Cardiovasc Med 2020;7:67. doi: 10.3389/fcvm.2020.00067
5. Xu D, Hu YH, Gou X, Li FY, Yang XYCh, Li YM, Chen F. Oxidative Stress and Antioxidative Therapy in Pulmonary Arterial Hypertension. Mo lecules 2022;27(12):3724. doi: 10.3390/molecules27123724
6. Kuchařová M, Hronek M, Rybáková K, Zadák Z, Štětina R, Josková V, Patková A. Comet assay and its use for evaluating oxidative DNA damage in some pathological states. Physiol Res 2019;68(1):1-15. doi: 10.33549/physiolres.933901
7. Williams B, Mancia G, Spiering W, Agabiti RE, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V, Desormais I; ESC Scientific Document Group. 2018 ESC/ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). Eur Heart J 2018;39:3021-04. doi: 10.1093/eurheartj/ehy339
8. Gur M, Yildiz A, Demirbag R, Yilmaz R, Koçyigit A, Celik H, Aksoy N. Relationship between left ventricle geometric patterns and lymphocyte DNA damage in patients with untreated essential hypertension. Clin Biochem 2007;40(7):454-9. doi: 10.1016/j.clinbiochem.2006.10.024
9. Yildiz A, Gür M, Yilmaz R, Demirbağ R, Celik H, Aslan M, Koçyiğit A. Lymphocyte DNA damage and total antioxidant status in patients with white-coat hypertension and sustained hypertension. Turk Kardiyol Dern Ars 2008;36(4):231-8.
10. Subash P, Premagurumurthy K, Sarasabharathi A, Cherian KM. Total antioxidant status and oxidative DNA damage in a South Indian population of essential hypertensives. J Hum Hypertens 2010;24(7):475-82. doi: 10.1038/jhh.2009.100
11. Gür M, Elbasan Z, Şahin DY, Koyunsever NY, Seker T, Ozaltun B, Caylı M, Kocyigit A. DNA damage and oxidative status in newly diagnosed, untreated, dipper and non-dipper hypertensive patients. Hypertens Res 2013;36(2):166-71. doi: 10.1038/hr.2012.156
12. Saiedullah M, Hayat S, Zamir R, Arif M, Howlader ZH, Rahman M. DNA damage in lymphocytes in hypertensive subjects in Bangladesh. J Sci Res 2013;5(3):535-43.
13. Subash P. Assessment of Oxidative DNA Damage by Alkaline Comet Assay in Human Essential Hypertension. Indian J Clin Biochem 2016;31(2):185-93. doi: 10.1007/s12291-015-0521-1
14. Negishi H, Njelekela M, Ikeda K, Sagara M, Noguchi T, Kuga S, Kanda T, Liu L, Nara Y, Tagami M, Yamori Y. Assessment of in vivo oxidative stress in hypertensive rats and hypertensive subjects in Tanzania, Africa. Hypertens Res 2000;23(3):285-9. doi: 10.1291/hypres.23.285
15. Negishi H, Ikeda K, Kuga S, Noguchi T, Kanda T, Njelekela M, Liu L, Miki T, Nara Y, Sato T, Mashalla Y, Mtabaji J, Yamori Y. The relation of oxidative DNA damage to hypertension and other cardiovascular risk factors in Tanzania. J Hypertens 2001;19(3 Pt 2):529-33. doi: 10.1097/00004872-200103001-00002
16. Lee J, Lee M, Kim JU, Song K II, Choi YS, Cheong S. Carvedilol Reduces Plasma 8-Hydroxy-2 '-Deoxyguanosine in Mild to Moderate Hypertension. Hypertension 2005;45(5):986-90. doi: 10.1161/01.HYP.0000164569.63160.24
17. Subash P, Gurumurthy P, Sarasabharathi A, Cherian KM. Urinary 8-OHdG: A marker of oxidative stress to DNA and total antioxidant status in essential hypertension with South Indian population. Indian J Clin Biochem 2010;25(2):127-32. doi: 10.1007/s12291-010-0024-z
18. Kotani K, Yamada T. Association between urinary 8-OHdG and pulse wave velocity in hypertensive patients with type 2 diabetes mellitus. Singapore Med J 2014;55(4):202-8. doi: 10.11622/smedj.2014053
19. Yavuzer S, Yavuzer H, Cengiz M, Erman H, Demirdag F, Doventas A, Balci H, Erdincler DS, Uzun H. The role of protein oxidation and DNA damage in elderly hypertension. Aging Clin Exp Res 2016;28(4):625-32. doi: 10.1007/s40520-015-0464-7
20. Yıldırım E, İpek E, Bavunoğlu I, Yıldırım N, Cengiz M, Yavuzer S, Yavuzer H, Erman H, Uzun H. The impact of protein oxidation on sustained and white coat hypertension. Anatol J Cardiol 2017;17(3):210-16. doi: 10.14744/AnatolJCardiol.2016.7174
21. Toljic M, Egic A, Munjas J, Orlic NK, Milovanovic Z, Radenkovic A, Vuceljic J, Joksic I. Increased oxidative stress and cytokinesis-block micronucleus cytome assay parameters in pregnant women with gestational diabetes mellitus and gestational arterial hypertension. Reprod Toxicol 2017;71:55-62. doi: 10.1016/j.reprotox.2017.04.002
22. Zhao Y, Liang W, Tian S, Shen L, Yang H. Impact of increased serum 8-hydroxy-2'-deoxyguanosine levels on extent of coronary artery lesions in elderly patients with type 2 diabetes. J Int Med Res 2020;48(7):300060520934653. doi: 10.1177/0300060520934653
23. Schupp N, Kolkhof P, Queisser N, Gärtner S, Schmid U, Kretschmer A, Hartmann E, Oli RG, Schäfer S, Stopper H. Mineralocorticoid receptor-mediated DNA damage in kidneys of DOCA-salt hypertensive rats. FASEB J 2011;25(3):968-78. doi: 10.1096/fj.10-173286
24. Queisser N, Amann K, Hey V, Habib SL, Schupp N. Blood pressure has only minor influence on aldosterone-induced oxidative stress

and DNA damage in vivo. Free Radic Biol Med 2013;54:17-25. doi: 10.1016/j.freeradbiomed.2012.10.549
25. Dias AT, Rodrigues BP, Porto ML, Gava AL, Balarini CM, Freitas FP, Palomino Z, Casarini DE, Campagnaro BP, Pereira TMC, Meyrelles SS, Vasquez EC. Sildenafil ameliorates oxidative stress and DNA damage in the stenotic kidneys in mice with renovascular hypertension. J Transl Med 2014;12:35. doi: 10.1186/1479-5876-12-35
26. Zimnol A, Amann K, Mandel P, Hartmann Ch, Schupp N. Angiotensin II type 1a receptor-deficient mice develop angiotensin II-induced oxidative stress and DNA damage without blood pressure increase. Am J Physiol Renal Physiol 2017;313(6):F1264-F1273. doi: 10.1152/ajprenal.00183.2017
27. Zimnol A, Spicker N, Balhorn R, Schröder K, Schupp N. The NADPH Oxidase Isoform 1 Contributes to Angiotensin II-Mediated DNA Damage in the Kidney. Antioxidants (Basel) 2020;9(7):586. doi: 10.3390/antiox9070586
28. Bruic M, Grujic-Milanovic J, Miloradovic Z, Jovovic D, Zivkovic L, Mihailovic-Stanojevic N, Karanovic D, Spremo-Potparevic B. DNA, protein and lipid oxidative damage in tissues of spontaneously hypertensive versus normotensive rats. Int J Biochem Cell Biol 2021;141:106088. doi: 10.1016/j.biocel.2021.106088
29. Negishi H, Ikeda K, Sagara M, Sawamura M, Yamori Y. Increased oxidative DNA damage in stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 1999;26(5-6):482-4. doi: 10.1046/j.1440-1681.1999.03055.x
30. Brand S, Amann K, Schupp N. Angiotensin II-induced hypertension dose-dependently leads to oxidative stress and DNA damage in mouse kidneys and hearts. J Hypertens 2013;31(2):333-44. doi: 10.1097/HJH.0b013e32835ba77e
31. Baban B, Liu JY, Mozaffari MS. Pressure overload regulates expression of cytokines, γH2AX, and growth arrest- and DNA-damage inducible protein 153 via glycogen synthase kinase-3β in ischemic-reperfused hearts. Hypertension 2013;61(1):95-104. doi: 10.1161/HYPERTENSIONAHA.111.00028
32. Brand S, Amann K, Mandel P, Zimnol A, Schupp N. Oxidative DNA damage in kidneys and heart of hypertensive mice is prevented by blocking angiotensin II and aldosterone receptors. PLoS One 2014;9(12):e115715. doi: 10.1371/journal.pone.0115715
33. Queisser N, Happ K, Link S, Jahn D, Zimnol A, Geier A, Schupp N. Aldosterone induces fibrosis, oxidative stress and DNA damage in livers of male rats independent of blood pressure ganges. Toxicol Appl Pharmacol 2014;280(3):399-407. doi: 10.1016/j.taap.2014.08.029
34. Schmid U, Stopper H, Schweda F, Queisser N, Schupp N. Angiotensin II induces DNA damage in the kidney. Cancer Res 2008;68(22):9239-46. doi: 10.1158/0008-5472.CAN-08-1310. doi: 10.1158/0008-5472.CAN-08-1310
35. Queisser N, Oteiza PI, Stopper H, Oli RG, Schupp N. Aldosterone induces oxidative stress, oxidative DNA damage and NF-κB-activation in kidney tubule cells. Mol Carcinog 2011;50(2):123-35. doi: 10.1002/mc.20710
36. Saheera S, Nair RR. Accelerated decline in cardiac stem cell efficiency in Spontaneously hypertensive rat compared to normotensive Wistar rat PLoS One 2017;12(12):e0189129. doi: 10.1371/journal.pone.0189129
37. Balhorn R, Hartmann C, Schupp N. Aldosterone Induces DNA Damage and Activation of Nrf2 Mainly in Tubuli of Mouse Kidneys. Int J Mol Sci 2020;21(13):4679. doi: 10.3390/ijms21134679