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Malignant brain tumors (brain cancer) in orchard farmers of Kashmir linked to pesticides

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Abstract

The objective of this hospital based study was to determine the relationship between the patients of primary malignant brain tumors and their occupation. Retrospectively case files (and death certificates) of 432 patients of primary malignant brain tumors and 457 controls (non-tumor neurologic diseases), admitted simultaneously over a period of 4 years from Jan., 2005 to Dec., 2008, to the Neurosurgery, Sher- I -Kashmir Institute of Medical Sciences (SKIMS), Kashmir were studied. Each passing year showed an increase in the incidence of new cases of highly malignant brain tumors like glioblastoma multiforme and medulloblastomas, most i.e. 29.30% (114 out of 389) patients in 2008 as compared to 20.82% (81 out of 389) patients in 2005. The pesticide use in the Kashmir-Valley has increased ten-fold for the last 20 years. Analysis revealed that 90.04% (389 out of 432) patients were orchard farm workers, pesticide mixers, sprayers, foggers, orchard residents and orchard playing children exposed to the high levels of multiple types of neurotoxic and carcinogenic (chlorpyriphos, dimethoate, mancozeb and captan) chemicals as pesticides, fungicides and insecticides for a duration of 10 to 30 years. About 61.18% had direct and 38.81% had indirect exposure. The 58.61% (228 out of 389) orchard-farm patients had pesticide exposure at an age of 19 to 40 years. Most of the orchard-farm patients i.e. 44.47% (173 out of 389) had spent 10 to 20 years of life exposed to pesticides directly and/or indirectly and 70.52% (122 out of 173) were males. About 73.00% (284 out of 389) orchard-farm patients presented with symptoms and signs related to brain cancer at the age of 21 to 60 years and most of these 56.33% (160 out of 284) at the age between 41 to 60 years of life. The 9.96% (43 out of 432) patients were not exposed to pesticides. On the other hand only 119 patients out of 457 controls had recorded history of pesticide exposure and 338 were unrelated to pesticides. Familial gliomas emerged in three families. All orchard-farm related 389 patients had high grade tumors as compared to the non-orchard farm tumors. Mortality in orchard-farmer tumors exposed to pesticides was 12%. The 31.9% (124 out of 389) orchard-farm workers with higher (> 6334 u/l} levels of SCE were below 40 years and had pesticide exposure of 10 to 20 years from an early age. This was postulated to be due to chronic exposure to organophosphates, exposure to many other carcinogenic pesticides and non-cholinergic mechanism of action of organophosphates than the one that depresses the levels of acetyl cholinesterase (AChE) as in acute poisoning. However exact mechanism by which pesticides cause brain cancer needs experimental models in future. The study shows significant Case / Control (Odds Ratio) OR of 0. 28 ; hospital controls SCE (serum cholinesterase) Odds Ratio of 1.1 and family control SCE OR of 1.5.

Key words: Brain Cancer, Orchard-Farmers, Pesticides, Kashmir.

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Introduction

Occupational health hazards are well known. The widespread use of pesticides in the agricultural industry, to control the insects, pests and fungus and to enhance the crop and fruit production, is recognized as a major chemical health hazard for the orchard workers, residents and children by the direct contact and by polluting the aerial, soil and water environment. The residual concentrations of these toxic chemicals in the farm-workers have lead to

a variety of neurological dysfunctions [1,2,3]. Because of the similarity in the brain biochemistry, the pesticides are particularly neurotoxic to the humans and most lethal are organophosphates, carbamates and ethylenebisdithiocarbamates [4]. The primary action of the organophosphates and carbamates is to irreversibly inhibit the activity of the enzyme acetylcholinesterase (AChE) that hydrolyses the neurotransmitter acetylchline in both the peripheral and central nervous systems. This causes accumulation of acetylcholine at cholinergic synapses, leading to overstimulation of muscarinic and nicotinic receptors and thus neurotoxicity [5]. A variety of occupational exposure of workers in industries like rubber, oil refinery, chemical plant and polyvinyl chloride, have been reported to have the elevated risk of developing brain tumors [6]. The etiologic importance of exposure to pesticides has been reported by case control studies on childhood brain tumors arising after exposure to the chlordane and heptachlor [7, 8]. This was confirmed by a report on patients who died from malignant tumors among whom a high level of organochlorine compounds was found in the adipose tissue of those who had glioblastomas [9]. A study reported 2 of 7 patients from a cluster of primary brain tumors who were exposed to the pesticides [10]. Pesticides are suspected to be the potent risk factors for the lethal brain tumors especially gliomas in the children and adults [11]. Annual European Union (EU) pesticide use includes 0.108 million tons of fungicides, 0.08 million tons of herbicides, 21,000 tons of insecticides and 7000 tons of growth regulators – amounting roughly to half a kilogram of active substances for every man, woman and child living within the European Union (EU) [12]. The fruit production of Kashmir province of J&K, India is 1.5 million metric tons annually from a total orchard area of 0.2 million hectares which is sprayed and fogged with 7750 metric tons of fungicides and 3186 metric tons of insecticides right from March to November every year in 10 recommended scheduled stages from green tip and pink budding of the trees (pre-bloom) upto and after the harvest of the fruits (post-harvest). Though un-official frequency of unscheduled sprays by farmers is increased to 15 to 20.The excessive use of synthetic pesticides for the last three decades and increase in admission of high grade malignant brain tumors, with history of pesticide exposure, to the Neurosurgical centre SKIMS, Kashmir in the last 10 years has elicited high degree of suspicion of a link between the pesticides and malignant brain tumors (brain cancer).

Material and Methods

The Department of Neurosurgery, Sher-i-Kashmir Institute of Medical Sciences (SKIMS) Srinagar, Kashmir province of J&K, India, caters about 7-8 million ethnic non-migratory population of Kashmir province as a single centre. Since Kashmir Valley has high potential to produce dry and fresh fruits, the pesticides have been used in huge quantities over the last 30 years. All patients with the history of contact with the pesticides are subjected to the serial measurements of serum cholinesterase (SCE) as a protocol. The case files, including death summaries, of 432 patients admitted from Jan., 2005 to Dec.,2008 (4 years) and proved histopathologically as primary malignant brain tumors, were studied. The controls, 457 patient files with non-tumor brain conditions like brain abscesses, tuberculomas, meningitis, strokes, multiple sclerosis and epilepsy, admitted during the same period were also studied. All metastatic lesions to brain were excluded. The history (exposure directly occupational; farm-workers and indirectly; residents and playing children), clinical, biochemical, radiological and histopathological findings were recorded. The patients and the families were contacted to collect further information, follow up and to select randomly the familial controls. Further 50 controls from the families were included and 50 more controls selected from general population randomly after age, sex and socio-economic matching, to make a total of 557 controls. The historical information collected was age, sex, socio-economic status, type of work exposure e.g., mixer-loader applicators, sprayer, fogger in case of adults, number and type of chemicals exposed to, lifelong jobs, orchard guards and supervisors, whether following pesticide applicator precautions like avoiding use of expired drugs or spurious drugs, use of hand gloves, hand wash, masks and goggles, head gear and uniform, eating unwashed fruits from the orchards, location of residential house, location of drinking water source, location of play grounds of children and frequency of female family members visiting and working in the orchards, age at exposure, duration of exposure, age at the onset of symptoms after exposure. The serum cholinesterase was measured at the admission, indoors and before discharge of patient and the control. The mean of the three estimates was calculated and recorded. The data support the use of sequential postexposure plasma cholinesterase analyses to confirm the diagnosis of organophosphate-induced illness in the absence of baseline values [13]. The serum cholinesterase activity was measured by kinetic/ DGKC calorimetric method and EDTA samples were sent to the laboratory. The results are expressed in KU / L which is U / L x 1000. The laboratory at SKIMS, Srinagar and Dr Lal PathLabs at New Delhi used a reference range for serum cholinesterase as: 3167 to 6333 U / L. Two major forms of cholinesterase exist in vertebrates which hydrolyze acetylcholine. The Plasma Cholinesterase (Pseudo- or Butyryl Cholinesterase) is found in plasma, liver, pancreas and intestinal mucosa (liver being the main organ). Variations occur due to liver disease, chronic inflammation, malnutrition, morphine, codeine, succinylcholine administration and hypersensitivity reactions. The RBC Cholinesterase (True, Specific Cholinesterase) is found in nervous tissue, erythrocytes, Lung, spleen and grey matter. It is decreased in pernicious anemia and after antimalarial therapy. The estimation of acetyl cholinesterase

level in circulation is theoretically preferred in organophosphorus poisoning since it would reflect the degree of inhibition of synaptic cholinesterase at motor end plates. But, in practice, estimation of serum cholinesterase has an advantage because the measurement is simpler and more accurate than estimation of the acetylcholinesterase [13, 14, 15, 16]. The Serum cholinesterase levels can indicate the prior presence of cholinesterase inhibition even after recovery of acetylcholinesterase activity by pralidoxime in organophosphorus poisoning [17]. Though, in acute poisoning of organophosphates, the confirmation of diagnosis depends on demonstrating reduced cholinesterase activity in the circulating blood and the activity is expressed as percentage of normality of healthy adults [13]. But in Kashmir study (with increased or high normal serum cholinesterase) the organophosphates may have compound specific effects (non-cholinergic) unrelated to the common AChE inhibition, as shown by the similar effects of two organophosphates like chlorpyriphos and diazinon on the gene expression of neonatal rat brain with the doses not inducing biologically significant AChE-inhibition and yet both have notable disparities. The disadvantages of serum cholinesterase estimations are that the normal values are widely variable from one person to another as well as in the same individual at different times and low levels have been observed in some disease states and may also be genetically determined. The serum acetylcholinesterase (AChE) levels of patients and hospitalized controls were recorded three times and the mean level was considered and similarly levels were checked in the family and general controls. The patients with history of exposure to radiation and anti-mitotic drugs for whatever disease were excluded. The patients and controls with carcinomas, metastasis, hepatitis, acute infection, cirrhosis, nephrotic syndrome, thyrotoxicosis, hemochromatosis, muscular dystrophies and psychiatric disorders were excluded. The data collected was compiled, the SPSS version 11.5 statistical programme was used to compute odds ratio (OR) adjusted for the matching variable (age, sex, orchard workers, non-pesticide exposed cases, serum cholinesterase). The law of variance was applied where ever required.

Results

Background

The atmosphere of Valley of Kashmir is ideal for fresh and dry fruit production, which is the major economic source of the Kashmiris (Table 1). The fruit production area spreads over around a 0.2 million hectares of land, of which 0.11 million hectares (> 50%) are under apple production, involving about 40% population of the Kashmir directly as orchard-farmers, chemical sprayers etc., and indirectly like children playing in and around orchards, residential houses in orchards etc. Millions of tons of pesticides, insecticides and fungicides (chemicals like chlorpyriphos, mancozeb, captan, dimethoate, phosalone etc) are being used by the orchard farmers to spray the plants, fruits and the leaves at different stages of growth to avoid the infestations and destruction of the fruits. For the last three decades the farmers have favoured and adapted to the newer synthetic but hazardous fungicides and pesticides, never applied before, to enhance the fruit production by replacing the older relatively non-hazardous inorganic sulphur (Table 1). The incidence of the malignant brain tumors in Kashmir has shown an upward surge in the last 10 years especially in the orchard farming districts.

Table1. *Kashmir Orchard Area, number of Orchard-Farm Patients, type of Pesticide Usage and approximate consumption*

Orchard District	**Orchard Area** (H)	**Pesticides Utilized (MT)**			**No. of Cases**
		Chlorpyriphos	Mancozeb	Captan	
Budgam	29572	Pink-Bud Stage	Fruit let Stage	Fruit let Stage	55
Anantnag	28697	50% 3 lit / H	30% 12 kg / H	10% 12 kg / Hec-	63
Barmaulla (Varmul)	28031			tare	88
Kupawara	25583	Fruit let Stage	Pre-harvest 30%	Pre harvest Stage	45
Shopian	24073	50% 4 lit / H	Stage 12 Kg / H	20% 12 Kg / Hec-	50
Kulgam	18926			tare	34
Pulwama	17664				25
Others (Srinagar etc)	20563				29
Total	**193109**	**3186 MT**	**3400 MT**	**4350 MT**	**389**

 # H = hectare, MT = metric ton (tonnes), lit = liter, Kg = kilogram
**Chlorpyriphos, Mancozeb and Captan are EU (European Union) labeled carcinogens*

Table 2. *Exposure data related to age and sex*

Age and Exposure		Males	Females	Total
Age at Exposure	Birth to 18 years	70	35	105
	19 to 40 years	166	62	228
	41 to 60 years	43	13	56
	61 to 80 years	0	0	0
Total		279	110	389
Duration of Exposure before onset of Symptoms				
	Upto 5 years	34	9	43
	5 to 10 years	78	17	95
	10 to 20 years	122	51	173
	20 to 30 years & more	45	33	78
Total		279	110	389
Age at onset of Symptoms	Birth to 10	7	4	11
	11 to 20 years	29	11	40
	21 to 40 years	87	37	124
	41 to 60 years	118	42	160
	61 to 80 years	38	16	54
Total		279	110	389

**Ages from an infant to 75 year old were involved.*
** 81 orchard resident families had 85 patients of brain cancer.*
** Familial brain cancer was found in three residential families: one family with brain tumors in mother and daughter, second family had tumors in 3 daughters and third had in two siblings, brother and sister.*
**23 pregnant females and 11 lactating mothers.*

Age and Sex

The 389 (90%) cases out of 432 primary malignant brain tumors (excluding metastatic lesions), proved by histopathology after open or closed biopsy, were orchard-farm workers in various ways while 43 had no pesticide exposure. Among 457 controls only 119 had pesticide exposure. Out of 389 (100%) patients there were 31 (7.9%) children, 304 (78.1%) adults and 54 (13.9%) elderly people (Table 2). A total of 279 (71.7%) males and 110 (28.3%) female patients were exposed to pesticides. The eldest patient was 75 years male with a hemispheric glioblastoma multiforme and the youngest was an infant female baby with medulloblastoma. A mortality of 12% (47 cases out of 389) was revealed among orchard (pesticide exposed) farm workers as compared to 7% (3 out of 43) deaths in non-pesticide workers.

Mode of Exposure

(a) Direct, 61.18% (238 out of 389) Orchard-farm patients

***** Pesticide mixers, sprayers, foggers and orchard tillers

* Females as residents or frequent visitors to help men, collect wood and part-time orchard helpers

* Men and women as weed handlers, fillers and orchard supervisors

* Avoiding use of hand gloves, hand wash, masks and goggles, head gear and uniform, while using short applicators.

(b) Indirect, 38.81% (151 out of 389) Orchard-farm patients

* Children either lived in the residential houses with their parents or had exposure by spending most of the time schooling and playing in and around orchards.

* The drinking water from the pesticide contaminated orchard wells was source of pesticide exposure for all ages and sexes

* Residential houses constructed in the orchard-areas are at risk of pesticide contamination due to unwashed foot wears of farm workers, storing farming tools, usage of orchard eatables like vegetables and fruits, hay stacks from orchards carried into homes to store for

cattle and contaminated orchard dust carried by the winds into orchard homes.

Age at Exposure

The 58.61% (228 out of 389) orchard-farm patients had pesticide exposure at an age of 19 to 40 years, including 23 pregnant women and 11 lactating mothers, and most (72.80% =166 out of 228) of these were males. While 26.99% (105 out of 389) patients, mostly children, had pesticide exposure at and below 18 years (Table 2). There was no history of exposure beyond 61 years of age in any orchard-farm patients.

Duration of Exposure before onset of symptoms

Most of the orchard-farm patients i.e. 44.47% (173 out of 389) had spent 10 to 20 years of life exposed to pesticides directly and/or indirectly and 70.52% (122 out of 173) were males. And 31.9% (124 out of 389) of these from both sexes were younger than 40 years initiating exposure at an early age and had higher (< 6334 u/L) SCE levels. While 20.05% (78 out of 389) orchard-farm patients had 20 to 30 years of history of pesticide exposure (Table 2). A group of 9.16% (54 out of 389) elderly (61 to 80 years) orchard-farm patients with high grade brain cancer had lifelong pesticide exposure mostly weed handlers, fillers and orchard supervisors.

Age at onset of symptoms

About 73.00% (284 out of 389) orchard-farm patients presented with symptoms and signs related to brain cancer at the age of 21 to 60 years and most of these 56.33% (160 out of 284) at the age between 41 to 60 years of life. Most of the males 73.47% (205 out of 279) presented in the age group of 21 to 60 years of life. While 35.48% (11 out of 31) children presented with the malignant brain lesions at the age of 10 years and below (Table 2). Only about 13.88% (54 out of 389) orchard-farm patients presented in the elderly age-group of 61 to 80 years, mostly males 70.37% (38 out of 54). The all elderly age-group orchard-farm patients had high grade brain cancer. There were 81 orchard residential families, 85 members of them suffered malignant brain tumors with 6 female (2 adults and 4 children) members from three families i.e. mother/daughter duo from one, three sisters from another family and brother / sister siblings from third family. The 31 children either lived in the residential houses with their parents or had exposure by spending most of the time schooling and playing in and around orchards.

Pesticide Abuse among Orchard-Farmers

The orchard area with most cases, 88 patients out of 389 cases, was Sopore, Baramulla (Varmul) and 63 cases from Anantnag, followed by the Budgam, Shopian, Kupwara, Srinagar etc. The orchard area of these Districts amounts to about 140 thousand hectares of a total of 0.193 million hectares, with an annual consumption of thousands of metric tonnes of pesticides when calculated at the officially recommended doses. The officially recommended dithiocarbamates fungicide (EU labelled carcinogen mancozeb) has been sprayed over in the apple orchards at the dose of 12.00Kg/hectare twice in a season {Plant Protection Spray Schedule, Information Office, Department of Horticulture Kashmir Division, Srinagar (Fig. 3**)}** in fruitlet stage (pen size) and pre-harvest stage, which alone amounts to about 700 MT (metric tonnes) per season (Table 1). The mancozeb is in use for the last 30 years in the Kashmir valley (Fig. 4). Meanwhile this fungicide is much abused unofficially by the farmers, by its excessive use on the apple trees in the stages not-recommended and on fruits not recommended, like walnut, almond, cherry etc (Fig. 5**)**. Similarly the use of captan – a dicarboximides fungicide, EU labeled carcinogen, used excessively by the orchard farmers than the recommended doses of 12.00Kg/hectare, is directly absorbed through skin, inhalation and ingestion. This was extensively sprayed in all stages and seasons of fruit growth without wearing any special body gear or uniform, bear handed with short applicators (Fig. 5). Among organophosphates, the most used chemicals were chlorpyriphos (dose 50% 4 liters/hectare) and dimethoate (Fig. Many of these are carcinogens e.g. chlorpyriphos, captan, mancozeb etc (Table 1). The 207 males out of 304 adults (age 19 – 50 years) were mostly pesticide mixers, sprayers, foggers and orchard tillers using short applicators with bare hands, naked eyes, without any body gear and airway protection (to protect mucous membranes). The 97 adult females were frequent visitors and part-time orchard workers. Of these 23 pregnant females had been exposed to the pesticides in their antenatal and postnatal periods and 11 were lactating mothers. Most adults (44.47% = 173 out of 389 orchard-farm patients) had more than 10 to 20 years working history in different (apple, walnut, almond, cherry, pear, grapes, peach, apricot etc.) orchards . (Table 2). Among organochlorine the endosulfan has been the choice of farmers which is a known convulsant, mutant and carcinogen. This is used on all trees in almost every stage of fruit growth (Fig. 3). The farmers have used cheaper and spurious drugs in greater quantities, than quality and officially sampled drugs (Fig. 7). The instructions labeled on the pesticide packs, including date of expiry, were neither attended to nor followed by the farmers. The drinking water from the pesticide contaminated orchard wells was source of pesticide exposure for all ages and sexes. Out of 432 cases, 43 (non-pesticide) primary malignant brain tumors were not associated in any way with orchards or pesticides. From 457 hospital controls only 119 controls had history of pesticide exposure and 338 had no relation to pesticides, OR = 0.28 significant. Residential houses constructed in the orchards are at risk of pesticide contamination due to unwashed foot wears of farm workers, farming tools, vegetables, fruits, hay stacks for cattle carried into homes and contaminated orchard dust carried by the winds. The drinking

water wells in the orchards and orchard residential houses are contaminated due to the direct spill of the chemical mixture into the wells while constituting the spray and indirectly by the contaminated soil washings drained by rain into the drinking wells. Thus drinking water site is the persistent source of pesticide exposure.

Symptoms and signs

The most common presenting symptom has been the headache, followed by the epilepsy, vomiting and the visual blurring. The most common sign found was papilloedema. The CT-Scan and MRI brain were the diagnostic tools of choice (Figs. 1 & 2). All patients were operated upon and histological diagnosis sought and recorded.

Incidence and Histological types of Malignant Brain Tumors

From Jan., 2005 to Dec., 2008, each passing year showed an increase in the incidence of new cases of highly malignant brain tumors like glioblastoma multiforme and medulloblastomas, most i.e. 29.30% (114 out of 389) patients in 2008 as compared to 20.82% (81 out of 389) patients in 2005. Such trend is not seen in case of non-pesticide tumors (Table 3). Most of the 389 orchard-farm workers with malignant brain tumors were having highly malignant astrocytomas, glioblastoma multiforme, anaplastic oligodendrogliomas, ependymomas, choroid plexus papillomas, medulloblastoma etc as compared to the 43

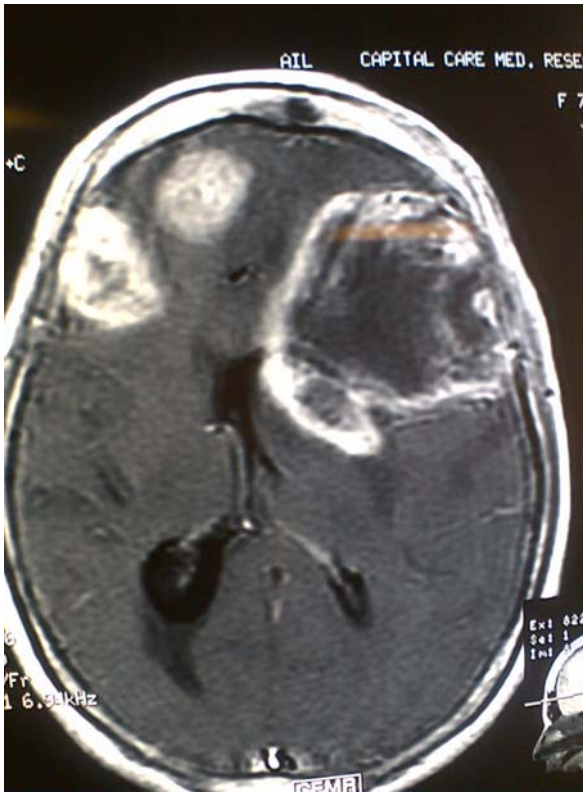


Figure 1. *T1WI Axial MRI Brain of a Kashmiri orchard-Farm worker showing Multicentric Glioma..*

non-pesticide malignant brain tumors. The children had mostly primitive neuroectodermal tumors. Some of the patients had high grade multicentric type of gliomas with worst prognosis (Fig. 1 & 2). The elderly age-group (61 – 80 years) of 54 orchard-farm workers had all worst grade (WHO- IV) of brain cancer (glioblastoma multiforme).

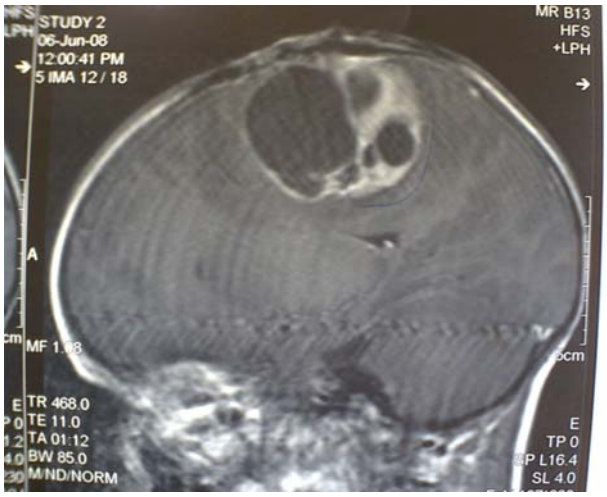


Figure 2. *Saggital MRI Brain with a Glioblastoma Multiforme in a male Kashmiri Orchard-Farm worker*

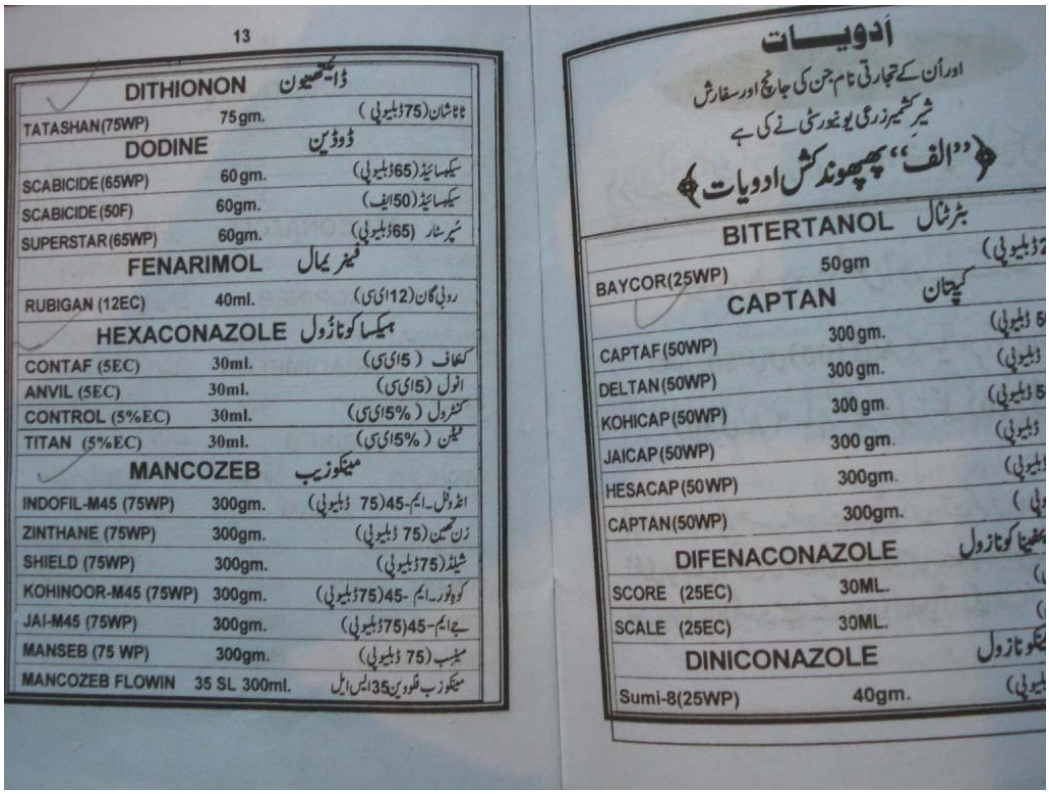
Pesticides and cholinesterase

The number of pesticides used by the orchard farmers in Kashmir was more than 30 fungicides, insecticides, acaricides etc most of these spurious. Among these chemical groups like organophosphates, organochlorines, carbamates, ethylenebisdithiocarbamates, pyrethroids, phosphines, dicarboximides, inorganics, ureas, dinitroanilines, etc. are used at large scales. The officially recommended dithiocarbamates fungicide (EU labelled carcinogen mancozeb) has been used in the apple orchards at the dose of 12.00Kg/hectare twice in a season (Fig. 3**)}** in fruitlet stage (pen size) and pre-harvest stage, which alone amounts to about 700 MT (metric tonnes) per season (Table 1). The mancozeb is in use for the last 30 years in the Kashmir valley (Fig. 4). Similarly the use of captan – a dicarboximides fungicide, EU labeled carcinogen, used excessively by the orchard farmers than the recommended doses of 12.00Kg/hectare, is directly absorbed through skin, inhalation and ingestion. Among organophosphates, the most used chemicals were chlorpyriphos (dose 50% 4 liters/hectare) and dimethoate (Fig. 6). Both are neurotoxic insecticides and depress the serum acetyl cholinesterase (AChE) levels (Table 3 & 4). However results revealed that only 45.3% (176 out of 389) patients of those exposed to pesticides 5 to 10 years had lower serum cholinesterase levels of < 3167 u/l. This also revealed normal SCE (3167 – 6333 u/l) in 22.8% (89 out of 389) patients and higher levels of > 6334 u/l in 31.9% (124 out of 389) patients equally in both the sexes (Table 4). The 31.9% orchard-farm workers with higher (> 6334 u/l}

levels of SCE were below 40 years and had pesticide exposure of 10 to 20 years from an early age. But the significant **Case / Control: O**R (Odds Ratio) = 0. 28; Hospital controls SCE (serum cholinesterase): OR= 1.1; Family control SCE: OR = 1.5 points the finger of suspicion towards the link between pesticides and brain cancer (Table 5). The reason for the altered levels of SCE enzyme may be either different, non-cholinergic, mechanism of action of organophosphates triggered through cyclic AMP or continuous chronic poisoning rather than acute which depresses the AChE levels or a mixture of different pesticides with different actions on the central nervous system. The causes may also be racial, genetic or immunological.

Figure 3. *Officially Recommended Spray-Schedules in the Orchard Farms of Kashmir have not been Followed usually by farmers.*

Figure 4. *The Fungicide Mancozeb (EthyleneBisdithiocarbamate), a Carcinogen, has been long in use in all orchards of Kashmir.*



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DITHIONON		ڈائتھیون
TATASHAN(75WP)	75gm.	ٹاٹاشان(75ڈبلیوپی)
DODINE		**ڈوڈین**
SCABICIDE(65WP)	60gm.	سکیبسائیڈ(65ڈبلیوپی)
SCABICIDE(50F)	60gm.	سکیبسائیڈ(50ایف)
SUPERSTAR(65WP)	60gm.	سپرسٹار (65ڈبلیوپی)
FENARIMOL		**فینریمال**
RUBIGAN (12EC)	40ml.	روبی گان(12ای سی)
HEXACONAZOLE		**ہیکساکوناژول**
CONTAF (5EC)	30ml.	کنٹاف (5ای سی)
ANVIL (5EC)	30ml.	انویل (5ای سی)
CONTROL (5%EC)	30ml.	کنٹرول (5%ای سی)
TITAN (5%EC)	30ml.	ٹیٹان (5%ای سی)
MANCOZEB		**مینکوزیب**
INDOFIL-M45 (75WP)	300gm.	انڈوفل-ایم-45 (75ڈبلیوپی)
ZINTHANE (75WP)	300gm.	زن تھین (75ڈبلیوپی)
SHIELD (75WP)	300gm.	شیلڈ(75ڈبلیوپی)
KOHINOOR-M45 (75WP)	300gm.	کوہنور-ایم-45(75ڈبلیوپی)
JAI-M45 (75WP)	300gm.	جےایم-45(75ڈبلیوپی)
MANSEB (75 WP)	300gm.	منسب (75 ڈبلیوپی)
MANCOZEB FLOWIN	35 SL 300ml.	میکوزیب فلووین35ایس ایل

ادویات

اور اُن کے تجارتی نام جن کی جانچ اور سفارش
شیرِ کشمیر زرعی یونیورسٹی نے کی ہے

"الف" پھپھوند کش ادویات

BITERTANOL		بٹرٹانل
BAYCOR(25WP)	50gm	(ڈبلیوپی
CAPTAN		**کپتان**
CAPTAF(50WP)	300gm.	(ڈبلیوپی
DELTAN(50WP)	300gm.	(ڈبلیوپی
KOHICAP(50WP)	300gm.	(ڈبلیوپی
JAICAP(50WP)	300gm.	(ڈبلیوپی
HESACAP(50WP)	300gm.	(ڈبلیوپی
CAPTAN(50WP)	300gm.	(ڈبلیوپی
DIFENACONAZOLE		**ڈیفینا کوناژول**
SCORE (25EC)	30ML.	
SCALE (25EC)	30ML.	
DINICONAZOLE		**ڈینکوناژول**
Sumi-8(25WP)	40gm.	(ڈبلیوپی

Figure 5. *All types of Pesticides, irrespective of their health hazardous activity, are used by the Kashmiri Orchard farmers.*

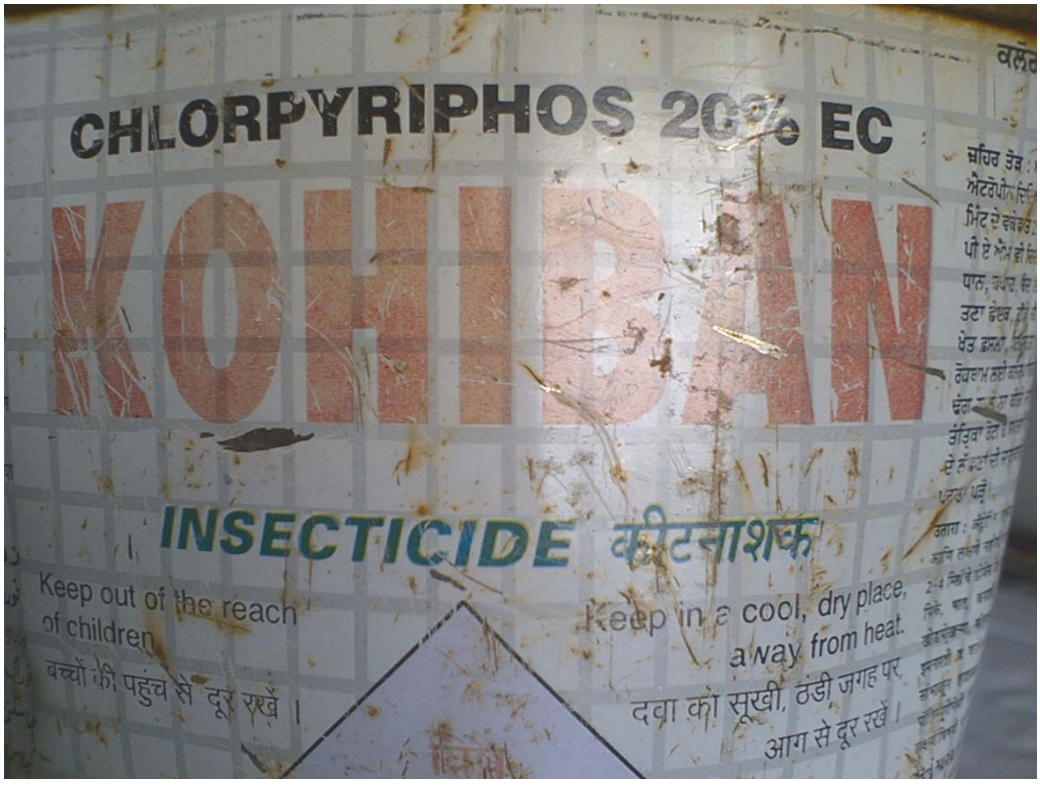

Figure. 6. *The Chlorpyriphos, most studied organophosphate, is known to act through non-cholinergic mechanisms to induce Brain Cancer*.

Spurious pesticides damage apple crop in Varmul

Growers say losses in crores of rupees; department waits for laboratory reports

UMER MAQBOOL DAR

Varmul, May 20: The use of spurious pesticides has wreaked havoc with the orchards in this north Kashmir district, fruit growers said.

A group of growers told Greater Kashmir on Tuesday that spraying of spurious and low graded pesticides has damaged the budding apple crop worth crores of rupees in different parts here.

"In some orchards the leaves of trees have burnt down and the apples have fallen 24 hours after these pesticides were sprayed on such trees," said Ghulam Rasool Bhat, president,

Figure 7. *The low quality, un-sampled, Spurious Pesticides - An ominous human health hazard.*

Table 3*. Histological Types of Primary Malignant Brain Tumors and incidence in Orchard–Farm Workers and Non – Pesticide patients from 2005 2008*

Histological Type	No. of Orchard Worker Patients/ Year					No. of Non-Pesticides Patients
	Total	Years				
	4 years	2005	2006	2007	2008	
Glioblastoma Multiforme (WHO gr. IV)	96	16	18	26	36	5
Anaplastic Astrocytomas (WHO grade–III)	67	13	15	18	21	4
Astrocytoma (WHO grade - II)	38	8	7	12	11	15
Anaplastic Oligodendroglioma (WHO grade-III)	28	6	8	7	7	2
Oligodendroglioma (WHO grade-II)	30	9	8	9	4	11
Anaplastic Ependymoma (WHO grade-III)	21	4	6	7	4	-
Ependymoma (WHO grade - II)	28	7	7	6	8	6
Anaplastic Oligo-astrocytoma (WHO grade III)	7	3	1	2	1	-
Mixed Oligo-astrocytoma (WHO grade II)	10	4	2	1	3	-
Gliosarcoma	11	4	4	3	-	
Gliomatosis Cerebri	6	-	1	2	3	-
Choroid plexus papilloma	19	5	5	4	5	-
Ganglioglioma	5	-	1	2	2	-
Esthesio Neuroblastoma	2	-	-	1	1	-
Pineocytoma	3	-	1	1	1	-
Medulloblastoma	15	2	4	4	5	-
Retinoblastoma	3	-	-	1	2	-
TOTAL	389	81	88	106	114	43

- *Some patients had high grade multicentric gliomas.*
- *Mortality in cases: 12% in orchard workers and 7% in non-pesticide tumors*
- *Glioblastoma multiforme and Medulloblastomas show increase in the incidence from the year 2005 to 2008*

Table 4. *Serum Cholinesterase (SCE) Levels in Orchard-Farm Worker Patients and non-pesticide Exposed Patients and controls.*

Cases / Controls	**Serum Cholinesterase (SCE) Levels (u/l)**			**Total**
Cases:	Decreased (< 3167)	Normal (3167-6333)	Increased (>6333)	
(a) Orchard-Farm Workers	176	89	124	389
(b) Non-Pesticide	1	38	4	43
Sub Total	*177*	*127*	*128*	*432*
Hospital Control:				
(a) Orchard-Farm Workers	2	10	7	19
(b) Non-Pesticide	17	354	67	438
Sub Total	*19*	*364*	*74*	*457*
Family Control				
(a) Orchard-Farm Workers	6	20	8	34
(b) Non-Pesticide	4	4	8	16
Sub Total	*10*	*24*	*16*	*50*
Generation Control				
(a) Orchard-Farm Workers	2	6	1	9
(b) Non-Pesticide	5	19	17	41
Sub Total	*7*	*25*	*18*	*50*
Grand Total	213	540	236	989

**P value = 0.0001 Hospital controls SCE: OR= 1.1; Family control= 1.5*

**Case/Control: OR= 0.28 ;*

**Depressed SCE levels in 82.6% (176 orchard-farmers out of 213 controls whether orchard-farmers or non-pesticide exposed) patients predicts the even more frequently in orchard-farm workers, though variations in Kashmiris are common.*

Table 5. *Retrospective case-control studies which evaluated the pesticide-brain tumor link*

Study	**No. & source of cases**	**No. & source of controls**	**Type of Exposure**	**Method**	**Results**
1. Thomas et al 1986	718 brain tumor deaths	738 controls	occupation	Death certificates	OR=0.8; 95%CI=0.4-1.8 (NO)
2. Speers et al 1988	202 Texas males died of gliomas	238 males	occupation	Death certificates	OR=0.61; 95%CI=0.3-1.22(NO
3. Musicco et al 1988	420 pts. of gliomas hospitalised	465 non-glioma brain tumors & 277 non-tumor pts. Of nerologic disorders	occupation and residence	interview	RR=1.6; 95%CI=1.06-2.42 (SIG)
4. Reif et al 1989	452 registered brain cancer pts.	19452 non-brain cancer pts.	occupation	interview	OR=1.3; 95%CI=1.0-1.7(SIG)
5. Schlehofer et al 1990	226 pts. With primary brain tumors in Germany	418 population controls	occupation	questionnaire	RR=1.1; 95%CI=0.7-1.9(NO)
6. Forastiere et al 1993	1674 male cancer deaths from Italian agricultural region	Random samples of 480 individuals selected from same regional mortality file as being deceased from all causes	occupation	Regional mortality file (death certificates)	OR=1.04; 95%CI=0.43-2.44 (NO)
7. Rashid et al (Kashmir study)	Out of 432 pts. of malignant	Of 457 hospital controls 119 orchard-	Occupation and resi-	Hospital files, medical records	Case/Control: OR= 0.28 ;

	brain tumor hospitalized, 389 orchard-farm workers	farmers, 50 family and 50 general controls	dence	& family/patient interaction	Hospital controls SCE: OR= 1.1; Family control= 1.5

Familial Gliomas

Three families among 81 orchard residential families had more than one member having primary malignant brain tumor. The first belonged to Baramulla (Varmul) and the two members were mother with anaplastic astrocytoma in left frontal lobe and the 10 year old daughter with a medulloblastoma in posterior fossa. The second family comprised of three sisters from the Srinagar District, who presented from eldest to youngest in a time span of 3 years. The eldest sister had ependymoma in right temporal lobe, the younger medulloblastomas in posterior fossa and the youngest had choroid plexus papilloma in left occipital lobe. The third family from Uri, Baramulla had two siblings, 10 year brother with ependymoma in fourth ventricle and 13 years sister with oligodendroglioma in right parital lobe. The reason for familial primary malignant brain tumors could be common stimulatory agent. These three families revealed extensive use of multiple pesticides including mancozeb, chlorpyriphos and captan. The history has revealed that about 16 female patients of primary brain cancer had multiple abortions, still births and babies delivered with congenital anomalies of brain and spinal cord.

Discussion

The present study on the orchard farmers of Kashmir shows an every year incidental increase in high grade brain cancer (glioblastoma multiforme and medulloblastomas) from Jan., 2005 to Dec., 2008. This study also revealed 31 children with brain cancer, the youngest being an infant. The prenatal, natal and postnatal exposure to pesticides has created enough of doubt concerning the toxicity and mitotic abnormalities in the developing brain of pregnant patients. The 16 female patients had delivered babies with congenital central nervous system malformations. The endosulfan has been one of the most used and abused pesticide. Chlorpyriphos is the most extensively studied organophosphate with respect to the neurotoxicity in the laboratory models where prenatal and neonatal exposure has lead to a variety of behavioural abnormalities in both the mice and rats. Chlorpyriphos exposure in rat embryo cultures at concentrations comparable to those found in human meconium, showed mitotic abnormalities and apoptosis during the neural tube development stage. However exposure during gestation lead to deficits in brain cell numbers, neuritic projections and synaptic communication, which emerged in adolescence and continued into adulthood. The deficits elicited by prenatal exposure to chlorpyriphos are evident even at exposures below the threshold for detectable AChE inhibition, i.e. far below the 70% inhibition of AChE required for systemic toxicity in adults. These findings suggest that chlorpyriphos also acts via non-AChE inhibition mechanisms to cause neurotoxicity [18, 19, 20, 21]. The Kashmir study shows difference in the levels of SCE among pesticide exposed farm workers patients (Table 4). The 31.9% (124 out of 389) orchard-farm workers with higher (> 6334 u/l} levels of SCE were below 40 years and had pesticide exposure of 10 to 20 years from an early age The non-cholinergic mechanisms of chlorpyriphos are not clear but a possible target may be the signalling cascades involved in neuronal and hormonal inputs, including the cyclic - AMP – protein kinase A cascade, receptor signalling through protein kinase C, and direct effects on the expression and function of nuclear transcription factors mediating the switch from proliferation to differentiation, including c-fos, p53, AP-1, SP 1 and CREB (Ca^{2+}/ cAMP response element binding protein) [22]. This has opened the possibility that organophosphates may have compound specific effects unrelated to the common AChE inhibition, as shown by the similar effects of two organophosphates chlorpyriphos and diazinon on the gene expression of neonatal rat brain with the doses not inducing biologically significant AChE-inhibition and yet both have notable disparities [23, 24, 25]. The pesticide-exposed orchard farmers of Kashmir with primary brain cancer showed a lot of variation in the levels of serum cholinesterase (SCE). Although 45.3% orchard-farm patients had depressed levels of SCE but 54.7% had normal and higher serum SCE levels with a significant Case / Control : OR = 0. 28 ; Hospital controls SCE (serum cholinesterase): OR= 1.1; Family control SCE: OR = 1.5 predicting decrease in SCE levels more often (Table 4 & 5). The non-cholinergic mechanism, slow and chronic poisoning with chlorpyriphos and mixed exposure to pesticides may be the probable causes for the neurotoxicity and stimulation of brain cancers in Kashmir. The primary malignant brain tumors in Kashmir are, reportedly, on a rise since 1999, especially among elderly population. A study showed glioblastomas multiforme accounting for 69.4% of all gliomas [26]. The headache and epilepsy has been the most common symptoms and signs as reported in a study [27]. This is similar to this hospital based case-control study. Epidemiological studies show associations with neurodevelopmental deficits on exposure to the mixed pesticides. Laboratory experimental studies using model compounds suggest that many pesticides currently used in Europe – including organophosphates, carbamates, pyrethroids, ethylenebisdithiocarbamates and chlorophenoxy herbicides – can cause neurodevelopmen-

tal toxicity [4]. The organochlorine pesticide, endosulfan, is reported to be lipophilc in nature and neurotoxic [28]. Endosulfan is a epileptogenic and experimentally a teratogenic, tumorogenic, carcinogenic and the human mutation is reported [29]. The manufacturer of chlorpyriphos, DowElanco, has agreed to restrict its recommended uses in fleas, ticks and pets [30]. A study that assessed mortality rates among vineyard workers in 89 geographical locations in France found a significantly higher incidence of brain cancer among those exposed to pesticides compared to the French population [31]. Similar to this study, the Kashmir study reveals the highest incidence of primary brain cancer in the geographical areas of Baramulla (Sopore, Varmul), Anantnag, Budgam, Shopian and Kupwara which comprises of most of the orchard areas of Kashmir (Table 1). A total mortality of 12% was recorded in the pesticide exposed orchard farmers as compared to 7% non-pesticide patients. Many farmers using fungicides reported the use of commercial compounds of copper sulphate, some of which contain methylurea - a carcinogen of nervous system in animals [32]. In Europe, the grapes receive 15% of total synthetic (active substance) pesticides applied to major crops. The synthetic fungicides applied to grapes include substances like dithiocarbamates, a family of chemicals in which pesticides like maneb and mancozeb are EU (European Union) classified carcinogens. Among hazardous pesticide list commonly found in the food items purchased in EU are proven carcinogens like maneb, procymidone, iprodione and captan. While procymidone has 93% and iprodione 100% transfer rate from grapes to wine, both are proven carcinogens as reported by French ministry of agriculture [33, 34]. Compared to the Europe, the Kashmir province of J&K state, India is 1/20th in area. The amount of pesticides and fungicides sprayed are amounting to the thousands of metric tonnes of mancozeb, captan, chlorpyriphos, dimethoate etc. Familial gliomas have been reported in many studies but not in pesticide workers. The present study recorded three families with 6 female members having deadly primary brain cancer and some of the cases even with multicentric high grade gliomas. There are many reports where brothers, parent and child in the families suffered similar types of brain cancer [35, 36]. Dithiocarbamates are non-cholinesterase inhibiting and sulphur-containing carbamates which are primarily used as fungicides and herbicides. There are four major classes, of which the ethylenebisdithiocarbamates (EBDC) like mancozeb, maneb and zineb are EU (European Union) labelled carcinogens. Mancozeb is linked to the uncoupling of the mitochondrial electron transport chain which generates reactive oxygen species leading to neuronal toxicity [37]. Owing to the rapid dermal, inhalational and oral absorption of the mancozeb, the un-gloved, un-masked and un-protectively clothed Kashmiri orchard workers who spray tonnes of this pesticide are much vulnerable to its toxicity and carcinogenic effects (Table 1). Epidemiologically it is difficult to study the risk of a specific pesticide as a cause of brain tumor, because the exposure is not limited to one chemical only but a mixture of multiple pesticides in a spray or a fog [32]. A case-control study revealed that among household pesticides, pest-strips have been reported to be the most consistent pesticides related to a variety of childhood cancers including brain cancer [38]. The childhood cancers in the pesticide workers of Kashmir study are 7.9% (31 out of 389) and most of these were primitive neuroectodermal tumors which have worst prognosis and fatal outcome (Table 2). However authors in an epidemiological review revealed that great majority of cohort studies of chemical workers employed in the manufacture of pesticides did not indicate an excess of brain cancer mortality. But few cohort studies of pesticide applicators showed elevated relative risk for excess mortality due to brain cancer [39]. The present Kashmir study finds substantial amount of evidence in favour of a relationship between the malignant brain tumors (brain cancer) and pesticide workers in the orchard farms of Kashmir with a significant Case / Control : OR = 0. 28 ; Hospital controls SCE (serum cholinesterase): OR= 1.1; Family control SCE: OR = 1.5 (Table 4 and 5). Evaluation of a series of retrospective case-control studies revealed significant link between occupation and the brain cancer. The studies of Musicco et al in 1988 showed a significant relative risk (RR) of 1.6 and 95% confidence interval (CI) of 1.06 to 2.42 and Reif et al 1989 reported a significant odds ratio (OR) of 1.3 and a 95% confidence interval (CI) of 1.0 to 1.7. However Thomas et al and others depicted non-significant relationship between the two [32,40,41,42,43,44].

Conclusion

The association between the malignant brain tumors and pesticide insult is still in dilemma.

This study provides many evidences to link primary malignant brain tumors in Kashmiri orchard-farm workers with pesticides. The study revealed a Case / Control OR (Odds Ratio) of 0. 28. Although chemically there appear prominent variations in the serum cholinesterase (SCE) levels between Kashmiris exposed to pesticides and people from other geographical locations but the SCE levels in Hospital controls depicts an Odds Ratio (OR) of 1.1 and SCE in Family controls shows an OR = 1.5 (Table 4 and 5) which predicts decreased levels in 45.3% orchard-farm workers more frequently than all controls and 31.9% orchard-farm patients. The SCE levels were higher than 6334 u/L in 31.9% (124 out of 389) orchard-farm patients younger than 40 years and exposed to pesticides for more than 10 to 20 years from an early age. This may be racial, genetic or else the non-cholinergic mechanisms of chlorpyriphos where the possible target may be the signalling cascades involved in neuronal and hormonal inputs, including the cyclic - AMP – protein kinase A cascade. Clinically the link between the pesticides and brain cancer appears quite strong and possible but accurate epidemiol-

ogical studies are yet to document this association. This is in part due to lack of study of action of a single pesticide in an individual case because of exposure to multiple pesticides in one time. However laboratory and animal studies are in favour of such a link. In the future, studies are needed to accurately localize the link. One more worry has been emergence of familial gliomas in pesticide handlers, orchard-farm workers and residents.

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