

Epidemiological studies of pesticide-exposed individuals and their clinical implications

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Epidemiological studies were conducted in pesticide-exposed agricultural workers along with an equal number of age- and sex-matched controls. All the 200 exposed volunteers were suffering from fever, nausea, headache and other abnormal symptoms and visited the hospital for general health check-up. These cases were taken up for more detailed studies. Five (2.5%) showed decrease in RBC, haemoglobin, and increase in WBC with a large number of immature cells. These volunteers were further diagnosed as Philadelphia-negative Chronic Myeloid Leukaemia (CML) cases based on clinical and pathological examinations.

In similar environs, about 100 children (aged between 1 and 17 years) were studied for the above parameters. About 3% of the exposed children showed signs of mental retardation and delayed milestones; these were compared with healthy children (age- and sex-matched) from pristine environment. It is therefore concluded that clinical evaluations supported by occupational epidemiology could determine CML in exposed individuals (adults).

Keywords: Chronic myeloid leukaemia, hematological and neurological parameters, occupational exposure, pesticides.

USAGE of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioural changes in individuals^{1,2}. The degree of toxicity depends upon the nature of the pesticides, their environmental concentration and factors such as temperature, humidity, pH, oxygen concentration, etc. Pesticides are chemicals designed to kill a variety of pests, such as weeds, insects, rodents and fungi. They can be characterized on the basis of their function as insecticidal, herbicidal, rodenticidal, fungicidal, etc. and also on the basis of chemical nature, i.e. organophosphates, organochlorines, S-triazines and pyrethroids. Potentially hazardous environmental toxicants like pesticides display a broad spectrum of biological effects, being toxic not only to target organisms but also to humans. In a broad sense, every disease is caused either by environmental factors or

genetic factors; the latter includes natural deterioration of the body with age.

The objective of pesticide usage to prevent crop loss from insects remains unachieved. Widespread use of synthetic chemicals after the Second World War has revolutionized agricultural practice. Initial studies of the possible health effects of these substances on humans were small and reassuring. During the 1960s, however, it became evident that persistent usage of pesticides had an adverse impact on the ecological communities³. This led to a number of extensive epidemiologic investigations exploring the possible impact of these exposures on human health. These studies faced numerous methodological problems common to environmental epidemiology, and even today, our understanding of the relationship between pesticides and human health is limited⁴.

Improper usage of pesticides and usage of banned pesticides lead to neurological and haematological complications in individuals^{5,6}. This is at a high incidence, especially in India where there is continuous usage of different pesticides, including banned ones, based on the season and crop produced. Household use of pesticides without prior knowledge of their toxic effects and use without safety precautions also lead to such incidents. Genotoxicity of pesticides on peripheral blood lymphocytes has been reported^{7,8}. Cytotoxicity of pesticides has also been reported⁹⁻¹². Current understanding of carcinogenesis favours the conclusion that even a tiny dose of a genotoxic agent can initiate the process of converting a normal cell to a malignant one. The aim of this study was to examine the health outcomes of a group of agricultural workers occupationally exposed to pesticides in Andhra Pradesh, India. Most of the exposed groups were apparently healthy when they came to the hospital with minor ailments. Therefore occupational epidemiology consists of recording the data of such groups.

Blood samples from 200 healthy adults, non-smoking, non-alcohol, i.e. with little or no exposure to pesticides were collected as controls. An equal number of blood samples was collected from male and female individuals (adults) exposed directly or indirectly to farm or indoor pesticide sprays and who came to the hospital with complaints and disease-like symptoms.

Similarly, blood samples from 100 children living in clean environs along with an equal number of samples from children living in pesticide-prevalent environments were collected in sterile vacutainers containing anti-coagulant for epidemiological studies. This study was carried out with the approval of the Ethical Committee of Mahavir Hospital and Research Centre and prior consent of the subjects (Annexures I and II). Blood samples obtained from the cases and controls were diagnosed for certain parameters.

Epidemiological parameters were recorded according to the WHO norms where age, sex, exposure period and clinical symptoms and other associated parameters were systematically recorded as given below.

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Chromosomal analysis is an extremely sensitive indicator of genotoxicity of harmful chemical and biological agents. This was carried out using the method described by Moorhead *et al.*¹³ with lymphocyte cultures. Lymphocytes were stimulated to divide in RPMI-1640 medium supplemented with 10% FCS. Subsequent cell division was arrested at the 70th hour by the addition of colchicine and slides were prepared. Results of the metaphase analysis were recorded by viewing under the light microscope with photographic or digital imaging. At least 100 metaphases were analysed and chromosomes were classified based on size and pattern of bands obtained after subjecting the slides to G-banding. Karyotyping was performed to check whether the individuals had any chromosomal aberrations as described by Moorhead *et al.*¹³.

DNA damage studies were carried out using the Single Cell Gel Electrophoresis method as described by Singh *et al.*¹⁴. This assay depicts the extent of DNA damage as comet tails in the nucleated cells of peripheral blood samples of the individuals. This assay is now widely used for detecting the extent of DNA damage and is also referred to as Comet Assay.

ADVIA-60-Cell Counter (auto analyser) was used to assess the percentage of haemoglobin, RBC and WBC count in all the 600 samples collected from adults and children. Differential leukocyte counting was done using Leishman's staining protocol. Thin smears of blood were spread on clean, non-greasy, dry slides and subsequently stained using Leishman's stain for 2 min and washed with buffered saline for 20 min. The smears were washed, air-dried, cover-slipped and observed under light microscope at 10 and 40x magnification. Blood samples of all individuals (exposed and unexposed to pesticides) were tested for ESR using Wintrobe's method. The rates of sedimentation of blood were recorded using the readings on the Wintrobe's tube.

Bone marrow tests for diagnosis of chronic myeloid leukaemia (CML) were done on subjects showing abnormal results during blood testing. Bone marrow aspirates were taken to confirm CML morphology in the subjects. The aspirates were cultured in RPMI-1640 medium and processed.

The results of chromosomal aberrations and comet tail lengths in the comet assay were subjected to statistical approaches like mean and standard deviation. *P* value (<0.05) was also checked for the significance in these analysis.

Organophosphorus (OP), organochlorine (OC), carbamate (CA) and pyrethroid pesticides are known to cause acute toxic effects in workers exposed during spraying on the fields for pest control regime relating to direct exposure. Pregnant women who are exposed during the spraying periods can also be affected and young children accompanying the adults are also affected. Indirect exposure here refers to the after-spray effects of vector control and repellants, etc. for indoor pests. Pristine environs include those places which are away from fields, generally referred to as urban areas.

The list of pesticides to which the adults, children and pregnant mothers were exposed is included in Annexures III and IV.

The occurrence of the possible causes of disease was compared between cases and controls. Individuals exposed to pesticides who came to the hospital had health problems like headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps and diarrhoea. Some had problems like excessive sweating and salivation while others complained of chest discomfort and tightness with involuntary urination and bowel movement and some had slowed heartbeat; all these are the symptoms of pesticide poisoning. Epidemiological investigations on the blood samples collected from these individuals are presented in Table 1. Most patients were asymptomatic at diagnosis and were discovered when a WBC count performed at the time of a routine medical examination was found to be abnormal.

Chromosomal analysis of individuals exposed to pesticides showed few aberrations like gaps and breaks, and there was significant increase in the satellite associations compared to the normal individuals. It was found that the aberrations were more in those with longer exposure. No chromosomal abnormalities in any of the patients showing neurological or haematological disorders were observed. There were no signs of genetic predisposition, consanguinity and clear absence of Philadelphia chromosome.

Comet assay for DNA damage studies showed significant increase in the tail lengths in farmers compared to the control groups and the damage is directly proportional to the duration of exposure.

The peripheral blood smear showed leukocytosis, with prominence of maturing myeloid precursors (Tables 2 and 3). Significant percentages of blasts and basophiles were seen. The bone marrow biopsy showed prominence of myeloid. There was significant damage in the indivi-

Table 1. Epidemiological data of subjects showing symptoms of CML

Observation	A	B	C	D	E
Age/sex	34 yrs/M	32 yrs/F	35 yrs/M	30 yrs/F	28 yrs/M
Kind of exposure	Direct	Indirect	Direct	Direct	Indirect
Length of exposure (yrs)	2	1	5	2-3	2
Other genetic abnormalities	Nil	Nil	Nil	Nil	Nil
Neurological complications	Nil	Nil	Nil	Nil	Nil

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Table 2. Clinical profile and CBP analysis of individuals showing symptoms of CML

Observation	A	B	C	D	E
Haemoglobin (g/dl)	10	12	Normal	9	10
RBC counts (million cells/cubic mm)	3	3.6	Normal	2.7	3
Platelets	Normal	Less than normal	Less than normal	Normal	Normal
ESR values	40/first hour 80/second hour	35/first hour 85/second hour	50/first hour 90/second hour	60/first hour 115/second hour	40/first hour 80/second hour
Leucocytosis	Negative	Positive	Positive	Negative	Negative
Splenomegaly	Positive	Negative	Positive	Positive	Negative
Hepatomegaly	Negative	Negative	Negative	Positive	Positive
Presence of myelocytes	Positive	Positive	Positive	Positive	Positive

Table 3. Differential leukocyte count of WBCs of subjects showing symptoms of CML

Type of cell	Individuals showing CML				
	A	B	C	D	E
Total leucocyte count (cells/cubic mm)	183,800	93,000	230,000	130,000	92,600
Neutrophils (%)	18	36	39	29	52
Lymphocytes (%)	15	21	14	28	23
Basophils (%)	6	7	4	4	7
Eosinophils (%)	3	2	4	6	1
Monocytes (%)	4	6	3	3	2
Promyelocytes (%)	8	7	6	4	5
Myelocytes (%)	19	12	19	15	14
Metamyelocytes (%)	16	3	7	4	4
Blast cells (%)	11	6	4	7	2

Table 4. Observations of children showing mental retardation

Observation	A	B	C
Length of exposure of mother during pregnancy (months)	3-4	6-7	4-5
Type of exposure	Indirect	Indirect	Indirect
Karyotyping	Normal	Normal	Normal
Consanguineous birth	No	No	No
Genetic predisposition	No	No	No
Mental retardation	Yes	Yes	Yes
Delayed milestones	Yes	Yes	Yes
Heart impairment	Negative	Positive	Negative
Respiratory complications	Negative	Positive	Negative
Other abnormalities	No	No	No

Individuals having hematological disorder. There was an increase of necrotic cells in these individuals.

Of the 200 exposed individuals approximately five showed morphologic features of CML, other than genotoxic effects and therefore, we carried out detailed investigations on these particular volunteers. The results are presented in Tables 2 and 3. No such effects were observed in controls and in exposed children. These five individuals gave information regarding pesticide exposure. The incidence of CML-like signs in both direct and indirect exposure to

pesticides in adults was about 2.5%. The results of bone marrow aspirates documented were taken to confirm CML-like signs in the subjects A, C and D – directly exposed to pesticides (usage at the farm fields), B – indirectly exposed to usage at home, E – live near farm lands (may be exposed to spray drift). The identities of persons were preserved for ethical reasons. One subject (33 yrs/male) who was directly exposed to pesticides showing CML-like signs expired recently due to neurological dysfunction prior to initiation of therapy.

Out of 100 children exposed to pesticides, three showed marked mental retardation and delayed milestones compared to age-matched siblings (Table 4). The incidence of such symptoms was 3%. All the 100 controls showed no such symptoms. The risk of mental retardation and delayed milestones is about 3% in children exposed to pesticides during foetal stages. A-C are subjects whose identities are preserved for ethical reasons. These subjects were all below the age of seven years.

In India, 70% of the population are farmers and they are the target group to be affected by the hazards of pesticide applications. Moreover, in developing countries the users are rather careless in handling pesticides. All pesticides in a given chemical group generally affect the human body in the same way; however, severity of the effects

varies depending on the formulation, concentration, toxicity and route of exposure of the pesticide. It is important, therefore, to know the type of pesticide associated with poisoning. Data covering more than one time point were collected. Many of the exposed individuals enrolled were already showing symptoms of headache, nausea, giddiness, restlessness and vomiting. These are retrospective studies since the investigations looked back from the disease to a possible cause. Also, confounding factors like age, social class, habits of smoking or drinking, or even high blood pressure or anaemia could influence the progression of the disease. Common findings at enrolment of the volunteers included fatigue, weight loss, anaemia and splenomegaly. There are several diseases linked with pesticide exposures, but in our clinical examination we found development of CML-like complications. Atypical presentations, including initial presentation in CML-BP without detectable chronic phase was seen. In this investigation epidemiology played a crucial role in identifying the cause of CML and linked it to pesticide exposure, since non-exposed persons remained unaffected.

Out of the 200 cases which we examined (pesticides-exposed), five individuals showed an increase in the total WBC counts (92,600–230,000 cells/cubic mm) compared to controls (4000–10,000 cells/cubic mm). There was also the presence of cells like promyelocytes, myelocytes, metamyelocytes and blast cells along with a decrease in the lymphocyte population. An increase in the total WBC count accompanied a decrease in RBCs, elevated ESR values and alterations in platelet counts. Vojdani *et al.*¹⁵ reported that pesticide exposure affected the functioning of the blood vascular system. Splenomegaly was also reported to have occurred due to hyperplasia and hepatomegaly in some subjects exposed to pesticides. The effect of the pesticides on the WBCs and liver function^{16–18} was also recorded.

Many of the signs and symptoms of CML occur because of undue proliferation of myeloid precursors replacing normal marrow cells. As a result, enough red blood cells are not produced, affecting the functioning of WBCs and blood platelets. Etiology of CML is not clear. Since factors predisposing to CML are not known, radiation or exposure to xenobiotics has been implicated in some cases. However, there does not appear to be an inherited disposition.

Shortage of normal WBCs (leucopenia) and, in particular, few mature granulocytes (neutropenia or granulocytopenia) increase the risk of infection. Neutropenia refers specifically to low levels of neutrophils (a type of granulocyte). Although patients with leukaemia may have very high WBC counts, the leukaemia cells do not protect against infection the way normal WBCs do. Chronic myelogenous leukaemia (also called CML, chronic myelocytic leukaemia, or chronic myeloid leukaemia) is a chronic disorder of the bone marrow, characterized by the overproduction of mostly mature WBCs of the granulocytic series. This is in contrast to the more serious condition of

acute myeloid leukaemia, characterized by the overproduction of immature (blast) cells of the granulocyte series.

The leukaemia-like condition observed here was not as a result of chromosomal aberrations as indicated by the normal karyotypes of the subjects. Moreover, all the cases showed a positive response to other treatment regimens like Hydroxyurea, Methotrexate, Cytarabine, Prednisone and Vincristine and have a currently normal health status. There was no recurrence of the leukaemia-like condition in the individuals, thereby indicating that pesticide toxicity can be reversed by proper treatment.

It is not clear whether exposure to pesticides is causally related to the rising rates of these cancers or due to other environmental toxicants. The mechanisms by which pesticides contribute to cancer vary¹⁸. Current understanding of carcinogenesis favours the conclusion that even a tiny dose of a genotoxic agent can initiate the process of converting a normal cell to a malignant one^{19,20}. Genotoxicity is one of the causes and in our study we found there was significant DNA damage in pesticide-exposed individuals with relation to the period of exposure. This reveals that the length of exposure also plays a significant role in DNA damage.

The study focuses primarily on human epidemiological evidence linking pesticide exposure and cancer. The pesticide specifically responsible for carcinogenesis²¹ has been reported in many human studies. Because occupations in agriculture involve use of multiple agents (including non-pesticidal chemicals), it is often difficult to determine what agent is linked to a specific end-point. The same problem occurs with home and environmental exposures, where multiple products are being used, their doses unmeasured, and their names long forgotten by those exposed.

Our observations also indicate that prenatal exposure to xenobiotics/pesticides might have led to mental retardation in 3% of the exposed children. Pedigree analyses of the children up to three generations revealed no genetic abnormalities and all the children showed normal karyotypes (Table 4). Neurological complications and delayed milestones can be attributed to maternal exposure to pesticides during pregnancy. Such pesticide toxicity could disrupt the cell cycle and cell differentiation leading to mental retardation and skeletal deformities in the embryos (teratogenic changes). One child with delayed milestones also showed congenital heart disease. Complications like congenital heart disease may occur based on time of exposure of the foetus to maternally circulating pesticide levels during pregnancy^{22,23}.

Some authors suggest that the susceptibility to pesticides depends mostly on the gene polymorphism at certain chromosomes on which the cancer genes are present, thus different individuals show varying degrees of response, while others believe that chronic exposures are critical^{24,25}. Therefore, it may be essential to study the subject at the individual level while assessing the risk of pesticide exposure. Since the subjects showing CML did not

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show specific genetic abnormality of the Philadelphia chromosome, it may also be concluded that pesticides might have caused point mutations in genes. Therefore, a more basic level of study concentrating on point mutations may further indicate the nature of pesticide toxicity. Our investigation strengthens the reports of others who diagnosed Philadelphia-negative CML²⁶⁻³¹.

The mechanisms by which pesticides contribute to cancer causation vary, and a pesticide may operate by more than one of the major mechanisms, which include genotoxic effects – producing direct changes in DNA. Whereas the usual concept of toxicity follows the principle that ‘the dose makes the poison’, genotoxic chemicals and hormone disruptors may have effects at very low doses without a true threshold below which no risk exists (the stochastic or probabilistic model). Current understanding of carcinogenesis favours the conclusion that even a tiny dose of a genotoxic agent can initiate the process of converting a normal cell to a malignant one.

Our investigation becomes more important because we report the clinical profile of CML at least in 2.5% of cases in relation to environmental pesticide exposures in

adult subjects. No such cases were reported in children of our study group below the age of 10 years. In view of the fact that pesticides can be mutagenic, teratogenic and carcinogenic, it is obvious that pesticide exposure to susceptible adults has caused CML and mental retardation along with delayed milestone in susceptible children. However, this study provides a direction to evaluate the general population exposed to pesticide, which might lead to cancers. Therefore, further studies on larger populations are warranted.

Although these studies have been limited to 600 (400 adults + 200 children, both exposed and unexposed) individuals, it is noteworthy that many of the reported increased risks are of greater magnitude than those just observed, suggesting that Indians living in rural areas are more exposed to the carcinogenic effects of pesticides. Future research on pesticides, especially in India, should include improved exposure assessment, evaluation of risk by the age at exposure, and investigation of possible gene–environment interactions. There is potential to prevent mental retardation and adulthood cancers by reducing or limiting exposure to pesticides.

Annexure I. Proforma/questionnaire for agricultural workers exposed to pesticides

Serial number	:	
Name	:	
Sex	:	Male/female
Age	:	
Address	:	
Location	:	
Period of exposure	:	
Workplace	:	
Status	:	
Marital status	:	Unmarried/Married
Number of pregnancies	:	
(a) Abortions	:	
(b) Miscarriages	:	
(c) Stillbirths	:	
(d) Infant mortality	:	
(i) Age	:	
(ii) Cause	:	
Number of normal children	:	
Number of abnormal children	:	
Type of abnormality seen	:	
Food habits	:	
	:	Vegetarian/Non-vegetarian
Staple food	:	Rice/wheat/any other
Source of drinking water	:	Well/borewell/tap water
Habits	:	Tea/coffee (no. of cups) :
	:	Non-smoker/smoker :
	:	Tobacco chewing/pan :
	:	Type/quantity :
	:	Non-alcoholic/alcoholic :
	:	Toddy/Arrack/IMEL :
Medical history of the subject	:	
Symptoms of toxicity	:	
Family history (pedigree)	:	
Signature of the Investigator and Date	:	

Annexure II. Patient consent form

I Confirm that	
I have read/understand/carefully considered the study	
I had a chance to ask questions and have been fully answered	
My participation is voluntary	
I agree to allow all study procedures as required	
I understand that I am free to discontinue at any stage	
I have received a copy of information and consent	
I am aware that the data and records are kept confidential	
This study has had a hospital ethics committee approval	
My address is	:
Patient name	:
Patient signature	:
Witness name	:
Signature	:
Name of the person taking consent	:
Signature	:

Annexure III.

- Pesticides used by subjects for household pests: Acephate, Chlorpyrifos, Chlordane, Dimethoate, Allethrin, Piperonyl Butoxide and Transfluthrin.
- Pesticides used on farm lands to control pests: Endosulfan, Chlorpyrifos, Profenofos, Monocrotophos, Carbofuran, Cypermethrin and Cyfluthrin.

Annexure IV.

Crops cultivated during exposure time:
Rice, wheat, jowar, barley, tomatoes, brinjals and chillies.

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Simulation of Antarctic sea ice

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Semtner's three-layer sea ice model and Winton's reformulated three-layer sea ice model have been applied to the Antarctic region (where there are chances of sea ice disappearing in the summer leading to open water) for simulating some features of sea ice using the ECMWF re-analysis data. The results of the simulation have been compared with the observation.

Keywords: Antarctica, ECMWF re-analysis, sea ice, Semtner model, Winton model.

It has been observed over the years that any change in the circulation pattern of the oceans, particularly the deep oceans, results in climatic changes. The characteristics and circulation of 50 to 60% of sub-surface waters in the oceans

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