Article

Automodified Poly(ADP-Ribose) Polymerase Analysis to monitor DNA Damage in Peripheral Lymphocytes of Floricoltorists occupationally exposed to Pesticides.

Serena Imperato [1], Carmela Mistretta [1], Maria Marone [1], Ilaria Migliaccio [1], Ilaria Pulcinelli [1] and Maria Rosaria Faraone Mennella [1,*]

[1] Department of Biology, University of Naples "Federico II", Via Cintia, 80126 Naples (Italy)
S.I.: serenaimperato@gmail.com
C.M.: carmela.mistretta@libero.it
M.M.: mariamarone1990@gmail.com
I.M.: ila.migliaccio@studenti.unina.it
I.P.: il.pulcinelli@studenti.unina.it
M.R.F.M.: faraone@unina.it

* Correspondence: faraone@unina.it; Tel.: +39 081679136; ORCID: 0000-0003-1947-4700

Abstract: Background: Increased DNA damage and the propension to cancer development, depend on the modulation of the mechanisms to control and maintain genomic integrity. Poly(ADP-Ribose)Polymerase activation and automodification are early responses to genotoxic stress. Upon binding to DNA strand breaks, the enzyme, a molecular DNA nick sensor, is hyperactivated: this is the first step in a series of events leading to either DNA repair or apoptosis. Enzyme hyperactivation and automodification can be easily measured and are widely used to look at DNA damage extent in the cell. We investigated whether these two markers (increased catalytic activity and auto modification), could help to monitor DNA damage in lymphocytes of flower growers from Southern Italy, occupationally exposed to pesticides. Methods: Peripheral lymphocyte lysates were analysed for Poly(ADP-Ribose) Polymerase activity, and by SDS-PAGE and anti-Poly(ADP-Ribose)Polymerase 1-antibody to measure automodified anti-Poly(ADP-Ribose) Polymerase levels by densitometry. Results: Poly(ADP-Ribose)Polymerase activity levels were consistent with those of enzyme auto-modification. Growers daily exposed to pesticides, showed both biomarkers very high, either in the presence or in the absence of pathologies. Conclusions: PARP activity and auto-modification in peripheral blood lymphocytes are possible,non-invasive, and routinar tools to monitor the healthy conditions of floricoltorists.

Keywords: Poly(ADP-Ribose)Polymerase; automodified PARP (PAR-PARP); DNA damage; pesticides; growers; greenhouses

1. Introduction

DNA structural alterations play a primary role in several pathologies, including neoplastic transformation [1, 2]. Many experimental evidences indicate that increased DNA damage, and then

propension to cancer development, is related to genetic factors as the modulation of the complex mechanisms involved in the control and maintainance of genomic integrity [3]. PARP-1 activation is an early response to genotoxic stress [4]. Infact, the enzyme acts as a molecular DNA nick sensor binding DNA strand breaks with high affinity [5]. This interaction stimulates PARP-1 catalytic activity and is the first step in a series of events leading to either DNA repair or apoptosis [reviewed in 5-7].

On such a background, it is clear that DNA damage, as well as alterations of some parameters of the poly-ADP-ribosylation system (PARP hyperactivation and automodification) can be considered as valuable biomarkers to assess the risk of cancer development in individuals exposed to genotoxic agents. In fact, a general and central task for worker protection is biomonitoring of occupationally exposed people [8,9]. For agriculturist category, hindrance to perform a right control of risk levels are several variables, i.e. mixing agrochemicals, loading equipment, spraying and application of insecticides, absorption from dermal exposure or inhalation, improper use of pesticides, use of mixtures of different compounds, etc. [10]. Acute toxic effects are easily recognized, whereas the effects resulting from long – term exposure to low doses are often difficult to distinguish. In growers disorders of cardiovascular, respiratory, nervous systems were reported together with skin disorders, as dermatitis, headache, nausea [11]. Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals [12]. However, the absence of a systemic surveillance of agriculturist health does not allow an actual estimation of exposure levels and pesticide-dependent diseases. Evaluation of DNA damage in agricultural workers exposed to pesticides has been performed by different methods (comet assay, chromosomal aberration, sister-chromatid exchanges, serum enzyme assays), the most frequently used being comet assay [13-16].

In Campania, a region in the South Italy, despite intervention programs for workers' health defence include biomonitoring subjects at occupational risk of diseases, particularly cancer, little attention has been payed to monitor workers in agriculture. This field is so dinamic and complex (high number of agrochemicals, greenhouses or open cultures, different times of exposure, etc.) to make difficult to follow it up.

From this area, we selected a group of volunteers (n=82) in a healthy population of both people involved in agricultural work (in particular floriculturists, n=42) and living/working close (n=10) or far (n=30, reference people) from greenhouses, to collect epidemiological data in order to determine prevalence of any disease, and study the possible correlation with pesticide-exposure by measuring PARP activity and automodification (PAR-PARP) in blood lymphocytes. The main advantage of using blood cells from exposed healthy individuals is the possibility to detect effects very early before clinical manifestation of disease allowing for intervention and prevention. Detection of PARP activity and automodification (PAR-PARP), known as biomarkers of DNA damage [17-19], might be easier than other molecular analyses [13-16], resulting possible, non-invasive, and routinar tools to monitor the healthy conditions of floriculturists.

2. Materials and Methods

2.1. Recruitment of volunteers

Among floriculturist farms selected randomly on the basis of the register of local farmer associations, 42 volunteers directly exposed to pesticides (E), were recruited; 10 subjects of other

working categories, including housekeeping women, indirectly exposed (IE), were asked to participate. The last group included 30 people enrolled at the Unit of Immunohematology and Transfusion of the Local Health Unit (ASL NA 3, Torre del Greco), and selected among blood donors (not exposed, negative controls). To increase the response rate, all volunteers, except those from the transfusional center, were visited at home.

This work was within the research project approved by the Bioetic Commettee "Carlo Romano", Department of Public Medicine and Social Security, University "Federico II" of Naples (Protocol N° 131/11).

2.2. Questionnaire

All volunteers authorized to treat personal data anonimously and answered a questionnaire to collect personal data (name, age, sex), life style (diet, smoke, alchool) and anamnesis (pharmacological treatments, recent infections, individual and familiar pathologies, etc.). Other items concerned occupational details. Subjects were asked to specify the exposure time inside greenhouses or storage areas per day and per month, the mixtures of employed agrochemicals, the way and frequency of pesticide spraying, and personal protective devices (gloves, boots, mask, etc.).

2.3 Clinical analyses and specimen collection

Volunteers were subjected to conventional diagnostic analyses (determination of hematic glucose, nitrogen, cholesterol, triglycerides, transaminase activities, blood cell count) to assess health status at the time of enrollment. Blood samples of volunteers were obtained by venipuncture during home visit. Routinely, blood specimen were transferred to the Chemical Clinical Laboratory (ASL NA3, Torre del Greco) within two hours from collection. The clinical analyses of blood donors were carried on at the Unit of Immunohematology and Transfusion (ASL NA3) .

2.4. Lymphocyte isolation and lysis

Total lymphocytes in the blood sample were counted by Fluorescence Activated Cell Sorting (FACS). Lymphocytes were prepared according to GE Healthcare (Italy) protocol, provided with Ficoll. Two aliquots (1mL) were used per each sample. Briefly each blood aliquot (1 mL) was layered on a Ficoll-Hypaque (GE Healthcare) cushion (1 : 0.7, v/v) and centrifuged at 250*g* for 10 minutes. Because of their lower density, the lymphocytes were found at the interface between the plasma and the Ficoll-Paque PLUS with other slowly sedimenting particles (platelets and monocytes). The lymphocytes were recovered from the interface and subjected to short washing steps to remove any platelets, Ficoll-Paque PLUS and plasma. Crude lymphocyte fraction was washed twice with 0.9% NaCl, followed by 10 minutes centrifugation at 250*g*. Pelleted pure lymphocytes were suspended in 0.9% NaCl (100 µL/blood mL). Few microliters of the suspension were used for counting recovered pure lymphocytes by FACS. In general a 20% loss was measured. Pure cells were often used as freshly prepared fraction or stored at -80°C until used. Cells from 1 mL blood were lysed by suspension in lysis buffer (300 µL; 10mM Tris-HCl pH 7.5, 1% Nonidet P40, 2mM Spermidine-HCl, 10mM Na_2EDTA, protease inhibitor cocktail 2µg/mL (Sigma), 1mM Phenyl Methyl Sulphonyl Fluoride, PMSF) and incubation for 30 minutes at 4°C. The whole lysate was further analysed. Protein content was determined by Bradford's reagent (Bio Rad,Milan, Italy) according to the provided instructions.

In order to determine whether increased PAR-PARP levels corresponded to damaged DNA, few cells from three normal and three PAR-PARP altered samples underwent to comet assay according to Bianchi et al. [19]. This technique is routinely performed on single cells by a research group at the Department of Biology (data not shown).

All analyses were performed blindly on blood samples, and thereafter the results were compared with anamnestic data.

2.5. PARP assay

PARP activity was assayed in duplicate on two cell preparations [20]. The reaction mixture (final volume 50 µL) contained 0.5 M Tris-HCl pH 8.0, 50 mM $MgCl_2$, 10mM DTT, 0.4 mM [^{32}P]NAD^+ (10,000 cpm/nmole) and a defined amount (20 µg protein) of whole lysates. After incubation for 15 minutes at 25°C, the reaction was stopped by transfer onto ice and addition of 20% (w/v) trichloroacetic acid (final concentration). The mixture was filtered through Millipore filters (HAWPP0001, 0.45 ◎m) and washed with 7% trichloroacetic acid. The activity was measured as acid-insoluble radioactivity by liquid scintillation in a Beckman counter (model LS 1701). Values were expressed per 10^6 cells.

2.6. SDS-PAGE, Immunochemical and Densitometric Analysis

SDS-Polyacrylamide (12%) gel electrophoresis was according to Bianchi et al. [19].

Immunochemical analysis was performed blindly on blood samples collected from both healthy people and volunteers [21]. Polyclonal anti-PARP1 catalytic site antibodies (H-250, Santa Cruz) were used to evidence PARPs, either in native or automodified state.

Checking automodification of PARP, alkali incubation of PAR-PARP was carried on in 10mM Tris-HCl pH 9.5, for three hours at room temperature, optimal conditions for completely removing

the polymer from the acceptor protein. The alkali mixture was analysed by SDS-PAGE and immunoblotting to evidence native PARP (data not shown).

Automodified PARP was quantified by densitometric analyses of immunobands with a Chemidoc apparatus (Bio Rad) and expressed as optical density (OD, i.e. intensity of a band)/mm^2.

To measure the densitometric value a rectangle was drawn encircling the band to quantify. The area of the rectangle was always constant. Therefore the variable parameter was the intensity of the band. Even if the Quantity One program software automatically subtracted a mean value of the densities measured all over the filter (blank), a background value was manually subtracted. This manual blank was measured by using the above described rectangle on a area of the filter without bands. In addition, the results were normalized by performing densitometric measures of images with the same time of development and chemiluminescent capture signals, as determined by preliminary analyses. In this way the results could be easily compared for all experiments.

2.7. Statistical Analyses

The results of PAR-PARP from the various groups were compared by box-plot analysis [22].

3. Results and Discussion

3.1.Volunteers

Eighty two people agreed to participate in the study. Forty two were floriculturists frequenting greenhouses (E, exposed people), ten were enrolled among subjects living close to or occasionally frequenting greenhouses (flower manipulators, transporters, housekeepings, students, etc.; IE, indirectly exposed), Table 1; thirty blood donors, involved neither directly nor indirectly with flower growth (NE, not exposed, or C, negative controls), were provided by the transfusional center of Local Health Unit. They were selected from other work categories, not involved in culturing, and living far from cultured fields.

Table 1 – General features of enrolled population

	EXPOSED[a]	**INDIRECTLY EXPOSED[b]**	**NOT EXPOSED (CONTROLS)**
Number (*n*)	42	10	30
Age Median (IQR)	43 (19)	34 (17)	42(14)
Males	17	7	20
Females	25	3	10
Smokers	26	4	8
Years of exposure, median (IQR)	15 (12)	-	-
Pesticide exposure (hours/day)	2-12	-	-
Preparation and spraying of pesticide mixtures	29	-	-
Full protective devices	14	-	-

(only for spraying)			
Daily use of gloves and/or mask	23	-	-
No protection	16	-	-

[a] All working in greenhouses with ornamental crops.

[b] Living/working daily (handling and packaging flowers) close to green houses. Most subjects were relatives of floriculturists and lived in the same house or building. Some volunteers had bedroom windows watching over greenhouses.

All volunteers were Caucasic and recruited within the same district to normalize some parameters (food sources, climatic factors, environmental conditions, etc.). Most interviewed people had mild life habits (mixed diet, limited smoke, low/medium alchool consume),Table 1.

Floriculturists (*n*=42) belonged to families working in the field from at least 3 generations and/or were daily exposed to pesticides (spraying and presence in greenhouses 6-12 hours a day). All of them used variable mixtures of the same pesticides (imidacloprid, abamectine, metomil, oxamil, deltametrine, endosulfan, spinosyns) and sprayed on Saturday to re-enter the greenhouses on Monday. 23 growers used gloves/ mask daily and only 14 full protective devices during spraying (Table 1).

3.2. Epidemiological data

Personal anamneses and clinical results of volunteers were first compared with familiar anamneses on the basis of declared personal and /or familiar pathologies (Table 2). Each volunteer was cited as many times as the number of declared diseases.

Anamnestic data were recorded back to granparents, since interviewed floriculturists belonging to the same families declared that culturing activity started at least two generations ahead.

Peer-reviewed version available at Cells 2019, 8, 137; doi:10.3390/cells8020137

Table 2 – Pathologies declared and diagnosed in Exposed and Indirectly Exposed People.

	Familiar Anamnesis		Personal Anamnesis	
	IE (*n*=10)	E (*n*=42)	IE (*n*=10)	E (*n*=42)
	Frequency[a]		Frequency[a]	
Pathology				
None	1	12	5	23
Lung disease[b]	0	2	1	3
Neuropathy[c]	1	12	1	0
Diabetis	3	13	0	3
Thyroidism	0	3	2	6
Cardiovasc. d.[d]	5	15	0	4
HCV[e]	1	5	0	1
Cirrosis	0	2	0	1
Gut d./*H.pylorii*	0	0	0	1
CANCER:	1	3	0	1
uterus	0	1	0	1
prostate	0	0	2	1
lung	1	6	0	0
pancreas	0	1	0	1
colon/gaster	0	1	0	0
bladder	0	1	0	0
liver	0	2	0	2
leucemia	0	1	0	0
brain	0	0	0	1

[a]Times of occurrence. Each volunteer was cited as many times as the number of declared diseases.

[b]Athsma, allergy

[c] Stroke, ischemia, depression

[d] Hypertension, angina, heart attack

[e]Hepathitis C Virus

3.3 PARP activity

3.3.1 PARP activity in controls

In Figure 1 PARP activity of controls is reported in mU/10^6 cells.

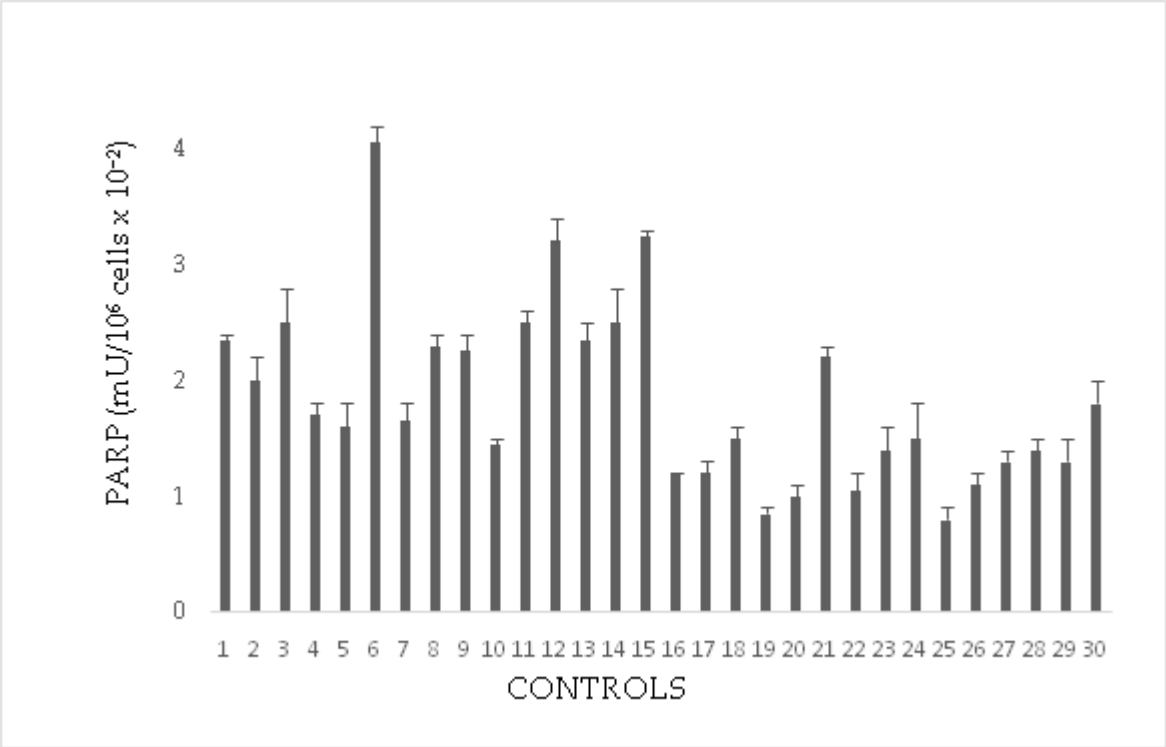

Figure 1. PARP activity in lymphocytes from blood donors.
The values are mean averages of duplicate measures from two different lysate preparations (20µg proteins per each assay).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 January 2019 doi:10.20944/preprints201901.0019.v1

Peer-reviewed version available at Cells 2019, 8, 137; doi:10.3390/cells8020137

Upper value is 4 mU/10^6 cells and is taken as *cut-off* in the following figures. It is slightly higher than that in the range of physiological PARP reported in the literature (1,8-3,0 mU/106 cells x 10^{-2}). This difference allows to consider individual variability, possibly due to factors like smoke, alcohol consumption, temporary stress, common to all analysed subjects.

3.3. 2 PARP activity in volunteers

PARP activity was evaluated by grouping floriculturists, daily exposed (E) to pesticides, on the basis of common anamnestic features (Figure 2).

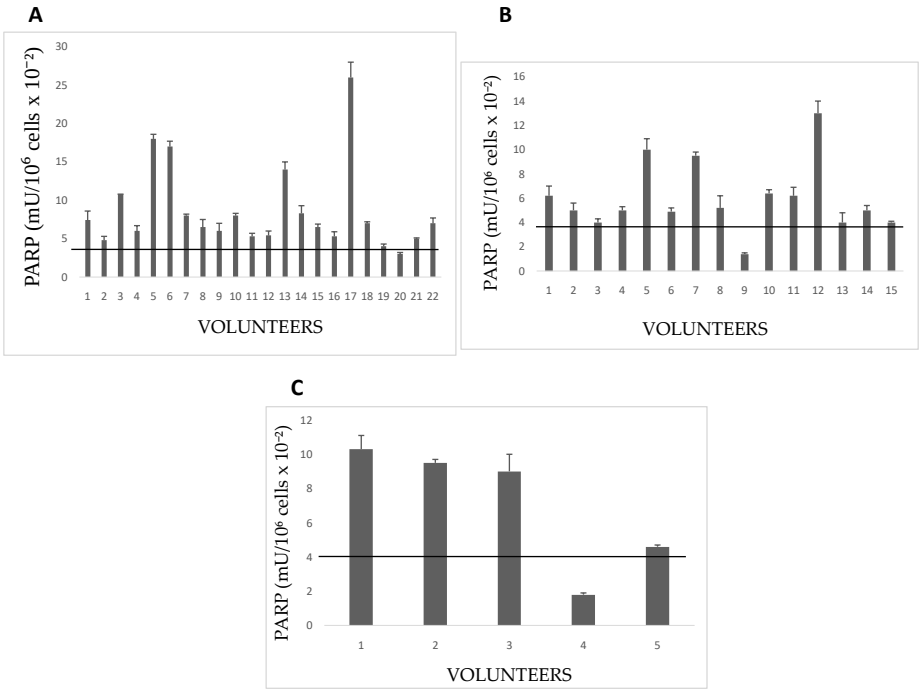

Figure 2. PARP activity in lymphocytes from Exposed People.
A, volunteers with both familiar and personal pathologies; B, subjects declaring only familiar diseases; C, healthy people with neither familiar nor personal pathologies. The values are mean averages of duplicate measures from two different lysate preparations (20µg Proteins per each assay). The black line is the *cut-off*, the upper limit of PARP activity in controls (Figure 1).

Group 1E included twenty two growers declaring both familiar and personal pathologies (Figure 2A). A number of these volunteers showed PARP activity levels above the *cut-off* of controls (no growers); in particular, hyperactivation of PARP in subjects 3, 5, 6, 13, 17 spanned from three to six times the upper physiological limit determined for not exposed people (4 mUx10^{-2}/10^6 cells). Other subjects, despite the declared diseases, showed a normal PARP activity. A possible explanation could be that once diagnosed the disease, these growers chose to monitor their health status by periodic checks and undergoing to appropriate therapies, which limited the dangerous effects of pesticides .

In Group 2E all fifteen people had a familiar anamnesis with pathologies, but they themselves were healthy (Figure 2B). However most floriculturists had altered PARP activity, increased to more than four times the control *cut-off*. By considering that those people were healthy on the basis of clinical

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 January 2019 doi:10.20944/preprints201901.0019.v1

Peer-reviewed version available at Cells 2019, 8, 137; doi:10.3390/cells8020137

and diagnostic reports, a high alteration of PARP might be interpreted as a response to a significant damage to DNA, possibly correlated with ex position topesticides.

Five volunteers (Group 3E) had neither familiar nor personal pathologies, but three of five subjects showed serious increase of PARP activity (Figure 2C). This result allows to hypothesize that, in the absence of diseases, possible causes of enzyme hyperactivation and, consequently, of DNA damage, might be both the prolonged (up to twelve hours a day), and long-lasting (10-40 years) exposure to pesticides in greenhouses.

PARP activity of indirectly exposed volunteers is shown in Figure 3.

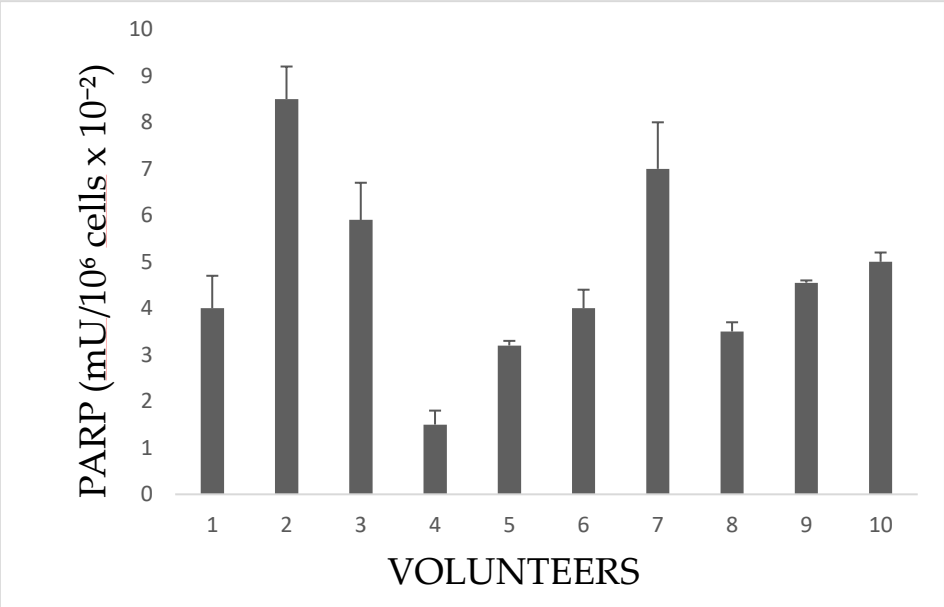

Figure 3. PARP activity in lymphocytes from Indirectly Exposed People.

The values are mean averages of duplicate measures from two different lysate preparations (20µg Proteins per each assay). The black line is the *cut-off*, the upper limit of PARP activity in controls (Figure 1).

These people frequent rooms near the greenhouses or live close to them. Subject 2 is a girl student, daughter of the farm owner, whose bedroom overlooks the greenhouse. Subject 3, 6, 7 handle and package flowers (they do not wear gloves). Subjects 1, 5, 8 are housekeepings living next to the greenhouse. Subjects 4 and 9 are parking attendant and secretary respectively, near greenhouses. Subject 10, housekeeping, is wife of a farm owner, and occasionally helping in packaging flowers.

3.4 Immunoblottings and PAR-PARP measure

3.4.1 Immunoblottings

Anti-PARP immunoblottings of all analysed samples were performed to confirm PARP activity results and to evidence and measure the levels of endogenous PARP automodification (PAR-PARP). Figure 4 shows some lymphocyte immunopatterns of controls, exposed and indirectly exposed people.

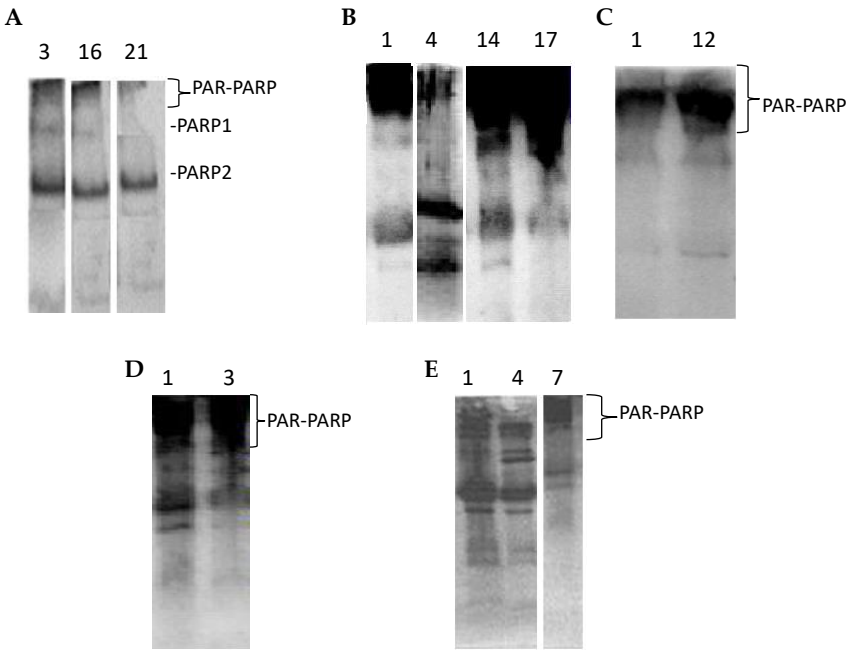

Figure 4. Anti-PARP Immunoblottings of lysate cell proteins.
A, Controls; volunteers of B, Group 1E; C, Group 2E; D, Group 3E; E, Group IE. Numbers indicate volunteers as in Figures 1-3. The same amount of proteins (20µg) was loaded for all samples.

In lymphocytes, under physiological conditions, PARP 2 is more abundant compared to PARP 1 (Figure 4A). Only traces of PAR-PARP are detectable in controls. Automodified PARP increases in immunoblots from both exposed and indirectly exposed people (Figure 4, B-E).

3.4.2 PAR-PARP measure

The results of densitometric analyses performed for all volunteers were grouped as for PARP activity (§3.3). In Figure 5 densitometries of controls are reported.

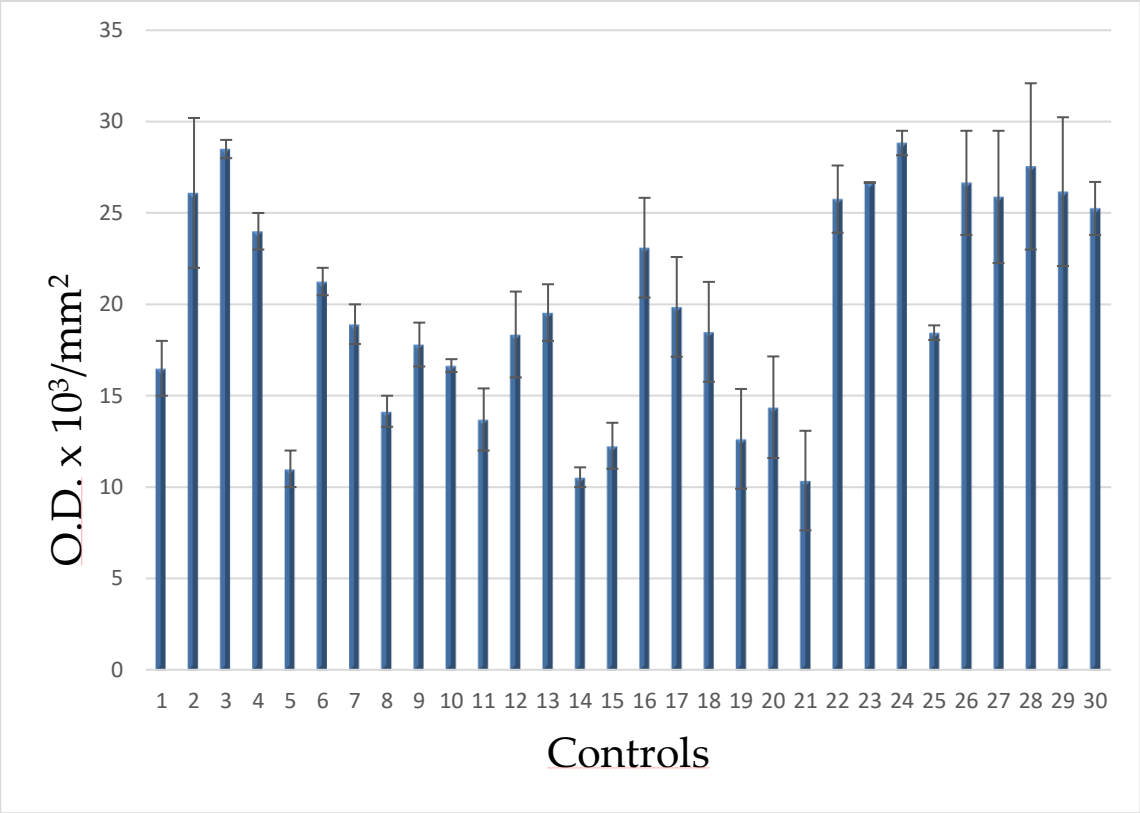

Figure 5. PAR-PARP of blood donors.

The range of these values allowed to calculate the upper limit (30 O.D. x 10^3/mm^2), taken as *cut-off* and reported in the following figures.

The values for the three groups of directly exposed people were consistent with those of PARP activity (Figure 6).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 January 2019 doi:10.20944/preprints201901.0019.v1

Peer-reviewed version available at Cells 2019, 8, 137; doi:10.3390/cells8020137

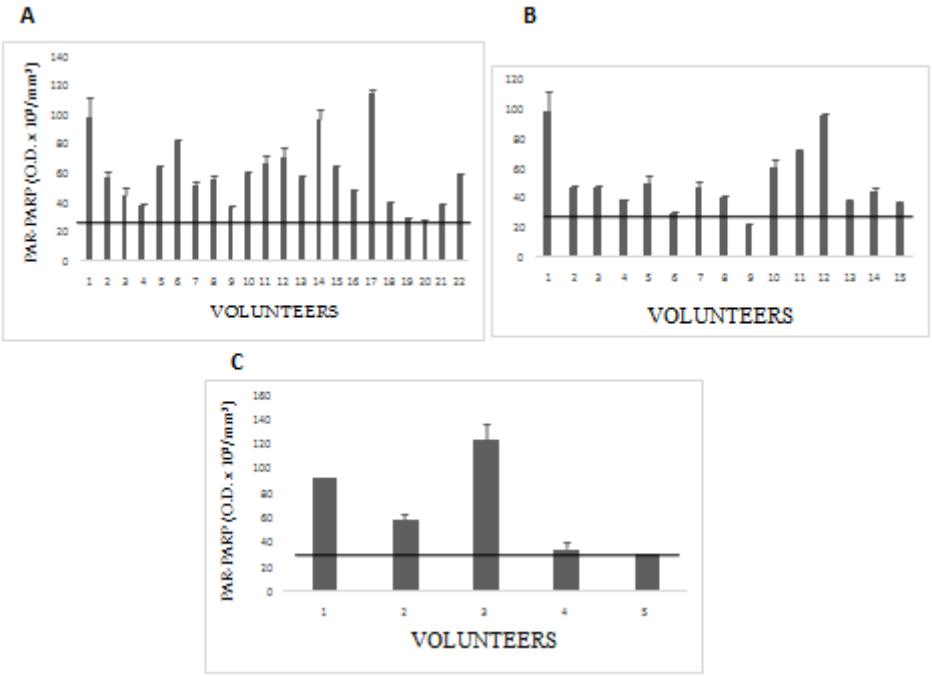

Figure 6. PAR-PARP values of Exposed subjects.
The black line is the *cut-off*. Mean values of duplicate measures from two different lysate preparations.

In Group 1E five people had PAR-PARP values slightly above the *cut-off* (Figure 6A). The other subjects showed PAR-PARP levels corresponding to DNA damage from moderate to excessive; in volunteers 5, 12, 15, 19, 22 PARP hyper-modification reached the maximal levels indicating an extensive DNA damage. Such high levels could not be explained on the basis of declared personal pathologies, since other people affected by the same deseases had moderate or even nearly physiological PAR-PARP. As an hypothesis PARP hyper-modification for subjects 5, 12, 15, 19, 22 might correlate to the years (10-40) of greenhouse frequency.

Group 2E included fifteen growers with many years of activity in greenhouses, and declaring no personal deseases, even if they have pathological familiar anamnesis (Figure 6B). Relevants were PAR-PARP results of people 2, 3, 8. In the absence of pathologies, such high levels might be correlated better with daily and long periods of greenhouse frequentation.

The five subjects of Group 3E declared to be healthy for both familiar and personal anamneses (Figure 6 C). However two of them had PAR-PARP above 60 O.D/mm^2. As for Group 2E it can be hypothesized that this PARP hypermodification might be due to an uninterrupted and prolonged exposition to pesticides.

Indirectly exposed volunteers, frequenting rooms next to greenhouses (storekeepers, secretary, staff for the packaging of flowers), or living close to them, are shown in Figure 7. In general most growers, expecially farm owners' families, have houses in proximity of greenhouses. Only the subject N° 2 showed a very high PAR-PARP. She was a student, daughter of a farm owner, whose bed room overlooks a greenhouse.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 January 2019 doi:10.20944/preprints201901.0019.v1

Peer-reviewed version available at Cells 2019, 8, 137; doi:10.3390/cells8020137

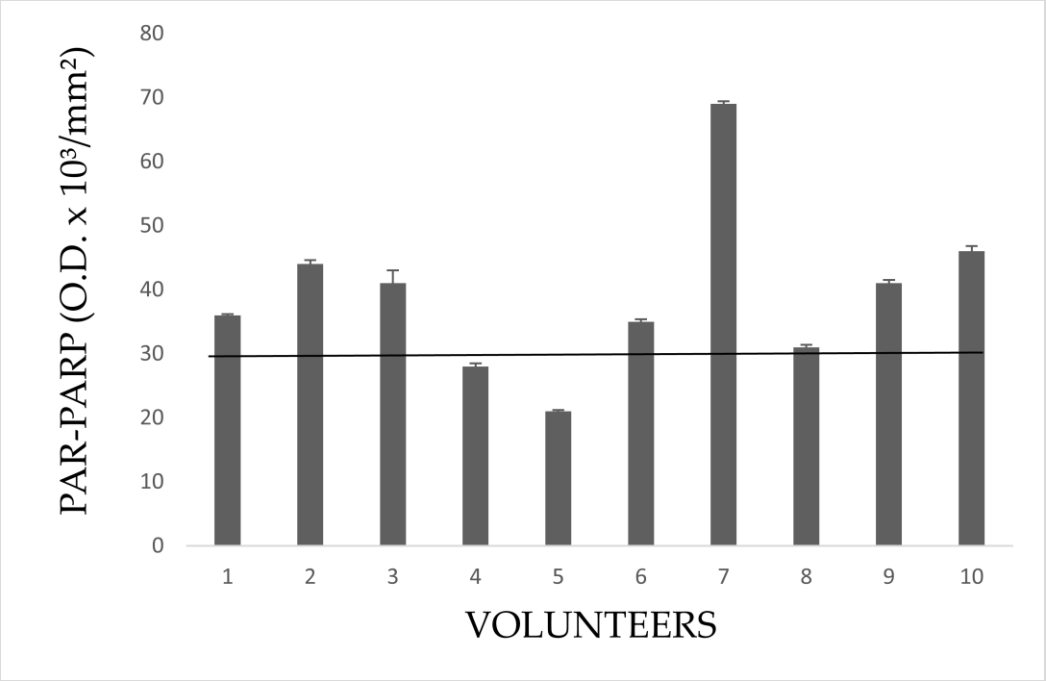

Figure 7. PAR-PARP in Indirectly Exposed subjects.
The black line is the *cut-off*. Mean values of duplicate measures from two different lysate preparations.

In order to evaluate better the collected data, the results of PAR-PARP measure were compared by the box plot analysis (Figure 8).

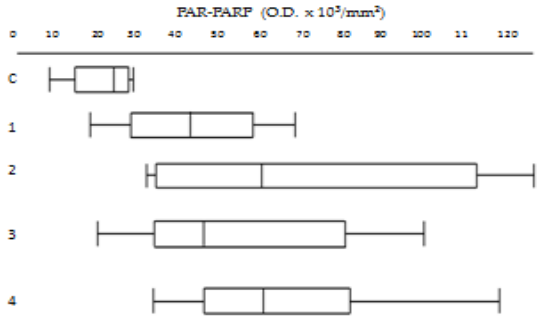

Figure 8. Box plots of PAR-PARP levels in different groups.
C, Controls; 1, indirectly exposed; 2, Group 3E; 3, Group 2 E; 4, Group 1E.

Compared to controls, all groups show PAR-PARP levels as for moderate to high DNA damage. In Group 1E the occurrence of both familiar and personal pathologies, and possible therapies, does not allow to correlate directly the damage with the use of pesticides (Figure 8, 4).

Most significant are the results of plots 2 and 3 (Group 2E and 3E, respectively) that suggest an extensive alteration of PAR-PARP levels and thereby a massive DNA damage. In the absence of personal pathologies, it is conceivable that such high values correlate with activity in greenhouses and prolonged exposition to pesticides. These are the most fitting examples that monitoring of growers with PAR-PARP as biomarker can be determinant in highlighting the altered conditions that clinical and diagnostic analyses are not able to evidence so early.

5. Conclusions

This research highlights the importance of PARP as biomarker of DNA damage, a simpler alternative to other methods to measure DNA damage [13-16]. We like to underline that the presented results do not allow to draw conclusive remarks as they need a larger sampling to get statistically significant data for both other workers and pesticide exposed people. However they suggest a trend: where there are no known pathologies, there are signs, namely PARP biomarkers (PARP hyperactivation and PAR-PARP), that may suggest more frequent controls of growers and possible therapeutic interventions. Thereby we propose PARP activity and auto-modification in peripheral blood lymphocytes as possible, non-invasive, and routinar tools to monitor the healthy conditions of floricoltorists.

In perspective, once validated these easy and not invasive molecular methods, it could be possible to plan a constant biomonitoring of workers, not only floriculturists, occupationally exposed to risk.

Author Contributions: conceptualization, M.R.F.M.; methodology, M.R.F.M.; validation, M.R.F.M. and C.M.; formal analysis, M.R.F.M., C.M., S.I. and M.M.; investigation, S.I., C.M., M.M., I.L. and I.P.; writing—original draft preparation, M.R.F.M.; supervision, M.R.F.M.; funding acquisition, M.R.F.M.;

Acknowledgments: This research was funded by Regione Campania (grant "Legge 5 / 07"). We wish to thank Dr Claudio Bufi, past Director of the Laboratory at ASL NA 3, and his staff, namely Dr Di Bernardo and Dr Sollo, for their support in clinical analyses. A special thank to the nurse Mrs Maria Cirillo for blood collection at growers' home, and to Mr. Ciro M. Aurilia, head of the flower growers' cooperative, for their recruitment.

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

References

1. Jackson, S.P.; Bartek, J. The DNA-damage response in human biology and disease. *Nature* **2009**, *461*(7267),1071–1078 DOI: 10.1038/nature08467.

2. Torgovnick, A.; Schumacher, B. DNA repair mechanisms in cancer development and therapy. *Front Genet.* **2015**, *6*, 157 DOI: 10.3389/fgene.2015.00157

3. Chakravarthi, B.V.S.K.; Nepal, S.; Varambally, S. Genomic and Epigenomic Alterations in Cancer. *Am J Pathol*. **2016**, *186*(7), 1724–1735, DOI: 10.1016/j.ajpath.2016.02.023

4. Sousa, F.G.; Matuo, R.; Soares, D.G.; Escargueil, A.E.; Henriques, J.A.; Larsen, A.K.; Saffi, J. PARPs and the DNA damage response. *Carcinogenesis* **2012**, *33*(8),1433–1440 DOI: 10.1093/carcin/bgs132

5. De Vos, M.; Schreiber, V.; Dantzer F. The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art. *Biochem. Pharmacol.* **2012**, *84*(2), 137–146, DOI: 10.1016/j.bcp.2012.03.018

6. Faraone Mennella, M. R. Mammalian Spermatogenesis, DNA Repair, Poly(ADP-ribose) Turnover: the State of the Art. In: DNA Repair − On the Pathways to Fixing DNA Damage and Errors, Storici, F. Ed.; IntechOpen 2011; Chapter 12, DOI: 10.5772/23337

7. Luo, X.; Kraus, W.L. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev.* **2012**, *26*(5), 417–432, DOI: 10.1101/gad.183509.111

8. Coronado, G. D.; Thompson, B.; Strong, L.; Griffith, W.C.; Islas, I. Agricultural task and exposure to organophosphate pesticides among farm workers. *Environmental Health Prospective* **2004**, *112*(2), 142–147, DOI: 10.1289/ehp.6412

9. Castillo-Cadena, J.; González-Mercado, A.L.; Hernández-Caballero, N.; Ramírez-San Juan, E.; Álvarez-González, I.; Madrigal-Bujaidar, E. Immunotoxic damage in floriculturists exposed to pesticide misture. *J. Environm. Sc. Health, Part B* **2013**, *48*(1), 33–39, DOI: 10.1080/03601234.2012.716690

10. Dosemeci, M.; Alavanja, M.C.; Rowland, A.S.; Mage, D.T.; Zahm, S.H.; Rothman, N.; Lubin, J.H.; Hoppin, J.A.; Sandler, D.P.; Blair, A. A Quantitative Approach for Estimating Exposure to Pesticides in the Agricultural Health Study. *Ann. Occupat. Hyg.* **2002**, *46*(2), 245-260, DOI: 10.1093/annhyg/mef011.

11. Darçın, E. S.; Darçın, M.; Alkan,M.; Doğrul, G. Occupational Risk Factors for Acute Pesticide Poisoning among Farmers in Asia. In: *Poisoning - From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis*; Malangu, N. Ed.; IntechOpen, 2017; Chapter 7, DOI: 10.5772/intechopen.72006.

12. Kisby, G.E.; Muniz, J.F.; Scherer, J.; M. R. Lasarev, M. R.; Koshy, M.; Yoke W. Kow, Y. W.; McCauley, L. Oxidative Stress and DNA Damage in Agricultural Workers. *J. Agromedic.* **2009**, *14*(2), 206–214.

13. Villarini, M.; Moretti, M.; Pasquini, R.; Scassellati-Sforzolini, G.; Fatigoni, C.; Marcarelli, M.; Monarca, S.; Rodriguez A.V. *In vitro* genotoxic effects of the insecticide deltamethrin in human peripheral blood leukocytes: DNA damage (comet assay) in relation to induction of sister-chromatid exchanges and micronuclei.*Toxicology* **1998**, *130*, 129-139.

14. Kaur, R.; Kaur, S.; Lata, M. Evaluation of DNA damage in agricultural workers exposed to pesticides using single cell gel electrophoresis (comet) assay," *Ind. J. Hum. Genet.* **2011**, *17*(3), pp. 179–187.

15. Jørs, E.; Gonzáles, A.R.; Ascarrunz, M.E.; Tirado, N.; Takahashi, C.; Lafuente, E.; Dos Santos, R.A.; Bailon, N.; Cervantes, R.; Huici, O.; Bælum, J.; Lander, F. Genetic Alterations in Pesticide Exposed Bolivian Farmers. An evaluation by analysis of chromosomal aberrations and the comet assay. *Biomarker Insights* **2017**, 2, 439-445.

16. Joksić, G.; Vidaković, A.; Spasojević-Tišma, V. Cytogenetic Monitoring of Pesticide Sprayers. *Environm. Res.* 1997, *75*(2), 113-118, DOI: 10.1006/enrs.1997.3753

17. Stover, E.H.; Konstantinopoulos, P.A.; Matulonis, U. A.; Swisher, E.M. Biomarkers of Response and Resistance to DNA Repair Targeted Therapies. *Clin. Cancer Res.* 2016, *22*(23), 5651-5660. DOI: 10.1158/1078-0432.CCR-16-0247

18. Schiewer, M. J.; Mandigo, A. C.; Gordon, N.; Huang, F.; Gaur, S.; de Leeuw, R.; Zhao, S.G.; Evans, J.; Han, S.; Parsons, T.; Birbe, R.; McCue, P.; McNair, C.; Chand,S.N.; Cendon-Florez, Y.; Gallagher, P.; McCann, J.J.; Neupane, N.P.; Shafi, A.A.; Dylgjeri, E.; Brand, L.J.; Visakorpi, T.; Raj, G.V.; Lallas, C.D.; Trabulsi, E.J.; Gomella, L.G.; Dicker, A.P.; Kelly, Wm. K.; Leiby, B.E.; Knudsen, B.; Feng, F.Y.; Knudsen, K.E. PARP-1 regulates DNA repair factor availability. *EMBO Mol. Med.* **2018**, *10*(12), e8816, DOI:10.15252/emmm.201708816

19. Bianchi, A.R.; Ferreri, C.; Ruggiero, S.; Deplano, S.; Sunda, V.; Galloro, G.; Formisano, C.; Faraone Mennella, M.R. Automodification of PARP and fatty acid-based membrane lipidome as a promising integrated biomarker panel in molecular medicine. *Biomark. Medic.* **2016**, *10*(3), 229-242, DOI: 10.2217/bmm.16.3

20. Faraone Mennella, M.R.; De Maio, A.; Petrella, A.; Syntichaki, E.; Kerbalaeva, A.M.; Nasmetova, S.M.; Goulyamova, T.G.; Farina, B. Yeast (ADPribosyl)ation: revisiting a controversial question. *J. Cell. Biochem.* **2005**, *94*, 1258-1266, DOI: 10.1002/jcb.20362

21.Faraone-Mennella, M.R.; Marini, M.; Ferone, A.; Cacace, O.; Liguoro, A.; Margonato, V.; Farina, B.; Veicsteinas, A. Physical exercise activates the poly(ADP-ribosyl)ation system in rat testes. *J. Biol. Regul. Homeost. Agents* 2010, *24*(3), 325-333,

22. https://www.statcan.gc.ca/edu/power-pouvoir/ch12/5214889-eng.htm