Evaluation of Genetic DNA damage in the agricultural workers exposed to combined action of pesticides in Jonia area of Barpeta district of Assam

Dr. Ranjit Hazarika[1§], Purbajyoti Deka[2]

[1]Associate Professor, Genotoxicology Laboratory, M. C. College, Barpeta (Assam) India
[2]Project Fellow, Genotoxicology Laboratory, M. C. College, Barpeta (Assam) India
[§]Corresponding author's Email: rranjit96@rediffmail.com

Abstract— *The use of pesticide is increasing day by day and it causes a serious concern to the human health, especially some pesticides causes' deleterious effect causing Cancer. Genotoxicity test has a special significance because it is one of the tests for cancer research and risk assessment. The sprayer has more risk because they directly cause in contact with pesticides. Pesticides may affect the DNA of the body cells of the sprayer and known to cause DNA damage. In this case control study DNA damage due to exposure of pesticides by the Agricultural workers of Jonia area of Barpeta district were assessed using the Comet assay. The result showed that the exposed workers had greater mean comet tail length than those of control i.e. 17.69±5.25 versus 10.72±7.89 (p=0.0034, t=3.8206). Among control group, smokers of exposed group have greater tail length than smokers of control group. When DNA damage in the form of comet tail length and frequency of blood leucocytes showing migration were further evaluated in study group. It was observed that although it is more in elders, vegetarian, alcoholics and smokers but it was it was found significant only in smokers than non smokers. Therefore the present studies suggest that exposure of pesticides and smoking habits may cause DNA damage.*

Keywords: Agricultural workers, Pesticide, DNA damage.

I. INTRODUCTION

The increasing use of pesticides drastically effect daily in the environment, these pesticides are release in the environment daily as a large scale and many of them have drastic effect on non-target species as well as represent a potential hazard to the human health.

Pesticides induced to breaking DNA and thus the affect of DNA replicate and its ability to carry information.[1,2] DNA damage cause instability in multiple pathways which can be considered as strategy for risk assessment and it is a reliable biomarker.

In the bio-monitoring studies, comet assay has been used rapidly as a sensitive tool that demonstrate damaging effect of different compounds on DNA at the individual cell level.[3] Cells with damaged DNA display increased migration of DNA fragments from nucleus, generating a comet shape.[4,5] At present comet assay has been used in occupational studies as well as molecular epidemiological studies to evaluate genotoxic effect of pesticides in human population. The aim of the present study is to find out the relation between the occupational exposure of different kinds of pesticides and DNA damage in individual directly spraying the pesticides in the agricultural field.

II. METHODOLOGY

This case control type of study was conducted in the department of Genotoxicology, M. C. College, Barpeta (Assam) India.

2.1 Population study

This study involved 60 participants, 30 from exposed and 30 from control groups in Jonia area of Barpeta district. Subjects for the control groups were selected from the area where exposed subjects were selected. They have almost similar as per demographic data. After taking written informed consent, all the subjects were asked to complete questationaries with standard demographic data (such as age and gender etc.) as well as other questationaries like hours of working per day, years of exposure, use of protective measures and other factors of the environment at the time of spraying ,wind direction, temperature etc. were also inquired.

2.2 Study design & Procedure

Comet assay or single cell gel electrophoresis was carried out according to the technique reported by Singh N, P. et. al[6]. Slides were randomly coded. All the slides were scored by a single person to avoid inter scorer variability. Blood samples were coded at the time of blood collection. DNA damage was studied from taking the blood sample from exposed and control subjects. Blood samples were collected from the vein and kept in ice and directly carried to the laboratory for analysis the Comet assay. Slides were prepared in duplicate. Clean and dry slides were coated by putting a small a small amount of 1% normal melting point agorose (NMPA) and dried at 37®C for 2 to 3 hours. 25µl of whole blood was mixed with 0.5% of 75µl low melting point agorose (LMPA 30°C) .100µl of this mixture was pipetted on to the recoated slides as second layer and covered with cover slip. For 30 min these sides were allow to solidify at 4°. After 30 min the cover slip were removed and third layer of 100µl of LMPA was pipetted on to the slides and spread with the help of cove slip. These slides were kept in 4°C for another 30 min. LMPA and NMPA were prepared in phosphate buffers line (136mM NaCl,2.68 Mm KCL, 8.10Mm Na_2 HPO_4, 1.47Mm KH_2 PO_4 pH-7.4). Slides were immerged in freshly prepared cold lying solution (2.5M NaCL, 100m MNa_2 EDTA, 10mMtris-HCL, pH 10, 1% Trition X-100 and 10%DMSO add freshly before use) for 2 hours. The slides were placed in alkaline buffer (300mM, NaOH and 1mM Na_2OH, pH-13) in horizontal electrophoretic chamber for 2 minutes to allow unwinding of DNA. Electrophoresis was conducted for 30 minutes at 25V (.66V/cm) and 300mA current. Current adjusted by lowering and rising the buffer level. After electrophoresis slides were dried placed in a tray and washed thrice for 10min each with neutralization buffer (.4M tris-HCL, pH 7.5). The whole procedure was carried out in the dim light to avoid additional DNA damage. The gel was dried for 1 hour at room tem and fixed for1o min at fixing solution. (15w/v trichloroactic acid, 5% glycerol) and dried at 37°for 1 hour. The staining solution was prepared freshly before use by mixing 34ml of solution A (0.2% silver nitrate and 5% formaldehyde) with solution B (5% sodium carbonate) and pored over sample gently. The slides immerged over 30 min. After staining slides were washed three times with deionizer water for 30 min, air dried and viewed under microscope.

Slides were examined by using a 400x objective with a Leica opt phase microscope equipped with an excitation filter of 515-560 nm and a barrier filter of 590 Nm. Slides were randomized and coded to blind the scorer. All slides were scored by one person to avoid inter scorer variability. Per subject, 50 cells were scored. Normal cells with intact nucleus and without tail and damaged cells with comet were measured. The comet tail length with DNA migration was measured using specific software CASP 1.1. (Figure 1-2).

2.3 Statistical analysis

For statistical analysis, frequency of DNA damage with mean and standard deviation of each parameter was calculated. The difference of DNA damage between control and exposed groups were measured and significance was inferred by using t-test. Multiple factor analysis (ANOVA) was used to check significant difference using p-value of 5%. All the analyses were performed with SPSS 19.0 version software packages (Trial version).

Figure 1
Photographs showing normal cells

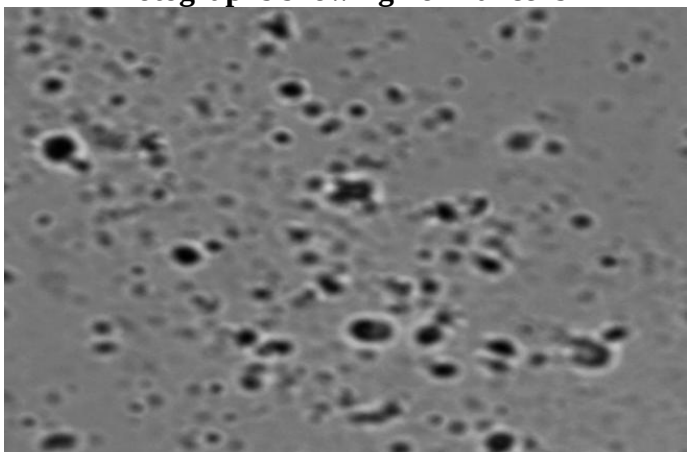
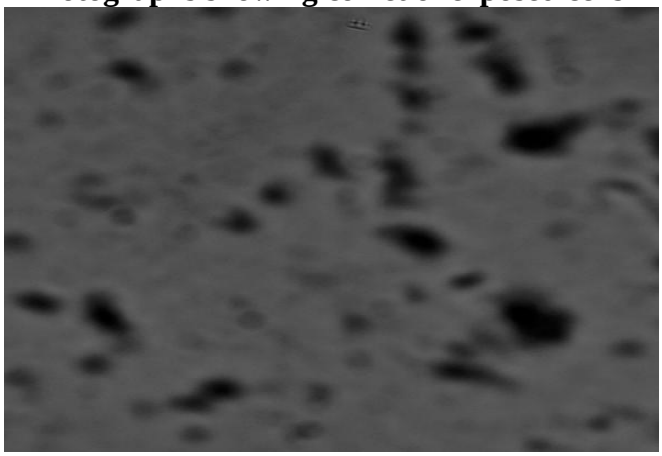


Figure 2
Photographs showing comet of exposed cells



III. RESULT

Both the groups i.e. study and control was compared as per age, sex & smoking status. It was found that although all the participants were males in both the group but subjects were significantly ($p<0.05$) more elder and more smokers in study group than control group. (Table 1)

Table 1
Comparison of study subjects of study and control group

Variables		Study Group (N=30)	Control Group (N=30)	P Value*
Age	**<35 Years**	0	10	**0.002**
	≥35 Years	30	20	
Sex	**Male**	30	30	**1**
	Female	0	0	
Smoking Status	**Smokers**	26	10	**<0.001**
	Non-smokers	4	20	
Years of Exposure	**<10 Years**	0	0	**NC**
	≥10 Years	30	0	

Chis quare Test

When DNA damage in the form of comet tail length in blood leucocytes was compared in study and control group it was found significantly more in study group than control group. (Table 2)

Table 2
Comparison of Comet tail length in study and control group

Parameter	Study Group (N=30)	Control Group (N=30)
Mean (μm)	**17.86**	**14.64**
SD (μm)	**4.81**	**3.43**

Unpaired 't' Test=2.985 at 58 DF P=0.004

When DNA damage in the form of comet tail length in blood leucocytes was compared in smokers and nonsmokers of both the groups. Although it was found more in smokers in both the groups but found significant in study group only. (Table 3)

Table 3
Comparison of Comet tail length in study and control group

	Study Group (N=30)	Control Group (N=30)
Smokers (Mean+SD) (µm)	17.69 ±5.25 (N=26)	10.72 ±7.89 (N=10)
Non-smokers (Mean+SD) (µm)	18.88±14.46 (N=4)	15.36±2.46 (N=20)
Unpaired 't' Test **P Value**	t= 3.8206 at 58 DF **p= 0.0034**	t= 2.0880 at 58 DF **p= 0.1720**

When DNA damage in the form of frequency of blood leucocytes showing migration was evaluated in study group. It was observed that although it is more in elders, vegetarian, alcoholics and smokers but it was it was found significant only in smokers than non smokers. (Table 4)

Table 4
Comparison of DNA Damage in various categories of study subjects (N=30)

Type of Categories		Frequency of cells showing migration		Comet tail length	
		Mean±SD	P Value*	Mean±SD	P Value*
Age	**<30 Years (N=27)**	18.03±3.10	0.116	9.08±5.12	0.099
	≥35 Years (N=3)	26.02±5.54		17.86±4.81	
Type of Diet	**Vegetarian (N=3)**	28.02±1.00	0.207	19.91±7.25	0.153
	Non-vegetarian (N=27)	26.02±5.54		17.86±4.81	
Alcoholic	**Yes (N=2)**	27.05±6.02	0.385	20.03±1.21	0.139
	No (N=28)	26.02±5.54		17.86±4.81	
Smoking Status	**Smokers (N=26)**	26.56±5.86	**0.031****	17.69±5.25	**0.003****
	Non-smokers (N=4)	22.78±0.00		18.88±14.46	
Years of Exposure	**<10 Years (N=4)**	15.09±1.12	0.054	13.01±7.12	0.077
	≥10 Years (N=26)	26.02±5.54		17.86±4.81	
Protective Measures	**Yes (N=3)**	24.02±5.4	0.663	20.81±4.31	0.163
	No (N=27)	26.02±5.54		17.86±4.81	

Chis quare Test** ****Significant at p<0.05

IV. DISCUSSION

In this study, level of DNA damage of 30 farmers of Jonia area of Barpeta district, compared with the 30 control subjects in Jonia area of Barpeta district of Assam.

The genotoxic investigation using comet assay is studied by very few authors. The assay was used to quantify the level of DNA damage in peripherial lymphocytes was conducted in agricultural areas, exposure to pesticides. Occupational exposure of number of pesticides at the level of DNA damage was investigated in French farmers out by Singh et al who reported the significant higher amount of genetic damage in exposed group as in resonance to this present study. Another investigation conducted by the same author suggested that increase level of DNA damage was observed in the farmers after one day spraying with mixture of pesticides.

In another study[7] it was found that lack of protective measure taken by the workers increased the amount of DNA damage or genotoxic damage. Almost similar was observed in this present study although it was not found significant.

Since DNA damage is an important step in the events in the leading carcinogenic pesticides exposed to cancer, Comet assay in the peripheral lymphocytes is an important potential risk evaluation of the monitoring of the potential genotoxic effect possibly due to cumulative effect of different mixtures of the pesticides, it is not possible to attribute damage to any particular agent. The DNA damage in agricultural workers may not due to any particular agent. It may be due to complex mixture of pesticides particularly in agricultural field at that particular time. The different studies suggested that the long time exposure of pesticides on agricultural workers effect on the DNA of peripheral lymphocytes. The genetic DNA damage in the blood leukocyte of the farmer of the present study could be due to the effect of pesticides molecules or its residue on the DNA molecules in the cell[3]. Increase in comet tail length could be possibly originated from DNA single stranded breaks, repair of DNA double stranded break, DNA adduct formation on RNA-DNA and DNA-Protein cross links[7]. Although there was more DNA damage in elders, vegetarian, alcoholics and smokers but it was it was found significant only in smokers than non smokers. Majority of authors[4,5,6] reported no significant relationship between the DNA damage was found in the workers with smoking habit, drinking habit.

V. CONCLUSION

It can be concluded that pesticides is the vital factor of DNA damage and it increases with its duration of exposure. Although there was more DNA damage in elders, vegetarian, alcoholics and smokers but it was it was found significant only in smokers than non smokers. So this present studies suggest that exposure of pesticides and smoking habits may cause DNA damage.

CONFLICT OF INTEREST

None declared till now.

REFERENCES

[1]. Garaj-Vrhovac, V. and Zeljezic, D.2000. Evaluation of DNA damage in workers occupationally exposed to pesticides using single –cell gel electrophoresis (SCGE) assay. Pesticide genotoxicity revealed by comet assay. Mutat. Res., 469,279-285.

[2]. Gontijo AM de MC,Elias FN,Salvadori DM,de Oliveira ML,Correa LA,Goldberg J,et al.Single(Comet) assay detects primary DNA damage in non-neoplastic urothelial cells of smokers and ex-smokers. Cancer Epidemiol Biomark Prev.2001; 10:987-93.

[3]. Islas-Gonzalez K,Gonzalez-Horta C,Sanchez-Ramirez B,Reyes-Aragon E,Levario-Carrillo M.I assessment of genotoxicity of ethyl paraoxon in newborns and adults. Human Exp Toxicol.2005; 24:319-24.

[4]. Lebailly,P.,Vigreux,C., Lechevrel, C., Ledemeney, D.,Godard, T.,Sichel, F.,LeTallaer, Y.,Henry-Amar, M. and Gauduchion, P.1998a. DNA damage in mononuclear leukocytes of farmers measured using the alkaline comet assay: discussion of critical parameters and evaluation of seasonal variations in relation to pesticide exposure. Cancer Epidemiol. Biomarkers Prev., 7,917-927.

[5]. Lebailly,P., Vigreux,C., Lechevrel,C., Ledemeney,D., Godard,T., Sichel,F., LeTalaer, J.Y.,Henry-Amar, M. and Gauduchon, P.1998b. DNA damage in mononuclear leucocytes of farmers measured using the alkaline comet assay: modification of DNA damage levels after a one day field spraying period with selected pesticides. Cancer Epidemiol.Biomarkers Prev., 7, 929-940.

[6]. Singh,N.P., McCopy, M.T..,Tice, R.R. and Schneider,E.L.1988 A simple technique for quantitation of low levels of DNA damage in individual cells. Exp.Cell Res., 175,184-191.

[7]. Velazquez AN,Xamena A,Macros R.Indication for weak mutagenicity of the organophosphorous insecticide dimethoate in drosophila melanogaster. Mutat Res.1986; 172:237-43.