EFFECT OF NANOPARTICLES ON HUMAN CELLS FROM HEALTHY INDIVIDUALS AND PATIENTS WITH RESPIRATORY DISEASES

Iham F. Osman [1], Badie K . Jacob [2] and Diana Anderson [1]

1. Division of Medical Sciences , University of Bradford , UK
2. Bradford Royal Infirmary, Bradford, UK

INTRODUCTION

- **Little is known about the effects of nanoparticles in human systems, let alone in diseased individuals.**

- **Therefore, titanium dioxide (TiO_2) - anatase nanoparticles were examined in peripheral blood lymphocytes from patients with respiratory diseases [lung cancer, chronic obstructive pulmonary disease (COPD) and asthma].**

- **They were compared to those in healthy individuals to determine any differences in sensitivity to nanochemical insult.**

MATERIALS AND METHODS

- **Ethical permission:**

  Bradford University Research Ethics Committee: Reference No.0405/8;

  Leeds (Central) Ethics Committee No.: 09/H1313/37 was obtained to collect blood samples.

- **The Comet assay was performed according to recommended guidelines (Tice et al, 2000).**

  The micronucleus assay was conducted according to Fenech (2000).

- ***Ras* p21 oncoprotein level detection was performed according to Anderson et al (1998b).**

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PREPARATION OF TiO_2 NANOPARTICLES

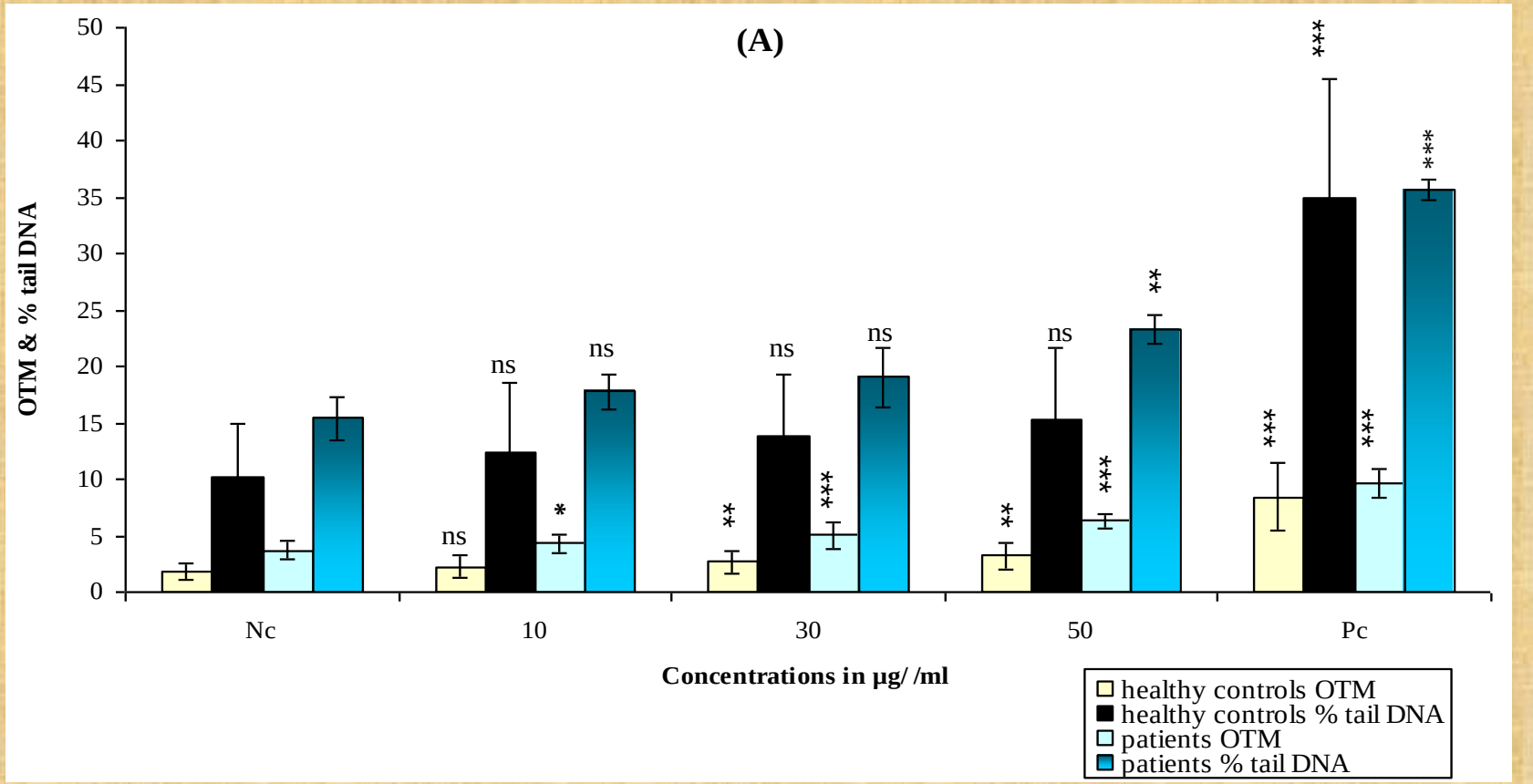
- Titanium (IV) oxide nanoparticles, 99.7%pure (CAS 1317-70-0 Sigma Aldrich).

- The particle size was ascertained using photon correlation spectometry (Zeta analyser, Malvern Instruments) and scanning electron microscopy (SEM).

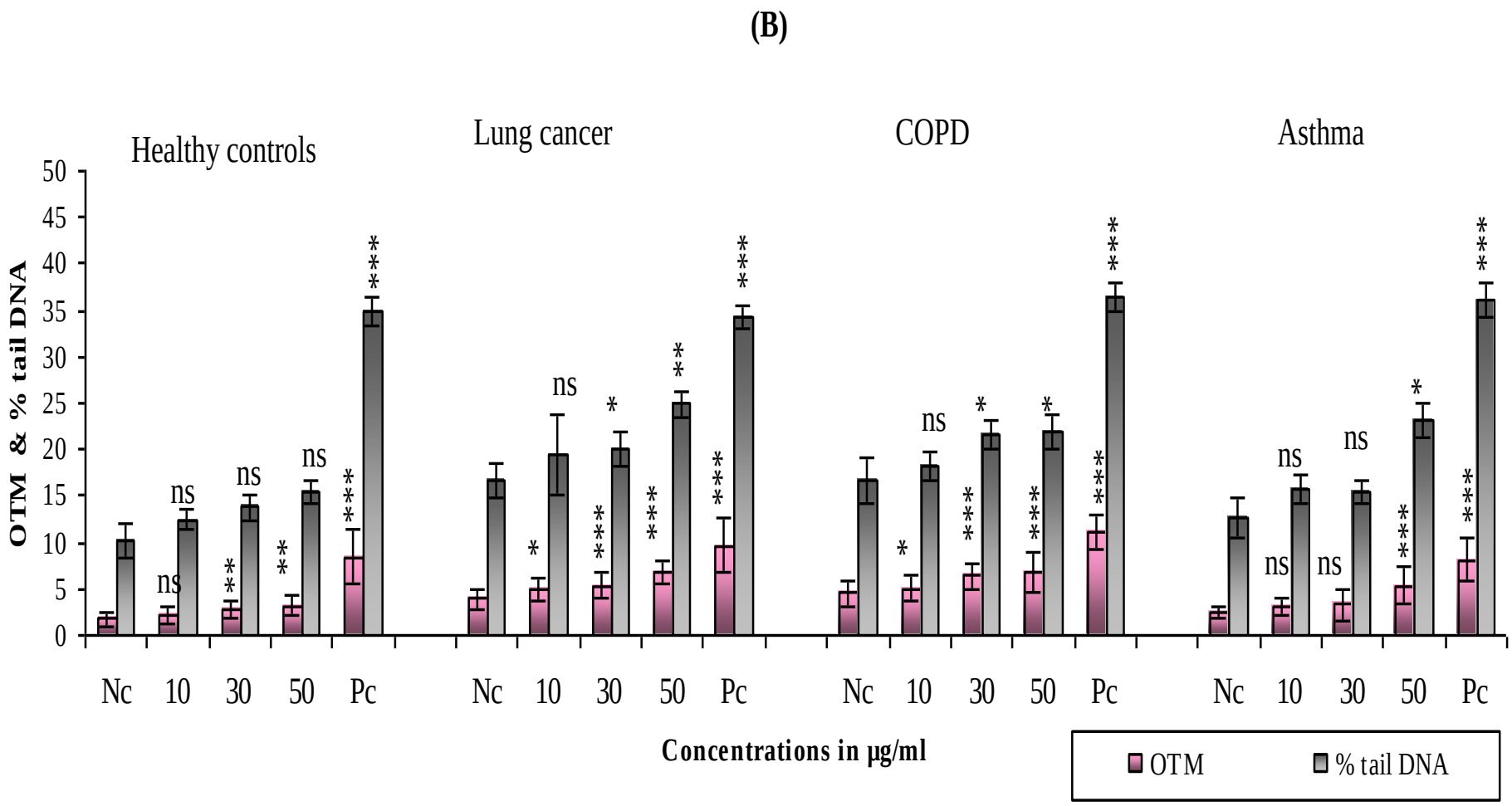
- For SEM analysis a drop of nano-suspension of TiO2 was placed and air-dried on separate mesh Formvar carbon coated (adhesive carbon stubs) and then quantified using SEM. The particle size was observed to be between the 50 nm and 150 nm range

RESULTS – COMET ASSAY

- **The results have shown statistically significant concentration-dependent genotoxic effects of TiO_2 in both respiratory patient and control groups in the Comet assay.**



Histogram showing the means of Olive tail moment and % tail DNA in lymphocytes of healthy controls, lung cancer, COPD and asthma patient groups in the Comet assay after treatment with different TiO_2 concentrations (10, 30 and 50 µg/ml), as well as the negative control of untreated lymphocytes (Nc) and the positive control of 80 µM (2.72 µg/ml) H_2O_2 (Pc) for 30 minutes. Bars indicate standard errors. Not significant: ns, *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$



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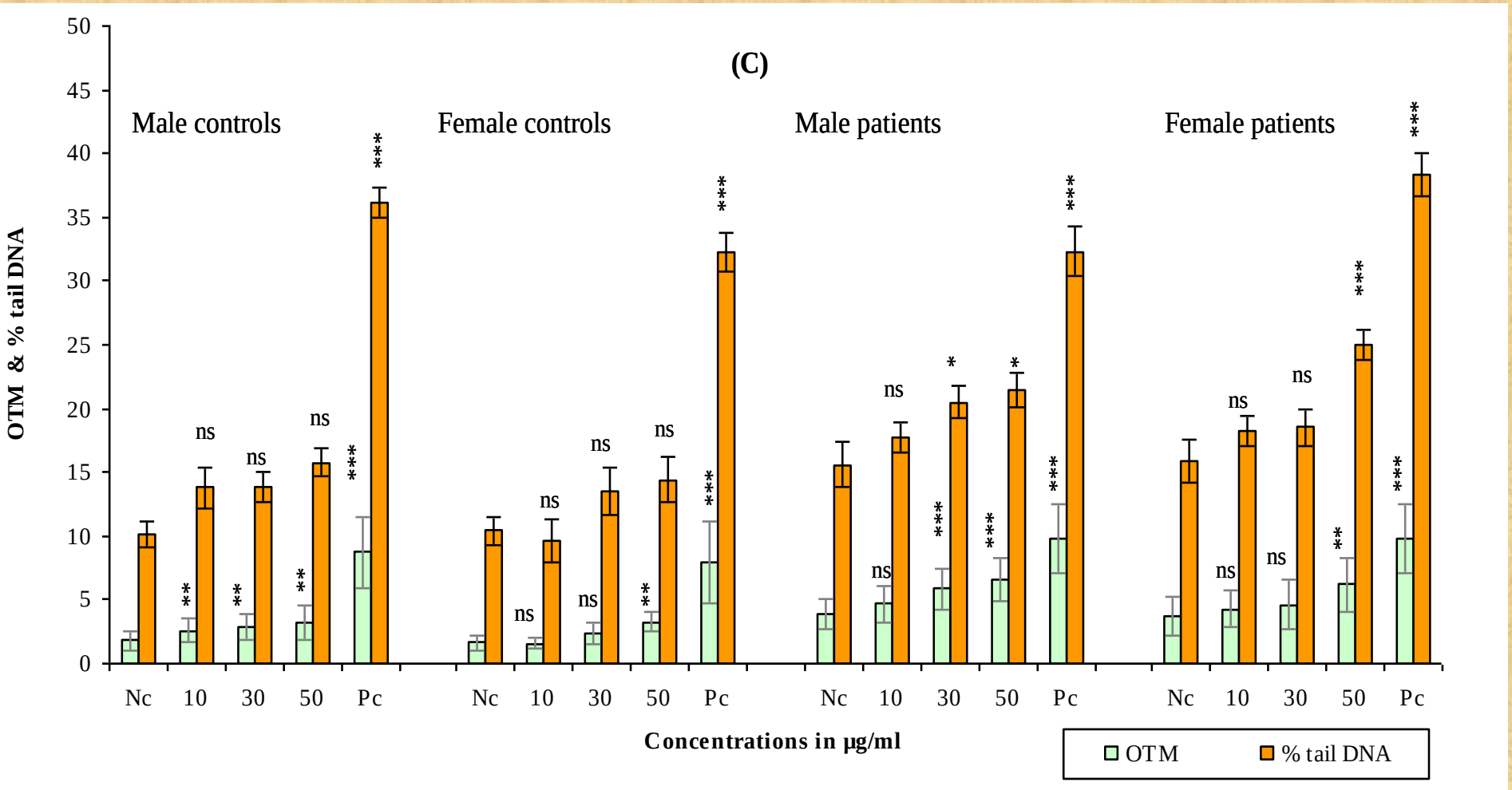

Histogram showing the means of Olive tail moment and % tail DNA in lymphocytes of male and female healthy controls, and male and female patient groups in the Comet assay after treatment with different TiO_2 concentrations (10, 30 and 50 µg/ml), as well as the negative control of untreated lymphocytes (Nc) and the positive control of 80 µM (2.72 µg/ml) H_2O_2 (Pc) for 30 minutes. Bars indicate standard errors. Not significant: ns, *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$

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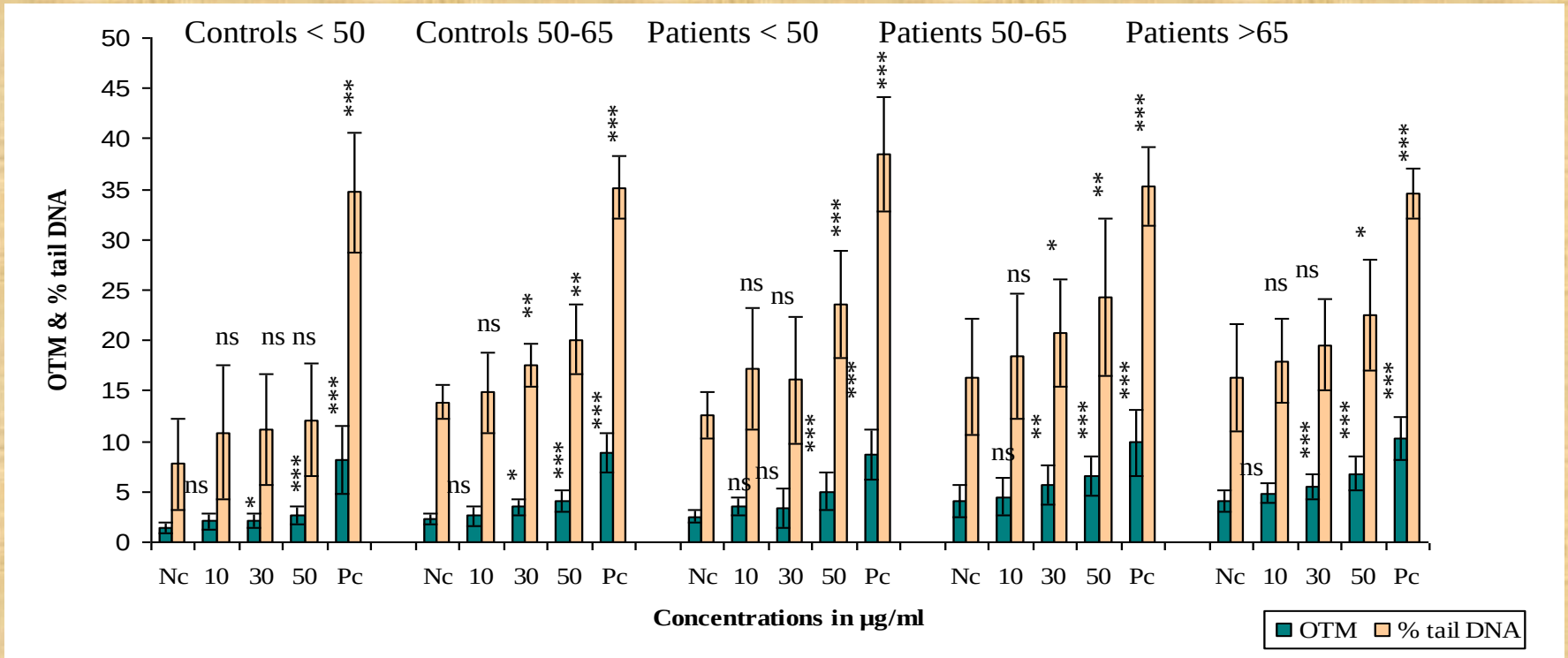
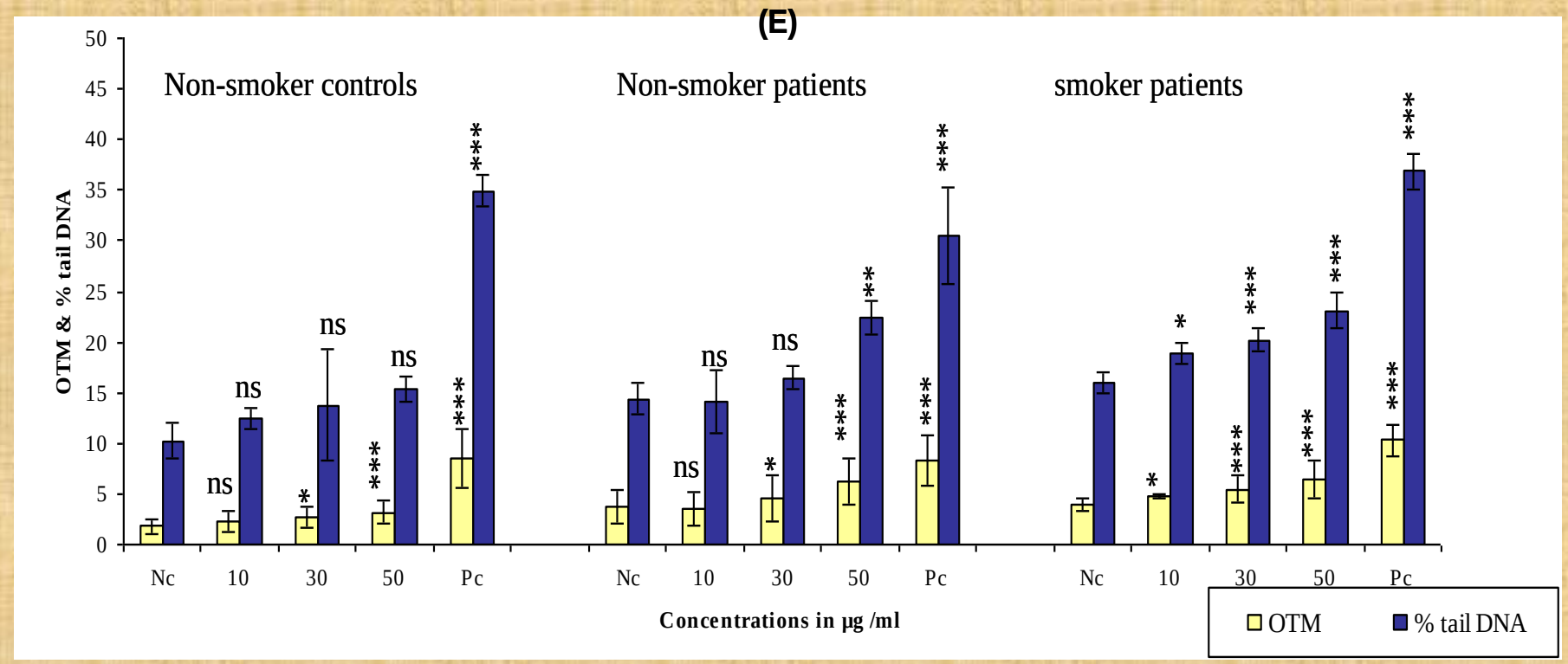
(D)



Histogram showing the means of Olive tail moment and % tail DNA in lymphocytes of healthy controls, aged < 50 and 50 -65 patients aged <50, 50 -65 years and >65 years in the Comet assay after treatment with different TiO_2 concentrations (10, 30 and 50 µg/ml), as well as the negative control of untreated lymphocytes (Nc) and the positive control of 80 µM (2.72 µg/ml) H_2O_2 (Pc) for 30 minutes. Bars indicate standard errors. Not significant: ns, *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$



Histogram showing the means of Olive tail moment and % tail DNA in lymphocytes of non smoker healthy controls and non smoker and smoker patient groups in the Comet assay after treatment with different TiO_2 concentrations (10, 30 and 50 µg/ml), as well as the negative control of untreated lymphocytes (Nc) and the positive control of 80 µM (2.72 µg/ml) H_2O_2 (Pc) for 30 minutes. Bars indicate standard errors. Not significant: ns, *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$

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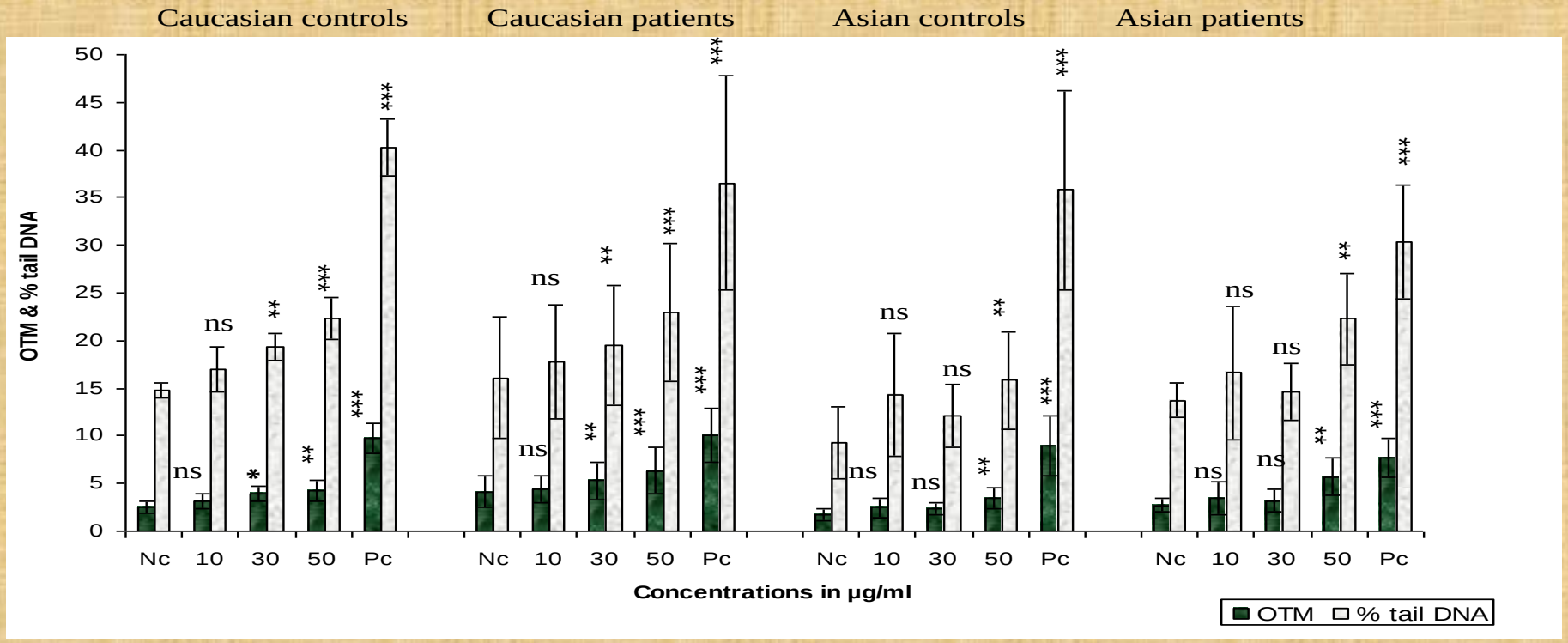

Histogram showing the means of Olive tail moment and % tail DNA in lymphocytes of Caucasian controls and patients and Asian controls andpatient groups in the Comet assay after treatment with different TiO_2 concentrations (10, 30 and 50 µg/ml), as well as the negative control of untreated lymphocytes (Nc) and the positive control of 80 µM (2.72 µg/ml) H_2O_2 (Pc) for 30 minutes. Bars indicate standard errors. Not significant: ns, *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$

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RESULTS – MICRONUCLEUS ASSAY

- **There was an increase in the pattern of cytogenetic damage measured in the Micronucleus assay without statistical significance except when compared to the negative control of healthy individuals**

Health condition as a factor in the CBMN. Means ± SD of different CBMN assay parameters of respiratory disease patients (lung cancer, COPD, asthma) and healthy controls in blood cultures treated with TiO_2 (5 and 10 µg/ml) as well as a negative control of untreated blood cultures (Nc) and a positive control (Pc) of 0.4 µM of mitomycin C (MMC). Symbols indicate level of significance when comparing each group to healthy individuals' untreated controls. * implies *P* < 0.05, ** *P* < 0.01 and $^{+}$denotes highly significant when compared each group to its own untreated control as well as to corresponding healthy individuals untreated control.

KEY:

NDI: indicates nuclear division index.

% Bi NC: indicates percentage binucleated cells in 1000 scored cells.

BiMN: indicates number of micronuclei in 1000 binucleated cells

Mono MN: indicates micronuclei in mononucleated cells in 1000 scored.

NPBs: indicates number of nucleoplasmic bridges in 1000 cells.

Group type and confounder		Nc	TiO_2 concentrations µg/ml		Pc
			5	10	
Control and patients groups	**Control group**				
	NDI	1.88 ± 0.03	1.84 ± 0.02	1.73 ± 0.02	1.66 ± 0.03
	%BiNC	58 .04 ± 4.42	56.35± 2.34	58.34 ± 3.45	46 ± 2.44
	BiMN	1.87 ± 1.63	4.47 ± 2.39	7.27 ± 1.69	29.87 ± 4.15^{+}
		1.93 ± 2.24	1.80 ± 1.51	3.67 ± 1.51	5.2 ± 2.56
	NPBs	0.33 ± 0.47	4.40 ± 2.95	4.80 ± 2.45	0.53 ± 0.81
	Nuclear buds	0.67 ± 0.79	2.13 ± 1.64	2.13 ± 1.5	0.72 ± 0.47
	Patients group				
	NDI	1.68 ± 0.03	1.54 ± 0.02	1.67 ± 0.08	1.52 ± 0.05
	%BiNC	48.50 ± 1.68	46.40 ± 3.45	49. 60 ± 2.87	43.45 ± 3.75
	BiMN	8.54 1.40±	8.29 ± 1.55	11.03 ± 1.70*	30.90 ± 12.21^{+}
		2.04 ± 0.48	3.06 ± 0.28	3.95 ± 1.33	1.21 ± 0.13
	NPBs	5.32 ± 0.06	5.39 ± 0.45	4.79 ± 6.65	5.42 ± 0.24
	Nuclear buds	2.58 ± 0.17	1.88 ± 0.73	1.96 ± 0.98	1.42 ± 0.27
	Lung cancer				
	NDI	1.65 ± 0.06	1.66 ± 0.03	1,65 ± 0.03	1.55 ± 5.2
	%BiNC	54. 70 ± 2.34	52. 50 ± 4.56	56.34 ± 3.42	37.35 ± 2.34
	BiMN	9.98 ± 2.40*	9.71 ± 3.35*	14.50 ± 3.01**	28.58 ± 4.39^{+}
		2.58 ± 2.60	3.33 ± 1.75	5.83 ± 2.11	1.08 ± 1.66
	NPBs	1.08 ± 1.11	5.67 ± 1.89	5.33 ± 2.53	1.50 ± 1.85
	Nuclear buds	0.83 ± 0.99	2.58 ± 2.26	1.33 ± 1.17	1.42 ± 1.04
	COPD				
	NDI	1.63 ± 0.02	1.59 ± 0.03	1.62 ± 0.04	1.38 ± 0.06
	%BiNC	49. 60 ± 2.35	52.25 ± 2.33	56 ± 3.44	46.34 ± 1.36
	BiMN	9.67 ± 2.61*	9.67 ± 3.25*	13.83 ± 1.95**	32.33 ± 4.37^{+}
		1.42 ± 1.38	3.17 ± 3.02	2.92 ± 2.66	1.17± 1.28
	NPBs	1.3 ± 1.43	5.75 ± 3.29	6.08 ± 2.69	2.58 ± 1.89
	Nuclear buds	1.17 ± 1.14	2.17 ± 2.94	3.42 ± 1.44	1.08 ± 1.11
	Asthma				
	NDI	1.86 ± 0.08	1.82 ± 0.02	1.67 ± 0.06	1.42 ± 0.06
	%BiNC	45.55 ± 2.06	65.23 ± 3.44	61.44 ± 2.44	45.2 ± 5.65
	BiMN	3.75 ± 1.48	6.13 ± 2.15	9.73 ± 1.54*	31.75 ± 4.92^{+}
		2.13 ± 1.69	2.6 ± 1.87	3.1 ± 2.15	1.38 ± 1.41
	NPBs	1.375 ± 1.5	4.75 ± 2.38	3.88 ± 3.22	2.63 ± 1.49
	Nuclear buds	0.75 ± 1.09	0.88 ± 1.05	1.13 ± 1.05	1.75 ± 0.97

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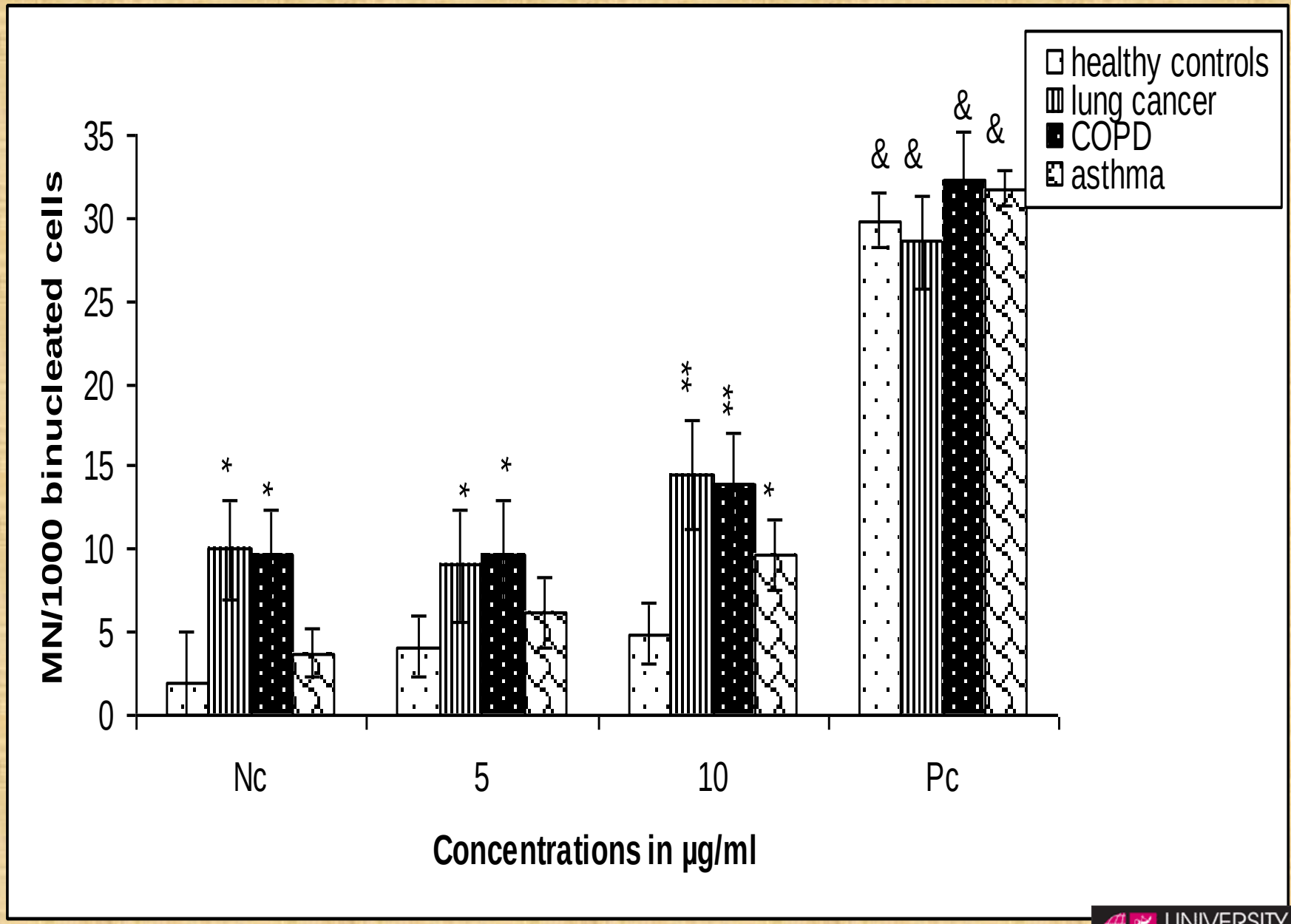
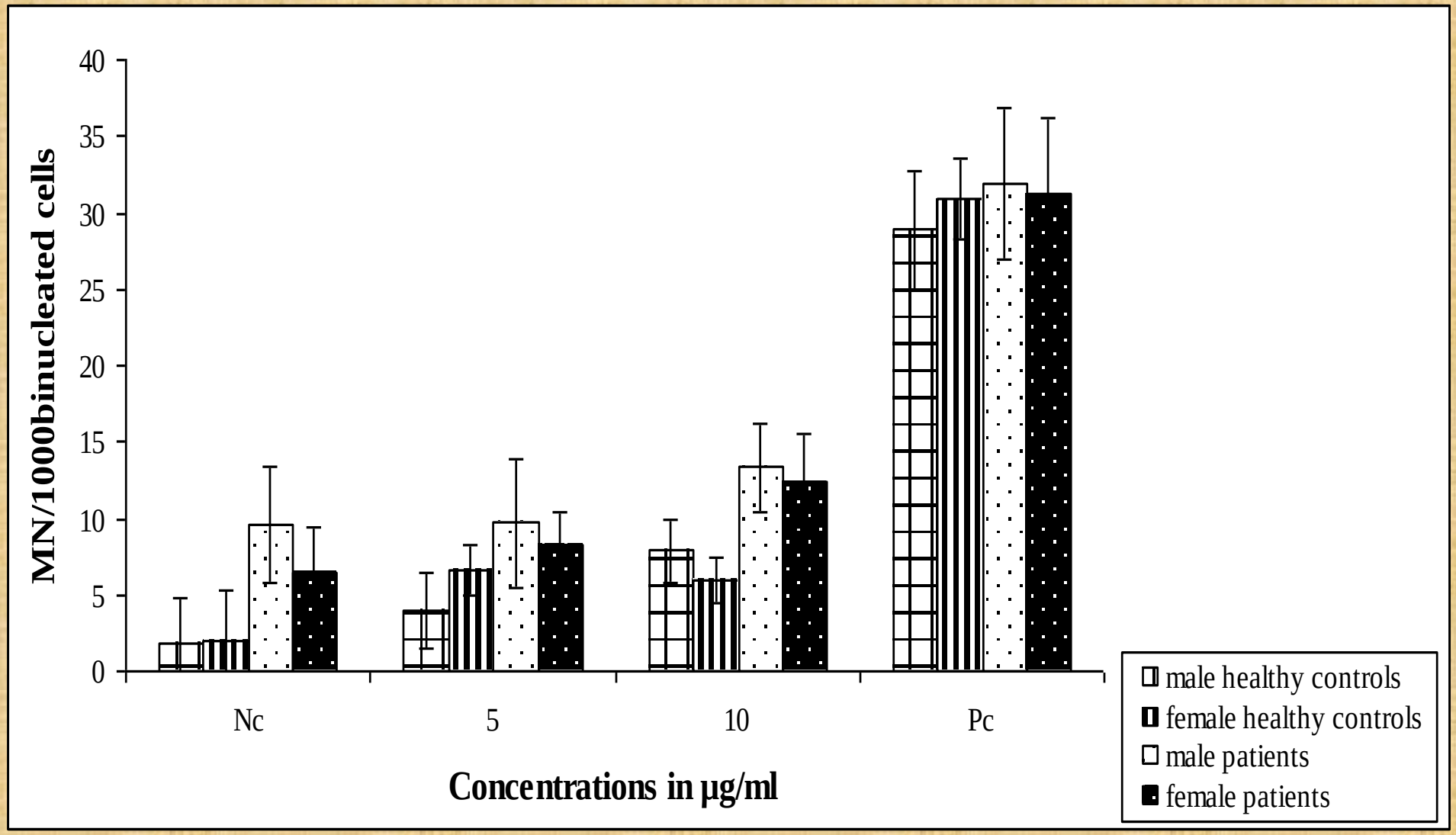
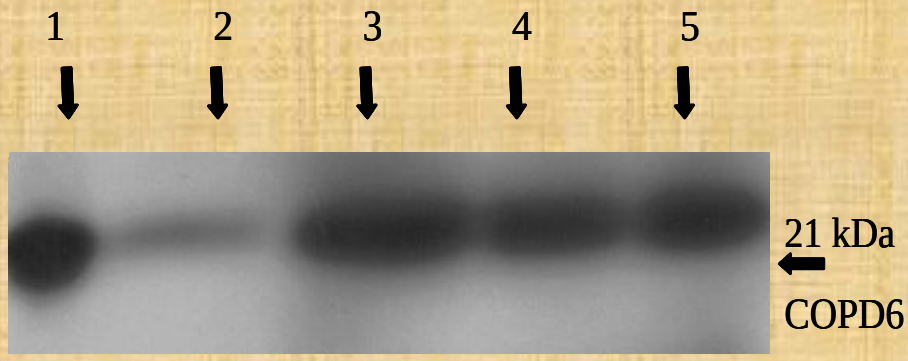

healthy controls
lung cancer
COPD
asthma
MN/1000 binucleated cells
0
5
10
15
20
25
30
35
Nc
5
10
Pc
Concentrations in µg/ml
*
**
&


MN/1000binucleated cells
40
35
30
25
20
15
10
5
0
Nc
5
10
Pc
Concentrations in µg/ml
male healthy controls
female healthy controls
male patients
female patients

RESULTS – *RAS* P 21 PROTEIN LEVELS

When modulation of *ras* p21 protein level expression was examined, regardless of TiO2 treatment, only lung cancer and COPD patients expressed measurable *ras* P21 levels.



Ras p21 protein expression in lymphocytes treated with different TiO_2 concentrations of (3)10 µg/ml, (4) 30µg/ml and (5) 50 µg/ml for 30 minutes, in addition to (1) SW 480 cell lysate (positive control) and (2) untreated

Ras p21 protein levels in human plasma from patients with chronic obstructive pulmonary disease (COPD) compared with lung cancer patients and healthy controls

D. Anderson [a,*], J.A. Hughes [a], A. Cebulska-Wasilewska [b], E. Nizankowska [c], B. Graca [c]

[a] *BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK*
[b] *Environmental and Radiation, Biology Department, INP, Radzikowskiego 152, 31-342 Krakow, Poland*
[c] *Department of Internal Medicine, Jagiellonian University, School of Medicine, Krakow, Poland*

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Cytogenetic damage and *ras* p21 oncoprotein levels from patients with chronic obstructive pulmonary disease (COPD), untreated lung cancer and healthy controls

A. Cebulska-Wasilewska [a,*], A. Wierzewska [a], E. Nizankowska [b], B. Graca [b], J.A. Hughes [c], D. Anderson [c]

[a] *Environmental and Radiation Biology Department, Institute of Nuclear Physics, Radzikowskiego 152, 31-342 Cracow, Poland*
[b] *Colleguim Medicum UJ, Cracow, Poland*
[c] *BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK*

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Group	Nc	TiO_2 concentration in µg/ml		
		10	30	50
COPD 2	217.6 ± 23.9	212.6 ± 26.8	214.4 ± 15.5	187.3 ± 17.3
COPD 5	187.0 ± 23.3	220.7 ± 14.0	223.0 ± 18.0	221.8 ± 18.3
COPD 6[$]	55.1 ± 10.1	187.0 ± 32.5	184.5 ± 40.4	178.0 ± 18.3
COPD **7**	227.0 ± 8.7	-	-	-
COPD	225.8 ± 5.8	222.0 ± 7.4	222.0 ± 7.4	219.6 ± 7.3
Lung cancer 1	222.0 ± 11.3	224.8 ± 15.6	226.6 ± 6.3	216.1 ± 9.4
Lung cancer 3*	227.1 ± 14.8	227.0 ± 22.7	-	-
Lung cancer 4	101.4 ± 25.4	-	-	-
Lung cancer 5	197.6 ± 9.3	196.7 ± 23.9	186.4 ± 32.6	-
Lung cancer 6	202.9 ± 18.0	201.5 ± 14.9	98.2 ± 32.9	197.2 ± 11.7
Lung cancer 7[*]	110.5 ± 20.3	75.2 ± 12.8	54.9 ± 11.6	43.9 ± 15.4
Lung cancer 8[*]	221.0 ± 22.7	217.7 ± 7.8	134.1 ± 13.9	-

Densitometry of ras p21 bands. The OD obtained with ImageJ software and standard deviations of scanned blots of ras p21 bands in respiratory disease patients and healthy controls lymphocyte protein extracts untreated (Nc) and treated with different TiO_2 concentrations (10, 30 and 50 µg/ml) for 30 minutes. All patients samples were coded * (OD means show ras p21 down regulation), $ (OD means show ras p21 up regulation) and – (ras p21 not detected or immeasurable bands). Negative responses have not been included.

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DISCUSSION

- **Respiratory disease patients have higher basal control levels of DNA damage than the healthy controls in the present study.**
- **In general in healthy individuals without confounding effects of smoking and respiratory disease show an increase in DNA damage.**
- **Confounding effects could work synergistically or antagonistically.**
- **Overall responses are still increased in the presence of TiO_2**

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